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Role of endothelin ET_A receptors in the hypertension produced by 4-day L-nitroarginine methyl ester and cyclosporine treatment

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Abstract

Studies were designed to examine the influence of endothelin type A receptor (ET_A) blockade on the hypertensive and renal response to 4 day treatment with the nitric oxide (NO) synthase inhibitor, L-nitroarginine methyl ester (L-NAME), and cyclosporine. In the first series of experiments, male Sprague–Dawley rats maintained in metabolic cages were given the L-NAME at 50 mg/100 ml in the drinking water with or without the ET_A receptor antagonist, A-127722 (2-(4-methoxyphenyl)-4-(1,3-benzodioxol-5-yl)-1-[[(dibutyl amino)carbonyl]methyl]-pyrrolidine-3-carboxylic acid; 10 mg kg^{-1} day^{-1}), in either food or water. After 4 days, tail cuff estimates of systolic arterial pressure and a blood sample were obtained. A-127722 prevented the rise in tail cuff pressure produced by L-NAME. In the second series of experiments, rats were given cyclosporine at 20 mg kg^{-1} day^{-1} (i.p.) or cyclosporine plus L-NAME. Control groups were given olive oil (1 ml/kg i.p.). Treatment with cyclosporine alone had no effect on tail cuff pressure or plasma creatinine, but significantly attenuated the normal increase in body weight over the 4-day period. The combination of cyclosporine plus L-NAME significantly increased both tail cuff pressure and plasma creatinine and completely prevented any gain in body weight. L-NAME plus olive oil produced a significant increase in tail cuff pressure but changed no other variable. To determine the role of ET_A receptors in this setting, a final series of rats were treated with cyclosporine and L-NAME along with A-127722 in the drinking water. ET_A receptor blockade had no effect on the increase in tail cuff pressure, plasma creatinine or the attenuated weight gain. These results indicate that subchronic (4-day) L-NAME hypertension is maintained, at least in part, by activation of ET_A receptors although the hypertensive and renal response to combined L-NAME and cyclosporine treatment does not involve ET_A receptor activation. These results support the hypothesis that endothelial dysfunction predisposes the kidney to functional derangements associated with cyclosporine. © 1998 Elsevier Science B.V.

Keywords: Cyclosporine; Endothelin; Endothelin ET_A receptors; Nitric oxide (NO)

1. Introduction

The endothelial-derived relaxing factor, nitric oxide (NO), appears to play a significant role in the regulation of renal and systemic hemodynamics. Acute inhibition of NO synthase, the enzyme responsible for production of NO from L-arginine and O_2, produces an increase in vascular resistance and blood pressure. Furthermore, it has been reported that chronic inhibition of NO synthase will produce a sustained hypertension in otherwise normotensive rats or dogs (Ribeiro et al., 1992; Salazar et al., 1992; Pollock et al., 1993). In addition to removing the vasodilating actions of NO, administration of the NO synthase inhibitor, L-nitroarginine methyl ester (L-NAME), may also result in an activation or potentiation of various vasoconstrictors.

Endothelin has been reported to be involved in the acute response to NO synthase inhibition. ET_A-selective or non-selective ET_A/ET_B receptor antagonists can attenuate the acute hypertensive and vasoconstrictor response to L-

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NAME (Qui et al., 1995; Richard et al., 1995; Thompson et al., 1995). These studies suggest that endothelin plays an important role in the hypertension and vasoconstrictor response to administration of NO synthase inhibitors. However, it is unclear whether or not endothelin plays a role in more chronic situations since endothelin receptor antagonists appear to have little effect on arterial pressure in rats chronically treated with the NO synthase inhibitors (Fujita et al., 1995; Moreau et al., 1997; Sventek et al., 1997). Thus, it is possible that endothelin plays a role only in the early phase of chronic NO synthase inhibition.

Endothelial cell dysfunction is thought to play a role in the development of hypertension and renal dysfunction that sometimes occurs during immunosuppressant therapy with cyclosporine. Possible mechanisms of cyclosporine-induced nephrotoxicity include an underlying inability to maintain sufficient vasodilator capacity through a lack of NO production and/or over-production of endothelin (Fogo et al., 1992; Hunley et al., 1995). We hypothesize that NO serves a protective role in attenuating cyclosporine-induced vasoconstriction and that an inability to produce NO increases cyclosporine-induced effects. Furthermore, when vasodilating ability is diminished, endothelin may serve to play a role in the vasoconstrictor response to cyclosporine.

The purpose of the current study was to examine the role of ET_A receptors in mediating the effects of NO synthase inhibition with L-NAME during subchronic cyclosporine treatment. More specifically, experiments were designed to (1) determine the role of ET_A receptors in the response to subchronic inhibition of NO synthase on blood pressure and renal function, (2) determine the effect of NO synthase inhibition on cyclosporine-induced effects, and (3) determine the effect of ET_A receptor blockade in rats treated with L-NAME plus cyclosporine. ET_A receptor blockade was achieved using the recently developed ET_A receptor antagonist, A-127722 (2-(4-methoxyphenyl)-4-(1,3-benzodioxol-5-yl)-1-[[(dibutyl amino) carbonyl]methyl]-pyrrolidine-3-carboxylic acid; Opgenorth et al., 1996; Winn et al., 1996).

2. Materials and methods

In all experiments, male Sprague–Dawley rats (Charles River, Cambridge, MA) weighing 200–250 g were placed in metabolic cages for 1–2 days prior to obtaining a baseline 24-h urine sample. Baseline estimates of systolic arterial pressure were then obtained using the tail cuff method as described below. Rats were maintained in metabolic cages for an additional period of 4 days while undergoing the treatment regimens as described below. At the end of the 4-day treatment period, rats were anesthetized with sodium pentobarbital (Abbott Laboratories, North Chicago, IL, 65 mg/kg i.p.) and an arterial blood sample obtained from the abdominal aorta.

2.1. Animal protocols

2.1.1. Series 1: L-NAME + A-127722

Five groups of rats were studied to determine the effect of the ET_A receptor antagonist, A-127722, on the hypertensive response to subchronic treatment with the NO synthase inhibitor, L-NAME. The first two groups were given L-NAME (Sigma Chemical, St. Louis, MO) in the drinking water at a concentration of 50 mg/100 ml (approximately 65 mg kg^{-1} day^{-1}) or L-NAME + A-127722 in drinking water at 8 mg/100 ml (approximately 10 mg kg^{-1} day^{-1}) ($n = 6$ in both groups). Since the combination of both L-NAME + A-127722 in the drinking water reduced water intake in these rats, several other groups were studied to determine whether the decreased intake had any effect on the outcome of the experiment. A third group of rats was given L-NAME without antagonist to drink at a lower concentration (approximately 40 mg kg^{-1} day^{-1}) adjusted to match the L-NAME dose of the original group drinking L-NAME + A-127722 ($n = 6$). A fourth group of rats was given L-NAME + A-127722 in the drinking water but the concentration of L-NAME was increased to compensate for lower water intake and deliver a higher dose of 65 mg kg^{-1} day^{-1}; A-127722 was given at 8 mg/100 ml ($n = 6$). A final group of rats was given L-NAME in the drinking water at a concentration of 50 mg/100 ml with A-127722 being mixed with the food at a concentration to deliver 10 mg kg^{-1} day^{-1} ($n = 5$).

2.1.2. Series 2: Cyclosporine + L-NAME

Experiments were conducted to determine the effect of cyclosporine (Sandoz Pharmaceuticals, East Hanover, NJ) on blood pressure and renal function during NO synthase inhibition with L-NAME. Following baseline measurements, cyclosporine was administered at 20 mg kg^{-1} day^{-1} by i.p. injection. Control groups received olive oil (1 ml/kg, i.p.). Additional groups of cyclosporine-treated and olive oil-treated rats were given L-NAME in the drinking water at a concentration of 50 mg/100 ml to deliver approximately 65 mg kg^{-1} day^{-1} ($n = 6$ in all groups).

2.1.3. Series 3: cyclosporine / L-NAME + A-127722

This series of experiments was conducted to determine the effect of ET_A receptor blockade in rats treated with cyclosporine and L-NAME. Rats were given cyclosporine and L-NAME in an identical fashion as Series 2. In addition, a separate group of rats were given the ET_A-selective receptor antagonist, A-127722, in drinking water at a concentration to deliver approximately 10 mg kg^{-1} day^{-1} (8 mg/100 ml beginning 3 days prior to starting cyclosporine and L-NAME treatment ($n = 6$ in both groups).

2.2. Measurement of tail cuff pressure

Rats were placed in a warmed restraining chamber and an inflatable cuff placed around the tail. A programmed electro-sphygmomanometer (Narco Bio-Systems, Austin, TX) was used to inflate and deflate the cuff and arterial pressure pulsations were detected using a pneumatic pulse transducer (Narco Bio-Systems, Austin, TX). Automatic collection of tail cuff pressure data was performed using a MacLab analog to digital converter (ADInstruments, Milford, MA) connected to a Macintosh SE/30 computer. Ten readings were taken for each rat with the highest and lowest being discarded. Readings with weak pressure pulsations or a high degree of noise produced by animal movement were also not used. The average of the remaining readings (a minimum of 5) was taken to generate a tail cuff pressure value for a given rat.

2.3. Urine and plasma analytical methods

Urinary electrolytes were measured using ion-selective electrodes (EL-ISE®, Beckman Instruments, Brea, CA). Urinary protein concentration, plasma and urine creatinine concentrations, and blood urea nitrogen were measured by colorimetric methods using the Abbott VP System (Abbott Laboratories, Abbott Park, IL). Standard formulas were used to calculate urinary excretion rates.

Total urinary nitrate and nitrite concentration (NO_x) was measured by the Griess reaction and used as a rough index of renal NO synthase activity. In 96-well microtiter plates, 50 μl of diluted urine or nitrate standards (0–75 μM) were incubated with 10 μl each of 1.0 U/ml nitrate reductase (Boehringer Mannheim, Indianapolis, IN), 1.0 mM NADPH (Boehringer Mannheim), and 0.1 mM FAD (Sigma). 20 μl of phosphate-buffered saline was then added to each well for a total volume of 100 μl followed by a 1-h incubation at room temperature. 200 μl of freshly prepared Griess reagent (0.05% *N*-(1-naphthyl)ethylenediamine) in 0.5% sulfanilamide; Sigma) or 0.5% sulfanilamide (blank controls) was then added before a 10-min incubation at room temperature. Absorbance was read at 540 nm. The absorbance of each sample was corrected using the corresponding blank and the total NO_x concentration (in μM) determined from the standard curve.

Statistical analysis to determine significant differences ($P < 0.05$) included Student's t-test for paired data, analysis of variance for repeated measures to determine day to day changes within a group, and one-way analysis of variance for comparison of group means (Statview II, Abacus Concepts, Berkeley, CA). Data sets were subjected to only a single statistical analysis appropriate to the experimental design. Animal protocols were approved by the Institutional Animal Care and Use Committee and are in full compliance with the NIH Guide.

3. Results

3.1. Series 1: L-NAME + A-127722

Treatment of rats with L-NAME in the drinking water ($\sim$ 65 mg kg^{-1} day^{-1}) for 4 days resulted in a significant increase in tail cuff pressure of about 25 mmHg (Fig. 1); tail cuff pressure was 106 ± 2 mmHg before and 130 ± 5 mmHg after L-NAME treatment. This increase was associated with a small decrease in food intake with no change in water intake (Table 1). A decrease in sodium excretion was noted in the initial 2 days of L-NAME treatment. Similar to our previous report (Pollock et al., 1993), water excretion was significantly increased by L-NAME. Simultaneous blockade of ET_A receptors by co-administration of A-127722 in drinking water significantly inhibited the hypertensive effect of L-NAME; tail cuff pressure was increased by about 12 mmHg (93 ± 4 mmHg before and 106 ± 5 mmHg after L-NAME + A-127722). However, in rats given both L-NAME and A-127722, water intake was significantly reduced thus decreasing the effective dose of both L-NAME and A-127722; urine volume was correspondingly reduced. ET_A receptor blockade had little effect on L-NAME-induced changes in food intake and sodium excretion.

To determine whether reduced L-NAME intake accounted for the attenuated hypertension in the L-NAME + A-127722 group, three additional treatment groups were studied (Fig. 1). First, rats were given L-NAME alone but at a lower concentration in order to deliver the same amount of L-NAME as the previous L-NAME + A-127722 group ($\sim$ 40 mg kg^{-1} day^{-1}). After 4 days of treatment with the lower dose of L-NAME, tail cuff pressure was significantly increased by roughly the same amount as the group receiving the higher dose of L-NAME, i.e. about 25 mmHg. Tail cuff pressure was 105 ± 5 mmHg before and

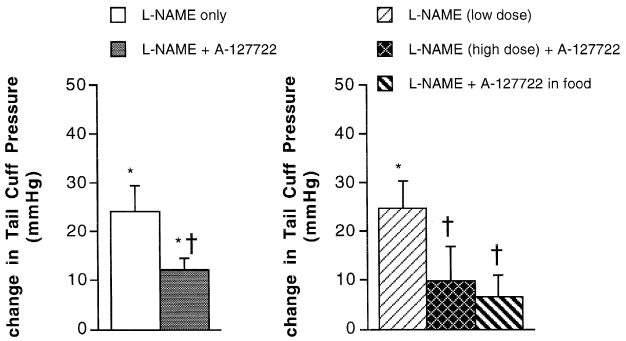

Fig. 1. Change in tail cuff pressure in conscious rats following 4 days treatment with L-NAME and A-127722 in the drinking water. Five separate groups of rats received (1) L-NAME only (50 mg/100 ml), (2) L-NAME (50 mg/100 ml) plus A-127722 (24 mg/100 ml), (3) L-NAME at a lower concentration (31 mg/100 ml), (4) L-NAME at a higher concentration (82 mg/100 ml) plus A-127722, and (5) L-NAME (50 mg/100 ml) plus A-127722 in food (36 mg/100 g; $n = 6$ in all groups). * denotes $P < 0.05$ compared to baseline and † denotes $P < 0.05$ vs. L-NAME only at either dose.

Table 1
Intake and excretion variables in rats treated with L-NAME and A-127722

Group	Day				
	0	1	2	3	4
Food intake (g / day)					
L-NAME	26.5 ± 0.6	20.5 ± 0.8[a]	24.7 ± 1.1	22.2 ± 0.7[a]	22.6 ± 1.2[a]
L-NAME + A-127722	26.3 ± 0.5	18.8 ± 1.2[a]	21.1 ± 0.9[a]	21.7 ± 1.7[a]	22.9 ± 1.2[a]
L-NAME (low dose)	24.4 ± 1.1	19.8 ± 0.5[a]	22.8 ± 0.7	21.6 ± 0.8[a]	21.8 ± 1.2[a]
L-NAME (high dose) + A-127722	25.3 ± 1.2	17.0 ± 0.5[a]	19.8 ± 1.2[a]	21.2 ± 1.2[a]	21.2 ± 1.0[a]
L-NAME + A-127722 via food	25.0 ± 1.2	19.2 ± 1.1[a]	20.4 ± 0.6[a]	20.7 ± 1.1[a]	21.3 ± 0.5[a]
Water intake (ml / day)					
L-NAME	33.5 ± 1.9	29.3 ± 1.8[a]	33.6 ± 2.9	33.8 ± 3.3	35.5 ± 3.0
L-NAME + A-127722	31.6 ± 1.7	15.9 ± 2.7[a]	20.1 ± 2.0[a]	25.0 ± 1.6[a]	21.5 ± 1.3[a]
L-NAME (low dose)	32.3 ± 2.3	28.9 ± 1.7	28.9 ± 1.1	27.0 ± 1.4[a]	28.2 ± 1.9
L-NAME (high dose) + A-127722	34.4 ± 1.9	18.5 ± 2.5[a]	24.0 ± 1.8[a]	23.2 ± 1.4[a]	22.0 ± 1.3[a]
L-NAME + A-127722 via food	29.8 ± 1.0	28.2 ± 1.2	28.3 ± 1.7	26.7 ± 1.0	26.4 ± 0.7
Urine volume (ml / day)					
L-NAME	13.8 ± 1.4	12.7 ± 1.4	18.0 ± 2.7[a]	19.5 ± 3.3[a]	20.2 ± 3.8[a]
L-NAME + A-127722	13.5 ± 1.1	6.5 ± 0.4[a]	7.7 ± 0.5[a]	7.6 ± 0.4[a]	7.6 ± 0.4[a]
L-NAME (low dose)	10.9 ± 1.0	9.6 ± 1.3	12.7 ± 1.1	13.5 ± 1.3[a]	12.4 ± 1.6
L-NAME (high dose) + A-127722	12.1 ± 0.9	7.1 ± 0.5[a]	8.7 ± 0.7[a]	9.3 ± 0.5[a]	8.1 ± 0.7[a]
L-NAME + A-127722 via food	9.5 ± 0.6	8.3 ± 0.5	10.5 ± 1.3	12.3 ± 0.7[a]	11.1 ± 0.4
Sodium excretion (mol / day)					
L-NAME	1.66 ± 0.04	1.29 ± 0.03[a]	1.27 ± 0.11[a]	1.42 ± 0.05	1.44 ± 0.12
L-NAME + A-127722	1.79 ± 0.09	1.44 ± 0.13[a]	1.23 ± 0.14[a]	1.16 ± 0.13[a]	1.78 ± 0.11
L-NAME (low dose)	1.87 ± 0.04	1.48 ± 0.08[a]	1.38 ± 0.10[a]	1.43 ± 0.08[a]	1.48 ± 0.09[a]
L-NAME (high dose) + A-127722	1.91 ± 0.05	2.50 ± 0.34[a]	1.70 ± 0.13	2.36 ± 0.27[a]	2.81 ± 0.32[a]
L-NAME + A-127722 via food	1.89 ± 0.12	1.35 ± 0.16[a]	1.19 ± 0.10[a]	1.31 ± 0.17[a]	1.61 ± 0.19

[a] Denotes $P < 0.05$ vs. day 0.

129 ± 4 mmHg after L-NAME. In another group of rats, both L-NAME and A-127722 were again administered via the drinking water. In this case, however, the concentration of L-NAME was adjusted to compensate for the reduced water intake, i.e., the actual L-NAME dose was similar to the initial group receiving L-NAME alone (~ 65 mg kg^{-1} day^{-1}). Again, the hypertensive effect of L-NAME was largely prevented by the ET_A receptor antagonist, A-127722. Tail cuff pressure was 115 ± 8 mmHg before and 124 ± 4 mmHg after L-NAME + A-127722 treatment. In a final group of rats, L-NAME was given in the drinking water at a concentration that delivered ~ 65 mg kg^{-1} day^{-1} while A-127722 was mixed with the powdered food to deliver similar concentrations as groups given A-127722 in the drinking water. A-127722 once again significantly diminished the rise in tail cuff pressure produced by L-NAME with tail cuff pressure being 98 ± 5 mmHg during baseline and 105 ± 7 mmHg after combined treatment with L-NAME and A-127722.

3.2. Series 2: Cyclosporine + L-NAME

Four-day treatment of rats with cyclosporine alone had no effect on tail cuff pressure, plasma creatinine, blood-urea nitrogen, or creatinine clearance compared to olive-oil-

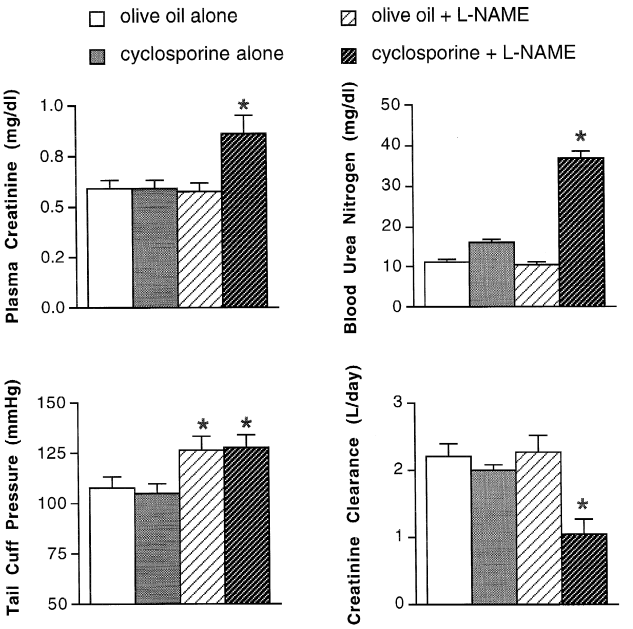

Fig. 2. Plasma creatinine, blood urea nitrogen, tail cuff pressure and creatinine clearance in rats treated for 4 days with either cyclosporine (20 mg kg^{-1} day^{-1} i.p.) or olive oil (1 ml/kg i.p.) with or without L-NAME in drinking water (50 mg/100 ml). * denotes $P < 0.05$ vs. cyclosporine alone.

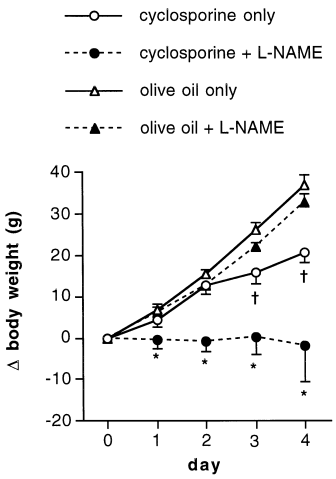

Fig. 3. Change in body weight over a 4-day period in rats treated with either cyclosporine or olive oil with or without L-NAME. * denotes $P < 0.05$ vs. cyclosporine alone and † denotes $P < 0.05$ vs. olive oil alone.

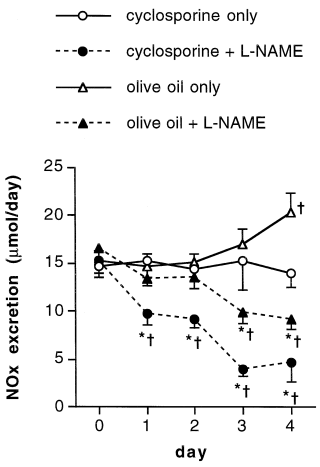

Fig. 5. Total urinary nitrate and nitrite (NO_x) excretion in rats treated with either cyclosporine or olive oil with or without L-NAME. * denotes $P < 0.05$ vs. without L-NAME and † denotes $P < 0.05$ vs. day 0.

treated controls (Fig. 2). However, when L-NAME was administered in the drinking water of rats being treated with cyclosporine, significant increases in tail cuff pressure, plasma creatinine, and blood urea nitrogen were observed as well as a significant decrease in creatinine clearance compared to rats treated with either olive oil or cyclosporine alone. Administration of L-NAME alone resulted in an elevation in tail cuff pressure while none of the indices of renal function were affected.

The combination of cyclosporine plus L-NAME prevented the normal increase in body weight observed over the 4-day period of treatment (Fig. 3). Rats receiving cyclosporine alone had normal increases in body weight over the first two days of treatment but gained significantly less weight than olive oil controls on days 3 and 4. L-NAME had no effect on body weight in rats being treated with olive oil.

Food and water intake were significantly less in the cyclosporine and L-NAME group compared to the other groups (Fig. 4). Food intake was significantly less in cyclosporine-treated rats on days 3 and 4 compared to olive-oil-treated rats which accounts for their attenuated gain in body weight. Sodium excretion roughly patterned food intake. However, urine volume was similar among all groups despite a significant reduction in water intake in the cyclosporine + L-NAME group indicating a significant negative water balance in this group (Fig. 4).

Urinary nitrate/nitrite (NO_x) excretion was reduced in the two groups receiving L-NAME indicating a reduction in NO production (Fig. 5). NO_x excretion was reduced to a larger extent in the cyclosporine + L-NAME group reflecting the decreased dietary nitrate as a consequence of the decrease in food intake.

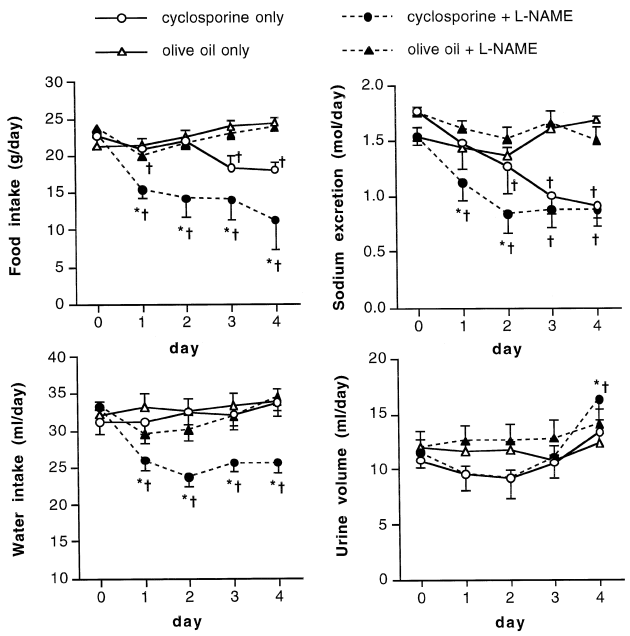

Fig. 4. Food and water intake and sodium and water excretion in rats treated with either cyclosporine or olive oil with or without L-NAME. * denotes $P < 0.05$ vs. without L-NAME and † denotes $P < 0.05$ vs. day 0.

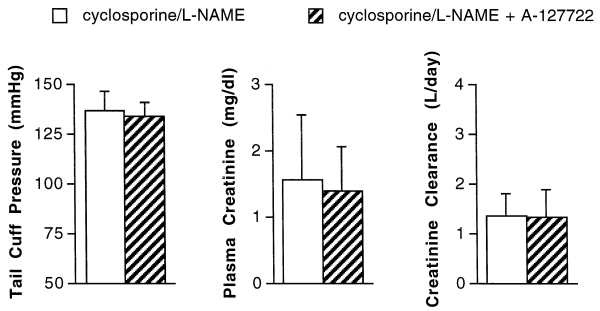

Fig. 6. Tail cuff pressure, plasma creatinine and creatinine clearance in conscious rats treated with cyclosporine (20 mg kg^{-1} day^{-1} i.p.) and L-NAME (50 mg/100 ml drinking water) for 4 days. A separate group of rats also received the ET_A receptor antagonist, A-127722 (8 mg/100 ml) in drinking water.

3.3. Series 3: Cyclosporine / L-NAME + A-127722

Administration of A-127722 had no effect on tail cuff pressure, plasma creatinine or creatinine clearance in rats also receiving cyclosporine and L-NAME (Fig. 6). Similarly, intake and excretory variables were unaffected by A-127722 in rats being treated with cyclosporine and L-NAME (data not shown).

4. Discussion

Endothelial dysfunction is now considered an important component of numerous vascular disorders and may be involved in determining the extent of nephrotoxicity produced by cyclosporine. Immunosuppressant therapy with cyclosporine is often limited by hypertension and renal vasoconstriction which can lead to the development of renal failure. Therefore, we examined the role of the endothelial-derived factors, NO and endothelin, in regulating arterial pressure and renal function and how these actions may influence the systemic and renal response to cyclosporine. The vascular effects of cyclosporine may be the result of several alterations in endothelial function including an inability to produce sufficient amounts of NO, an overproduction of endothelin, or an imbalance between these two opposing agents.

The present study was conducted based on several objectives. First, we sought to determine the role of endothelin, more specifically, ET_A receptors, in the hypertensive and renal response to subchronic inhibition of NO synthase by determining the influence of the ET_A receptor antagonist, A-127722, on the response to the NO synthase inhibitor, L-NAME. We have previously reported that the ET_A receptor antagonist, A-127722, has no effect on intake or excretory variables in untreated rats over a 4-week period (Pollock and Polakowski, 1997). Secondly, we determined the influence of removing NO synthase activity on blood pressure and renal responses to cyclosporine. And finally, experiments were designed to examine the role of ET_A receptors in the responses to cyclosporine during NO synthase blockade.

A role for endothelin in producing the hypertension associated with subchronic NO synthase inhibition is supported by our initial series of experiments demonstrating that ET_A receptor blockade significantly attenuated the pressor response to 4 days of L-NAME treatment. These results suggest an interaction between endothelin and NO systems and support the hypothesis that removing NO generating ability results in a hypertension that is maintained, at least in part, by activation of ET_A receptors. These observations are qualitatively similar to our previous findings that inhibition of the renin–angiotensin system reverses L-NAME hypertension (Pollock et al., 1993). Thus it appears as though removal of NO serves to stimulate multiple vasoconstrictor systems to maintain vascular tone.

Several laboratories have reported that the systemic and renal hemodynamic response to acute NO synthase inhibition can be attenuated with ET_A receptor antagonists in the rat (Qui et al., 1995; Richard et al., 1995; Thompson et al., 1995). In contrast, acute ET_A receptor blockade had no effect on rats made hypertensive by chronic NO synthase inhibition (Fujita et al., 1995). More recently, Moreau and colleagues observed that bosentan, a non-selective ET_A/ET_B receptor antagonist, had little effect on hypertension produced by L-NAME treatment for 6 weeks (Moreau et al., 1997). However, the antagonist did significantly attenuate the development of hypertension during the first week of treatment. Sventek et al. (1997) have reported that simultaneous treatment with L-NAME and A-127722 for 3 weeks had absolutely no effect on tail cuff pressure even during the initial week of treatment. It is not readily apparent why their results do not agree with those of the present study but it may be related to their use of higher doses of L-NAME. It is also possible that the source and/or strain of rat could influence the degree to which endothelin plays a role in L-NAME hypertension. Nonetheless, there now appears to be sufficient evidence to suggest that endothelin plays an important role only during the developing phase of L-NAME hypertension.

The next question we addressed was whether a deficiency in NO-generating ability would compromise the ability of the kidney to prevent cyclosporine-induced nephrotoxicity. Daily treatment of rats with cyclosporine at 20 mg/kg had no discernible effect on blood pressure or renal excretory function over a 4-day period. Further, rats receiving L-NAME without cyclosporine developed a significant degree of hypertension over the 4-day period, but again, without any associated changes in renal function. In marked contrast, cyclosporine along with L-NAME produced increases in plasma creatinine and blood-urea nitrogen indicating that NO synthase inhibition enhances cyclosporine-induced nephrotoxicity. In acute infusion studies, Bobadilla et al. (1994) provided evidence that renal vasoconstriction produced by cyclosporine is not specifically mediated by NO deficiency. These investigators observed enhanced renal vasoconstrictor responses to L-NAME in rats chronically treated with cyclosporine suggesting an increased role for NO in maintaining tone during cyclosporine treatment. Consistent with functional data in the current study, Gardner et al. (1996) recently observed that L-NAME plus low-dose cyclosporine (2.5 mg kg^{-1} day^{-1}) treatment resulted in vascular abnormalities not observed with cyclosporine or L-NAME alone.

Although cyclosporine has been shown to inhibit NO-generating ability in vitro (Akita et al., 1994; Fast et al., 1994; Gallego et al., 1994; Takenaka et al., 1992), our studies are consistent with the hypothesis that NO is serving to attenuate potential hypertensive and/or vasoconstrictor actions of cyclosporine in vivo. We would

further suggest that an inability to generate NO could predispose a patient to the vasoconstrictor actions of cyclosporine. Consistent with this idea is the ability of L-arginine treatment to help maintain renal production of NO during cyclosporine treatment (Bobadilla et al., 1994; De Nicola et al., 1993; Gallego et al., 1993). In the current study, it is also possible that a contributing factor to the decline in renal function observed in rats treated with both cyclosporine and L-NAME may be the decrease in L-arginine intake associated with the decreased food intake observed in this group. NO synthase activity has been reported to be elevated in renal tissue homogenates from cyclosporine-treated rats (Amore et al., 1995). However, we did not detect any changes in urinary NO_x excretion when cyclosporine was given alone indicating that basal NO synthase activity may be sufficient to counteract much of the renal effects of cyclosporine in our rats. An ability to generate NO during cyclosporine treatment appears to be important for maintaining and preventing cyclosporine-induced hypertension and renal failure.

Many studies have now provided evidence that the endothelin system is 'activated' in response to cyclosporine treatment (Abassi et al., 1996; Nassar and Badr, 1994). This includes elevated plasma endothelin immunoreactivity as well as increased mRNA expression of preproendothelin-1 and the ET_A receptor in a variety of tissues. Several studies have suggested that endothelin plays an important role in cyclosporine-induced renal vasoconstriction but the degree to which ET_A receptors are involved is not completely understood. Conger et al. (1994) observed that the ET_A receptor antagonist, BQ-123, can only partially inhibit the direct renal vasoconstrictor effects of acute i.v. cyclosporine. Studies by Fogo et al. (1992) show that BQ-123 attenuates the vasoconstrictor response to acute i.v. cyclosporine when the antagonist was administered directly into the renal artery but not when given systemically. Similar results were observed when anti-endothelin antibody was injected directly into the renal artery (Kon et al., 1990). In contrast, it has been reported that the hypertensive and renal vasoconstrictor responses to acute i.v. cyclosporine was not influenced by either ET_A-selective (BQ-123) or non-selective ET_A/ET_B (PD 142893) antagonists (Davis et al., 1994). The mechanisms responsible for renal vasoconstrictor responses to acute vs. chronic cyclosporine may be different since the functional but not structural damage produced by cyclosporine can be ameliorated by chronic, subcutaneous administration of BQ-123 (Hunley et al., 1995).

It was unexpected that A-127722 inhibited the pressor response to subchronic L-NAME treatment but did not inhibit the response to combined L-NAME plus cyclosporine. These findings suggest that the mechanisms responsible for cyclosporine/L-NAME-induced hypertension are not the same as NO synthase inhibition alone. This would further suggest that an inability to synthesize NO is not a primary mechanism of the hypertension associated with cyclosporine. Nonetheless, the combination of L-NAME and cyclosporine may provide a useful model for studying mechanisms underlying the vascular effects of cyclosporine.

In summary, results from the current study allow us to conclude that the hypertension produced by 4-day treatment with L-NAME is maintained, at least in part, by activation of ET_A receptors, and that the hypertensive and vasoconstrictor response to cyclosporine + L-NAME does not involve ET_A receptor activation. These findings provide support for the hypothesis that endothelial dysfunction predisposes the kidney to functional derangements associated with administration of cyclosporine.

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