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Assessment of renal dopaminergic system activity during cyclosporine A administration in the rat

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1 Administration of cyclosporine A (CsA; 50 mg kg^{-1} day^{-1}, s.c.) for 14 days produced an increase in both systolic (SBP) and diastolic (DBP) blood pressure by 60 and 25 mmHg, respectively. The urinary excretion of dopamine, DOPAC and HVA was reduced from day 5-6 of CsA administration onwards (dopamine from 19 to 46%, DOPAC from 16 to 48%; HVA from 18 to 42%). In vehicle-treated rats, the urinary excretion of dopamine and DOPAC increased (from 7 to 60%) from day 5 onwards; by contrast, the urinary excretion of HVA was reduced (from 27 to 60%) during the second week.

2 No significant difference was observed between the V_{max} and K_m values of renal aromatic L-amino acid decarboxylase (AAAD) in rats treated with CsA for 7 and 14 days or with vehicle.

3 K_m and V_{max} of monoamine oxidase types A and B did not differ significantly between rats treated with CsA for 7 and 14 days or with vehicle.

4 Maximal catechol-*O*-methyltransferase activity (V_{max}) in homogenates of renal tissues obtained from rats treated with CsA for 7 or 14 days was significantly higher than that in vehicle-treated rats; K_m (22.3 ± 1.5 μM) values for COMT did not differ between the three groups of rats.

5 The accumulation of newly-formed dopamine and DOPAC in cortical tissues of rats treated with CsA for 14 days was three to four times higher than in controls. The outflow of both dopamine and DOPAC declined progressively with time and reflected the amine and amine metabolite tissue contents. No significant difference was observed between the DOPAC/dopamine ratios in the perifusate of renal tissues obtained from CsA- and vehicle-treated rats. In addition, no significant differences were observed in k values or in the slope of decline of both DA and DOPAC between experiments performed with CsA and vehicle-treated animals.

6 The V_{max} for the saturable component of L-3,4-dihydroxyphenylalanine (L-DOPA) uptake in renal tubules from rats treated with CsA was twice that of vehicle-treated animals. K_m in CsA- and vehicle-treated rats did not differ.

7 The decrease in the urinary excretion of sodium and an increase in blood pressure during CsA treatment was accompanied by a reduction in daily urinary excretion of dopamine. This appears to result from a reduction in the amount of L-DOPA made available to the kidney and does not involve changes in tubular AAAD, the availability of dopamine to leave the renal cells and dopamine metabolism. The enhanced ability of the renal tissues of CsA-treated animals to synthesize dopamine, when exogenous L-DOPA is provided, results from an enhanced activity of the uptake process of L-DOPA in renal tubular cells.

Keywords: Dopamine; monoamine oxidase; catechol-*O*-methyltransferase; kidney; cyclosporine A

Introduction

Cyclosporine A (CsA), a potent imunosuppressant with relative lack of myelotoxic effects, has been hailed as a major advance in the field of organ transplantation. Major adverse effects of CsA, which constitute a limitation to its clinical utilization, are nephrotoxicity and the development of hypertension (Sturrock & Struthers, 1994). CsA causes a dose-dependent impairment in renal function and it is now recognized that CsA-induced nephrotoxicity mainly manifests through three different syndromes: (1) acute decrease in glomerular filtration rate (GFR); (2) acute microvascular disease with the pattern of thrombotic microangiopathy; (3) chronic irreversible renal damage (Remuzzi & Bertani, 1989). The reduction in GFR is initially reversible and appears to be related to vasoconstriction of the afferent arterioles (Kopp & Klotman, 1990); this effect has its origin in disturbances of renal blood flow as induced by an imbalance between vasodilator and vasoconstrictor eicosanoids and also from the release of endothelin (Kon *et al.*, 1990). The CsA-induced nephrotoxicity is accompanied by an increase in tubular sodium reabsorption in proximal tubules, as shown by a reduction in lithium clearance (Whiting *et al.*, 1988). The nature of the mechanisms involved in sodium retention during CsA administration appear to result from stimulation of the renin-angiotensin-aldosterone system as a consequence of the renal hypoperfusion (Ishikawa *et al.*, 1991), stimulation of the tubulo-glomerular feedback mechanism (Siegl & Ryffel, 1982) or increased renal nerve activity (Murray & Paller, 1986). As a consequence of renal vasoconstriction and increased tubular sodium reabsorption, all forms of CsA nephrotoxicity may be accompanied by some degree of hypertension.

Renal tubular cells, namely those of proximal convoluted tubules are endowed with a high aromatic-L-amino acid decarboxylase (AAAD) activity and synthesize dopamine from filtered or circulating L-3,4-dihydroxyphenylalanine (L-DOPA) (Soares-da-Silva, 1994). A considerable body of evidence favours the view that dopamine of renal origin plays a role in the regulation of central blood volume by reducing the tubular reabsorption of sodium as a paracrine or autocrine substance (Siragy *et al.*, 1989). The mechanisms through which renal dopamine is thought to produce natriuresis involve the inhibition of Na^+-K^+ ATPase and Na^+-H^+ exchanger activities (Bertorello *et al.*, 1988; Felder *et al.*, 1989). The role of renal

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dopamine in the renal handling of sodium has been empahsized recently by the suggestion that disturbances in the activity of the renal dopaminergic system may contribute to the development of some forms of hypertension, namely in subjects with sodium-sensitive hypertension (for review see Lee, 1993). The antinatriuretic effects of CsA have been demonstrated to result in part from an increase in the reabsorption of sodium, this is particularly evident at the level of the proximal tubules. This nephron segment is precisely that presenting the highest AAAD activity and is thought to be the area where the tubular actions of dopamine are particularly relevant. Thus, one possible explanation for the antinatriuretic effects of CsA is that of a reduced activity of the renal dopaminergic system. The fact that dopamine (Conte *et al.*, 1988; Sabbatini *et al.*, 1989) and dopamine agonists (Brooks *et al.*, 1990) have been found, both in man and rat, to reverse completely the haemodynamic and tubular disturbances induced by CsA, favours this hypothesis.

The aim of the present work was to evaluate the activity of the renal dopaminergic system in rats submitted to long-term (7 and 14 days) administration of CsA, in a dose known to produce nephrotoxic effects (Petric *et al.*, 1990). In one group of experiments (*in vivo* studies), the daily urinary excretion of dopamine and its deaminated (3,4-dihydroxyphenylacetic acid; DOPAC) and deaminated plus methylated (homovanillic acid; HVA) metabolites were monitored. In another group of experiments (*in vitro* studies), the uptake of L-DOPA, the synthesis, metabolism and fate of dopamine were also evaluated. Preliminary accounts of some of these findings have been presented (Pestana *et al.*, 1993; 1994; Pestana & Soares-da-Silva, 1994a).

Methods

In vivo *studies*

Normotensive male Wistar rats (Biotério do Instituto Gulbenkian de Ciências, Oeiras, Portugal) 45–60 days and weighing 250–320 g were selected after a 7 day period of stabilization and following adaptation to blood pressure measurements. Animals were kept in metabolic cages under controlled environmental conditions (12 h light/dark cycle and room temperature 24°C); liquid intake and food consumption was monitored daily throughout the study. All animals were fed throughout the study with ordinary rat chow (Letica, Barcelona, Spain, sodium, potassium and protein contents, 0.1%, 0.75% and 17%, respectively). The daily sodium intake averaged 0.5 mmol 100 g^{-1} of body weight. Twenty-four hours urine was collected in vials containing 1 ml of 6 M HCl to prevent the spontaneous decomposition of monoamines and amine metabolites. Thereafter, CsA (50 mg kg^{-1} day^{-1}, s.c.) was administered for 14 ($n=4$) consecutive days. Another group of rats ($n=4$), was treated with the vehicle of CsA for 14 days (olive oil; 0.5 ml kg^{-1} day^{-1}, s.c.); this group of rats was allowed a daily food consumption similar to that observed in CsA-treated rats. Blood pressure (systolic, SBP; diastolic, DBP) and heart rate were measured in conscious restrained animals between 07 h 00 min to 10 h 00 min, with a photoelectric tail cuff pulse detector (LE 5000, Letica, Barcelona, Spain) on the fourth day of the stabilization period and on the fourteenth day of CsA administration.

After completion of these studies, rats were anaesthetized with sodium pentobarbitone (60 mg kg^{-1}, i.p.), blood from the vena cava was collected and the kidneys were rapidly removed through an abdominal midline incision. The blood was collected in ice tubes containing heparin for later determination of plasma catecholamines and biochemical parameters.

Results are means ± s.e.mean of values for the indicated number of determinations. Within-group analysis of data was performed by Friedman's test comparing all values to the baseline state (the last four days of stabilization period) or values of interest to the corresponding controls. Significance of differences between one control and several experimental groups was evaluated by the Tukey-Kramer method (Sokal & Rohlf, 1981). Differences between two means were estimated by Student's t test for unpaired data; a P value less than 0.05 was assumed to denote a significant difference.

In vitro *studies*

Renal tissues were obtained from five different experimental groups of rats: (1) animals given CsA (50 mg kg^{-1} day^{-1}; s.c.; $n=12$) for 7 consecutive days; (2) animals given CsA (50 mg kg^{-1} day^{-1}; s.c.; $n=20$) for 14 consecutive days; (3) animals given olive oil for 7 consecutive days (0.5 ml kg^{-1} day^{-1}; s.c.; $n=12$); (4) animals given olive oil for 14 consecutive days (0.5 ml kg^{-1} day^{-1}; s.c.; $n=20$). In these four groups of rats, the food consumption was allowed *ad libitum*. In a fifth group of rats, olive oil (0.5 ml kg^{-1} day^{-1}, s.c.; $n=4$) was given for 14 days, but the allowance of daily food consumption was restricted to the amount observed in CsA-treated rats.

AAAD activity In experiments designed to study the decarboxylation of L-DOPA, homogenates of renal tubules obtained from animals given CsA for 7 ($n=4$) and 14 ($n=4$) days and from animals given olive oil for 14 ($n=4$) days were used. The preparation of renal tubules was based on the technique described previously (Soares-da-Silva *et al.*, 1994). The renal tubules were homogenized in the medium used in the decarboxylation reaction with Thomas Teflon homogenizers and kept continuously on ice. The composition of the incubation medium was as follows (in M): NaH_2PO_4 0.35, Na_2HPO_4 0.15, $Na_2B_4O_7$ 0.1 and pyridoxal phosphate 0.12; pargyline (100 μM) and tolcapone (1 μM) were also added to the incubation medium in order to inhibit the enzymes monoamine oxidase (MAO) and catechol-*O*-methyltransferase (COMT), respectively. AAAD activity was determined as previously described (Soares-da-Silva *et al.*, 1995). In some experiments, tubular homogenates were incubated with L-DOPA (100 μM) in the presence of different concentrations of pyridoxal phosphate (0 to 3000 μM). K_m and V_{max} values for the metabolism of L-DOPA were calculated from linear regression analysis (V against V/S).

MAO activity MAO activity was determined with [^{3}H]-5-hydroxytryptamine ([^{3}H]-5-HT) as a preferential substrate for MAO-A and [^{14}C]-β-phenylethylamine ([^{14}C]-β-PEA) as a preferential substrate for MAO-B, as previously described (Fernandes & Soares-da-Silva, 1992). The deaminated products were extracted with ethyl and measured by liquid scintillation counting. K_m and V_{max} values for the metabolism of L-[^{3}H]-5-HT and [^{14}C]-β-PEA were calculated from linear regression analysis (V against V/S).

COMT activity COMT activity was determined in renal tissue from rats treated with CsA for 7 ($n=4$) and 14 ($n=4$) days and from animals treated with olive oil for 14 days ($n=4$). Homogenates of renal cortex were prepared in 0.5 M phosphate buffer, pH 7.8, at 4°C with a Duall-Kontes homogenizer. COMT activity was determined by evaluating the ability of kidney homogenates to methylate adrenaline into metanephrine. Aliquots of 0.5 ml of the homogenate were preincubated for 20 min with 0.5 ml of phosphate buffer (0.5 M); thereafter, the reaction mixture was incubated for 30 min with increasing concentrations of adrenaline (5 to 500 μM) in the presence of a methyl donor (S-adenosylmethionine; 100 μM); the incubation medium contained also pargyline (100 μM), $MgCl_2$ (100 μM) and EGTA (1 mM). The preincubation and incubation were carried out at 37°C, in conditions of light protection, with continuous shaking and without oxygenation. At the end of the incubation period the tubes were transferred to ice and the reaction was stopped by the addition of 50 μl of perchloric acid (2 M). The samples were then centrifuged (200 g, 4 min, 4°C), and 500 μl aliquots

of the supernatant filtered on Millipore microfilters (MF1) were used for the assay of metanephrine. K_m and V_{max} values for the metabolism adrenaline were calculated from linear regression analysis (V against V/S).

Outflow of dopamine The kinetic parameters of the outflow of dopamine and DOPAC in renal tissues of rats treated with CsA ($n=4$) or olive oil ($n=4$) for 14 days were determined as previously described (Pestana & Soares-da-Silva, 1994b). Briefly, on the 14th day of CsA administration, rats were given L-DOPA (30 mg kg^{-1}, i.p.) and killed by decapitation under ether anaesthesia 15 min after the injection. The kidneys were removed, rinsed free of blood with saline (NaCl 0.9%), placed on an ice-cold glass plate and the kidney poles removed; thereafter, slices approximately 2 mm thick were obtained and cortical fragments weighing about 60 mg were prepared with a scalpel. Some cortical fragments were immediately introduced into vials containing 2 ml perchloric acid (0.2 M) for the quantification of dopamine and DOPAC. The remaining four cortical fragments were placed in glass perifusion chambers; the process of preparing the renal fragments and placing them in the perifusion chambers took no more than 15 min.

The preparations were perifused with warm (37°C) and gassed (95% O_2 and 5% CO_2) Krebs solution at a rate of 350 $\mu l\ min^{-1}$ and allowed a 30 min stabilization period. Thereafter, five consecutive 10 min perifusate samples were collected into glass test tubes kept on ice and containing 500 μl perchloric acid (2 M). The composition of the Krebs solution was as follows (in mM): NaCl 118, KCl 4.7, $CaCl_2$ 2.4, $MgSO_4$ 1.4, $NaHCO_3$ 25, KH_2PO_4 1.2, EDTA 0.4 and glucose 11; tolcapone (1 μM) was added to the Krebs solution in order to inhibit COMT. At the end of the perifusion period, renal fragments were collected, blotted with filter paper and placed in 2 ml of 0.2 M perchloric acid. The acidified perifusates and tissue fragments maintained in perchloric acid were kept at 4°C for the next 24 h till quantification of catecholamines.

The levels (in nmol g^{-1} 10 min^{-1}) of dopamine and DOPAC in the perifusate were logarithmically transformed, plotted against the time of perifusion and the slope of decline

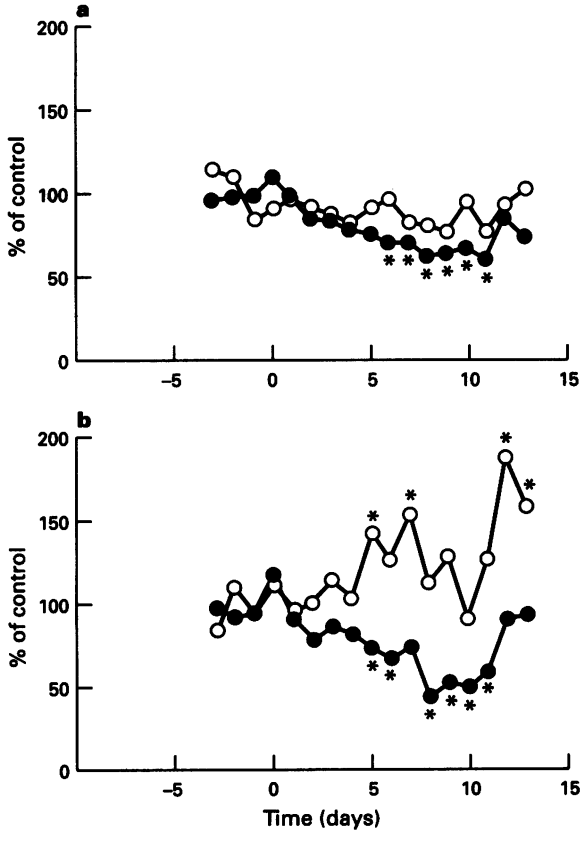

Figure 1 Liquid ingestion (a) and urinary volume (b) of rats during the last four days of stabilization period (from day −4 till day 0) and during administration of CsA (50 mg kg^{-1} day^{-1}, s.c.; day 0 till day 14; ●) or olive oil (0.5 ml kg^{-1} day^{-1}, s.c.; day 0 till day 14; ○). Results are percentage of baseline values considering individual determinations. Each point represents the mean of 4 determinations per group; s.e.mean values were less than 10% of the corresponding means. Significantly different from corresponding baseline values (*$P<0.05$).

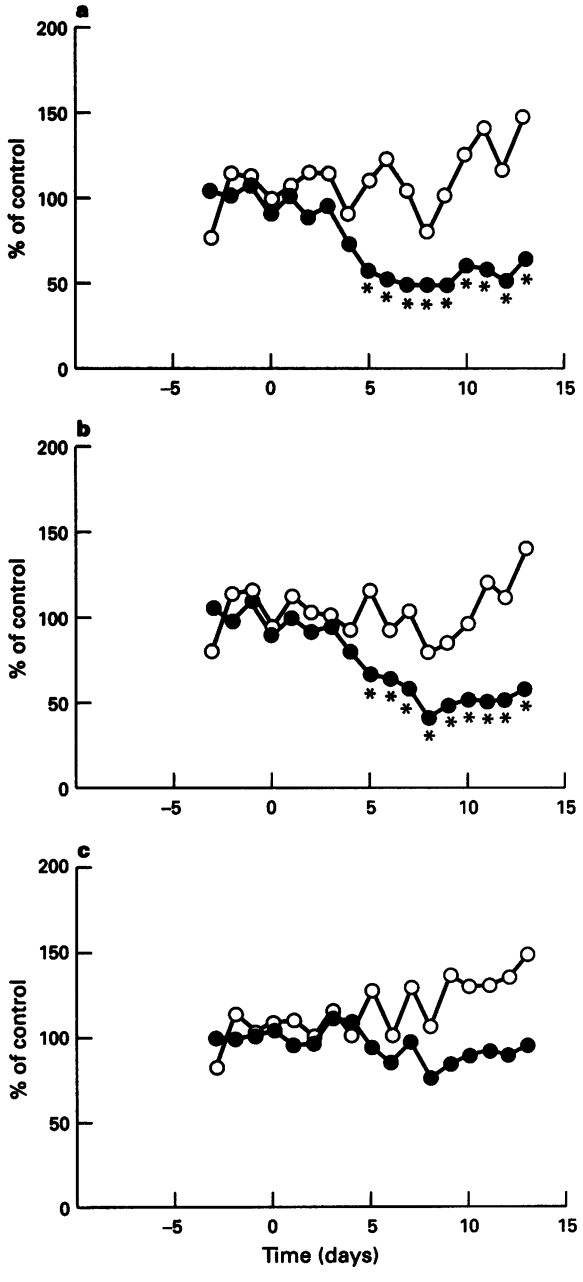

Figure 2 Daily urinary excretion of (a) sodium, (b) potassium and (c) creatinine during the last four days of stabilization period (from day −4 till day 0) and during administration of CsA (50 mg kg^{-1} day^{-1}, s.c.; day 0 till day 14; ●) or olive oil (0.5 ml kg^{-1} day^{-1}, s.c.; day 0 till day 14; ○). Results are percentage of baseline values considering individual determinations. Each point represents the mean of 4 determinations per group; s.e.mean values were less than 10% of the corresponding means. Significantly different from corresponding baseline values (*$P<0.05$).

calculated by linear regression analysis. The rate constants of efflux of dopamine or DOPAC were calculated by dividing the levels of the amine or amine metabolite (nmol g^{-1} min^{-1}) in the last efflux sample by the tissue content (nmol g^{-1}) at the end of the experiment (k = rate of efflux/tissue content; see Trendelenburg *et al.*, 1979). Results are means ± s.e.mean of values for the indicated number of experiments. Values for the rate constant of loss (k) are geometric means with 95% confidence intervals. Statistical significance was determined using the Tukey-Kramer method. A P value less than 0.05 was assumed to denote a significant difference.

Uptake of L-DOPA in tubular epithelial cells In some experiments the tubular uptake of L-DOPA was determined in isolated renal tubules obtained from rats treated with CsA ($n=4$) or olive oil ($n=4$) for 14 days and from animals treated with olive oil ($n=4$) for 14 days and allowed an amount of daily food consumption similar to that observed in CsA-treated rats. The rats were given benserazide (10 mg kg^{-1}, i.p.) in order to inhibit AAAD (DaPrada *et al.*, 1987) and killed by decapitation under ether anaesthesia 60 min after the injection. The renal tubules were prepared as described above and the non-saturable and saturable components of L-DOPA uptake determined as previously described (Soares-da-Silva *et al.*, 1994). The V_{max} and K_m values of the saturable component of L-DOPA uptake in renal tubules were calculated by linear regression analysis. The diffusion constant was determined by the slope of the accumulation of L-DOPA measured in experiments carried out at 4°C (Neame & Richards, 1972).

Quantification of catecholamines, sodium, potassium and creatinine The quantification of catecholamines and its metabolites in urine (dopamine, DOPAC and HVA), plasma samples (L-DOPA, noradrenaline, adrenaline and DOPEG), renal tissues (dopamine and DOPAC) and samples of the perifusion medium (dopamine and DOPAC) was performed by high performance liquid chromatography (h.p.l.c.) with electrochemical detection, as previously described (Soares-da-Silva *et al.*, 1993; 1995).

Urinary sodium and potassium were measured by flame photometry (model FML3) connected to a dilutor (model A 6241, Radiometer, Copenhagen, Denmark) and urine and plasma osmolality by means of an osmometer (Advanced Instruments, Inc., MA, U.S.A., model 3 MO). Urinary and plasma creatinine and plasma urea were measured by a wavelength photometer (Hitashi Automatic Analizer, model 717, or a Beckman Analyzer II).

The protein content in kidney homogenates (approximately 4 mg ml^{-1}) was determined by the method of Bradford (1976) with human serum albumin as standard.

Drugs

CsA oral solution was purchased from Sandoz, Basel, Switzerland. L-3,4-dihydroxyphenylalanine (L-DOPA), pargy-

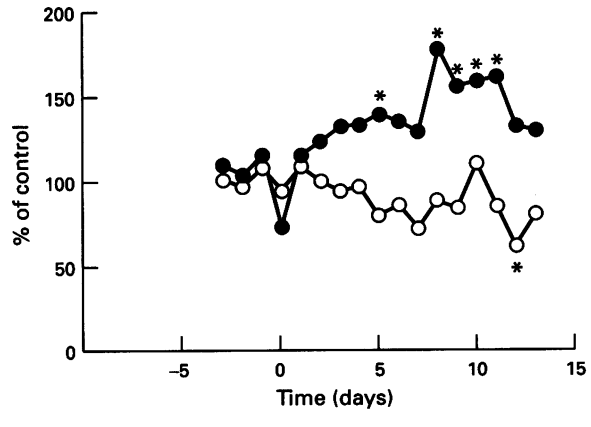

Figure 3 Daily urine osmolality of rats during the last four days of stabilization period (from day −4 till day 0) and during administration of CsA (50 mg kg^{-1} day^{-1}, s.c.; day 0 till day 14; ●) or olive oil (0.5 ml kg^{-1} day^{-1}, s.c.; day 0 till day 14; ○). Results are percentage of baseline values considering individual determinations. Each point represents the mean of 4 determinations per group; s.e.mean values were less than 10% of the corresponding means. Significantly different from corresponding baseline values (*$P<0.05$).

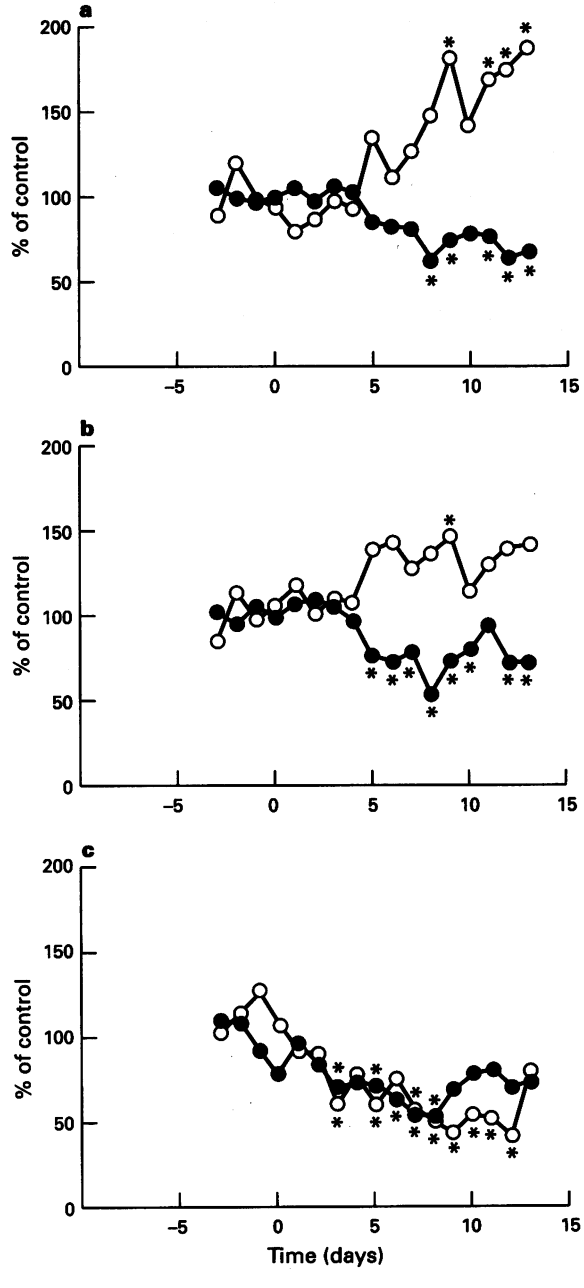

Figure 4 Daily urine excretion of (a) dopamine, (b) 3,4-dihydroxyphenylacetic acid (DOPAC) and (c) homovanillic acid (HVA) during the last four days of stabilization period (from day −4 till day 0) and during administration of CsA (50 mg kg^{-1} day^{-1}, s.c.; day 0 till day 14; ●) or olive oil (0.5 ml kg^{-1} day^{-1}, s.c.; day 0 till day 14;○). Results are percentage of baseline values considering individual determinations. Each point represents the mean of 4 determinations per group; s.e.mean values were less than 10% of the corresponding means. Significantly different from corresponding baseline values (*$P<0.05$).

Table 1 Plasma concentrations of L-3,4-dihydroxyphenylalanine (L-DOPA), noradrenaline (NA), adrenaline (Ad) and 3,4-dihydroxyphenylglycol (DOPEG), Na^+, K^+, urea, creatinine and plasma osmolality in rats treated with cyclosporine A (CsA; 50 mg kg^{-1} day^{-1}, s.c., $n=4$) for 14 days and in rats treated with olive oil (0.5 ml kg^{-1} day^{-1}, s.c., $n=4$) for 14 days

	Olive oil	*CsA* (14 days)
L-DOPA (pmol ml^{-1})	4.8±0.5	2.6±0.5#
NA (pmol ml^{-1})	3.0±0.7	8.9±1.9**
Ad (pmol ml^{-1})	3.8±1.9	5.7±2.6
DOPEG (pmol ml^{-1})	5.5±0.5	7.3±0.8
Na^+ (mmol l^{-1})	137±0.7	131±1**
K^+ (mmol l^{-1})	4.95±0.5	5.28±0.46
Urea (mg dl^{-1})	27.8±2.3	74.3±7.7*
Creatinine (mg dl^{-1})	0.38±0.05	0.48±0.03
Osmolality (mO smol kg^{-1} H_2O)	286.3±9.4	292±4.5

Values are means±s.e.mean of four determinations per group. Significantly different from corresponding control values (**$P<0.01$; *$P<0.02$; #$P<0.05$) using Student's t test.

Table 2 Kinetic parameters (V_{max}, in nmol mg^{-1} of protein h^{-1}; K_m, in μM) of aromatic L-amino acid decarboxylase (AAAD) activity in homogenates of isolated renal tubules obtained from rats treated with cyclosporine A (CsA; 50 mg kg^{-1} day^{-1}, s.c., $n=4$) for 7 and 14 days and in rats treated with olive oil (0.5 ml kg^{-1} day^{-1}, s.c., $n=4$) for 14 days

	V_{max}	K_m
Olive oil ($n=4$)	305±24	3580±630
CsA 7 days ($n=4$)	326±91	3338±1466
CsA 14 days ($n=4$)	316±13	5892±779

Values are means±s.e.mean of four determinations per group.

line hydrochloride, dopamine hydrochloride, pargyline hydrochloride, homovanillic acid (HVA), noradrenaline bitartrate, 3,4-dihydroxyphenylacetic acid (DOPAC), 1-α-methyl-*p*-tyrosine, trypan blue and olive oil were purchased from Sigma Chemical Company (St. Louis, Mo, U.S.A.); tolcapone was kindly donated by the producer (Hoffman La Roche, Basle, Switzerland).

Results

In vivo *studies*

Administration of CsA (50 mg kg^{-1} day^{-1}, s.c.) for 14 days was accompanied by an increase in blood pressure. Average baseline values of systolic (SBP) and diastolic (DBP) blood pressure were 127±2 mmHg and 86±1 mmHg, respectively. On the 14th day of CsA administration, both SBP and DBP were significantly increased to 183±4 mmHg ($P<0.01$) and 110±3 mmHg ($P<0.05$), respectively; in contrast, heart rate did not change significantly (412±1 vs 427±41 beats min^{-1}).

In CsA-treated rats, the increase in body weight throughout the study (8.0% increase) was found to be similar to that observed in controls (7.9% increase). During the stabilization period, daily liquid intake and urinary volume (ml kg^{-1} day^{-1}) averaged 142.0±13.2 and 65.2±10.4, respectively. In rats receiving CsA for 14 days, a significant reduction in daily liquid intake (up to 40% reduction; $P<0.05$) and urinary volume (up to 56% reduction, $P<0.01$) was observed (Figure 1). In contrast, in rats treated with olive oil, the urinary volume increased up to 86% ($P<0.01$); daily average liquid intake was also found to increase slightly (Figure 1).

Baseline daily urinary excretion of sodium, potassium and creatinine averaged 6.5±0.7 mmol kg^{-1} day^{-1}, 10.0±0.6 mmol kg^{-1} day^{-1} and 30.6±1.0 mg kg^{-1} day^{-1}, respectively. In the group of rats treated with CsA, a progressive decline in the urinary excretion of sodium (from 24% to 52%; $P<0.01$), potassium (from 32% to 53% reduction; $P<0.01$) and creatinine (from 11% to 27% reduction; $P=0.08$) was observed (Figure 2). In contrast, in olive oil-treated rats, daily urinary excretion of sodium, potassium and creatinine did not change.

During the stabilization period, daily urine osmolality averaged 1409.9±142.6 mOsm kg^{-1} H_2O. In CsA-treated rats, the urine osmolality progressively increased (up to 77%; $P<0.01$). In contrast, a slight reduction in urine osmolality was observed in olive oil treated animals (Figure 3).

Daily urinary excretion of dopamine, DOPAC and HVA (nmol kg^{-1} day^{-1}) during the four day period of stabilization averaged 55.5±6.2, 145.6±20.7 and 739.7±78.7, respectively. In CsA-treated rats, a progressive reduction was observed in daily urinary excretion of dopamine (from 19 to 46%; $P<0.01$), DOPAC (from 16 to 48%; $P<0.01$) and HVA (from 18 to 42%; $P<0.05$) (Figure 4). However, the reduction in the urinary excretion of dopamine, DOPAC and HVA was observed only from the 5th-6th day of CsA administration onwards. In vehicle-treated rats, the urinary excretion of dopamine and DOPAC increased (dopamine from 7 to 60%; $P<0.02$; DOPAC from 6 to 36%; $P<0.05$) from day 5 of olive oil administration onwards (Figure 4). By contrast, the urinary excretion of HVA was reduced (from 27 to 60%; $P<0.02$) during the second week of olive oil administration (Figure 4).

The concentrations in plasma of L-DOPA, noradrenaline,

Table 3 Kinetic parameters (V_{max}, in nmol mg^{-1} protein h^{-1}; K_m, in μM) of type A and B monoamine oxidase (MAO) activities in homogenates of renal cortex obtained from rats treated with cyclosporine A (CsA; 50 mg kg^{-1} day^{-1}, s.c., $n=4$) for 7 and 14 days and in rats treated with olive oil (0.5 ml kg^{-1} day^{-1}, s.c., $n=4$) for 14 days

	MAO-A		*MAO-B*	
	V_{max}	K_m	V_{max}	K_m
Olive oil ($n=4$)	44.7±1	1254±26	23.5±1	42±6
CsA 7 days ($n=4$)	41.2±1	1217±11	24.6±1	42±3
CsA 14 days ($n=4$)	43.2±2	1217±24	22.7±1	40±4

Values are means±s.e.mean of four determinations per group.

adrenaline, DOPEG, sodium, potassium, urea, creatinine and plasma osmolality in a terminal blood sample obtained from the vena cava of rats treated with CsA for 14 days and rats treated with olive oil for 14 days are shown in Table 1. Noradrenaline levels were found to be increased in rats treated with CsA for 14 days, whereas L-DOPA concentration was significantly reduced. No significant differences were observed in concentrations of creatinine, potassium, and plasma osmolality between CsA and olive oil-treated rats (Table 1). Urea concentration was significantly higher in rats treated with CsA for 14 days than in controls. In addition, sodium concentration was significantly lower in CsA-treated rats than controls.

In vitro *studies*

AAAD activity In experiments performed on homogenates of renal tubules, the decarboxylation of L-DOPA to dopamine was found to be dependent on the concentration of L-DOPA used (50 to 20000 μM). No significant difference was observed between the V_{max} values (in nmol mg^{-1} protein h^{-1}) of renal AAAD in rats treated with CsA for 7 and 14 days and olive oil-treated animals (Table 2). K_m values for AAAD were also found not to differ between the three groups of rats: CsA for 7 and 14 days and olive oil-treated animals. In addition, no significant differences were observed between CsA and vehicle-treated rats in experiments performed with increasing concentrations of pyridoxal phosphate (data not shown).

MAO activity The kinetic parameters (V_{max} and K_m) of the two forms of MAO, types A and B, in homogenates of renal tissues obtaind from rats treated with CsA for 7 and 14 days and rats treated with olive oil for 14 days, are shown in Table 2. The values of both K_m and V_{max} towards [^{3}H]-5-HT (MAO-A) were similar in all three experimental groups. In addition, no significant differences were observed between values of both K_m and V_{max} towards [^{14}C]-β-PEA (MAO-B) in CsA and olive oil treated rats (Table 3).

COMT activity Maximal COMT activity (V_{max}) in homogenates of renal tissues obtained from rats treated with CsA for 7 or 14 days was significantly higher than that observed in rats treated with olive oil for 14 days (Table 4). However, no significant difference was observed between V_{max} values for COMT in renal tissues of rats treated with CsA for 7 and 14 days. In addition, K_m values for COMT did not differ between the three groups of rats.

Table 4 Kinetic parameters (V_{max}, in pmol mg^{-1} of protein h^{-1}; K_m, in μM) of catechol-*O*-methyltransferase (COMT) activity in homogenates of renal cortex obtained from rats treated with cyclosporine A (CsA; 50 mg kg^{-1} day^{-1}, s.c., $n=4$) for 7 and 14 days and in rats treated with olive oil (0.5 ml kg^{-1} day^{-1}, s.c., $n=4$) for 14 days

	V_{max}	K_m
Olive oil ($n=4$)	2282±230	22.3±1.5
CsA 7 days ($n=4$)	3778±377*	24.1±1.5
CsA 14 days ($n=4$)	3791±539*	22.8±1.3

Values are means±s.e.mean of four determinations per group. Significantly different from corresponding control values (*$P<0.05$) using Tukey-Kramer method.

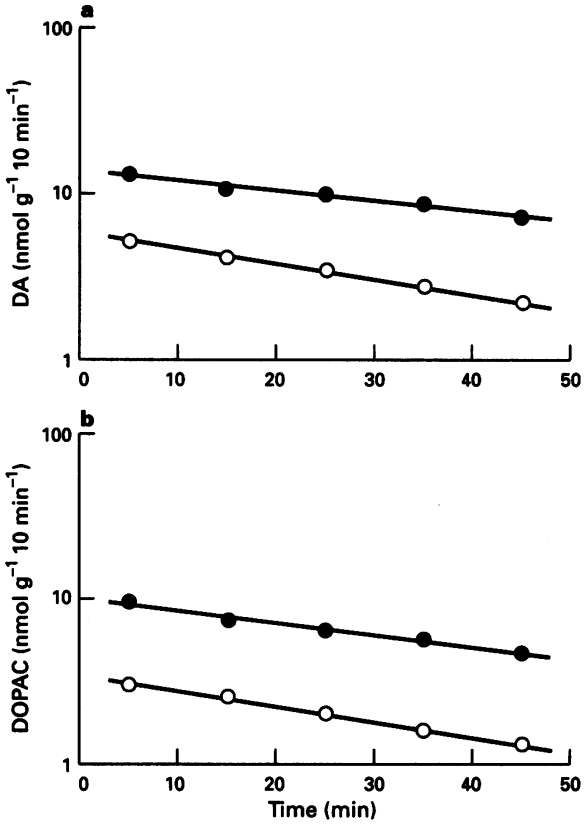

Figure 5 Outflow (in nmol g^{-1} 10 min^{-1}) of (a) dopamine (DA) and (b) DOPAC in perifusate samples of renal cortical slices obtained from rats injected with L-DOPA (30 mg kg^{-1}). The efflux curves are from animals treated for 14 days with CsA (50 mg kg^{-1} day^{-1}, s.c., ●) or the vehicle (0.5 ml kg^{-1} day^{-1}, s.c., ○). Each point represents the mean of four experiments per group; s.e.mean values were less than 10% of respective mean values. Linear coefficient values were in rats treated with CsA: DA, $r=0.9879$, $n=20$; DOPAC, $r=0.9907$, $n=20$; and in rats treated with olive oil: DA, $r=0.9988$, $n=20$, DOPAC, $r=0.9982$, $n=20$.

Table 5 Tissue levels of dopamine (DA) and DOPAC (in nmol g^{-1}) and DOPAC/DA ratios in slices of renal cortex of rats treated with cyclosporine A (CsA; 50 mg kg^{-1} day^{-1}, s.c., $n=4$) for 14 days and in rats treated with olive oil (0.5 ml kg^{-1} day^{-1}, s.c., $n=4$) for 14 days and given L-DOPA (30 mg kg^{-1}, i.p.) 15 min before they were killed

	DA	*DOPAC*	*DOPAC/DA*
Before perifusion			
CsA	315±47*	143±28*	0.45±0.05*
Control	138±23	33±5	0.24±0.02
After perifusion			
CsA	109±16*	42±5*	0.39±0.01*
Control	41±3	11±1	0.26±0.02

The results presented show the levels of DA and DOPAC observed before and after the perifusion of the renal tissues. Values are means±s.e.mean of four determinations per group. Significantly different (*$P<0.01$) from corresponding control values using Student's *t* test.

Outflow of dopamine and DOPAC The accumulation of newly-formed dopamine and its deaminated metabolite DOPAC in cortical tissues of rats treated for 14 days with CsA was three to four times higher than that observed in olive oil-treated animals (Table 5). This was found to occur in renal tissues collected immediately after the rats had been killed and in the tissues collected at the end of the perifusion period. In addition, DOPAC/DA ratios in renal tissues of CsA-treated animals were higher ($P<0.01$) than those observed in the corresponding controls (Table 5).

The outflow of both dopamine and DOPAC in renal tissues of CsA and vehicle-treated animals declined progressively with time and reflected the amine and amine metabolite tissue contents (Figure 5). The slope of decline of dopamine was similar to that for DOPAC in experiments performed with both CsA or vehicle-treated animals (Figure 5). The rate constant of loss (k; min^{-1}) of DOPAC was 2 fold that observed for dopamine in the two experimental groups. No significant difference was observed between the DOPAC/dopamine ratio in the perifusate of renal tissues obtained from CsA and olive oil treated rats (Figure 6). In addition, no significant differences were observed in k values or in the slope of decline of both dopamine and DOPAC between experiments performed with CsA and olive oil-treated animals (Table 6).

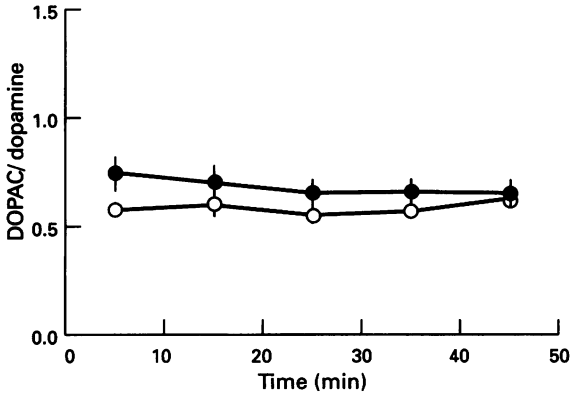

Figure 6 Proportion of DOPAC to dopamine in 10 min perifusate samples collected from renal cortical fragments obtained from rats injected with L-DOPA (30 mg kg^{-1}). The results are from animals treated for 14 days with CsA (50 mg kg^{-1} day^{-1}, s.c., ●) or the vehicle (0.5 ml kg^{-1} day^{-1}, s.c., ○). Each point represents the mean of four experiments per group; vertical lines show s.e.mean.

Table 6 Rate constant of loss (k, min^{-1}) of dopamine and DOPAC in renal cortical slices of rats treated with cyclosporine A (CsA; 50 mg kg^{-1} day^{-1}, s.c., $n=4$) for 14 days and in rats treated with olive oil (0.5 ml kg^{-1} day^{-1}, s.c., $n=4$) for 14 days and given L-DOPA (30 mg kg^{-1}, i.p.) 15 min before they were killed

	Dopamine k	*DOPAC* k
CsA	0.0064 (0.0042; 0.0096)	0.0106 (0.0057; 0.0197)
Control	0.0058 (0.0046; 0.0073)	0.0137 (0.0086; 0.0218)

Values are geometric means with 95% confidence limits of four experiments per group.

Uptake of L-DOPA in isolated tubules Incubation of renal tubules at 4°C in the presence of increasing concentrations of L-DOPA resulted in a concentration-dependent accumulation of the substrate. When the experiments were carried out at 37°C, the accumulation of L-DOPA in renal tubules was greater than that occurring at 4°C, and showed a trend for saturation. The saturable component of L-DOPA uptake was derived from the total amount of L-DOPA accumulated in renal tubules at 37°C with the values obtained in experiments conducted at 4°C subtracted. The V_{max} for the saturable component of L-DOPA uptake in renal tubules from rats treated with CsA was significantly higher than that observed in vehicle-treated animals, restriction of food consumption to a level similar to that observed in CsA treated rats did not change the kinetics of L-DOPA uptake in renal tubules (Table 7). No significant difference was observed between K_m values for the saturable component of L-DOPA uptake in CsA and vehicle-treated rats. The diffusional rate of transfer of L-DOPA was found to be similar in the three experimental groups of rats.

Discussion

The present studies show that chronic administration of CsA produces a decrease in the urinary excretion of sodium and an increase in blood pressure. The antinatriuretic and hypertensive effects of CsA were accompanied by a reduction in daily urinary excretion of dopamine, which appears to result from a reduction in the amount of L-DOPA made available to the kidney. The administration of CsA was not accompanied by changes in either the activity of the enzyme responsible for the formation of dopamine in tubular epithelial cells, AAAD, or the kinetic parameters of the efflux of newly-formed dopamine in renal tissues. It is interesting to mention, however, that the decrease in daily urinary excretion of dopamine during CsA administration was accompanied by an enhanced ability of renal tissues to synthesize the amine when exogenous L-DOPA was provided. It is suggested that this phenomenom might result from an enhanced activity of the uptake process of L-DOPA in renal tubular cells of CsA-treated animals.

A parallel reduction in daily urinary excretion of both dopamine and DOPAC was observed from the 5th-6th day of

Table 7 Kinetic parameters (V_{max}, nmol mg^{-1} protein h^{-1}; K_m, in μM; diffusion constant, in μmol^{-1}) for the uptake of L-DOPA in isolated renal tubules from rats treated with cyclosporine A (CsA; 50 mg kg^{-1} day^{-1}, s.c., $n=4$) for 14 days and in rats treated with olive oil (0.5 ml kg^{-1} day^{-1}, s.c., $n=4$) for 14 days with and without restrictive diet

	V_{max}	K_m	*Diffusion constant*
Olive oil (14 days)	6.39 ± 0.57	272 (249; 298)	0.0012 (0.0010; 0.0016)
Olive oil + restrictive diet (14 days)	7.32 ± 0.18	215 (116; 279)	0.0012 (0.0010; 0.0014)
CsA (14 days)	12.37 ± 0.53*	219 (166; 299)	0.0012 (0.0009; 0.0015)

Values are means ± s.e.mean of four determinations per group. Significantly different from corresponding values observed in animals treated with olive oil (*$P<0.01$) Tukey-Kramer method.

CsA administration onwards. Daily urinary excretion of HVA was also found to be reduced during the 2nd week of CsA administration. These results provide evidence favouring the view that the synthesis of dopamine may have been reduced during CsA treatment and the decrease in dopamine synthesis may have occurred, not only in renal tissues, but also in extrarenal ones. In fact, urinary levels of HVA have been suggested to result mainly from the metabolism of dopamine originating in brain (Kopin, 1985), whereas the urinary excretion of both free dopamine and DOPAC is thought to reflect mainly the excretion of dopamine of renal origin (Wolfowitz *et al.*, 1993; Vieira-Coelho *et al.*, 1994). The administration of olive oil was also found to be accompanied by a reduction in the urinary excretion of HVA and the magnitude of this reduction was found to be similar to that observed in CsA treated animals. In contrast, daily urinary excretion of both dopamine and DOPAC were found to increase during the 2nd week of olive oil administration. These findings suggest that changes in the urinary excretion of HVA observed in CsA-treated rats may not be a CsA-mediated effect, but most probably are related to the properties of the vehicle. This, on the other hand, reinforces the view that the mechanisms responsible for the reduction of the urinary excretion of both dopamine and DOPAC during CsA administration are different from those involved in the reduction of urinary HVA.

Assuming urinary creatinine gives a rough estimation of glomerular filtration rate and since a slight decrease was observed during the second week of CsA administration, it can be hypothesized that differences in the urinary excretion of dopamine and amino metabolites may result from changes in glomerular filtration rate. The fact that the plasma levels of dopamine are too low to change the urinary excretion of the amine (Van Loon, 1983) may argue against this suggestion; furthermore, urinary DOPAC, as well as urinary dopamine, appear to have their origin mainly in the kidney (Soares-da-Silva *et al.*, 1995). Another argument against this hypothesis is that urinary levels of HVA were found similarly reduced in CsA and vehicle-treated rats, in spite of a slight increase in urinary creatinine in olive oil-treated animals.

Plasma levels of L-DOPA were significantly lower in CsA-treated rats than in vehicle-treated animals. This finding may explain the reduction in the urinary excretion of both dopamine and DOPAC during the 2nd week of CsA administration. Urinary dopamine is believed to be derived mainly from the tubular decarboxylation of filtered or circulating L-DOPA, therefore, a decrease in the amount of L-DOPA made available to the cellular compartments where dopamine is produced is expected to result in a decrease in the urinary excretion of the amine. In fact, recent studies provided evidence favouring the view that a large amount (up to 77%) of plasma L-DOPA is removed by the kidneys and about 80% of urinary dopamine derives from circulating L-DOPA. Sympathetic neurones are believed to be a major source of plasma L-DOPA and the rate of noradrenaline synthesis in sympathetic nerve terminals has been suggested to determine the rate at which L-DOPA appears in plasma. A good correlation has been observed between L-DOPA plasma levels and the plasma concentration of 3,4-dihydroxyphenylglycol (DOPEG), the most important metabolite of noradrenaline (Eisenhofer *et al.*, 1989; Goldstein *et al.*, 1991). Assuming that plasma levels of noradrenaline reflect the release of this catecholamine from sympathetic neurones and since CsA-treated rats were found to present significantly higher plasma levels of this catecholamine in comparison with vehicle-treated animals, it might be hypothesized that there was an increased sympathetic tone in CsA-treated rats. The increased sympathetic drive in CsA-treated rats is in agreement with the observations of other authors (Murray & Paller, 1986) and it would be expected to be accompanied by an increased neuronal spillover of L-DOPA into the plasma. However, quite unexpectedly, plasma levels of L-DOPA in CsA-treated animals were found to be markedly reduced. The results presented here offer no explanation for the reduction of L-DOPA plasma levels in CsA-treated animals. However, it can be suggested that CsA might have disturbed the synthetic chain of catecholamine synthesis in sympathetic neurones or the processes controlling the availability of L-DOPA to extraneuronal tissues (Goldstein *et al.*, 1991).

An alternative explanation for the reduction in the urinary excretion of dopamine in CsA-treated animals could be that of a reduced ability of the amine to leave the cellular compartment where the synthesis has occurred. In studies performed in order to examine such a possibility, the kinetics of dopamine and DOPAC outflow were studied in renal tissues obtained from CsA and vehicle-treated rats injected with exogenous L-DOPA. CsA administration was not found to be associated with changes in the kinetic parameters of efflux of either dopamine or DOPAC. However, quite unexpectedly, the tissue accumulation and outflow levels of both dopamine and DOPAC in CsA treated animals were 3 to 4 fold greater than in vehicle-treated rats, reflecting an increased ability of renal tissues from CsA-treated rats to accumulate dopamine from exogenous L-DOPA.

The increased accumulation of dopamine in renal tissues of CsA-treated rats cannot be explained by an increased activity of the enzyme involved in the decarboxylation of L-DOPA, since AAAD activity in homogenates of isolated proximal tubules from CsA-treated rats did not differ from that observed in vehicle-treated animals. Furthermore, the increased ability of renal tissues from CsA-treated rats to accumulate dopamine from exogenous L-DOPA cannot be explained by a decrease in the metabolism of the amine. In fact, the activity of the most important enzyme involved in the metabolism of renal dopamine, type A MAO, did no differ between CsA and vehicle-treated rats; similar findings were observed for type B MAO. By contrast, COMT activity was higher in rats treated with CsA for 7 and 14 days than in control animals. This also does not explain the increased ability of renal tissues from CsA treated rats to accumulate dopamine from exogenous L-DOPA, but is an important argument against the view that the decrease in the urinary excretion of HVA, the methylated metabolite of dopamine, is not related to a decreased activity of renal COMT.

It is possible that the increased ability of renal tissues from CsA-treated rats to accumulate dopamine, when injected with exogenous L-DOPA, might result from an increased uptake of L-DOPA into the tubular epithelial cells. The data presented here show that L-DOPA is taken up into renal tubules of CsA and vehicle-treated rats through saturable and non-saturable mechanisms. The maximum rate of L-DOPA uptake through the saturable transporter, as shown by V_{max} values, was significantly higher in renal tubules from CsA-treated rats than controls, whether or not they have been submitted to diet restriction. In addition, both the affinity for the transporter unit, as shown by K_m values, and the diffusion constant, as determined in experiments performed at 4°C, were similar in CsA and vehicle-treated rats. These results show that L-DOPA is taken up more easily in renal tubular cells from CsA-treated rats than from controls. Assuming this increase in the tubular uptake of L-DOPA was a consequence of a direct effect of CsA, then the urinary excretion of dopamine would follow the increased ability of renal tissues to take up L-DOPA and decarboxylate it to dopamine. However, the urinary excretion of dopamine progressively decreased during CsA administration. Therefore, it might be hypothesized that the increase in the tubular uptake of L-DOPA observed in CsA-treated animals may have resulted from a compensatory mechanism in response to the reduced availability of L-DOPA to renal tubular cells. This is in line with the observation that plasma L-DOPA levels of CsA treated rats were lower than the corresponding controls.

Continuous administration of CsA produced a significant reduction in the urinary excretion of sodium, from the 5th day onwards. This finding agrees with the antinatriuretic effect of CsA described by others, both in man and the rat (Kaskel *et al.*, 1987; Dieperink *et al.*, 1988; Petric *et al.*, 1990). The anti-

natriuretic effect induced by CsA has been suggested to result from reductions in sodium filtered load and also from an increase in sodium reabsorption, namely in proximal tubules. The mechanisms involved in the increase of the proximal reabsorption of sodium during CsA administration are as yet unknown, although it has been suggested to result from haemodynamic effects produced by endogenous vasoconstrictor substances. Dopamine of renal origin has been demonstrated to produce natriuresis as a result of the activation of specific receptors located in proximal renal tubules and a reduced activity of the renal dopaminergic system has been suggested as of importance in the development of some forms of hypertension (Lee, 1993). In experiments reported here, the decrease in daily urinary excretion of sodium in CsA treated rats paralleled the decrease in the urinary excretion of dopamine and DOPAC. These results suggest that the decrease in the renal synthesis of dopamine during CsA administration might constitute an additional factor in the development of the antinatriuretic and hypertensive effects of CsA. The fact that dopamine and dopamine agonists have been found to reverse completely the renal vasoconstriction and antinatriuresis resulting from CsA administration (Brooks *et al.*, 1990) may represent an additional argument in favour of this possibility.

In conclusion, the results presented here show that chronic administration of CsA produced a decrease in the urinary sodium excretion and an increase in blood pressure accompanied by a reduction in daily urinary excretion of dopamine. This appears to result from a reduction in the amount of L-DOPA made available to the kidney and does not involve changes in either the activity of the enzyme responsible for the formation of dopamine in tubular epithelial cells, the kinetic parameters of the efflux of dopamine newly-formed in renal tissues or in its metabolism. The enhanced ability of the renal tissues of CsA-treated animals to synthesize the dopamine when exogenous L-DOPA is provided, appears to result from an enhanced activity of the uptake process of L-DOPA in renal tubular cells.

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References

BERTORELLO, A., HÖKFELT, T., GOLDSTEIN, M. & APERIA, A. (1988). Proximal tubule Na^+-K^+-ATPase activity is inhibited during high-salt diet: evidence for DA-mediated effect. *Am. J. Physiol.*, **254**, F795–F801.

BRADFORD, M. (1976). A rapid method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal. Biochem.*, **72**, 248–254.

BROOKS, D., DRUTZ, D. & RUFFOLO, R. (1990). Prevention and complete reversal of cyclosporine A induced renal vasoconstriction and nephrotoxicity in the rat by fenoldopam. *J. Pharmacol. Exp. Ther.*, **254**, 375–379.

CONTE, G., SABBATINI, M., NAPODANO, P., DE NICOLA, L., GIGLIOTTI, G., FUIANO, G., TESTA, A., RUSSO, D., ESPOSITO, C., LIBETTA, F., DAL CANTON, A. & ANDEUCCI, V. (1988). Dopamine counteracts the acute renal effects of cyclosporine in normal subjects. *Transplant Proc.*, **3**, 563–567.

DAPRADA, M., KETTLER, R., ZURCHER, G., SCHAFFNER, R. & HAEFLEY, W.E. (1987). Inhibition of decarboxylase and levels of dopa and 3-O-methyldopa: A comparative study of benserazide versus carbidopa in rodents and of madopar standard versus madopar HBS in volunteers. *Eur. Neurol.*, **27** (suppl. 1), 9–20.

DIEPERINK, H., LEYSSAC, P., STARKLINT, H. & KEMP, E. (1988). Long term cyclosporin nephrotoxicity in the rat: effects on renal function and morphology. *Nephrol. Dial. Transplant*, **3**, 317–326.

EISENHOFTER, G., MEREDITH, I. & FERRIER, C. (1989). Increased plasma dihydroxyphenylalanine and total body and regional noradrenergic activity in humans. *J. Clin. Endocrinol. Metab.*, **68**, 247–255.

FELDER, R.A., FELDER, C.C., EISNER, G.M. & JOSE, P.A. (1989). The dopamine receptor in adult and maturing kidneys. *Am. J. Physiol.*, **257**, F315–F327.

FERNANDES, M.H. & SOARES-DA-SILVA, P. (1992). Type A and B monoamine oxidase activities in the human and rat kidney. *Acta Physiol. Scand.*, **145**, 363–367.

GOLDSTEIN, D., CANNON, R. & QUYUMI, I. (1991). Regional extraction of circulating norepinephrine, dopa, and dihydroxyphenylglycol in humans. *J. Autonom. Nerv. Syst.*, **34**, 17–36.

ISHIKAWA, A., SAKAGUCHI, M., NAKANO, M., USHIYAMA, T., OHTA, N., OHTAWARA, Y., TAJIMA, A., KAWABE, K. & ASO, Y. (1991). Cyclosporine induced nephrotoxicity and justaglomerular apparatus in mice. *Nippon Hinyokika Gakkai Zasshi*, **82**, 1286–1291.

KASKEL, F., DEVARAJAN, P., ARBEIT, L., PARTIN, J. & MOORE, L. (1987). Cyclosporine nephrotoxicity: sodium excretion, autoregulation and angiotensin II. *Am. J. Physiol.*, **252**, F733–F742.

KON, V., SUGIURA, M., INAGAMI, T., HARVIE, B.R., ICHIKAWA, I. & HOOVER, R.L. (1990). Role of endothelin in cyclosporine-induced glomerular dysfunction. *Kidney Int.*, **37**, 1487–1491.

KOPIN, I.J. (1986). Catecholamine metabolism: basic aspects and clinical significance. *Pharmacol. Rev.*, **37**, 334–364.

KOPP, J.B. & KLOTMAN, P.E. (1990). Cellular and molecular mechanisms of cyclosporin nephrotoxicity. *J. Am. Soc. Nephrol.*, **1**, 162–179.

LEE, M.R. (1993). Dopamine and the kidney-ten years on. *Clin. Sci.*, **84**, 357–375.

MURRAY, B. & PALLER, M. (1986). Benefitial effects of renal denervation and prazosin on GFR and renal blood flow after cyclosporine in rats. *Clin. Nephrol.*, **25**, 193–198.

NEAME, K. & RICHARDS, T. (1972) *Elementary Kinetics of Membrane Carrier Transport*. Oxford: Blackwell Scientific Publications.

PESTANA, M., FERNANDES, M.H. & SOARES-DA-SILVA, P. (1993). Urinary excretion of dopamine and amine metabolites during chronic administration of cyclosporine A. *J. Am. Soc. Nephrol.*, **3**, P458 (abstract).

PESTANA, M., FERNANDES, M.H. & SOARES-DA-SILVA, P. (1994). Urinary excretion of dopamine and reduction in urinary dopamine excretion in an experimental model of cyclosporine A (CsA) nephrotoxicity. *Kidney Int.*, **45**, P290 (abstract).

PESTANA, M. & SOARES-DA-SILVA, P. (1994a). Urinary excretion of dopamine and renal handling of newly-formed dopamine in a rat model of cyclosporine A induced nephrotoxicity. *J. Auton. Pharmacol.*, **14**, 25 (abstract).

PESTANA, M. & SOARES-DA-SILVA, P. (1994b). The renal handling of dopamine originating from L-DOPA and γ-glutamyl-L-DOPA. *Br. J. Pharmacol.*, **112**, 417–422.

PETRIC, R., FREEMAN, D., WALLACE, C., MCDONALD, J., STILLER, C. & KEOWN, P. (1990). Modulation of experimental cyclosporine A nephrotoxicity by inhibition of thromboxane synthesis. *Transplantation*, **50**, 558–563.

REMUZZI, G. & BERTANI, T. (1989). Renal vascular and thrombotic effects of cyclosporine. *Am. J. Kidney Dis.*, **13**, 261–272.

SABBATINI, M., ESPOSITO, C., DE NICOLA, L., UCCELLO, F., ALTOMONTE, M., CONTE, G., DAL CANTON, A. & ANDREUCCI, V.E. (1989). Reversibility of acute cyclosporin nephrotoxicity by dopamine. Micropuncture study. *Nephrol. Dial. Transplant.*, **4**, 327–333.

SIEGL, H. & RYFFEL, B. (1982). Effect of cyclosporin on renin-angiotensin-aldosterone system. *Lancet*, **i**, 1274.

SIRAGY, H.M., FELDER, R.A., HOWELL, N.L. & CAREY, R.M. (1989). Evidence that intrarenal dopamine acts as a paracrine substance at the renal tubule. *Am. J. Physiol.*, **257**, F469–F477.

SOARES-DA-SILVA, P. (1994). Source and handling of renal dopamine: Its physiological importance. *News Physiol. Sci.*, **9**, 128–134.

SOARES-DA-SILVA, P., FERNANDES, M.H. & PESTANA, M. (1993). Studies on the role of sodium on the synthesis of dopamine in the rat kidney. *J. Pharmacol. Exp. Ther.*, **264**, 406–414.

SOARES-DA-SILVA, P., FERNANDES, M.H. & PINTO-DO-Ó, P.C. (1994). Cell inward transport of L-DOPA and 3-O-methyl-L-DOPA in rat renal tubules. *Br. J. Pharmacol.*, **112**, 611–615.

SOARES-DA-SILVA, P., PESTANA, M., VIEIRA-COELHO, M.A., FERNANDES, M.H. & ALBINO-TEIXEIRA, A. (1995). Assessment of renal dopaminergic system activity in the nitric oxide-deprived hypertensive rat model. *Br. J. Pharmacol.*, **114**, 1403–1413.

SOKAL, R. & ROHLF, F. (1981). Biometry. *The Principles and Practice of Statistics in Biological Research*. New York: Freeman and Company.

STURROCK, N.D.C. & STRUTHERS, A.D. (1994). Hormonal and other mechanisms involved in the pathogenesis of cyclosporyn-induced nephrotoxicity and hypertension in man. *Clin. Sci.*, **86**, 1–9.

TRENDELENBURG, U., BöNISCH, H., GRAEFE, K.H. & HENSELING, M. (1979). The rate constant of the efflux of metabolites of catecholamines and phenylethylamines. *Pharmacol. Rev.*, **31**, 179–213.

VAN LOON, G. (1983). Plasma dopamine: regulation and significance. *Fed. Proc.*, **42**, 3012–3018.

VIEIRA-COELHO, M.A., FERNANDES, M.H. & SOARES-DA-SILVA, P. (1994). In vivo effects of the monamine oxidase inhibitors Ro 41-1049 and Ro 19-6327 on the production and fate of renal dopamine. *J. Neural. Trans.*, (Suppl 41), 365–370.

WHITING, P.H., PROPPER, D.J., SIMPSON, J.G., MCKAY, J., JONES, M.C. & CATTO, G.R. (1988). The use of lithium clearance measurements to assess renal tubular function in experimental and clinical cyclosporine nephrotoxicity. *Transplant. Proc.*, **20** (Suppl 3), 675–680.

WOLFOVITZ, E., GROSSMAN, E., FOLIO, C., KEISER, H.R., KOPIN, I. & GOLDSTEIN, D.S. (1993). Derivation of urinary dopamine from plasma dihydroxyphenylalanine in humans. *Clin. Sci.*, **84**, 549–557.

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