THE INFLUENCE OF CALCIUM CHANNEL

BLOCKERS ON CYCLOSPORIN A NEPHROTOXICITY

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Intrarenal vasoconstriction is a characteristic feature of cyclosporin A nephrotoxicity. This thesis investigates the effect of nifedipine, a dihydropyridine calcium channel blocker and potent renal vasodilator, on various aspects of experimental and clinical cyclosporin A nephrotoxicity.

In the surgically intact spontaneously hypertensive rat (two-kidney model), short-term administration of cyclosporin A (14 days) induced a marked reduction in glomerular filtration rate and effective renal plasma flow, and an increase in renal vascular resistance. These changes were both reversible on stopping treatment and without histological evidence of renal cell injury. Concomitant nifedipine from day 1 prevented these adverse alterations in renal haemodynamics. However, administering nifedipine after cyclosporin A (from day 7) failed to improve renal function. By contrast, neither renal denervation nor nifedipine prevented cyclosporin A-induced renal dysfunction in uninephrectomized rats. The mechanism mediating these alterations in renal vascular tone is unclear, however, studies in isolated blood vessels demonstrate that it is independent of phosphoinositide hydrolysis.

In man, short-term administration of nifedipine (28 days) to stable cyclosporin A-treated renal allograft recipients led to a significant, albeit small, increase in glomerular filtration rate, without a parallel increase in effective renal plasma flow or reduction in renal vascular resistance. Using cell culture techniques, this beneficial effect was not secondary to a reduction in the uptake of cyclosporin A into proximal tubular cells.

Thus, these results suggest that nifedipine can under certain experimental and clinical conditions ameliorate cyclosporin A-induced renal dysfunction. The protective mechanism afforded appears to involve both vascular and non-vascular mechanisms. Adaptive changes in renal haemodynamics occurring after uninephrectomy may account for the poor response in this model. Finally, the failure of renal denervation to preserve renal function infers that the sympathetic nervous system is not mediating cyclosporin A-induced alterations in renal vascular tone.

To the memory of my father

PREFACE

Cyclosporin A, a potent immunosuppressive drug, has afforded immense benefit in transplantation and in the treatment of several immune-related diseases. Nonetheless, initial enthusiasm has been tempered by its propensity to induce acute and chronic nephrotoxicity. Although the acute nephrotoxic effects are rapidly reversible with a reduction in the daily cyclosporin A dose, concern has been expressed regarding the reversibility of the long-term effects on the kidney. The precise pathophysiological mechanism(s) underlying cyclosporin A nephrotoxicity is presently unclear, however, experimental and clinical research has suggested that haemodynamic factors play a pivotal role.

Therapeutic options to limit cyclosporin A nephrotoxicity are limited, since low-dose protocols retain their nephrotoxic potential and conversion to other immunosuppressive regimens may precipitate allograft rejection. However, pharmacological intervention with renal vasodilators to ameliorate the intrarenal vasoconstriction that characterises cyclosporin A therapy, may improve and potentially prolong renal function.

The present series of experiments investigates the effect of nifedipine, a dihydropyridine calcium channel blocker and potent renal vasodilator, on renal haemodynamics in both an experimental rat model and in renal allograft recipients receiving cyclosporin A. Further studies explore the mechanisms activating the alterations in vascular tone and the basis of the nephroprotection conferred by calcium channel blockers.

ACKNOWLEDGEMENTS

CONTENTS

iv

ABBREVIATIONS

^{51}Cr-EDTA	Chromium^{51} - labelled ethylenediaminetetra-acetate
AA	Arachidonic acid
AZA	Azathioprine
Ca^{2+}	Calcium
C_K	Clearance of potassium
C_{Na}	Clearance of sodium
CYA	Cyclosporin A
ERPF	Effective renal plasma flow
FE_K	Fractional excretion of potassium
FE_{Na}	Fractional excretion of sodium
FF	Filtration fraction
GFR	Glomerular filtration rate
i.m.	intramuscular
i.p.	Intraperitoneal
i.v.	Intravenous
IP_3	Inositol 1,4,5 - trisphosphate
K_f	Glomerular ultrafiltration coefficient
MAP	Mean arterial pressure
NAG	N-acetyl-β-D-glucosaminidase
NIF	Nifedipine
NIF veh	Nifedipine vehicle
Oil	Olive oil
PG	Prostaglandin
PFR	Proximal fractional reabsorption
RAS	Renin-angiotensin system
RBF	Renal blood flow
RFRC	Renal functional reserve capacity
RVR	Renal vascular resistance
s.c.	Subcutaneously
SHR	Spontaneously hypertensive rat
SNGFR	Single nephron glomerular filtration rate
$U_K V$	Absolute potassium excretion
$U_{Na} V$	Absolute sodium excretion

SECTION I

GENERAL INTRODUCTION

CHAPTER 1: THE DISCOVERY OF CYCLOSPORIN A

The isolation of CYA from two strains of fungi imperfecti,

Cylindrocarpon lucidium Booth and Tolypocladium inflatum Gams

(originally classified as Trichoderma polysporum Rifae) during routine

microbiological screening of soil samples collected in Wisconsin

(U.S.A.) and the Hardinger Vidda (Norway) in 1970, heralded a major

addition to the immunosuppressive armamentarium (Dreyfuss et al.,

1976). From these fungal isolates two crude metabolites with

antifungal properties were extracted and characterised as novel neutral

polypeptides. After routine pharmacological screening, Borel in 1972

discovered the marked immunosuppressive properties and specificity of

this crude preparation, suppressing the production of haemoagglutinin

titres of mice to sheep red blood cells (Borel, 1976; Borel et al.,

1977). Purification of these fungal metabolites into single components

was achieved by 1973, yielding pure CYA (Ruegger et al., 1976).

Subsequently, the structure, conformation and absolute configuration

was elucidated by chemical degradation and X-ray crystallography of an

iodo-derivative (Ruegger et al., 1976; Petcher et al., 1976). Further

purification processes led to the isolation of a plethora of

structurally related congeners, designated cyclosporin B to cyclosporin

Z (Von Wartburg and Traber, 1988). These structurally related

oligopeptides classified as neutral cyclic undecapeptides, consist of

ten trivial aminoacids and a unique C_9-aminoacid [(4R)-4-((E)-2-

butenyl)-4, N-dimethyl-L-threonine]. The presence of this latter

aminoacid at position 11 is paramount for maximal immunosuppressive

activity, as modification or substitution at this position with similar

chemical structures attenuates the biological activity of pure CYA (Von Wartburg and Traber, 1988).

The specificity of CYA immunosuppression and lack of myelotoxicity was rapidly communicated by Borel to the British Society for Immunology in 1976. This stimulated Calne and co-workers in Cambridge to initiate successful immunosuppressive studies in animal models of transplantation (Kostakis et al., 1977a; Kostakis et al., 1977b; Calne and White, 1977; Calne et al., 1978a). Although their initial studies in a rat heterotopic heart transplant model were complicated by an excessive number of deaths from the alcohol vehicle and infection associated with the high dose of CYA administered [30 to 150 mg/Kg daily via i.m. route], in the surviving rats not a single instance of cardiac rejection was observed (Kostakis et al., 1977a). Similar experiments repeated in this model using a lower dose of CYA [20 mg/Kg i.m. on days 0, 2, 4 and 6 only] dissolved in an olive oil vehicle were successful in prolonging rat heart allograft survival in most animals for an average of 36 days after the final injection, without excessive infection (Kostakis et al., 1977b). Later studies undertaken in mongrel dogs with a renal allograft (Calne and White, 1977) and in pigs receiving an MHC-incompatible orthotopic heart allograft (Calne et al., 1978a) confirmed CYA as a successful immunosuppressive agent in differing species. These preliminary animal experiments culminated in the first successful clinical trials of CYA in renal allograft (Calne et al., 1978b) and bone marrow transplantation (Powles et al., 1978). However, despite its ability to selectively suppress one arm of the immune response and improve graft

2

survival rates, it only became apparent from these pilot trials in man

that CYA was a nephrotoxic agent.

CHAPTER 2: CYA NEPHROTOXICITY IN MAN

Nephrotoxicity emerged as the major limiting side-effect

associated with the administration of CYA to man. In their preliminary

studies performed in renal allograft recipients receiving CYA [25

mg/Kg/day orally], Calne and co-workers observed an unexpected increase

in the number of cases of primary and secondary anuria (Calne et al.,

1978b; Calne et al., 1979). Furthermore, histological examination of

renal tissue obtained by transplant biopsy were of interest for either

their normality or mild degree of proximal tubular cell injury in nine

of fifteen patients with impaired renal function. Substantial

rejection was observed in only four patients, with mild changes in the

remaining two patients. The improvement in renal function that

followed a reduction in the daily dose of CYA or conversion to

conventional immunosuppressive regimens contributed further evidence to

implicate CYA as a nephrotoxic agent. Starzl et al (1980) concluded

that the abnormalities in renal function associated with CYA [17.5

mg/Kg/day orally] most likely represented varying degrees of

unrecognised rejection, although they too reported an improvement in

renal function in a significant proportion (in 7 of 22 patients) with a

reduction in the daily CYA dose. Other groups also observed an early

deterioration in renal function after administering CYA [17 mg/Kg/day

orally] to renal allograft recipients (Carpenter et al., 1981). In

this latter series immediate graft function was evident in all renal

allograft recipients, however, five of nine patients subsequently

developed oliguria requiring intervention with haemodialysis. In

addition, renal function was markedly impaired in a further two

patients with functioning allografts.

A similar pattern of events evolved in patients receiving CYA for non-renal transplant immunosuppression. Powles and co-workers reported a definite, but reversible increase in blood urea and serum creatinine in bone marrow transplant recipients administered high dose CYA [25 mg/Kg/day i.v.] (Powles et al., 1978; Powles et al., 1980). In their first study they described one patient who had developed moderate renal impairment and a second patient developing de novo haemolytic uraemic syndrome (HUS). HUS was also considered as a possible toxic effect of CYA after further reports in bone marrow recipients (Gluckman et al., 1981).

Renal impairment was also a feature of CYA therapy in patients with normal healthy native kidneys receiving CYA as an immunosuppressive agent and not undergoing organ transplantation. Increased serum creatinine and blood urea concentrations were reported in patients with primary biliary cirrhosis (Routhier et al., 1980) and an elevated blood urea in five of six patients with rheumatoid arthritis receiving CYA (Herramann and Mueller, 1979). More recent studies continue to document impaired renal function in patients with primary biliary cirrhosis, despite receiving low dose CYA therapy [4 mg/Kg/day] (Wiesner et al., 1990).

Despite the propensity of CYA to induce renal impairment, a marked increase in actuarial graft survival was reported by several large multicentre trials at 1 year (European Multicentre Trial Group, 1983; Canadian Multicentre Transplant Study Group, 1983). Actuarial graft survival at 1 year was 72 per cent in the European Multicentre

Study, compared to 52 per cent in the conventionally treated group; and in the Canadian Multicentre Study 1 year graft survival was 84 per cent compared to 67 per cent respectively. Later analysis of these studies confirmed a sustained benefit, although less striking; actuarial 3 year survival was 69 per cent in the CYA group compared to 58 per cent in the control group in the Canadian study, and actuarial 5 year survival 55 per cent versus 40 per cent in the European Study (Canadian Multicentre Transplant Study Group, 1986; European Multicentre Trial Group, 1987). The clinical experience with CYA in organ transplantation has recently been reviewed (Morris, 1988; Kahan, 1989).

CYA has achieved first-line immunosuppressive status in most transplant centres despite nephrotoxicity. Recently, attention has been directed at developing immunosuppressive protocols that incorporate a lower dose of CYA to minimise the risks of nephrotoxicity. Although this has been achieved to a certain extent, nephrotoxicity is still a major clinical problem, despite the use of low-dose CYA regimens (Morris, 1988; Myers et al., 1988a; Wiesner et al., 1990).

CHAPTER 3: CLINICAL AND PATHOLOGICAL FEATURES OF CYCLOSPORIN A

NEPHROTOXICITY IN MAN.

The widespread use of CYA since 1978 has allowed the prevalence

and severity of CYA nephrotoxicity in man to be more accurately

defined. At present the clinical spectrum of nephrotoxicity can be

classified into four discrete categories:

(i)   delayed renal allograft function,

(ii)   acute dose-dependent reversible nephrotoxicity,

(iii) acute arteriolopathy,

(iv)   chronic irreversible nephrotoxicity.

(i) delayed renal allograft function

Several groups reported an increased incidence of ischaemic

post-transplant oligoanuria, as well as a more prolonged recovery

period in renal allograft recipients receiving CYA (Canadian

Multicentre Transplant Study Group, 1983; Flechner et al., 1983a). In

many cases primary non-function was associated with initial high dose

intravenous therapy in the first week following transplantation

(Powell-Jackson et al., 1983; McGiffen et al., 1985). In addition, a

high incidence of acute renal failure was observed in patients with

poor cardiac function receiving CYA (Oyer et al., 1983; McGiffen et

al., 1985). These observations and a lower frequency of oligoanuria in

patients administered conventional immunosuppression inferred that CYA

was exacerbating renal ischaemic injury (Powell-Jackson et al., 1983;

McGiffen et al., 1985).

The relationship between renal ischaemia and primary non-

function was clearly demonstrated in studies performed in Australia. The incidence of primary non-function due to acute tubular necrosis was already high (often greater than 50 per cent) in certain parts of Australia, as harvesting of organs for transplantation was only possible after the donor heart had stopped beating, due to the absence of brain death legislation. Shiel et al (1984) observed that the incidence of primary non-function increased from 60 to 83 per cent after the introduction of CYA, and the mean duration to onset of renal allograft function increase from 12 days in patients receiving azathioprine and prednisolone immunosuppression to 23 days with CYA. Furthermore, renal function did not resume until CYA was replaced by conventional immunosuppression in up to one-quarter of patients developing protracted oligoanuria.

Although most patients with primary non-function regain function, overall graft survival was reported to be worse in patients whose kidneys had long (greater than 24 hours cold ischaemia) before CYA administration (Canadian Multicentre Transplant Study Group, 1983). The possibility that CYA was potentiating renal ischaemia was considered, although other groups had not witnessed an increase in the incidence of primary non-function, despite using similar CYA protocols (European Multicentre Trial Group, 1983; Milford et al., 1986).

The pathological features associated with CYA-induced oligoanuria in the early period post-transplant are non-specific (Mihatsch et al., 1988). Histology may disclose features of acute tubular necrosis, while diffuse interstitial fibrosis may develop in patients with protracted oligoanuria of more than 2 to 3 weeks

(Mihatsch et al., 1983; Farnsworth et al., 1984; Hall et al., 1985).
Excess collagen deposition and oedema may develop, leading to
separation of the tubules.

(ii) Acute reversible renal functional impairment

Acute, but reversible renal impairment is the most common
clinical manifestation of CYA nephrotoxicity (Kahan, 1989). The first
description of this phenomenon was in a bone marrow recipient
administered high dose CYA [25 mg/Kg/day] as a prophylactic agent
against graft-versus-host disease (Powles et al., 1978). It commonly
presents in the first few weeks to months after transplantation with
oliguria and a rising serum creatinine (Myers, 1986). Renal impairment
is usually mild, with serum creatinine concentrations
characteristically raised by less than 25 per cent, compared to
patients receiving conventional immunosuppressive regimens (Kahan,
1986). A characteristic feature is its transient nature and rapid
reversibility with a reduction in the daily dose of CYA (Flechner et
al., 1983b; Von Willebrand and Hayry, 1983). Normalisation of renal
function coinciding with lower CYA blood levels is observed in most
cases (Myers, 1986).

Acute nephrotoxicity is not unique to renal allograft
recipients, and is frequently observed in non-renal transplants (Myers
et al., 1984) and non-transplant settings in patients with previously
healthy native kidneys receiving CYA immunosuppression (Herramann and
Mueller, 1979; Routhier et al., 1980; Palestine et al., 1986a; Austin
et al., 1989).

9

Differentiating CYA-induced renal impairment from acute
rejection presents difficulties in renal allograft recipients, as
similar clinical features prevail. In CYA nephrotoxicity blood levels
are frequently high, although in the wake of prolonged cold ischaemia,
rejection or additional nephrotoxic agents, renal impairment may
develop within the therapeutic range (Mihatsch et al., 1983). In many
cases the diagnosis of nephrotoxicity is only established
retrospectively after an improvement in renal function is documented
with a reduction in the daily dose of CYA (Flechner et al., 1983b; Von
Willebrand and Hayry, 1983). Heterogeneity in individual sensitivity
may account for the development of tubular toxicity at low CYA trough
levels (Opelz, 1986).

A variety of non-specific morphological tubular abnormalities
also characterise acute CYA nephrotoxicity. These abnormalities
include giant mitochondria, isometric vacuolisation and
microcalcification. The giant mitochondria tend to predominate in the
convoluted section of the proximal tubule, whereas isometric
vacuolisation is mainly limited to the thick descending limb of the
loop of Henle. Furthermore, these changes may be focal or even absent
in the presence of clinical nephrotoxicity (Mihatsch et al., 1988).

(iii) Acute arteriolopathy.

CYA-induced vascular injury was recognised after vessel wall
abnormalities and a haemolytic uraemic-like syndrome with glomerular
capillary thrombosis were reported in bone marrow (Shulman et al.,
1981), liver (Bonser et al, 1984) and renal transplant recipients

10

(Mihatsch et al., 1983; Farnsworth et al., 1984; Neild et al., 1985).

Sommer et al (1985) described irreversible oligoanuria in renal allograft recipients, characterised by an obliterative arteriopathy involving interlobular and arcuate renal vessels. Serial histology in these patients demonstrated initial fibrin and platelet adherence to the vascular endothelium, followed by progressive intimal thickening and intraluminal narrowing. The absence of cellular infiltration led Sommer and co-workers to propose that CYA was directly responsible for these changes.

The morphological features of vascular-interstitial injury have been clearly characterised by Mihatsch and co-workers (Mihatsch et al., 1985; 1988). Three major abnormalities are recognised to occur either alone or in combination, including glomerular and arteriolar thrombi (Neild et al., 1985), arteriolopathy and interstitial fibrosis with tubular atrophy (Mihatsch et al., 1983). The vascular changes in arteriolopathy predominate in the afferent arterioles and may extend to the vascular pole of the glomerulus. Circular nodular protein deposits permeate the arteriolar wall, narrowing or occluding the vascular lumen. The protein deposits have been shown to consist of immunoglobulin and complement components and replace necrotic myocytes. Mucoid intimal thickening may occur and also contribute to luminal narrowing. All these abnormalities can precede or accompany stripe interstitial fibrosis, diffuse interstitial and tubular atrophy in the renal cortex. However, these abnormalities are again non-specific, with similar changes reported in other conditions associated with thrombotic microangiopathy. Although differentiation from vascular

11

rejection may present difficulties, in general the changes in CYA-associated arteriopathy predominate in the afferent arteriolar vessels, in contrast to vascular rejection.

(iv) chronic nephropathy

The most controversial aspect of CYA therapy relates to its propensity to induce chronic nephropathy, characterised by diffuse interstitial fibrosis and progressive irreversible renal impairment (Myers, 1986; Mihatsch et al., 1988). Although renal impairment is characteristically reversible after short-term CYA therapy (Sweny et al., 1981; Chapman et al., 1985), several groups reported that treatment for six months or longer induced a sustained elevation in the serum creatinine concentration in renal allograft recipients (Morris et al., 1983; Canadian Multicentre Transplant Study Group, 1983; European Multicentre Trial Group, 1983; Merion et al., 1984). Klintmalm et al (1984) demonstrated that histological analysis of serial renal biopsies taken one to four years post-transplant from patients without clinical episodes of rejection or acute CYA nephrotoxicity, revealed substantially greater degrees of interstitial fibrosis and tubular atrophy in the CYA group, in contrast to the azathioprine treated recipients. In addition, the severity of interstitial fibrosis correlated with the cumulative dose of CYA administered during the first six months.

To circumvent the complicating factor of differentiating renal allograft rejection from CYA-induced injury, Myers and co-workers studied cardiac allograft recipients with previously healthy native

kidneys (Myers et al., 1984; Myers et al., 1988a; Myers et al., 1988b).
Using accurate isotopic measurements of renal function a 50 per cent
reduction in glomerular filtration rate was documented after 12 to 24
months of treatment with CYA, as well as histological evidence of
atrophic tubular injury and interstitial fibrosis (Myers et al., 1984).
After 48 months markedly impaired renal function persisted, despite a
reduction in the daily dose or even complete withdrawal of CYA in some
patients (Myers et al., 1988b). Histological evidence of progressive
renal injury was documented without exception, with increased
glomerular sclerosis, ischaemic collapse of the glomerular tuft,
tubular atrophy and interstitial fibrosis. Furthermore, three patients
initially studied in 1984 had progressed to end-stage renal failure.
Detailed studies by other groups in patients with normal renal function
receiving CYA immunosuppression for uveitis have confirmed these
findings (Palestine et al., 1986a; Svenson et al., 1986; Austin et al.,
1989). Palestine and co-workers performed percutaneous renal biopsy in
17 patients receiving CYA for autoimmune uveitis for eleven to thirty-
nine months [mean CYA dose 4.2 to 15.4 mg/Kg/ day orally]. In all
patients biopsied evidence of interstitial fibrosis and/or tubular
atrophy was present. Later studies by this group also report that
progressive renal injury may occur, despite reducing the dose of CYA
administered (Austin et al., 1989).

In contrast, although most acknowledge that impaired renal
function often develops during the first six months of treatment in
renal allograft recipients receiving CYA, several groups have observed
stable renal function with continued administration for up to five

13

years (Kahan et al., 1985; Post et al., 1987; Scolari et al., 1987; European Multicentre Trial Group, 1987; Lewis et al., 1989). In addition, an excess rate of attrition of cadaveric renal allografts beyond the first post-transplant year has not been demonstrated by some groups (Gordon et al., 1987). However, these studies have been criticised for the insensitive techniques used to assess renal function, including serum creatinine, reciprocal of serum creatinine against time and creatinine clearance (Myers, 1986; Bennett and Porter, 1988).

In summary, the acute reduction in renal function observed in the early phase after introducing CYA to man is well recognised and appears to respond to dose reduction. However, the long-term effects of CYA on the kidney are currently controversial and will only be resolved with the continued surveillance of both renal function and morphology.

CHAPTER 4: PATHOPHYSIOLOGICAL MECHANISMS OF CYCLOSPORIN A

NEPHROTOXICITY

A rich blood supply and vast endothelial surface area relative to weight inherent in the kidney contribute to its susceptibility to injury from chemicals and drugs (Brenner and Stein, 1980). All elements of renal tissue are susceptible to injury, including glomerular, tubular and vascular tissue. Furthermore, the medullary countercurrent mechanism responsible for generating medullary and papilla hypertonicity, and the unique transport mechanisms of the tubular epithelium are able to concentrate potentially toxic compounds and thus, induce interstitial and tubular injury.

Three major pathophysiological mechanisms have been proposed to explain CYA-induced renal impairment:

(i)   direct tubular toxicity,

(ii)   altered intrarenal haemodynamics,

(iii) altered glomerular capillary permeability.

(i) TUBULAR TOXICITY

The final common pathogenetic pathway for the development of either ischaemic or nephrotoxic acute renal failure, is via renal tubular cell injury (Humes et al., 1985). Tubular cell damage culminating in impaired tubular function or intraluminal obstruction has been shown to play a major pathophysiological role in the reduction of GFR in a variety of experimental models of acute renal failure, including ischaemic acute renal failure (Donohoe et al., 1978), gentamicin (Avasthi et al., 1980), glycerol (Richards and DiBona, 1974)

15

and myoglobin induced renal impairment (Finckh, 1957).

Proximal tubular injury was an early morphological abnormality ascribed to CYA therapy. Borel et al (1980) originally described both swelling and vacuolisation of tubular cells, tubular necrosis with areas of active regeneration in rats administered high doses of CYA. Later studies by Whiting and co-workers also reported marked light microscopic abnormalities in the proximal tubular epithelium of Sprague-Dawley rats administered CYA for three weeks [50 mg/Kg/48 hours, 100 mg/Kg/48 hours and 100 mg/Kg/day via gavage]. These changes predominantly involved the epithelium lining the thick descending limb of Henle and incorporated varying degrees of cytoplasmic vacuolisation (Whiting et al., 1982; Blair et al., 1982). At the highest administered dose occasional necrotic tubular cells were evident. Electron microscopy helped clarify the tubular abnormalities further, demonstrating dilatation of both the smooth and rough endoplasmic reticulum, giant mitochondria and increased numbers of eosinophilic inclusion bodies (Ryffel et al., 1983; Siegl et al., 1983). These tubular abnormalities were not unique to rodents, and were similarly described in other species (Thomson et al., 1984) and man (Mihatsch et al., 1983).

Biochemical evidence of tubular damage in the form of enzymuria offered further support for CYA-induced tubular cell injury. A dose-dependent increase in the excretion of the proximal tubular cell brush border enzyme, N-acetyl-β-D-glucosaminidase (NAG) was initially documented in Sprague-Dawley rats administered CYA over a three week period (Whiting et al., 1982; Blair et al., 1982). Later studies by

the same group (Cunningham et al., 1984) and others (Perico et al., 1986a) demonstrated a direct relationship between the extent of tubular cell injury and urinary NAG excretion. Increased urinary NAG was also documented in renal allograft recipients receiving CYA, thus making a strong case for CYA behaving as a tubular toxin (Sweny et al., 1981).

By contrast, several groups have not been able to consistently demonstrate morphological abnormalities, despite inducing overt renal impairment. Indeed, earlier studies performed by Whiting and co-workers in Sprague-Dawley rats, administered CYA [100 mg/Kg/48 hours via gavage] for 21 days had failed to induce morphological abnormalities in the kidney despite an elevated serum urea and urinary NAG concentration (Thomson et al., 1981). Similar studies in Fischer rats administered CYA [25 to 40 mg/Kg/day by gavage] for 14 days by Schwass et al (1986) were not associated with histological evidence of proximal tubular cell damage, although serum urea and creatinine were also elevated, as well as a markedly depressed inulin clearance (by 59 per cent). Studies performed in other species contributed to the controversy. The acute administration of CYA [15 mg/Kg i.v.] to dogs subjected to unilateral renal ischaemia was associated with increased urinary excretion of the brush border enzyme gamma-glutamyl transferase and NAG from the contralateral normal kidney, even though renal function remained normal (Devineni et al., 1983). Other investigators have also emphasised the mild degree of morphological damage frequently observed in animal and clinical studies despite the presence of overt renal impairment (Hamilton et al., 1982; Gerkens et al., 1984). These disparate findings led the Aberdeen group to conclude that the degree

17

of proximal tubular cell damage induced by CYA was not only dose dependent, but also species, strain and sex dependent (Duncan et al., 1986).

The possibility that the tubular abnormalities witnessed after CYA may be non-specific artefacts associated with histological preparation of sections and/or the carrier vehicle used has also been entertained (Dieperink et al., 1983). In order to minimise fixation artifacts in vivo perfusion fixation was utilised by Dieperink et al (1986a). Using this technique no pathological changes were attributable to CYA.

In accord with observations made in animal models, tubular vacuolisation was shown to occur in the absence of renal dysfunction in man (Dieperink et al., 1987). Furthermore, the histological abnormalities characterising CYA nephrotoxicity, including tubular vacuolisation, eosinophilic inclusions and necrosis also were shown to be induced by the carrier vehicle, polyoxyethylated castor oil derivative, Cremophor EL (Kone et al., 1986; Chow et al., 1986). Thus, it is unclear to what extent some of the renal effects may be due to the vehicle and/or histological preparation.

The effect of CYA on tubular function has been investigated using different clearance markers, including sodium and lithium. In general drugs that induce renal injury tend to affect both the reabsorptive and secretory functions of the renal tubules. Characteristically, tubular injury depresses the reabsorption of both sodium and filtered fluid along the entire nephron. Thus, both the percentage of filtered sodium and volume of urine produced increases,

18

leading to an overall decrease in the concentration of the urine excreted. However, increased fractional excretion of sodium has not been a consistent feature with CYA therapy, with unchanged (Dieperink et al., 1986b; English et al., 1987; Dieperink et al., 1988; Whiting and Simpson, 1988a) or only mild increases in sodium excretion documented in animals (Bertani et al., 1987). Similar observations are reported in man (Dieperink et al., 1987; Whiting et al., 1988b; Morales et al., 1988; Vincent et al., 1988).

Most information regarding tubular integrity has been gleaned from studies utilizing lithium as a clearance marker for proximal tubular function (Dieperink et al., 1986a; Dieperink et al., 1986b; Dieperink et al., 1987; Whiting and Simpson, 1988a; Propper et al., 1989). Lithium is reabsorbed by the proximal tubule in parallel with sodium and water, whereas no detectable lithium reabsorption occurs in the distal tubule and collecting ducts. Lithium clearance therefore represents a measure of the delivery of proximal tubular fluid from the end of proximal tubule to the ascending limb of the loop of Henle. Using this technique Dieperink and co-workers demonstrated a dose-dependent increase in proximal fractional reabsorption (PFR) in the presence of CYA, indicating a functionally intact, but hypoperfused nephron. Decreased fractional lithium clearance has been consistently documented by this group in rat (Dieperink et al., 1983; Dieperink et al., 1986a; Dieperink et al., 1986b; Dieperink et al., 1988) and clinical studies (Dieperink et al., 1987). Although the validity of lithium clearance as a measure of proximal tubular function was questioned, a comparison with the standard occlusion time/transit time

19

direct method produced comparable data to the lithium clearance method
(Dieperink et al., 1986a). Their observations conflicted with the
general notion that CYA nephrotoxicity was primarily due to proximal
tubular cell damage, on the basis that if tubular injury was the
primary mechanism a decrease in PFR would be expected, and hence an
increase in lithium clearance. This salutary finding led them to
postulate that CYA nephrotoxicity was entirely functional, with the
physiological effects documented being compatible with a reduction in
filtration pressure, possibly secondary to preglomerular
vasoconstriction. However, they also intimated that a reduction in the
net ultrafiltration pressure may also result from a decrease in the
glomerular ultrafiltration coefficient (K_f), via an effect on the
hydraulic permeability or glomerular capillary surface area.

Recent studies by Whiting and Simpson (1988a) have derived
similar data in male Sprague-dawley rats administered CYA [50 mg/Kg/day
by gavage] for 14 days. They too documented a reduction in lithium
clearance, increased PFR, decreased total clearance and absolute distal
reabsorption of sodium and potassium, and reabsorption of water in the
distal tubule. However, absolute isosmotic reabsorption in the
proximal tubule and reabsorption in the distal tubule did not change,
although urine flow rate increased. Differences in experimental design
may partially explain the discrepancy in electrolyte and water
handling. Whiting and Simpson concluded from these studies that CYA-
induced nephrotoxicity is most likely a combination of tubular
toxicity, manifested by an increase in urinary NAG excretion,
glycosuria and urine flow rate, with afferent arteriolar

vasoconstriction accounting for the increased PFR.

Tubular obstruction at a site distal to the proximal tubule was also considered a potential mechanism to account for the reduction in GFR (Dieperink et al., 1986a). However, low intratubular pressures and absence of histological evidence of tubular obstruction was documented in micropuncture studies, excluding obstruction distal to the proximal tubule as the mechanism for the increase in PFR, reduced inulin clearance and increased RVR.

Clinical studies also indicate that the functional integrity of the renal tubules remains intact in the presence of CYA. Palestine et al (1986b) assessed renal tubular function in 32 consecutive patients treated with CYA for auto-immune uveitis (mean dose after three months was 9 mg/kg and after six months 10 mg/kg). No evidence of amino-aciduria, glycosuria, hypokalaemia, hypophosphataemia or hypouricaemia indicative of a Fanconi's syndrome, was detected during the three to six months observation period.

Distal tubular functional characteristics have received scant attention. Hyperkalaemia and metabolic acidosis are an occasional feature of cyclosporin toxicity in allograft recipients and may result from a primary defect in distal tubular function (Adu et al., 1983; European Multicentre Trial Group, 1983). Adu et al (1983) postulated that aldosterone deficiency associated with hyporeninism was a potential mechanism for these changes. However, a defect in distal tubular acidification and impaired potassium excretion has been documented in rats treated with CYA [25 mg/kg by gavage or i.p] for 8 days (Batlle et al., 1986).

21

In summary, the majority of data gleaned from tubular studies indicate that functional integrity is largely preserved. Thus, these findings lend support to the notion that an alteration in the renal haemodynamic circulation is the primary pathophysiological factor underlying CYA-induced renal impairment, and not tubular injury.

(ii) ALTERED RENAL HAEMODYNAMICS

Two distinct observations derived from animal models led to the suggestion that CYA directly influenced renal haemodynamics. First, morphological changes were evident in the renal vasculature of several species treated with CYA (Neild et al., 1983a; Neild et al., 1983b: Ryffel, 1986). Secondly, an immediate increase in RVR was consistently documented after intravenous CYA (Sullivan et al., 1985; Murray et al., 1985). CYA administration to rabbits was shown to induce glomerular capillary thrombosis and cortical necrosis, similar to the vascular changes frequently observed in the thrombotic microangiopathies, including haemolytic-uraemic syndrome (Neild et al., 1983a; 1983b). Exudative and proliferative vascular lesions were also observed in SHR administered CYA (Ryffel, 1986). This constellation of vascular changes were also reported in occasional patients receiving CYA immunosuppression. Mihatsch et al (1985) described an arteriolopathy in renal transplant patients recipients, consisting of intimal thickening, proliferation and hyalinosis. Farnsworth et al (1984) also described intimal proliferation and vascular thromboses in CYA treated patients with impaired renal function, although most patients had no evidence of vascular injury.

A major observation was the demonstration that intravenous CYA

led to an immediate increase in RVR and a concomitant reduction in GFR

and RBF, indicating that tubular toxicity was not the primary

pathophysiological mechanism (Sullivan et al., 1985; Murray et al.,

1985). Sullivan et al (1985) observed that the reduction in RBF in

rats infused with CYA [10 mg/kg] occurred without a reduction in mean

arterial pressure, therefore indicating that RVR had increased.

Furthermore, these perturbations in renal haemodynamics returned to

normal values 10 minutes after the infusion was stopped, inferring that

CYA had induced a reversible vasoconstriction of the renal vascular

bed. Furthermore, these alterations in renal haemodynamics persisted

with continued CYA administration. In chronic studies performed over 7

days [10 mg/Kg daily i.p. or i.v.] the renal abnormalities persisted,

with RBF decreased by 15 per cent, inulin clearance decreased by 43 per

cent and RVR increased by 13 per cent. Similarly, Murray et al (1985)

reported that in conscious rats administered either a single

intravenous infusion [20 mg/Kg] or administered CYA for 7 days [20

mg/Kg daily i.p], a marked reduction in RBF (47 and 49 per cent

respectively) and increase in RVR (79 and 82 per cent respectively)

occurred, supporting the notion that CYA-induced renal impairment was

mediated by an alteration in intrarenal haemodynamics. These changes

were dose-dependent, as low dose CYA infusions [10 mg/Kg] did not

influence renal haemodynamics.

Recently, visual confirmation of the changes in renal vascular

tone were presented by English et al (1987). Using morphometric

analysis of renal vascular perfusion casts, they demonstrated a

progressive reduction in luminal diameter of the afferent arteriole

with continued CYA therapy. After 7 days of CYA therapy [50 mg/Kg via

gavage] the afferent arteriole showed constriction near the glomerular

tuft, while the entire afferent arteriole was narrowed to 34 per cent

of control values after 14 days.

Altered renal haemodynamics is also a feature of CYA

nephrotoxicity in man. Curtis et al (1986) demonstrated in a group of

14 renal transplant recipients converted from CYA to azathioprine for

financial reasons 244±22 days after transplantation, a significant

increase in ERPF and reduction in RVR. In accord with animal studies,

immediate alterations in renal haemodynamics have been shown to occur

in healthy volunteers administered CYA. Conte et al (1989) documented

a rapid decrease in both inulin (27 per cent) and PAH clearance (41 per

cent), and a marked increment in RVR 60 minutes after an oral dose of

CYA [12 mg/Kg]. Similar observations are reported by others groups

(Messana et al., 1990; Weir et al., 1990).

The vasoconstrictor effects associated with CYA administration

are not solely confined to the renal vascular bed. Increased systemic

blood pressure has been documented in both animals (Lustig et al.,

1987; Whitworth et al., 1987; Nahman et al., 1988) and man receiving

CYA (Bellett et al., 1985; Curtis et al., 1986; Schachter, 1988;

Curtis, 1990). Furthermore, decreased liver perfusion has been

documented in man (Leunissen et al., 1987), while in the rat decreased

blood flow to the kidney, pancreas and spleen is described (Petric et

al., 1988).

The possibility that the carrier vehicle cremophore contained in

the intravenous preparation of CYA is responsible for the haemodynamic alterations has been addressed. Following the observation that tubular abnormalities were present in vehicle treated rats, Thiel et al (1986a) demonstrated that equivalent reductions in RBF could be induced by CYA or cremophore alone. Other studies in in vivo and ex vivo preparations either support (Racusen et al., 1987; Besarab et al., 1987; Finn et al., 1989) or refute these observations (Murray et al., 1985; Sullivan et al., 1985; Sumpio, 1988; Zimmerhackl et al., 1990). However, retinoleic acid, a component of cremophore is known to possess vasoactive properties in animals by virtue of its ability to induce histamine release (Lorenz et al., 1982). It is conceivable that cremophore contributes to the haemodynamic effects via histamine release, which in rodents exerts a predominant vasoconstrictive effect on the renal arterioles (Douglas, 1980).

Thus, the almost instantaneous reduction in GFR and RBF after intravenous CYA, together with abnormal vascular morphology strongly support the proposition that CYA nephrotoxicity is mediated via an alteration in intrarenal haemodynamics.

(iii) CHANGES IN GLOMERULAR PERMEABILITY

Micropuncture studies have contributed further insight into the glomerular haemodynamic alterations induced by CYA and other nephrotoxins. Reduction in glomerular capillary permeability is known to contribute to the renal impairment induced by other nephrotoxins, including gentamicin and uranyl nitrate-induced acute renal failure (Blantz, 1975; Baylis et al., 1977). Barros et al (1987a) intimated

that CYA may reduce glomerular permeability after documenting

significant reductions in whole kidney GFR, single nephron GFR and

glomerular capillary perfusion in euvolemic Munich-Wistar rats treated

with intravenous CYA [50 mg/Kg]. These alterations in intrarenal

haemodynamics were accompanied by a disproportionate increase in

efferent compared to afferent arteriolar resistance (360 per cent

versus 188 per cent respectively), leading to a significant rise in

glomerular capillary pressure and a 70 per cent reduction in glomerular

K_f. This latter observation led them to postulate that a CYA-induced

decline in glomerular K_f may be contributing to the overall reduction

in GFR. They suggested CYA might influence mesangial cell

contractility, thereby reducing glomerular surface area, and hence

glomerular K_f. Similar conclusions were reached by Myers several years

earlier in cardiac allograft recipients treated with CYA, after

demonstrating a 36 per cent reduction in glomerular membrane pore

density using various sized neutral dextrans (Myers et al., 1984).

Experimental studies using cultured mesangial cells support the

proposition that CYA has the capacity to directly influence glomerular

K_f by altering mesangial cell contractility. Meyer-Lehnert and Schrier

(1988) and Rodriguez-Puyol et al (1989) have both recently demonstrated

direct mesangial contraction with local application of CYA. In

addition, CYA enhanced mesangial contractility to vasoconstrictor

stimuli, including arginine vasopressin and angiotensin II. These

changes in vascular smooth muscle tone and mesangial cell contractility

appear to be accompanied by augmented transmembrane Ca^{2+} influx and

intracellular Ca^{2+} mobilisation (Pfeilschifter and Ruegg, 1987;

Meyer-Lehnert and Schrier, 1988; Pfeilschifter, 1988). These latter workers postulate that the perturbations in Ca^{2+} homeostasis within the mesangial cell are able to potentiate the contractile response in the presence of CYA, thereby contributing to the reduction in glomerular K_f, and ultimately in GFR. Recently, Wiegmann and co-workers have contributed further evidence to support the notion that CYA influences mesangial contractility, after demonstrating mesangial contraction and a significant reduction in glomerular K_f in isolated rat glomeruli (Wiegmann et al., 1990). Furthermore, as glomerular volume decreased to a minor degree, they concluded that the majority of the change in glomerular K_f was due to a decrease in hydraulic conductivity (L_p).

WHERE IS CYCLOSPORIN A ACTING WITHIN THE INTRARENAL CIRCULATION?

The major intrarenal target for CYA is still uncertain, although the weight of evidence favours the afferent arteriole and/or the glomerular mesangial cell. The majority of investigators report a reduction in both GFR and ERPF following either acute or chronic CYA administration to animals. Perico and co-workers are the only group to report a reduction in GFR without a fall in RBF (Bertani et al., 1987; Perico et al., 1986b; Perico et al., 1986c). Further analysis reveals that GFR and renal plasma flow tend to both decrease in parallel so that filtration fraction remains unchanged in the majority of animal studies (Murray et al., 1985; Sullivan et al., 1985; Dieperink et al., 1986b; Jackson et al., 1987; Kaskel et al., 1988) or decreases only slightly at higher doses (Jackson et al., 1987). Similarly in man, GFR and ERPF usually decrease in parallel leaving filtration fraction

unchanged (Bantle et al., 1987; Wheatley et al., 1987; Hoyer et al., 1987; Tegzess et al., 1988; Heering et al., 1988; Myers et al., 1988b) or slightly decreased (Myers et al., 1984; Tomlanovich et al., 1986; Myers et al., 1988a). Thus, this pattern of renal haemodynamic changes indicates that the increase in RVR following CYA is predominately due to preglomerular vasoconstriction.

Although the weight of evidence supports a preglomerular location for the vasoconstrictor effects, studies by Zimmerhackl et al (1990) suggest that the vasoconstrictor effects occur in the more proximal vessels of the preglomerular circulation. By directly visualising the vascular circulation of the hydronephrotic rat kidney model after acute CYA administration [30 to 50 mg/Kg i.v], they were able to demonstrate an immediate decrease in renal blood flow, secondary to marked vasoconstriction of the larger arcuate arteries, with a smaller decrease in the diameter of the efferent, but, not the afferent arteriole (Zimmerhackl et al., 1990). An explanation for the disparate findings reported in this latter study is unclear.

The mesangial cell is also considered a target following the demonstration that CYA decreases glomerular K_f (Barros et al., 1987a). However, later observations by the same group conflicted with these findings (Barros et al., 1987b). In their earlier studies the reduction in glomerular K_f was associated with a predominant vasoconstrictor effect on the efferent arteriole in Munich-Wistar rats administered CYA [50 mg/Kg intravenously]. Using a lower, but comparable dose to that used in clinical transplantation [25 mg/Kg i.v.], a predominant effect on the afferent arteriolar circulation was

observed, leading to a reduction in glomerular capillary pressure, but no alteration in glomerular K_f (Barros et al., 1987b). Similarly, conflicting data is also reported by other groups utilizing micropuncture preparations, with unchanged segmental resistances (Winston et al., 1987), predominately increased efferent (Kaskel et al., 1987a) or afferent arteriolar resistance documented (Thomson et al., 1989). In contrast, several groups using different methodology implicate changes in mesangial contractility as a significant factor accounting for the perturbations in intrarenal haemodynamics (Meyer-Lehnert and Schrier, 1988; Rodriguez-Puyol et al., 1989; Wiegmann et al., 1990). The conflicting data presented is most likely the result of the differing dose schedules and experimental models employed.

Thus, both micropuncture data and indirect evidence from animal and clinical studies suggest that CYA is acting both at the level of the afferent arteriole and the mesangial cell.

CHAPTER 5. THE FACTORS THAT MAY PLAY A ROLE IN MEDIATING

CYCLOSPORIN A-INDUCED VASOCONSTRICTION

The mechanism(s) underlying CYA nephrotoxicity is still a subject of intense debate and investigation (Thiel, 1986b; Humes et al., 1988). However, most workers agree that the reduction in RBF and increase in RVR is haemodynamically mediated. Although the factor(s) inducing renal arteriolar vasoconstriction and/or mesangial cell contraction has not yet been defined, several pathophysiological mechanisms have been proposed from data derived from experimental and clinical studies:

(i)   excess adrenergic nerve stimulation,

(ii)   excess renin-angiotensin system stimulation,

(iii)   altered eicosanoid production,

(iv)   intrinsic vasoconstrictor activity of CYA,

(v)   endothelin.

(i) EXCESS ADRENERGIC NERVE STIMULATION

The renal circulation is richly innervated with adrenergic nerve fibres (Kon, 1989). During low levels of adrenergic nerve stimulation in rats, equivalent constriction of the afferent and efferent arterioles is evident, leading to a parallel reduction in single nephron GFR and glomerular plasma flow rates and unchanged glomerular capillary pressure (Hermansson et al., 1981). However, higher frequencies of nerve stimulation induce a predominant increase in afferent arteriolar tone, thus reducing glomerular capillary pressure (Kon and Ichikawa, 1983). In addition, a marked reduction in

glomerular K_f following sympathetic nerve stimulation suggests that the renal nerves actively contribute to the regulation of glomerular capillary surface area (Kon, 1989).

Siegl et al (1983) initially proposed that the tremor, tachycardia, dysaesthesiae, hyperglycaemia, hypertension, increased plasma renin activity and reduction in sodium and potassium clearance induced by CYA in the SHR treated with CYA were compatible with stimulation of the sympathetic nervous system. Murray and co-workers also supported a role for CYA-induced sympathetic nerve activity modulating renal function following the demonstration that both renal denervation and the administration of the α-adrenergic blockers, phenoxybenzamine and prazosin, ameliorated both the CYA-induced increase in RVR and decrease in RBF, when administered to rats prior to CYA (Murray et al., 1985; Murray and Paller, 1986). They also demonstrated that in rats with unilateral renal denervation (achieved by painting the renal pedicle with a 10 % phenol: 90 % ethanol solution), vasoconstriction occurred only in the innervated kidney in the presence of CYA. In contrast, CYA did not influence RBF and RVR in the denervated kidney (Murray et al., 1986). Moss et al (1985) also support a role for the sympathetic nervous system after demonstrating marked sympathetic nerve stimulation in rats subjected to unilateral renal denervation receiving CYA. After infusion of CYA [10 mg/Kg] afferent and efferent renal nerve activity increased in the innervated kidney only. Increased efferent genitofemoral nerve activity was also documented, suggesting generalised sympathetic nerve stimulation occurs. Furthermore, urine flow rate, absolute sodium excretion and

fractional sodium excretion decreased only in the innervated kidney. This led them to postulate that CYA induced excess efferent renal nerve activity, and hence, sodium and water retention.

In contrast, CYA nephrotoxicity in man often presents early in the post-transplant period with reductions in GFR, RBF and sodium excretion. It has therefore been argued, however, that the denervated transplanted kidney should be protected from the nephrotoxic effects of CYA, if the sympathetic nerves mediate CYA-induced vasoconstriction. This observation led Moss et al (1985) to postulate that in the transplanted kidney other mechanisms must be invoked to account for the observed nephrotoxicity, including possible adrenergic supersensitivity to circulating catecholamines, a phenomenon that is known to occur within the chronically denervated kidney (Kline and Mercer, 1980). Although plasma catecholamines are only an indirect measure of sympathetic nerve activity, experimental (Gerkens et al., 1989) and clinical studies (Bellett et al., 1985; Steigerwalt et al., 1987; Thompson et al., 1986) have failed to demonstrate elevated circulating levels after CYA therapy. Furthermore, whereas Thomson et al (1989) reported that renal denervation increased single nephron GFR and glomerular perfusion in CYA-treated rats, it failed to restore glomerular capillary pressure to control levels, with significant haemodynamic differences still present between CYA-treated and control pair-fed rats.

Thus, the ability of CYA to induce renal vasoconstriction in the acutely denervated transplanted kidney in man is not compatible with excess adrenergic stimulation being the factor mediating

vasoconstriction.

(ii) EXCESS RENIN-ANGIOTENSIN SYSTEM ACTIVITY.

Glomeruli possess receptors for a variety of vasoactive hormones capable of modulating glomerular haemodynamics (Dworkin et al., 1983). In the renal vascular bed angiotensin II exerts potent vasoconstrictor effects on arterioles and glomerular mesangial cells, leading to a reduction in RBF and GFR. Angiotensin II appears to preferentially constrict the efferent arteriole and thus preserve SNGFR in the face of falling glomerular perfusion (Heller, 1987). Data from micropuncture studies also support an effect on the glomerular ultrafiltration coefficient by directly influencing mesangial cell contractility (Blantz et al., 1976; Ichikawa et al., 1979), although this has not been universally observed (Arendshorst and Navar, 1987). The role of angiotensin II in the control of intrarenal haemodynamics has recently been reviewed (Arendshorst and Navar, 1987).

Divergent opinion has evolved regarding the role of the renin-angiotensin system (RAS) in CYA nephrotoxicity. Although activation of the RAS is a consistent feature of both acute and short-term administration of CYA in animal models (Siegl et al., 1983; Murray and Paller, 1985; Perico et al., 1986a; Perico et al., 1986b), in man chronic administration is consistently associated with suppressed plasma renin activity (Myers et al., 1984; Bantle et al., 1985; Deray et al., 1988). Initial studies by Siegl et al (1983) in SHR administered varying doses of CYA [0, 20, 50 and 100 mg/Kg/day via gavage] for 4 weeks documented intense stimulation of the RAS. The co-

existence of a tachycardia, hyperglycaemia and elevated blood pressure

in the 20 mg/Kg group led them to speculate that CYA-induced

stimulation of the RAS was secondary to stimulation of the sympathetic

nervous system. Later studies by Baxter et al (1984) in vitro and

Duggin et al (1986) in vivo supported this proposition after

demonstrating that CYA augmented renin release from rat kidney slices.

Recent studies corroborate these findings using isolated rat

juxtaglomerular cells, demonstrating a dose-dependent increase in renin

secretion and intracellular prorenin production (Kurtz et al., 1988).

Pharmacological manipulation of the RAS axis with angiotensin

converting enzyme inhibitors (ACE) has allowed further insight into its

role in CYA nephrotoxicity. Although beneficial effects on renal

haemodynamics have been reported with ACE inhibitors, this has not been

a universal finding. Jao et al (1986) and Barros et al (1987a) both

reported that captopril completely abolished CYA-induced renal

vasoconstriction in rats. Conversely, Murray et al (1985) observed

that pretreatment of rats with captopril [50 mg/Kg i.v] failed to

preserve renal haemodynamics after CYA administration [20 mg/Kg i.v.].

Similarly, captopril failed to improve either renal or tubular function

in rats treated with CYA for short-term periods [12.5 and 25 mg/Kg via

gavage for 13 days] (Dieperink et al., 1986b).

Clinical studies do not lend support for a major role of the RAS

in CYA nephrotoxicity. Most studies document either unchanged (Bellett

et al., 1985; Stanek et al., 1987), or decreased activity of the RAS

(Bantle et al., 1985; Bantle et al., 1987). Furthermore, both Bantle

et al (1985) and Stanek et al (1987) noted a relatively unresponsive

RAS axis in response to stimulatory manoeuvres, including changes in posture, captopril and diuretic administration. Measurement of extracellular volume in renal and cardiac allograft recipients using isotopic methods has shown a trend for increased extracellular and plasma volume, which may account for suppression of RAS activity in man, via CYA-induced sodium retention (Bellett et al., 1985; Stanek et al., 1987; Bantle et al., 1987; Curtis et al., 1988).

The disparate findings regarding the role of the RAS in CYA nephrotoxicity in animal and clinical studies may simply reflect the model and duration of treatment employed. In most of the animal studies investigating the effect of CYA on the RAS, pair-feeding between the control and treated rats was not performed (Siegl et al., 1983; Perico et al., 1986a; Perico et al., 1986b). Short-term administration of CYA to animals tends to be associated with reduced food and water intake (Dieperink et al., 1983; Thiel, 1986), therefore it is unclear whether the stimulus for renin secretion is volume depletion. A number of studies support this contention. Gerkens et al (1984) reported less renal impairment and an unstimulated RAS in rats treated with high dose CYA [100 mg/Kg/48 hours via gavage] for 3 weeks, but fed a high salt diet, compared to equivalent low salt fed rats. Several groups have also reported that inhibition of the RAS with captopril (Kaskel et al., 1987) or sympathetic nervous blockade with alpha-adrenergic blockers (Murray and Paller, 1986) leads to a marked reduction in systemic blood pressure in chronically CYA-treated rats. These observations imply that increased RAS and sympathetic nerve activity play a physiological role in maintaining arterial pressure in

animals with chronic CYA nephrotoxicity. Thus, renal vasoconstriction may be a physiological response to depletion in circulating volume. Recently, Devarajan et al (1989) specifically addressed this area in a rat model. In Sprague-Dawley rats administered CYA [10 mg/Kg i.m. for 7 days] they were able to demonstrate that acute volume expansion completely normalised RBF and GFR, inferring that CYA-induced a reduction in circulating volume, thus appropriately stimulating both RAS and sympathetic nerve activity directly. This observation led them to conclude that renal vasoconstriction in experimental models of CYA nephrotoxicity is in part pre-renal in origin and related to reduced circulating volume. By contrast, chronic CYA administration to man tends to be associated with volume expansion (Bellett et al., 1985; Bantle et al., 1987; Stanek et al., 1987; Curtis et al., 1988).

The role of the tubuloglomerular feedback (TGF) mechanism in modulating the reduction in GFR induced by CYA has also been addressed. The macula densa region of the early distal tubule is in close proximity to the glomerulus and the afferent and efferent arterioles of the same nephron. It is postulated that this anatomical arrangement allows intra-nephron feedback, with GFR inversely related to both the delivery of sodium and chloride ions to this region and luminal flow rates (Schnermann et al., 1976). Whiting et al (1982) initially proposed that CYA decreased GFR by reducing the reabsorptive capacity of the proximal tubule, increasing the delivery of sodium ions to the distal tubule and thus, stimulating TGF activity, thereby decreasing GFR. However, subsequent studies by Dieperink and co-workers suggest TGF is unlikely to be playing a role, as proximal tubular reabsorption

is enhanced and the distal delivery of sodium decreased in both CYA-treated rats (Dieperink et al., 1986a) and man (Dieperink et al., 1987).

(iii) PROSTAGLANDINS

An intrinsic component in the intrarenal control of glomerular haemodynamics is the fatty acid products of arachidonic acid (AA). Multiple sites of arachidonic acid metabolism exist within the kidney, including arterial and arteriolar smooth muscle, endothelial cells and glomerular mesangial and epithelial cells (Arendshorst and Navar, 1987). GFR and RBF are regulated via their direct effects on vascular smooth muscle and mesangial cells, and indirectly by modulating the action of other hormones or neural stimuli. A potent stimulus for the release of endogenous vasodilatory prostaglandins include, renal vasoconstriction, volume depletion and glomerular hypoperfusion. The vasodilator prostaglandins, PGE_2 and PGI_2 thus serve as an important protective mechanism and homeostatically balance the haemodynamic effects of vasoconstrictor substances (Baer and McGriff, 1980).

The role of vasodilator and vasoconstrictor prostaglandins in CYA nephrotoxicity has been extensively investigated since the demonstration of an interaction between CYA and the metabolism of AA in peripheral smooth muscle cells in culture and peripheral blood monocytes (Lindsey et al., 1983; Whistler et al., 1984). Neild et al (1983c) and others (Egel et al., 1983) postulated that CYA-induced nephrotoxicity was related to inhibition of the synthesis of renal vasodilatory prostaglandins, particulary PGI_2. Later studies

confirmed a dose-dependent reduction in production of PGI_2 from human endothelium cells, possibly by a direct inhibitory effect on cyclo-oxygenase (Brown and Neild, 1987; Brown et al., 1988). These findings were recently corroborated by Voss et al (1988) following the demonstration that CYA induced a dose-dependent and reversible reduction of basal, Ca^{2+} ionophore A23187 stimulated and arachidonate stimulated PGI_2 release from cultured human umbilical vein endothelial cells.

The in vivo effect of CYA on prostaglandin production was initially studied by Kawaguchi et al (1985). The administration of CYA [15 mg/Kg/day i.m] to surgically intact Fischer rats and rats with heterotopic cardiac isografts increased immunoreactive urinary thromboxane B_2 (stable metabolite of thromboxane A_2) production. In contrast, Murray et al (1985) reported increased 6-Keto $PGF_{1\alpha}$, but not $PGE_{2\alpha}$ production after both acute and chronic CYA therapy. They considered increased production of vasodilator prostaglandins an appropriate physiological response to CYA-induced hypoperfusion in order to maintain RBF. Furthermore, pharmacological intervention with cyclo-oxygenase inhibitors compromised RBF, inferring that vasodilator prostaglandin production attenuated CYA-induced vasoconstriction.

In contrast, other workers have documented unchanged or decreased vasodilator prostaglandin production after CYA (Duggin et al., 1986; Perico et al., 1986a; 1986b). Duggin et al (1986) administered CYA to rats for 3 to 7 days before measuring prostaglandin production and renin secretion in an ex vivo preparation of kidney cortical slices. Although increased renin secretion was evident,

release of 6-Keto $PGF_{1\alpha}$, thromboxane B_2, PGE_2 and $PGF_{1\alpha}$ was unchanged. Similarly, Perico et al (1986b) documented unchanged glomerular synthesis of PGE_2 and 6-Keto $PGF_{1\alpha}$; however, a progressive increase in glomerular thromboxane A_2 production (measured as urinary thromboxane B_2) was observed over the 45 day study period in rats receiving CYA [25 mg/Kg/day orally]. Systemic serum thromboxane B_2 levels were similar to control animals. In addition, in vitro production of PGE_2 from rat papillae and isolated glomeruli was reduced in rats administered CYA [25 or 50 mg/Kg/day orally] for 3 and 6 weeks (Stahl and Kudelka, 1986). Conversely, Brown et al (1988) failed to demonstrate a change in prostaglandin production from renal papillae with CYA, suggesting no direct effect on cyclo-oxygenase activity. Recent observations by Bunke et al (1988) support these findings. In rats treated with CYA for 4 days, they demonstrated that glomerular PGE_2 and thromboxane B_2 production were unchanged, while 6-Keto $PGF_{1\alpha}$ decreased, suggesting that a CYA-induced decrease in renal vasodilator prostaglandin production may play a role in the renal haemodynamic alterations.

The effect of CYA on renal prostaglandin production in man has received little attention. Interpretation of data in renal transplant recipients is complicated by the effect of graft rejection, since a significant rise in urinary thromboxane B_2 excretion occurs prior to rejection episodes (Foegh et al., 1982). Despite this caveat, Klassen and co-workers have recently demonstrated that within the first hour post-transplant, 6-Keto $PGF_{1\alpha}$ concentration increases significantly in the renal vein effluent, without changes in PGE_2 or thromboxane B_2. In addition, production of renin is low, suggesting that acute CYA-induced

alterations in renal vascular tone are independent of the RAS in man

(Klassen et al., 1989). However, as blood flow rates were not measured

the precise production rate of these arachidonic metabolites was

unknown.

The apparent reduction in vasodilator prostaglandin production

reported by several groups has prompted studies investigating the

effect of exogenous vasodilatory prostaglandins on CYA-induced

vasoconstriction. The concomitant administration of the methylated

prostaglandin analogue 16,16-dimethyl PGE_2 to Wistar-Furth rats treated

with high dose CYA [100 mg/Kg/day via gavage for 7 days] improved

survival, renal morphology and function (Makowka et al., 1986).

Although similar studies performed in SHR treated with CYA for 28 days

[20 mg/Kg/day via gavage] accord with these observations,

pharmacokinetic data suggested that the bioavailability of oral CYA was

reduced in the presence of the PGE analogue, thus accounting for the

improved function and morphology (Ryffel et al., 1986b).

Attention also focused on the role of thromboxanes in CYA

nephrotoxicity as thromboxane A_2 plays a significant role in the

regulation of glomerular haemodynamics. Activation of thromboxane

receptors within the renal circulation leads to a reduction in GFR and

RBF, via afferent and efferent arteriolar vasoconstriction and

glomerular mesangial contraction (Ballerman et al., 1986;

Arendshorst and Navar, 1987). Duarte et al (1985) first hinted that

thromboxane A_2 may play a role in CYA nephrotoxicity by promoting

vasoconstriction and capillary microthrombosis, after de novo CYA-

induced haemolytic uraemic syndrome was reported in man (Shulman et

al., 1981) and experimental animals (Neild et al., 1984a; Neild et al. 1984b).

Kawaguchi et al (1985) reported increased urinary thromboxane B_2 excretion in rats receiving CYA. Similarly, in an ex vivo isolated rat kidney preparation [treated with 50 mg/Kg/day via gavage for 14 days], Coffman et al (1987) documented a significant increase in basal thromboxane B_2 and arachidonate stimulated thromboxane B_2 production. Further support to implicate thromboxane A_2 was provided by Perico et al (1986b), demonstrating a progressive rise in the glomerular synthesis and urinary excretion of thromboxane B_2, which preceded the deterioration in renal function in rats treated with CYA for 45 days. Furthermore, PGE_2 levels remained unchanged despite an increase in plasma renin activity. An inverse relationship between GFR and urinary thromboxane B_2 levels, but not with RBF, led them to suggest an effect on glomerular K_f may also be involved.

Although the cellular source of renal thromboxane production is uncertain, the macrophage is thought to be the most likely candidate (Rogers et al., 1988). Increased synthesis of thromboxane A_2 was documented in kidney and peritoneal macrophages from rats treated with CYA [12.5 mg/Kg/day i.p. for 14 days], whereas 6-Keto PGF_{1a} and PGE_2 synthesis remained unchanged.

By contrast, excess thromboxane A_2 production has not been a consistent finding in man (Stahl et al., 1985; Foegh et al., 1986; Humes et al., 1988). However, a significant fall in urinary thromboxane B_2 levels was documented in renal transplant recipients when converted from CYA to azathioprine (Humes et al., 1988).

Recently, the effects of pharmacological intervention with selective thromboxane inhibitors on CYA nephrotoxicity have been reported in both animal and clinical studies. Improved GFR followed the administration of UK-38,485, a selective thromboxane synthesis inhibitor, to rats treated with CYA [50 mg/Kg day orally for 7 weeks], albeit to subnormal levels, suggesting other mechanisms must participate in loss of renal function (Perico et al., 1986c). Other studies confirm this beneficial effect (Smeesters et al., 1988). However, Coffman et al (1988) hinted that studies with thromboxane synthetase inhibitors should be viewed with caution, as they result in accumulation of the endoperoxide intermediates PGG_2 and PGH_2, which not only possess agonist activity at the thromboxane receptor, but may also be processed to vasodilator prostaglandins. Nevertheless, a preliminary report by Coffman et al (1990) reported that the acute administration of CGS 13080, a thromboxane synthetase inhibitor, to CYA-treated renal allograft recipients reduced urinary excretion of thromboxanes, while increasing GFR and PAH clearance. Prostacyclin metabolite excretion was not affected. Coffman and co-workers have also investigated the response to specific thromboxane receptor antagonists. The acute administration of GR 32191, a specific thromboxane receptor antagonist, to rats pretreated with CYA (50 mg/Kg/day i.p) for 14 days, induced a 33 per cent improvement in GFR and an 18 per cent increase in RBF (Coffman, 1989). Similar observations have recently been reported by Rossini et al (1990). However, GR 32191 did not improve renal function in a placebo controlled clinical trial involving CYA-treated renal allograft

recipients (Mobb, 1990).

In summary, although altered eicosanoid production has been documented in CYA nephrotoxicity, it is unclear whether this is a primary or secondary effect as a consequence of renal vasoconstriction.

(iv). INTRINSIC VASOCONSTRICTOR ACTIVITY

The possibility that CYA might possess intrinsic vasoconstrictor activity has been addressed by several groups (Xue et al., 1987; Lamb and Webb, 1987; Golub and Berger, 1987). Xue et al (1987) initially demonstrated an increase in vascular tension in rat aortic ring segments when incubated in vitro with CYA [5×10^{-6} M]. Furthermore, concomitant incubation with the irreversible α-adrenergic blocker phenoxybenzamine inhibited the contractile response, suggesting that CYA-induced contraction was secondary to noradrenaline release from the sympathetic nerve terminal. Subsequent studies by Golub et al (1989) confirmed these observations and additionally demonstrated that CYA augmented the contractile response to transmural nerve stimulation in a dose-dependent fashion in isolated SHR caudal artery ring segments. Since exogenous noradrenaline in this model did not affect the responses to stimulation they postulated that CYA enhanced adrenergic nerve stimulation by a presynaptic mechanism. Later studies by this group in SHR pretreated with CYA (5 and 20 mg/Kg/day by gavage) for 14 days demonstrated that ex vivo contractile responses to nerve stimulation and exogenous noradrenaline were augmented (Golub et al., 1989). In contrast, Lamb and Webb (1987) showed that prolonged incubation of unstimulated rat tail artery strips in the presence of

CYA failed to induce a contractile response, although the contractile response to transmural nerve stimulation and exogenous noradrenaline was augmented. In addition, incubating the vascular strips with both CYA and increasing concentrations of potassium chloride, suggested that CYA influenced the resting membrane potential, thereby lowering the threshold for contraction.

Recently, several groups have reported impaired relaxation responsiveness of vessels exposed to CYA. Rego et al (1988) demonstrated in Lewis rat aortic rings that CYA [5 or 10 mg/Kg/day i.m. for 3 weeks] augmented the maximal tension generated by phenylephrine and $PGF1_{2\alpha}$, while attenuating the relaxation response to acetylcholine and sodium nitroprusside in a dose-dependent and reversible fashion. They postulated the increased contractile response to CYA may be mediated by increased Ca^{2+} flux across muscle plasmalemma. An attenuated contractile response to noradrenaline in the absence of Ca^{2+} in the incubation media supports this hypothesis (Lamb and Webb, 1987). Furthermore, verapamil was able to inhibit the contractile response to CYA in aortic strips (Xue et al., 1987). Rego et al (1988) postulated that CYA attenuated relaxation by decreasing guanosine 3': 5'-cyclic monophosphate (cGMP) levels in the endothelium or by interfering with endothelium-derived relaxing factor (EDRF) release. However, studies using isolated perfused kidneys obtained from rabbits treated with CYA in vivo [15 mg/Kg/day s.c. for 20 days] suggest that CYA does not influence vascular tone solely by an effect on EDRF-dependent vasodilatation, with a direct effect on vascular smooth muscle being more likely (Cairns et al., 1989).

Recent studies in human subcutaneous resistance arterioles
support an effect on vascular relaxation (Richards et al., 1989).
Using a myograph preparation capable of measuring isometric tension
(Mulvany and Halpern, 1977) CYA did not induce a contractile response
in unstimulated arterioles despite co-incubating for 1 hour. However,
CYA decreased the rate of spontaneous relaxation and inhibited
endothelium-dependent relaxation to acetylcholine. In contrast to the
earlier reports, CYA reduced the contractility to noradrenaline and
potassium stimulation, and augmented endothelium-independent relaxation
to sodium nitroprusside. More recent studies from this group suggest
that CYA inhibits endothelium-dependent relaxation in vitro by
inhibition of the release or action of vasodilatory eicosanoids
(Richards et al., 1990). Observations by Bossaller et al (1990)
corroborate these findings using rat aortic rings.

(v). ENDOTHELIN

Attention has recently focused on the role of endothelin, a
potent vasoconstrictor, in CYA-induced renal impairment (Kon et al.,
1990; Dadan et al., 1990). Endothelin, the most potent vasoconstrictor
yet characterised, is released from endothelial cells by a variety of
mechanical and neurohumeral stimuli and stimulates excess Ca^{2+} influx
and the release of intracellular Ca^{2+} (King et al., 1990). Within the
renal microcirculation endothelin release induces a prolonged increase
in RVR, and marked reduction in GFR and RBF at high concentrations
(Firth et al., 1988). Since endothelial cell injury is a feature of
CYA toxicity both in vitro (Zoja et al., 1986; Makowka et al., 1986;

Brown et al., 1986) and in vivo (Shulman et al., 1981) studies, Cairns et al (1988) postulated that endothelin may be involved in CYA-induced renal dysfunction. An increase in the density of the specific binding sites for endothelin was recently documented in mouse cardiac membranes after receiving CYA therapy in vivo for 21 days (Nayler et al., 1989). It is presently unknown whether CYA increases endothelin binding sites in other tissues, but such an increase in the peripheral and renal vasculature may contribute to CYA-induced alterations in vascular resistance.

Recent preliminary studies demonstrate that concomitant infusion of antiporcine endothelin antibodies ameliorate the vasoconstrictor effects of CYA in both an isolated rat kidney model and in in vivo rat studies (Kon et al., 1990; Dadan et al., 1990). Furthermore, CYA stimulated endothelin release from cultured bovine pulmonary artery endothelial cells and increased circulating endothelin levels in rats infused with CYA (Kon et al, 1990). Thus, these recent reports suggest a role for endothelin in CYA-induced nephrotoxicity.

CHAPTER 6: THE RENAL HAEMODYNAMIC RESPONSE TO CALCIUM CHANNEL
BLOCKERS

CALCIUM HOMEOSTASIS AND VASCULAR SMOOTH MUSCLE CONTRACTION

The entry of Ca^{2+} ions into excitable cells plays a pivotal role in signal transduction between the extracellular and intracellular environment. This second messenger system is paramount for a variety of physiological processes including cardiac and vascular smooth muscle contraction, excitation-secretion coupling in endocrine glands, neurotransmitter release and for numerous other biological processes (Exton, 1987).

The essential trigger for vascular smooth muscle cell contraction is an increase in cytosolic Ca^{2+} concentration. Ca^{2+} ions may gain access into the cytosol of vascular smooth muscle cells by several discrete portals in the plasma membrane; one activated by membrane depolarisation (voltage-sensitive channel), one activated by agonist-interaction with its membrane receptor (receptor-operated channel), and a third channel activated by mechanical stretch (Meisheri et al., 1981; Bevan et al., 1982). Ca^{2+} may also gain access to the cytosol by a route that is independent of activation of Ca^{2+} channels, via a channel termed the leak channel. This poorly characterised channel is sensitive to lanthanum and other inorganic non-specific calcium channel blockers (Cauvin et al., 1983), but not to the selective voltage-sensitive organic calcium channel blockers.

Ca^{2+} channels are complex glycoprotein macromolecules filled with water, spanning the plasma membrane and possess the unique ability

47

to function as ion-selective gates (Schwartz et al., 1988). In the

open state Ca^{2+} ions are permitted to advance in the direction of its

electrochemical gradient from the extracellular to intracellular

compartment. In vascular smooth muscle cells, the changing

concentration of free intracellular Ca^{2+}, from 0.1 μM in the resting

state to 10 μM after stimulation, activates the contractile mechanism

of the cell (Godfraind et al., 1986; Van Nueten et al., 1988).

Vascular smooth muscle cells also possess receptors for a

variety of vasoactive agents and are richly innervated, and thus may be

activated by either agonist-receptor interactions or via membrane

depolarisation (Greenberg et al., 1983). Certain vascular smooth

muscle cells depend more on an influx of Ca^{2+} via their Ca^{2+} channels

located in the plasma membrane for activation, in contrast to other

vascular smooth muscle cells that are activated predominantly by

intracellular mobilisation of sequestered Ca^{2+} from storage sites,

including the endoplasmic reticulum and mitochondria (Cauvin et al.,

1983; Loutzenhiser and Van Breemen, 1983). Thus, cells that rely more

on an influx of Ca^{2+} ions across the plasma membrane for activation

tend to be more sensitive to Ca^{2+} channel blockade than cells activated

by intracellular Ca^{2+} mobilisation (Cauvin et al., 1983; Bell et al.,

1985).

Binding of vasoconstrictor hormones or agents to their plasma

membrane receptor increases intracellular Ca^{2+} concentration by their

ability to open agonist-controlled Ca^{2+} channels in the plasma

membrane, or by mobilising Ca^{2+} from sequestered stores via the

generation of Ca^{2+} releasing intracellular messengers from inositol

lipids residing in the inner lamellar of the plasma membrane (Heagerty
and Ollerenshaw, 1987). Occupation of the receptor by the vasoactive
agent activates phospholipase C (PLC) using a transducing guanine
trisphosphate binding protein as a receptor-effector couple (Cockcroft
and Gomperts, 1985). Active PLC generates a water soluble product
inositol 1,4,5 trisphosphate (IP_3) which is released into the cytosol
and a hydrophobic product 1,2 diacylglycerol that is retained in the
membrane. IP_3 is able to stimulate release of organelle bound Ca^{2+}
stores from the endoplasmic reticulum and other intracellular sites to
raise intracellular Ca^{2+} ion concentration to the contractile threshold
(Streb et al., 1983; Prentki et al., 1985). It is this acceleration of
Ca^{2+} into the cytosol that triggers vascular smooth muscle cell
contraction (Van Neuten et al., 1988).

The increase in free cytosolic Ca^{2+} that is generated by either
mechanism interacts with calmodulin, a specific binding protein, to
form a Ca^{2+}-calmodulin complex that activates myosin light chain
kinase, transferring phosphate from adenosine triphosphate (ATP) to the
light chains of the muscle protein myosin. This phosphorylation
permits myosin to interact with actin. The attachment of myosin heads
to actin induces a change in their orientation and allows the filaments
to slide past each other to shorten the vascular smooth muscle cell
(Greenberg et al., 1983). The process is terminated by simultaneous
stimuli to lower the cytosolic Ca^{2+} through energy requiring pumps to
extrude Ca^{2+} from the cell either via the plasma membrane or
endoplasmic reticulum. Ca^{2+} may also be buffered by other
intracellular organelles, including mitochondria (Braunwald, 1982).

49

Experimental observations using different vascular beds have demonstrated that some vasoactive agents have the capacity to activate vascular smooth muscle contraction in one region, but not in another. This heterogeneity most likely relates to the ability of contraction to be elicited by either membrane depolarisation opening Ca^{2+} channels in the plasma membrane (Sperelakis, 1984), or activation of receptor-operated Ca^{2+} channels by agonists (Loutzenhiser and Van Breeman, 1983), or as a result of a hormone-receptor-complex activating intracellular biochemical messengers, such as the phosphoinositide system. Recent experimental studies suggest that the afferent and efferent arteriolar vessels in the renal vascular bed may be activated independently via these differing mechanisms (Meisheri et al., 1981; Loutzenhiser and Epstein, 1984). This feature may account for the variety of haemodynamic responses produced by differing vasoconstrictor agents within the renal microcirculation.

INTRARENAL CONTROL OF GLOMERULAR FILTRATION

A major factor determining the intrarenal control of glomerular ultrafiltration is the contractile state of the preglomerular and postglomerular segmental resistances. Their independent activity and differing sensitivities to vasoconstrictor stimuli regulate blood flow and intraglomerular hydrostatic pressure by constantly regulating their luminal diameter. Although smooth muscle surrounds all vascular tissue from the main renal artery to individual afferent arterioles, it is the latter that principally determine preglomerular resistance (Arendshorst and Navar, 1987). The afferent arteriole branches into fine capillary

loops in the glomerulus and intermingles with the glomerular mesangial cells. These loops subdivide further into a complicating series of branching channels before reforming into the efferent arteriole. The reduction in perfusion pressure along the arterial tree to the glomerular capillary tuft is much less when compared to other vascular beds. This high perfusion pressure accounts for the large hydrostatic pressure generated in the glomerular capillaries, which is able to overcome plasma colloid osmotic pressure, generating ultrafiltration of fluid into Bowman's space. Thus, this unique architecture allows the independent activity of the afferent and efferent arteriole to regulate glomerular blood flow and glomerular hydrostatic pressure, and hence single nephron GFR (Arendshorst and Navar, 1987; Brenner et al., 1986).

A third component influencing glomerular ultrafiltration is the glomerular mesangial cell. These cells reside in the intercapillary spaces of the glomerulus and also possess the cytoplasmic filaments, actin and myosin, essential components of the contractile machinery (Foidart et al., 1979). Recent studies indicate that the mesangial compartment is capable of de novo synthesis of a number of vasoactive substances including cAMP, cGMP, prostaglandins and angiotensin II (Brenner et al., 1986). Local generation of these vasoactive substances may profoundly influence renal haemodynamics in the absence of alterations in systemic haemodynamics. Receptors for numerous vasoactive hormones capable of inducing a contractile response are also present on the mesangial plasma membrane, including specific receptors for angiotensin II, vasopressin, parathormone, dopamine, histamine, prostaglandins (PGE_2 and PGI_2), thromboxane, leukotrienes, serotonin

and atrial natriuretic peptide (Arendshorst and Navar, 1987). These
characteristics suggest that the mesangial compartment participates in
the intrarenal regulation of haemodynamics via their contractile nature
(Kreisberg et al., 1985).

The mesangial cell may alter intraglomerular haemodynamics by
its ability to vary the glomerular ultrafiltration coefficient (K_f).
Glomerular K_f is determined by the capillary surface area available for
filtration and the hydraulic conductivity across the endothelial cell
(Schor et al., 1981). An alteration in the size of the capillaries may
reduce the available filtering surface area and thus, alter K_f.
Alternatively, hydraulic conductivity may be altered by adjustment in
the size and number of the endothelial fenestrations, the thickness or
permeability of the basement membrane and/or the number or structural
configuration of the slit pores between the foot processes. Although
changes in glomerular K_f may be due to alterations of either variable,
considerable experimental evidence infers that glomerular K_f is
predominately influenced by alterations in available capillary surface
area (Schor et al., 1981). Reduction in hydraulic conductivity is
difficult to quantify and ultrastructural alterations of the glomerular
wall in response to a variety of glomerular K_f lowering agents have not
been observed (Baylis et al., 1977; Ichikawa and Brenner, 1979).

As in vascular smooth muscle, membrane depolarisation and
agonist-receptor interactions in the mesangial cell induce a rapid rise
in cytosolic Ca^{2+} from either sequestered stores and/or opening of
plasma membrane Ca^{2+} channels (Takeda et al., 1988). There is also
evidence to suggest that IP_3 acts as a second messenger for Ca^{2+}

mobilisation in mesangial cells (Troyer et al., 1985).

In addition to the exogenous factors that can regulate glomerular ultrafiltration the kidney possesses the capacity to adjust intrarenal haemodynamics by intrinsic mechanisms; a phenomenon termed renal autoregulation (Brenner et al., 1986; Arendshorst and Navar, 1987). An alteration in renal arteriolar perfusion pressure is normally counterbalanced by a change in afferent and efferent arteriolar resistances to maintain or autoregulate intraglomerular blood flow. Thus, even in the face of a decrease in systemic blood pressure (within the autoregulatory range) RBF and GFR will be maintained by an decrease in renal vascular resistance. The precise mechanism responsible for intrinsic autoregulation is unclear. Two major mechanisms have been proposed; the myogenic (Bayliss, 1902) and the tubuloglomerular feedback mechanisms (Guyton et al., 1964). The myogenic theory postulates that an increase in transmural pressure will induce a rapid increase in arteriolar resistance to prevent over perfusion. Conversely, in response to a decrease in perfusion pressure myogenic responses decrease vascular tone to maintain RBF and GFR. In contrast, the tubuloglomerular feedback hypothesis was proposed in view of the close proximity of the distal tubule to the afferent arteriole, ideally located to act as a communication link between the distal tubular region and the afferent arteriole. This theory proposes that alterations in the chemical composition of the ultrafiltrate are rapidly sensed by the macula densa region and the information transmitted to the afferent arteriolar circulation. Thus, increased tubular fluid solute concentration or urine flow will lead to

vasoconstrictor signals being transmitted to the afferent arteriole to

maintain a stable renal blood flow and GFR, or vice versa.

CALCIUM CHANNELS, CALCIUM CHANNEL BLOCKADE AND RENAL HAEMODYNAMICS

Agents may selectively interact with the voltage-dependent Ca^{2+}

channel to inhibit Ca^{2+} influx. This class of drug were termed Ca^{2+}

antagonists or calcium channel blockers by Fleckenstein, following the

demonstration in vitro that verapamil and prenylamine inhibited

contractility in guinea pig papillary muscle, thus mirroring the

cardiac effects of removing Ca^{2+} from the incubation media

(Fleckenstein, 1983). These drugs selectively interact with the

voltage-dependent Ca^{2+} channel by binding to discrete receptor sites

located on or near the channels (Vaghy et al. 1987; Triggle and Janis,

1987). This drug-receptor interaction inhibits the opening or function

of the channel, impeding the influx of Ca^{2+} into the cell. The

sensitivity of the Ca^{2+} channel to blockade with voltage-sensitive

calcium channel blockers can vary between or even within a vascular

bed. For example, in most vascular preparations calcium channel

blockers depress the contractile response to exogenous noradrenaline,

although the degree of inhibition may vary among different vessels. In

some vessels (canine saphenous vein, guinea pig aorta, ear artery of

the rabbit and rat caudal artery) concentrations of calcium channel

blockers that are able to inhibit Ca^{2+}-induced contractions of

depolarised tissue, are only able to depress moderately the contractile

response to noradrenaline (Vanhouette et al., 1983). This observation

indicates that part of the activation process in certain vascular

smooth muscle, in response to noradrenaline, is due to enhanced mobilisation of intracellular Ca^{2+} and/or differences in the properties of the vascular smooth muscle in differing regions (Casteels and Droogmans, 1981; Vanhouette et al., 1983). This heterogeneity relates also to the dependency of the vascular tissue on the influx of Ca^{2+} for normal function and/or the binding affinity of the drug. Calcium channel blockers exhibit differing binding affinities depending on the membrane potential of the cell and the frequency of channel opening, demonstrating a high affinity for channels in the inactivated state. Because the resting membrane potential differs widely among tissues, the sensitivity to calcium channel blockers varies. Thus, recruitment of calcium channel blocker-sensitive Ca^{2+} channels in vascular smooth muscle cell vary with differing vasoconstrictors substances and consequently respond to calcium channel blockers to differing degrees (Meischeri et al., 1981; Loutzenhiser and Epstein, 1984). The unique architecture of the renal vasculature with its independent control of pre- and postglomerular resistances is a setting in which regional heterogeneity may exert a predominant influence on the pharmacologic response to calcium channel blockers.

Experimental and clinical data indicate that calcium channel blockers exert unique effects on renal haemodynamics compared to other vasodilator drugs (Baer and Navar, 1973; Ono et al., 1974; Ichikawa et al., 1979; Bell and Lindner, 1984). Vasodilators including acetylcholine, PGE_2 and papaverine are able to augment renal blood in vivo, although GFR generally remains unchanged (Baer and Navar, 1973). In contrast, calcium channel blockers possess the unique ability to

induce substantial increments in both GFR and RBF under certain experimental and clinical situations, maintaining or even increasing filtration fraction (Dietz et al., 1983; Bell and Lindner, 1984; Arend et al., 1984). This contrasts with the reduction in filtration fraction that is often observed with other vasodilator agents (Baer and Navar, 1973; Baylis et al., 1976; Thomas et al., 1983). In a review of the current literature Loutzenhiser and Epstein (1985a) noted that calcium channel blockers tended to exert a beneficial effect on GFR when administered to either hypertensive animals or man. Furthermore, increments in GFR were consistently documented in experimental models in the presence of exogenous vasoconstrictor agents (angiotensin II and noradrenaline) (Loutzenhiser and Epstein, 1985a). Beneficial effects were observed after both short and long-term administration of calcium channel blockers to hypertensive animals (Ichikawa et al., 1979; Huelsemann et al., 1985; Nordlander et al., 1985; Loutzenhiser et al., 1985b) and patients (Guazzi et al., 1983; Bauer et al., 1985; Loutzenhiser and Epstein, 1985a). These salutary effects were exerted even when administered to animals or man with both pre-existing hypertension and renal impairment (Ichikawa et al., 1979; Guazzi et al., 1983; Huelsemann et al., 1985; Bauer et al., 1985; Loutzenhiser and Epstein, 1985a). In contrast, administration of calcium channel blockers to normotensive man did not appear to influence GFR or RBF (Austin et al., 1983; Loutzenhiser and Epstein, 1985a). These observations led Loutzenhiser and Epstein to postulate that the unique renal haemodynamic effects of calcium channel blockers are elicited only under conditions in which renal vascular resistance is increased

(Loutzenhiser and Epstein, 1987; 1988).

To avoid the complicating factor of blood pressure, endogenous vasoactive substances and renal neural activity, Loutzenhiser and co-workers investigated the renal haemodynamic actions of calcium channel blockers using the isolated perfused kidney preparation (Loutzenhiser et al., 1985b; Loutzenhiser and Epstein, 1987; Loutzenhiser et al., 1988). In this model, in the absence of exogenous vasoconstrictor agents, calcium channel blockers did not influence renal haemodynamics. Since the isolated perfused kidney preparation in the basal state possesses little intrinsic vascular tone, the uncoupling of excitation-contraction with calcium channel blockers is unlikely to influence vascular resistance any further in this setting. However, marked effects were observed in the presence of increased vascular resistance. Furthermore, they demonstrated that the renal response to calcium channel blockers in the presence of exogenous vasoconstrictors was also dependent on the vasoconstrictor agent, confirming that not all vasoconstrictors mechanisms are inhibited by calcium channel blockers. For example, contraction of isolated rings of human renal artery activated by potassium chloride-depolarisation is completely blocked by nitrendipine, whereas the contractile response to noradrenaline is relatively unaffected, suggesting that noradrenaline-induced vasoconstriction is mediated by mechanisms not dependent on voltage-dependent Ca^{2+} channels. Loutzenhiser and co-workers demonstrated in this model that if three dissimilar vasoconstrictors (angiotensin II, noradrenaline and potassium chloride) are made to elicit comparable reductions in renal plasma flow, differing effects on

57

GFR are observed; GFR fell to 3 per cent of control values with potassium chloride, whereas with noradrenaline and angiotensin II, GFR only fell to 24 and 51 per cent of control levels respectively. They interpreted these effects as reflecting different relative effects on afferent and efferent arteriolar tone, in addition to possible differences in the effects of these agents on the non-vascular determinants of GFR. The addition of nitrendipine or other voltage-sensitive calcium channel blockers (Loutzenhiser et al., 1984; Steele and Challoner-Hue, 1984; Loutzenhiser et al., 1985b) to this model in the presence of these vasoconstrictor agonists, exerted effects on GFR that were quantitatively dissociated from its effects on the renal plasma flow. In presence of angiotensin II and noradrenaline, nitrendipine elicited an increase in GFR that greatly exceeded its effects on renal plasma flow. In contrast, this preferential effect on GFR was not observed with potassium chloride, where a parallel increase in GFR and renal plasma flow occurred. Furthermore, this type of renal haemodynamic response was not observed with the inorganic nonspecific calcium channel blocker, manganese. These observations were interpreted to represent a predominant relaxing effect of calcium channel blockers on the preglomerular resistance vessels, leading to a rise in glomerular hydrostatic pressure and hence, in GFR. The selective augmentation of GFR by calcium channel blockers thus requires the presence of an agonist that acts upon afferent and efferent resistance arterioles by different activating mechanisms. Such agonists may elicit membrane depolarisation and activate voltage-dependent Ca^{2+} channels in the afferent but not the efferent arteriolar

smooth muscle (Loutzenhiser and Epstein, 1988). Since organic calcium channel blockers selectively inhibit the function of this Ca^{2+} channel, they increase GFR by preferentially attenuating afferent arteriolar tone. Evidence supporting these observations in vivo followed similar observations using a hydronephrotic rat kidney model, where direct observation of the renal microcirculation is possible. The afferent arteriole was shown to more sensitive to calcium channel blockers than the efferent arteriole (Fleming et al., 1987). Thus, the ability of calcium channel blockers to preferentially augment GFR is seen only with the selective organic calcium channel blockers which act on specific voltage-sensitive channels, and not with the inorganic calcium channel blockers that block a wider variety of Ca^{2+} channels (Deth and Lynch, 1981).

Although in the studies by Loutzenhiser and co-workers the effects of calcium channel blockers on glomerular K_f were not examined, it is possible that a preferential improvement in GFR was the result of an increase in glomerular K_f. Micropuncture studies by Ichikawa et al (1979) demonstrated that during angiotensin II infusion in the rat, GFR was decreased primarily by a decline in glomerular K_f. However, verapamil was able to reverse the angiotensin II-induced increase in afferent and efferent arteriolar resistance and augment GFR by increasing glomerular K_f. They postulated that the improvement in glomerular K_f by verapamil was due to an effect on mesangial cell contractility.

Experimental studies also suggest that calcium channel blockers have the capacity to inhibit the ability of the kidney to autoregulate

59

RBF (Ono et al., 1974; Cohen and Fray, 1982; Navar et al., 1986). As autoregulatory function is predominantly the role of the afferent arteriolar circulation (Thurau, 1966; Navar, 1970; Robertson et al., 1972), this contributes further evidence to suggest that calcium channel blockers act principally on the afferent arteriolar circulation. Interference with tubuloglomerular feedback has also been suggested as a potential mechanism for disrupting autoregulation (Haberle et al., 1987).

Calcium channel blockers stimulate release of renin and theoretically may lead to an elevation in glomerular hydrostatic pressure via angiotensin II-induced efferent vasoconstriction. However, since calcium channel blockers abolish the intrarenal effects of angiotensin II by inhibiting Ca^{2+} influx, it is unlikely that this mechanism contributes to the increase in GFR observed with this class of drug (Goldberg and Schrier, 1984).

A variety of non-vascular effects related to Ca^{2+} channel blockade may also influence renal haemodynamics by modulating the renal inflammatory response to injury. Ca^{2+} has been shown to play a pivotal role in lymphocyte proliferation and function following the demonstration that the removal of extracellular Ca^{2+} from the culture medium decreased lymphocyte transformation (Kay, 1971; Whitney and Sutherland, 1972) and proliferation to mitogenic lectins (Maino et al., 1974; Freedman et al,. 1981). Later studies revealed calcium channel blockers to possess immunosuppressive activity by virtue of their ability to inhibit the proliferation of T cells after mitogen stimulation via a reduction in mitogen induced Ca^{2+} influx (Birx et

al., 1984; McMillen et al., 1985), and production of interleukin 2

(Bruserud, 1985). The activity of various inflammatory cells,

including macrophages (Wright et al., 1985) granulocytes (Jouvin-

Marche et al., 1983) and platelets (Mehta, 1985) are inhibited,

therefore interfering with the renal inflammatory response to injury.

The potential for an additive or synergistic immunosuppressive effect

between CYA and calcium channel blockers was confirmed by McMillen and

co-workers, demonstrating that the combination of verapamil and CYA did

indeed potentiate the immunosuppressive activity of CYA on murine

(McMillen et al., 1985) and human lymphocytes in vitro (Tesi et al.,

1985). The ability to potentiate the immunosuppressive effect of

CYA by calcium channel blockers in vivo has since been reported in both

animal (Tesi et al., 1987) and clinical studies (Mandreoli et al.,

1990). This synergistic effect may account for the reduced number of

rejection episodes reported by several groups in renal allograft

recipients receiving calcium channel blockers in the early post-

transplant period (Wagner et al., 1987; Solez et al., 1988; Dawidson et

al., 1989).

Ca^{2+} also plays a role in the pathogenesis of ischaemic cell

injury (Schanne et al.,1979; Humes, 1986; Schrier et al., 1987).

Calcium channel blockers have been shown to confer a beneficial effect

on renal function in the setting of renal hypoperfusion. An ischaemic

insult renders the plasma membrane permeable to Ca^{2+}, allowing the

concentration in the cytosol to rise. Normally, the cell membrane

actively pumps Ca^{2+} out of the cell, maintaining a significant

extracellular to intracellular gradient. Massive Ca^{2+} overload in the

cytosol disrupts mitochondrial function, activates endoperoxidases, enhances myofibrillar contraction, leading to intense vasospasm and cell death. Several studies have demonstrated that calcium channel blockers have the capacity to ameliorate ischaemic injury in a variety of organs including the myocardium (Cheung et al., 1984) liver (Farber et al., 1978) and kidney (Burke et al., 1984). Burke et al (1984) suggested that the protective effect of calcium channel blockers relates to their ability to prevent uptake of Ca^{2+} into the mitochondria, preserving mitochondrial function. Other renal vasodilator agents that do not influence cellular Ca^{2+}, such as serotonin and acetylcholine, are not protective. Thus, in the setting of renal hypoperfusion, calcium channel blockers appear to be nephroprotective, preserving renal function.

In summary, voltage-sensitive calcium channel blockers exert unique effects within the renal vascular bed, with the capacity to augment both GFR and RBF. The demonstration that their effects are predominately exerted on the afferent arteriolar vessels makes this class of vasodilator an appropriate agent to counteract CYA-induced afferent arteriolar vasoconstriction.

CHAPTER 7: CYCLOSPORIN A: MODE OF ACTION, PHARMACOKINETICS AND

           METABOLISM.

MODE OF ACTION

Borel initially demonstrated the reversible inhibition induced by CYA on T-cell mediated alloimmune and autoimmune responses (Borel, 1976). In addition, generation of interleukin 2 (IL-2) which initiates proliferation of T-cells by binding to high affinity receptors on activated T-cells, and the synthesis of gamma interferon by the T-helper cell, that activates macrophages and monocytes are inhibited by CYA (Hess et al., 1982; Azogui et al., 1983). The selective effect of CYA on T-helper cells is demonstrated by the normal production of α-interferon by leucocytes and β-interferon by fibroblasts. Experimental studies suggest that it is via inhibition of IL-2 and these other lymphokines that CYA induces immunosuppression.

The precise mechanism(s) responsible for inhibiting lymphokine production is unclear. The initial plasma membrane events of signal transduction and Ca^{2+} influx are not disturbed (Kahan, 1989). However, it is postulated that CYA directly influences Ca^{2+} related cytoplasmic processes, inhibiting enzymatic generation of activation protein(s) that mediate signal transduction from the cytoplasm to the nucleus. The generation of the cytoplasmic activation signal that triggers DNA synthesis by resting lymphocyte nuclei is reduced in the presence of CYA (Citterio and Kahan, 1989). CYA may act by inhibiting the activity of the biologically important enzyme effector molecule calmodulin (Colombani et al., 1985) or cyclophilin, an uncharacterised cytosolic

protein related to calmodulin, by its immunologic epitope and its binding sites for CYA (Handschumacher et al., 1984).

A further potential site of activity is within the nucleus. CYA selectively inhibits the capacity of nuclei isolated from human or rat lymphocytes to incorporate ^{3}H-thymidine (Citterio and Kahan, 1989). These observations suggest that CYA binds to a nuclear receptor. At therapeutic concentrations CYA specifically disrupts lymphokine and proto-oncogene C-myc mRNA synthesis, but not non-lymphokine antigen signal-dependent HT-3 or IL-2-receptor mRNA (Kahan, 1989).

In contrast, CYA spares T-suppressor lymphocyte generation both in vitro and in vivo. Furthermore, chemotactic and phagocytic activity of neutrophils is preserved. The intracellular mechanisms of immunosuppression by CYA have recently been reviewed (Aszalos, 1988; Kahan, 1989).

PHARMACOKINETICS

CYA is extremely hydrophobic, therefore for clinical use it is necessary to stabilise in olive oil or polyoxylated castor oil (cremophore) vehicles for oral or intravenous administration, respectively. After oral administration the absorption of CYA is slow, attaining peak levels after 3 to 4 hours. Furthermore, a three fold difference in the bioavailability (range 20-50 per cent) is documented between patients, possibly due to individual differences in the ability of the intestinal chyme to disperse CYA from its vehicle (Kahan, 1989). Within the circulation CYA is bound to erythrocytes (50 per cent), leucocytes (10-20 per cent) and lipoprotein fractions (30-40 per cent),

including high density lipoprotein [HDL], low density lipoprotein [LDL] and very low density lipoprotein [VLDL] (Ryffel et al., 1988). Although a small proportion of CYA circulates free in the plasma, this fraction is not correlated with adverse effects (Lindholm et al., 1988). CYA is widely distributed throughout the body due to its lipophilic nature, with high tissue concentrations in fat, pancreas, adrenal glands and liver (Lemaire et al., 1986). A similar pattern of distribution is observed in rats (Niederberger et al., 1983).

Studies in animals and man using tritiated CYA demonstrate that extensive metabolism takes place in the liver by the microsomal enzymes of the cytochrome P-450 family, via hydroxylation and N-demethylation biotransformations into structures that retain the oligopeptide ring structure (Maurer, 1985; Moochhala and Renton, 1986; Kronbach et al., 1988). The metabolites possess low immunosuppressive activity and do not appear to be responsible for nephrotoxicity (Cunningham et al., 1983). Other drugs that are similarly metabolised by the hepatic microsomal system may alter CYA blood levels, either decreasing immunosuppression or increasing the risk of nephrotoxicity (Kahan, 1989).

The majority of CYA is eliminated as metabolites in the bile, with only a small proportion (4-6 per cent) excreted in the urine. A small proportion (0.1-0.2 per cent) of the parent drug is excreted unchanged in the urine (Maurer et al., 1983). Detailed reviews on the pharmacokinetics and metabolism of CYA have recently been published (Lemaire et al., 1986; Kahan, 1989).

PHARMACOKINETICS AND METABOLISM OF CYCLOSPORIN A IN THE RAT

Initial reports intimated that the absorption, distribution, metabolism and elimination of CYA were similar in man and rat (Sullivan et al., 1985; Sangalli et al., 1988). However, recent reports cast doubt on this proposition as CYA and metabolite concentrations in rat blood differ significantly from concentrations in man (Venkataramanan et al., 1988). CYA concentration predominates over metabolites in rats, while the converse is true in man. Furthermore, metabolite M17 is the principal fraction in man, compared to M1 in the rat (Pell et al., 1988). It is possible that the resistance to nephrotoxicity in the rat relates to these differences in metabolism. However, experimental studies suggest that it is the parent compound that is responsible for the nephrotoxicity (Pell et al., 1988). Modulation of CYA metabolism with hepatic enzyme inducers to increase conversion to metabolites reduces nephrotoxicity (Cunningham et al., 1983). In addition, the administration of metabolite M17 [20 mg/Kg/day orally for 28 days or 10 mg/Kg/day i.p. for 10 days] did not induce functional or morphological abnormalities in the rat kidney, inferring that the parent compound is the active nephrotoxin (Ryffel et al., 1988).

The use of the rat to study CYA nephrotoxicity has also been criticised on the basis of the supraimmunosuppressive doses required to induce renal impairment compared to man. However, a reduced ratio of kidney to body weight and higher GFR per gram of kidney weight in the rat may explain the disparity in toxicity and relative resistance to CYA-induced renal impairment (Sullivan et al., 1985). Despite these caveats, the effects of CYA on the kidney have been most extensively

66

studied in the rat.

CHAPTER 8: NIFEDIPINE: PHARMACOKINETICS AND METABOLISM

Calcium channel blockers have emerged as a major class of therapeutic agents in the last twenty years, with a wide spectrum of activity in view of their ability to block Ca^{2+} influx into cells. Their major site of action is centred on the slow or voltage-sensitive Ca^{2+} channel located in the plasma membrane, where they inhibit Ca^{2+} influx into the cell (see chapter 6). Electrophysiological studies in isolated cardiac and vascular smooth muscle cells have demonstrated that the fast upstroke of the action potential (phase 0), due to the rapid influx of sodium, is not affected by nifedipine. However, the plateau phase of the action potential (phase 2), important for excitation-contraction coupling is sustained by a slow inward Ca^{2+} influx, and is inhibited by these drugs. The ionic flux through the slow channels is controlled by an outside and inside gating mechanism. The exact mechanisms whereby calcium channel blockers inhibit these channels is unclear, but experimental evidence supports the following possibilities: (i) physical blockade of the Ca^{2+} channel outside the cell, (ii) competing with Ca^{2+} at the outside gate, (iii) deforming the channels or interfering with the passage of Ca^{2+}, or (iv) interfering with the release of activated Ca^{2+} on the inside of the cell membrane (Sorkin et al., 1985). Although these agents all possess the ability to inhibit Ca^{2+} influx, there is great variation in the chemical structure between the major calcium channel blockers classes: verapamil is structurally similar to papaverine, diltiazem is a benzothiazepine derivative and nifedipine a dihydropyridine derivative. Individual compounds also exhibit varying propensities to inhibit myocardial slow

channel and Ca^{2+} fluxes in vascular smooth muscle. The renal
haemodynamic actions of calcium channel blockers are discussed in
chapter 6.

The pharmacokinetics and metabolism of nifedipine have recently
been extensively reviewed (Sorkin et al., 1985). The pharmacokinetics
of nifedipine in man were studied with ^{14}C-nifedipine. Absorption
following oral nifedipine is more than 90 per cent complete, with oral
capsules producing peak plasma concentrations between 0.5 to 2.2 hours
with 45 to 68 per cent bioavailability. The reduced bioavailability is
accounted for by a substantial first pass hepatic clearance.
Nifedipine is extensively bound to plasma proteins (92 to 98 per cent)
and is therefore distributed widely in the tissues. This extensive
binding to albumin contributes to its long duration of action, with an
elimination half life between 6 and 11 hours after oral administration.

Nifedipine is also extensively metabolised by the hepatic P450
cytochrome enzyme system via oxidative biotransformation. The first
step involves dehydrogenation of the dihydropyridine nucleus into its
corresponding pyridine derivative. This step is followed by hydrolysis
of the ester moiety. The major metabolite in the urine is the
pyridine-carboxyl metabolite (M-I), which accounts for 60-80 per cent
of the dose, whereas the pyridine-carboxyl-hydroxyl metabolite (M-II)
represents less than 6 per cent of the dose. Because it is highly
lipophilic, the parent drug is excreted in the urine in only trace
amounts.

Studies in rat liver demonstrate that nifedipine, in common with
verapamil and diltiazem, inhibits microsomal drug metabolism (Maenpaa

69

et al., 1989). This route of metabolism accounts for the relatively large number of clinically important interactions with other drugs, in particular CYA, which is also metabolised via this system (Bourbigot et al., 1986; Wagner et al., 1988; Maenpaa et al., 1989).

CHAPTER 9: CONCLUSIONS

The rapid changes in renal haemodynamics and paucity of tubular abnormalities that follow intravenous CYA together infer that the primary actions of CYA on the kidney are haemodynamically mediated. Although the factor(s) inducing this alteration in vascular tone are uncertain, several candidates have been proposed, including CYA itself, the RAS, altered eicosanoid production and excess sympathetic nerve activity. The possibility that CYA increases vascular sensitivity to humoral and neurogenic stimuli has also been entertained. It is likely that the varying degrees of tubular injury and interstitial fibrosis described after chronic CYA administration are the result of tubular hypoperfusion and focal tubular collapse, secondary to afferent arteriolar vasoconstriction.

Therapeutic intervention with renal vasodilator agents to oppose the vasoconstrictor effects of CYA is an attractive proposition that may protect the renal circulation. Unlike the specific receptor blockers phenoxybenzamine and prazosin, and the angiotensin converting enzyme inhibitor captopril, calcium channel blockers including nifedipine are able to inhibit a wide range of vasoconstrictors acting on the vascular smooth muscle cell, via their salutary effects on Ca^{2+} influx into the cytosol. Furthermore, the choice of calcium channel blockers to oppose the vasoconstrictor effects of CYA is most appropriate, as both act predominately on the preglomerular circulation. Thus, the following series of experiments have been designed to investigate the effects of nifedipine, a dihydropyridine calcium channel blocker, on CYA nephrotoxicity in an animal model and

in renal allograft recipients.

This series of experiments addresses the following specific questions:

(a) Can nifedipine successfully reverse CYA-induced renal dysfunction in the surgically intact (two-kidney) spontaneously hypertensive rat (SHR)?

(b) Can nifedipine successfully reverse CYA-induced renal dysfunction in SHR with reduced renal mass?

(c) Is nifedipine beneficial if administered after a period of CYA therapy?

(d) What are the renal haemodynamic changes induced by nifedipine in CYA-treated SHR and renal allograft recipients?

(e) Will nifedipine reverse the morphological abnormalities associated with chronic CYA administration?

(f) How does CYA initiate vascular smooth muscle activation?

(g) What is the mechanism underlying the preservation of renal function associated with concomitant nifedipine and CYA administration?

(h) Is nifedipine beneficial when administered prospectively to long-term renal allograft recipients receiving CYA?

SECTION II

CYCLOSPORIN A NEPHROTOXICITY IN THE

SPONTANEOUSLY HYPERTENSIVE RAT

INTRODUCTION

The renal impairment observed in the pilot studies performed in renal and bone marrow transplant recipients treated with CYA was unexpected and had not been predicted from preclinical toxicology studies, in which comparative immunosuppressive doses to man had been administered (Calne et al., 1978b; Powles et al., 1978). Subsequent experimental studies emphasised the inherent resistance possessed by most animal species to CYA nephrotoxicity (Ryffel et al., 1983). For example, the administration of CYA orally to Rhesus monkeys aged 3 to 6 years at doses of 20, 60 and 200 mg/Kg per day for 13 weeks did not induce renal impairment, despite increasing the dose to 300 mg/Kg per day for the last 4 weeks in the high dose group. Similarly, renal impairment was not evident in Beagle dogs treated with CYA [up to 45 mg/Kg per day] for 1 year (Ryffel et al., 1983). However, it became apparent from these and other studies that various strains of rat were relatively sensitive to CYA nephrotoxicity, albeit at supraimmunosuppressive doses (Homan et al., 1980; Ryffel et al., 1983).

In developing a suitable rat model in which to study CYA nephrotoxicity, it was demonstrated that certain strains of rat were intrinsically more susceptible to CYA-induced renal impairment (Ryffel et al., 1984). Although several rodent strains, including Sprague-Dawley, Fischer and Wistar rats developed functional and morphological lesions after acute and short-term CYA therapy, only the SHR demonstrated the full spectrum of impaired renal function, tubular and arteriolar lesions as observed in man (Ryffel et al., 1984; Ryffel et al., 1986a).

In view of the known sensitivity of the SHR to CYA nephrotoxicity, this strain of rat was chosen to investigate the effect of nifedipine on CYA nephrotoxicity in the following series of experiments. Initial experiments were performed in surgically intact (two-kidney) rats to characterise the functional and morphological effects of CYA in locally bred SHR. A dose of 25 mg/Kg body weight per day was administered, as a similar dose was reported by other workers to consistently induce nephrotoxicity in the SHR, without the unwanted side-effects of anorexia, weight loss or hypotension (Ryffel et al., 1986a). In the following series of experiments CYA [25 mg/kg per day] was administered via several routes for varying durations, in an attempt to develop both an acute and chronic model of CYA nephrotoxicity.

MATERIAL AND METHODS

ANIMALS

Female SHR (bred in the Department of Biomedical Services, University of Leicester) weighing 180 to 230 g were studied. The rats were individually caged and fed a standard wet mash chow (Special Diets Services Ltd, Essex, U.K.) containing 0.3 % (w/w) sodium and 23 % (w/w) protein and allowed free access to tap water (see appendix 1). All experiments were performed in pair-fed animals because of the known anorectic effect of CYA (Farthing et al., 1981).

DRUG PREPARATION AND ADMINISTRATION

All injections were performed in the morning between 0900 and 1100 hours. CYA (Sandoz Pharmaceuticals, Surrey, U.K.) was dissolved in an olive-oil vehicle (Boots, Nottingham, U.K.) at 40-50° C to achieve a final concentration of 25 mg/ml and administered at a dose of 25mg/kg body weight per day. CYA or its vehicle was administered once daily via the following routes:

(i) gavage using a stainless steel tube with a round bodied
    end (volume of injection 0.15-0.26 ml),

(ii) mixed in with the wet mash chow (the amount added to
     the feed was adjusted daily so the rats consumed
     approximately 25 mg/Kg per day),

(iii) subcutaneous injection (0.15-0.26 ml) using an 18
      gauge sterile needle.

(iv) intraperitoneal injection (0.15-0.26 ml) using a 25
     gauge sterile needle.

EXPERIMENTAL GROUPS

The following experiments were performed in surgically intact (two-kidney) SHR. Olive-oil or CYA or was administered as follows:

(A) via gavage for 2 weeks (n=12).

(B) via feed for 12 weeks (n=8).

(C) via subcutaneous injection

   (i) for 2 weeks [C2] (n=20)

   (ii) for 4 weeks [C4] (n=16)

   (iii) for 2 weeks, then olive-oil for 2 weeks [C2/2] (n=12)

(D) via intraperitoneal injection for 7 days (n=8).

EXPERIMENTAL PROTOCOL

All animals were allowed at least one full week after transfer to the Leicester General Hospital Animal Unit to acclimatise, before changing their diet from standard rat chow logs to a wet mash chow. Age and weight matched rats were then pair-fed for at least 7 days prior to commencing the study to acclimatise the animals to their new diet. Group D rats (i.p route) were not pair-fed in order to assess fully the effects of CYA on appetite. The rats were weighed every 3 to 4 days and inspected daily for local or systemic side-effects. Two days before commencing the study the rats were transferred to individual metabolic cages and a 24 hour urine collection obtained. In preliminary studies both food intake and urine volumes did not change significantly over a 7 day period while continuously housed in metabolic cages, therefore rats were not routinely acclimatised to the metabolic cages prior to urine collection (see appendix 2). The day

before starting injections the rats were fasted for 24 hours and a baseline fasting tail vein blood sample (1.5 ml) was taken to measure serum electrolytes and urea under a light ether anaesthesia (May and Baker Limited, Dagenham, Essex, U.K.). The blood was allowed to clot for 1 hour before centrifugation. A 24 hour urine collection and blood sample (for serum electrolytes only) were repeated at the end of the study period.

SURGICAL PROCEDURES FOR CLEARANCE STUDIES

In an attempt to avoid anaesthetic and surgery-induced alterations in renal haemodynamics that are recognised to occur in rats studied shortly after preparation (Walker et al., 1983), all clearances and haemodynamic measurements were performed in conscious unrestrained rats 18-24 hours after surgery and anaesthesia. Animals were anaesthetised using a halothane and oxygen mixture (4% halothane for induction and 1.5 % for maintenance) delivered via a Fluotec anaesthetic machine (Fluotec Mark 2, Cyprane, Keighley, U.K.) and operations performed on a heated operating mat (Pet Warmer, model 1715, Johnson and Calverley, West Yorkshire, U.K.). The left carotid artery and right jugular vein were cannulated with polythene catheters (Portex Ltd, Hythe, Kent). The carotid artery catheter had an internal diameter of 0.58 mm with an external diameter of 0.96 mm (P50). The jugular catheter had an internal diameter of 0.28 mm and external diameter of 0.61 mm (P10). Through a midline submental incision the vessels were located by blunt dissection and carefully dissected free. Three sutures were then passed around each vessel and the distal suture

tied. The proximal suture was used as a sling to temporarily retard the flow of blood while the catheter was passed down the vessel. The catheters were secured in the vessel by tightening all three sutures around the catheter. The catheters were exteriorized between the scapulae via a tunnel created from the anterior triangle of the neck to the shoulder. The catheters were housed in a protective stainless steel spring 40-50 cm in length and an internal diameter of 5 mm. At the end of the spring was a metal flange with four equidistant holes so that this could be sutured to the skin surrounding the exit site. A linen jacket was secured around the rat at this site to offer further support. The spring was attached at the opposite end to a steel rod (60 cm) and pivoted at its midpoint to a vertical stand, which was 45 cm from the bench and 6 cm from the top of the perspex cage. The weight of the spring and rat was counterbalanced by an adjustable weight at the opposite end (Figure 1). This arrangement would allow the rat free movement about the cage while the catheters were protected from damage and tangling. On recovery from the anaesthetic (usually within 5 minutes) the rats were placed in a perspex cage (30x30x40 cm) and allowed free access to water. The internal carotid artery cannula was kept patent by a constant infusion of 0.9 % saline containing heparin (10 units/ml) delivered at 0.6 ml/hr (Braun Perfusor VI, Braun Medical Ltd, Aylesbury, U.K.) and the rats left overnight to recover from the anaesthetic. Systemic blood pressure was measured via the carotid line (Washington 400 MD2 channel blood pressure transducer, Palmer Bioscience, Kent, U.K.) in the morning before clearance studies
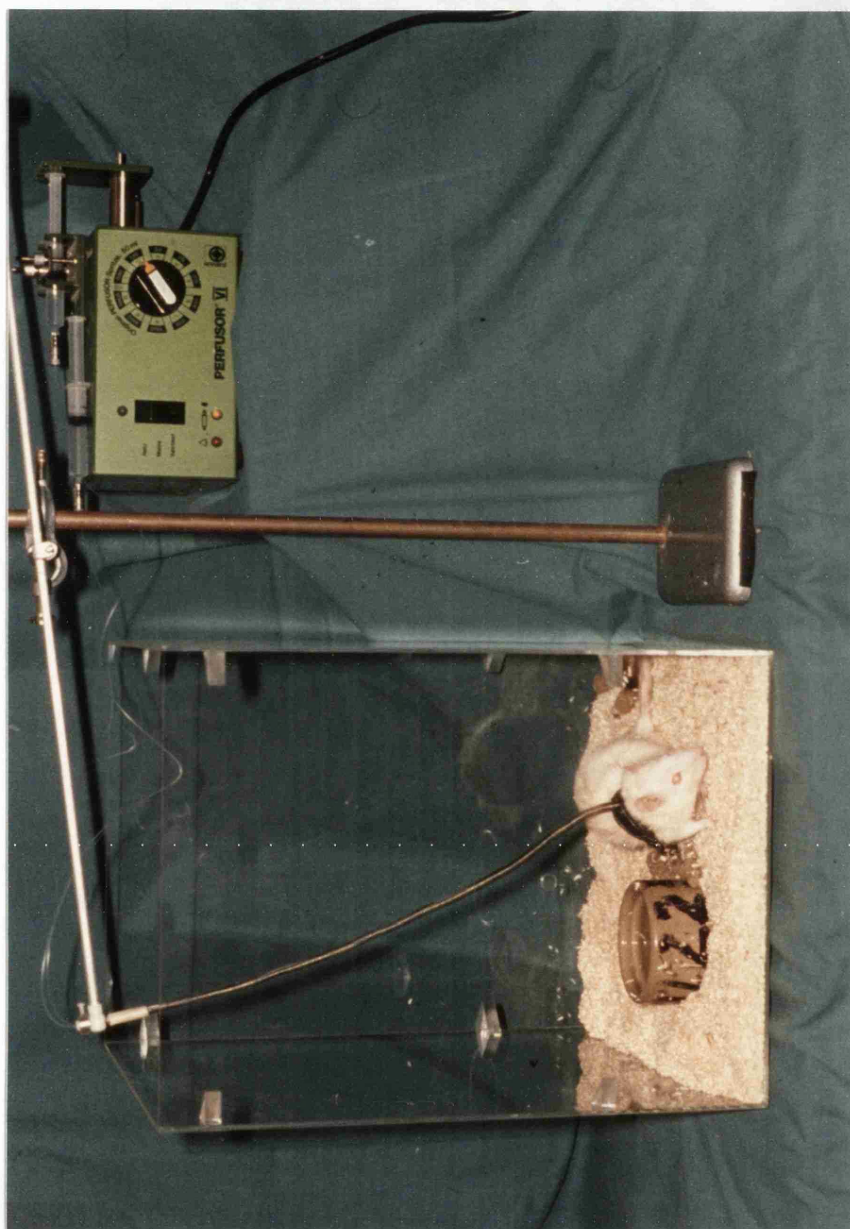
Figure 2.1. Clearances performed in conscious unrestrained rats 18 to 24 hours post-anaesthesia via an isotopic constant infusion technique. The coiled spring allows the rat free movement around the cage and access to water, as well as preventing the rat from gnawing the venous and arterial catheters.

were performed and at the end of the clearance study shortly before culling the animal.

GFR and ERPF were estimated by an isotopic constant infusion technique utilising inulin [^{14}C] carboxylic acid and para-amino [^{3}H] hippuric acid respectively (Amersham International PLC, Buckinghamshire, U.K.). Following loading doses of Inulin [^{14}C] carboxylic acid (0.0264 MBq) and para-amino [^{3}H] hippuric acid (0.264MBq) in 0.5 ml of 0.9% saline, a constant infusion of both isotopes was commenced at 0.8ml/hour for 3 hours (0.0529 MBq hour^{-1} and 0.529 MBq hour^{-1} respectively). A 2 hour equilibrium period was observed before a 0.3 ml blood sample was taken from the carotid line to measure haematocrit (heparinized microhaematocrit capillary tubes, Lancer, County Kildare, Eire) and activity of both isotopes in duplicate 50 µl aliquots of plasma. A second sample was taken at 3 hours followed by a terminal bleed to measure trough CYA whole blood levels and serum electrolytes. For accuracy the syringes were weighed pre- and post infusion to determine the volume of isotope infused (1g was equivalent to 1 ml of infusion). The animal was culled with 0.5 ml pentobarbitone forte [200mg/ml] (Product Division, Veterinary Drug Company PLC, York, U.K.) injected via the carotid line and the kidneys removed for weighing (wet weight) and histological examination.

CALCULATION OF CLEARANCES

The calculation of GFR and ERPF was based on a technique described by Earle and Berliner (1946), eliminating the need for urine collection (see appendix 3). Renal blood flow (RBF) was calculated as

ERPF/(1-haematocrit). FF as the ratio of GFR/ERPF. RVR was calculated using Gomez's formula (Gomez, 1951): MAP/RBF x 80,000 and expressed in dynes/second/cm^{-5}/10^3. MAP was calculated from diastolic pressure plus 1/3 of the pulse pressure. $U_{Na}V$, U_KV, C_{Na} and C_K were calculated using standard formulae. FE_{Na} and FE_K was calculated as C_{Na}/GFR and C_K/GFR respectively.

ANALYTICAL METHODS.

Sodium and potassium were measured by flame photometry (Corning 480; Corning Medical and Scientific Ltd, Essex, U.K.). Serum urea was measured by the enzyme urease method (Dri Stat, BUN rate reagent, Beckman Instruments Inc., High Wycombe, Bucks, U.K.; coefficient of variation, between batch 13.9% and within batch 1.5%). Trough CYA levels (pre-injection) was measured in duplicate samples where possible using high performance liquid chromatography (Waters Wisp 710B, Waters Millipore Ltd, Watford, U.K.; intraassay coefficient of variation, 10 % and interassay coefficient of variation, 16%). Isotopic activity was measured in duplicate 50 μl plasma samples (corrected for quenching and cross-over) by scintillation counting (LKB 1215 Rackbeta Counter, LKB Instruments Ltd, Surrey, U.K.; counting coefficient of variation, 1.3%) after mixing with 5 ml scintillation fluid (Ecoscint LS 271, National Diagnostics, Aylesbury, U.K.).

HISTOLOGY

The kidneys were removed for histology immediately after clearance studies and fixed in 10% formalin. Tissue sections (5μm)

were examined under light microscopy after staining with haematoxylin and eosin and periodic acid Schiff. Sections were examined without prior knowledge of treatment received for evidence of arteriolopathy, tubular vacuolisation, tubular necrosis and interstitial fibrosis.

STATISTICS

All results are expressed as mean$\pm$SEM. Comparisons across groups were made using two-way analysis of variance with Scheffe's correction (Scheffe, 1953). Pair-fed animals were compared using paired and unpaired Students t-test where appropriate. Significance is defined as $p < 0.05$.

RESULTS

Pair-feeding was successfully performed in all groups of
animals. However, group D rats (not pair-fed) receiving CYA via the
intraperitoneal route ate substantially less food daily than their
controls (Figure 2.2). Table 2.1 summarises the renal and systemic
haemodynamic data for groups B and C. Tables 2.2 and 2.3 summarise the
serum and urinary electrolyte data for groups B and C respectively.

GROUP A.

The administration of CYA via gavage proved unsuccessful. There
was a high mortality rate associated with this technique due to either
acute respiratory inhalation of the olive-oil solution (n=3) or
pneumonia developing (n=2). Functional and morphological studies were
not performed in the surviving rats.

GROUP B

The administration of CYA in the feed for 12 weeks did not
induce functional or morphological renal changes. Mean daily weight
gain was similar in CYA- and olive-oil-treated SHR (Figure 2.3).
Although the dose of CYA in the feed was adjusted to deliver
approximately 25 mg/Kg per day, the mean whole blood CYA levels
achieved were significantly lower when compared to the subcutaneous
route (Table 2.1). Serum and urinary electrolytes were similar in
olive-oil- and CYA-treated SHR (Tables 2.2 and 2.3).
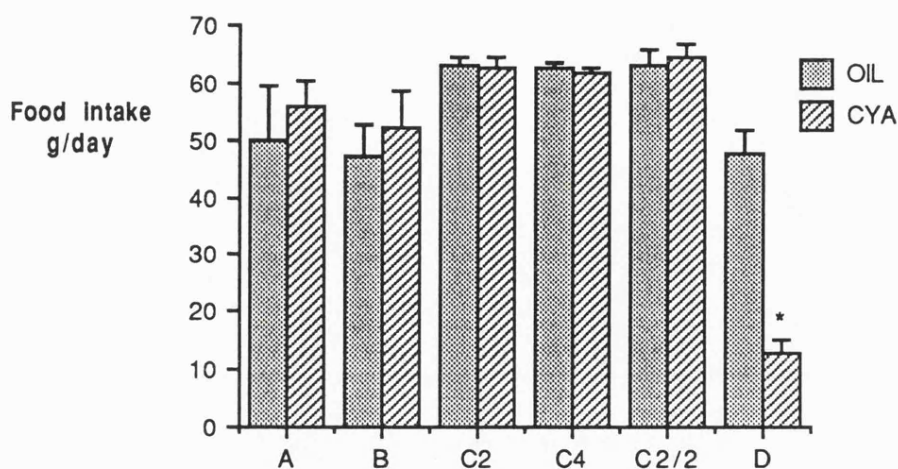
Figure 2.2. Mean daily food intake (g/day) in olive-oil-or CYA-treated SHR (groups A to D). Group A treated via gavage, group B via feed, group C2 via s.c. route for 2 weeks, group C4 via s.c. route for 4 weeks, group C2/2 via s.c route for 2 weeks, then olive-oil via s.c. route for another 2 weeks and group D via i.p. route for 7 days. Group D rats were not pair-fed. * p<0.001.
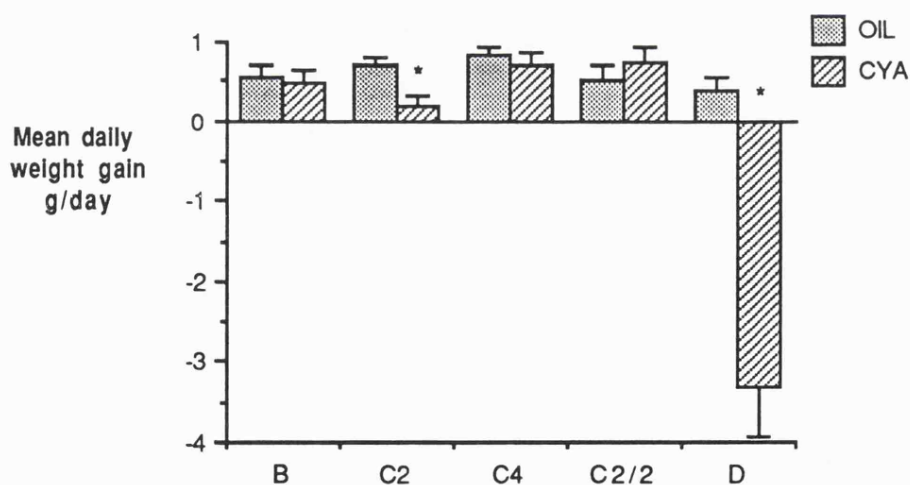
Figure 2.3. The effect of olive-oil or CYA on mean daily weight gain (g/day) in group B, C and D rats. * p <0.001.

TABLE 2.1. EFFECT OF ADMINISTERING OLIVE-OIL OR CYA IN THE FEED (GROUP B) OR VIA THE SUBCUTANEOUS ROUTE (GROUP C) ON CYA WHOLE BLOOD LEVELS, SYSTEMIC AND RENAL HAEMODYNAMICS.

Results are mean±SEM. GFR and ERPF are expressed per 100 g body weight. Abbreviations: Oil, olive-oil and CYA, cyclosporin A. Number of animals in each group used to derive the data is shown in parentheses. Significant differences between groups are indicated by: $C2_{oil}$ versus $C2_{CYA}$, $^a p<0.01$, $^b p<0.001$, $^c p<0.05$. $C4_{oil}$ versus $C4_{CYA}$. $^d p<0.01$, $^e p<0.05$. B_{CYA} versus $C2_{oil}$. $^f p<0.001$, $^g p<0.001$.

| Group... Treatment...	B Oil (n=4)	B CYA (n=4)	C2 Oil (n=9)	C2 CYA (n=9)	C4 Oil (n=10)	C4 CYA (n=10)	C2/2 Oil (n=5)	C2/2 CYA (n=5)
CYA (ng/ml)	-	132.5±32.0^g	-	440±66.5	-	311.6±31.8	-	42.6±10.5
MAP (mmHg)	101.7±6.7	141.2±24.5	157.1±2.8	168.7±2.9^a	142.5±4.7	164.0±4.7^d	163.7±7.4	151.3±5.3
GFR (ml/min)	1.14±0.13	1.15±0.18	1.33±0.05	0.86±0.11^b	1.14±0.05	0.77±0.04^e	1.18±0.06	1.28±0.07
ERPF (ml/min)	3.01±0.11	2.68±0.22	3.11±0.13	1.71±0.16^b	2.84±0.13	1.84±0.19^e	2.44±0.18	2.68±0.20
RVR dynes/sec /cm^{-5}/10^3	8.23±0.87	11.9±1.27	12.7±0.4	27.8±4.8^c	12.3±0.7	24.5±3.1^f	15.0±1.0	14.0±2.5
FF	0.38±0.06	0.42±0.03	0.43±0.01	0.50±0.03^c	0.41±0.01	0.45±0.04	0.50±0.06	0.49±0.07

TABLE 2.2. BASELINE SERUM UREA AND THE EFFECT OF OLIVE-OIL OR CYA ADMINISTERED IN THE FEED (GROUP B) OR VIA THE SUBCUTANEOUS ROUTE (GROUP C) ON SERUM ELECTROLYTES.

Results are mean±SEM and are shown for pre and post treatment. Abbreviations: Oil, olive-oil and CYA, cyclosporin A. Number of animals in each group used to derive the data is shown in parentheses.

Group...Treatment		B Oil (n=4)	B CYA (n=4)	C2 Oil (n=10)	C2 CYA (n=10)	C4 Oil (n=10)	C4 CYA (n=10)	C2/2 Oil (n=5)	C2/2 CYA (n=5)
Baseline Urea (mmol/l)		10.0±1.2	9.6±0.8	7.9±0.5	8.0±0.5	8.6±0.5	8.0±0.4	7.7±0.5	7.0±0.4
Serum Na$^+$ (mmol/l)	Pre..	140.1±0.9	146.3±1.1	143.0±0.9	143.2±0.5	145.1±0.9	141.4±1.5	142.1±1.3	139.1±1.6
	Post.	142.1±1.1	144.6±1.3	144.8±0.9	144.1±2.7	146.3±1.3	146.6±1.0	146.1±0.9	141.4±1.3
Serum K$^+$ (mmol/l)	Pre..	6.1±0.8	6.3±0.8	6.1±0.2	6.1±0.1	5.8±0.4	5.9±0.6	6.1±0.8	6.7±0.6
	Post..	6.0±0.5	5.9±1.5	5.8±0.4	5.4±0.6	5.7±0.6	5.5±0.4	6.4±0.4	6.3±0.3

TABLE 2.3. EFFECT OF OLIVE-OIL OR CYA ADMINISTERED IN THE FEED (GROUP B) OR VIA THE SUBCUTANEOUS ROUTE (GROUP C) ON ON URINE PRODUCTION (V), ABSOLUTE SODIUM ($U_{Na}V$) AND POTASSIUM (U_KV) EXCRETION, SODIUM (C_{Na}) AND POTASSIUM CLEARANCE (C_K) AND FRACTIONAL SODIUM (FE_{Na}) AND POTASSIUM (FE_K) EXCRETION.

Results are mean±SEM and are shown for before and after treatment. Abbreviations: Oil, olive-oil and CYA, cyclosporin A. V, $U_{Na}V$, C_{Na}, U_KV and C_K are expressed per 100 g body weight. Number of animals in each group used to derive the data is shown in parentheses.

Group... Treatment		B Oil (n=4)	B CYA (n=4)	C2 Oil (n=10)	C2 CYA (n=10)	C4 Oil (n=10)	C4 CYA (n=10)	C2/2 Oil (n=5)	C2/2 CYA (n=5)
V (ml/min)	pre..	13.1±2.5	10.4±3.7	9.4±0.9	8.0±0.6	9.7±1.7	8.6±0.8	8.4±1.0	6.8±0.5
	post.	10.1±0.4	12.4±2.7	7.7±0.6	6.8±0.7	7.0±0.8	7.3±0.6	7.1±1.7	6.1±1.4
$U_{Na}V$ (µmol/min)		0.62±0.04	0.47±0.09	0.61±0.05	0.57±0.03	0.62±0.03	0.64±0.03	0.57±0.05	0.49±0.05
		0.58±0.02	0.60±0.09	0.56±0.06	0.48±0.05	0.46±0.06	0.59±0.40	0.51±0.09	0.48±0.10
U_KV (µmol/min)		0.79±0.03	0.63±0.07	0.79±0.04	0.71±0.03	0.82±0.07	0.79±0.03	0.70±0.06	0.65±0.03
		0.64±0.09	0.70±0.03	0.75±0.09	0.56±0.07	0.75±0.04	0.72±0.04	0.69±0.05	0.64±0.09
C_{Na} (ml/min)		4.34±0.27	3.24±0.06	4.29±0.35	3.96±0.25	4.33±0.21	4.49±0.19	3.98±0.36	3.46±0.39
		3.61±0.29	4.15±0.70	3.84±0.41	3.13±0.39	3.23±0.39	3.33±0.26	3.31±0.53	3.27±0.64
C_K (ml/min)		0.15±0.01	0.19±0.02	0.14±0.01	0.12±0.01	0.15±0.01	0.14±0.02	0.15±0.01	0.13±0.01
		0.12±0.04	0.16±0.01	0.17±0.02	0.13±0.02	0.16±0.01	0.16±0.01	0.14±0.02	0.15±0.03
FE_{Na} (%)		0.32±0.01	0.37±0.05	0.31±0.03	0.40±0.06	0.38±0.04	0.45±0.05	3.31±0.53	3.27±0.64
FE_K (%)		10.9±4.7	15.2±3.2	13.0±2.1	15.4±2.4	16.8±1.4	21.9±2.1	12.3±2.2	11.5±2.1

GROUP C

The administration of CYA via the subcutaneous route was simple
to perform, with all rats tolerating the injection regimen without
local or systemic complications developing. Although the daily food
intake was similar in olive-oil- and CYA-treated rats in the two week
group [C2], the mean daily weight gain was significantly lower in rats
receiving CYA (Figure 2.3). In contrast, those rats receiving olive-
oil or CYA for 4 weeks (both C4 and C2/2) had a similar food intake and
daily weight gain. CYA whole blood levels achieved in both the 2 and 4
week treatment groups were effective in inducing functional CYA
nephrotoxicity (Table 2.1).

The effect of CYA on systemic and renal haemodynamics

CYA induced a significant increase in MAP and RVR in both the 2
and 4 week treatment groups. Both GFR and ERPF fell significantly in
the CYA-treated groups. Their was a small but significant increase in
FF ($p < 0.05$) in the 2 week treated group only. The degree of functional
CYA nephrotoxicity induced was comparable in both the 2 and 4 week
treatment groups. However, functional nephrotoxicity was fully
reversible on stopping CYA for a 2 week period. Figure 2.4 summarises
the renal haemodynamic data for group C.

The effect of CYA on serum and urine biochemistry

Baseline serum sodium, potassium and urea concentration were
similar in both olive-oil- and CYA-treated rats. Neither serum sodium
nor potassium levels were influenced by olive-oil or CYA treatment.
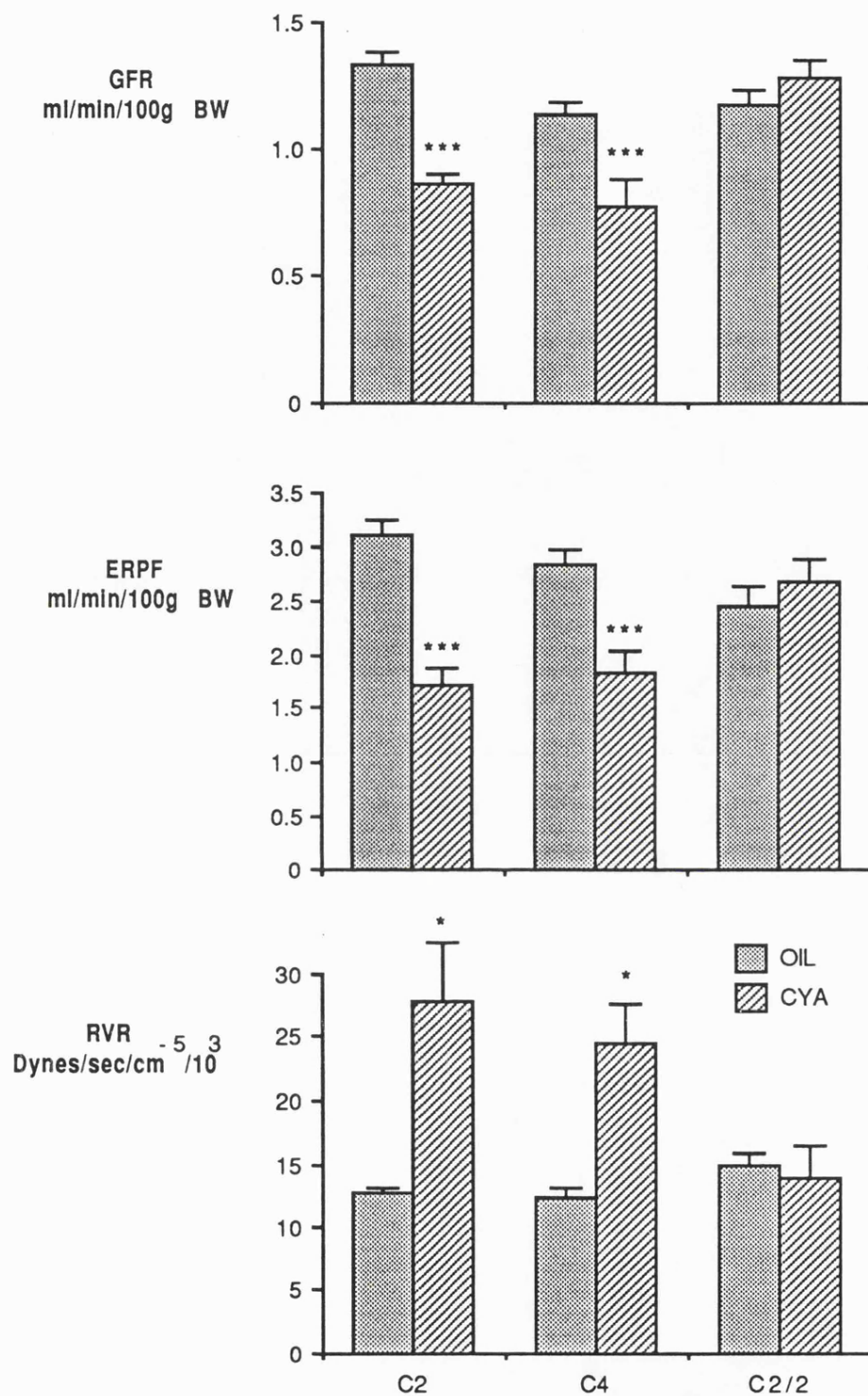
88

Figure 2.4. The effect of olive-oil or CYA administered via the s.c. route on GFR, ERPF and RVR; C2 (2 weeks), C4 (4 weeks) and C2/2 (2 weeks olive-oil or CYA followed by olive-oil for 2 weeks. * p <0.05 and *** p <0.001.

Urine production, $U_{Na}V$, U_KV, C_{Na}, C_k, FE_{Na} and FE_K did not change with CYA therapy (Table 2.2).

The effect of CYA on renal morphology

Light microscopy did not demonstrate any differences in proximal tubular cell, vascular, glomerular or interstitial morphology between olive-oil- and CYA-treated SHR in either the 2 or 4 week groups.

GROUP D.

The administration of CYA via the intraperitoneal route yielded exceedingly high whole blood levels (3946±547 ng/ml). However, these high levels were not tolerated by the rats. All became profoundly anorexic (mean daily food intake 12.6±2.6 g/day) and lost excessive weight (> 10 % compared to baseline) over the 7 day study period. The animals were generally unwell and subdued. Two rats lost greater than 20 % of their baseline weights and were therefore culled after day 4 and 6 respectively. Post-mortem studies on all the rats in this group revealed 1-2 mls of oily material in the peritoneal cavity, which was foul-smelling and purulent in 1 case. Functional and morphological studies were therefore not performed in this group due to the general poor condition of the rats.

DISCUSSION

These preliminary studies were performed in an attempt to develop models for acute and chronic CYA nephrotoxicity in locally bred SHR. The administration of CYA via the subcutaneous route proved most successful in producing satisfactory CYA blood levels, which led to reversible functional CYA nephrotoxicity in both the 2 and 4 week treatment groups, characterised by a significant increase in MAP and RVR, and a decrease in GFR and ERPF. The reversibility of these alterations in systemic and renal haemodynamics is comparable to the acute and reversible CYA nephrotoxicity observed in man (Kahan, 1985). CYA was administered via the subcutaneous route as it proved technically easier than gavage, yet produces a similar pharmacokinetic profile and higher bioavailability (Wassef et al., 1985). Pair-feeding was instituted in view of the anorectic effect of CYA documented at high doses in rodent models to eliminate the effects of differences in protein and sodium intake on renal function (Farthing et al., 1981). However, in common with other studies (Farthing et al., 1981; Dieperink et al., 1983; Dieperink et al., 1983; Jackson et al., 1987; Kaskel et al., 1987b; Winston et al., 1989) weight gain was significantly less in the CYA-treated SHR despite successful pair-feeding, and similar urine and electrolyte excretion. Although the rats in this study were not pair-watered, poor weight gain was also observed by Dieperink et al (1983) in Sprague-Dawley rats consuming similar food and water quantities after administering a comparable dose of CYA for 14 days. Although the cause for this effect is not apparent, CYA-induced protein catabolism may account for the poor weight gain (Farthing et al.,

91

1981).

The SHR is a comparable model to man in which to study CYA nephrotoxicity for several reasons. First, a large proportion of patients treated with CYA develop de novo hypertension, which may have a bearing on the renal effects of this agent (Myers, 1986). Secondly, hypertension is not a consistent feature of CYA administration in other animal models of CYA nephrotoxicity (Murray et al., 1985; Sullivan et al., 1985; Dieperink et al., 1986b; Jackson et al., 1987). However, similar doses of CYA to that used in the present study have been shown to consistently increase MAP in SHR (Siegl et al., 1983; Nahman et al., 1988; Ryffel et al., 1986a; Golub et al., 1989). Much higher doses of CYA [50-100 mg/Kg per day] are associated with hypotension, which is most likely due to volume depletion secondary to food and water anorexia (Ryffel et al., 1986a). Thus, the validity of systemic and renal haemodynamic data derived from extremely toxic animals is doubtful. Thirdly, this strain demonstrates a marked sensitivity to the renal effects of CYA (Ryffel et al., 1986c). The presence of afferent arteriolar vasoconstriction (Arendshorst and Beierwaltes, 1979), increased renal nerve activity (Lunden et al., 1984) and renin-dependent hypertension may account for its sensitivity to CYA nephrotoxicity (Sen et al., 1972), albeit at a comparatively higher dose compared to man. Supraimmunosuppressive doses have been found necessary to induce nephrotoxicity in the rat, despite a similar pharmacokinetic profile to man (Wassef et al., 1985). A reduced ratio of kidney to body weight and higher GFR per gram of kidney weight in the rat may explain the disparity in toxicity and its relative

92

resistance to CYA-induced renal impairment (Sullivan et al., 1985). Finally, the reduction in GFR and ERPF, and the increase in MAP and RVR, induced by CYA correspond to the alterations in renal haemodynamics observed in man (Curtis et al., 1986).

In common with other studies the demonstration that the decline in GFR and ERPF occurred without overt histological evidence of tubular cell damage infers that CYA is not a direct tubular toxin (English et al., 1987; Jackson et al., 1987; Nahman et al., 1988). The disparity between my findings and a previous report documenting morphological abnormalities in an SHR model may relate to the sex and age of the rats studied (Ryffel et al., 1986a). In their study, older male rats (20 weeks) were found to develop more marked morphological abnormalities than comparable females, although it is likely this was due to differences in the pharmacokinetics of CYA between sexes, as lower levels have been shown to occur in females administered comparable doses to males (Faraci et al., 1988). Our results accord with observations made in young female SHR by Nahman et al (1988), who similarly demonstrated an absence of vascular, glomerular or tubular changes under light microscopy, despite administering a similar dose of CYA to SHR by gavage for 4 or 8 weeks. Periodic acid Schiff positive material observed in the preglomerular arterioles was characterised as renin granules and not degenerative or proliferative vascular lesions under electron microscopy. Although the use of higher doses appears to consistently induce morphological damage, significant decreases in food and water intake, weight loss and hypotension are a major handicap and confuse systemic and renal haemodynamic data.

Although hormonal variations in female rats may influence renal blood flow (Evans et al., 1986), the timing of the oestrous cycle was not determined prior to clearance studies. However, as the rats were age and weight matched before randomization to their treatment group, it is unlikely that different oestrous cycles influenced the overall results.

Attempts to induce a chronic model of CYA nephrotoxicity were unsuccessful. The high mortality associated with gavage was unexpected. Olive-oil is viscous and tended to overflow into the lungs. Despite repeated practice this route of administration was abandoned after discussion with the Home Office Liaison Officer for Animal Welfare. The administration of CYA in the feed failed to achieve adequate blood levels and therefore this route was abandoned. The intra-peritoneal injection of CYA successfully produced high whole blood levels, however, these levels were not tolerated by the rats. Gillum et al (1988) induced chronic nephrotoxicity in Sprague-Dawley rats by administering CYA in an olive-oil vehicle [25 mg/Kg per day] via this route for 28 days, with all but 2 rats surviving the study period. However, despite pair-feeding CYA-treated rats gained only 9.4 g, compared to 69 g in olive-oil controls (food intake was not stated). Furthermore, these high levels (4712±500 ng/ml; measured by radioimmunoassay) were associated with a grand mal seizure in 1 rat. In the light of these experiments and the present study permission to administer CYA via this route using lower doses was not granted by the Home Office Inspector.

In conclusion, a model of acute reversible CYA nephrotoxicity

has been characterised in SHR by administering CYA for 2 and 4 weeks via the subcutaneous route. This route proved technically easier than gavage and has been shown to produce a similar pharmacokinetic profile and higher bioavailability (Wassef et al., 1985). The rapid onset of renal dysfunction following acute CYA infusions and the paucity of morphological damage together infer that the renal impairment is primarily a haemodynamic phenomenon, due to changes in RVR and renal blood flow. The failure to produce a chronic model is disappointing, but highlights the resistance to CYA-induced morphological damage in the kidneys of experimental animals. As the degree of functional CYA nephrotoxicity was qualitatively and quantitatively similar in the 2 and 4 week subcutaneous group, all subsequent studies were performed using the 2 week model.

SECTION III

EFFECT OF NIFEDIPINE ON

CYCLOSPORIN A NEPHROTOXICITY IN THE SPONTANEOUSLY HYPERTENSIVE RAT

INTRODUCTION

Intrarenal vasoconstriction has been shown to be a characteristic feature of CYA nephrotoxicity in both animal and clinical studies (Murray et al., 1985; Dieperink et al., 1986a; Curtis et al., 1986; English et al., 1987; Rooth et al., 1988a; Myers et al., 1988a; Conte et al., 1989). If CYA-induced renal arteriolar vasoconstriction is the forerunner of chronic irreversible renal impairment, then measures to ameliorate vasoconstriction with concomitant renal vasodilators may prevent or retard renal injury and preserve renal function. The present series of experiments were designed to investigate the effect of nifedipine, a dihydropyridine calcium channel blocker, with renal vasodilator actions on CYA nephrotoxicity in the SHR. Studies have been performed in surgically intact (two-kidney) SHR, in uninephrectomized SHR and in SHR undergoing uninephrectomy with contralateral renal denervation, to explore the effects of CYA and nifedipine in animals with reduced renal mass, in an attempt to mimic the clinical situation in patients undergoing renal allograft transplantation. Uninephrectomy with contralateral denervation can be considered a comparative model for the transplantation of a kidney from a living donor (Provoost et al., 1986), without the complicating factor of rejection on renal function.

MATERIAL AND METHODS

Animals.

Pair-fed female SHR aged 12 to 16 weeks and weighing 160 to 230 g were used throughout. The animals were individually caged and fed the same diet as described in Section II (appendix 1).

DRUG PREPARATION AND ADMINISTRATION

All injections were performed in the morning between 0900 and 1100 hours. CYA was administered subcutaneously at a dose of 25mg/kg body weight per day as previously described.

Nifedipine (Bayer UK Limited, Berkshire, U.K.) was dissolved in polyethylene glycol, ethanol and water (1.5:1.5:7, v/v) to achieve a final concentration of 0.1 mg/ml and administered at a dose of 0.1 mg/Kg body weight daily. Nifedipine or its vehicle was administered by intraperitoneal injection once daily (0.15-0.26 ml). Preparation of nifedipine and all injections were performed under sodium lighting (Thorn Lighting, Leicester, U.K.) to prevent photodegradation (Tucker et al., 1985).

EXPERIMENTAL GROUPS

The following experimental groups were studied:

(i)   surgically intact SHR (Groups I_{in}-IV_{in}),

(ii) uninephrectomized SHR (Groups I_{nx}-III_{nx}),

(ii) uninephrectomized SHR with contralateral renal denervation (Groups I_{nx+d}-II_{nx+d}).

Pair-fed rats were injected with either olive-oil or CYA for 14

days. Nifedipine or its vehicle was administered as follows:

(i) Surgically intact rats

Group I_{in}a.   Olive-oil (n=10)

Group I_{in}b.   CYA alone (n=10)

Group II_{in}a.   Olive-oil and nifedipine (from day 1; n=10).

Group II_{in}b.   CYA and nifedipine (from day 1; n=10)

Group III_{in}a.   Olive-oil and nifedipine (from day 7 only;

             n=8).

Group III_{in}b.   CYA and nifedipine (from day 7 only; n=8)

Group IV_{in}a.   Olive-oil and nifedipine vehicle

             (from day 1; n=4)

Group IV_{in}b.   CYA and nifedipine vehicle

             (from day 1; n=4)

(ii) Uninephrectomized rats

Group I_{nx}a.   Olive-oil (n=8)

Group I_{nx}b.   CYA alone (n=8)

Group II_{nx}a.   Olive-oil and nifedipine (from day 1; n=8).

Group II_{nx}b.   CYA and nifedipine (from day 1; n=8)

Group III_{nx}a.   Olive-oil and nifedipine vehicle

             (from day 1; n=8)

Group III_{nx}b.   CYA and nifedipine vehicle (from day 1; n=8)

98

(iii) Uninephrectomized SHR with contralateral renal denervation

Group I_{nx+d}a.   Olive-oil (n=8)

Group I_{nx+d}b.   CYA alone (n=8)

Group II_{nx+d}a.   Olive-oil and nifedipine (from day 1; n=8).

Group II_{nx+d}b.   CYA and nifedipine (from day 1; n=8)

EXPERIMENTAL PROTOCOL

All experiments were performed over a 14 day period. Animals were age and weight matched and pair-fed for at least 7 days prior to study. Two days before starting the injections rats were transferred to individual metabolic cages and a 24 hour urine collection obtained. The day before starting injections the rats were fasted for 24 hours and a baseline fasting tail vein blood sample (1.5 ml) was taken to measure serum electrolytes and serum urea. On day 12 the rats were returned to the metabolic cages and a further 24 hour urine collection performed.

SURGICAL PROCEDURES

All operations were performed on a heated operating mat.

(a) Uninephrectomy: anaesthesia for uninephrectomy and renal denervation was induced by an intraperitoneal injection of 2.7 mls/kg of a 2:1:1 mixture by volume of sterile water, midazolam [5mgs/ml] (Roche Products Limited, Welwyn Garden City, U.K.) and Hypnorm [fentanyl 0.315 mgs/ml, fluanisone 10mgs/ml] (Janssen Pharmaceuticals Limited, Oxford, U.K.). The left kidney was exposed by a 3 cm

retroperitoneal flank incision, parallel to the subcostal margin. The kidney was lifted out of the peritoneal cavity by grasping the perinephric fat with a forceps and the capsule incised and retracted to preserve the adrenal gland. The hilar vessels and ureter were then tied off using 3/0 mersilk ligatures (x2) and the kidney removed and the pedicle returned to the abdominal cavity (Figure 3.1). The peritoneum and skin incisions were closed with interrupted sutures (3/0 Supramid pseudofilament polyamide; B. Braun Melsungen AG, W.G.). Each rat was injected post-operatively with buprenorphine hydrochloride (0.3 mg/Kg once daily; Rickitt and Colman Pharmaceuticals Division, Hull, U.K.) subcutaneously for 48 hours for analgesia. The rats were examined daily following the procedure for 1 week for signs of discomfort and dislodged sutures replaced. All sutures were removed after this period.

(b) Unilateral nephrectomy with contralateral renal denervation:
a right uninephrectomy with renal denervation of the left kidney was performed. The left kidney was denervated as it was technically easier to perform on that side in view of the longer renal pedicle. Denervation was achieved by stripping all visible nerves along the renal artery and vein from the aorta to the hilus of the kidney using a watchmakers forceps (Richardsons and Son, Evington, Leicester, U.K.) and cotton wool buds (Boots Limited, Beeston, Nottingham, U.K.). The connective tissue was also stripped away from the hilus to each pole of the kidney. Both renal artery and vein were then painted with 10% phenol solution solubilized in 90% ethanol over a 1 cm zone

Figure 3.1. Uninephrectomy performed via a lateral flank incision under general anaesthesia (i.p. midazolam/Hypnorm).

101

circumferentially towards the middle of the pedicle. Extreme care was taken not to splash phenol onto the ureters as in preliminary studies this led to ureteric scarring and eventual ureteric obstruction. The kidney was returned to the peritoneal cavity and the incision closed. The effectiveness of the denervation procedure was assessed at the end of the study period by comparing tissue noradrenaline content in the denervated kidney to that in the innervated nephrectomy specimen removed at operation (see below).

(c) **Insertion of catheters:** internal carotid and jugular lines were inserted as described in Section II.

MEASUREMENT OF CLEARANCES

The technique used to estimate clearances was as described in Section II.

MEASUREMENT OF TISSUE CATECHOLAMINE CONTENT IN DENERVATED KIDNEYS

The redundant innervated kidney removed at operation and the denervated kidney removed on completion of the clearance studies were cleaned of adherent fat and connective tissue by blunt dissection. The kidney was washed in distilled water and blotted dry before being weighed. The kidney was placed in a 10 ml measuring cylinder and ice-cold distilled water added until the total volume including the tissue was 8 mls. The distilled water and kidney was then thoroughly homogenised on ice using a glass tissue grinder (TKW-300-030T, Gallenkamp Ltd, Loughborough, Leicestershire, U.K.). The

homogenate was transferred to a 20 ml universal bottle (Sterilin Ltd,

Hounslow, Middlesex, U.K.) and dihydroxybenzylamine hydrobromide (1.5

nmol DHB) in 1 ml of 0.1 M perchloric acid added as an internal

standard. The homogenate was acidified with 1 ml of 1.0 M perchloric

acid to prevent loss of noradrenaline from oxidation. Finally, the

homogenate was thoroughly mixed on a vortex mixer and stored at - 70 °C

until assayed for noradrenaline.

The tissue noradrenaline was extracted from the homogenate via

an alumina extraction procedure (Anton and Sayre, 1962) to purify and

concentrate the sample prior to analysis. The samples were allowed to

thaw at room temperature, centrifuged at 2000 g for 10 minutes and then

placed on ice. A 1 ml aliquot was pipetted into a 5 ml test tube and

the pH adjusted to 7.2-8.6 with 2% tris-buffer (250 μl added) and 25 mg

of acid washed alumina added (Bioanalytical Systems, Anachem Ltd.,

Luton, U.K.). The tubes were sealed and mixed on a spiral mixer (Denly

Spiramix, Sussex, U.K.) for 15 minutes. During mixing the

catecholamines are selectively absorbed onto the alumina. After mixing

the suspension was allowed to settle and the supernatant aspirated off

and discarded. The alumina was washed 3 times with ice cold distilled

water (3 mls) and each wash discarded. On the final wash as much

liquid as possible was removed before adding 250 μl of 0.1 M

perchloric acid to elute the catecholamines from the alumina. The

tubes were agitated on ice for 15 minutes and then transferred to a 4 °C

fridge. The supernatant extract was assayed for noradrenaline 24 hours

later by high performance liquid chromatography (Electrochemical

detector LC-3A with an LC-17 Flowcell, Bioanalytical Systems, Purdue

Research Park, West Lafayette, Illinois, U.S.A. Pump delivery system;

model 101A, Altex Scientific Incorporated, Berkeley, California,

U.S.A.). Calibration of equipment and injecting of samples was

performed by Pauline Quinn, Department of Anaesthetics, Leicester Royal

Infirmary). The internal standard added prior to the extraction

procedure allowed correction for loss of noradrenaline during storage

and recovery from the alumina to be made automatically. Calibration

was achieved by comparing the noradrenaline and DHB peak heights

between unknown samples and standards of known concentration. The

chromatogram was recorded on an integrator (Shimadzu C-RIB Chromatopac

integrator; Dyson Instruments, Tyne and Wear, U.K.), which was

programmed to calculate the concentration of noradrenaline per sample

in pmol per 100 µl injected. The total amount of noradrenaline was

expressed in nmol/g kidney.

MEASUREMENT OF URINARY N-ACETYL-β-D-GLUCOSAMINIDASE (NAG)

Biochemical evidence of proximal tubular cell damage was

assessed by measuring urinary NAG activity before and after CYA therapy

in groups $I_{nx+d}a$ and $I_{nx+d}b$ using a colorimetric assay (Boehringer

Mannheim Biochemica, Boehringer Corporation Ltd, Sussex, U.K.).

COMPENSATORY CHANGES IN RENAL GROWTH AFTER UNINEPHRECTOMY (GROUP $I_{nx+d}a$ versus $I_{nx+d}b$ only)

As an indirect measure of the effect of olive-oil or CYA on

renal growth, changes in kidney wet weight were measured by comparing

the weight of the right kidney removed at nephrectomy (cleaned of

adherent fat and washed in saline) with the weight of the left kidney removed after the study had been completed (28 days after uninephrectomy had been performed).

HISTOLOGY

The remaining kidney in the uninephrectomized rats were removed for histology immediately after clearance studies and fixed in 10% formalin. Paraffin tissue sections (5μm) were examined under light microscopy after staining with haematoxylin and eosin and periodic acid Schiff. Sections were examined without prior knowledge of treatment received for evidence of arteriolopathy, tubular vacuolisation, tubular necrosis and interstitial fibrosis.

Histology was not performed in SHR undergoing uninephrectomy with contralateral renal denervation.

STATISTICS

All results are expressed as mean±SEM. Comparisons across groups were made using two-way analysis of variance with Scheffe's correction (Scheffe, 1953). Pair-fed animals were compared using paired and unpaired Students t-test where appropriate. Significance is defined as $p < 0.05$.

RESULTS

(i) SURGICALLY INTACT SHR (Group I_{in}-IV_{in})

Data for rats treated with olive-oil or CYA alone (group $I_{in}a$ and $I_{in}b$) has been transferred from Section II (group C2) for ease of comparison with nifedipine data. Pair-feeding was successfully performed in all groups of animals (Figure 3.2; overall mean daily food consumption [groups I_{in}-IV_{in} inclusive]: olive-oil, 64.3 ± 1.0 versus CYA, 63.5 ± 1.1 g/day, not significant). However, mean daily weight gain was significantly lower in the CYA-treated rats, except for group $III_{in}b$ (Figure 3.3; groups I_{in}-IV_{in} inclusive: olive-oil, 1.10 ± 0.1 versus CYA, 0.49 ± 0.1 g/day, $p < 0.001$).

All rats tolerated the injection regimen without local or systemic sepsis. Systemic and renal haemodynamic data is missing for 3 pairs of rats, due to technical problems during the cannulation or clearance period.

EFFECT OF OLIVE-OIL OR CYA ON SYSTEMIC AND RENAL HAEMODYNAMICS (GROUP $I_{in}a$ versus $I_{in}b$).

Data for CYA whole blood levels, MAP, GFR, ERPF, RVR and FF for groups I_{in}-IV_{in} is documented in Table 3.1. Mean CYA whole blood levels were similar in groups $I_{in}b$, $II_{in}b$ and $III_{in}b$. In group $IV_{in}b$ CYA levels were higher, although only significantly different from group $III_{in}b$. CYA-induced a significant increase in MAP ($p < 0.01$) and RVR ($p < 0.05$) compared with olive-oil-treated SHR after 14 days. CYA also induced a significant reduction in both GFR (35.3 %, $p < 0.001$) and ERPF (45.0 %, $p < 0.001$), leading to a small increase in FF ($p < 0.05$).
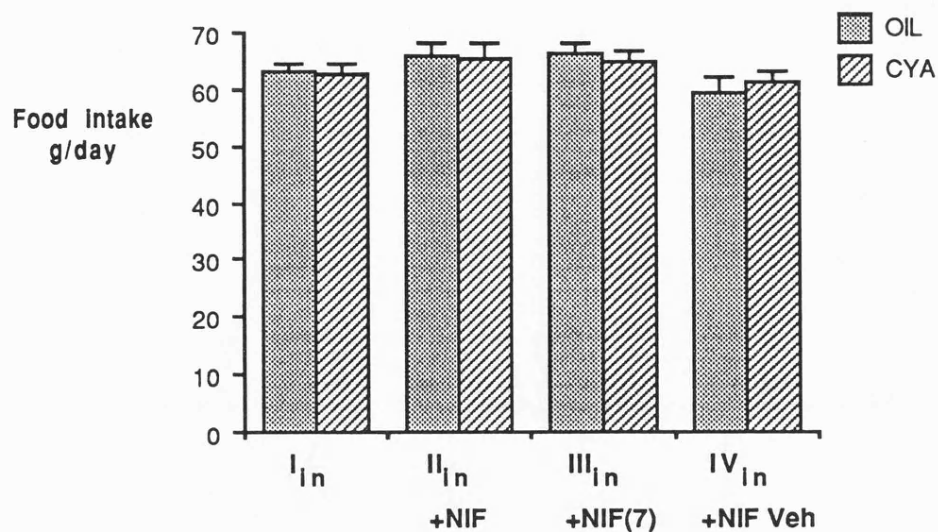
Figure 3.2. The effect of olive-oil or CYA on mean daily food intake (g/day). Group I_{in} received either olive-oil or CYA for 14 days; group II_{in} received either olive-oil or CYA plus nifedipine for 14 days; group III_{in} received either olive-oil or CYA plus nifedipine from day 7 only; group IV_{in} received olive-oil or CYA plus nifedipine vehicle for 14 days.
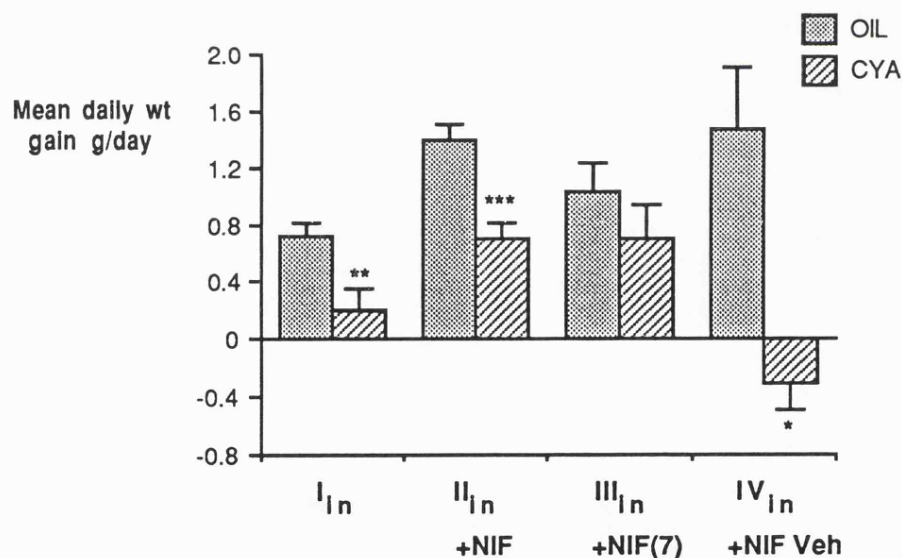
Figure 3.3. The effect of olive-oil or CYA on mean daily weight gain (g/day). * $p < 0.05$, ** $p < 0.01$ and *** $p < 0.001$.

107

TABLE 3.1. EFFECT OF NIFEDIPINE ON CYA WHOLE BLOOD LEVELS, SYSTEMIC AND RENAL HAEMODYNAMICS IN SURGICALLY INTACT SHR RECEIVING OLIVE-OIL OR CYA (GROUPS I_{in}-IV_{in}).

Results are mean±SEM. GFR and ERPF are expressed per 100g body weight. Abbreviations: Oil, olive-oil; NIF, nifedipine; NIF veh, nifedipine vehicle. Significant differences between groups are indicated by: Ia vs Ib, [a]$p<0.001$, [b]$p<0.01$ and [c]$p<0.05$; IIb vs Ib, [d]$p<0.001$, [e]$p<0.02$ and [f]$p<0.01$; IIa vs IIb, [g]$p<0.05$; IIIa vs IIIb, [h]$p<0.01$ and [i]$p<0.02$; IVb vs IIIb, [j]$p<0.01$; IVa vs IVb, [k]$p<0.001$ and [l]$p<0.05$. IIIb vs IIb, [m]$p<0.01$. The number of animals in each group used to derive the data shown in parentheses.

Group... Treatment...	I_{in}^{a} Oil (n=9)	I_{in}^{b} CYA (n=9)	II_{in}^{a} Oil+NIF (n=9)	II_{in}^{b} CYA+NIF (n=9)	III_{in}^{a} Oil+NIF (day 7) (n=7)	III_{in}^{b} CYA+NIF (day7) (n=7)	IV_{in}^{a} Oil+NIF veh (n=4)	IV_{in}^{b} CYA+NIF veh (n=4)
CYA whole blood level (ng/ml)	-	440.1±66.5	-	396.3±103.2	-	334.0±50.5	-	606.0±48.7[j]
MAP (mmHg)	157.1±2.8	168.7±2.9[b]	142.2±6.6	164.4±6.2[g]	141.0±4.2	153.3±6.6	127.9±8.0	150.8±13.2
GFR (ml/min)	1.33±0.05	0.86±0.11[a]	1.34±0.06	1.38±0.06[f]	1.21±0.06	0.88±0.09[im]	1.26±0.05	0.86±0.11[l]
ERPF (ml/min)	3.11±0.13	1.71±0.16[a]	3.10±0.18	2.95±0.19[d]	2.61±0.07	1.74±0.24[hm]	2.90±0.14	1.64±0.05[k]
RVR (dynes/sec /cm^{-5}/10^{3})	12.7±0.4	27.8±4.8[c]	11.6±1.1	15.4±1.08[e]	11.7±0.6	19.1±2.5[l]	11.5±1.3	20.0±2.0[l]
FF	0.43±0.01	0.50±0.03[c]	0.43±0.02	0.47±0.02	0.54±0.03	0.53±0.03	0.56±0.03	0.52±0.05

EFFECT OF NIFEDIPINE ON SYSTEMIC AND RENAL HAEMODYNAMICS IN OLIVE-OIL-OR CYA-TREATED SHR (GROUP II_{in}-IV_{in}).

In olive-oil-treated SHR neither nifedipine (group II_{in}a versus I_{in}a) nor nifedipine vehicle (group IV_{in}a versus I_{in}a) influenced MAP, GFR, ERPF, RVR or FF. However, nifedipine commenced simultaneously with CYA from day 1 prevented the reduction in GFR and ERPF, which remained at comparable levels to their olive-oil-treated counterparts (group II_{in}b versus II_{in}a). Nifedipine also attenuated the increase in RVR, which remained significantly lower than in the rats treated with CYA alone (group II_{in}b versus I_{in}b, $p < 0.02$), although still elevated compared to group II_{in}a ($p < 0.05$).

In contrast, commencing nifedipine 7 days after CYA failed to improve renal function, with GFR ($p < 0.02$) and ERPF ($p < 0.01$) remaining significantly depressed compared with the olive-oil treated controls (group III_{in}b versus III_{in}a) and group II_{in}b ($p < 0.01$ for both parameters). Delayed nifedipine therapy also failed to restore RVR to control levels and remained elevated in the CYA-treated SHR (group III_{in}b versus III_{in}a, $p < 0.02$), comparable to the level documented in the group treated with CYA alone (group III_{in}b versus I_{in}b, not significant). MAP was similar in both groups treated with nifedipine (group II_{in}b versus III_{in}b, not significant). Figure 3.4 summarises the renal haemodynamic data for groups I_{in}-IV_{in}.
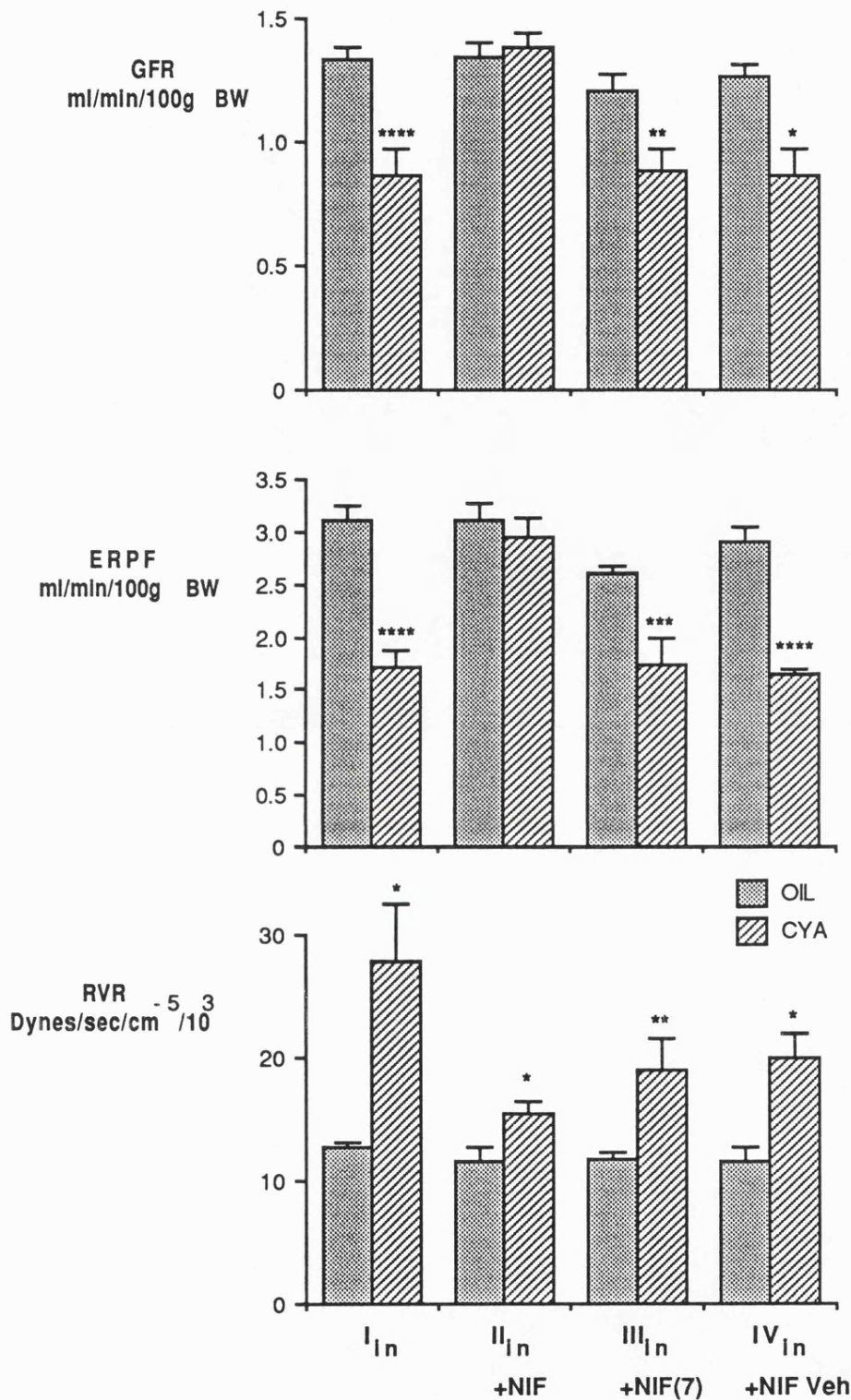
Figure 3.4. The effect of nifedipine on GFR, ERPF and RVR in surgically intact (two-kidney) SHR receiving either olive-oil or CYA (groups I_{in} to IV_{in}). * $p < 0.05$, ** $p < 0.02$, *** $p < 0.01$ and **** $p < 0.001$.

EFFECT OF OLIVE-OIL OR CYA ON SERUM ELECTROLYTES, URINE PRODUCTION AND ELECTROLYTE EXCRETION (GROUP $I_{in}a$ and $I_{in}b$).

Table 3.2 and 3.3 document the data on serum and urinary electrolytes respectively for all rats studied (groups I_{in}-IV_{in}). Baseline serum sodium and potassium were similar in olive-oil- and CYA-treated SHR and did not change following treatment. Baseline serum urea was similar in olive- oil- and CYA-treated SHR. Furthermore, urine production, $U_{Na}V$, U_KV, C_{Na} and C_K did not change following olive-oil (group $I_{in}a$) or CYA (group $I_{in}b$). FE_{Na} and FE_K were similar in olive-oil- and CYA-treated SHR (group $I_{in}a$ versus $I_{in}b$).

EFFECT OF NIFEDIPINE ON SERUM ELECTROLYTES, URINE PRODUCTION AND ELECTROLYTE EXCRETION (GROUP II_{in}-IV_{in}).

Baseline serum sodium and potassium were similar in both olive-oil- and CYA-treated SHR and were not influenced by treatment. Baseline serum urea was also comparable in all groups. In olive-oil-treated SHR neither nifedipine (group $II_{in}a$, paired t-test) nor nifedipine vehicle (group $IV_{in}a$, paired t-test) influenced urine production, $U_{Na}V$, U_KV, C_{Na} or C_K. Similarly, FE_{Na} and FE_K in the olive-oil-treated SHR did not change after nifedipine (group $II_{in}a$ versus $I_{in}a$) nor after nifedipine vehicle (group $IV_{in}a$ versus $I_{in}a$). However, nifedipine introduced simultaneously with CYA from day 1 significantly increased $U_{Na}V$ ($p<0.01$), U_KV ($P<0.001$), C_{Na} ($p<0.02$) and C_K ($p<0.001$) [group $II_{in}b$, paired t-test]. However, these changes in urine flow rate and electrolyte excretion were not accompanied by a change in FE_{Na} or FE_K (Group $II_{in}b$ versus $I_{in}b$). Conversely, the

TABLE 3.2. BASELINE SERUM UREA AND THE EFFECT OF NIFEDIPINE ON SERUM ELECTROLYTES IN OLIVE-OIL- OR CYA-TREATED SHR (GROUPS I_{in}-IV_{in}).

Results are means±SEM and are shown for before and after treatment. Abbreviations: Oil, olive-oil; NIF, nifedipine; NIF veh, nifedipine vehicle. The number of animals in each group used to derive data shown in parentheses.

Group... Treatment...		I_{in}^a Oil (n=10)	I_{in}^b CYA (n=10)	II_{in}^a Oil+NIF (n=10)	II_{in}^b CYA+NIF (n=10)	III_{in}^a Oil+NIF (day 7) (n=8)	III_{in}^b CYA+NIF (day7) (n=8)	IV_{in}^a Oil+NIF veh (n=4)	IV_{in}^b CYA+NIF veh (n=4)
Baseline Urea (mmol/l)		7.9±0.5	8.0±0.5	9.4±0.5	9.5±0.5	9.7±0.6	9.4±0.3	7.7±0.5	7.9±0.6
Serum Na$^+$ (mmol/l)	pre..	143.0±0.9	143.2±0.5	150.5±2.3	149.3±1.9	147.0±1.2	148.1±1.3	144.5±0.7	145.0±2.2
	post..	144.8±0.9	144.1±2.7	144.1±1.7	140.1±1.8	141.7±1.2	140.5±0.4	141.0±2.9	140.0±1.2
Serum K$^+$ (mmol/l)	pre..	6.1±0.2	6.1±0.1	6.5±0.2	6.8±0.3	5.5±0.2	5.2±0.2	5.0±0.2	5.0±0.2
	post..	5.8±0.2	5.4±0.2	5.7±0.4	6.4±0.2	4.9±0.1	5.4±0.1	4.7±0.2	4.7±0.3

TABLE 3.3. EFFECT OF NIFEDIPINE ON URINE PRODUCTION (V), ABSOLUTE SODIUM ($U_{Na}V$) AND POTASSIUM (U_KV) EXCRETION, SODIUM (C_{Na}) AND POTASSIUM CLEARANCE (C_K) AND FRACTIONAL SODIUM (FE_{Na}) AND POTASSIUM (FE_K) EXCRETION (GROUPS $I_{in}-IV_{in}$).

Results are means±SEM per 100 g body weight and are shown for before and after treatment. Abbreviations: Oil, olive-oil; NIF, nifedipine; NIF veh, nifedipine vehicle. The number of animals in each group used to derive data shown in parentheses. Statistical significance (paired t-test):*p<0.01,**p<0.02,***p<0.001.

Group Treatment		I_{in}^a Oil (n=10)	I_{in}^b CYA (n=10)	II_{in}^a Oil+NIF (n=10)	II_{in}^b CYA+NIF (n=10)	III_{in}^a Oil+NIF (day 7) (n=8)	III_{in}^b CYA+NIF (day7) (n=8)	IV_{in}^a Oil+NIF veh (n=4)	IV_{in}^b CYA+NIF veh (n=4)
V (µl/min)	pre.	9.35±0.94	7.98±0.62	8.14±0.47	9.49±1.03	9.10±0.60	10.2±0.51	6.87±0.84	8.70±0.84
	post.	7.65±0.61	5.84±0.71	8.75±0.45	10.01±0.77	7.60±0.90	9.11±0.53	10.15±0.91	6.70±0.75
$U_{Na}V$ (µmol/min)		0.61±0.05	0.57±0.03	0.65±0.03	0.65±0.03*	0.87±0.04	0.82±0.05	0.73±0.07	0.78±0.07
		0.56±0.06	0.43±0.05	0.67±0.03	0.77±0.04	0.75±0.10	0.56±0.10	0.69±0.06	0.67±0.04
C_{Na} (ml/min)		4.29±0.35	3.96±0.25	4.53±0.23	4.55±0.23**	5.91±0.30	5.53±0.33	5.07±0.53	5.36±0.46
		3.84±0.41	3.13±0.39	4.63±0.23	5.62±0.29	5.24±0.70	3.98±0.71	4.90±0.52	4.91±0.29
FE_{Na} (%)		0.31±0.03	0.40±0.06	0.35±0.03	0.41±0.01	0.26±0.05	0.51±0.11	0.39±0.03	0.32±0.02
U_KV (µmol/min)		0.79±0.04	0.71±0.03	0.78±0.02	0.81±0.04***	1.07±0.05	1.00±0.06	0.80±0.08	0.78±0.07
		0.75±0.09	0.56±0.07	0.77±0.03	0.91±0.03	0.84±0.06	0.86±0.07	0.81±0.05	0.41±0.05
C_K (ml/min)		0.13±0.01	0.12±0.01	0.12±0.01	0.12±0.01***	0.20±0.01	0.20±0.02	0.18±0.02	0.16±0.02
		0.17±0.02	0.13±0.02	0.16±0.02	0.21±0.01	0.20±0.02	0.20±0.02	0.21±0.02	0.10±0.02
FE_K (%)		13.0±2.1	15.4±2.4	12.6±2.2	15.5±1.4	16.8±1.0	25.8±3.9	16.9±1.00	11.3±1.53

delayed administration of nifedipine did not influence urine

production, $U_{Na}V$, U_KV, C_{Na} and C_K (Group $III_{in}b$, paired t-test), or

FE_{Na} and FE_K (group $III_{in}b$ versus $I_{in}b$).

LIGHT MICROSCOPY

Light microscopy did not demonstrate any differences in proximal

tubular cell, vascular, glomerular or interstitial morphology between

olive-oil- and CYA-treated SHR.

(ii) UNINEPHRECTOMIZED SHR (Group I_{nx}-III_{nx})

All rats survived the uninephrectomy procedure and none

developed local or systemic sepsis. Although several rats managed to

remove their sutures during the first 24 hours post-operatively, they

were re-sutured under halothane:oxygen anaesthesia without

complications developing. Post-operative analgesia was continued for

48 hours.

Pair feeding was successfully performed in all groups studied

(Figure 3.5; overall mean food intake [groups I_{nx}-III_{nx} inclusive]:

olive-oil, 64.8±1.3 versus CYA, 67.3±0.9 g/day, not significant).

However, mean daily weight gain was significantly lower in CYA compared

with olive-oil-treated SHR for all groups studied (Figure 3.6; groups

I_{nx}-III_{nx} inclusive: olive-oil, 1.27±0.20 versus CYA, 0.70±0.06 g/day,

$p < 0.05$).

Systemic and renal haemodynamic data is missing for 2 pairs of

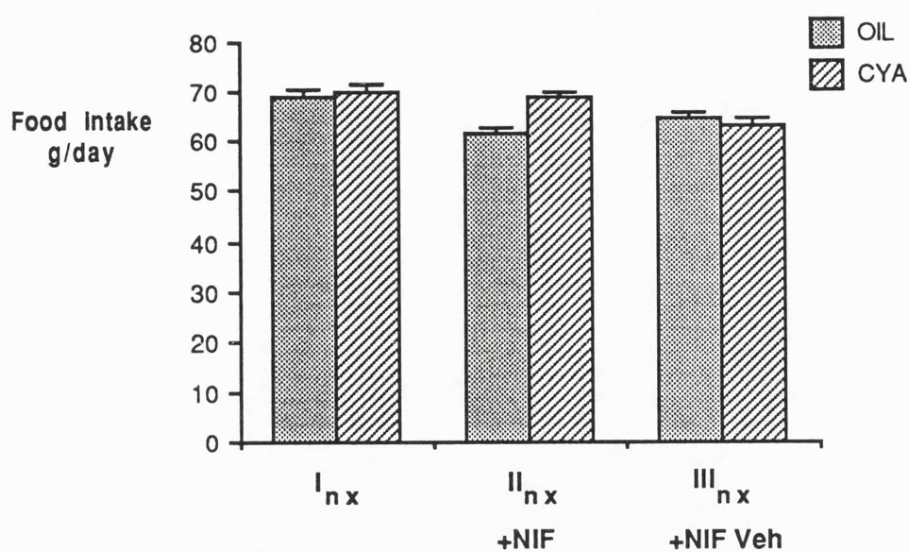rats due to technical problems during the cannulation or clearance

Figure 3.5. The effect of olive-oil or CYA on mean daily food intake (g/day). Group I_{nx} received either olive-oil or CYA for 14 days; group II_{nx} received either olive-oil or CYA plus nifedipine for 14 days; group III_{nx} received either olive-oil or CYA plus nifedipine vehicle for 14 days.
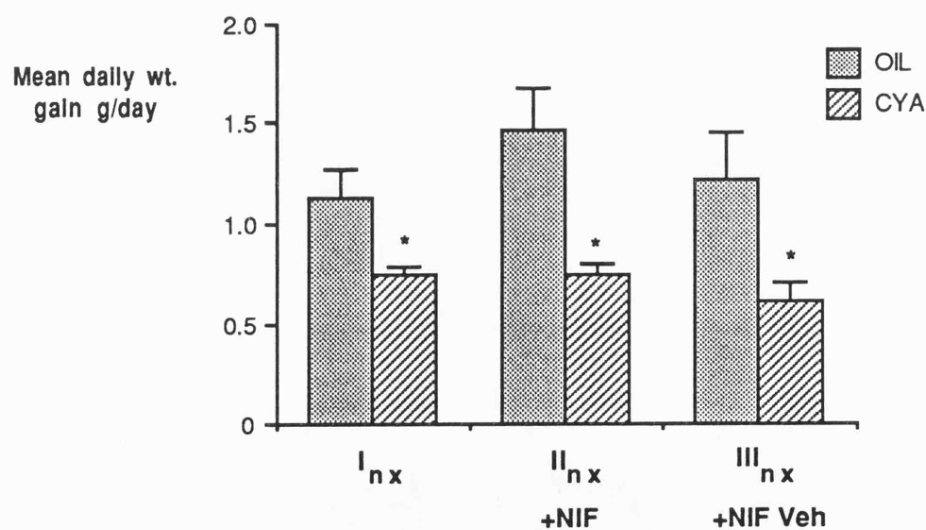

Figure 3.6. The effect of olive-oil or CYA on mean daily weight gain (g/day). * $p < 0.05$.

period. No episodes of local or systemic sepsis occurred secondary to
the injections.

EFFECT OF OLIVE-OIL OR CYA ON SYSTEMIC AND RENAL HAEMODYNAMICS (GROUP $I_{nx}a$ versus $I_{nx}b$).

Table 3.4 summarises the data for CYA whole blood levels, MAP,
GFR, ERPF, RVR and FF for all groups studied. Mean CYA whole blood
levels were similar in all CYA-treated groups. CYA administration for
14 days led to a significant increase in MAP ($p < 0.05$) and RVR ($p < 0.02$)
compared to olive-oil-treated SHR. A significant reduction in GFR
(27.1 per cent, $p < 0.01$) and ERPF (28.9 per cent, $p < 0.01$) was documented
in the CYA-treated SHR, without a change in FF.

EFFECT OF NIFEDIPINE ON SYSTEMIC AND RENAL HAEMODYNAMICS IN OLIVE-OIL- OR CYA-TREATED SHR (GROUPS II_{nx} and III_{nx})

Neither nifedipine (group $II_{nx}a$ versus $I_{nx}a$) nor nifedipine
vehicle (group $III_{nx}a$ versus $I_{nx}a$) influenced systemic or renal
haemodynamics (Table 3.4). The concomitant administration of
nifedipine to CYA-treated SHR did not prevent the reduction in GFR or
ERPF, which were significantly lower when compared with their pair-fed
olive-oil controls (group $II_{nx}b$ versus $II_{nx}a$). In addition, RVR
remained elevated ($p < 0.02$) in the in the CYA-treated SHR receiving
nifedipine (group $II_{in}b$ versus $II_{in}a$), remaining at comparable levels
to SHR receiving CYA (group $I_{in}b$). MAP was not influenced by treatment
with nifedipine (group $II_{in}a$ versus $I_{in}a$, not significant). Figure 3.7

TABLE 3.4. EFFECT OF NIFEDIPINE ON CYA WHOLE BLOOD LEVELS, SYSTEMIC AND RENAL HAEMODYNAMICS IN UNINEPHRECTOMIZED SHR RECEIVING OLIVE-OIL OR CYA (GROUPS I_{nx}-III_{nx}).

Results are mean±SEM. GFR and ERPF are expressed per 100g body weight. Abbreviations: Oil, olive-oil; NIF, nifedipine; NIF veh, nifedipine vehicle. Significant differences between groups are indicated by: Ia vs Ib; [a]p<0.05, [b]p<0.01, [c]p<0.02. IIa vs IIb; [d]p<0.05, [e]p<0.02. IIIa vs IIIb; [f]p<0.05. IIb vs Ib; [g]p>0.1. The number of animals in each group used to derive the data is shown in parentheses.

| Group... Treatment... Number of animals...	I_{nx}[a] Oil (n=7)	I_{nx}[b] CYA (n=7)	II_{nx}[a] Oil+NIF (n=8)	II_{nx}[b] CYA+NIF (n=8)	III_{nx}[a] Oil+NIF Veh (n=7)	III_{nx}[b] CYA+NIF Veh (n=7)
CYA whole blood level (ng/ml)	-	323±32.8	-	308.2±10.2	-	335±34.9
MAP (mmHg)	146.7±6.7	167.2±2.9[a]	148.6±2.8	168.6±6.1[e]	155.0±5.1	162.2±3.3
GFR (mls/min)	0.96±0.04	0.70±0.06[b]	0.96±0.05	0.73±0.06[d]	0.92±0.08	0.68±0.06[f]
ERPF (mls/min)	1.94±0.10	1.38±0.13[b]	1.63±0.09	1.32±0.10[d]	1.77±0.20	1.28±0.12[f]
RVR (Dynes/s/cm^{-5}/10^3)	20.2±1.8	31.6±3.3[c]	17.6±1.16	24.3±2.2[eg]	17.7±2.0	25.9±3.6[f]
FF	0.50±0.02	0.50±0.01	0.59±0.03	0.56±0.03	0.53±0.04	0.53±0.02
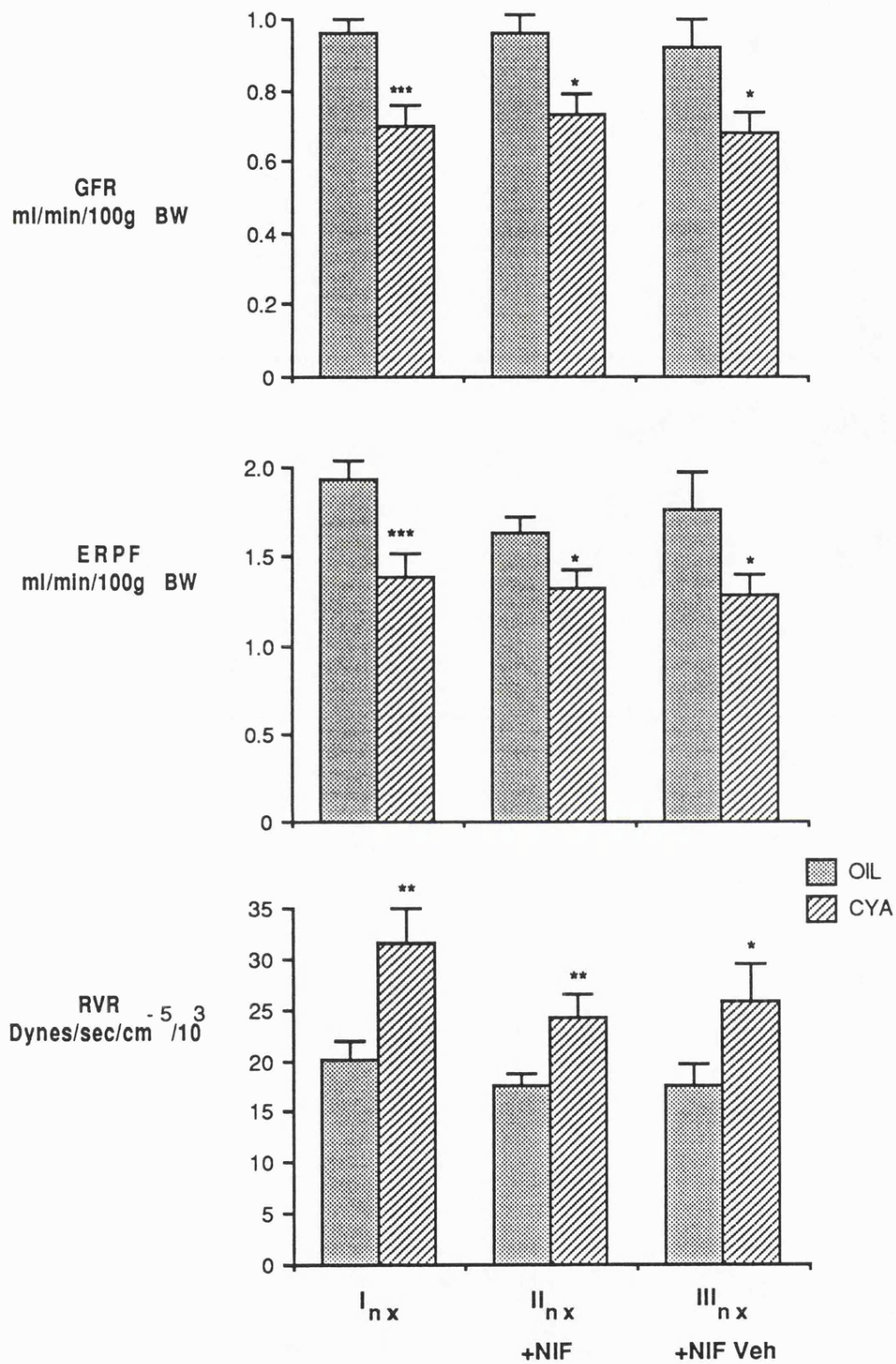
Figure 3.7. The effect of nifedipine on GFR, ERPF and RVR in uninephrectomized SHR receiving either olive-oil or CYA (groups I_{nx} to III_{nx}). * $p < 0.05$, ** $p < 0.02$ and *** $p < 0.01$.

summarises the renal haemodynamic data for groups I_{nx}-III_{nx}.

EFFECT OF OLIVE-OIL OR CYA ON SERUM ELECTROLYTES AND UREA, URINE PRODUCTION AND ELECTROLYTE EXCRETION (GROUP I_{nx}a and I_{nx}b).

Tables 3.5 and 3.6 document data for serum and urinary electrolytes respectively for all rats undergoing uninephrectomy. Plasma sodium and potassium were similar at baseline in olive-oil- and CYA-treated SHR and did not change after nifedipine nor after nifedipine vehicle. Baseline serum urea was similar for all groups studied. Urine production, $U_{Na}V$, $U_K V$, C_{Na} and C_K did not change after olive-oil (group I_{nx}a, paired t-test). However, CYA therapy led to a significant decrease in $U_{Na}V$ ($p<0.05$) and $U_K V$ ($p<0.02$), although C_{Na}, C_K, FE_{Na} and FE_K did not change.

EFFECT OF NIFEDIPINE ON URINE PRODUCTION AND SODIUM EXCRETION (GROUP II_{nx} and III_{nx}).

In olive-oil-treated SHR neither nifedipine (group II_{nx}a, paired t-test) nor nifedipine vehicle (group III_{nx}a, paired t-test) influenced urine production, $U_{Na}V$, U_K, C_{Na} or C_K. FE_{Na} and FE_K in the olive-oil-treated SHR did not change after nifedipine (group II_{nx}a versus I_{nx}a) nor after nifedipine vehicle (group III_{nx}a versus group II_{nx}a). Furthermore, concomitant nifedipine treatment did not alter urine production, $U_{Na}V$, $U_K V$ or C_{Na}, although C_K increased significantly (group II_{nx}b, $p<0.01$). FE_{Na} and FE_K were similar in olive-oil- and CYA-treated SHR receiving nifedipine (group II_{nx}a versus II_{nx}b).

TABLE 3.5. BASELINE UREA AND SERUM ELECTROLYTES BEFORE AND AFTER NIFEDIPINE THERAPY (GROUPS I_{nx} - III_{nx}).

Results are mean±SEM. Abbreviations: Oil, olive-oil; NIF, nifedipine; NIF veh, nifedipine vehicle. The number of animals in each group used to derive the data is shown in parentheses.

Group.... Treatment... Number of animals		I_{nx}^a Oil (n=7)	I_{nx}^b CYA (n=7)	II_{nx}^a Oil+NIF (n=8)	II_{nx}^b CYA+NIF (n=8)	III_{nx}^a Oil+NIF veh (n=7)	III_{nx}^b CYA+NIF veh (n=7)
Baseline urea (mmol/1)		10.5±0.3	11.0±0.3	10.6±0.23	11.1±0.6	10.8±0.2	11.3±0.5
Serum Na$^+$ (mmol/1)	pre..	147.9±1.0	146.8±0.6	145.5±0.4	149.6±1.3	149.1±1.0	145.8±0.2
	post..	148.3±1.5	153.3±1.1	143.1±1.2	140.0±1.8	143.8±1.3	143.5±0.7
Serum K$^+$ (mmol/1)	pre..	5.8±0.2	5.9±0.1	5.0±0.1	5.3±0.2	5.3±0.2	5.2±0.2
	post..	5.4±0.1	5.7±0.1	5.2±0.2	4.6±0.3	4.9±0.3	5.4±0.1

TABLE 3.6. EFFECT OF NIFEDIPINE ON URINE PRODUCTION (V), ABSOLUTE SODIUM ($U_{Na}V$) AND POTASSIUM (U_KV) EXCRETION, SODIUM (C_{Na}) AND POTASSIUM CLEARANCE (C_K) AND FRACTIONAL SODIUM (FE_{Na}) AND POTASSIUM (FE_K) EXCRETION IN UNINEPHRECTOMIZED SHR (GROUPS I_{nx}-III_{nx}).

Results are mean±SEM per 100g body weight. Abbreviations: Oil, olive-oil; NIF, nifedipine; NIF veh, nifedipine vehicle. Significant differences between groups are indicated by: Ia vs Ib; [a]p<0.05, [b]p<0.02. IIa vs IIb; [c]p<0.01. The number of animals in each group used to derive the data is shown in parentheses.

Group Treatment Number of animals		I_{nx}[a] Oil (n=7)	I_{nx}[b] CYA (n=7)	II_{nx}[a] Oil+NIF (n=8)	II_{nx}[b] CYA+NIF (n=8)	III_{nx}[a] Oil+NIF veh (n=7)	III_{nx}[b] CYA+NIF veh (n=7)
V (μl/min)	pre..	20.9±1.4	24.0±2.1	21.4±1.3	19.4±1.4	19.0±1.4	22.1±1.2
	post..	19.2±1.6	21.8±2.4	18.5±2.2	21.2±0.5	20.8±1.9	19.0±1.5
$U_{Na}V$ (μmol/min)		1.79±0.12	1.87±0.07	1.88±0.09	1.68±0.12	1.41±0.08	1.90±0.10
		1.53±0.15	1.57±0.09[a]	1.48±0.15	1.86±0.09	1.43±0.14	1.70±0.15
C_{Na} (ml/min)		12.1±0.8	12.8±0.5	12.9±0.6	11.2±0.7	9.5±0.5	13.0±0.6
		10.8±1.3	10.2±0.7	10.3±1.0	13.3±0.8	10.0±0.1	11.8±1.0
FE_{Na} (%)		0.55±0.08	0.73±0.10	0.52±0.09	0.74±0.09	0.42±0.07	0.64±0.16
C_K (ml/min)		0.37±0.01	0.38±0.01	0.46±0.02	0.39±0.02	0.35±0.14	0.46±0.02
		0.45±0.05	0.45±0.02	0.48±0.04	0.54±0.03[c]	0.47±0.03	0.53±0.04
U_KV (μmol/min)		2.13±0.08	2.23±0.08	2.31±0.11	2.08±0.15	1.85±0.11	2.40±0.10
		1.89±0.14	1.97±0.09[b]	2.00±0.21	2.40±0.06	2.02±0.17	2.20±0.18
FE_K (%)		23.3±2.8	31.8±4.4	23.5±3.6	33.7±3.2	25.2±3.7	35.0±5.3

LIGHT MICROSCOPY.

There were no differences in proximal tubular cell, vascular, glomerular or interstitial morphology observed between olive-oil- and CYA-treated SHR.

(c) UNINEPHRECTOMIZED SHR WITH CONTRALATERAL DENERVATION (groups I_{nx+d}-II_{nx+d}).

All animals survived nephrectomy and contralateral renal denervation without sepsis developing. Pair feeding was successful (Figure 3.8; overall mean food intake [groups I_{nx+d} and II_{nx+d} inclusive]: olive-oil, 69.9±1.5 versus CYA, 69.3±1.2 g/day). However, mean daily weight gain was lower in the CYA-treated SHR (Figure 3.9: [groups I_{nx+d} and II_{nx+d} inclusive]: olive-oil, 1.37±0.14 versus CYA, 0.79±0.10 g/day, p<0.01). Denervation reduced tissue noradrenaline content in the denervated kidney to approximately 7 per cent compared to the innervated kidney content (9.63±1.4 versus 0.68±0.13 nmol/g kidney respectively, p<0.001). All rats completed the study.

EFFECT OF OLIVE-OIL OR CYA ON SYSTEMIC AND RENAL HAEMODYNAMICS (GROUP I_{nx+d}a versus I_{nx+d}b).

Table 3.7 shows data for CYA whole blood levels, MAP, GFR, ERPF, RVR and FF for both groups studied. CYA whole blood levels were similar in the CYA-treated SHR. Compared to uninephrectomy alone, renal denervation did not ameliorate the adverse effects of CYA on GFR, ERPF or RVR (I_{nx+d}b versus I_{nx}b, not significant). Although there was a small increase in MAP (not significant) in the CYA-treated SHR, there
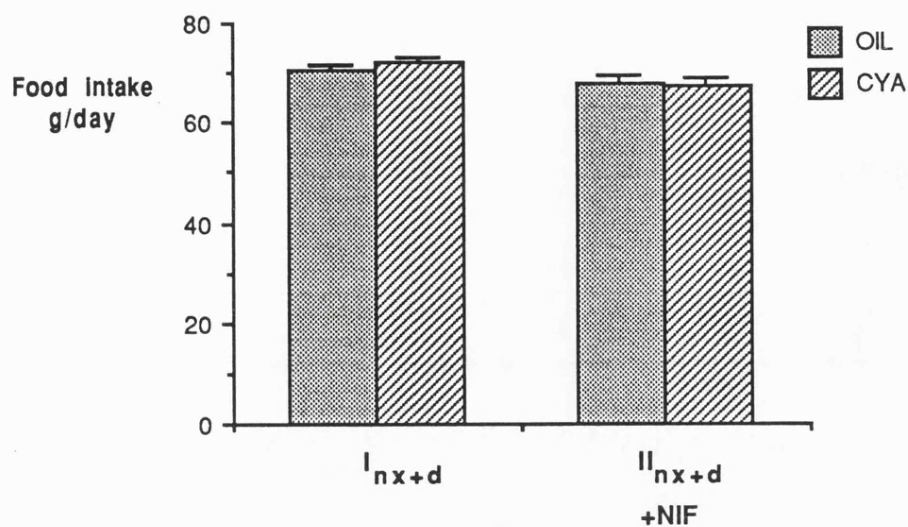
Figure 3.8. The effect of olive-oil or CYA on mean daily food intake (g/day). Group I_{nx+d} received either olive-oil or CYA for 14 days; group II_{nx+d} received either olive-oil or CYA plus nifedipine for 14 days.
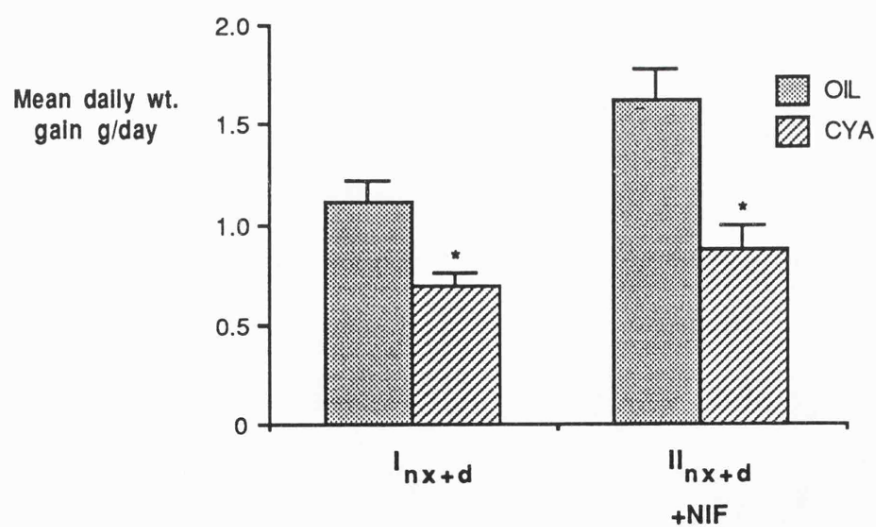

Figure 3.9. The effect of olive-oil or CYA on mean daily weight gain (g/day). * $p < 0.01$.

TABLE 3.7. EFFECT OF NIFEDIPINE ON CYA WHOLE BLOOD LEVELS, SYSTEMIC AND RENAL HAEMODYNAMICS IN UNINEPHRECTOMIZED SHR WITH CONTRALATERAL RENAL DENERVATION RECEIVING OLIVE-OIL OR CYA (GROUPS I_{nx+d} and II_{nx+d}).

Results are mean±SEM. GFR and ERPF are expressed per 100g body weight. Abbreviations: Oil, olive-oil and NIF, nifedipine. Significant differences between groups are indicated by: Ia vs Ib; [a]$p<0.05$, [b]$p<0.02$. IIa vs IIb; [c]$p<0.05$, [d]$p<0.01$, [e]$p<0.02$. The number of animals in each group used to derive the data is shown in parentheses.

Group... Treatment... Number of animals	I_{nx+d}[a] Oil (n=8)	I_{nx+d}[b] CYA (n=8)	II_{nx+d}[a] Oil+NIF (n=8)	II_{nx+d}[b] CYA+NIF (n=8)
CYA whole blood levels (ng/ml)	-	320±68.3	-	404.9±46.9
MAP (mmHg)	144.1±7.0	152.4±5.5	143.8±7.1	148.1±7.0
GFR (ml/min)	0.78±0.07	0.55±0.05[a]	0.77±0.04	0.66±0.03[c]
ERPF (ml/min)	1.61±0.06	1.23±0.13[a]	1.74±0.09	1.30±0.06[d]
RVR (Dynes/sec/cm^{-5}/10^3)	16.9±1.2	25.8±2.8[b]	17.5±1.7	24.7±1.8[e]
FF	0.48±0.03	0.45±0.02	0.44±0.01	0.51±0.01[d]

was a marked increment in RVR (group I_{nx+d}b versus I_{nx+d}a, $P<0.02$). FF did not change.

EFFECT OF NIFEDIPINE ON SYSTEMIC AND RENAL HAEMODYNAMICS IN OLIVE-OIL- OR CYA-TREATED SHR (Group II_{nx+d}a versus II_{nx+d}b)

Nifedipine did not influence systemic or renal haemodynamics (group II_{nx+d}a versus I_{nx+d}a). Administration of nifedipine failed to prevent CYA-induced decreases in GFR or ERPF, or attenuate the rise in

RVR that characterised CYA administration in this model. MAP was
similar in both olive-oil- and CYA-treated SHR. FF increased in SHR
receiving nifedipine (p<0.01). Figure 3.10 summarises the renal
haemodynamic data for uninephrectomized SHR with contralateral renal
denervation receiving olive-oil or CYA.

**EFFECT OF OLIVE-OIL OR CYA ON SERUM ELECTROLYTES AND UREA, URINE
PRODUCTION AND ELECTROLYTE EXCRETION (Group $I_{nx+d}a$ and $I_{nx+d}b$)**

Data on serum and urinary electrolytes for all rats undergoing
uninephrectomy and contralateral renal denervation are shown in Tables
3.8 and 3.9 respectively. Plasma sodium and potassium were similar in
olive-oil- and CYA-treated SHR before and after treatment. Baseline
serum urea was similar in olive-oil- and CYA-treated SHR. Urine
volume, $U_{Na}V$ U_KV, C_{Na} and C_K did not change after olive-oil (group
$I_{nx+d}a$, paired t-test) nor after CYA (group $I_{nx+d}b$, paired t-test). In
addition, FE_{Na} and FE_K were similar in olive-oil- and CYA-treated SHR.
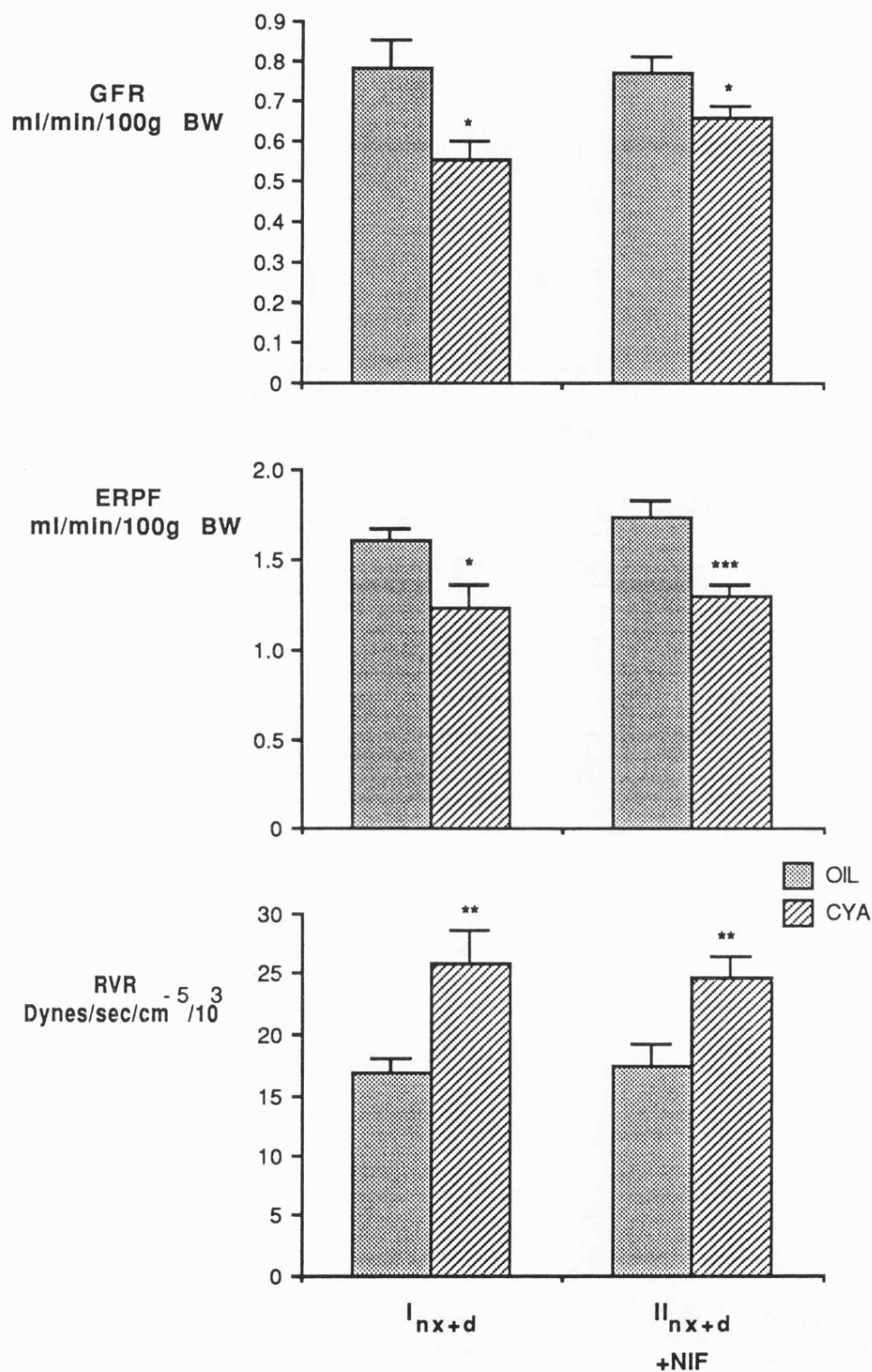
Figure 3.10. The effect of nifedipine on GFR, ERPF and RVR in uninephrectomized SHR with contralateral renal denervation receiving either olive-oil or CYA (groups I_{nx+d} and II_{nx+d}). *p <0.05, **p <0.02 and ***p <0.01.

TABLE 3.8. BASELINE UREA AND SERUM ELECTROLYTES BEFORE AND AFTER NIFEDIPINE THERAPY (GROUPS I_{nx+d} and II_{nx+d}).

Results are mean±SEM. Abbreviations: Oil, olive-oil and NIF, nifedipine. The number of animals in each group used to derive the data is shown in parentheses.

Group... Treatment.. Number of animals...	I_{nx+d}[a] Oil (n=8)	I_{nx+d}[a] CYA (n=8)	II_{nx+d}[a] Oil+NIF (n=8)	II_{nx+d}[b] CYA+NIF (n=8)
Baseline serum Urea (mmol/l)	9.5±0.2	9.9±0.4	10.2±0.4	9.8±0.5
Serum Na^+ pre.. (mmol/l) post..	143.2±1.3 144.3±2.6	144±1.9 146±3.1	145.5±3.2 147.3±3.7	141.3±1.9 143.7±3.0
Serum K^+ pre.. (mmol/l) post..	5.2±0.2 4.9±0.3	5.7±0.4 5.1±0.2	5.1±0.2 4.6±0.3	5.5±0.4 5.2±0.3

EFFECT OF NIFEDIPINE ON SERUM ELECTROLYTES, URINE PRODUCTION AND ELECTROLYTE EXCRETION (GROUPS II_{nx+d}[a] versus II_{nx+d}[b])

Nifedipine did not influence serum electrolytes, $U_{Na}V$, U_KV, C_{Na}, C_K (group II_{nx+d}[a] versus I_{nx+d}[a]). Furthermore, concomitant nifedipine treatment did not alter urine production, $U_{Na}V$, U_KV, C_{Na}, C_K, FE_{Na} or FE_K in either the olive-oil- or CYA-treated SHR.

URINARY N-ACETYL-β-D-GLUCOSAMINIDASE EXCRETION (GROUP I_{nx+d}[a] versus I_{nx+d}[b])

Urinary NAG excretion did not change after treatment with olive-oil or CYA (Figure 3.11).

TABLE 3.9. EFFECT OF NIFEDIPINE ON URINE PRODUCTION (V), ABSOLUTE SODIUM ($U_{Na}V$) AND POTASSIUM (U_KV) EXCRETION, SODIUM (C_{Na}) AND POTASSIUM CLEARANCE (C_K) AND FRACTIONAL SODIUM (FE_{Na}) AND POTASSIUM (FE_K) EXCRETION (GROUPS I_{nx+d} and II_{nx+d}).

Results are mean±SEM per 100g body weight before and after treatment. Abbreviations: Oil, olive-oil and NIF, nifedipine. The number of animals in each group used to derive the data is shown in parentheses.

Group... Treatment.. Number of animals...	I_{nx+d}^a Oil (n=8)	I_{nx+d}^a CYA (n=8)	II_{nx+d}^a Oil+NIF (n=8)	II_{nx+d}^b CYA+NIF (n=8)
V pre..	17.8±2.1	17.8±1.5	17.4±2.0	16.2±2.2
(µl/min) post.	14.9±1.8	16.6±1.5	15.2±1.0	9.5±1.3
$U_{Na}V$	1.83±0.14	1.86±0.16	1.89±0.18	1.79±0.12
(µmol/min)	1.79±0.11	1.62±0.13	1.80±0.20	1.74±0.16
C_{Na}	12.5±0.8	10.6±0.7	11.1±0.8	11.9±0.5
(ml/min)	11.9±0.9	9.4±0.9	10.1±0.6	12.4±0.9
FE_{Na} (%)	0.69±0.10	0.73±0.13	0.82±0.11	0.77±4.4
U_KV	2.30±0.10	2.21±0.13	2.09±0.17	2.02±0.16
(µmol/min)	2.10±0.08	2.3±0.14	2.14±0.18	2.40±0.18
C_K	0.37±0.06	0.42±0.04	0.48±0.09	0.39±0.08
(ml/min)	0.39±0.05	0.47±0.07	0.41±0.10	0.44±0.09
FE_K (%)	26.3±5.3	32.1±5.4	28.1±6.1	21.6±4.4

COMPENSATORY RENAL GROWTH AFTER UNINEPHRECTOMY (GROUP I_{nx+d}^a versus I_{nx+d}^b)

Kidney growth (measured as a change in wet weight from the time of uninephrectomy to culling at day 28) increased after both olive-' oil or CYA to comparable levels (baseline kidney weight: olive-oil versus CYA, 0.76±0.02 versus 0.79±0.02 g, not significant; after treatment: 0.96±0.04 versus 0.89±0.03 g, not significant).
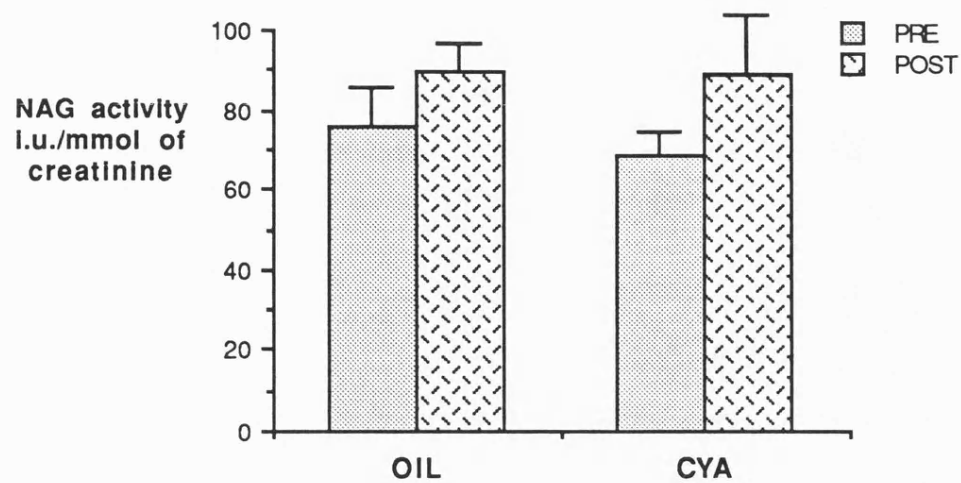
Figure 3.11. The effect of olive-oil or CYA on urinary N-acetyl-β-D-glucosaminidase (NAG) excretion in rats undergoing uninephrectomy and contralateral renal denervation.

DISCUSSION

The present series of experiments demonstrate marked differences in the ability of nifedipine to prevent CYA-induced renal dysfunction between surgically intact SHR (two-kidney model) and SHR with reduced renal mass after uninephrectomy. Introducing nifedipine with CYA from day 1 in the two-kidney model was able to prevent the characteristic decline in renal function associated with CYA administration. However, this protective effect was lost if the administration of nifedipine was delayed until 7 days after commencing CYA. By contrast, the concomitant administration of nifedipine to SHR subjected to uninephrectomy or uninephrectomy with contralateral renal denervation did not influence CYA-induced alterations in renal haemodynamics, with both GFR and ERPF remaining depressed, and RVR elevated.

In order to overcome the complicating factor of blood pressure influencing renal haemodynamics, a non-hypotensive dose of nifedipine (0.1 mg/Kg intraperitoneally) was administered (Iriuchijima, 1980). Although maximal pharmacological and haemodynamic effects may not have been induced using this regimen, the dose administered did lead to complete normalisation of GFR and ERPF in the two-kidney model, implying a satisfactory pharmacological effect was achieved at this dose. Although all animals in this series of experiments were successfully pair-fed, weight gain was significantly less in all CYA-treated groups. The mechanism responsible for poor weight gain is unclear, but does not appear to be due to increased urine production, electrolyte excretion or gastrointestinal losses.

The precise pathophysiological mechanism(s) underlying CYA-

induced impaired renal function remains unclear. The demonstration
that the decline in GFR and ERPF occurred without overt histological
evidence of tubular cell damage in either the two-kidney or
uninephrectomized models mitigates CYA being a direct tubular toxin.
The present findings accord with observations made in young female SHR
by Nahman et al (1988), who similarly demonstrated no vascular,
glomerular or tubular changes under light microscopy, despite
administering a similar dose of CYA by gavage for 4 or 8 weeks to both
surgically intact (two-kidney) and uninephrectomized SHR. Furthermore,
in contrast to previous studies (Whiting et al., 1982; Blair et al.,
1982; Perico et al., 1986a) the demonstration that urinary NAG
concentration did not increase after CYA (measured in group $I_{nx+d}a$ and
$I_{nx+d}b$ only) suggests that CYA is not behaving as a tubular toxin.
Similar observations were recently reported (Elzinga et al., 1989).

Thus, the rapid onset of renal dysfunction after acute CYA
infusions, the paucity of morphological damage evident on light
microscopy and a normal urinary NAG excretion together infer that CYA-
induced renal impairment is primarily a haemodynamic phenomenon, due to
changes in RVR and renal blood flow.

Experimental studies indicate that the major target for CYA is
the afferent arteriole (English et al., 1987; Thomson et al., 1989).
Morphological studies in rats treated with CYA for up to 14 days
demonstrate disproportionate and progressive narrowing of the afferent
compared to efferent arteriolar vessels (English et al., 1987).
Similarly, micropuncture studies report disproportionate increases in
afferent arteriolar resistance in Munich-Wistar rats (Thomson et al.,

1989). The protective effect conferred by administering nifedipine to the two-kidney model from day 1 might be due to the intrinsic ability of nifedipine to counteract CYA-induced afferent arteriolar vasoconstriction. Calcium channel blockers, including nifedipine, have been shown to be potent renal vasodilators, disrupting excitation-contraction coupling by inhibiting calcium influx through voltage-sensitive channels in the plasmalemma (Loutzenhiser and Epstein, 1987). Their capacity to induce renal vasodilatation has been demonstrated in isolated perfused kidney preparations to be predominately limited to the afferent arteriolar vessels and furthermore, is elicited only if the vascular tone is increased (Loutzenhiser and Epstein, 1987). If the vasoconstrictor effects of CYA are limited to the afferent arteriolar vessels, then preferential vasodilatation of the preglomerular vessels could account for the observed parallel increase in GFR and ERPF documented in the two-kidney studies. This type of haemodynamic response would be expected to increase glomerular capillary pressure and ultimately GFR.

Alternatively, the protection afforded by nifedipine in the two-kidney model may relate to its intrinsic ability to antagonize the vasoconstrictor effects of both noradrenaline and angiotensin II acting on the afferent and efferent arteriolar vessels respectively (Goldberg and Schrier, 1984). Both vasoconstrictor agonists have been implicated as potential mediators for the vasoconstrictor effects of CYA (Murray et al., 1985; Siegl et al., 1983). However, it is unlikely nifedipine is acting in this manner as a major role for either agonist in CYA nephrotoxicity has not been substantiated. Acute CYA-induced renal

impairment often presents in the denervated transplanted kidney in the immediate post-transplant period (Myers, 1986), and normal (Stanek et al., 1987) or reduced (Bantle et al., 1985) activity of the renin-angiotensin system has been documented in clinical studies. Furthermore, variable effects on renal haemodynamics have been reported with both converting enzyme inhibitors and α-adrenoreceptor blocking agents. Although Barros et al (1987a) demonstrated complete reversal of CYA-induced vasoconstriction in rats treated with captopril, others have failed to demonstrate a beneficial response, even in the presence of overt overstimulation of the renin-angiotensin system (Murray et al., 1985; Dieperink et al., 1986b). Similarly, whereas α-adrenoreceptor blocking agents have proved successful in animal studies (Murray et al., 1985; Murray and Paller, 1986), the results from clinical studies have been disappointing (Nussenblatt et al., 1986; Vanrenterghem et al., 1988).

Altered eicosanoid production in favour of vasoconstriction has also been entertained as a potential mediator for CYA-induced renal impairment (Benigni et al., 1989). However, Bunke and Wilder (1988) recently demonstrated that the beneficial response conferred by verapamil is not the result of an increase in vasodilatory prostaglandins. They reported that the improvement in renal function with verapamil in CYA-treated rats was associated with an absolute reduction in vasoconstrictor and vasodilator glomerular prostaglandins (prostaglandin E_2, 6-Keto-Prostaglandin $F_{1\alpha}$ and thromboxane B_2), including a decrease in the prostaglandin E_2/thromboxane B_2 ratio.

The degree of reduction in GFR and ERPF documented in both the

two-kidney and uninephrectomized models was comparable, indicating that the major increase in vascular resistance after CYA is predominantly due to afferent arteriolar vasoconstriction. However, other workers have documented significantly greater reductions in GFR compared to ERPF (Barros et al., 1987a; Perico et al., 1986c). Barros et al (1987a) suggested that this disproportionate decline in GFR was due to a concomitant reduction of the glomerular ultrafiltration coefficient (Kf). Since glomerular surface area is the major factor determining glomerular Kf (Kreisberg et al., 1985), it was postulated that CYA decreased glomerular Kf by reducing the glomerular surface area via mesangial cell contraction. Subsequent studies have demonstrated direct mesangial cell contraction after local application of CYA (Meyer-Lehnert and Schrier, 1988; Rodriguez-Puyol et al., 1989). Furthermore, CYA has been shown to enhance the contractile response induced by vasoconstrictor agonists acting on the mesangial cell (Meyer-Lehnert and Schrier, 1988), as well as augmenting transmembrane calcium influx and intracellular mobilisation in mesangial (Meyer-Lehnert, 1988) and vascular smooth muscle cells (Pfeilschifter and Ruegg, 1987) after stimulation with angiotensin II or arginine vasopressin. These perturbations in calcium homeostasis may potentiate mesangial and vascular smooth muscle contractility and thereby contribute to the reduction in glomerular Kf, and ultimately in GFR. Nonetheless, despite these documented alterations in cellular calcium kinetics, experimental studies in cultured mesangial and vascular smooth muscle cells suggest that the improvement in renal function with calcium channel blockers is not due to their intrinsic ability to block

transmembrane calcium influx. Recently, Rodriguez-Puyol et al (1989) demonstrated only partial inhibition of CYA-induced mesangial cell contraction with verapamil. Similarly, the perturbations in cellular calcium kinetics documented in mesangial and vascular smooth muscle cells were not abrogated by verapamil or nifedipine (Meyer-Lehnert and Schrier, 1988; Pfeilschifter and Ruegg, 1987).

The failure of nifedipine to improve renal function in the two-kidney model if commenced after a period of CYA therapy accords with earlier observations in experimental models (Dieperink et al., 1986b; Rooth et al., 1988b). Rooth et al (1988b) demonstrated that the cessation of subcapsular kidney blood flow induced by an acute CYA infusion in mice was restored to normal only with pre- and not post-treatment with verapamil. Recently, this group has confirmed similar findings in cadaveric renal allograft recipients (Dawidson et al., 1989). They demonstrated that verapamil, administered for 48 hours prior to introducing CYA therapy (80 mg t.d.s.) and for a total of 10 days after CYA (120 mg slow release t.d.s.) was able to prevent both CYA-induced decreases in renal parenchymal blood flow (measured by duplex Doppler scanning) and CYA-induced acute nephrotoxicity, as well as decreasing the number of early rejection episodes. Unfortunately, this study did not investigate the effect of delaying verapamil until after CYA therapy had been introduced. Dieperink et al (1986b) also failed to document an improvement in renal function after administering a single intravenous dose of nifedipine to Sprague-Dawley rats treated with CYA for 13 days, in contrast to the protection afforded by concomitant therapy over the same period. However, in their study the

dose of nifedipine infused induced a significant reduction in MAP, thus it was unclear whether the lack of effect was due to the reduction in MAP adversely influencing renal haemodynamics. It is apparent from the present two-kidney studies that prior administration of CYA interferes with the protection afforded by concomitant nifedipine administration. However, the factor(s) underlying the failure of nifedipine to improve renal haemodynamics if administered to a pre-existing CYA-induced nephrotoxicity are speculative. It is conceivable that unopposed CYA might impair vascular reactivity, preventing a vasodilatory response to nifedipine occurring. Although no vascular structural abnormalities were seen on light microscopy in these studies, others have demonstrated exudative and proliferative vascular lesions, but only at higher doses and after more prolonged administration (Siegl et al., 1983). Alternatively, the capacity of the renal vessels to relax may be impaired by CYA. Richards et al (1989) recently observed impaired endothelium-dependent relaxation to acetylcholine in human subcutaneous resistance arterioles incubated with CYA. Furthermore, earlier studies performed in proximal tubular cells reported a reduction in the cellular uptake of CYA in the presence of calcium channel blockers (Nagineni et al., 1987). Although similar studies have not been performed with vascular smooth muscle, it is possible that a greater accumulation of CYA occurs in the vascular tissue in the delayed nifedipine group, interfering with smooth muscle contraction or relaxation.

In rats and dogs both verapamil or nifedipine pretreatment have been shown to significantly counteract intrarenal damage induced by

renal ischaemia (Burke et al., 1984). However, attenuation of cellular ischaemic injury is unlikely to be a major factor contributing to the nephroprotection afforded by concomitant nifedipine therapy, as tubular cell damage was not a feature of CYA nephrotoxicity in the present studies.

In conflict with previous studies that report a beneficial effect of renal denervation on CYA nephrotoxicity (Murray et al., 1985; Murray and Paller, 1986; Thomson et al., 1989), renal denervation in the present uninephrectomy studies failed to ameliorate CYA-induced decreases in GFR and RBF, or reduce RVR in the rat. Similar observations to the present findings were recently reported by Friedman et al (1988) using a sheep model. This salutary observation that renal denervation has no protective effect on the vascular responses of the kidney to CYA infers that the sympathetic nervous system does not play an active role in CYA nephrotoxicity. This finding parallels clinical observations, since CYA-induced renal dysfunction frequently presents in the immediate post-transplant phase, at a time when the transplanted kidney is denervated. Although studies in animals with α-adrenoreceptor blockers have also been shown to ameliorate CYA nephrotoxicity (Murray et al., 1985; Murray and Paller, 1986), administration of similar agents to man have not proved beneficial (Nussenblatt et al., 1986; Vanrenterghem et al., 1988). Nussenblatt et al (1986) reported that the administration of Hydergine, an α-adrenoreceptor blocker with central and peripheral blocking effects, to patients treated with CYA for uveitis did not favourably influence nephrotoxicity. Although renal re-innervation after surgical

137

denervation has been shown to occur in the rat,it appears to be only partial between days 24 and 32 days (Kline and Mercer, 1980) and may remain incomplete for up to 12 months (Premen et al., 1985). Nonetheless, the demonstration that tissue noradrenaline concentration 28 days after denervation was significantly lower (approximately 7 per cent of innervated kidneys) confirms successful denervation had taken place in the present studies.

The reason(s) for the failure of nifedipine to prevent the deterioration in renal function after CYA administration to uninephrectomized rats, in contrast to the two-kidney model, was not explored by the present studies and remain speculative. Several workers have intimated that the kidney may be more susceptible to the toxic effects of CYA after loss of renal mass, particularly in the early stages while undergoing compensatory haemodynamic changes and hypertrophy (Whiting et al., 1985; Schurek et al., 1986). Whiting and co-workers reported that the administration of CYA [25 mg/Kg/day via gavage for 28 days] to uninephrectomized DA rats led to more severe renal impairment compared with control (two-kidney) rats, inducing extensive tubular vacuolisation and frank nuclear pyknosis in the proximal straight tubules after uninephrectomy, despite comparable CYA levels. In the present study CYA and nifedipine were administered 14 days after uninephrectomy, at a time when compensatory changes in renal function and growth are virtually complete (Hayslett, 1979; Lopez-Novoa et al., 1982; Brenner, 1985). An indirect analysis of kidney growth (by a comparison of wet weight) after uninephrectomy and CYA therapy confirms that the weight of the intact remaining kidney was equal in

magnitude to that in the olive-oil-treated SHR, indicating that while CYA reduced the GFR and ERPF, it did not interfere with any remaining renal growth that was taking place from days 14 to 28 of the study period. Whether CYA interferes with the adaptive changes in renal haemodynamics and hypertrophy after reduction in renal mass is currently a contentious area, as normal uninhibited (Nahman et al., 1988; Winston et al., 1989; Gillum et al., 1989) or inhibited growth (Schurek et al., 1986; Logan and Bensen, 1987; Batlle et al., 1990) is reported. Nevertheless, the functional abnormalities that accompany CYA therapy have been shown to still occur after uninephrectomy (Nahman et al., 1988; Winston et al., 1989; Gillum et al., 1989; Batlle et al., 1990), suggesting that CYA may interfere with adaptive factor(s) that influence renal haemodynamics, rather than actual tissue growth. In the present uninephrectomy studies there were no features to suggest that the extent of nephrotoxicity was more severe than that induced in the two-kidney model, as the degree of functional nephrotoxicity (percentage reduction in GFR and ERPF) was less in the uninephrectomized SHR compared to the surgically intact SHR (two-kidney). In addition, uninephrectomy did not induce overt histological injury. A direct comparison of the values obtained for GFR and ERPF in the uninephrectomized SHR with a derived single kidney value obtained in the two-kidney studies (computed simply by dividing the two-kidney value by two) demonstrates that a degree of compensatory functional hypertrophy did take place in the presence of CYA in the present studies (single kidney GFR and ERPF derived from surgically intact animals receiving CYA was 0.43 and 0.86 ml/min/100 g BW respectively,

compared to 0.70 and 1.38 ml/min/100 g BW in the uninephrectomized SHR

receiving CYA).

Recently, Gillum et al (1989) reported that uninephrectomy alone

afforded a degree of protection against CYA-induced interstitial injury

by virtue of the renal vasodilatation that accompanies the adaptive

changes to reduction in renal mass. However, despite a reduction in

the degree of histological injury after uninephrectomy, there remains a

component of functional CYA nephrotoxicity that is resistant to

adaptive compensatory haemodynamic changes. Furthermore, this

component of functional CYA nephrotoxicity also remains resistant to

nifedipine-induced renal vasodilatation. An alteration in the vascular

reactivity of the renal vessels after uninephrectomy, by the factors

inducing compensatory alterations in renal haemodynamics, may

conceivably modify CYA-induced vasoconstriction and hence, interfere

with nifedipine-induced vasodilatation. Although evidence to support

changes in vascular reactivity after uninephrectomy is lacking,

structural changes in the intrarenal arterioles, including an increase

cross-sectional area of the renal arterioles, have been shown to occur

following uninephrectomy, which might conceivably modify vasodilatory

responses (Tucker and Blantz, 1977). Whether the adaptive changes in

the intrarenal haemodynamics influences tissue distribution of CYA or

concentration delivered to the renal vascular tissue after nephrectomy

is not known.

GFR and ERPF were markedly depressed by CYA in the present

studies. However, in common with other studies (English et al., 1987;

Dieperink et al., 1986b; Dieperink et al., 1988) the ability to

conserve sodium and potassium was preserved in all CYA-treated animals, inferring that functional integrity of the proximal tubular cell was maintained. These observations contribute further evidence to support the notion that CYA nephrotoxicity is entirely functional, and secondary to a reduction in filtration pressure via afferent arteriolar vasoconstriction. In the two-kidney studies the increase in $U_{Na}V$, U_KV, C_{Na} and C_K with nifedipine was not associated with a change in FE_{Na} or FE_K, suggesting that the improvement in renal function was secondary to an increase in renal blood flow. Nevertheless, other workers have documented CYA-induced increases in proximal tubular absorption of sodium and water, and impaired distal tubular sodium reabsorption in experimental (Dieperink et al., 1986b) and clinical studies (Propper et al., 1989). In the latter study, nifedipine was reported to improve distal tubular sodium and water excretion in renal allograft recipients treated for 8 days (10 mg three times daily), presumed to be via improved renal blood flow.

Although CYA is metabolised by the same discrete hepatic isozyme P450PCN1 as nifedipine (Kronbach et al., 1988), CYA levels were comparable after nifedipine in all groups suggesting that the improvement in renal function was not the result of lower CYA levels. The higher CYA levels documented in the nifedipine vehicle treated rats in the two-kidney studies (group $IV_{in}b$) are difficult to interpret due to the small number of animals in this group. However, examination of the actual levels achieved in each group using the subcutaneous route, demonstrated a wide variation in the blood concentrations, indicating individual differences in absorption and/or metabolism. In man

nifedipine is reported not to alter CYA levels, although other calcium channel blockers including, verapamil, nicardipine and diltiazem increase CYA whole blood levels (Wagner et al., 1988).

The demonstration that other classes of drug with either direct vasodilator (Dieperink et al., 1986b) or antivasoconstrictive activity (Murray et al., 1985; Dieperink et al., 1986b) can ameliorate CYA-induced alterations in renal haemodynamics, suggests that the beneficial vascular effects ascribed to nifedipine and other calcium channel blockers (Rooth et al., 1988b) are not specific to this agent and its receptors. However, it is not known at present whether the vasoconstrictor effect of CYA is the sole mechanism responsible for inducing acute and chronic nephrotoxicity, or whether other intracellular toxic effects are involved. Thus, calcium channel blockers may also confer protection via non-vascular mechanisms not possible with other classes of drug, by interfering with cellular calcium homeostasis.

In conclusion, these studies demonstrate that nifedipine is nephroprotective only in surgically intact (two-kidney) SHR. Furthermore, the afforded protection pertains only when nifedipine administration is commenced simultaneously with CYA. The factor(s) accounting for the failure of nifedipine to attenuate CYA nephrotoxicity in the uninephrectomized animals were not explored in these studies, but might conceivably relate to adaptive changes in vascular reactivity and intrarenal haemodynamics induced by uninephrectomy.

SECTION IV

EFFECT OF CYCLOSPORIN A ON PHOSPHOINOSITIDE

HYDROLYSIS IN RAT AORTA AND RESISTANCE ARTERIES

INTRODUCTION

The administration of CYA to animals and man is associated with an increase in both renal (Murray et al., 1985; Curtis et al., 1986) and peripheral vascular resistance (Thompson et al., 1986; Steigerwalt et al., 1987). Although the precise pathophysiological mechanism responsible for this increase in vascular reactivity is unclear, changes in the activity of the renin-angiotensin system (Siegl et al., 1983), prostaglandin metabolism (Benigni et al., 1989) and sympathetic nervous system activity have been proposed (Murray et al., 1985; Moss et al., 1985). Recently, several workers have also raised the possibility that CYA might possess intrinsic vasoconstrictor activity after demonstrating a contractile response to CYA in isolated blood vessels (Golub and Berger, 1987; Lamb and Webb, 1987; Xue et al., 1987; Golub et al., 1989), cultured vascular smooth muscle (Pfeilschifter and Ruegg, 1987; Meyer-Lehnert and Schrier, 1989) and mesangial cells (Pfeilschifter, 1988; Meyer-Lehnert and Schrier, 1988). Furthermore, CYA has recently been shown to induce alterations in cellular Ca^{2+} homeostasis in a variety of cell types, including increased transmembrane Ca^{2+} influx and mobilization from intracellular storage sites in cultured vascular smooth muscle cells (Pfeilschifter and Ruegg, 1987; Meyer-Lehnert and Schrier, 1989), cultured mesangial cells (Pfeilschifter, 1988; Meyer-Lehnert and Schrier, 1988) and isolated hepatocytes (Nicchitta et al., 1985).

An increase in intracellular free Ca^{2+} concentration in arterial smooth muscle cells has been implicated in the pathogenesis of essential hypertension (Zidek et al., 1982; Losse et al., 1984). The

143

mechanism responsible for this abnormality in Ca^{2+} homeostasis is unclear. However, the initial steps in contraction of vascular smooth muscle and mesangial cells after the binding of various vasoactive agents including angiotensin II, arginine vasopressin and noradrenaline, have been shown to involve the generation of a group of intracellular second messengers with Ca^{2+} releasing properties from inositol lipids residing in the plasma membrane (Heagerty and Ollerenshaw, 1987). Receptor binding of the agonist or hormone stimulates phospholipase C located in the plasma membrane, which is able to cleave phosphatidyl 4,5-bisphosphate (IP_2) to yield inositol 1,4,5-trisphosphate (IP_3) and 1,2 diacylglycerol (1,2 DAG). The release of IP_3 into the cytosol is a potent stimulus for the release of Ca^{2+} from intracellular storage sites, including the endoplasmic reticulum. In both vascular smooth muscle and mesangial cells this early signal increasing free cytosolic Ca^{2+} is the trigger for contraction. The mechanism underlying the perturbations in Ca^{2+} homeostasis associated with CYA are not clear, however, increased generation of IP_3 by CYA is a potential mechanism that may account for the increase in Ca^{2+} mobilization and hence, an augmented contractile response precipitating changes in peripheral and RVR.

The present investigation was designed to determine the effect of CYA on phosphoinositide metabolism in vascular tissue isolated from the SHR.

MATERIALS AND METHODS

Female SHR (200-240g body weight) were studied (n=16). The rats
were individually caged and fed the same standard wet mash chow as rats
in Section II (appendix 1).

Tissue preparation and [^{3}H]-inositol phosphate accumulation

The method used to measure total inositol phosphate hydrolysis
was a modification of the technique described by Berridge et al (1982).

On the day of study rats were killed by cervical dislocation and
the thoracic aorta and renal vessels with both kidneys still attached
dissected free and placed in tissue culture medium (M199) containing
(mM): NaCl 137, KCl 5.4, MgSO$_4$7H$_2$O 0.81, Na$_2$HPO$_4$7H$_2$O 0.36, CaCl$_2$ 1.26,
NaHCO$_3$ 4.2, KH$_2$PO$_4$ 0.44, Fe(NO$_3$)9H$_2$O 0.02. Subcutaneous resistance
vessels (<300 µm internal diameter) dissected from the skin were also
studied. The vascular tissue was dissected free of adherent fat and
divided into segments (aorta approximately 4 mm and resistance vessels
20 mm). Basal phosphoinositide hydrolysis was measured in tissue
segments incubated at 37° C for 120 min in 200 µl M199 containing CYA
(10^{-5} and 10^{-6} M) or vehicle (10^{-5} and 10^{-6} M ethanol), with lithium
Chloride 10^{-2} M, bovine serum albumin 4 mg/ml and [^{3}H]-myoinositol 10^{-6}
M. The addition of lithium chloride prevents the hydrolysis of
inositol monophosphates back to inositol by inhibiting the enzyme myo-
inositol 1-phosphatase. Thus, total inositol phosphate accumulation is
measured.

Phosphoinositide hydrolysis was also studied after stimulation
with noradrenaline 10^{-4} M over the final 15 minutes of incubation with

CYA. After 120 minutes the vessels were homogenised in 0.5 ml

chloroform: methanol: HCl (20: 40: 1 v/v) at 4°C. The homogenate was

left on ice for 10 minutes before adding 0.5 ml chloroform and 0.5 ml

water. The tubes were agitated on a vortex mixer and centrifuged at

1800g to separate the aqueous and organic phases. The upper aqueous

phase (containing the inositol phosphates) was transferred to a 10 ml

test-tube and a further 0.5 ml water added to the organic phase and the

separation process repeated. The second aqueous phase was pooled with

the first and neutralized with 43 μl 2M NaOH and 0.5 ml Bio-Rad 1-x8

anion exchange resin in the formate form added. The resin was agitated

and washed 5 times with 5mM myoinositol. The bound [^{3}H]-inositol

phosphates were subsequently eluted from the resin with 0.1 M formic

acid and 1 M ammonium formate (resin washed 3 times). Duplicate 2 ml

aliquots of the eluate were added to 16 ml Hi Salt scintillation fluid

and the radioactivity counted (corrected for quenching) using a Packard

Scintillation counter (Scintillation Analyzer 2200 CA Tri-Carb,

Canberra Packard, Berkshire, U.K.).

The protein content of the vascular segments was determined by

the method of Lowry et al (1951) and the amount of [^{3}H]-inositol

phosphate accumulated expressed as pmol [^{3}H]-inositol phosphate/mg

protein per 120 min. The intraassay coefficient of variation was 15.8

per cent. Data has been compared using Student's unpaired t-test.

Drugs and Isotopes

[^{3}H]-myoinositol (specific activity 16.5 Ci/mmol) was obtained

from New England Nuclear, Boston, U.S.A., lithium chloride from Fisons,

Loughborough., myoinositol and noradrenaline hydrochloride from Sigma

Chemical Company, Poole, Dorset., tissue culture medium (M199) from

Gibco Biocult Limited, Paisley, Scotland., anion-exchange resin AG1-x8

(in the formate form) from Bio-Rad Laboratories, Richmond, California,

U.S.A., bovine serum albumin from Miles Chemicals, Slough., Hi Salt

scintillation fluid from Lab Logic Systems Limited, Sheffield.

RESULTS

The administration of CYA did not influence basal inositol

phosphate hydrolysis in either aorta or resistance arteries compared to

vehicle (Figure 4.1). Incubation of vessels with noradrenaline

(10^{-4} M) stimulated inositol phosphate accumulation, however, an

augmented response was not observed in the presence of CYA (Figure
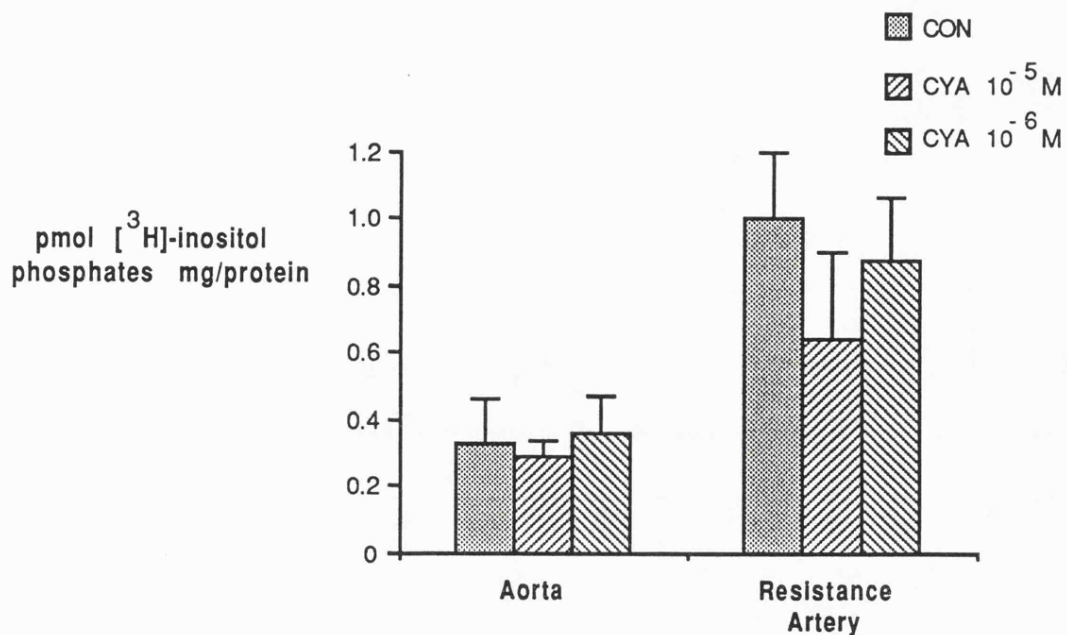
4.2).

Figure 4.1. The effect of CYA (10^{-5} and 10^{-6} M) or vehicle (10^{-4} M ethanol) on basal phosphoinositide hydrolysis in rat aorta and subcutaneous resistance arteries.
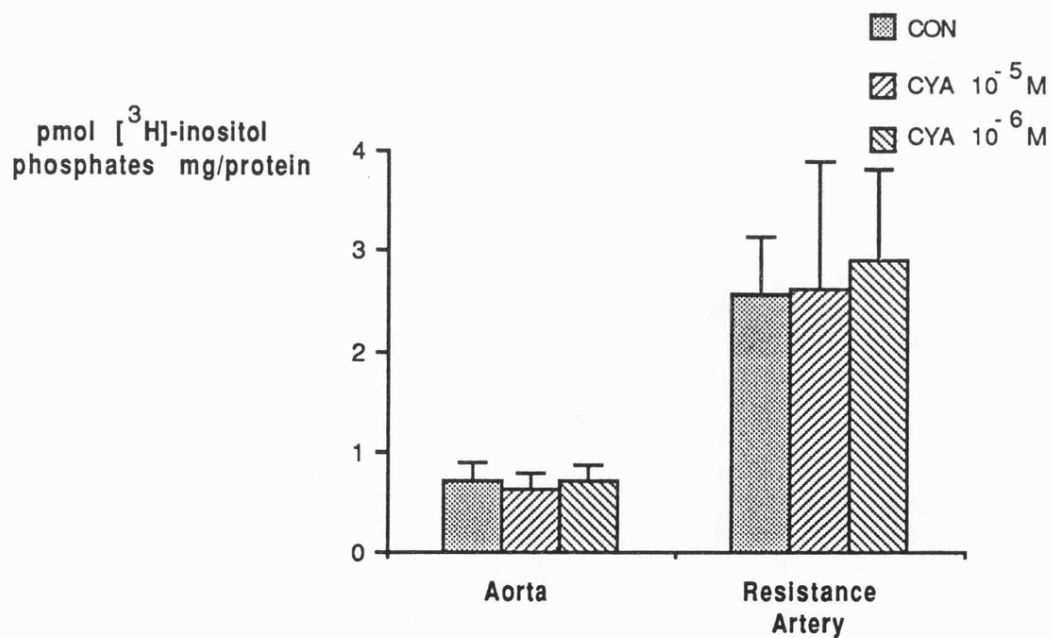

Figure 4.2. The effect of noradrenaline stimulation (10^{-4} M) on phosphoinositide hydrolysis in the presence of vehicle or CYA (10^{-5} and 10^{-6} M).

DISCUSSION

This study demonstrates that an acute two hour exposure of isolated aorta and resistance arteries to 10^{-5} and 10^{-6} M CYA does not directly influence either basal or agonist stimulated inositol phosphate hydrolysis. Although the major phosphoinositide measured by this technique is inositol monophosphate, any transient changes in IP_3 would be reflected by an increase in total inositol phosphate accumulation, as lithium ions prevent the enzymatic degradation of inositol monophosphate to inositol and thus allow the rate of water soluble inositol phosphate accumulation to be determined (Berridge et al., 1982). Thus, it is unlikely that the increased mobilization of Ca^{2+} from intracellular stores reported by several groups in differing cell types is a direct effect of CYA increasing inositol phosphate production, in particular augmented IP_3 production (Nicchitta et al., 1985; Meyer-Lehnert and Schrier, 1988; Pfeilschifter, 1988).

Alternatively, the changes in cellular Ca^{2+} homeostasis that lead to increased intracellular free Ca^{2+} concentration may result from either increased influx or decreased efflux of Ca^{2+} across the plasma membrane, or via increased release of stored Ca^{2+} from mitochondrial and endoplasmic reticulum storage sites. A reduction in Ca^{2+} efflux is unlikely as most workers have observed increased Ca^{2+} efflux in the presence of CYA (Nicchitta et al., 1985; Meyer-Lehnert and Schrier, 1988; Pfeilschifter and Ruegg, 1987). However, increased Ca^{2+} influx may conceivably occur via changes in the activity of one or more of the plasma membrane Ca^{2+} transporters, including the Ca^{2+}/H^+ exchanger, Na^{2+}/Ca^{2+} exchanger, or via modulation of receptor-operated Ca^{2+}

channels. Although receptor binding of CYA to vascular smooth muscle or mesangial cells has not been demonstrated (Mason, 1989), it is possible that CYA may interact with a plasma membrane Ca^{2+} transporter as studies indicate that CYA alters the physical properties of the dipalmitoyl-phosphatidylcholine bilayer in cell membranes (O'Leary et al., 1986). Furthermore, incorporation of CYA into the lipid membrane of lymphocytes has recently been shown to interfere with plasma membrane potential and transmembrane Ca^{2+} flux (Matyus et al., 1986). It is unlikely that Ca^{2+} influx occurs via voltage-dependent Ca^{2+} channels as neither verapamil nor nifedipine blocked Ca^{2+} influx in vascular smooth muscle or mesangial cell culture preparations (Pfeilschifter and Ruegg, 1987; Meyer-Lehnert and Schrier, 1988). Thus, transmembrane flux of Ca^{2+} appears to traverse via non-voltage-sensitive channels.

The raised intracellular free Ca^{2+} concentration may also arise by CYA liberating stored Ca^{2+} into the cytosol by a mechanism that is independent from inositol phosphate production. Zidek et al (1990) recently demonstrated in permeabilized neutrophils that CYA is able to induce a rapid increase in free Ca^{2+} concentration in the absence of extracellular Ca^{2+}, suggesting that the release of stored Ca^{2+} is the principal cause of the increase in intracellular free Ca^{2+}. Furthermore, re-uptake of liberated Ca^{2+} was attenuated and failed to achieve baseline concentration, inferring that CYA may decrease Ca^{2+} binding and re-uptake into cellular organelles concerned with Ca^{2+} sequestration, in particular mitochondria. Toxic effects of CYA on renal mitochondrial structure and function have previously been

demonstrated and may account for the reduction in Ca^{2+} uptake and hence, contribute to the increased intracellular free Ca^{2+} concentration (Hay et al., 1986). Thus, these alterations in intracellular free Ca^{2+} may play a pivotal role in the pathogenesis of CYA-induced changes in renal and peripheral vascular tone.

Whether chronic dosing with CYA in in vivo models leads to augmented phosphoinositide hydrolysis and Ca^{2+} mobilization is not known. However, augmented phosphoinositide production may be occurring in vivo by stimulation of an intermediary that acts via the inositol second messenger cascade system, such as CYA-induced stimulation of the sympathetic nervous system (Murray et al., 1985; Moss et al., 1985), the renin-angiotensin system (Siegl et al., 1983) or other, as yet unknown circulating factors.

In summary, this in vitro study suggests that CYA does not influence Ca^{2+} homeostasis by stimulation of phosphoinositide production.

SECTION V

EFFECT OF NIFEDIPINE ON CYCLOSPORIN A UPTAKE

INTO HUMAN PROXIMAL TUBULAR CELLS

INTRODUCTION

CYA-induced tubular injury has been proposed as a major mechanism contributing to the reduction in GFR and RBF (Whiting et al., 1982; Blair et al., 1982). This hypothesis was formulated following a number of experimental and clinical observations. First, a variety of proximal tubular morphological abnormalities were frequently observed in both animals and man receiving CYA, including vacuolisation, giant mitochondria, atrophy and interstitial fibrosis (Whiting et al., 1982; Blair et al., 1982; Ryffel et al., 1983; Mihatsch et al., 1983). Secondly, deposits of CYA were demonstrated by indirect immunofluorescence in human proximal tubules obtained by fine needle aspiration biopsy from renal allograft recipients (Von Willebrand and Hayry, 1983). Thirdly, a direct correlation existed between the quantity of CYA present in the transplant biopsy and the degree of clinical nephrotoxicity, implicating CYA as a direct tubular toxin (Von Willebrand and Hayry, 1983).

Although the protective mechanism afforded by nifedipine and other calcium channel blockers in CYA nephrotoxicity is uncertain, most workers favour a haemodynamic effect, with calcium channel blockers ameliorating CYA-induced vasoconstriction (Dieperink et al., 1986b; Rooth et al., 1988b). However, an alternative mechanism has been postulated following the demonstration that several different classes of calcium channel blockers inhibited the uptake of CYA into renal tubular cells (Nagineni et al., 1987; Nagineni et al., 1988). Using isolated renal proximal tubular cells obtained from New Zealand white rabbits the uptake of CYA was shown to be mediated via a rapid and

152

concentrative process, which was significantly blocked by both

inorganic and organic calcium channel blockers, including verapamil and

diltiazem. This led them to speculate that the portal in the plasma

membrane by which CYA enters the cytosol of the tubular cell is located

at or close to the voltage-sensitive calcium channel, so that

conformational changes induced by occupation of the voltage-sensitive

calcium receptor impedes the transmembrane passage of CYA.

The present study investigates the effect of nifedipine on CYA

uptake into cultured human renal proximal tubular cells using ^{3}H-CYA.

TISSUE CULTURE OF HUMAN PROXIMAL TUBULAR CELLS

All tissue culture preparation and characterisation was performed by Dr. T. Horsburgh, Clinical Scientist in Transplant Immunology, Department of Surgery, Leicester General Hospital.

Proximal tubular cells were isolated from renal cortical tissue obtained from patients undergoing investigation for microscopic haematuria (n=8). Renal tissue was finely chopped in 10mls of culture medium (containing; RPMI tissue culture medium 1640, foetal calf serum 10%, penicillin 50μg/ml, streptomycin 50μg/ml, glutamine 2mM, hydrocortisone 50ng/ml, insulin 5μg/ml, transferrin 5μg/ml, selenite 5ng/ml and epidermal growth factor 50ng/ml) and initially pressed through a coarse steel sieve to remove large tissue fragments. The suspension was allowed to settle for 5 minutes before resuspending in fresh medium. The suspension was then plated into culture flasks and left undisturbed for 4-7 days at 37°C in humidified air with 5% CO_2. When the growths became confluent the cells were trypsinised and aliquoted (1 ml) into a culture dish containing 28 wells. The cells were left to grow to confluence for 3-4 days, which was confirmed by phase contrast microscopy (Figure 5.1).

The viability of isolated proximal tubular cells was periodically assessed by staining with fluorescein diacetate to demonstrate living cells and propidium iodide to demonstrate dead cells. The cultured cells were characterised as proximal tubular cells using morphological criteria (electron microscopy: dense microvilli, junctional complexes, abundant rough endoplasmic reticulum and free polysomes, cytoplasmic filaments, basal cell membrane interdigitations

154

Figure 5.1. Phase contrast microscopy appearance of human proximal tubular cells in culture (x 200).

and well developed endocytotic apparatus) and monoclonal antibody

staining (keratin negative, factor VIIIRAg negative, fibronectin

negative).

CYCLOSPORIN A UPTAKE STUDIES USING ^{3}H-CYA.

All CYA uptake studies were performed in cultures that had

reached confluence. Preliminary studies were performed to document the

time course of ^{3}H-CYA uptake into cultured proximal tubular cells. The

culture medium was removed from each well and the cell monolayers

incubated for 30 seconds, 1, 5, 10, 15, 30 and 60 minutes, in 0.5 ml of

10 % fetal calf serum and 90 % physiological saline solution (PSS)

[NaCl 119 mM, KCl 4.7 mM, $CaCl_2.2H_2O$, 2.5 mM, $MgSO_4.7H_2O$ 1.17 mM,

$NaHCO_3$ 25mM, KH_2PO_4 1.18 mM, EDTA 0.026 mM and glucose 5.5 mM]

containing 0.0185 MBq ^{3}H-CYA and cold CYA (10 µg/ml). At the end of

the incubation period the incubation medium was removed and the cells

gently rinsed with physiological saline containing cold CYA (1 µg/ml)

at 4° C five times. The final wash was saved for counting (background

activity). Intracellular radioactivity was extracted with 1 ml of

sodium dodecyl sulphate (SDS) in an alkaline milieu (0.1 % SDS, 2 %

Na_2CO_3, 0.1 N NaOH) and measured by liquid scintillation counting (LKB

1215 Rackbeta Counter, LKB Instruments Ltd, Surrey, U.K.) after mixing

with 5 ml scintillation fluid (Ecoscint LS 271, National Diagnostics,

Aylesbury, U.K.) and corrected for quenching. The protein content of

each well was determined by the method of Lowry et al (1951) on an

aliquot of the extract and the results expressed as cpm/mg protein.

Uptake studies were performed in the presence or absence of

nifedipine over a 30 minute period, as uptake of CYA into tubular cells

reached a maximum after this period (Figure 5.2). The cells were

preincubated with 0.5 ml of nifedipine (10^{-4} M and 10^{-6} M) or vehicle

(0.1 % ethanol in PSS) for 5 minutes at 37° C before adding ^{3}H-CYA. In

preliminary studies phase contrast microscopy confirmed that the cell

monolayer remained intact and did not detach during the incubation

period.

STATISTICS

Data has been expressed as mean±SEM (mean of 6 separate cell

cultures per patient for each experiment) and compared using Student's

t-test for unpaired data.

RESULTS

CYA uptake into tubular cells was rapid with 80 per cent of

total uptake achieved after 10 minutes, reaching a plateau after 30

minutes; thus all subsequent uptake studies with nifedipine were

performed over a 30 minute period (Figure 5.2). Incubation with

nifedipine did not influence the uptake of 3H-CYA into cultured tubular

cells (Figure 5.3).
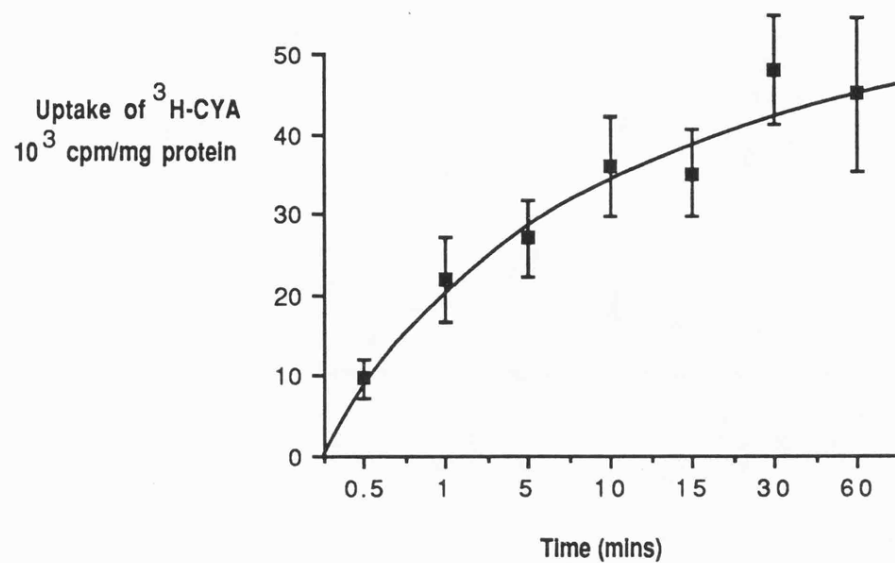
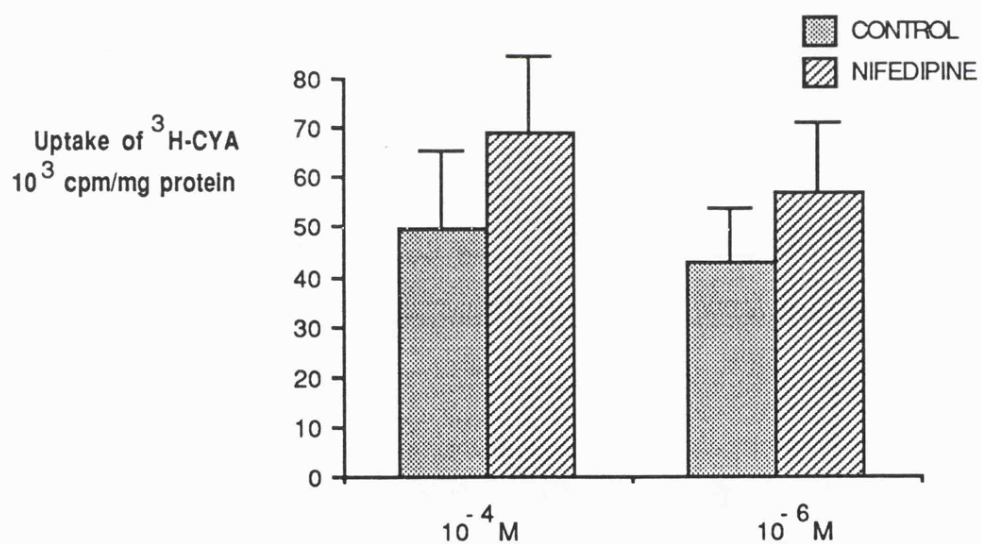**Figure** 5.2. Time course for the uptake of ^{3}H-CYA into human cultured proximal tubular cells.

Figure 5.3. The effect of nifedipine (10^{-4} and 10^{-6} M) on ^{3}H-CYA uptake into human cultured proximal cells.

158

DISCUSSION

This study demonstrates that the uptake of CYA into cultured human proximal tubular cells is not influenced by nifedipine, and infers that the protection afforded by nifedipine in the SHR (Section III; surgically intact two-kidney studies) is not due to a reduction in tubular cell CYA concentration. Similar conclusions were recently reached by Scoble et al (1989), after demonstrating that verapamil did not ameliorate CYA-induced injury of LLC-PK$_1$ cells, an epithelial cell line which express many proximal tubular characteristics. The mechanism postulated by Nagineni et al (1987) to account for the reduced uptake of CYA by calcium channel blockers into proximal tubular cells is unclear, as renal tubular cells have not been shown to possess voltage-sensitive calcium channels (Schrier et al., 1987).

Whether CYA is toxic to tubular cells is controversial. Although several groups have documented both overt biochemical and histological evidence of injury (Whiting et al., 1982; Cunningham et al., 1984; Perico et al., 1986a), others have reported normal tubular morphological and functional characteristics, despite continuous CYA administration to both man and animals (Schwass et al., 1986; Dieperink et al., 1986b; Palestine et al., 1986b; Dieperink et al., 1987; English et al., 1987; Jackson et al., 1987; Nahman et al., 1988).

In conclusion, this study suggests that nifedipine does not influence CYA uptake into proximal tubular cells. It seem more likely that the nephroprotection associated with nifedipine is mediated via a vascular effect, possibly by counteracting CYA-induced renal arteriolar vasoconstriction.

SECTION VI

EFFECT OF NIFEDIPINE ON RENAL FUNCTION IN

NORMOTENSIVE CYCLOSPORIN A TREATED RENAL ALLOGRAFT RECIPIENTS

INTRODUCTION

Nephrotoxicity is a major disadvantage of CYA immunosuppression in renal allograft recipients (Canadian Multicentre Transplant Study Group, 1986; Myers, 1986; European Multicentre Trial Group, 1987). Although it has been suggested that conversion to azathioprine several weeks or months after transplantation will avoid nephrotoxicity, this therapeutic manoeuvre is associated with an increased risk of rejection (Morris et al., 1983). Thus, CYA therapy remains the major form of immunosuppression in the maintenance period.

Prevention of nephrotoxicity by the use of low dose protocols has reduced, but not eliminated the threat of nephrotoxicity (Morris, 1988; Myers et al., 1988a). In experimental animals (Murray et al., 1985; Sullivan et al., 1985) and man (Conte et al., 1989; Weir et al., 1990), the acute haemodynamic effects of CYA occur almost immediately after intravenous infusion. It is conceivable that these haemodynamic changes contribute solely to the tubulo-interstitial injury induced by CYA. The morphological abnormalities recognised in CYA nephrotoxicity are entirely non-specific (Mihatsch et al., 1988), with a similar histological pattern described after renal ischaemia, following renal vasoconstriction induced by a variety of factors (Andreucci et al., 1984).

Reversal of CYA-induced intrarenal vasoconstriction with renal vasodilators is an attractive therapeutic manoeuvre that may prevent both adverse renal haemodynamic alterations and ischaemic-induced injury, and thus, prolong graft function. To date no prospective clinical studies have been performed in patients administered

concomitant calcium channel blockers and CYA. However, a large retrospective study of renal transplant recipients in Leicester receiving antihypertensive medication for CYA-associated hypertension identified a cohort of patients receiving nifedipine who had better graft function than equivalent hypertensive patients treated with other agents. This effect occurred despite shorter graft duration, higher CYA dosage and higher whole blood CYA levels, factors that are usually associated with a greater risk of nephrotoxicity (Feehally et al., 1987).

The present study was designed to investigate the effect of short-term nifedipine administration on renal haemodynamics in long-term CYA treated renal allograft recipients, comparing them with the effects of nifedipine in a group of long-term azathioprine (AZA) treated recipients.

PATIENTS AND METHODS

PATIENTS

Twenty renal allograft recipients with stable renal function were studied. Eleven were receiving CYA and prednisolone and nine AZA and prednisolone maintenance immunosuppression. All were at least 6 months post-transplant and normotensive (<140/90 mmHg) without antihypertensive therapy, nor were they receiving other vasoactive therapy. Daily CYA dose and whole blood CYA levels were stable. All patients had a stable protein intake for at least 2 months prior to the study (19 normal protein and 1 low protein diet). All studies were performed with informed consent and Local Ethical Committee approval.

RENAL FUNCTION STUDIES

Study 1. The effect of nifedipine on renal function.

All studies were performed in the morning following a light breakfast (< 10 g protein). Patients attended with a baseline 24 hour urine collection to measure urinary sodium, urea and protein excretion. Lying and standing blood pressure was recorded in the non-fistula arm, after 15 minutes rest (mean of 3 readings). Baseline GFR was measured using chromium ^{51}-labelled ethylenediaminetetra-acetate (Cr^{51}-EDTA) and ERPF measured using I^{125}-Hippuran. Clearances were estimated using the methods of Chantler and Barratt (1972) and Wagoner et al (1964) respectively. A baseline serum biochemical profile and 12 hour trough CYA level were taken before injecting a single bolus dose of Cr^{51}-EDTA (5MBq) and I^{125}-Hippuran (5 MBq) intravenously. Serial blood samples were taken after 10, 20, 30, 40, 50, 60 minutes and hourly thereafter

for 4 hours from an indwelling cannula in the opposite limb. Height (m) and weight (Kg) were recorded and the results corrected for body surface area (1.73 m2). RBF was calculated as ERPF/(1-haematocrit). FF as the ratio of GFR/ERPF. RVR was calculated using Gomez's formula (Gomez, 1951): RVR = MAP/RBF x 80,000 and expressed in dynes/second/ $cm^{-5}/10^3/1.73$ m^2. MAP was calculated from diastolic pressure plus 1/3 of the pulse pressure.

After baseline studies patients were commenced on nifedipine slow release (Adalat retard, Bayer U.K. Limited, Newbury, Berkshire) 20 mg twice daily for 28 days and instructed to remain on the same diet for the duration of the study. Clearance studies were repeated at a second visit and trough whole blood CYA levels and serum nifedipine and its major metabolite M-I measured (2 hours after the morning dose).

Study 2. Measurement of renal functional reserve capacity (RFRC).

Eighteen patients from the original group were studied (10 CYA and 8 AZA-treated). RFRC was assessed using a 80 g oral protein load in the form of cooked red meat. Studies were performed in the morning following an overnight fast. On arrival patients were water loaded orally (20 ml/Kg) over 30 minutes, to achieve a urine output between 3 and 10 mls/minute. Hydration was maintained by replacing urine losses with an equivalent volume of water for the duration of the experiment. Following loading doses of Cr^{51}-EDTA (1 MBq) and I^{125}-Hippuran (1 MBq) a sustaining infusion of both isotopes was maintained for 6 hours (0.053-0.48 MBq/hour and 0.25-1.23 MBq/hour respectively). Infusion doses were calculated individually from baseline Cr^{51}-EDTA clearances

obtained in study 1 by the method of Liedtke and Duarte (1980). After

a 60 minute equilibrium period two baseline 45 minute urine collections

were performed. Complete bladder emptying was confirmed by assessing

the radioactivity (using a gamma counter) over the bladder region

before and after voiding and comparing the radioactivity to that

obtained over the cardiac region (background level). The patient was

instructed to void again if the radioactivity remained high over the

bladder region. The volume of urine passed was recorded and a 20 ml

aliquot saved for counting. Blood samples were taken at the beginning

and end of each collection period and the plasma saved for counting.

Baseline clearances were derived from the mean count of the two 45

minute urine collections and mean count of the two plasma samples using

the standard clearance formula. Clearance periods with urinary flow

rates outside the range 3 to 10 mls/minute were discarded because of

the effects of dehydration and overhydration on renal function.

After baseline collections patients ingested an 80 g protein

meal and a further 60 minute equilibrium period was observed. Three

further 45 minute urine collections were performed with blood sampling

before and after each urine sample. The results were corrected for

body surface area (1.73 m^2) and expressed as a percentage of the

baseline renal function.

ANALYTICAL METHODS

Serum and urinary electrolytes, serum creatinine, urinary urea

and protein were measured using standard laboratory techniques by the

Department of Clinical Chemistry, Leicester General Hospital. Whole

blood CYA levels were measured by high performance liquid chromatography (mean of 3 measurements). Serum nifedipine and its major metabolite M-I were measured by automated electron-capture capillary gas chromatography (Schmid et al., 1988) [Nifedipine levels were kindly measured by Prof. JR Idle, Department of Pharmacogenetics, University of Newcastle-Upon-Tyne]. Radioactivity was counted in duplicate 2 ml aliquots of serum and urine, using a Philips PW4800 automatic gamma counter.

STATISTICS

Results are expressed as mean±SEM. Statistical comparisons were made using Wilcoxon matched pairs signed rank test and Mann-Whitney U test for paired and unpaired data respectively. Correlation was assessed using Spearman's rank correlation test. Statistical significance was taken as $p < 0.05$.

RESULTS

Nineteen patients (10 CYA and 9 AZA) completed the study (1 patient on CYA was withdrawn because of an unrelated pyrexial illness during the study period). The mean age of the patients was similar in both groups (Table 6.1). However, duration of graft was significantly longer in the AZA-treated group. The mean daily CYA dose and CYA whole blood level at baseline is shown in Table 6.1. All patients were receiving 10 mg of prednisolone on alternate days at the time of the study. At baseline, serum creatinine, Cr^{51}-EDTA clearance and ERPF were not significantly different, although FF was significantly lower in the CYA treated patients (Table 6.2). Twenty-four hour urinary urea did not change throughout the study period in either the AZA (300.7±35.3 versus 277.8±41.4 mmols/24 hours) or CYA- treated patients (270.1±32.9 versus 286.0±31.3), suggesting a constant protein intake during the study.

Mean whole blood CYA levels at the end of the study were unchanged (256.9±22.5 versus 256.5±16.8 ng/ml). Mean serum nifedipine and its major metabolite M-I were similar in both the CYA and AZA-treated patients (66.9±14.8 versus 66.9±13.2 and 35.1±5.6 versus 27.7±7.3 ng/ml respectively).

Twenty-four hour urinary protein, urine production, absolute sodium excretion and fractional excretion of sodium did not differ between groups at baseline and were not influenced by nifedipine therapy (Table 6.3).

TABLE 6.1. CHARACTERISTICS OF RENAL TRANSPLANT RECIPIENTS STUDIED

Variable	Azathioprine group (n=9)	CYA group (n=10)
M:F ratio	5:4	5:5
Age (years)	45.4±3.7	54.3±3.0
Duration of graft (months)	79.2±12.9*	23.4±4.5
Mean daily CYA dose (mg/Kg/day) (range)	-	5.1 (2.8-6.6)
CYA whole blood level (ng/ml)	-	256.9±22.5

* $p < 0.001$ compared to CYA group

TABLE 6.2. EFFECT OF NIFEDIPINE ON SERUM CREATININE, Cr^{51}-EDTA CLEARANCE, ERPF, RVR AND FF IN AZA- AND CYA-TREATED PATIENTS.

	AZA (n=9)		CYA (n=10)	
	Baseline	After	Baseline	After
MAP (mmHg)	94.1±2.7	93.3±2.2	90.1±1.7	89.7±2.0
Serum creatinine (μmol/1)	156±40	156±40	145±16	146±16
Cr^{51}-EDTA (ml/min/1.73m^2)	58.5±10.3	56.5±9.3	39.3±5.3	44.1±6.2*
ERPF (ml/min/1.73m^2)	262.4±42.9	274.9±48.9	213.7±22.2	229.9±21.3
RVR (dynes/sec/cm^{-5} /10^3/1.73m^2)	23.5±7.0	23.3±6.9	22.8±2.5	20.8±2.2
FF (%)	22±1	21±1	18±2**	19±2

* $p < 0.02$ compared to baseline CYA Cr^{51}-EDTA clearance
** $p < 0.05$ compared to baseline AZA FF

TABLE 6.3. EFFECT OF NIFEDIPINE ON URINE PRODUCTION, $U_{Na}V$, FE_{Na} AND 24 HOUR URINARY PROTEIN EXCRETION.

	AZA (n=9)		CYA (n=10)	
	Baseline	After	Baseline	After
Urine production (ml/min/1.73m^2)	1.35±0.14	1.40±0.14	1.46±0.18	1.42±0.19
$U_{Na}V$ (μmol/min/1.73m^2)	119.9±12.2	104.1±22.3	123.6±21.4	110.3±12.2
FE_{Na} (%)	1.77±0.24	1.64±0.38	2.29±0.46	1.92±0.29
24 hour urinary protein (mg)	0.32±0.10	0.59±0.20	0.62±0.18	0.68±0.18

STUDY 1: SYSTEMIC AND RENAL HAEMODYNAMIC RESPONSE TO NIFEDIPINE

Isotopic studies.

Table 6.2 documents the effect of nifedipine on MAP, serum creatinine, renal haemodynamics and FF for both groups. MAP was not influenced by nifedipine in either the AZA or CYA-treated patients. There was an increase in Cr^{51}-EDTA clearance in CYA-treated patients (mean; +14.8 % [range; -4 to 38 %], $p < 0.02$), although ERPF, RVR and FF did not change. In AZA-treated patients, Cr^{51}-EDTA clearance, ERPF, RVR and FF were unchanged. Serum creatinine concentration did not change after treatment in the CYA-treated patients. However, a poor correlation between the prevailing serum creatinine concentration and the Cr^{51}-EDTA clearance was demonstrated in this group ($r_s = 0.06$, not significant), as opposed to the AZA group ($r_s = -0.83$, $p < 0.001$). The percentage increase in Cr^{51}-EDTA clearance in the CYA-treated patients following nifedipine therapy did not correlate with baseline Cr^{51}-EDTA clearance (Figure 6.1), CYA daily dose or whole blood level, donor age, duration of graft or number of rejection episodes.

STUDY 2: RENAL FUNCTIONAL RESERVE CAPACITY (RFRC)

To investigate whether the increase in Cr^{51}-EDTA clearance following nifedipine therapy in the CYA-treated patients related to RFRC, the renal response to an 80 g oral protein load was documented. Eighteen patients were studied (10 CYA and 8 AZA). Cr^{51}-EDTA clearance and ERPF did not change following the protein load in the CYA-treated patients. However, there was a small but significant increase in both Cr^{51}-EDTA clearance and ERPF in the AZA-treated patients (12.1±3.4 per
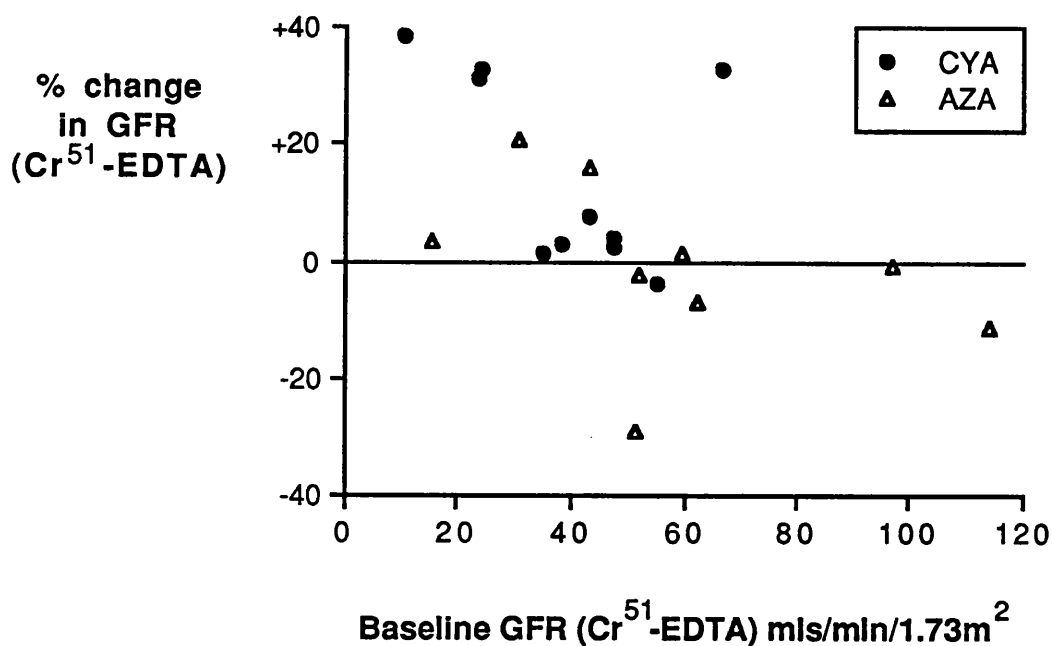
Figure 6.1. The percentage change in Cr^{51}-EDTA clearance from baseline versus baseline Cr^{51}-EDTA clearance following nifedipine in AZA- and CYA-treated recipients.

cent, p<0.02 and 13.1±3.3 per cent, p<0.02 respectively). Individual patients in the CYA group did, however, demonstrate a RFRC (Figure 6.2). The maximum change in Cr^{51}-EDTA clearance and ERPF in CYA-treated patients did not correlate with baseline Cr^{51}-EDTA clearance, CYA daily dose or whole blood levels, donor age, duration of graft or the number of rejection episodes.
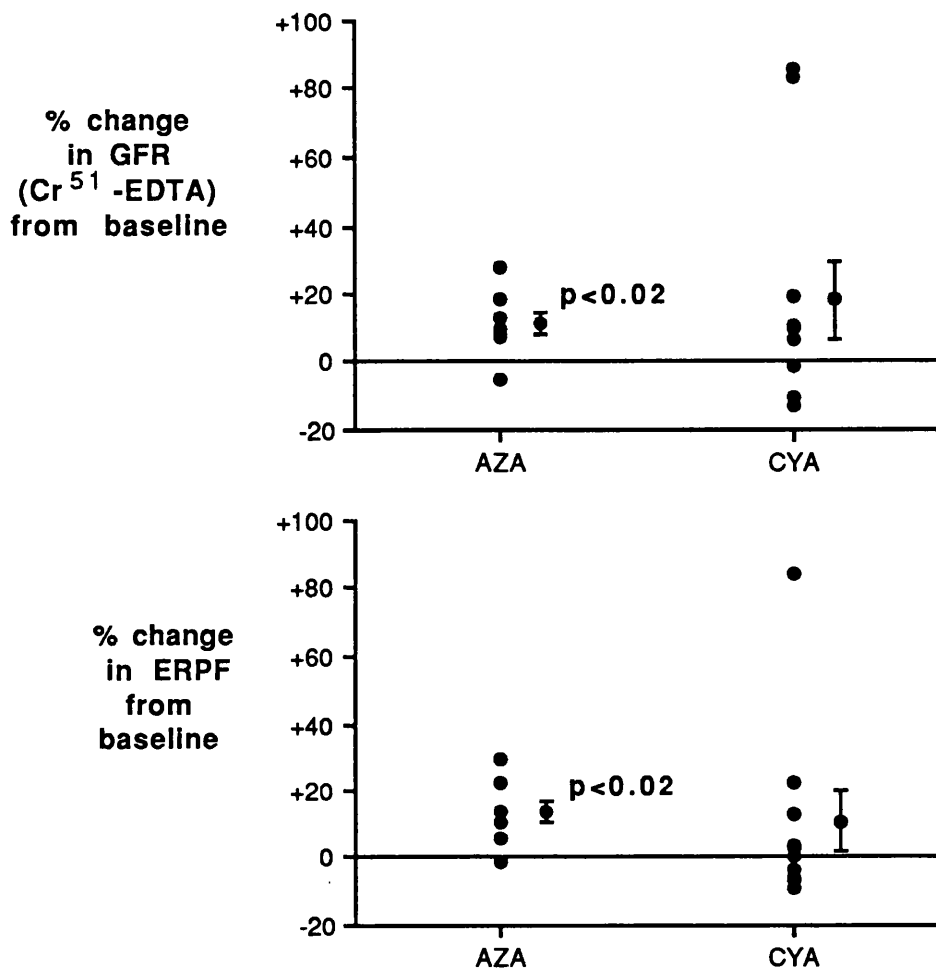
Figure 6.2. The percentage change (maximum) in Cr^{51}-EDTA clearance from baseline following an 80 g protein load in AZA- and CYA-treated patients.

ADVERSE SIDE-EFFECTS OF NIFEDIPINE

Adverse side-effects related to nifedipine were reported by five patients, although none were serious, unexpected or necessitated cessation of therapy. These included headaches during the first few days of treatment (n=3), mild ankle oedema (n=3) and dizziness (n=2).

DISCUSSION

The administration of nifedipine (20 mg twice daily) for 28 days to long-term normotensive CYA-treated renal allograft recipients led to a significant increase in Cr^{51}-EDTA clearance (+14.8%). This increase was not associated with a parallel increase in ERPF, or a reduction in either MAP or RVR, suggesting nifedipine improved renal function via a non-haemodynamic mechanism. If nifedipine-induced afferent arteriolar vasodilatation is the principal mechanism responsible for the observed increase in GFR, then the predicted rise in glomerular filtration pressure would lead to a preferential increase in GFR, without necessarily altering renal blood flow. However, these changes in renal haemodynamics were not associated with a significant reduction in RVR, making it unlikely that nifedipine-induced afferent arteriolar vasodilatation is the major factor contributing to the increase in GFR.

The improvement in GFR induced by nifedipine in this study is compatible with an increase in glomerular K_f, possibly via an increase in available glomerular filtration surface area, which is mainly regulated by mesangial cell contractility (Kreisberg et al., 1985). CYA-induced decreases in glomerular K_f have been reported (Myers et al., 1984; Barros et al., 1987a). It is possible that nifedipine may act by either inducing mesangial cell relaxation via direct interference with voltage-sensitive calcium channels on the plasma membrane or via antagonism of angiotensin II, via inhibition of receptor-operated calcium channels (Ausiello et al., 1980; Kreisberg et al., 1985). Experimental studies suggest that CYA is able to both induce mesangial cell contraction and augment contractility in the

presence of agonists (Meyer-Lehnert and Schrier, 1988; Rodriguez-Puyol et al., 1989), with similar observations reported in vascular smooth muscle cells (Meyer-Lehnert and Schrier, 1989). These effects may be secondary to CYA-enhanced transmembrane calcium influx and mobilisation from intracellular stores (Pfeilschifter and Ruegg, 1987; Meyer-Lehnert and Schrier, 1988; Pfeilschifter, 1988; Meyer-Lehnert and Schrier, 1989). These changes in calcium homeostasis may possibly potentiate mesangial contractility and thereby exaggerate the reduction in glomerular K_f and ultimately in GFR. Although disruption of excitation-contraction coupling within the mesangial cell may account for the increase in glomerular K_f and hence in GFR, verapamil was recently shown to be only able to partially inhibit CYA-induced mesangial contraction (Rodriguez-Puyol et al., 1989). Furthermore, the perturbations in cellular calcium kinetics induced by CYA were not abrogated by nifedipine or verapamil (Pfeilschifter and Ruegg, 1987; Meyer-Lehnert and Schrier, 1988). Thus, in vitro studies infer that the improvement in GFR conferred by nifedipine in this study may not be entirely due to a direct effect on mesangial cell contractility.

Nifedipine may also operate by antagonising the actions of several vasoactive hormones acting on the mesangial cell, particularly angiotensin II and the vasodilatory prostaglandins. In both in vivo and in vitro studies CYA has been shown to stimulate angiotensin II and renin release (Siegl et al., 1983; Baxter et al., 1982), the effects of which may be blocked by nifedipine, since its actions are mediated via calcium dependent pathways (Ichikawa et al., 1979; Goldberg and Schrier, 1984). However, a major role for angiotensin II in CYA

174

nephrotoxicity is doubted, as normal (Stanek et al., 1987) or reduced

activity (Bantle et al., 1985) has been reported in clinical studies

and conflicting results are reported with converting enzyme inhibition

in experimental models of CYA nephrotoxicity (Murray et al., 1985;

Dieperink et al., 1986b; Barros et al., 1987a).

Several recent studies accord with the present findings

(Dawidson et al., 1989; Kiberd, 1989). A study performed in the peri-

transplant period confirmed the beneficial effects of calcium channel

blockers on renal function in cadaveric renal allograft recipients

(Dawidson et al., 1989). In this latter study, pretreatment of

recipients for 48 hours with verapamil (80 mg t.d.s.) and then 120 mg

slow release (t.d.s.) for a total of 10 days after introducing CYA

prevented CYA-induced decreases in renal parenchymal blood flow

(measured by duplex Doppler scanning), ameliorating CYA-induced acute

nephrotoxicity, as well as decreasing the number of early rejection

episodes. Nifedipine has also been shown to have a beneficial effect

on renal function in renal allograft recipients, increasing GFR and

ERPF acutely by 14 % and 17 % respectively (Kiberd, 1989).

Although CYA is metabolised by the hepatic microsomal cytochrome

P-450 isozyme P450PCN1 (Kronbach et al., 1988), neither trough whole

blood CYA levels or serum nifedipine and its major metabolite M-I

levels changed. Inhibition of this system by the calcium channel

blockers, verapamil (Lindholm and Henricsson, 1987), diltiazem (Pochet

and Pirson, 1986) and nicardipine (Cantarovich et al., 1987), also

metabolised by hepatic oxidation, has been reported to increase

cyclosporin whole blood levels in renal allograft recipients. Although

in vitro, nifedipine inhibits cytochrome P-450 metabolism of

cyclosporin in human liver microsomal preparations (Henricsson and

Lindholm, 1988; Maenpaa et al., 1989), the present results suggest that

in vivo this interaction is clinically less important. In animal

studies high liver tissue concentrations of verapamil (Hamman et al.,

1983) but not nifedipine (Ptazschke et al., 1975) have been

demonstrated, which may partly explain the absence of a clinically

significant interaction between cyclosporin and nifedipine.

No serious adverse side-effects related to nifedipine were

reported that necessitated withdrawal of treatment. Significant

hypotension was not induced, corroborating previous reports that

normotensive individuals are relatively insensitive to the hypotensive

effects of nifedipine, in contrast to hypertensive subjects (Corea et

al., 1979). Although gingival hyperplasia is a recognised complication

of both CYA and nifedipine therapy alone, no cases developed over the

duration of the study. However, a previous study from this unit

reported that concurrent administration of nifedipine and CYA resulted

in an increased rate of gingival hyperplasia, compared to CYA alone

(Slavin and Taylor, 1985). Thus, nifedipine should be avoided in

patients with pre-existing gingival hyperplasia receiving CYA and

cessation of nifedipine therapy considered in those developing the

complication after treatment is initiated.

Most CYA-treated patients (9/10) in this study responded with an

increase in Cr^{51}-EDTA clearance, albeit small in 4 patients. However,

it is unclear from this study which factors predict a marked response

to nifedipine, as the percentage increase in GFR did not correlate with

baseline renal function, donor age, CYA daily dose, CYA whole blood levels or renal reserve capacity. Although the duration since transplantation was significantly longer in the AZA-treated patients it is unlikely that the failure to respond to nifedipine was related to chronic scarring or vascular damage preventing changes in renal vascular tone, since the AZA-treated patients were able to increase ERPF and GFR after the protein load. The response to the protein load in the CYA-treated patients was variable, with two patients demonstrating a significant renal reserve capacity. However, again there were no features to distinguish these patients from the CYA-treated patients who did not have a renal reserve capacity. Similarly, Cairns et al (1988) also did not demonstrate a RFRC in CYA-treated recipients after infusion with an aminoacid load, suggesting that the increase in afferent vascular tone induced by CYA impaired the vasodilatory response to a protein load.

The absence of a change in serum creatinine despite an increase in Cr^{51}-EDTA clearance highlights the inadequacy of a single serum creatinine value in quantifying the prevailing level of renal function. Furthermore, in common with other workers a poor correlation between the prevailing serum creatinine and Cr^{51}-EDTA clearance was documented (Tomlanovich et al., 1986). Thus, markedly impaired renal function may be associated with normal or only mildly elevated serum creatinine concentration in CYA-treated patients. Augmented tubular secretion of creatinine has been suggested as a possible mechanism for the reduced serum creatinine levels documented (Tomlanovich et al., 1986). Nevertheless, it is likely serial measurements in individual patients

will allow changes in renal function to be recognised, although the degree of renal impairment may be underestimated.

In summary, this study suggests nifedipine may confer a beneficial effect on renal function in selected CYA-treated renal allograft recipients, even after long-term therapy. However, the clinical features distinguishing those patients demonstrating a clinically relevant response are unclear from this study. Further studies are required to determine whether prolonged concomitant therapy with CYA and nifedipine immediately post-transplant will preserve renal function and mitigate the pathological changes associated with chronic CYA therapy. The pattern of haemodynamic changes induced by nifedipine in the present study suggest both vascular and non-vascular mechanisms may be involved in the nephroprotection conferred by nifedipine.

CONCLUSIONS

CONCLUSIONS

The experimental and clinical studies reported in this thesis were designed to investigate the effects of nifedipine, a dihydropyridine calcium channel blocker and potent renal vasodilator, on CYA nephrotoxicity. Further experiments were performed to identify the mechanism(s) activating the change in renal vascular resistance and the basis of the nephroprotection conferred by this class of drug in combination with CYA.

The first series of experiments (Section III) were performed initially in surgically intact (two-kidney) SHR and subsequently in uninephrectomized SHR and in SHR undergoing uninephrectomy with contralateral renal denervation, to explore the effects of nifedipine on CYA nephrotoxicity in animals with reduced renal mass, in an attempt to mimic the clinical situation in patients undergoing renal allograft transplantation. The findings that the alterations in renal haemodynamics associated with CYA administration occurred without overt histological evidence of proximal tubular cell, glomerular or vascular injury or an increase in N-acetyl-β-D-glucosaminidase excretion, in either the two-kidney or uninephrectomized models suggests CYA is not a direct tubular toxin, adding support to the notion that CYA nephrotoxicity is haemodynamically mediated. The observation that concomitant nifedipine and CYA from day 1 in the two-kidney model prevented the characteristic reductions in GFR and ERPF, and the increase in RVR associated with CYA, infer that nifedipine counteracts CYA-induced renal arteriolar vasoconstriction. However, this beneficial effect was lost when nifedipine was administered to animals

179

previously exposed to CYA for a period of 7 days, suggesting that alterations in vascular reactivity secondary to unopposed CYA administration might impair the vasodilatory capacity of the renal vasculature.

Several pathophysiological mechanisms have been proposed as mediators for the changes in renal vascular tone, including the renal sympathetic nerves. However, the demonstration that renal denervation failed to ameliorate CYA nephrotoxicity excludes the renal sympathetic nervous system as playing a major role. This finding, although at variance with some workers, parallels observations made in man, that in the early posttransplant period the acute functional abnormalities associated with CYA administration occur in a denervated allograft. The failure of nifedipine to influence renal function in the uninephrectomy models might relate to the adaptive changes that take place in intrarenal haemodynamics after a reduction in renal mass, interfering with the vasodilatory effects of nifedipine.

The abnormalities in cellular Ca^{2+} homeostasis reported by several groups, including increased transmembrane Ca^{2+} influx and mobilization of Ca^{2+} from intracellular stores was shown to be independent of phosphoinositide metabolism (Section IV). Thus, alternative mechanisms must be invoked to account for the alterations in cellular Ca^{2+} homeostasis, such as changes in activity of the plasma membrane Ca^{2+} transporter mechanisms or the capacity of intracellular organelles to bind and release stored Ca^{2+} in presence of CYA.

A potential mechanism proposed to account for the nephroprotection conferred by calcium channel blockers is reduced

uptake of CYA into proximal tubular cells. However, the combined observations that histological evidence of renal tubular cell injury is absent in vivo, and secondly that nifedipine does not inhibit the uptake of CYA into cultured human proximal tubular cells makes it unlikely that nifedipine-induced nephroprotection is secondary to reduced tubular cell injury (Section V).

Finally, in the clinical studies performed in stable long-term renal allograft recipients receiving CYA, short-term nifedipine administration was shown to improve Cr^{51}-EDTA clearance, albeit by a small degree (Section VI). However, this beneficial effect was not accompanied by a parallel increase in ERPF, or a reduction in either MAP or RVR, intimating that the improvement in renal function after nifedipine was predominantly due to a non-vascular mechanism. This pattern of renal haemodynamic changes could be compatible with an increase in glomerular K_f, secondary to nifedipine-induced changes in mesangial cell contractility. Further studies are needed to determine whether prolonged concomitant therapy with CYA and nifedipine immediately post-transplant will preserve renal function and mitigate the pathological changes associated with chronic CYA therapy in man.

Thus, the present series of studies contribute further support to the notion that acute CYA nephrotoxicity is a primarily a haemodynamic phenomenon, with the varying degrees of tubular injury and interstitial fibrosis described after chronic CYA administration most likely the result of tubular hypoperfusion and focal tubular collapse, secondary to afferent arteriolar vasoconstriction. The observation that under certain conditions nifedipine is able to ameliorate CYA

nephrotoxicity offers a potential therapeutic option to prevent chronic

nephrotoxicity in man.

APPENDICES

APPENDIX 1

ANIMAL DIET

Analysis of dietary composition.

The animal diet used in all experimental studies was supplied by Special Diets Services Limited, Witham, Essex. The following dietary composition was supplied by the manufacturers and expressed as a percentage of total weight.

Constituent	% by weight
Moisture	10.2
Crude fat	4.9
Crude Protein	21.9
Maize starch	45.0
Crude fibre	2.6
Ash	6.6
Calcium	1.13
Phosphorus	0.75
Sodium	0.26
Chloride	0.44
Potassium	0.86
Magnesium	0.20
Other substances (vitamins,inorganic ions etc..)	5.19

APPENDIX 2

THE EFFECT OF HOUSING IN METABOLIC CAGES ON FOOD INTAKE AND URINE PRODUCTION

To obtain reliable measurements of urine production over a period of time animals need to be housed in metabolic cages. These units consist of a perspex surround with a metal grill floor. Housing animals in these unfamiliar surroundings may in the short-term be stressful and directly influence food intake and urine production. To determine whether prolonged acclimatisation under these conditions would provide more representative data, daily food intake, body weight and urine output were measured before and during a prolonged period in the metabolic cage.

MATERIALS AND METHODS

Daily food intake (g/day), body weight (g) and 24 hour urine production (ml) were repeatedly measured in eight control (two-kidney) SHR over a period of 3 days prior and for 5 days during housing in metabolic cages. Data was analysed by two-way analysis of variance for multiple comparisons.

RESULTS

Mean daily food intake, body weight and urine volume per 24 hours did not change over the duration of the study (Table A.2.1.).

DISCUSSION

These preliminary studies demonstrate that housing in metabolic

184

cages did not influence any of the parameters measured. Thus, all urine collections were performed over a single 24 hour period without acclimatising the animal to the metabolic cage conditions.

TABLE A.2.1. The effect of housing in metabolic cages on food intake, body weight and urine production in two-kidney SHR.

Variable measured	Before metabolic caging (day)			Metabolic cage period (day)				
	-3	-2	-1	1	2	3	4	5
Mean daily weight (g)	169.2±2.0	168.5±1.7	171±1.9	175±2.7	175.6±2.6	179.4±2.7	176.9±1.9	178.1±1.3
Mean daily food intake (g/day)	65.2±3.1	62.2±2.1	58.7±3.2	60.0±1.6	55.5±3.6	55.9±3.1	58.6±4.3	65.5±4.7
Urine production (ml/24 hours)	22.1±2.1	24.4±1.5	21.5±2.2	23.4±1.5	20.8±1.2	22.1±1.7	20.5±3.2	22.8±1.4

APPENDIX 3

CLEARANCE PROTOCOL

GFR and ERPF were determined by calculating inulin and para-aminohippurate clearances using an isotopic constant infusion technique based on a method described by Earle and Berliner (1946). To obviate the need for urine collection the rate of excretion (UV) of a test substance is substituted for the rate of infusion (IV) after steady state conditions have been obtained, as indicated by a constant plasma level. Thus, at equilibrium the rate of infusion of both isotopes $(I_x R)$ is equal to the rate of excretion $(U_x V_x)$, where I_x is the number of counts/min infused per unit volume, R is the rate of infusion (ml/min), U_x is the number of counts/min per unit volume of urine and V_x is the urinary flow rate (ml/min) for isotope (x).

GFR is calculated as follows:

$$\text{GFR (ml/min)} = \frac{^{14}C_{inf} \times R_{inf} \text{ ml.min}^{-1}}{^{14}C_p}$$

and ERPF as:

$$\text{ERPF (ml/min)} = \frac{^{3}H_{inf} \times R_{inf} \text{ ml.min}^{-1}}{^{3}H_p}$$

where $^{14}C_{inf}$ = infusion ^{14}C counts min^{-1} ml^{-1}, R_{inf} = rate of infusion (ml/min), $^{14}C_p$ = plasma ^{14}C counts min^{-1} ml^{-1}, $^{3}H_{inf}$ = infusion ^{3}H counts min^{-1} ml^{-1} and $^{3}H_p$ = plasma ^{3}H counts min^{-1} ml^{-1}.

Preliminary studies were performed to establish the appropriate priming dose and systemic infusion activity and rate of administration

to assure steady state conditions [based on a modification of the technique described by Lote et al (1985)]. The plasma level was assumed to be constant after time (x) when the measured concentrations differed by less than 10 % of the mean at that moment. Low activity infusions failed to achieve steady state conditions, with the measured plasma activity continuing to rise over the 5 hour study period. Conversely, high activity infusions led to increasing plasma activity. By maintaining the priming dose at a constant activity, steady state conditions were achieved by minor adjustment of the infusion activity and rate. A loading dose of Inulin [^{14}C] carboxylic acid (0.0264 MBq) and para-amino [^{3}H] hippuric acid (0.264MBq) in 0.5 ml of 0.9% saline and a constant infusion of both isotopes at 0.8ml/hour for 3 hours (0.0529 MBq/ml and 0.529 MBq/ml respectively) reliably maintained stable plasma activity. Table A.3.1. documents the variation in plasma activity achieved over a five hour period for six control SHR using this protocol. To reduce the coefficient of variation, a 2 hour equilibrium phase was observed in all experimental studies (coefficient of variation ranging from 5.7 to 11.3 % for ^{3}H-PAH and 2.4 to 12.4 % for ^{14}C-inulin).

TABLE A.3.1. The variability of plasma activity using the standard protocol established for all clearance studies. Studies were performed over 5 hours to confirm that steady state conditions prevailed. Coefficient of variation has been calculated for samples including and excluding the 1 hour measurement.

Time (hours)	Counts/min for rats 1-6					
	1	2	3	4	5	6
1 ^{3}H-PAH	875	1002	990	1090	1111	943
2	1056	1200	1090	1209	1324	1005
3	987	1134	1178	1190	1001	1200
4	1180	1134	1140	1070	1209	1194
5	997	1134	1140	1207	1204	1076
CV (%) 1-5 h	10.9	8.2	7.6	5.9	10.3	10.5
CV (%) 2-5 h	8.4	7.6	6.7	5.7	11.3	8.5
1 C^{14}-Inulin	560	679	550	569	554	689
2	670	734	679	589	623	798
3	634	777	678	666	600	599
4	710	756	788	677	566	651
5	601	762	790	701	643	673
CV (%) 1-5 h	9.2	5.2	14.2	9.0	6.3	10.7
CV (%) 2-5 h	7.2	2.4	8.7	7.4	5.4	12.4

REFERENCES

REFERENCES

ADU D, MICHAEL J, TURNEY J, MCMASTER P. Hyperkalaemia in cyclosporine-treated renal allograft recipients. Lancet 1983; ii: 370-371.

ANDREUCCI VE. Pathophysiology of ischemic/toxic acute renal failure. In: Acute renal failure, edited by Andreucci VE, Boston, Martinus Nijjhoff Publishing. 1984; pp 1-50.

ANTON AH, SAYRE DF. A study of the factors affecting the aluminium-oxide-hydroxyindole procedure for the analysis of catecholamines. The Journal of Pharmacology and Experimental Therapeutics 1962; 138: 360-374.

AREND LJ, HARAMATI A, THOMPSON CI, SPIELMAN WS. Adenosine-induced decrease in renin release: dissociation from haemodynamic effects. American Journal of Physiology 1984; 247(Renal Fluid Electrolyte Physiol. 16): F447-F452.

ARENDSHORST WJ AND BEIERWALTES WH. Renal and nephron haemodynamics in spontaneously hypertensive rats. American Journal of Physiology 1979; 236(Renal Fluid Electrolyte Physiol. 5(3): F246-F251.

ARENDSHORST WJ, NAVAR LG. Renal circulation and glomerular haemodynamics. In: Diseases of the kidney. Edited by Schrier RW and Gottschalk CW. Little, Brown and Company, Boston/Toronto 1988. p65-118.

ASZALOS A. Cyclosporin: some aspects of its mode of action. A review. Journal of Medicine 1988; 19: 297-316.

AUSIELLO DA, KREISBERG JI, ROY C, KARNOVSKY MJ. Contraction of cultured rat glomerular mesangial cells of apparent mesangial origin after stimulation with angiotensin II and arginine vasopressin. Journal of Clinical Investigation 1980; 65: 754-760.

AUSTIN HA, PALESTINE AG, SABNIS SG, BALOW JE, PREUSS HG, NUSSENBLATT RB, ANTONOVYCH TT. Evolution of ciclosporin nephrotoxicity in patients treated for autoimmune uveitis. American Journal of Nephrology 1989; 9: 392-402.

AUSTIN MB, ROBSON RA, BAILEY RR. Effect of nifedipine on renal function of normal subjects and hypertensive patients with renal functional impairment. New Zealand Medical Journal 1983; 96: 829-831.

AVASTHI PS, EVAN AP, HAY D. Glomerular endothelial cell in uranyl nitrate-induced acute renal failure in rats. Journal of Clinical Investigation 1980; 65: 121-127.

AZOGUI O, GLUCKMAN E, FRADELIZI D. Inhibition of IL-2 production after human allogeneic bone marrow transplantation. Journal of Immunology

1983; 131: 1205-1208.

BAER PG, MCGRIFF JC. Hormonal systems and renal haemodynamics. Annual Reviews of Physiology 1980; 42: 589-601.
BAER PG, NAVAR LG. Renal vasodilation and uncoupling of blood flow and filtration rate autoregulation. Kidney International 1973; 4: 12-21.

BALLERMAN BJ, LEVINSON DJ, BRENNER BM. The renal prostanoid-thromboxane system. In: Brenner BM, Rector FC (eds). The Kidney, WB Saunders; 1986: 301-340.

BANTLE JP, NATH KA, SUTHERLAND DER, NAJARIAN JS, FERRIS TF. Effects of cyclosporine on the renin-angiotensin-aldosterone system and potassium excretion in renal transplant recipients. Archives of Internal Medicine 1985; 145: 505-508.

BANTLE JP, BOURDREAU RJ, FERRIS TF. Suppression of plasma renin activity by cyclosporine. American Journal of Medicine 1987; 83: 59-64.

BARROS EJG, BOIM MA, AJZEN H, RAMOS OL, SCHOR N. Glomerular hemodynamics and hormonal participation on cyclosporine nephrotoxicity. Kidney International 1987a; 32: 19-25.

BARROS EJG, BOIM MA, MOURA LAR, RAMOS OL, SCHOR N. Effects of acute and chronic cyclosporine administration on glomerular haemodynamics in rats. In: Bach PH and Lock EA (eds). Nephrotoxicity: extrapolation from in vitro to in vivo and from animals to man. Plenum Press, 1987b.

BATLLE DC, GUTTERMAN C, TARKA J, PRASAD R. Effect of short-term cyclosporine A administration on urinary acidification. Clinical Nephrology 1986; 25 (suppl 1): S62-S69.

BATLLE DC, GUTTERMAN C, KEILANI T, PECES R, LAPOINTE M. Effect of cyclosporin A on renal function and kidney growth in the uninephrectomized rat. Kidney International 1990; 37: 21-28.

BAUER JH, SUNDERRAJAN S, REAMS G. Effects of calcium entry blockers on renin-angiotensin-aldosterone system, renal function and haemodynamics, salt and water excretion and body fluid composition. American Journal of Cardiology 1985; 56: 62H-67H.

BAXTER CE, DUGGIN GG, WILLIS N, HALL BM, HORNATH JS, TILLER DJ. Cyclosporin A-induced increases in renin storage and release. Res Commun Chem pathol Pharmacol 1982; 37: 305-312.

BAXTER CR, DUGGIN GG, HALL BM, HORVATH JS, TILLER DJ. Stimulation of renin release from rat renal cortical slices by cyclosporin A. Research Communications in Chemical Pathology and Pharmacology 1984; 43(3): 417-423.

BAYLIS C, DEEN WM, MYERS BD, BRENNER BM. Effects of some vasodilator drugs on transcapillary fluid exchange in renal cortex. American

Journal of Physiology 1976; 230(4): 1148-1158.

BAYLIS C, RENNKE HR, BRENNER BM. Mechanisms of the defect in glomerular ultrafiltration associated with gentamicin administration. Kidney International 1977; 12: 344-353.

BAYLISS WM. On the local reactions of arterial wall to change in internal pressure. Journal of Physiology 1902; 28: 220.

BELL DR, WEBB RC, BOHR DF. Functional bases for individualities among vascular smooth muscles. Cardiovascular Pharmacology 1985; 7(suppl 3): S1-S11.

BELL AJ, LINDNER A. Effects of verapamil and nifedipine on renal function and haemodynamics in the dog. Renal Physiology 1984; 7: 329-343.

BELLETT M, CABROL C, SASSANO P, LEGER P, CORVOL P, MENARD J. Systemic hypertension after cardiac transplantation: effect of cyclosporine on the renin-angiotensin-aldosterone system. American Journal of Cardiology 1985; 56: 927-931.

BENIGNI A, PERICO N, REMUZZI G. Abnormalities of arachidonate metabolism in experimental ciclosporin nephrotoxicity. American Journal of Nephrology 1989; 9(suppl 1): 72-77.

BENNETT WM, PORTER GA. Cyclosporine-associated hypertension. The American Journal of Medicine 1988; 85: 131-133.

BERRIDGE MJ, DOWNES CP, HANLEY MR. Lithium amplifies agonist dependent phosphatidylinositol responses in brain and salivary glands. Biochemical Journal 1982; 206: 587-595.

BERTANI T, PERICO N, ABBATE M, BATTAGLIA C, REMUZZI G. Renal injury induced by long-term administration of CYA to rats. American Journal of Pathology 1987; 127: 569-597.

BESARAB A, JARRELL BE, HIRSCH S, CARABASI RA, CRESSMAN MD, GREEN P. Use of the isolated perfused kidney model to assess the acute pharmacologic effects of cyclosporine and its vehicle, cremophor EL. Transplantation 1987; 44(2): 195-201.

BEVAN JA, BEVAN RD, HWA JJ, OWEN MP, TAYO FM, WINQUIST RJ. Calcium, extrinsic and intrinsic (myogenic) vascular tone. In Calcium modulators. Godfraind T, Albertini A, Pauletti R, Eds: Elsevier Biomedical Press, Amsterdam, Holland, 1982; 125-132.

BIRX DL, BERGER M, FLEISHER TA. The interference of T cell activation by calcium channel blocking agents. The Journal of Immunology 1984; 133: 2904-2909.

BLAIR JT, THOMSON AW, WHITING PH, DAVIDSON RJL AND SIMPSON JG. Toxicity of the immune suppressant cyclosporin A in the rat. Journal of Pathology 1982; 138: 163-178.

BLANTZ RC. The mechanism of acute renal failure after uranyl nitrate. Journal of Clinical Investigation 1975; 55: 621-635.

BLANTZ RC, KONNEN KS, TUCKER BJ. Angiotensin II effects upon the glomerular microcirculation and ultrafiltration coefficient of the rat. Journal of Clinical Investigation 1976; 57:419-434.

BONSER RS, ADU D, FRANKLIN I, MCMASTER P. Cyclosporin-induced haemolytic uraemic syndrome in a liver allograft recipient. Lancet 1984; ii: 1337.

BOREL JF. Comparative study of in vitro and in vivo drug effects on cell mediated cytotoxicity. Immunology 1976; 31: 631-641.

BOREL JF, FEURER C, MAGNEE C, STAHELIN H. Effects of a new anti-lymphocytic peptide cyclosporin A in animals. Immunology 1977; 32: 1017-1025.

BOREL JF. Cyclosporine. Trends Pharmacol Sci 1980; 2: 146.

BOSSALLER C, FORSTERMANN U, HERTEL R, OLBRICHT C, RESCHKE V, FLECK E. Ciclosporin A inhibits endothelium-dependent vasodilatation and vascular prostacyclin production. European Journal of Pharmacology 1989; 165; 165-169.

BOURBIGOT B, GUISERIX J, AIRIAU J, BRESSOLLETTE L, MORIN JF, CLEDES J. Nicardipine increases cyclosporine blood levels. Lancet 1986; i: 1447.

BRAUNWALD E. Mechanism of action of calcium-channel-blocking agents. New England Journal of Medicine 1982; 307: 1618-1627.

BRENNER BM, STEIN J. Nephrotoxic-induced acute renal failure. In Acute Renal Failure. (Eds) Brenner BM and Stein J. Churchill Livingstone, New York 1980; 123-162.

BRENNER BM. Nephron adaptation to renal injury or ablation. American Journal of Physiology 1985; 249 (Renal Fluid Electrolyte Physiol. 18): F324-F337.

BRENNER BM, DWORKIN LD, ICHIKAWA I. Glomerular filtration. In: The Kidney (3rd Edition). Edited by Brenner BM and Rector FC. Ardmore Medical Books. WB Saunders Company, Philadelphia. 1986: p124-144.

BROWN Z, NEILD GH, WILLOUGHBY JJ, SOMIA N, CAMERON SJ. Increased factor VIII as an index of vascular injury in cyclosporine nephrotoxicity. Transplantation 1986; 42(2): 150-153.

BROWN Z, NEILD GH. Cyclosporine inhibits prostacyclin production by cultured human endothelial cells. Transplantation Proceedings 1987; 19: 1178-1180.

BROWN Z, NEILD GH, LEWIS G. Mechanism of cyclosporine inhibition of prostacyclin synthesis by cultured human umbilical vein endothelial cells. Transplantation Proceedings 1988; 20(3, suppl 3): 654-657.

BRUSERUD O. Effect of verapamil on T-lymphocyte activation in vitro. Scandinavian Journal of Immunology 1985; 21: 73-79.

BUNKE M, WILDER L. Effect of verapamil on glomerular filtration rate and prostaglandin production during cyclosporine administration. Transplantation 1988; 46: 919-921

BURKE TJ, ARNOLD PE, GORDON JA, BULGER R, DOBYAN DC, SCHRIER RW. Protective effect of intrarenal calcium membrane blockers before or after renal ischemia. Journal of Clinical Investigation 1984; 74: 1830-1841.

CAIRNS HS, ROGERSON M, FAIRBANKS LD, WETWICK J, NEILD GH. Endothelin and cyclosporin nephrotoxicity. Lancet 1988a; ii: 1496-1497.

CAIRNS HS, RAVAL U, NEILD GH. Failure of cyclosporine treated renal allograft recipients to increase glomerular filtration rate following an amino acid infusion. Transplantation 1988b; 46: 79-82.

CAIRNS HS, FAIRBANKS LD, WESTWICK J, NEILD GH. Cyclosporin therapy in vivo attenuates the response to vasodilators in the isolated perfused kidney of the rabbit. British Journal of Pharmacology 1989; 98: 463-468.

CALNE RY, WHITE DJG. Cyclosporin A - a powerful immunosuppressant in dogs with renal allografts. IRCS Medical Sciences 1977; 5: 595.

CALNE RY, ROLLES K, SMITH DP, HERBERTSON BM. Prolonged survival of pig orthotopic heart grafts treated with cyclosporin A. Lancet 1978a; i: 1183-1185.

CALNE RY, WHITE DJG, THIRU S, EVANS DB, MCMASTER P, DUNN DC, CRADDOCK GN, PENTLOW BD AND ROLLES K. Cyclosporin in patients receiving renal allografts from cadaver donors. Lancet 1978b; ii: 1323-1327.

CALNE RY, ROLLES K, WHITE DJG, THIRU S, EVANS DB, MCMASTER P, DUNN DC, CRADDOCK GM, HENDERSON RG, AZIZ S, LEWIS P. Cyclosporin A initially as the only immunosuppressant in 34 recipients of cadaveric organs: 32 kidneys, 2 pancreases, and 2 livers. Lancet 1979; ii: 1033-1036.

CANADIAN MULTICENTRE TRANSPLANT STUDY GROUP: A randomized clinical trial of cyclosporine in cadaveric renal transplantation. New England Journal of Medicine 1983; 309: 809-815.

CANADIAN MULTICENTRE TRANSPLANT STUDY GROUP. A randomized clinical trial of cyclosporine in cadaveric renal transplantation at three years. New England Journal of Medicine 1986; 314: 1219-1225.

CANTAROVICH M, LOCKIEC F, HIESSE C, CHARPENTIER B, FRIES D. Confirmation of the interaction between cyclosporine and the calcium channel blocker nicardipine in renal transplant patients. Clinical Nephrology 1987; 28(4): 190-193.

CARPENTER BJ, TILNEY NL, STROM TB, GARAVOY MR AND LAZARUS JM. Cyclosporin A in cadaver renal allografts. Kidney International 1981; 19: 265A.

CASTEELS R, DROOGMAN G. Exchange characteristics of the noradrenaline-sensitive calcium store in vascular smooth muscle cells of the rabbit ear artery. Journal of Physiology (London) 1981; 317: 263-279.

CAUVIN C, LOUTZENHISER R, VAN BREEMEN C. Mechanisms of calcium antagonist-induced vasodilation. Annual Reviews of Pharmacology and Toxicology 1983; 23: 373-96.

CHANTLER C, BARRATT TM. Estimation of glomerular filtration rate from plasma clearance of 51-chromium edetic acid. Archives of Diseases in Childhood 1972; 47: 613-617.

CHAPMAN JR, HARDING NGL, GRIFFITHS D, MORRIS PJ. Reversibility of cyclosporin nephrotoxicity after three months treatment. Lancet 1985; i: 128-130.

CHEUNG JY, LEAF A, BONVENTRE JV. Mechanism of protection by verapamil and nifedipine from anoxic injury in isolated cardiac myocytes. American Journal of Physiology 1984; 246: C323-C329.

CHOW SS, THORNER P, BAUMAL R AND WILSON DR. Cyclosporine and experimental renal ischaemic injury. Transplantation 1986; 41: 152-156.

CITTERIO F, KAHAN BD. The inhibitory effect of cyclosporine on the nuclear proliferative response to a variety of T cell activators. Transplantation 1989; 47: 334-338.

COCKCROFT S, GOMPERTS BD. Role of guanine nucleotide binding protein in the activation of polyphosphoinositide phosphodiesterase. Nature 1985; 314: 534-536.

COFFMAN TM, CARR DR, YARGER WE, KLOTMAN PE. Evidence that renal prostaglandin and thromboxane production is stimulated in chronic cyclosporine nephrotoxicity. Transplantation 1987; 43(2): 282-285.

COFFMAN TM. In: Humes HD, Coffman T, Halderman H, Mihatsch M, Henry M, Porter GA. Cyclosporine nephrotoxicity: A workshop to discuss mechanisms, diagnosis, and treatment. Transplantation Proceedings 1988;

20 (3): 833-840.

COFFMAN TM, MAUROS SM, COLLINS DM, RUIZ P, YARGER WE, KLOTMAN PE.
Administration of thromboxane receptor antagonist improves renal
function in rats with cyclosporine nephrotoxicity. Kidney
International 1989; 35: 505A.

COFFMAN TM, SMITH SR, CREECH EA, SCHAFFER AV, MARTIN LL, RAKHIT A,
DOUGLAS FL, KLOTMAN PE. The thromboxane synthetase inhibitor CGS 13080
improves renal allograft function in patients taking cyclosporine A.
Kidney International 1990; 36: 416A.

COHEN AJ, FRAY JCS. Calcium ion dependence of myogenic renal plasma
flow autoregulation: evidence from isolated perfused rat kidney.
Journal of Physiology (London) 1982; 330: 449-460.

COLOMBANI PM, ROBB A, HESS AD. Cyclosporin A binding to calmodulin: A
possible site of action on T Lymphocytes. Science 1985; 228: 337-339.

CONTE G, CANTON AD, SABBATINI M, NAPODANO P, DE NICOLA L, GIGLIOTTI G,
FUIANO G, TESTA A, ESPOSITO C, RUSSO D AND ANDREUCCI VE. Acute
cyclosporine renal dysfunction reversed by dopamine infusion in healthy
subjects. Kidney International 1989; 36: 1086-1092.

COREA L, MIELE N, BENTIVOGLIO M, BOSCHETTI E, AGABITI-ROSEI E, MUIESAN.
Acute and chronic effects of nifedipine on plasma renin activity and
plasma adrenaline and noradrenaline in controls and hypertensive
patients. Clinical Science 1979; 57: 115S-117S.

CUNNINGHAM C, WHITING PH, BURKE MD, WHEATLEY DN, SIMPSON JG.
Increasing the hepatic metabolism of cyclosporine abolishes
nephrotoxicity. Transplantation Proceedings 1983; 15: 2712-2715.

CUNNINGHAM C, GAVIN MP, WHITING PH, BURKE MD, MACINTYRE F, THOMSON AW,
SIMPSON JG. Serum cyclosporin levels, hepatic drug metabolism and
renal tubulotoxicity. Biochem Pharmacol 1984; 33: 2857-2861.

CURTIS JJ, LUKE RG, DUBOVSKY E, DIETHELM AG, WHELCHEL JD, JONES P.
Cyclosporine in therapeutic doses increases renal allograft vascular
resistance. Lancet 1986; ii: 477-479.

CURTIS JJ, LUKE RG, JONES P, DIETHELM AG. Hypertension in
cyclosporine-treated renal transplant recipients is sodium dependent.
The American Journal of Medicine 1988; 85: 134-138.

CURTIS JJ. Cyclosporine-induced hypertension. In, Hypertension:
Pathophysiology, diagnosis, management. Edited by Laragh JH and
Brenner BM. Ravens Press Limited, New York, 1990. p1829-1835.

DADAN J, PERICO N, REMUZZI G. Role of endothelin in cyclosporine-
induced renal vasoconstriction. Kidney International 1990; 36 :291A.

DAWIDSON I, ROOTH P, FRY WR, SANDOR Z, WILLMS C, COORPENDER L, ALWAY C, REISCH J. Prevention of acute cyclosporine-induced blood flow inhibition and improved immunosuppression with verapamil. Transplantation 1989; 48(4): 575-580.

DERAY G, LE HOANG P, CACOUB P, HORNYCH A, LEGRAND S, AUPETIT B, ZOGBI F, LANDAULT C, CARAYON A, BAUMELOU A, JACOBS C. Effects of cyclosporin on plasma renin activity, catecholamines and prostaglandins in patients with idiopathic uveitis. American Journal of Nephrology 1988; 8: 298-304.

DETH D, LYNCH C. Inhibition of alpha-receptor induced Ca^{++} release and Ca^{++} influx by Mn^{++} and Ln^{3+}. European Journal of Pharmacology 1981; 71: 1-11.

DEVARAJAN P, KASKEL FJ, ARBEIT LA, MOORE LC. Cyclosporine nephrotoxicity: blood volume, sodium conservation, and renal hemodynamics. American Journal of Physiology 1989; 256 (Renal Fluid Electrolyte Physiol. 25): F71-F78.

DEVINENI R, MCKENZIE N, DUPLAN J, KEOWN P, STILLER C, WALLACE AC. Renal effects of cyclosporin: clinical and experimental observations. Transplantation Proceedings 1983; 15 (suppl 1): 2695-2698.

DIEPERINK H, STARKLINT H, LEYSACC PP. Nephrotoxicity of cyclosporine - an animal model: study of the nephrotoxic effect of cyclosporine on an overall renal and tubular function in conscious rats. Transplantation Proceedings 1983; 15(4): 2736-2741.

DIEPERINK H, LEYSSAC PP, STARKLINT H, KEMP E. Nephrotoxicity of cyclosporin A. A lithium clearance and micropuncture study in rats. European Journal of Clinical Investigation 1986a; 16: 69-77.

DIEPERINK H, LEYSACC PP, STARKLINT H, JORGENSEN KA, KEMP E. Antagonist capacities of nifedipine, captopril, phenoxybenzamine, prostacyclin and indomethacin on cyclosporin A induced impairment of rat renal function. European Journal of Clinical Investigation 1986b; 16: 540-548.

DIEPERINK H, LEYSACC PP, KEMP E, STARKLINT H, FRANDSEN NE, TVEDES N, MOLLER J, BUCHLER P, ROSSING N. Nephrotoxicity of cyclosporine A in humans: effects on glomerular filtration and tubular reabsorption rates. European Journal Of Clinical Investigation 1987; 17: 493-496.

DIEPERINK H, LEYSACC PP, STARKLINT H, KEMP E. Long-term cyclosporine A nephrotoxicity in the rat. Effects on renal function and morphology. Nephrology, Dialysis and Transplantation 1988; 3: 317-326.

DIETZ JR, DAVIS JO, FREEMAN RH, VILLARREAL D, ECHTENKAMP SF. Effects of intrarenal infusion of calcium entry blockers in anaesthetised dogs. Hypertension 1983; 5: 482-488.

DONOHOE JF, VENKATACHALAM MA, BERNARD DB, LEVINSKY NG. Tubular leakage

and obstruction in acute ischaemic renal failure. Kidney International 1978; 13: 208-222.

DOUGLAS WW. Histamine and 5-hydroxytryptamine (serotonin) and their antagonists. In Goodman A and Gilman A (eds). The pharmacological basis of therapeutics. Macmillan Publishing Co. new York, 1980; p609.

DREYFUSS M, HARRI E, HOFMANN H, KOBEL H, PACHE W, TSCHERTER H. Cyclosporin A and C. New metabolites from Trichoderma polysporum (Link ex Pers.) Rifai. European Journal of Applied Microbiology 1976; 3: 125-133.

DUGGIN GC, BAXTER C, HALL BM, HORVATH JS, TILLER DJ. Influence of cyclosporine A (CSA) on intrarenal control of GFR. Clinical Nephrology 1986; 25(suppl 1): S43-S45.

DUNCAN JI, THOMSON AW, ALDRIDGE RD, SIMPSON JG AND WHITING PH. Cyclosporine-induced renal structural damage: influence of dosage, strain, age and sex with reference to the rat and guinea pig. Clinical Nephrology 1986; 25 (suppl 1): S14-S17.

DWORKIN LD, ICHIKAWA I, BRENNER BM. Hormonal modulation of glomerular function. American Journal of Physiology 1983; 244 (Renal Fluid Electrolyte Physiol.13): F95-F104.

EARLE DP AND BERLINER RW. A simplified clinical procedure for measurement of glomerular filtration rate and renal plasma flow. Proc Soc Exper Biol Med 1946; 62: 262-264.

EGEL J, GREENBERG A, THOMPSON ME, HARDESTY HL, GRIFFITH BP, BAHNSON HT, BERNSTEIN RI, PUSCHETT JB. Renal failure in heart transplant patients receiving cyclosporin. Transplantation Proceedings 1983; 15: 2706-2707.

ELZINGA LA, MELA-RIKER LM, WIDENER LL, BENNETT WM. Renal cortical mitochondrial integrity in experimental cyclosporine nephrotoxicity. Transplantation 1989; 48(1): 102-108.

ENGLISH J, EVAN A, HOUGHTON DC, BENNETT WM. Cyclosporine-induced acute renal dysfunction in the rat. Transplantation 1987; 44(1): 135-141.

EUROPEAN MULTICENTRE TRIAL GROUP: Cyclosporine in cadaveric renal transplantation: one year follow-up of a multicentre trial. Lancet 1983; ii: 986-989.

EUROPEAN MULTICENTRE TRIAL GROUP. Cyclosporine in cadaveric renal transplantation: 5-year follow-up of a multicentre trial. Lancet 1987; 2: 506-507.

EVANS JK, NAISH PF, ABER GM. Oestrogen-induced changes in renal haemodynamics in the rat: influence of plasma and intrarenal renin. Clinical Science 1986; 71: 613-619.

EXTON JH. Calcium signalling in cells - molecular mechanisms. Kidney International 1987; 32 (suppl 23): S68-S76.

FARACI M, VIGEANT C, YALE JF. Pharmacokinetic profile of cyclosporine A and G and their effects on cellular immunity and glucose tolerance in male and female Wistar rats. Transplantation 1988; 45(3): 617-621.

FARBER JL, MARTIN JT, CHIEN KR. Irreversible ischaemic cell injury: prevention by chlorpromazine of the intramembranous particles of rat liver plasma membranes. American Journal of Pathology 1978; 92: 713-732.

FARNSWORTH A, HORVATH JS, HALL BM, SHEIL AGR, NG ABP, TILLER DJ, DUGGIN GG. Renal biopsy morphology in renal transplantation. American Journal of Surgical Pathology 1984; 8: 243-252.

FARTHING MJG, CLARK ML, PENDRY A, SLOEAN J AND ALEXANDER P. Nature of the toxicity of cyclosporin A in the rat. Biochem Pharmacol 1981; 30: 3311-3316

FEEHALLY J, WALLS J, MISTRY N, HORSBURGH T, TAYLOR J, VEITCH PS, BELL PRF. Does nifedipine ameliorate cyclosporin A nephrotoxicity?. British Medical Journal 1987; 295: 310.

FINCKH ES. Experimental acute tubular necrosis following subcutaneous injection of glycerol. J Pathol Bact 1957; 73: 69-85.

FINN WF, MCCORMACK AJ, SULLIVAN BA, HAK LJ, CLARK RL. Influence of rate of infusion on cyclosporine nephrotoxicity in the rat. Renal Failure 1989; 11(1): 3-15.

FIRTH JD, RATCLIFFE PJ, RAINE AEG, LEDINGHAM JGG. Endothelin: an important factor in acute renal failure? Lancet 1988; ii: 1179-1181.

FLECHNER SM, VAN BUREN C, KERMAN RH, KAHAN DB. The nephrotoxicity of cyclosporine in renal transplant recipients. Transplant Proceedings 1983a;15(suppl 1):2689-2694.

FLECHNER SM, VAN BUREN CT, KERMAN R, KAHAN BD. The effect of conversion from cyclosporine to azathioprine immunosuppression for intractable nephrotoxicity. Transplantation Proceedings 1983b; 15 (suppl 1): 2869-2873.

FLECKENSTEIN A. History of calcium antagonists. Circulation Research 1983; 52(suppl I): I3-I16.

FLEMING JT, PAREKH N, STEINHAUSEN M. Calcium antagonists preferentially dilate preglomerular vessels of the hydronephrotic kidney. American Journal of Physiology 1987 (Renal Fluid Electrolyte Physiol.22) 253: F1157-F1163.

FOEGH ML, WINCHESTER JF, ZMIJDKA M, HELFRISCH GB, RAMWELL PW, SCHREINER GE. Aspirin inhibition of thromboxane release in thrombosis and renal transplant rejection. Lancet 1982; i: 48-49.

FOEGH ML, ALIJANI MR, HELFRICH GB, KHIRABADI BS, LIM K, RAMWELL PW. Lipid mediators in organ transplantation. Transplantation Proceedings 1986; 17 (4): 20-22.

FOIDART JB, DECHENNE CA, MAHIEU P, CREUTZ CE, MEY JP. Tissue cultures of normal rat glomerular: isolation and morphological characterization of two homogenous cell lines. Invest Cell Pathol 1979; 2: 15-26.

FREEDMAN MH, KHAN NR, TREW-MARSHALL BJ, CUPPLES CG, MELY-GOUBERT B. Early biochemical events in lymphocyte activation. Cell Immunology 1981; 58: 134-146.

FRIEDMANN AL, KAHNG KU, MONACO DO, ROSEN BD, WAIT RB. Cyclosporine nephrotoxicity in conscious sheep. Transplantation Proceedings 1988; 20(3, suppl 3): 595-602.

GERKENS JF, BHAGWANDKEN SB, DOSEN PJ, SMITH AJ. The effect of salt intake on cyclosporine-induced impairment of renal function in rats. Transplantation 1984; 38(4): 412-417.

GERKENS JF. Cyclosporine treatment of normal rats produces a rise in blood pressure and decreased renal vascular responses to nerve stimulation, vasoconstrictors and endothelium-dependent dilators. The Journal of Pharmacology and Experimental Therapeutics 1989; 250(3): 1105-1112.

GILLUM DM TRUONG L, TASBY J, MIGLIORE P, SUKI WN. Chronic cyclosporine nephrotoxicity: a rodent model. Transplantation 1988; 46: 285-292.

GILLUM DM, TRUONG L, TASBY J, SUKI WN. Effects of uninephrectomy and high protein feeding in cyclosporine nephrotoxicity. Kidney International 1989; 36: 194-200.

GLUCKMAN E, ARCESE W, DEVERGIE A, BOLTON M. Cyclosporin A prophylactic treatment of graft-versus-host disease in human allogeneic bone-marrow transplantation: preliminary results. Transplantation Proceedings 1981; 13: 368-370.

GODFRAIND T, MILLER R, WIBO M. Calcium antagonism and calcium entry blockade. Pharmacological Reviews 1986; 321-416.

GOLDBERG JP, SCHRIER RW. Effect of calcium membrane blockers on in vivo vasoconstrictor properties of norepinephrine, angiotensin II and vasopressin. Mineral Electrolyte Metab 1984;10:178-183

GOLUB MS AND BERGER ME. Direct augmentation by cyclosporin A of the vascular contractile response to nerve stimulation. Hypertension 1987; 9(suppl III): III-96-III-100.

GOLUB MS, LUSTIG S, BERGER ME AND LEE DBN. Altered vascular responses in cyclosporine-treated rats. Transplantation 1989; 48: 116-118.

GOMEZ DM. Evaluation of renal resistances with special reference to changes in essential hypertension. Journal of Clinical Investigation 1951; 30: 1143-1155.

GORDON RD, STARZL TE, HAKALA TR ET AL. Long-term results of cyclosporine-steroid therapy in 131 non-matched cadaveric renal transplants. Clinical Transplantation 1987; 1: 44.

GREENBERG S, CURRO FA, TANAKA TP. Regulation of vascular smooth muscle of the circulation. In The physiology and pharmacology of the microcirculation. N Mortillaro (ed). New York, Academic Press, 1983.

GUAZZI MD, POLESE A, FIORENTINI C, BARTORELLI A AND MORUZZI P. Treatment of hypertension with calcium antagonists. Hypertension 1983; 5 (suppl 2): II85-II90.

GUYTON AC, LANGSTON JB, NAVAR G. Theory for renal autoregulation by feedback at the juxtaglomerular apparatus. Circulation Research 1964; 15 (suppl 1): I-187-I-196.

HABERLE DA, KAWATA T, DAVIS JM. The site of action of nitrendipine in the rat kidney. Journal of Cardiovascular Pharmacology 1987; 9(suppl 1): S17-S23.

HALL BM, TILLER DJ, DUGGIN GG, HORVATH JS, FARNSWORTH A, MAY J, JOHNSON JR, SHEIL AGR. Post-transplant acute renal failure in cadaver renal transplant recipients treated with cyclosporine. Kidney International 1985; 28: 178-186.

HAMILTON DV, EVANS DB, THIRU S. Toxicity of cyclosporin A in organ grafting. In Cyclosporin A, Proceedings of an international conference on Cyclosporin A. DJG White (ed). Elsevier Press, Amsterdam 1982; 393-411.

HAMMAN SR, TODD GD, MCALLISTER RG. The pharmacology of verapamil. Pharmacology 1983; 27: 1-8.

HANDSCHUMACHER RE, HARDING NW, RICE J, DRUGGE RJ, SPEICHER DW. Cyclophilin: a specific cytosolic binding protein for cyclosporin A. Science 1984; 226: 544-547.

HAY R, TAMMI K, RYFFEL B, MIHATSCH MJ. Alterations in molecular structure of renal mitochondria associated with cyclosporin A treatment. Clinical Nephrology 1986; 25 (suppl 1): S23-S26.

HAYSLETT JP. Functional adaptation to reduction in renal mass. Physiological Reviews 1979; 59: 137-164.

HEAGERTY AM, OLLERENSHAW JD. The phosphoinositide signalling system and hypertension. Journal of Hypertension 1987; 5: 515-524.

HEERING P, WESTHOFF A, BACH D, SPRENGER KBG, PASSLICK J, HELMCHEN U, GRABENSEE B. Renin-aldosterone system and renal function under cyclosporin A. Transplantation Proceedings 1988; 20(3, suppl 3): 556-562.

HELLER J. Effect of vasoactive mediators on renal haemodynamics. Nephrol Dial Transplant 1987;2:197-204.

HENRICSSON S, LINDHOLM A. Inhibition of cyclosporine metabolism by other drugs in vitro. Transplantation Proceedings 1988; 20(suppl 2): 569-571.

HERMANSSON K, LARSON M, KALLSKOG O, WOLGAST M. Influence of renal nerve activity on arteriolar resistance, ultrafiltration dynamics and fluid reabsorption. Pfluegers Arch 1981; 389(2): 85-90.

HERRAMANN B, MUELLER W. Die therapie der chronischen polyarthritis mit cyclosporin A, einen neuen immunosuppressivum. Akt Rheumatol 1979; 4: 173-186.

HESS AD, TUTSCHKA PJ, SANTOS GW. Effect of cyclosporin A on human lymphocyte responses in vitro. III. CsA inhibits the production of T lymphocyte growth factors in secondary mixed lymphocyte responses but does not inhibit the response of primed lymphocytes to TCGF. Journal of Immunology 1982; 128: 355-339.

HOMAN WP, FABRE JW, WILLIAMS KA, MILLARD PR, MORRIS PJ. Studies of the immunosuppressive properties of cyclosporin A in rats receiving renal allografts. Transplantation 1980; 29: 361-366.

HOYER PF, KROHN HP, OFFNER G, BYRD DJ, BRODEHL J, WONIGEIT K, PICHLMAYR R. Renal function after kidney transplantation in children. Transplantation 1987; 43: 489-493.

HUELSEMANN JL, STERZAL RB, MCKENZIE DE, WILCOX CS. Effects of a calcium entry blocker on renal function during angiotensin-induced hypertension in the rat. Hypertension 1985; 7: 374-379.

HUMES HD, JACKSON NM, O'CONNOR RP, HUNT DA, WHITE MD. Pathogenetic mechanisms of nephrotoxicity: Insights into cyclosporine nephrotoxicity. Transplantation Proceedings 1985; 17(4): 51-62.

HUMES HD. Role of calcium in pathogenesis of acute renal failure. American Journal of Physiology 1986; 250: F579-F589.

HUMES HD, COFFMAN TM, HALDERMAN H, MIHATSCH MJ, HENRY M, PORTER GA. Cyclosporine nephrotoxicity: a workshop to discuss mechanisms, diagnosis and treatment. Transplantation Proceeding 1988; 20(3, suppl 3): 833-840.

ICHIKAWA I, MEILE F, BRENNER B. Reversal of renal cortical actions of angiotensin II by verapamil and manganese. Kidney International 1979; 16: 137-147.

IRIUCHIJIMA J. Effect of calcium antagonist, nifedipine, on blood pressure of various hypertensive rats. Hiroshima Journal of Medical Sciences 1980; 29(1): 15-19.

JACKSON NM, HSU CH, VISSCHER GE, VENKATACHALAM MA AND HUMES HD. Alterations in renal structure and function in a rat model of cyclosporine nephrotoxicity. The Journal of Pharmacology and Experimental Therapeutics 1987; 242: 749-756.

JAO S, WALTZER W, ARBEIT LA. Acute cyclosporin decrease in GFR is mediated by changes in renal blood flow and renal vascular resistance. Kidney International 1986; 29: 431A.

JOUVIN-MARCHE E, CERRINA J, COEFFIER E, DUROUX P, BENVENISTE J. Effect of the calcium antagonist nifedipine on the release of platelet-activating factor (PAF-acetether), slow-reacting substance and beta-glucuronidase from neutrophils. European Journal of Pharmacology 1983; 89: 19-26.

KAHAN BD, KERMAN RH, WIDEMAN CA ET AL. The impact of cyclosporin on renal transplant practice at the University of Texas Medical School at Houston. American Journal of Kidney Diseases 1985; 5(6): 288-295.

KAHAN BD. Cyclosporine nephrotoxicity: pathogenesis, prophylaxis, therapy and prognosis. American Journal of Kidney Diseases 1986; 8(5): 323-331.

KAHAN BD. Cyclosporine. New England Journal of Medicine 1989; 321: 1725-1738.

KASKEL FJ, DEVARAJAN P, ARBEIT LA, PARTIN JS, MOORE LC. Cyclosporine nephrotoxicity: sodium excretion, autoregulation, and angiotensin II. American Journal of Physiology 1987a; 252 (Renal Fluid Electrolyte Physiol. 21): F733-F742.

KASKEL FJ, MOORE LC, DEVARAJAN P, ARBEIT LA. Cyclosporin A increases afferent and efferent resistance in rats. Proceedings of the 10th International Congress of Nephrology 1987b; 10: 467.

KASKEL F, DEVARAJAN P, ARBEIT LA, MOORE LC. Effects of cyclosporine on renal haemodynamics and autoregulation in rats. Transplantation Proceedings 1988; 20(suppl 3): 603-609.

KAWAGUCHI A, GOLDMAN MH, SHAPIRO R, FOEGH ML, RAMWELL PW, LOWER RR. Increase in urinary thromboxane B2 in rats caused by cyclosporine. Transplantation 1985; 40: 214-216.

KAY JE. Interaction of lymphocytes and phytohemagglutinin inhibition by chelating agents. Experimental cell Research 1971; 68: 11-16.

KIBERD BA. Cyclosporine-induced renal dysfunction in human renal allograft recipients. Transplantation 1989; 48(6): 965-969.

KING AJ, MARSDEN PA, BRENNER BM. Endothelin: a potent vasoactive peptide of endothelial origin. In: Hypertension: Pathophysiology, diagnosis and management. Edited by Laragh JH and Brenner BM. Raven Press Limited, New York. p649-660.

KLASSEN DK, SOLEZ K, BURDICK JF. Effects of cyclosporine on human renal allograft renin and prostaglandin production. Transplantation 1989; 47: 1072-1075.

KLINE RL, MERCER PF. Functional re-innervation and development of supersensitivity to norepinephrine after renal denervation in rats. (Regulatory Integrative Comp. 17). American Journal of Physiology 1980; 238: R353-R358.

KLINTMALM G, SUNDELIN B, BOHMAN SO, WILCZEK H. Interstitial fibrosis in renal allografts after 12 to 46 months of cyclosporin treatment: beneficial effect of low doses in early post-transplantation period. Lancet 1984; ii: 950-954.

KON V, ICHIKAWA I. Effector loci for renal nerve control of cortical microcirculation. American Journal of Physiology 1983 (Renal Fluid Electrolyte Physiol. 14); 245: F545-553.

KON V. Neural control of renal circulation. Mineral Electrolyte Metabolism 1989; 15: 33-43.

KON V, SUGIURA M, INAGAMI T, HOOVER RL, FOGO A, HARVIE BR, ICHIKAWA I. Cyclosporine causes endothelin-dependent acute renal failure. Kidney International 1990; 33: 298A.

KONE BC, RACUSEN LC, WHELTON A, SOLEZ K. Acute renal failure produced by combining cyclosporine and brief renal ischaemia in the Munich Wistar. Clinical Nephrology 1986; 25(suppl 1) S171-S174.

KOSTAKIS AJ, WHITE DJG, CALNE RY. Toxic effect in the use of cyclosporin A in alcoholic solution as an immunosuppressant of rat heart allografts. IRCS Medical Science 1977a; 5: 243.

KOSTAKIS AJ, WHITE DJG, CALNE RY. Prolongation of rat heart allograft survival by cyclosporin A. IRCS Medical Science 1977b; 5: 280.

KREISBERG JI, VENKATACHALAM M, TROYER D. Contractile properties of glomerular mesangial cells. American Journal of Physiology 1985;249 (Renal Fluid Electrolyte Physiol.18): F457-F463.

KRONBACH T, FISCHER V, MEYER UA. Cyclosporine metabolism in human liver: Identification of a cytochrome P-450III gene family as the major cyclosporine-metabolizing enzyme explains interactions of cyclosporine with other drugs. Clin Pharmacol Ther 1988; 43: 630-635.

KURTZ A, DELLA BRUNA R, KUHN K. Cyclosporine A enhances renin secretion and production in isolated juxtaglomerular cells. Kidney International 1988; 947-953.

LAMB FS, WEBB C. Cyclosporine augments reactivity of isolated blood vessels. Life Sciences 1987; 40: 2571-2578.

LEMAIRE M, MAURER G, WOOD AJ. Pharmacokinetics and metabolism. Progress Allergy 1986; 38: 93-107.

LEUINISSEN KML, TEULE J, DEGENAAR CP, KHO TL, FRENKEN LAM. Impairment of liver synthetic function and decreased liver flow during cyclosporin A therapy. Transplantation Proceedings 1987; 19: 1822-1824.

LEWIS RM, JANNEY RP, GOLDEN DL, KERR NB, VAN BURREN CT, KERMAN RH, KAHAN BD. Stability of renal allograft function associated with long-term cyclosporine immunosuppressive therapy-five year follow-up. Transplantation 1989; 47(2): 266-272.

LIEDTKE RR, DUARTE CG. Laboratory protocols and methods for the measurement of glomerular filtration rate and renal plasma flow. In Renal function tests. Clinical laboratory procedures and diagnosis. Edited by CG Duarte. Published 1980. Little, Brown and Company. Boston. pp 49-63.

LINDHOLM A, HENRICSSON S. Verapamil inhibits cyclosporin metabolism. Lancet 1987;i:1262-1263.

LINDHOLM A, HENRICSSON S, GANG P. The free fraction of cyclosporin in the plasma; clinical findings with a new method. Transplantation Proceedings 1988; 20 (suppl 2): 377-381.

LINDSEY JA, MORISAKI N, STITTS JM, ZAGER RA, CORNWELL DG. Fatty acid metabolism and cell proliferation. IV. Effect of prostanoid biosynthesis from endogenous fatty acid release with cyclosporin A. Lipids 1983; 18: 566-569.

LOGAN JL, BENSEN B. Retardation of renal growth and orthinine decarboxylase activity by cyclosporine after uninephrectomy in rats. Transplantation 1987; 44: 559-562.

LOPEZ-NOVOA JM, RAMOS B, MARTIN-OAR JE, HERNANDO L. Functional compensatory changes after unilateral nephrectomy in rats. Renal Physiology 1982; 5: 76-84.

LORENZ W, SCHMAL A, SCHULT H, LANG S, OHMAN C, WEBER D, KAPP B, LUBEN L, DOENICKE A. Histamine release and hypotensive reactions in dogs by

solubilizing agents and fatty acids: analysis of various components in Cremophor EL and development of a compound with reduced toxicity. Agents Actions 1982; 12: 64-80.

LOSSE H, ZIDEK W, VETTER H. Intracellular sodium and calcium in vascular smooth muscle of spontaneously hypertensive rats. Journal of Cardiovascular Pharmacology 1984; 6: S32-S34.

LOTE CJ, MCVICAR AJ, YARDLEY CP. Renal extraction and clearance of p-aminohippurate during saline and dextrose infusion in the rat. Journal of Physiology 1985; 363: 303-313.

LOUTZENHISER R, VAN BREEMEN C. The influence of receptor occupation on calcium influx mediated vascular smooth muscle contraction. Circulation Research 1983; 52(suppl I): 97-103.

LOUTZENHISER R, EPSTEIN M. Effects of calcium antagonists on renal haemodynamics. American Journal of Physiology 1985a; 249: F619-F629.

LOUTZENHISER R, HORTON C, EPSTEIN M. Effects of diltiazem and manganese on renal haemodynamics: studies in the isolated perfused rat kidney. Nephron 1985b; 39: 382-388.

LOUTZENHISER R, EPSTEIN M. Activation mechanisms of human renal artery: Effects of KCL, norepinephrine and nitrendipine upon tension development and ^{45}Ca influx. European Journal of Pharmacology 1985c; 106: 47-52.

LOUTZENHISER R, EPSTEIN M. Modification of the renal haemodynamic response to vasoconstrictors by calcium antagonists. American Journal of Nephrology 1987; 7(suppl 1):7-16.

LOUTZENHISER R, EPSTEIN M. Calcium antagonists and the renal hemodynamic response to vasoconstrictors. Annals of the New York Academy of Sciences 1988; 522; 771-784.

LOWRY OH, ROSEBROUGH NJ, FARR AL, RANDALL RJ. Protein measurement with the folin-phenol reagent. J Biol Chem 1951; 193: 265-275.

LUNDEN S, RICKSTEN SE, THOREN P. Renal sympathetic activity in spontaneously hypertensive rats with normotensive controls, as studied by 3 different methods. Acta Physiol Scand 1984; 120: 265-272.

LUSTIG S, STERN N, EGGENA P, TUCK ML, LEE DBN. Effect of cyclosporin on blood pressure and renin-aldosterone axis in rats. American Journal of Physiology 1987; 253 (Heart Circulation Physiol. 22): H1596-H1600.

MAENPAA J, RUSKOAHO H, PELKONEN O. Inhibition of hepatic microsomal drug metabolism in rats by five calcium antagonists. Pharmacology and Toxicology 1989; 64: 446-450.

MAINO VC, GREEN NM, CRUMPTON MJ. The role of calcium ions in
initiating transformation of lymphocytes. Nature 1974; 251: 324-327.

MAKOWKA L, LOPATIN W, GILAS, FALK J, PHILLIPS MJ, FALK R. Prevention
of cyclosporine nephrotoxicity by synthetic prostaglandins. Clinical
Nephrology 1986; 25(suppl 1); S89-S94.

MANDREOLI M, ESPOSTI ED, COCCHI R, FABBRI A, GUERRINI L, FUSAROLI M.
Do calcium channel blockers have any influence on the immunological
status of renal graft recipients on ciclosporin therapy? American
Journal of Nephrology 1990; 10: 58-60.

MASON J. Pathophysiology and toxicology of cyclosporine in humans and
animals. Pharmacological Reviews 1989; 41(3): 423-431.

MATYUS L, BALAZS M, ASZALOS A, MULHERN S, DAMJANOVICH S. Cyclosporin A
depolarizes cytoplasmic membrane potential and interacts with Ca^{2+}
ionophores. Biochimica et Biophysica Acta 1986; 886: 353-360.

MAURER G. Metabolism of cyclosporine. Transplantation Proceedings
1985; 17(suppl 1): 19-26.

MCGIFFEN DC, KIRKLIN JK, NAFTEL DC. Acute renal failure after heart
transplantation and cyclosporine therapy. Heart Transplant 1985; 4:
396.

MCMILLEN MA, LEWIS T, JAFFE BM, WAIT RB. Verapamil inhibition of
lymphocyte proliferation and function in vitro. Journal of Surgical
Research 1985; 39: 76-80.

MEHTA JL. Influence of calcium channel blockers on platelet function
and arachidonic acid metabolism. American Journal of Cardiology 1985;
56: 158B-164B.

MEISHERI KD, HWANG O, VAN BREEMEN C. Evidence for two separate calcium
pathways in smooth muscle plasmalemma. Journal of Membrane Biology
1981; 59: 19-25.

MERION RM, WHITE DJG, THIRU S, EVANS DB, CALNE RY. Cyclosporine: five
years' experience in cadaveric renal transplantation. New England
Journal of Medicine 1984; 310: 148-154.

MESSANA JM, ROCHER LL, ELLIS C, FRADIN M, VOORHEIS J. Acute effects of
oral cyclosporine (CSA) on glomerular filtration rate in patients with
normal renal function. Kidney International 1990; p301A.

MEYER-LEHNERT H, SCHRIER RW. Cyclosporine A enhances vasopressin-
induced Ca^{2+} mobilisation and contraction in mesangial cells. Kidney
International 1988; 34: 89-97.

MEYER-LEHNERT H, SCHRIER RW. Potential mechanism of cyclosporine A-
induced vascular smooth muscle contraction. Hypertension 1989; 13:

352-360.

MIHATSCH MJ, THIEL G, SPICHTIN HP, OBERHOLZER M, BRUNNER FP, HARDER F, OLIVIERI V, BRENNER R, RYFFEL B, STOCKLER E, TORHORST J, GUDAT F, ZOLLINGER HU, LOERTSCHER R. Findings in kidney transplant after treatment with cyclosporine. Transplantation proceedings 1983; 15: 2821-2035.

MIHATSCH MJ, THIEL G, BOSLER V, RYFFEL B, LANDMANN J, VON OVERBECK J, ZOLLINGER HU. Morphological patterns in cyclosporine-treated renal transplant recipients. Transplantation Proceedings 1985; 17: 101-116.

MIHATSCH MJ, THIEL G, RYFFEL B. Morphologic diagnosis of cyclosporine nephrotoxicity. Seminars in Diagnostic Pathology 1988; 5(1): 104-121.

MILFORD EL, KIRKMAN RL, TILNEY NL, STROM TB, CARPENTER CB. Clinical experience with cyclosporin and azathioprine at Brigham and Womens Hospital. American Journal of Kidney Diseases 1986; 5: 313.

MOBB G. Cyclosporin A and renal function in renal transplant recipients. MD Thesis. 1990.

MOOCHHALA SM, RENTON KW. Inhibition of hepatic microsomal drug metabolism by the immunosuppressive agent cyclosporin A. Biochemical Pharmacology 1986; 35(9): 1499-1503.

MORALES JM, ANDRES AS, PRIETO C, DIAZ DE TUESTA I, ALCAZAR JM, RUILOPE LM, OLIET A, PRAGA M, RODICIO JL. Usefulness of fractional excretion of sodium as index of cyclosporine nephrotoxicity in renal transplantation. Transplantation Proceedings 1988; 20(suppl 3): 691-699.

MORRIS PJ, FRENCH ME, DUNNILL MS, HUNNISETT AGW, TING A, THOMPSON JF, WOOD RFM. A controlled trial of cyclosporine in renal transplantation with conversion to azathioprine and prednisolone after three months. Transplantation 1983; 36(3): 273-277.

MORRIS PJ. Cyclosporine. In Morris PJ (ed) Kidney Transplantation. Principles and practice 3rd Edition. Philadelphia, Harcourt Brace Jovanovich Inc. 1988; 285-317.

MOSS NG, POWELL SL, FALK RJ. Intravenous cyclosporine activates afferent and efferent renal nerves and causes sodium retention in innervated kidneys in rats. Proc. Natl. Acad. Sci. USA 1985; 82: 8222-8226.

MULVANY MJ, HALPERN W. Contractile properties of small arterial resistance vessels in spontaneously hypertensive and normotensive rats. Circulation Research 1977; 41: 19-26.

MURRAY BM, PALLER MS, FERRIS TF. Effect of cyclosporine administration on renal hemodynamics in conscious rats. Kidney International 1985;

28: 767-774.

MURRAY BM, PALLER MS. Beneficial effect of renal denervation and prazosin on GFR and renal blood flow after cyclosporine in rats. Clinical Nephrology 1986; 25(suppl 1): S37-S39.

MYERS BD, ROSS J, NEWTON L, LUETSCHER J, PERLROTH M. Cyclosporine-associated chronic nephropathy. New England Journal of Medicine 1984; 311: 699-705.

MYERS BD. Cyclosporine nephrotoxicity. Kidney International 1986; 30: 964-974.

MYERS BD, NEWTON L, BOSHKOS C, MACOVIAK JA, FRIST WH, DERBY GC, PERLROTH MG AND SIBLEY R. Chronic injury of human renal microvessels with low-dose cyclosporine therapy. Transplantation 1988a; 46: 694-703.

MYERS BD, SIBLEY R, NEWTON L, TOMLANOVICH SJ, BOSHKOS C, STINSON E, LEUTSCHER JA, WHITNEY DJ, KRASNY D, COPLON NS, PERLROTH MG. The long-term course of cyclosporine-associated chronic nephropathy. Kidney International 1988b; 33: 590-600.

NAGINENI CN, MISRA BC, LEE DBN, YANAGAWA N. Cyclosporine A - Calcium channels interaction: a possible mechanism for nephrotoxicity. Transplantation Proceedings 1987; 19(1): 1358-1362.

NAGINENI CN, LEE DBN, MISRA BC, N YANAGAWA. Cyclosporine-A Transport in isolated renal proximal tubular cells: inhibition by calcium channel blockers. Biochemical and Biophysical Research Communications 1988; 157(3): 1226-1230.

NAHMAN NS, COSIO FG, MAHAN JD, HENRY ML, FERGUSON RM. Cyclosporine nephrotoxicity in spontaneously hypertensive rats. Transplantation 1988; 45(4): 768-772.

NAVAR LG. Minimal preglomerular resistance and calculation of normal glomerular pressure. American Journal of Physiology 1970; 219: 1658-1664.

NAVAR LG, CHAMPION WJ, THOMAS CE. Effects of calcium channel blockade on renal vascular resistance responses to changes in perfusion pressure and angiotensin-converting enzyme inhibition in the dogs. Circulation Research 1986; 58: 874-881.

NAYLER WG, GU XH, CASLEY DJ, PANAGIOTOPOULOS S, LIU J, MOTTRAM PL. Cyclosporine increases endothelin-1 binding site density in cardiac cell membranes. Biochemical and Biophysical Research Communications 1989; 163(3): 1270-1274.

NEILD GH, IVORY K, WILLIAMS DG. Glomerular thrombi and infraction in rabbits with serum sickness following cyclosporine therapy.

Transplantation Proceedings 1983a; 15 (suppl 1): 2782-2786.

NEILD GH, IVORY K, H, WILLIAMS DG. Cortical infarction in rabbits with serum sickness following cyclosporin A therapy. Kidney International 1983b; 24: 416A.

NEILD GH, ROCCHI G, IMBERTI L, FUMAGALLI F, BROWN Z, REMUZZI G, WILLIAMS DG. Effect of cyclosporin A on prostacyclin synthesis by vascular tissue. Thrombosis Research 1983c; 32: 373-379.

NEILD GH, IVORY K, WILLIAMS DG. Glomerular thrombosis and cortical infarction in cyclosporin-treated rabbits with acute serum sickness. British Journal of Experimental Pathology 1984a; 65: 133-144.

NEILD GH, IVORY K, WILLIAMS DG. Severe systemic vascular necrosis in cyclosporin-treated rabbits with acute serum sickness. British Journal of Experimental Pathology 1984b; 65: 731-743.

NEILD GH, REUBEN R, HARTLEY RB, CAMERON JS. Glomerular thrombi in renal allografts associated with cyclosporin treatment. Journal of Clinical pathology 1985; 38: 253-258.

NICCHITTA CV, KAMOUN M, WILLIAMSON JR. Cyclosporine augments receptor-mediated cellular Ca^{2+} fluxes in isolated hepatocytes. J Biol Chem 1985; 260: 13613-13618.

NIEDERBERGER W, LEMAIRE M, MAURER G, NUSSBAUMER K, WAGNER O. Distribution and binding of cyclosporine in blood and tissues. Transplantation Proceedings 1983; 15: 2419-2421.

NORDLANDER M, DIBONA GF, LIUNGB, YAO T, THOREN P. Renal and cardiovascular effects of acute and chronic administration of felodipine to SHR. European Journal of Pharmacology 1985: 113: 25-36.

NUSSENBLATT RB, AUSTIN HA, PALESTINE AG, PREUSS HG. Hydergine and cyclosporine nephrotoxicity. Lancet 1986; i: 1220-1221.

O'LEARY TJ, ROSS PD, LIEBER MR, LEVIN IW. Effect of cyclosporine A on biomembranes. Vibrational, spectroscopic, calorimetric, and hemolysis studies. Biophys J 1986; 49: 795-801.

ONO H, KOKUBUN H, HASHIMOTO K. Abolition by calcium antagonists of the autoregulation of renal blood flow. Naunyn Schmiedebergs Arch Pharmacol 1974; 285: 201-207.

OPELZ G. Multicenter impact of ciclosporin on cadaver kidney graft survival. Progress Allergy 1986; 38: 329-345.

OYER PE, STINSON EB, JAMIESON SW ET AL. Cyclosporine in cardiac transplantation: a two and a half year follow-up. Transplantation Proceedings 1983; 15(suppl 1): 2546.

PALESTINE AG, AUSTIN HA, BALOW JE, ANTONOVYCH TT, SABNIS SG, PREUSS HG, NUSSENBLATT RB. Renal histopathologic alterations in patients treated with cyclosporine for uveitis. New England Journal of Medicine 1986a; 314: 1293-1298.

PALESTINE AG, AUSTIN HA, NUSSENBLATT RB. Renal tubular function in cyclosporine-treated patients. The American Journal of Medicine 1986b; 81: 419-424.

PELL MA, ROSSANO TG, BRAYMAN KL, FREED BM, SHAW LM, LEMPERT N. Predominance of native cyclosporine over metabolites in rat blood and tissue. Transplantation Proceedings 1988; 20(2, suppl2): 674-679.

PERICO N, BENIGNI A, BOSCO E, ROSSINI M, ORISIO S, GHILARDI F, PICCINELLI A, REMUZZI G. Acute cyclosporine A nephrotoxicity in rats: which role for renin-angiotensin system and glomerular prostaglandins? Clinical Nephrology 1986a; 25(suppl 1): S83-S88.

PERICO N, ZOJA C, BENIGNI A, GHILARDI F, GUALANDRIS L, REMUZZI G. Effect of short-term cyclosporine administration in rats on renin-angiotensin and thromboxane A2: possible relevance to the reduction in glomerular filtration rate. The Journal of Pharmacology and Experimental Therapeutics 1986b; 239: 229-235.

PERICO N, BENIGNI A, ZOJA C, DELAINI F, REMUZZI G. Functional significance of the exaggerated renal thromboxane A2 synthesis induced by cyclosporin A. American Journal of Physiology 1986c; 251: F581-F587.

PETCHER TJ, WEBER HP AND RUEGGER A. Crystal and molecular structure of the cyclic undecapeptide cyclosporin A. Helv Chim Acta 1976; 59(5): 1480-1489.

PETRIC R, FREEMAN D, WALLACE C, MCDONALD J, STLLER C, KEOWN P. Effect of cyclosporine on urinary prostanoid excretion, renal blood flow and glomerulotubular function. Transplantation 1988; 45: 883-889.

PFEILSCHIFTER J, RUEGG UT. Cyclosporin A augments angiotensin II-stimulated rise in intracellular free calcium in vascular smooth muscle cells. Biochem J 1987;248:883-887.

PFEILSCHIFTER J. Cyclosporin A augments vasoconstrictor-induced rise in intracellular free calcium in rat renal mesangial cells. Biochemical Pharmacology 1988; 37: 4205-4210

POCHET JM, PIRSON Y. Cyclosporin-diltiazem interaction. Lancet 1986; i: 979.

POST GR, MOYER TP, ANDERSON CF, STERIOFF S. Long-term cyclosporine for renal allograft patients does not cause nephrotoxicity. Transplantation Proceedings 1987; 19(1): 1761-1763.

POWELL-JACKSON PR, YOUNG B, CALNE RY, WILLIAMS R. Nephrotoxicity of parenterally administered cyclosporine after orthotopic liver transplantation. Transplantation 1983; 36: 505-509.

POWLES RL, BARRETT AJ, CLINK H, KAY HE, SLOANE J, MCELWAIN TJ. Cyclosporin A for the treatment of graft-versus-host disease in man. Lancet 1978; ii: 1327-1331.

POWLES, CLINK H, SPENCE D, MORGENSTERN G, WATSON JG, SELBY PT, WOODS M, JAMESON B, SLOANE J, LAWLER SD, KAY HEM, LAWSON D, MCELWAIN TJ, ALEXANDER P. Cyclosporin A to prevent graft versus host disease in man after allogeneic bone marrow transplantation. Lancet 1980; i: 327-329.

PREMEN AJ, HALL JE, SMITH MJ. Postprandial regulation of renal haemodynamics: role of pancreatic glucagon. American Journal of Physiology 1985; 248: F656.

PRENTKI M, CORKEY BE, MATSCHINSKY FM. Inositol 1,4,5-trisphosphate and the endoplasmic reticulum calcium cycle of a rat insulinoma cell line. Journal of Biological Chem 1985; 260: 9185-9190.

PROPPER DJ, WHITING PH, POWER DA, EDWARD N AND CATTO GRD. The effect of nifedipine on graft function in renal allograft recipients treated with cyclosporin A. Clinical Nephrology 1989; 32: 62-67.

PROVOOST AP. Cyclosporine nephrotoxicity in rats with an acute reduction of renal function. American Journal of Kidney Diseases 1986; 8(5): 314-318.

PTAZSCHKE K, DUHM B, MAUL W, MEDENWALD H, WEGNER LA. Second International Adalat Symposium. Editors: Lochner W, Braasch W, Kroneberg G. Springer-Verlag, Berlin, Heidelberg, New York. 1975; p27-32.

RACUSEN LC, KONE BC, SOLEZ K. Early renal pathophysiology in an acute model of cyclosporine nephrotoxicity in rats. Renal failure 1987; 10(1): 29-37.

REGO A, VARGAS R, FOEGH ML, RAMWELL PW. Effect of cyclosporine A treatment on vascular reactivity of the rat thoracic aorta. Transplantation Proceedings 1988; 20 (3): 572-577.

RICHARDS NT, POSTON L, HILTON PJ. Cyclosporine A inhibits relaxation but doe not induce vasoconstriction in human subcutaneous resistance vessels. Journal of Hypertension 1989;7:1-3.
RICHARDS NT, POSTON L, HILTON PJ. Cyclosporin A inhibits endothelium-dependent, prostanoid-induced relaxation in human subcutaneous resistance vessels. Journal of Hypertension 1990; 8: 159-163.

RICHARDS CJ, DI BONA GF. Acute renal failure: structural-functional correlation. Proc Soc Exp Biol Med 1974; 146: 880-884.

ROBERTSON CR, DEEN WM, TRAY JL, BRENNER BM. Dynamics of glomerular ultrafiltration in the rat. III. Hemodynamics and autoregulation. American Journal of Physiology 1972; 223: 1191-1200.

RODRIGUEZ-PUYOL D, LAMAS S, OLIVERA A, LOPEZ-FARRE P, ORTEGA G, HERNANDO L, LOPEZ-NOVOA J. Actions of cyclosporin A on cultured rat mesangial cells. Kidney International 1989; 35: 632-637.

ROGERS TS, ELZINGA L, BENNETT WM, KELLEY VE. Selective enhancement of thromboxane in macrophages and kidneys in cyclosporine-induced nephrotoxicity. Transplantation 1988; 45: 153-156.

ROOTH P, DAWIDSON I, CLOTHIER N, DILLER K. In vivo fluorescence microscopy of kidney subcapsular blood flow in mice. Transplantation 1988a; 46: 566-569

ROOTH P, DAWIDSON I, DILLER K, TALJEDAL I-B. Protection against cyclosporine-induced impairment of renal microcirculation by verapamil in mice. Transplantation 1988b:45:433-436.

ROSSINI M, BELLONI A, REMUZZI G, PERICO N. Thromboxane receptor blockade attenuates the toxic effect of cyclosporine in experimental renal transplantation. Circulation 1990; 81(suppl I): I-61-I-67.

ROUTHIER G, EPSTEIN O, JANOSSYM G, THOMAS HC, SHERLOCK S. Effects of cyclosporin A on suppressor and inducer T lymphocytes in primary biliary cirrhosis. Lancet 1980; ii: 1223-1226.

RUEGGER A, KUHN M, LICHTI H, LOOSLI HR, HUGUENIN R, QUIQUEREZ CH, VON WARTBURG A. Cyclosporin A, ein immunosuppressiv wirksamerpeptide metabolit aus trichoderma polysporum (Link ex. Pers.) Rifai. Helv Chim Acta 1976; 59(4): 1075-92.

RYFFEL B, DONATSCH P, MADORIN M, MATTER BE, RUTTIMANN G, SCHON H, STOLL R, WILSON J. Toxicological evaluation of cyclosporin A. Arch. Toxicol 1983; 53: 107-141.

RYFFEL B, SIEGL H, MULLER S, DONATSCH P, WILSON JT, MIHATSCH MJ. The rat as an experimental model of cyclosporine nephrotoxicity. Human Toxicol 1984; 3: 417.

RYFFEL B, SIEGL H, PETRIC R, MULLER AM, HAUSER R, MIHATSCH MJ. Nephrotoxicity of cyclosporine in spontaneously hypertensive rats; effects on blood pressure and vascular lesions. Clinical Nephrology 1986a; 25 (suppl 1): S193-S198.

RYFFEL B, DONATSCH P, HIESTAND P, MIHATSCH MJ. PGE_2 reduces nephrotoxicity and immunosuppression of cyclosporine in rats. Clinical Nephrology 1986b; 25(suppl 1): S95-S99

RYFFEL B. Toxicology-experimental studies. Progress in Allergy 1986c; 38: 181-197.

213

RYFFEL B. FOXWELL BM, MIHATSCH MJ, DONATSCH P, MAURER G. Biologic significance of cyclosporine metabolites. Transplantation Proceedings 1988; 20 (suppl 2): 575-584.

SANGALLI L, BORTOLOTTI A, JIRITANO L, BONATI M. Cyclosporine pharmacokinetics in rats and interspecies comparison in dogs, rabbits, rats, and humans. Drug metabolism and disposition 1988; 16(5): 749-753.

SCHACHTER M. Cyclosporine A and hypertension. J Hypertension 1988; 6: 511-516.

SCHANNE FX, KANE AB, YOUNG EE, FARBER JL. Calcium dependence of toxic cell death: a final common pathway. Science 1979; 206: 700-702.

SCHEFFE H. A method for judging all contrasts in the analysis of variance. Biometrika 1953; 40: 87-104.

SCHMID BJ, PERRY HE, IDLE JR. Determination of nifedipine and its three principal metabolites in plasma and urine by automated electron-capture capillary gas chromatography. Journal of Chromatography 1988; 425(1): 107-19.

SCHNERMANN J, PLOTH DW, HERMLE M. Activation of tubuloglomerular feedback by chloride transport. Pfluegers Arch 1976; 362: 229-240.

SCHOR N, ICHIKAWA I, BRENNER BM. Mechanism of action of various hormones and vasoactive substances on glomerular ultrafiltration in the rat. Kidney International 1981; 20: 442-451.

SCHRIER RW, ARNOLD PE, VAN PUTTEN VJ, BURKE TJ. Cellular calcium in ischemic acute renal failure: role of calcium entry blockers. Kidney International 1987; 32: 313-321.

SCHUREK HJ, NEUMANN KH, JESINGHAUS WP, AEIKENS B, WONIGEIT K. Influence of cyclosporine A on adaptive hypertrophy after unilateral nephrectomy in the rat. Clinical Nephrology 1986; 25 (suppl 1); S144-S147.

SCHWARTZ A, MCKENNA E, VAGHY PL. Receptors for calcium antagonists. The American Journal of Cardiology 1988; 62: 3G-6G.

SCHWASS DE, SASAKI AW, HOUGHTON DC, BRENNER KE, BENNETT WM. Effect of phenobarbitol and cimetidine on experimental cyclosporine nephrotoxicity; preliminary observations. Clinical Nephrology 1986: 25 (suppl 1) S117-S120.

SCOBLE JE, SENIOR JCM, CHAN P, VARGHESE Z, SWENY P, MOORHEAD JF. In vitro cyclosporine toxicity. The effect of verapamil. Transplantation 1989; 47: 647-650.

SCOLARI F, SANDRINI S, SAVOLDI S, CRISTINELLI L, BRUNORI G, PRATI E, SACCHI G, TARDANICO R, CANCARINI GC, MAIORCA R. Is cyclosporine-induced chronic nephrotoxicity inevitable? Transplantation Proceedings 1987;19(1) :1745-1748.

SEN S, SMEBY RR AND BUMPUS FM. Renin in rats with spontaneous hypertension. Circulation research 1972; 31: 876.

SHIEL AGR, HALL BM, TILLER DJ, STEPHEN MS, HARRIS JP, DUGGIN GG, HORVATH JS, JOHNSON JR, ROBERTS JR, BOULAS J. In Cyclosporine: Biological activity and clinical applications. Kahan BD (ed). Grune and Stratton, Orlando, Florida. 1984; p387.

SHULMAN H, STRIKER G, DEEG HJ, KENNEDY M, STORB R, THOMAS ED. Nephrotoxicity of cyclosporin A after allogeneic marrow transplantation: glomerular thromboses and tubular injury. New England Journal of Medicine 1981; 305: 1392-1395.

SIEGL H, RYFFEL B, PETRIC R, SHOEMAKER P, MULLER A, DONATSCH P, MIHATSCH M. Cyclosporine, the renin-angiotensin-aldosterone system, and renal adverse reactions. Transplantation Proceedings 1983; 15(4): 2719-2725.

SLAVIN J, TAYLOR J. Cyclosporin, nifedipine, and gingival hyperplasia. Lancet 1985; ii: 739.

SMEESTERS C, CHALAND P, GIROUX L, MOUTQUIN JM, ETIENNE P, DOUGLAS F, CORMAN J, ST LOUIS G, DALOZE P. Prevention of acute cyclosporine nephrotoxicity by a thromboxane synthesis inhibitor. Transplantation Proceedings 1988; 20 (suppl 3): 658-660.

SOLEZ K, RACUSEN LC, VAUGHAN WK. Effect of nifedipine, propranolol and other antihypertensive drugs on renal function of transplant recipients treated with cyclosporine. Kidney International 1988; 33: 367A.

SOMMER BG, INNES JT, WHITEHURST RM, SHARMA HM, FERGUSON RM. Cyclosporine-associated renal arteriopathy resulting in loss of allograft function. American Journal of Surgery 1985; 149: 756-764.

SORKIN EM, CLISSOLD SP, BROGDEN RN. Nifedipine: A review of its pharmacodynamic and pharmacokinetic properties, and therapeutic efficacy, in ischaemic heart disease, hypertension and related cardiovascular disorders. Drugs 1985; 30: 182-274.

SPERELAKIS N. Electrophysiology of vascular smooth muscle. In N Sperelakis (ed), Physiology and pathophysiology of the heart. Boston, The Hague: Martimus Nijjboff, 1984.

STAHL RAK, KUDELKA S. Chronic cyclosporine A treatment reduces prostaglandin E_2 formation in isolated glomeruli and papilla of rat kidneys. Clinical Nephrology 1986; 25 (suppl 1): S78-S82.

STANEK J, KOVARIK J, RASOUL-ROCKENSCHAUB S, SILBERBAUER K. Renin-angiotensin-aldosterone system and vasopressin in cyclosporine-treated renal allograft recipients. Clinical Nephrology 1987; 28: 186-189.

STARLZ TE, WEIL R, IWATSUKI S, KLINTMALM G, SCHROTER GPJ, KOEP LJ, IWAKI T, TERASAKI PI, PORTER KA. The use of cyclosporin A and prednisolone in cadaver kidney transplantation. Surg Gynae Obstet 1980; 151: 17-26.

STEELE T, CHALLONER-HUE L. Renal interactions between norepinephrine and calcium antagonists. Kidney International 1984; 26: 719-724.

STEIGERWALT SP, MCCURDY R, SMITH S, LOCKETTE W. Role of sympathetic nervous system in cyclosporine hypertension following orthotopic cardiac transplant in man. Kidney International 187; 31: 309A.

STREB H, IRVINE RF, BERRIDGE MJ, SCHULZ I. Release of calcium from a non-mitochondrial store in pancreatic acinar cells by inositol 1,4,5-trisphosphate. Nature 1983; 306: 67-69.

SULLIVAN BA, HAK LJ AND FINN WF. Cyclosporine nephrotoxicity: studies in laboratory animals. Transplantation Proceedings 1985; 17(4): 145-154.

SUMPIO BE. Cyclosporine toxicity in the isolated perfused rat kidney. Transplantation Proceedings 1988; 20(3, suppl 3): 712-716.

SVENSON K, BOHMAN SO, HALLGREN R. Renal interstitial fibrosis and vascular changes. Occurrence in patients with autoimmune diseases treated with cyclosporine. Arch Intern Med 1986; 146:2007-2010.

SWENY P, HOPPER J, GROSS M, VARGHESE Z. Nephrotoxicity of cyclosporin A. Lancet 1981; 1: 663.

TAKEDA K, MEYER-LEHNERT H, KIM JH, SCHRIER RW. Effect of angiotensin on ca++ kinetics and contraction of cultured glomerular mesangial cells. American Journal Physiology 1988; 254 (Renal Fluid electrolyte Physiol. 23): F254-F266.

TEGZESS AM, DOORENBOS BM, MINDERHOUD JM, DONKER AMJ. Prospective serial renal function studies in patients with non renal disease treated with cyclosporine A. Transplantation Proceedings 1988; 20(suppl 3): 530-533.

TESI RJ, WAIT RB, BUTT KMH, JAFFE BM, MCMILLEN MA. Calcium antagonists potentiate cyclosporine effects on human lymphocytes. Surgical Forum 1985: 36: 339-340.

TESI RJ, HONG J, BUTT KMH, JAFFE BM, MCMILLEN MA. In vivo potentiation of cyclosporine immunosuppression by calcium antagonists. Transplantation Proceedings 1987; 19(1): 1382-1384.

216

THIEL G, HERMLE M, BRUNNER FP. Acutely impaired renal function during intravenous administration of cyclosporine A: a cremophore side-effect. Clinical Nephrology 1986a; 25 (suppl 1): S40-S42.

THIEL G. Experimental cyclosporine A nephrotoxicity: a summary of the International Workshop (Basle, April 24-26, 1985). Clinical Nephrology 1986b; 25 (suppl 1): S205-S210.

THOMAS CE, OTT CE, BELL PD, KNOX FG, NAVAR LG. Glomerular filtration dynamics during vasodilation with acetylcholine in the dog. American Journal of Physiology 1983 (Renal Fluid Electrolyte Physiol. 13); 244: F606-F611.

THOMPSON ME, SHAPIRO AP, JOHNSON AM, ITZKOFF JM, HARDISTY RL, GRIFFITH BP, BAHNSON HT, MCDONALD RJ Jr, HASTILLO A, HESS M. The contrasting effects of cyclosporine A and azathioprine on arterial blood pressure and renal function following cardiac transplantation. Int J Cardiol 1986; 11: 219-229.

THOMSON AW, WHITING PH, BLAIR JT, DAVIDSON RJL, SIMPSON JG. Pathological changes developing in the rat during a 3 week course of high dosage cyclosporin A and their reversal following drug withdrawal. Transplantation 1981; 32: 271-277.

THOMSON AW, WHITING PH, SIMPSON JG. Cyclosporine: Immunology, toxicity and pharmacology in experimental animals. Agents and Actions 1984; 15: 306-327.

THOMSON SC, TUCKER BJ, GABBAI F, BLANTZ RC. Functional effects on glomerular hemodynamics of short-term chronic cyclosporine in male rats. Journal of Clinical Investigation 1989; 83: 960-969.

THURAU K. Nature of autoregulation of renal blood flow. Proceedings, Third International Congress of nephrology, Washington D.C. New York, Karger, 1966; 162-173.

TOMLANOVICH S, GOLLBETZ H, PERLROTH M, STINSON E, MYERS BD. Limitations of creatinine in quantifying the severity of cyclosporine-induced chronic nephropathy. American Journal of kidney diseases 1986; 8: 332-337.

TRIGGLE DJ, JANIS RA. Calcium channel ligands. Annu Rev Pharmacol Toxicol 1987; 27: 347-369.

TROYER DA, KREISBERG JI, SCHWERTZ DW, VENKATACHALAM MA. Effects of vasopressin on phosphoinositides and prostaglandin production in cultured mesangial cells. American Journal of Physiology 1985; 249 (Renal Fluid Electrolyte Physiol 18): F139-F147.

TUCKER BJ, BLANTZ RC. Factors determining superficial nephron filtration in the mature growing rat. American Journal of Physiology 1977; 232: F97-F104.

217

TUCKER FA, MINTY PSB AND MACGREGOR GA. Study of nifedipine
photodecomposition in plasma and whole blood using capillary gas liquid
chromatography. Journal of Chromatography 1985; 342: 193-198.

VAGHY PL, WILLIAMS JS, SCHWARTZ A. Receptor pharmacology of calcium
entry blocking agents. American Journal of cardiology 1987; 59: 9A-
17A.

VAN NEUTEN JM, JANSSENS WJ, VANHOUTTE PM. Calcium antagonism and
vascular smooth muscle. Annals of the New York Academy of Sciences
1988; 522; 234-247.

VANHOUTTE PM, RIMELE TJ, VAN NEUTEN JM. Calcium entry blockers and
contractile responses of vascular smooth muscle. In Calcium
antagonists: state of the art and role in cardiovascular disease.
Symposium. Frontiers Pharmacol 1983; 2: 65-75.

VANRENTERGHEM Y, WAER M, DE KEYSER P, ROELS L, MICHIELSEN P.
Controlled trial of the protective effect of dihyroergotoxine
(Hydergine) on cyclosporine-associated nephrotoxicity in renal graft
recipients. Transplantation Proceedings 1988; 20(3), suppl 3: 615-617.

VENKATARAMANAN R, WANG CP, HABUCKY K, PTACHCINSKI RJ, BURCKART GJ,
KONERU B, BAKER R, TODO, S, STARZL TE. Species-specific cyclosporine
metabolism. Transplantation Proceedings 1988; 20 (suppl 2): 680-683.

VINCENT HH, WENTING GJ, VERSLUIS DJ, WEIMAR W, SCHOLENKAMP MADH.
Impaired fractional excretion of lithium: an early marker of
cyclosporine induced changes in renal haemodynamics. Transplantation
Proceedings 1988; 20(suppl 3): 681-687.

VON WILLEBRAND E, HAYRY P. Cyclosporin-A deposits in renal allografts.
Lancet 1983;ii:189-192

VON WARTBURG A, TRABER R. Cyclosporins, fungal metabolites with
immunosuppressive activities. Progress in Medicinal Chemistry 1988;
25: 1-33.

VOSS BL, HAMILTON KK, SCOTT SAMARA EN, MCKEE PA. Cyclosporine
suppression of endothelial prostacyclin generation. Transplantation
1988; 45(4): 793-796.

WAGNER K, ALBRECHT S, NEUMAYER HH. Prevention of posttransplant acute
tubular necrosis by the calcium antagonist diltiazem: a prospective
randomized study. American Journal of Nephrology 1987;7:287-291.

WAGNER K, HENKEL M, HEINEMEYER G, NEUMAYER HH. Interaction of calcium
blockers and cyclosporine. Transplantation Proceedings 1988; 20 (suppl
2):561-568.

WAGONER RD, NEWLON TAUXE W, MAHER FT, HUNT JC. Measurement of
effective renal plasma flow with sodium iodohippurate I 131. Journal

of the American Medical Association 1964; 187: 811-813

WALKER LA, BUSCEMI-BERGIN M, GELLAI M. Renal haemodynamics in
conscious rats: effects of anaesthesia, surgery and recovery. American
Journal of Physiology 1983; 245 (Renal Fluid Electrolyte Physiol.14):
F67-F74.

WASSEF R, COHEN Z AND LANGER B. Pharmacokinetic profiles of
cyclosporine in rats. Transplantation 1985; 40: 489-493.

WEIR MR, KLASSEN DK, SHEN SY, SULLIVAN D, BUDDEMEYER EU, HANDWERGER BS.
Acute effects of intravenous cyclosporine on blood pressure, renal
haemodynamics, and urine prostaglandin production of healthy humans.
Transplantation 1990; 49: 41-47.

WHEATLEY HC, DATZMAN M, WILLIAMS JW, MILES DE, HATCH FE. Long-term
effects of cyclosporine on renal function in liver transplant
recipients. Transplantation 1987; 43(5): 641-647.

WHISTLER RL, LINDSEY JA, PROCTOR KVM, MORISAKI N, CORNWELL DG.
Characteristics of cyclosporine induction of increased prostaglandin
levels from human peripheral blood monocytes. Transplantation 1984;
38: 377-381.

WHITING PH, THOMSON AW, BLAIR JT, SIMPSON JG. Experimental cyclosporin
A nephrotoxicity. British Journal of Experimental Pathology 1982; 63:
88-94.

WHITING PH, DUNCAN JI, GAVIN MP, HEYS SD, SIMPSON JG, ASFAR SK, THOMSON
AW. Renal function in rats treated with cyclosporin following
unilateral nephrectomy. British Journal of Pathology 1985; 66: 535-542.

WHITING PH, SIMPSON G. Lithium clearance measurements as an indication
of cyclosporine A nephrotoxicity in the rat. Clinical Science 1988a;
74: 173-178.

WHITING PH, PROPPER DJ, SIMPSON JG, MCKAY J, JONES M C, CATTO GRD. The
use of lithium clearance measurements to assess renal tubular function
in experimental and clinical cyclosporine nephrotoxicity.
Transplantation Proceedings 1988b; 20(suppl 3): 675-680.

WHITNEY RB AND SUTHERLAND RM. Requirement of calcium ion in lymphocyte
transformation stimulated by phytohemagglutin. Journal of Cell
Physiology 1972; 80: 329-337.

WHITWORTH JA, MILLS EH, COGHLAN JP, MCDOUGALL JG, NELSON MA, SPENCE CD,
TRESHAM JJ, SCOGGINS BA. The haemodynamic effects of cyclosporin A in
sheep. Clinical and Experimental Pharmacology and Physiology 1987; 14:
573-580.

WIEGMANN TB, SHARMA R, DIEDERICH DA, SAVIN VJ. In vitro effects of
cyclosporine on glomerular function. The American Journal of The

Medical Sciences 1990; 299(3): 149-152.

WIESNER RH, LUDWIG J, LINDOR KD, JORGENSEN RA, BALDUS WP, HOMBURGER HA, DICKSON ER. A controlled trial of cyclosporine in the treatment of primary biliary cirrhosis. New England Journal of Medicine 1990; 322: 1419-1424.

WINSTON AJ, SAFIRSTEIN R. Determinants of glomerular filtration in uninephrectomized rats treated with cyclosporin A. Clin Res 1987; 35: 561A.

WINSTON JA, FEINGOLD R, SAFIRSTEIN R. Glomerular hemodynamics in cyclosporine nephrotoxicity following uninephrectomy. Kidney International 1989; 35: 1175-1182.

WRIGHT B, ZEIDMAN I, GREIG R, POSTE G. Inhibition of macrophage activation by calcium channel blockers and calmodulin antagonists. Cell Immunology 1985; 95: 46-53.

XUE H, BUKOSKI RD, MCCARRON DA, BENNETT WH. Induction of contraction in isolated rat aorta by cyclosporine. Transplantation 1987; 43(5): 715-718.

ZIDEK W, VETTER H, DORST KG, ZUMKLEY H, LOSSE H. Intracellular Na^+ and Ca^{2+} activities in essential hypertension. Clinical Science 1982; 63: 41S-43S.

ZIDEK W, NEUMANN KH. Ca^{2+} release in permeabilized human neutrophils induced by ciclosporin. Nephron 1990; 56: 30-34.

ZIMMERHACKL LB, FRETSCHNER M, STEINHAUSEN M. Cyclosporin reduces renal blood flow through vasoconstriction of arcuate arteries in the hydronephrotic rat model. Klin Wochenschr 1990; 68: 166-174.

ZOJA C, FURCI L, GHILARDI F, ZILIO P, BENIGNI A, REMUZZI G. Cyclosporin-induced endothelial cell injury. Laboratory Investigation 1986; 55: 455-462.

PUBLICATIONS BASED ON EXPERIMENTS IN THIS THESIS

McNally PG, Feehally J and Walls J.

The effect of an oral protein load on renal haemodynamics in

cyclosporin A and azathioprine treated renal allograft recipients.

Clinical Science 1989; 76 (suppl 20): 62A.

Presented to the Medical Research Society, London, January 1989.

McNally PG, Feehally J and Walls J.

Nifedipine increases GFR in chronic cyclosporin A (CYA) treated

renal transplant recipients.

Nephrology, Dialysis and Transplantation 1989; 4(5): 515 A.

Presented to the European Dialysis and Transplant Association,

Goteborg, June 1989.

McNally PG, Mistry N, Idle JR, Walls J and Feehally J.

Calcium channel blockers and cyclosporin A metabolism. Transplantation

1989; 48(6): 1071 (Brief Communication).

McNally PG, Walls J and Feehally J.

Effect of nifedipine on glomerular haemodynamics in an animal model of

cyclosporin A nephrotoxicity.

Clinical Science 1990; 79: 259-266.

Work contained in this paper presented to the British Association of

Renal Physicians, Glasgow, May 1989 and Amsterdam, October 1989.

European Dialysis and Transplant Association, Goteborg, June 1989 and

The American Society of Nephrology, Washington DC, December 1989.

McNally PG, Heagerty AM, Walls J and Feehally J.

The effect of cyclosporin A (CYA) on phosphoinositide (PI) hydrolysis

in rat aorta and resistance arterioles.

Clinical Science 1989; 77 (suppl 21): 28A.

Presented to the Medical Research Society, Leeds, June 1989.

McNally PG, Walls J and Feehally J.

Is serum creatinine a reliable index of impaired renal function in

cyclosporin (CYA) treated renal allograft recipients?

Clinical Science 1989; 77 (suppl 21): 28.

Presented to the Medical Research Society, Leeds, June 1989.

McNally PG, Horsburgh T, Walls J and Feehally J.

The effect of nifedipine (NIF) on [3H]-cyclosporin A (CYA) uptake into

human proximal tubular cells (PTC).

Clinical Science 1990; 78 (suppl 22): 34.

Presented to the Medical Research Society, London, January 1990.

McNally PG, Walls J and Feehally J.

The effect of nifedipine on renal function in normotensive cyclosporin

A treated renal allograft recipients.

Nephrology, Dialysis and Transplantation (In press).

Presented to the British Transplantation Society, London, December

1988.

McNally PG, Walls J and Feehally J.

The effect of nifedipine on cyclosporin A nephrotoxicity in the spontaneously hypertensive rat after uninephrectomy (U_{nx}) or U_{nx} with contralateral renal denervation.

Nephrology Dialysis Transplantation 1990; 5(8): 660A.

Presented to the European Dialysis and Transplant Association, Vienna, 1990.