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Enalapril and valsartan improve cyclosporine A-induced vascular dysfunction in spontaneously hypertensive rats

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Abstract

Cyclosporine A causes hypertension and nephrotoxicity in spontaneously hypertensive rats (SHR). In the present study, arterial function was investigated using in vitro vascular preparations after long-term treatment with cyclosporine A. SHR received cyclosporine A (5 mg kg^{-1} day^{-1} s.c.) and high-Na^+ diet for 6 weeks during the developmental phase of hypertension. Part of the rats were treated concomitantly either with the angiotensin converting enzyme inhibitor enalapril (30 mg kg^{-1} day^{-1} p.o.) or with an angiotensin AT_1 receptor antagonist valsartan (3 or 30 mg kg^{-1} day^{-1} p.o.). In renal arteries, contractile responses to noradrenaline and angiotensin II, as well as relaxation responses to acetylcholine (endothelium-dependent) and to sodium nitroprusside (endothelium-independent), were severely impaired by cyclosporine A-treatment. There was also a trend for the dysfunction of the mesenteric arteries, but the impairment did not reach statistical difference. Enalapril and valsartan improved the impaired renal arterial functions. Cyclosporine A-induced hypertension and nephrotoxicity seem to be associated with renal arterial dysfunction in SHR on high-Na^+ diet. Antagonism of the renin–angiotensin system protects from vascular toxicity of cyclosporine A.

Keywords: Cyclosporine A; Na^+; Enalapril; Valsartan; Angiotensin II; Vascular response

1. Introduction

Cyclosporine A is an immunosuppressive drug which is used to prevent rejection of transplanted organs and to treat autoimmune diseases (Faulds et al., 1993). Cyclosporine A therapy is often limited due to the development of hypertension and nephrotoxicity (Mason, 1989; Textor et al., 1994). In animal studies, the harmful effects of cyclosporine A are associated with either the increased contractility or decreased relaxation of arteries (Stephan et al., 1995; Epstein et al., 1998). However, some studies report that endothelium-dependent relaxations of renal arteries of normotensive rats after long-term cyclosporine A treatment remain normal even though high doses of cyclosporine A have been used (Mikkelsen et al., 1992; Verbeke et al., 1995). We have recently found a detrimental interaction between cyclosporine A and high intake of Na^+ in spontaneously hypertensive rats (SHR) observed as an increase in blood pressure and renal dysfunction (Mervaala et al., 1997), as well as morphological nephrotoxicity with arteriolopathy and glomerular damage (Pere et al., 1998).

The exact mechanism of cyclosporine A-induced hypertension and nephrotoxicity is not known, but several lines of evidence suggest an involvement of the renin–angiotensin system. Cyclosporine A stimulates renin production and release in isolated juxtaglomerular cells (Kurtz et al., 1988). An increase in plasma renin activity has been demonstrated in cyclosporine A-treated rats either on Na^+ depletion (Burdmann et al., 1995), on standard chow (Abassi et al., 1996), or during high intake of Na^+ (Mervaala et al., 1997, 1999). In addition, cyclosporine A has been reported to elevate plasma angiotensin II levels (Edwards et al., 1994). Furthermore, long-term treatment with cyclosporine A upregulates angiotensin AT_1 receptors in vascular and renal tissue (Iwai et al., 1993; Regitz-Zagrosek et al., 1995) and increases vasoconstrictive effect of angiotensin II in vitro (Auch-Schwelk et al., 1993;

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Takeda et al., 1995). Recently, we have shown that cyclosporine A-induced rise in blood pressure and kidney dysfunction in SHR on high-Na^+ diet was prevented by angiotensin converting enzyme inhibition with enalapril (Mervaala et al., 1999).

The present study was aimed to investigate in more detail the arterial functions in relation to the cyclosporine A-induced hypertension and kidney dysfunction of SHR on high-Na^+ diet, as well as the role of the renin–angiotensin system in these adverse effects of cyclosporine A.

2. Materials and methods

2.1. Experimental protocol

Forty-eight 8–9-week-old male SHR (243–314 g, Harlan Sprague Dawley; Indianapolis, IN, USA) were used. The protocol of the study was approved by the Animal Experimentation Committee of the Institute of Biomedicine, University of Helsinki, Finland.

The rats received high-Na^+ diet (Na 2.6%, Mg 0.2%, K 0.8%, Ca 1.0%, P 0.75% of the dry weight of the chow; R36, Finnewos Aqua, Helsinki, Finland) and tap water ad libitum. In the beginning of the study, the blood pressure- and body weight-matched SHR were divided into five different drug regimen groups for 6 weeks ($n = 9–10$ in each).

1. Control (vehicle s.c.)
2. Cyclosporine A (5 mg kg^{-1} day^{-1} s.c.)
3. Cyclosporine A and enalapril (30 mg kg^{-1} day^{-1} p.o.)
4. Cyclosporine A and valsartan (3 mg kg^{-1} day^{-1} p.o.)
5. Cyclosporine A and valsartan (30 mg kg^{-1} day^{-1} p.o.)

The dose of enalapril was chosen on the basis of our previous studies to prevent the hypertensive effect of cyclosporine A in SHR (Mervaala et al., 1999). The higher dose of valsartan (30 mg kg^{-1} day^{-1}) has been reported to produce a marked antihypertensive effect in SHR, whereas the lower dose (3 mg kg^{-1} day^{-1}) caused only a slight antihypertensive effect during long-term administration (Yamamoto et al., 1997). Cyclosporine A was administered subcutaneously at a dose of 5 mg kg^{-1} day^{-1}, which in our previous studies (Mervaala et al., 1997), produced plasma concentrations similar to those measured in cyclosporine A-treated patients. The control rats received the same volume of the vehicle (1 ml kg^{-1} s.c.). The rats were weighed daily during the experiment.

To clarify whether part of the enalapril effect is due to the inhibition of bradykinin degradation, another set of experiments with twelve 8-week-old SHR (246–286 g) was performed. The rats received high-Na^+ diet and cyclosporine A (5 mg kg^{-1} day^{-1} s.c.) and were concomitantly treated with enalapril (30 mg kg^{-1} day^{-1} p.o.). After 4 weeks of treatment, the rats were divided into two groups:

1. Enalapril + bradykinin B_2 receptor antagonist icatibant (HOE 140, 500 μg kg^{-1} day^{-1}) by a subcutaneous minipump for 2 weeks, and
2. Enalapril + saline by minipump for 2 weeks.

For the implantation of the subcutanous osmotic minipumps (Alzet®, model 2002, Alza, Palo Alto, CA, USA), the rats were anaesthetised with pentobarbital (50 mg kg^{-1} i.p.). The dose of icatibant was chosen on the basis of the previous studies to antagonise the antihypertensive effects of angiotensin converting enzyme inhibition (Linz and Schölkens, 1992). Two weeks administration was chosen due to the limited capacity of the minipump and to avoid repeated operation procedures.

2.2. Measurement of systolic blood pressure

Systolic blood pressure and heart rate were measured using a tail cuff blood pressure analyser (Apollo-2AB Blood Pressure Analyser, Model 179-2AB, IITC Life Science, Woodland Hills, CA, USA). Measurements were performed at the same day of the week and at the same time of the day by the same person.

2.3. Renal and mesenteric arterial responses

After the 6 weeks treatment, the animals were made unconscious with CO_2/O_2 (AGA, Riihimäki, Finland) and decapitated 24 h after the last cyclosporine A administration. A 3-mm long section of the mesenteric artery was cut 5-mm distally from the mesenteric artery–aorta junction. Likewise, a 2-mm long section of the right renal artery was cut. The rings were placed between stainless steel hooks and mounted in an organ bath chamber in Krebs–Ringer buffer (pH 7.4) of the following composition (mM): NaCl 119.0, $NaHCO_3$ 25.0, glucose 11.1, $CaCl_2$ 1.6, KCl 4.7, KH_2PO_4 1.2, $MgSO_4$ 1.2 and aerated with 95% O_2 and 5% CO_2. The rings were equilibrated for 30 min at 37°C, with a resting tension of 0.2 g for renal and 1.0 g for mesenteric arteries. The force of contraction was measured with an isometric force displacement transducer and registered with a polygraph (FTO3 transducer, Model 7P122E Polygraph; Grass Instrument, Quincy, MA, USA).

Cumulative concentration–response curves for noradrenaline (1×10^{-9}–1×10^{-6} M, renal artery; 1×10^{-9}–1×10^{-5} M, mesenteric artery) and the response to a single administration of angiotensin II (1×10^{-6} M) were determined. Angiotensin II was administered only once to avoid tachyphylaxis (Khairallah et al., 1966). Cumulative relaxation responses to acetylcholine ($1 \times$

Table 1
Effects of cyclosporine A, enalapril and valsartan on body weight gain and systolic blood pressure of SHR on high-Na^+ diet
CsA, cyclosporine A. Values are means ± S.E.M. $n = 9–10$.

Variable	Control	CsA (5 mg kg^{-1} day^{-1})	CsA + enalapril (30 mg kg^{-1} day^{-1})	CsA + valsartan (3 mg kg^{-1} day^{-1})	CsA + valsartan (30 mg kg^{-1} day^{-1})	P
Body weight, g						
Week 0	284 ± 5	288 ± 6	284 ± 4	289 ± 5	288 ± 7	0.923
Week 6	350 ± 8	301 ± 9 *	311 ± 7 *	318 ± 6	320 ± 9	< 0.01
Systolic blood pressure, mm Hg						
Week 0	152 ± 10	155 ± 9	155 ± 7	156 ± 7	155 ± 8	0.998
Week 6	195 ± 3	242 ± 6 * *	196 ± 4 * * *	205 ± 6 * * *	173 ± 5 * * *	< 0.001

* $P < 0.01$ vs. control.
* * $P < 0.001$ vs. control.
* * * $P < 0.001$ vs. CsA.

10^{-9}–1×10^{-7} M, renal artery; 1×10^{-9}–1×10^{-6} M, mesenteric artery) and sodium nitroprusside (1×10^{-10}–1×10^{-6} M, renal artery; 1×10^{-8}–1×10^{-5} M, mesenteric artery) were examined after precontraction with noradrenaline (1×10^{-6} M). Between each set of measurements, the rings were washed three times and were allowed to equilibrate at least 20 min before generating the next response curve.

2.4. Drugs

Cyclosporine A (Sandimmun® infusion concentrate 50 mg ml^{-1}) and valsartan were generous gifts from Novartis (Basel, Switzerland). Enalapril was kindly donated by Leiras (Turku, Finland). Icatibant (D-Arg[Hyp^3, Thi^5, Oic^8] bradykinin) was a generous gift from Dr. Klaus Wirth, Hoechst (Frankfurt am Main, Germany). Cyclosporine A was diluted in a lipid solution (Intralipid®, Kabi Pharmacia, Stockholm, Sweden).

The following drugs were used for renal and mesenteric arterial responses: acetylcholine chloride, noradrenaline bitartrate (Sigma Chemical, St. Louis, MO, USA), sodium nitroprusside dihydrate (F. Hoffmann–La Roche, Basel, Switzerland). The compounds were dissolved in water. All solutions were freshly prepared before use and protected from light.

2.5. Statistical analysis

The results for cumulative arterial responses were analysed using two-way analysis of variance (ANOVA) with repeated measures for overall treatment effect. The maximal relaxation and contraction responses, systolic blood pressure and body weight were analysed with one-way ANOVA. The Tukey's test was made for pairwise comparisons between the treatment groups. $P < 0.05$ was considered significant. The results are expressed as means ± S.E.M.

3. Results

3.1. Body weight and systolic blood pressure

Cyclosporine A (5 mg kg^{-1} day^{-1}) decreased the body weight gain during the 6 weeks treatment period when compared to the control group (Table 1). There were no differences in the body weight between cyclosporine A group and cyclosporine A groups receiving either enalapril or valsartan. The body weight gain was not affected by the coadministration of icatibant during cyclosporine A and enalapril treatment (Table 2).

Systolic blood pressure was significantly higher after the 6 weeks experimental period with cyclosporine A than in the control group (Table 1). Concomitant enalapril (30 mg kg^{-1} day^{-1}) or valsartan (3 or 30 mg kg^{-1} day^{-1}) treatment attenuated the development of the cyclosporine A-induced hypertension. Bradykinin B_2 receptor antagonist icatibant seemed to have a slight but not significant systolic blood pressure-lowering effect during enalapril administration in cyclosporine A-treated rats (Table 2).

Table 2
Effects of bradykinin B_2 receptor antagonist icatibant on body weight gain, and systolic blood pressure of cyclosporine A and enalapril treated SHR on high-Na^+ diet
CsA, cyclosporine A. Values are means ± S.E.M. $n = 5–7$.

Variable	CsA 5 mg kg^{-1} day^{-1} + enalapril 30 mg kg^{-1} day^{-1}	CsA + enalapril + + icatibant 500 μg kg^{-1} day^{-1}	P
Body weight, g			
Week 0	263 ± 4		
Week 6	308 ± 8	306 ± 8	0.826
Systolic blood pressure, mm Hg			
Week 0	134 ± 5		
Week 4	160 ± 5		
Week 6	206 ± 8	195 ± 4	0.208

3.2. Renal arterial responses

Contractile responses to noradrenaline (1×10^{-9}–1×10^{-6} M) were impaired in the cyclosporine A group compared to the control group (Fig. 1a). Treatment with enalapril or valsartan at both doses normalised the noradrenaline contractions.

Contractile response to angiotensin II (1×10^{-6} M) was also decreased in the group receiving cyclosporine A alone when compared to the control group (Fig. 2a). Concomitant treatment with enalapril or valsartan normalised the angiotensin II contractions.

The endothelium-dependent relaxation responses to acetylcholine (1×10^{-9}–1×10^{-7} M) after noradrenaline precontraction (1×10^{-6} M) were impaired in the cyclosporine A group (Fig. 3a). The endothelium-dependent relaxations were improved significantly in the group receiving cyclosporine A and valsartan at 30 mg kg^{-1} day^{-1} compared to the cyclosporine A alone ($P < 0.05$). Enalapril also tended to improve the endothelium-dependent relaxations, but the difference was not significant ($P = 0.06$). The relaxations in the groups receiving cyclosporine A with enalapril or valsartan did not differ from the response in the control group.

The endothelium-independent relaxations to sodium nitroprusside (1×10^{-10}–1×10^{-6} M) were attenuated in the cyclosporine A group compared to the control group (Fig. 4a). Enalapril and valsartan at both doses protected from the cyclosporine A-induced impairment of endothelium-independent relaxations.

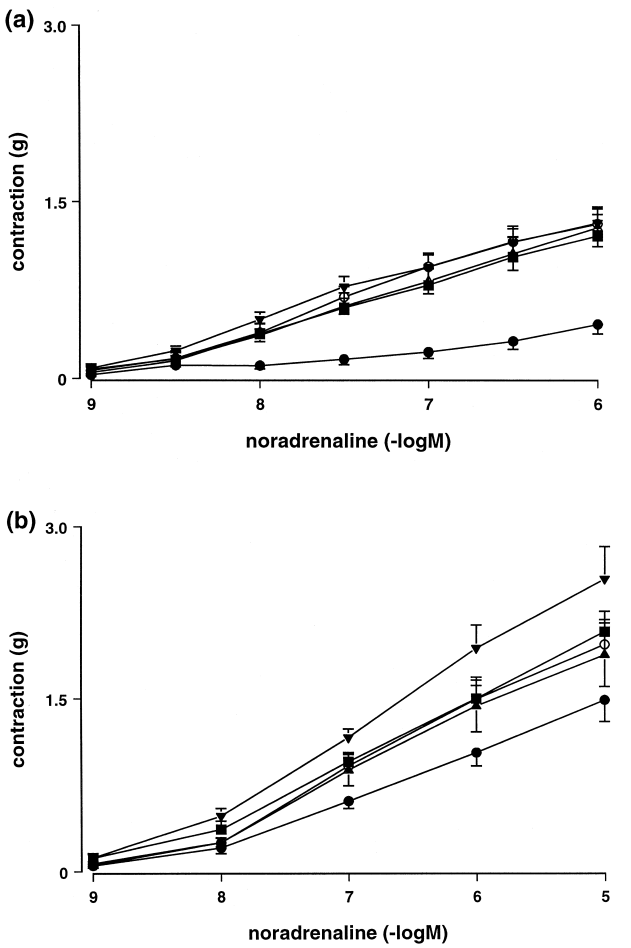

Fig. 1. Concentration response curves to noradrenaline-induced contractions in renal (a) and mesenteric (b) artery rings of SHR on high-Na^+ diet after the 6 weeks treatment period. (○) Control, (●) cyclosporine A 5 mg kg^{-1} day^{-1}, (■) cyclosporine A + enalapril 30 mg kg^{-1} day^{-1}, (▲) cyclosporine A + valsartan 3 mg kg^{-1} day^{-1}, (▼) cyclosporine A + valsartan 30 mg kg^{-1} day^{-1}. Renal arteries: cyclosporine A vs. control ($P < 0.001$); cyclosporine A + enalapril 30 mg kg^{-1} day^{-1} and cyclosporine A + valsartan 3 mg kg^{-1} day^{-1} vs. cyclosporine A ($P < 0.05$); cyclosporine A + valsartan 30 mg kg^{-1} day^{-1} vs. cyclosporine A ($P < 0.001$). Mesenteric arteries: cyclosporine A + valsartan 30 mg kg^{-1} day^{-1} vs. cyclosporine A ($P < 0.01$). The results are expressed as means ± S.E.M. $n = 7$–9 each group.

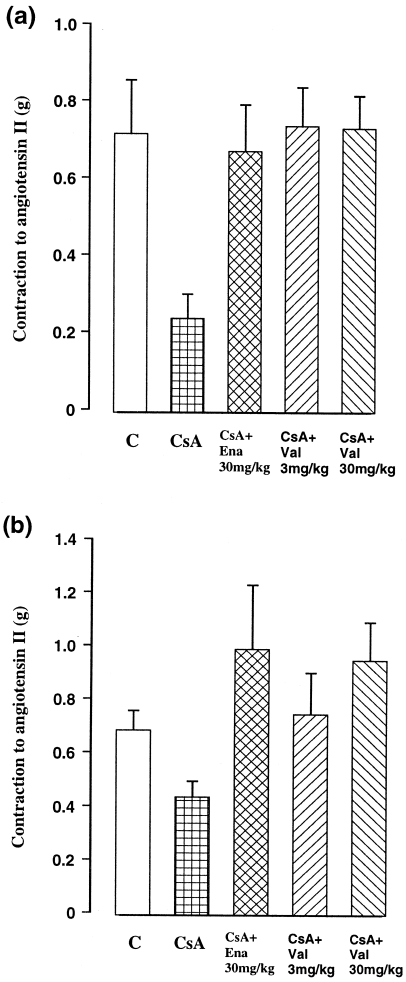

Fig. 2. Contractions to angiotensin II (1×10^{-6} M) in renal (a) and mesenteric (b) artery rings of SHR on high-Na^+ diet after the 6 weeks treatment period. (C) Control, (CsA) cyclosporine A 5 mg kg^{-1} day^{-1}, (CsA + Ena 30 mg/kg) cyclosporine A + enalapril 30 mg kg^{-1} day^{-1}, (CsA + Val 3 mg/kg) cyclosporine A + valsartan 3 mg kg^{-1} day^{-1}, (CsA + Val 30 mg/kg) cyclosporine A + valsartan 30 mg kg^{-1} day^{-1}. Renal arteries: cyclosporine A vs. control ($P < 0.05$); cyclosporine A + enalapril 30 mg kg^{-1} day^{-1}, cyclosporine A + valsartan 3 mg kg^{-1} day^{-1} and cyclosporine A + valsartan 30 mg kg^{-1} day^{-1} vs. cyclosporine A ($P < 0.05$). The results are expressed as means ± S.E.M. $n = 7$–9 each group.

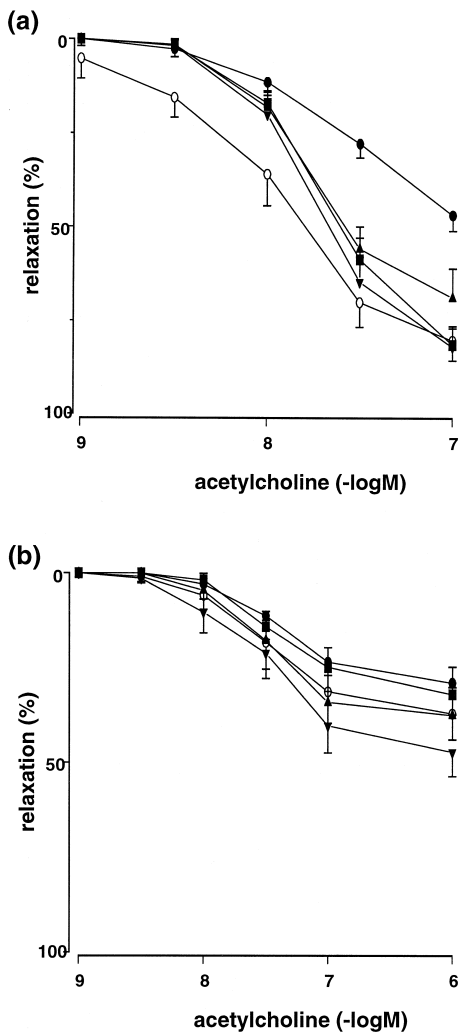

Fig. 3. Relaxations to acetylcholine in noradrenaline-precontracted artery rings from renal (a) and from mesenteric (b) arteries of SHR on high-Na^+ diet after the 6 weeks treatment period. (○) Control, (●) cyclosporine A 5 mg kg^{-1} day^{-1}, (■) cyclosporine A + enalapril 30 mg kg^{-1} day^{-1}, (▲) cyclosporine A + valsartan 3 mg kg^{-1} day^{-1}, (▼) cyclosporine A + valsartan 30 mg kg^{-1} day^{-1}. Renal arteries: cyclosporine A vs. control ($P < 0.001$); cyclosporine A + valsartan 30 mg kg^{-1} day^{-1} vs. cyclosporine A ($P < 0.05$). The results are expressed as means ± S.E.M. $n = 7–9$ each group.

Coadministration of the bradykinin B_2-receptor antagonist icatibant did not significantly affect renal arterial relaxation or contractile responses after cyclosporine A and enalapril treatment (Table 3).

3.3. Mesenteric arterial responses

Cyclosporine A slightly reduced the noradrenaline-induced (1×10^{-9}–1×10^{-5} M) contractions compared to the control group, but the difference was not significant ($P = 0.40$; Fig. 1b). The contraction responses were strongest after valsartan treatment at 30 mg kg^{-1} day^{-1} ($P < 0.01$, vs. cyclosporine A group). Valsartan at 3 mg kg^{-1} day^{-1} or enalapril at 30 mg kg^{-1} day^{-1} did not markedly affect the noradrenaline-induced contractions.

Cyclosporine A tended to impair the angiotensin II-induced (1×10^{-6} M) contractions ($P = 0.73$; Fig. 2b). Enalapril and the higher dose of valsartan seemed to cause a slightly more pronounced contractile response to angiotensin II compared to the cyclosporine A group ($P = 0.10$). However, there were no significant differences between the experimental groups in the contractions elicited by angiotensin II.

Cyclosporine A did not significantly impair the acetylcholine-induced (1×10^{-9}–1×10^{-6} M) relaxations ($P = 0.93$; Fig. 3b). There were no differences between the

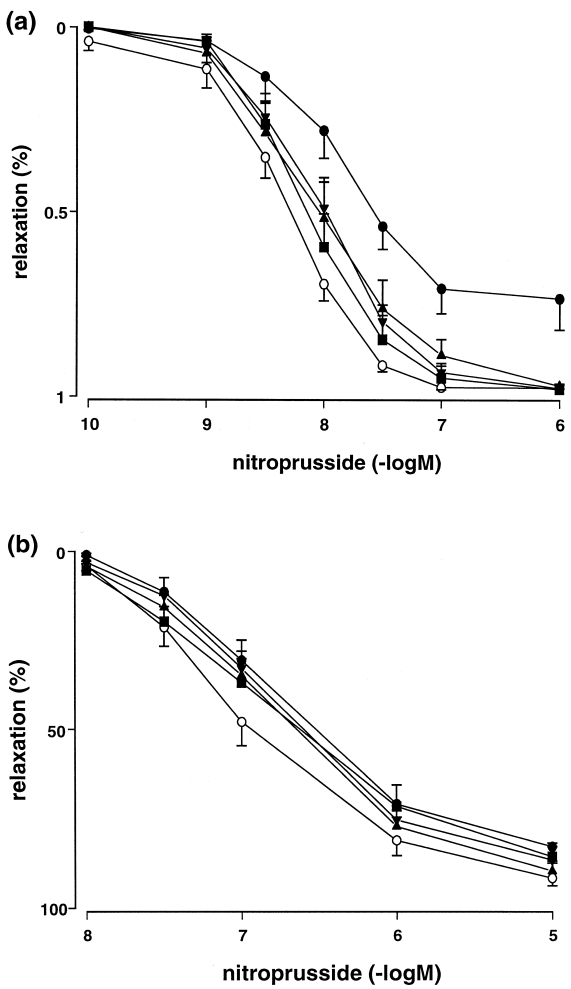

Fig. 4. Relaxations to sodium nitroprusside in noradrenaline (10^{-6} M) precontracted artery rings from renal (a) and from mesenteric (b) arteries of SHR on high-Na^+ diet after the 6 weeks treatment period. (○) Control, (●) cyclosporine A 5 mg kg^{-1} day^{-1}, (■) cyclosporine A + enalapril 30 mg kg^{-1} day^{-1}, (▲) cyclosporine A + valsartan 3 mg kg^{-1} day^{-1}, (▼) cyclosporine A + valsartan 30 mg kg^{-1} day^{-1}. Renal arteries: cyclosporine A vs. control ($P < 0.01$); cyclosporine A + enalapril 30 mg kg^{-1} day^{-1}, cyclosporine A + valsartan 3 mg kg^{-1} day^{-1} and cyclosporine A + valsartan 30 mg kg^{-1} day^{-1} vs. cyclosporine A ($P < 0.05$). The results are expressed as means ± S.E.M. $n = 7–9$ each group.

Table 3
Effects of bradykinin B_2 receptor antagonist icatibant (500 μg kg^{-1} day^{-1}) on cyclosporine A (5 mg kg^{-1} day^{-1})- and enalapril (30 mg kg^{-1} day^{-1})-treated SHR on high-Na^+ diet on mesenteric and renal arterial function
CsA, cyclosporine A. Values are means ± S.E.M. $n = 5–7$.

Variable	enalapril + saline	enalapril + icatibant	P
Renal arteries			
Maximal contraction responses, g			
Noradrenaline	0.9 ± 0.2	1.2 ± 0.2	0.325
Angiotensin II	0.54 ± 0.09	0.71 ± 0.12	0.222
Maximal relaxation responses (%)			
Acetylcholine	74 ± 8	85 ± 4	0.214
Sodium nitroprusside	100 ± 1	98 ± 1	0.527
Mesentric arteries			
Maximal contraction responses, g			
Noradrenaline	1.4 ± 0.2	1.7 ± 0.2	0.309
Angiotensin II	0.37 ± 0.08	0.54 ± 0.09	0.217
Maximal relaxation responses (%)			
Acetylcholine	45 ± 12	33 ± 9	0.424
Sodium nitroprusside	93 ± 2	86 ± 3	0.091

experimental groups in sodium nitroprusside-induced (1×10^{-8}–1×10^{-5} M) relaxations (Fig. 4b).

Coadministration of icatibant did not affect mesenteric arterial relaxation or contractile responses after cyclosporine A and enalapril treatment (Table 3).

4. Discussion

In the present study, long-term treatment with cyclosporine A increased blood pressure and caused an impairment of arterial functions in SHR on high-Na^+ diet during the developmental phase of hypertension. The dysfunction included both relaxations, as well as contractile responses. The functions of renal artery are more sensitive to cardiovascular dysfunction induced by cyclosporine A than those of mesenteric artery. Enalapril and valsartan, which attenuated the blood pressure elevating effect of cyclosporine A, were also able to improve the cyclosporine A-induced deterioration of vascular responses.

Cyclosporine A has been shown to be toxic to endothelial cells in vitro (Zoja et al., 1986). The toxic effects of cyclosporine A on endothelium may be potentiated in SHR during high intake of Na^+, because we found that the endothelium-dependent relaxations elicited by acetylcholine in renal arteries were impaired after long-term treatment with cyclosporine A. Acetylcholine is known to relax arterial smooth muscle by releasing nitric oxide, prostacyclin and endothelium-derived hyperpolarising factor from the vascular endothelium (Busse and Fleming., 1994). Repeated administration of cyclosporine A has also been found to impair acetylcholine responses in rat aorta (Mikkelsen et al., 1992; Auch-Schwelk et al., 1994; Verbeke et al., 1995, Kim et al., 1996), in femoral arteries (Gallego et al., 1994) and in mesenteric arteries (Rego et al., 1990) when fairly high doses of cyclosporine A were used. On the contrary, in some studies, the endothelium-dependent relaxations in renal arteries remained normal after long-term cyclosporine A treatment (Mikkelsen et al., 1992; Verbeke et al., 1995), whereas impaired relaxation to acetylcholine has been reported in other studies (Diederich et al., 1992). Differences in experimental conditions, such as rat strain, dose of cyclosporine A, or duration of the treatment may explain these differences.

Acute administration of cyclosporine A contracts renal arterioles in vitro (Lanese et al., 1994; Epstein et al., 1998). Furthermore, there are studies in which cyclosporine A, given acutely (Epstein et al., 1998) or repeatedly (Mikkelsen et al., 1992), sensitised renal arteries to contractile responses. Cyclosporine A has been reported to increase (Rego et al., 1990) or decrease (Roullet et al., 1995) or have no effect (Ressureicao et al., 1995) on contractile responses in mesenteric arteries. However, in the present study cyclosporine A did not strengthen but reduced the contractile responses to both noradrenaline and angiotensin II, especially in renal arteries.

Hypertension is associated with structural changes of the arteries, such as hypertrophy and an increase in extracellular matrix, especially in collagen content (Chrysant, 1998). The vascular reactivity may be reduced because of increased stiffness of arteries. Although these alterations are partly related to elevated blood pressure, other factors (e.g. angiotensin II) have been found to stimulate vascular hypertrophy and collagen accumulation (Chrysant, 1998). Beyond lowering blood pressure, antagonists of the renin–angiotensin system may improve vascular functions by reducing the direct effects of angiotensin II on artery wall (Gohlke et al., 1996). This may at least partly explain the positive effects of the renin–angiotensin system antagonism during cyclosporine A treatment.

There is evidence that administration of cyclosporine A leads to stronger contractility because of the accumulation of intracellular free calcium in smooth muscle cells via influx through voltage-gated calcium channels (Rossi et al., 1989). In addition to contraction, the rise in intracellular calcium may lead to necrosis in vascular smooth muscle cells (Kristian and Siesjö, 1998). Chronic administration of cyclosporine A during high intake of Na^+ leads to the accumulation of calcium in the kidneys concomitantly with morphological changes in renal arterioles (Pere et al., 1998). Thus, the impairment of contractile responses in the present study may be caused by vascular smooth muscle damage rather than by a direct effect of cyclosporine A on vascular contractility.

Chronic treatment with cyclosporine A also impaired the endothelium-independent relaxations to sodium nitroprusside in renal arteries. Earlier, it has been reported that endothelium-independent relaxations were preserved in rat renal artery (Lanese et al., 1994), were unaffected (Auch-

Schwelk et al., 1993) or inhibited (Kim et al., 1996) in rat aorta during repeated cyclosporine A-administration. The impairment of the endothelium-independent relaxations in the present study may be seen, analogously with the impaired contractile responses, as a result of cyclosporine A-induced vascular smooth muscle damage.

In the present study, the harmful effects of cyclosporine A were more evident in renal than in mesenteric arteries. Regional differences in vasculotoxic effects of cyclosporine A have also been described earlier (Verbeke et al., 1995; Epstein et al., 1998). It has been shown that the contractile effect of acute administration of cyclosporine A was markedly stronger in bovine renal than in mesenteric arteries (Epstein et al., 1998). Thus, it may be suggested that in the acute phase, cyclosporine A causes vasoconstriction, which is more evident in renal than in mesenteric arteries. During long-term administration of cyclosporine A, the vasoconstriction may lead to hypoxia-induced artery damage in renal vessels which can account for the functional and morphological changes of kidneys in cyclosporine A-treated SHR on high-Na^+ diet.

Antagonism of the renin–angiotensin system has been reported to prevent cyclosporine A-induced hypertension (Mervaala et al., 1999) and nephrotoxicity (Burdmann et al., 1995; Pichler et al., 1995) and preserve the endothelium-dependent relaxation responses in rat aorta (Auch-Schwelk et al., 1994). In the present study, both the angiotensin converting enzyme inhibitor enalapril and the angiotensin AT_1 receptor antagonist valsartan improved cyclosporine A-induced impairment of endothelium-dependent and -independent relaxations, as well as contractile responses to noradrenaline and angiotensin II in renal arteries. On the other hand, coadministration of bradykinin B_2 receptor antagonist did not markedly influence any of the arterial responses during cyclosporine A and enalapril treatment. These results point to the direction that inhibition of angiotensin II mediates the beneficial effects of angiotensin converting enzyme inhibition and the role of bradykinin is not important. It seems that activation of the renin–angiotensin system is a major mechanism in vascular toxicity of cyclosporine A in SHR during high intake of Na^+.

In addition to angiotensin II, other vasoactive substances may be involved in cyclosporine A toxicity. One of these is endothelin, which has been suggested to have an important role in the cyclosporine A-induced vasoconstriction of the renal afferent arterioles (Lanese and Conger, 1993). An increased production of endothelin has been reported after the administration of cyclosporine A (Takeda et al., 1993; Abassi et al., 1996). Moreover, endothelin receptor antagonists attenuated the cyclosporine A-induced vasoconstriction (Lanese and Conger, 1993) and hypertension (Takeda et al., 1995; Oriji and Keiser, 1998). However, the tubulointerstitial fibrosis associated with cyclosporine A was not suppressed by an endothelin receptor antagonist (Kon et al., 1995), whereas enalapril was able to prevent the development of interstitial fibrosis (Kon et al., 1995; Burdmann et al., 1995). The vascular dysfunction by cyclosporine A has also been linked with enhanced production of superoxide, which could lead to increased destruction of nitric oxide (Diederich et al., 1994).

In summary, the most important finding in our study was that cyclosporine A, at a dose which produces clinically relevant plasma concentrations, during high intake of Na^+ caused a severe impairment of arterial function especially in renal arteries that included both endothelium-dependent and -independent relaxations, as well as contractile responses. Thus, cyclosporine A-induced hypertension and nephrotoxicity in SHR on high-Na^+ diet seem to be associated mainly with renal arterial dysfunction. Enalapril and valsartan protected from cyclosporine A induced arterial dysfunction pointing to a major role of the renin–angiotensin system in cyclosporine A-induced vascular toxicity in SHR.

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