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Testosterone depletion contributes to cyclosporine-induced chronic impairment of acetylcholine renovascular relaxations

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Abstract

The immunosuppressant drug cyclosporine causes nephrotoxicity mainly via alterations of renovascular reactivity. This study investigated whether this effect of cyclosporine is modulated by the male gonadal hormone testosterone. The endothelium-dependent and -independent relaxations evoked by acetylcholine and sodium nitroprusside, respectively, were evaluated in phenylephrine-preconstricted isolated perfused kidneys obtained from sham-operated, castrated, and testosterone-replaced castrated (CAS + T) male rats in the absence and presence of cyclosporine. Compared with sham-operated values, short-term (10 days) castration or cyclosporine treatment caused significant and equivalent reductions in plasma testosterone levels and vasorelaxant responses to acetylcholine. Treatment of castrated rats with cyclosporine caused no further attenuation of acetylcholine relaxations. Testosterone replacement of castrated (CAS + T) or cyclosporine-treated castrated (CAS + CyA + T) rats restored plasma testosterone and acetylcholine relaxations to near-sham-operated levels. On the other hand, castration caused significant increases in nitroprusside relaxations versus no effect for cyclosporine. The relaxant responses to nitroprusside in castrated rats were restored to sham-operated levels after testosterone replacement. Plasma urea and creatinine were not affected by castration but were significantly increased by cyclosporine. These findings suggest that testosterone exerts directionally opposite modulatory effects on endothelium-dependent and -independent renal relaxations. Further, the results demonstrate that testosterone depletion may contribute, at least partly, to the inhibitory effect of cyclosporine on renovascular endothelial function. These data are clinically important because endothelial dysfunction contributes to vascular abnormalities associating cyclosporine therapy.
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1. Introduction

Cyclosporine has been widely accepted as the immunosuppressant drug of choice in preventing organ graft rejection and reducing mortality rates in transplantation patients (Cohen et al., 1984). Nonetheless, the therapeutic benefit of cyclosporine is appreciably hampered by the development of several side effects of which nephrotoxicity is the most troublesome (Myers, 1986; Mason, 1989). The deleterious effect of cyclosporine on renal function was first reported by Calne et al. (1978) who demonstrated a putative acute nephrotoxic syndrome in about 35% of patients treated with cyclosporine. The cyclosporine-induced renal injury ranges from a reversible rise in serum creatinine and blood urea nitrogen progressing to a late irreversible chronic renal failure (Myers, 1986; Gillum et al., 1988). These effects of cyclosporine have been reported not only in kidney transplant recipients but also in patients with healthy kidneys receiving cyclosporine for nonrenal disorders (Merion et al., 1984).

It is generally believed that the initial event in cyclosporine-induced nephrotoxicity comprises an intense renal afferent or preglomerular vasoconstriction (Myers, 1986; Mason, 1989). The exact mechanism underlying this effect of cyclosporine has been a subject of extensive studies. It has been suggested that cyclosporine nephrotoxicity results from an imbalance between renal vasoconstrictor and vasodilator mechanisms. For instance, a growing body of evidence suggests that cyclosporine increases renal sympathetic and

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angiotensin activities, which play fundamental roles in cyclosporine-induced renal vasoconstriction (Moss et al., 1985; Kon et al., 1995). The vascular endothelium and its derived vasoconstrictor (e.g. endothelin) and vasodilator (e.g. nitric oxide and prostanoids) factors also play a considerable role in the renal vascular spasm induced by cyclosporine. An increased release of endothelin (Zoja et al., 1986) and reduced nitric oxide (NO) activity (Gossmann et al., 2001) are known to associate cyclosporine therapy. Moreover, cyclosporine alters the renal metabolism of arachidonic acid favoring a vasoconstrictor prostanoid profile (Perico et al., 1986).

Several studies including our own have shown that cyclosporine impairs testicular function and reduces plasma testosterone levels (Krueger et al., 1991; El-Mas et al., 2002a), effects that can be reversed by testosterone replacement therapy (Seethalakshmi et al., 1990; El-Mas et al., 2002a). These findings may suggest that at least some of the adverse effects of cyclosporine might be due to the reduced testosterone availability. Recent reports from our laboratory established a link between testosterone depletion and the depressant effect of cyclosporine on arterial baroreflex function (El-Mas et al., 2001, 2002a,b). These studies provided circumstantial evidence that inhibition of the facilitatory effect of testosterone on baroreflex function underlies the deleterious effect of cyclosporine on baroreflexes (El-Mas et al., 2002a,b). Whether a similar interaction between cyclosporine and testosterone might occur at the kidney level is not clear. The need for such a study is justified as testosterone elicits vasodilatation and enhances vascular endothelial activity (Honda et al., 1999; Worboys et al., 2001). This effect relates to the ability of testosterone to release endothelium-derived relaxing factors including NO (Tatchum-Talom et al., 2002) and hyperpolarizing factor (Honda et al., 1999; Ding and Stallone, 2001). Moreover, NO synthase activity is reduced by castration and restored to intact levels after testosterone replacement (Baba et al., 2000). The vasodilatory effect of testosterone may also involve endothelium-independent mechanisms such as inhibition of the voltage-gated calcium channels (Jones et al., 2002). Together, these findings suggest a favorable effect of testosterone on endothelial activity and raise the possibility that the cyclosporine-induced renovascular dysfunction may be due to the reductions in plasma levels and vasodilatory effects of the hormone.

The present study therefore sought evidence to implicate the male gonadal hormone, testosterone, in the cyclosporine-induced impairment of renovascular reactivity. Accordingly, ex vivo studies were undertaken to evaluate the responsiveness of the renal vasculature to endothelium-dependent and -independent relaxations evoked by acetylcholine and nitroprusside, respectively, in isolated perfused kidneys obtained from sham-operated, castrated, and CAS + T rats treated chronically with cyclosporine or the vehicle. These protocols allowed the determination of the individual and combined modulatory effects of testosterone and cyclosporine on the tested functions. Plasma levels of urea and creatinine, as indices of kidney function, and testosterone were measured and correlated to changes in renovascular reactivity. Notably, acetylcholine relaxations are largely mediated via releasing endothelial factors including NO, prostaglandins, and hyperpolarizing factor (Vargas et al., 1994; Stephan et al., 1995) and endothelium removal abolishes completely acetylcholine responses (Stephan et al., 1995). On the other hand, nitrovasodilators such as nitroprusside act independent of the endothelium via generating NO, which directly relaxes smooth muscle through activation of guanylate cyclase (Ignarro et al., 1981; Rapoport and Murad, 1983).

2. Methods

Male Wistar rats (230–280 g; High Institute of Public Health, University of Alexandria, Alexandria, Egypt) were used in the present study. Experiments were performed in accordance with the European Community guidelines for the use of experimental animals and were approved by the institutional ethics committee.

2.1. Castration

Castration was performed as described in our previous studies (El-Mas et al., 2001, 2002a). A single 2–3 cm incision was made in the scrotum. The testes were isolated, tied-off with sterile suture and removed. Sham operation involved exposure of the testes without isolation. Each rat received an intramuscular injection of 60,000 U of penicillin G benzathine and penicillin G procaine (Penicid) and was housed in a separate cage. The ex vivo studies on the isolated perfused kidneys were performed 10 days later.

2.2. Rat isolated perfused kidney

The rat kidney was isolated and perfused according to the method described in previous studies including ours (Mohy El-Din and Malik, 1988; Vargas et al., 1994). Briefly, rats were anesthetized with thiopental sodium (50 mg/kg, i.p.), the abdomen was opened by a midline incision, and the left kidney was exposed. The left renal artery with its sympathetic nerve plexus was dissected free from its surrounding tissues. Loose ties were made around the renal artery and the abdominal aorta, proximal and distal to the renal artery. A beveled 18-gauge needle connected to a 5-ml syringe filled with heparinized saline (100 U/ml) was used for cannulation. The aorta was ligated, and the left renal artery was cannulated via an incision made in the aorta. The cannula was immediately secured with ligatures and the kidney was flushed with heparinized saline and rapidly excised from its surrounding tissues.

The kidney was transferred into a jacketed glass chamber maintained at 37 °C and continuously perfused with Krebs'

solution (NaCl 120, KCl 5, $CaCl_2$ 2.5, $MgSO_4 \cdot 7H_2O$ 1.2, KH_2PO_4 1.2, $NaHCO_3$ 25, and glucose 11 mM) maintained at 37 °C and gassed with 95% O_2 and 5% CO_2. Kidney perfusion was adjusted at a constant flow rate of 5 ml/min using a peristaltic pump (Model P3-Pharmacia Fine Chemicals®). The pump delivered a pulsatile flow, and an open circuit was used so that the venous effluent was allowed to drain freely. The kidney perfusion pressure was continuously monitored by means of a Gould–Statham pressure transducer distal to the pump and recorded on a Grass polygraph (model 7D). Inasmuch as the renal flow was kept constant, changes in perfusion pressure were indicative of alterations in renal vascular resistance.

2.3. Biochemical determinations

A blood sample (0.4 ml) was withdrawn from each rat on the experiment day. Plasma levels of urea and creatinine were determined using the Blood Urea Nitrogen Reagent Kit P/N 667530 and the Creatinine Reagent Kit P/N 668306, respectively (Beckman Instruments, Galway, Ireland). The plasma testosterone level was measured by the radioimmunoassay (Diagnostic Products Corporation, Los Angeles, CA).

2.4. Protocols and experimental groups

Six groups of male rats (sham-operated, castrated, CAS + T, cyclosporine, CAS + CyA, and CAS + CyA + T; $n = 7–9$) were used in this study to evaluate the modulatory effects of testosterone on the endothelium-dependent and -independent relaxations in the renal vasculature and its possible involvement in cyclosporine-induced impairment of renovascular reactivity. Cyclosporine (20 mg/kg, dissolved in olive oil) or equal volume of the vehicle was injected subcutaneously in single daily doses for 10 consecutive days. This dose of cyclosporine has been shown to produce nephrotoxicity and impair renal vascular reactivity (Gerkens, 1989). Testosterone (1 mg dissolved in olive oil) was injected subcutaneously in single daily doses for five consecutive days starting 5 days before the experiment day as described in our previous studies (El-Mas et al., 2001, 2002a). The last dose of cyclosporine, testosterone, or the vehicle was injected on the morning of the experiment day.

On the day of the experiment, the left kidney was isolated from thiopental-anesthetized rats and perfused as detailed above in Methods. A 30-min equilibration period was allowed at the beginning of the experiment, which has been shown adequate for the stabilization of the perfusion pressure (Vargas et al., 1994). Two consecutive dose–response curves were established in each kidney to determine the effect of acetylcholine and nitroprusside on the renal perfusion pressure. To study the vasorelaxant effects of acetylcholine or nitroprusside, the renal vascular tone was elevated by a continuous infusion of the α_1-adrenoceptor agonist phenylephrine (10 μM). The infusion of phenylephrine into the renal vasculature produced an abrupt increase in perfusion pressure, which was slowly decreased and stabilized within 20 min at a higher level for the remainder of the experiment as in previous studies (Vargas et al., 1994). This concentration of phenylephrine was sufficient to increase the renal perfusion pressure by 125–150 mm Hg.

Under conditions of sustained elevations in renovascular tone, dose–response curves to bolus injections of acetylcholine (0.03–2 nmol, at 2-min intervals) and nitroprusside (0.001–10 μmol, at 5-min intervals) were established consecutively. Generally, a given dose of either of the two relaxants was injected when the response to the previous dose disappeared and the vascular tone returned to the pretreatment levels. After the completion of acetylcholine dose–response curve, a washout period of 10 min was allowed before nitroprusside testing. In order to standardize the vasorelaxant responses to acetylcholine or nitroprusside, a bolus dose of papaverine (50 nmol) was injected into each preparation and the effects of acetylcholine or nitroprusside were expressed as a percentage of the papaverine relaxation (Heuzé-Joubert et al., 1992). Bolus doses of all drugs were administered at a constant volume of 100 μl. The injected volume caused a small and transient increase in renal perfusion pressure, which preceded the drug-evoked response. A control injection of saline was given to each preparation to verify that the responses to drug injections were not artifacts.

2.5. Drugs

Testosterone (Organon, Oss, Netherlands), phenylephrine hydrochloride, sodium nitroprusside, (Sigma Chemical, St. Louis, MO, USA), papaverine (Recordati, Milano, Italy), acetylcholine chloride (Roche, Basel, Switzerland), thiopental (Thiopental, Biochemie, Vienna, Austria), povidone–iodine solution (Betadine, Nile Pharmaceutical, Cairo, Egypt), and Penicid (Cid Pharmaceutical, Cairo, Egypt) were purchased from commercial vendors. Cyclosporine was a gift from Novartis Pharma (Basel, Switzerland). A fresh solution of cyclosporine in olive oil was prepared every 3 days and kept in the refrigerator.

2.6. Statistical analysis

Values are expressed as mean ± S.E.M. The vasorelaxant responses to acetylcholine and nitroprusside were measured as the maximum decreases in the renal perfusion pressure in tissues preconstricted with phenylephrine, and expressed as a percentage of the relaxation caused by papaverine (50 nmol). The E_{max} (maximum relaxation) and ED_{50} (dose which caused 50% of the maximum relaxation) values of acetylcholine or nitroprusside were determined for individual experiments. The ED_{50} was computed by regression analysis of the dose–response curves of acetylcholine or nitroprusside using the Graph PAD Instat software (version

1.13). The repeated measures analysis of variance (ANOVA) followed by a Newman–Keuls post hoc analysis was used for multiple comparisons with the level of significance set at $P < 0.05$.

3. Results

3.1. Cyclosporine–testosterone interaction on endothelium-dependent relaxations

Figs. 1 and 2 illustrate the effects of castration, cyclosporine, or their combination on the falls in renal perfusion pressure evoked by the endothelium-dependent vasodilator, acetylcholine, in the isolated perfused kidneys preconstricted with phenylephrine. Continuous infusion of phenylephrine (10 μM) at a rate of 5 ml/min caused a sustained elevation in the perfusion pressure that reached a steady level within 20 min. Bolus injections of acetylcholine (0.03–2 nmol) into the renal vasculature of kidneys obtained from sham-operated rats induced dose-dependent decreases in the renal perfusion pressure (Fig. 1), with a maximum relaxation of 101.6 ± 4.9% and an ED_{50} value of 0.27 ± 0.04 nmol (Table 1). Testosterone depletion evoked by castration or cyclosporine treatment (20 mg/kg/day) for 10 days caused significant ($P < 0.05$) and equivalent reductions in the acetylcholine-induced relaxations as compared with sham-operated values (Fig. 1). As shown in Table 1, castration or cyclosporine caused significant and similar decreases in E_{max} (64.6 ± 9.5% and 62.6 ± 6.5%, respectively) versus no effect on ED_{50}. Treatment of castrated rats with cyclosporine caused no further decreases in the vasorelaxant responses to acetylcholine (Fig. 1). The E_{max} and

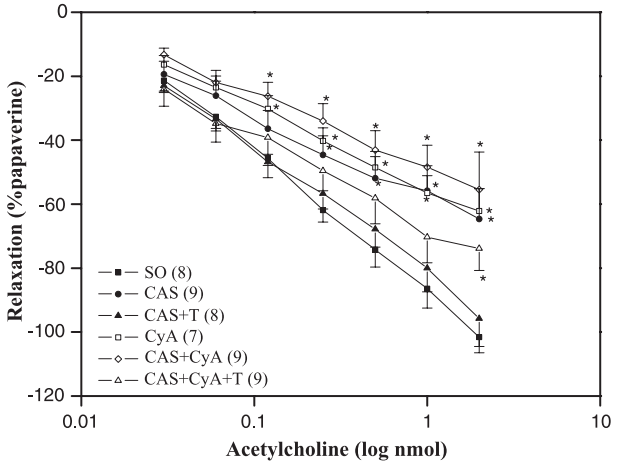

Fig. 1. Vasorelaxant effects of acetylcholine in phenylephrine (10 μM)-preconstricted isolated perfused kidneys obtained from sham-operated (SO), castrated (CAS), and testosterone-replaced castrated (CAS + T) rats in the presence and absence of cyclosporine A (CyA, 20 mg/kg/day for 10 days, s.c.). Values are mean ± S.E.M. and expressed as percentages of papaverine (50 nmol)-induced relaxations. *$P < 0.05$ compared with SO values.

Table 1
ED_{50} and E_{max} values of acetylcholine and sodium nitroprusside in phenylephrine (10 μM)-preconstricted isolated perfused kidneys obtained from sham-operated (SO), castrated (CAS), and testosterone-replaced castrated (CAS + T) rats in the presence and absence of cyclosporine (CyA)

Group	Acetylcholine		Nitroprusside	
	ED_{50} (nmol)	E_{max} (percentage of relaxation)	ED_{50} (nmol)	E_{max} (percentage of relaxation)
SO	0.27 ± 0.04	101.6 ± 4.9	35.9 ± 8.7	64.1 ± 3.6
CAS	0.28 ± 0.08	64.6 ± 9.5[a,b]	41.4 ± 9.2	90.2 ± 10.4[a,b]
CAS + T	0.31 ± 0.05	95.8 ± 8.7	24.6 ± 6.1	67.2 ± 7.5
CyA	0.31 ± 0.06	62.6 ± 6.5[a]	26.8 ± 6.3	57.8 ± 4.2
CAS + CyA	0.23 ± 0.06	57.7 ± 9.8[a]	26.9 ± 11.2	74.6 ± 7.8
CAS + CyA + T	0.21 ± 0.04	80.4 ± 8.3[c]	33.1 ± 17.5	62.7 ± 3.9

Values are mean ± S.E.M. ([a, b, c]) $P < 0.05$ compared with SO, CAS + T, and CAS + CyA values, respectively.

ED_{50} of acetylcholine were similar in castrated, cyclosporine, and CAS + CyA rats (Table 1). Fig. 2 depicts some representative tracings of the dose-related vasorelaxant effects of acetylcholine in kidneys obtained from sham-operated rats and the comparable attenuation of these effects by castration or cyclosporine treatment.

Subcutaneous injections of testosterone (1 mg/day for 5 days) to castrated rats fully restored acetylcholine relaxations to sham-operated levels (Fig. 1, Table 1). In cyclosporine-treated castrated rats, testosterone replacement significantly increased acetylcholine relaxations (Fig. 1).

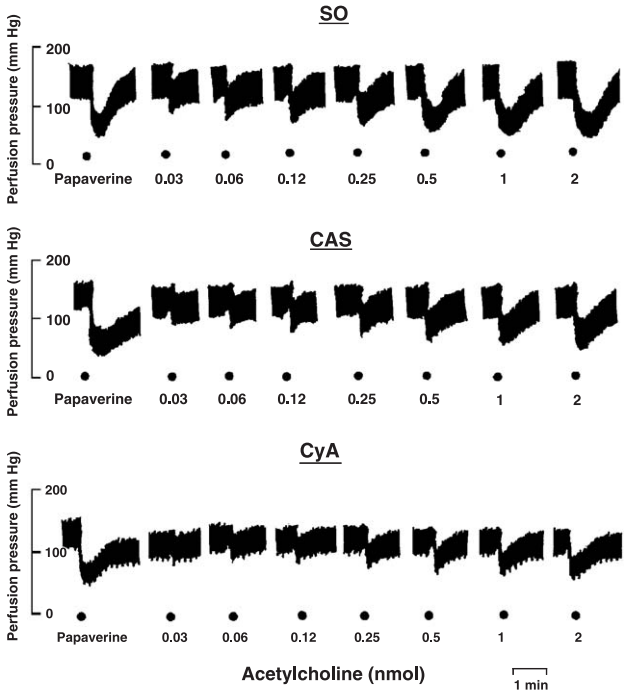

Fig. 2. Representative tracings showing the dose-dependent vasorelaxant effects of acetylcholine in phenylephrine (10 μM)-preconstricted isolated perfused kidneys obtained from sham-operated (SO), castrated (CAS), and cyclosporine A (CyA)-treated rats. Note that, compared with SO responses, CAS or CyA elicited equivalent reductions in acetylcholine relaxations.

The E_{max} of acetylcholine in CAS + CyA + T rats was also significantly higher than CAS + CyA values (Table 1).

3.2. Cyclosporine–testosterone interaction on endothelium-independent relaxations

The effect of the endothelium-independent vasorelaxant nitroprusside on the perfusion pressure of phenylephrine-preconstricted kidneys obtained from sham-operated, castrated, or CAS + T rats treated with cyclosporine or the vehicle are shown in Fig. 3. Bolus injections of nitroprusside (0.001–10 μmol) into the renal vasculature of kidneys obtained from sham-operated rats induced dose-dependent decreases in the renal perfusion pressure (Fig. 3). Castration caused significant ($P<0.05$) increases in the nitroprusside-induced relaxations compared with sham-operated values (Fig. 3). The E_{max} of nitroprusside was significantly increased in kidneys of castrated rats compared with sham-operated values whereas the ED_{50} remained unchanged (Table 1). Testosterone replacement of castrated rats reduced the relaxant responses to nitroprusside (Fig. 3) and restored the E_{max} of nitroprusside to sham-operated levels (Table 1). Treatment with cyclosporine had no effect on the relaxant responses to nitroprusside in all rat groups receiving the drug (Fig. 3, Table 1).

3.3. Biochemical determinations

Changes in plasma levels of testosterone, urea, and creatinine evoked by castration, testosterone replacement, cyclosporine, or their combinations are shown in Table 2. Compared with sham-operated values, plasma testosterone levels were significantly ($P<0.05$) reduced by castration, cyclosporine, or their combination and testosterone replacement restored the physiological levels of the hormone (Table 2). Plasma urea and creatinine levels were not affected by castration or testosterone replacement but showed significant increases by cyclosporine (Table 2). The increases in urea and creatinine levels were demonstrated in all rat groups receiving cyclosporine (i.e. cyclosporine, CAS + CyA, and CAS + CyA + T; Table 2).

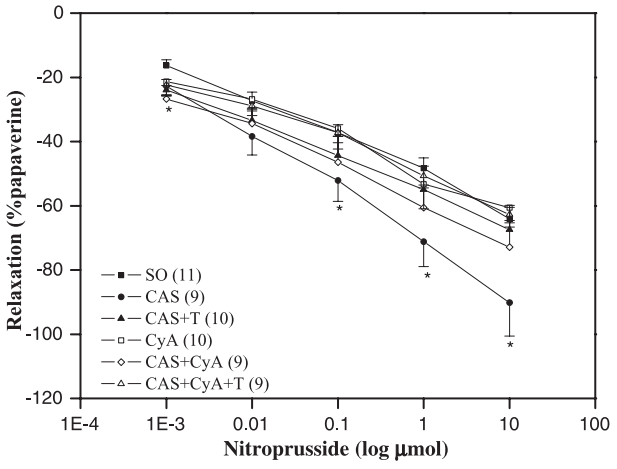

Fig. 3. Vasorelaxant effects of sodium nitroprusside in phenylephrine (10 μM)-preconstricted isolated perfused kidneys obtained from sham-operated (SO), castrated (CAS), and testosterone-replaced castrated (CAS + T) rats in the presence and absence of cyclosporine A (CyA, 20 mg/kg/day for 10 days, s.c.). Values are mean ± S.E.M. and expressed as percentages of papaverine (50 nmol)-induced relaxations. *$P<0.05$ compared with SO values.

Table 2
Plasma levels of testosterone, urea, and creatinine in sham-operated (SO), castrated (CAS), and testosterone-replaced castrated (CAS + T) rats in the presence and absence of cyclosporine (CyA)

Group	Testosterone (ng/dl)	Urea (mg/dl)	Creatinine (mg/dl)
SO	98.8 ± 20.1	13.21 ± 0.75	0.29 ± 0.01
CAS	19.0 ± 5.8[a,b]	15.55 ± 1.00	0.34 ± 0.03
CAS + T	85.5 ± 18.7	14.93 ± 0.97	0.35 ± 0.02
CyA	34.6 ± 7.6[a]	51.00 ± 10.49[a]	0.47 ± 0.04[a]
CAS + CyA	22.3 ± 5.5[a]	52.87 ± 8.37[a]	0.46 ± 0.03[a]
CAS + CyA + T	122.0 ± 23.8[c]	58.45 ± 10.68[a]	0.54 ± 0.06[a]

Values are mean ± S.E.M. ([a, b, c]) $P<0.05$ compared with SO, CAS + T, and CAS + CyA values, respectively.

4. Discussion

The present study addressed two important questions pertinent to the modulatory effects of testosterone on endothelium-dependent and -independent relaxations in the isolated perfused kidney and their role in the development of cyclosporine-induced renovascular impairment. Because castration caused remarkable decreases and increases in acetylcholine and nitroprusside vasorelaxant responses, respectively, and testosterone replacement restored these responses to sham-operated levels, it is concluded that the male gonadal hormone exerts opposite tonic modulatory effects on endothelium-dependent (facilitation) and -independent (inhibition) relaxations in the renal vasculature. The present study also tested the hypothesis that lowering plasma testosterone level mediates the inhibitory effect of cyclosporine on renovascular reactivity. Castration, cyclosporine, or a combination of the two interventions elicited comparable reductions in plasma testosterone and acetylcholine relaxations. In addition, restoration of physiological testosterone levels in castrated or cyclosporine-treated castrated rats enhanced acetylcholine responses. These findings suggest that cyclosporine impairs endothelium-dependent relaxations via, at least partly, reducing the availability of testosterone and inhibiting its facilitatory effect on cholinergically mediated renovascular relaxations.

The present study provided evidence that supports a favorable effect of testosterone on endothelium-dependent relaxations in the isolated perfused kidney. This conclusion is supported by the observations that: (i) testosterone depletion evoked by surgical elimination of the testes caused significant reductions in the relaxant responses to acetylcho-

line, and (ii) testosterone replacement of castrated rats regained the physiological levels of the hormone and restored the relaxant responses of acetylcholine to sham-operated levels. These findings are consistent with earlier observations that highlighted a positive modulation by testosterone of endothelial function in nonrenal vascular beds (Honda et al., 1999; Ding and Stallone, 2001; Tatchum-Talom et al., 2002).

Contrary to its facilitatory effect on renal endothelial function, the present study demonstrated a downregulatory effect of testosterone on endothelium-independent relaxations. Decreases in the renal perfusion pressure evoked by nitroprusside were potentiated by castration and restored to intact (sham-operated) levels after testosterone replacement. The reason for the opposite modulatory effects of testosterone on endothelium-dependent and -independent relaxations is not known. The enhanced nitroprusside effect in castrated rats does not appear to be directly related to the reduction in plasma testosterone because cyclosporine, like castration, caused dramatic decreases in testosterone levels but had no effect on nitroprusside relaxations. The enhancement of nitroprusside relaxations in response to castration may represent a counterregulatory mechanism to compensate for the castration-induced inhibition of endothelial activity. Interestingly, earlier reports support the presence of a mutual functional interaction between vascular endothelium and smooth muscle mechanisms to maintain vascular tone and homeostasis. For instance, endothelium removal increases nitroprusside-induced vasorelaxation in rat aortic rings (Shirasaki and Su, 1985). Also, the inhibition of NO synthase by l-NAME increases the responsiveness of nitroprusside in the renal vasculature (Vargas et al., 1994) and in other vascular beds (Bellan et al., 1991). It is suggested that the increased reactivity to nitroprusside, which acts by generating NO (Ignarro et al., 1981) and stimulating cGMP (Rapoport and Murad, 1983), may be secondary to the endogenous suppression of NO evoked by l-NAME. This would ultimately result in the upregulation of the receptors for NO in the soluble guanylate cyclase of vascular smooth muscle cells (Moncada et al., 1991).

The primary focus of the present study was to investigate the role of androgens in cyclosporine-induced renovascular dysfunction. The present finding that cyclosporine attenuated the vasorelaxant effects of acetylcholine in the isolated perfused kidney supports the view that cyclosporine impairs endothelium activity in renal and nonrenal vasculatures (Gerkens, 1989; Rego et al., 1990; Stephan et al., 1995). The current study established convincing evidence that supports a role for the male gonadal hormone in the adverse effects of cyclosporine on renovascular responsiveness. This conclusion is supported by several observations. First, the reduction in plasma testosterone levels evoked chemically (by cyclosporine) or surgically (by castration) was associated with a similar attenuation of the vasorelaxant effects of acetylcholine in renal tissues. Second, the effects of these two interventions (cyclosporine and castration) were not additive, i.e., treatment of castrated rats with cyclosporine elicited no further reductions in acetylcholine relaxations. In effect, the significant decreases in acetylcholine relaxations were similarly demonstrated in isolated kidneys obtained from castrated, cyclosporine, or CAS + CyA rats compared with sham-operated values. Third, testosterone replacement of CAS + CyA rats regained the physiological plasma levels of the hormone and increased the vasorelaxant effects of acetylcholine to near-sham-operated levels, suggesting the ability of testosterone to enhance endothelial muscarinic receptor responsiveness to cholinergic stimuli. It should be mentioned that the alterations in acetylcholine relaxations by cyclosporine or castration cannot be explained by differences in the tone achieved by prior infusion of phenylephrine because the latter produced similar increases in the renal perfusion pressure in all preparations. Taken together, the data presented in this study may suggest a possible link between cyclosporine-induced endothelial dysfunction and the reduction in circulating testosterone levels and support the hypothesis that cyclosporine alters renovascular reactivity via, at least partly, compromising the favorable effect of testosterone on endothelial function. These findings may be clinically important as endothelium dysfunction is a common etiologic factor in the pathogenesis of serious adverse effects of cyclosporine such as nephrotoxicity (Gossmann et al., 2001) and microvascular thromboses (Shulman et al., 1981; Sommer et al., 1985).

The present study measured plasma levels of urea and creatinine, biochemical indices of kidney function (Rock et al., 1986), to gain more insights into cyclosporine–testosterone renovascular interaction. Notably, elevated levels of these parameters are taken as early signs of renal insult (Klintmalm et al., 1981). The present demonstration that impairment of acetylcholine relaxations by cyclosporine was paralleled with significant increases in urea and creatinine levels is consistent with previous reports that suggested a role for endothelium dysfunction in cyclosporine-evoked nephrotoxicity (Gerkens, 1989; Stephan et al., 1995). Nonetheless, the enhancement by testosterone of renal acetylcholine responsiveness in CAS + CyA rats was not coupled with normalization of urea and creatinine levels, suggesting a maintained renal damage. The inability of testosterone to improve kidney function, despite the enhancement of acetylcholine relaxations, may be accounted for by the complex and widespread effects of cyclosporine on renal structure and homeostasis, which eventually mediate its nephrotoxicity (Moss et al., 1985; Jackson et al., 1987; Gerkens, 1989; Rego et al., 1990; Kon et al., 1995). Clearly, more studies are needed to determine the potential beneficial effect of a longer-duration and possibly higher dose level of testosterone in the control of cyclosporine adverse effects.

Finally, it is important to comment on the differential effects of cyclosporine on endothelium-dependent and -independent vasorelaxations. The current investigation showed that acetylcholine relaxations were significantly

reduced by cyclosporine whereas nitroprusside relaxations were preserved. The lack of an effect of cyclosporine on nitroprusside relaxations, which is consistent with previous studies (Gerkens, 1989; Stephan et al., 1995), may suggest a specific interaction of cyclosporine with vascular endothelial pathways and argue against a direct effect of cyclosporine on smooth muscle guanylate cyclase. It is noteworthy that contradictory reports of increases (Richards et al., 1989; Hosogai et al., 2001) and decreases (Cairns et al., 1989; Rego et al., 1990) in nitroprusside relaxations and cyclic GMP by cyclosporine are available. These discrepancies may relate to differences in species, vascular bed, dose, and duration of cyclosporine treatment.

In conclusion, the present study attempted to characterize the role of testosterone in cyclosporine-induced renovascular dysfunction. The results showed that testosterone exerts directionally opposite effects on endothelium-dependent (facilitation) and -independent (inhibition) relaxations evoked by acetylcholine and nitroprusside, respectively. The impairment of endothelial activity by chronically administered cyclosporine appears to be mediated, at least in part, via inhibition of the endothelial facilitatory effect of testosterone because: (i) castration or cyclosporine produced comparable reductions in plasma testosterone levels and acetylcholine relaxations, (ii) treatment of castrated rats with cyclosporine caused no additional reductions in acetylcholine relaxations, and (iii) restoration of physiological testosterone levels in cyclosporine-treated castrated rats enhanced acetylcholine responses.

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