JACC Vol. 25, No. 3
March 1, 1995:761–7

Selective Downregulation of Rat Cardiac Beta$_1$-Adrenoceptors by Cyclosporine A: Prevention by Diltiazem or Angiotensin-Converting Enzyme Inhibitors

OTTO-ERICH BRODDE, PHD,* MELANIE ADAMCZYK, FRAUKE BUSCH, CLAUS BOSSALLER, MD,† ELLEN DUSKE,† ECKART FLECK, MD,† STEPHAN GÖTZE, MD,† WOLFGANG AUCH-SCHWELK, MD†

Essen and Berlin, Germany

Objectives. **This study attempted to determine whether long-term treatment with cyclosporine A in rats affects cardiac beta$_1$-adrenoceptors and whether this can be prevented by angiotensin-converting enzyme inhibitors or calcium-entry blocking agents.**

Background. **In the transplanted human heart the density of beta$_1$-adrenoceptors decreases with time after transplantation, whereas that of beta$_2$-adrenoceptors does not. Because heart transplant recipients are treated with cyclosporine A, we studied whether administration of cyclosporine A in rats might cause this beta$_1$-adrenoceptor downregulation.**

Methods. **We performed two studies. First, we treated groups of 10 male normotensive Wistar rats orally with 30 mg/kg body weight per day of cyclosporine A, 10 mg/kg per day of enalapril and 60 mg/kg per day of diltiazem, alone or in combination, for 6 weeks each. Second, we treated groups of 15 male normotensive Wistar rats orally with 15 mg/kg per day of cyclosporine A and 10 mg/kg per day of lisinopril, alone or in combination, for 6 weeks each. At the end of each treatment regimen, cardiac beta-adrenoceptor density and subtype distribution were assessed by (−)-[^{125}I]iodocyanopindolol binding.**

Results. **Both doses of cyclosporine A caused a significant decrease in cardiac beta$_1$-adrenoceptor density without affecting beta$_2$-adrenoceptor density. Although diltiazem and the angiotensin-converting enzyme inhibitors alone did not affect cardiac beta-adrenoceptors, they prevented the cyclosporine A–induced downregulation of beta$_1$-adrenoceptors.**

Conclusions. **In normotensive Wistar rats, cyclosporine A causes a significant decrease in cardiac beta$_1$-adrenoceptors without affecting beta$_2$-adrenoceptors. This can be prevented by diltiazem or angiotensin-converting enzyme inhibitors. In heart transplant recipients, who undergo long-term treatment with cyclosporine A, there is a very similar beta$_1$-adrenoceptor downregulation with time after transplantation. Thus, administration of cyclosporine A may cause these beta-adrenoceptor subtype alterations.**

(J Am Coll Cardiol 1995;25:761–7)

With the introduction of the immunosuppressive agent cyclosporine A, graft survival in organ transplantation has markedly improved (1). However, a number of unwanted side effects are associated with cyclosporine A treatment, including tremor, tachycardia (2,3), nephrotoxicity (4) and dysfunction of endothelial and smooth muscle cells in the arterial wall (5,6), that might be involved in the development of hypertension and renal failure (7,8).

We recently observed that 7 weeks of treatment with cyclosporine A (20 mg/kg body weight orally) in young spontaneously hypertensive rats induced a marked increase in the elevated blood pressure (9,10) and caused a selective downregulation of cardiac beta$_1$-adrenoceptors without altering cardiac beta$_2$-adrenoceptors (11). Very similar decreases in beta$_1$-adrenoceptors without any alteration in beta$_2$-adrenoceptors are found in the transplanted human heart with increasing time after transplantation (12–14). Because heart transplant recipients undergo long-term treatment with cyclosporine A, we hypothesized that cyclosporine A might be the cause of the cardiac beta$_1$-adrenoceptor downregulation. We therefore studied the effects of cyclosporine A (30 and 15 mg/kg orally for 6 weeks) on cardiac beta-adrenoceptors in normotensive Wistar rats, which do not develop hypertension during cyclosporine A treatment (10). Furthermore, cyclosporine A in the rat increases afferent and efferent sympathetic nerve activity and activates the renin–angiotensin system, as demonstrated by increased renin release (15 [and references

From the Biochemical Research Laboratory, Department of Internal Medicine, University of Essen, Essen and †Department of Cardiology, German Heart Institute, Berlin, Germany. This study was supported by Grant DFG Br 526/3-2 from the Deutsche Forschungsgemeinschaft, Bonn, Germany, to Dr. Brodde. Cyclosporine A was kindly donated by Sandoz, Nürnberg; diltiazem by Gödecke, Berlin; enalapril by Merck Sharp & Dohme, Munich; and lisinopril by Zeneca, Plankstadt, Germany. Ciba-Geigy, Basel, Switzerland, kindly donated the 2 CGP 20712 A (1-[2-((3-carbamoyl-4-hydroxy)phenoxy)ethylamino]-3-[4-(1-methyl-4-trifluoromethyl-2-imidazolyl)phenoxy]-2-propanol methanesulfonate) and (±)-CGP 12177 hydrochloride (4-(3-tertiarybutylamino-2-hydroxypropoxy)-benzimidazole-2-on).

Manuscript received May 5, 1994; revised manuscript received September 23, 1994, accepted September 28, 1994.

*Present address and address for correspondence: Institute of Pharmacology and Toxicology, Martin-Luther-University of Halle-Wittenberg, Magdeburger Strasse 4, D-06097 Halle (Saale), Germany.

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0735-1097/95/$9.50
0735-1097(94)00425-P

therein]). We therefore also studied the effects of angiotensin-converting enzyme inhibitors on possible cyclosporine A–induced changes in cardiac beta-adrenoceptor subtypes. Finally, because calcium-entry blocking agents have been found to be very protective against cyclosporine A–induced renal ischemic injury (16–18) and are therefore frequently used in renal transplant recipients (19,20), we also studied the effects of diltiazem on possible cyclosporine A effects on cardiac beta-adrenoceptor subtypes.

Methods

Study protocol. We performed two studies. *First study.* Eighty male Wistar rats were randomly assigned to six groups. In addition to their standard diet (Altromin, Altromin GmbH & Co KG, Lange, Germany), the rats received daily treatment with one of the following: 1 ml of olive oil (n = 20), 30 mg/kg per day of cyclosporine A dissolved in olive oil (n = 20), 10 mg/kg per day of the angiotensin-converting enzyme-inhibitor enalapril dissolved in distilled water (n = 10), 60 mg/kg per day of the calcium-entry blocker diltiazem dissolved in distilled water (n = 10), cyclosporine A plus diltiazem (n = 10) or cyclosporine A plus enalapril (n = 10) administered through an oral gastric tube for 6 weeks. Two rats in the cyclosporine A group, two rats in the cyclosporine A plus diltiazem group and four rats in the cyclosporine A plus enalapril group died during the treatment period. On the basis of the data obtained with these treatment regimens (see Results), we decided to perform a second study.

Second study. Sixty male adult Wistar rats were treated with one of the following: 1 ml of olive oil (n = 15), 15 mg/kg per day of cyclosporine A (n = 15), 10 mg/kg per day of the angiotensin-converting enzyme inhibitor lisinopril dissolved in distilled water (n = 15) or cyclosporine A plus lisinopril (n = 15) orally for 6 weeks, as described in the first study.

Twenty-four hours after the last treatment, the rats were anesthetized with ether; blood pressure was measured invasively in the abdominal aorta; and 5 ml of blood was withdrawn from the abdominal aorta for laboratory tests. Blood levels of cyclosporine A were measured by a fluorescence-polarizing immunoassay used for routine drug monitoring in heart transplant recipients (Abbott Laboratories). Laboratory tests for renal function (serum creatinine and serum nitrogen urea levels) were performed with an automatic analyzer (BM/Hitachi System 717, Boehringer, Mannheim, Germany). After the rats had been exsanguinated, the aortas and hearts were rapidly excised. The aortas were used to study the effects of cyclosporine A on vascular function (18,21,22), and the hearts were cleaned of connective tissue and quickly frozen in liquid nitrogen.

Beta-adrenoceptor determination. For preparation of membranes, the hearts were minced with scissors and homogenized with a glass–Teflon homogenizer (Braun, Melsungen, Germany) with 10 strokes at 1,500 rpm. The homogenate was diluted up to 50 ml with buffer and centrifuged at 1,000 *g* for 15 min. Pellets were discarded, and the supernatant was filtered through four layers of medical gauze and centrifuged at 21,000 *g* for 45 min. Final pellets were resuspended in incubation buffer (10 mmol/liter of Tris, 154 mmol/liter of sodium chloride and 0.01% ascorbic acid at a pH of 7.4), rehomogenized with six strokes at 800 rpm and diluted to 100 ml/g of wet weight. The protein concentration of the final membrane preparation was assessed by the method of Bradford (23) using bovine immunoglobulin G as standard.

Beta-adrenoceptor density was assessed by (−)-[^{125}I] iodocyanopindolol (ICYP) binding at six concentrations of ICYP ranging from 10 to 200 pmol/liter (ICYP specific activity 2,000 Ci/mmol, New England Nuclear, Dreieich, Germany). An aliquot of the membrane suspension (150 μl) was incubated with ICYP in a final volume of 250 μl for 90 min at 37°C. Incubation was stopped by adding 10 ml of incubation buffer to the entire incubation mixture, followed by rapid vacuum filtration over Whatman GF/C glass fiber filters. Each filter was washed with an additional 10 ml of incubation buffer, and the remaining radioactivity was determined in a Beckman gamma counter at an efficiency of 80%. Nonspecific binding of ICYP was defined as the radioactivity bound in the presence of a high concentration of (±)-CGP 12177 (1 μmol/liter). Specific binding was defined as total binding minus nonspecific binding and usually amounted to >80% at 20 pmol/liter of ICYP.

To determine the relative amount of $beta_1$- and $beta_2$-adrenoceptors, membranes were incubated with ICYP (100 pmol/liter) in the presence or absence of eight concentrations (range 10^{-10} to 10^{-3} mol/liter) of the highly selective $beta_1$-adrenoceptor antagonist CGP 20712 A (24), and specific binding was determined as described earlier. Details have been described elsewhere (25). The CGP 20712 A competition curves were analyzed by the iterative curve-fitting program InPlot (GraphPad software). Statistical analysis was performed using the F ratio test to measure the goodness of fit of the competition curves for either one or two sites.

All chemicals used in this study were of the highest purity grade commercially available.

Statistical evaluations. Results are presented as mean value ± SEM of n experiments. The equilibrium dissociation constant (K_D) and the maximal number of binding sites (B_{max}) for ICYP were calculated from plots according to Scatchard (26). The significance of differences in the number of binding sites in two different groups was estimated by an unpaired two-tailed Student *t* test; $p < 0.05$ was considered significant.

Results

Effects of 30 mg/kg per day of cyclosporine A. After 6 weeks of treatment with cyclosporine A (30 mg/kg per day), blood levels were between 3,000 and 5,000 ng/ml (Table 1). In the cyclosporine A–treated rats, mean blood pressure was unchanged, but weight gain was significantly retarded. In addition, serum nitrogen urea levels were significantly elevated, and serum creatinine levels showed a tendency to increase. Enalapril and diltiazem did not prevent retardation

Table 1. Laboratory Tests in Rats After 6 Weeks of Treatment With 30 mg/kg per Day of Cyclosporine A

	Control (n = 20)	CSA (n = 18)	Diltiazem (n = 10)	Enalapril (n = 10)	Diltiazem + CSA (n = 8)	Enalapril + CSA (n = 6)
Body weight (g)						
Before treatment	358 ± 5	316 ± 7	317 ± 10	335 ± 10	324 ± 7	328 ± 7
End of treatment	402 ± 9	306 ± 15*	386 ± 7	383 ± 13	323 ± 13*	343 ± 5
Mean blood pressure (mm Hg)	81 ± 4	81 ± 3	80 ± 5	76 ± 3	77 ± 5	75 ± 6
CSA blood levels (ng/ml)		5,313 ± 913			5,615 ± 888	3,180 ± 610
Serum urea (mg/100 ml)	47.6 ± 1.7	82.2 ± 4.5*	47.1 ± 1.7	45.0 ± 2.0	86.3 ± 13.5*	80.0 ± 5.1*
Serum creatinine (mg/100 ml)	0.7 ± 0.02	0.8 ± 0.02	0.6 ± 0.01	0.6 ± 0.02	0.7 ± 0.1	0.8 ± 0.1

*$p < 0.01$ versus olive oil-treated rats (Control). Data presented are mean value ± SEM. CSA = cyclosporine A.

in weight gain and increase in serum nitrogen urea levels (Table 1).

Cyclosporine A significantly decreased cardiac beta-adrenoceptor density by ~20 to 25% (Fig. 1), a result of a selective decrease in $beta_1$-adrenoceptors but no significant alteration in $beta_2$-adrenoceptors (Fig. 1). Enalapril alone did not affect rat cardiac beta-adrenoceptor density or subtype distribution (Fig. 2). However, administration of enalapril in addition to cyclosporine A completely prevented the cyclosporine A–induced decrease in $beta_1$-adrenoceptor density (Fig. 2).

Similar to enalapril, administration of the calcium-entry blocker diltiazem alone did not affect cardiac beta-adrenoceptor density or subtype distribution (Fig. 3) but completely prevented the cyclosporine A–induced decrease in cardiac $beta_1$-adrenoceptor density (Fig. 3).

Effects of 15 mg/kg per day of cyclosporine A. In the first study, the 30-mg/kg cyclosporine A dose resulted in markedly higher blood levels of cyclosporine A compared with the therapeutic range used in humans (27). Because the observed changes in cardiac beta-adrenoceptors might express toxic side effects of cyclosporine A, we therefore decided to study a 15-mg/kg per day dose of cyclosporine A for 6 weeks that would result in more therapeutically relevant blood levels (~400 to 500 ng/ml) (Table 2). Moreover, in this study we used lisinopril as the angiotensin-converting enzyme inhibitor to determine whether it might have beta-adrenoceptor–protecting effects in the rat similar to those in the failing human heart (28).

As shown in Table 2, the 15-mg/kg dose of cyclosporine A did not cause weight gain retardation or increases in serum creatinine or nitrogen urea levels. Serum nitrogen urea was increased only in the cyclosporine A plus lisinopril group (Table 2).

Treatment with 15 mg/kg of cyclosporine A caused only a mild reduction in rat cardiac beta-adrenoceptor density (p = NS) (Fig. 4). However, after 6 weeks of treatment with

Figure 1. Effects of 6 weeks of treatment with cyclosporine A (CSA) (30 mg/kg per day orally) on cardiac beta-adrenoceptors in rats. **Ordinate** = total beta-, $beta_1$- and $beta_2$-adrenoceptor density in cardiac membranes in fmol of ICYP specifically bound/mg of protein. Data are mean value ± SEM (number of experiments); dissociation constant values for ICYP are 8.3 ± 1.1 pmol/liter (Control) and 8.9 ± 1.4 pmol/liter (cyclosporine A). Control = olive oil-treated rats; ICYP = (−)-[^{125}I]iodocyanopindolol.

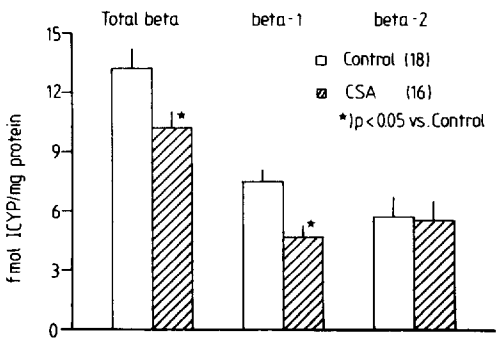
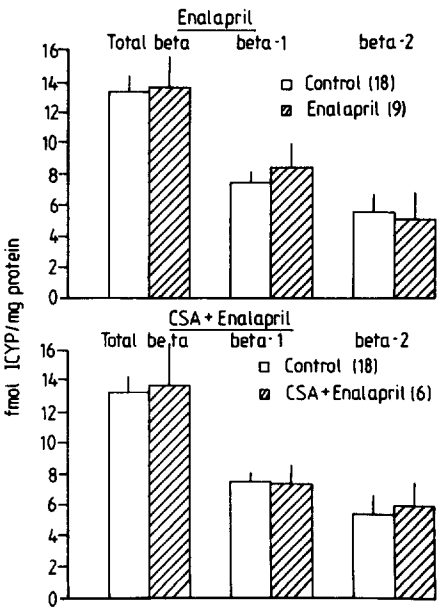


Figure 2. Effects of 6 weeks of treatment with enalapril (10 mg/kg per day orally) **(top)** or cyclosporine A (CSA) (30 mg/kg per day) plus enalapril (10 mg/kg per day orally) **(bottom)** on cardiac beta-adrenoceptors in rats. **Ordinates** = total beta-, $beta_1$- and $beta_2$-adrenoceptor density in cardiac membranes in fmol of ICYP specifically bound/mg of protein. Data are mean value ± SEM (number of experiments); dissociation constant values for ICYP are 9.1 ± 0.9 pmol/liter (enalapril) and 8.8 ± 1.7 pmol/liter (enalapril plus cyclosporine A). Other abbreviations as in Figure 1.



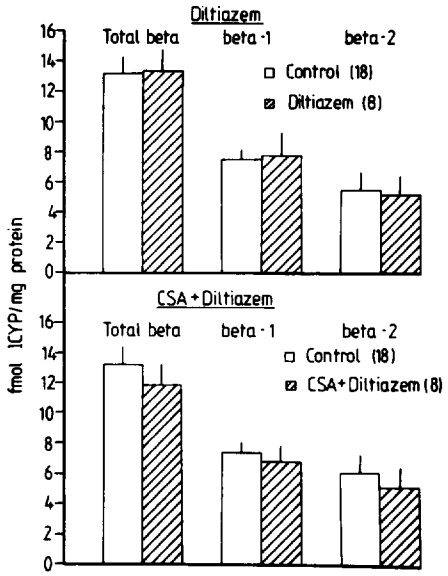


Figure 3. Effects of 6 weeks of treatment with diltiazem (60 mg/kg per day orally) **(top)** or cyclosporine A (CSA) (30 mg/kg per day) plus diltiazem (60 mg/kg per day orally) **(bottom)** on cardiac beta-adrenoceptors in rats. **Ordinates** = total beta-, $beta_1$- and $beta_2$-adrenoceptor density in cardiac membranes in fmol of ICYP specifically bound/mg of protein. Data are mean value ± SEM (number of experiments); dissociation constant values for ICYP are 7.9 ± 1.2 pmol/liter (diltiazem) and 8.4 ± 0.8 pmol/liter (diltiazem plus cyclosporine A). Other abbreviations as in Figure 1.

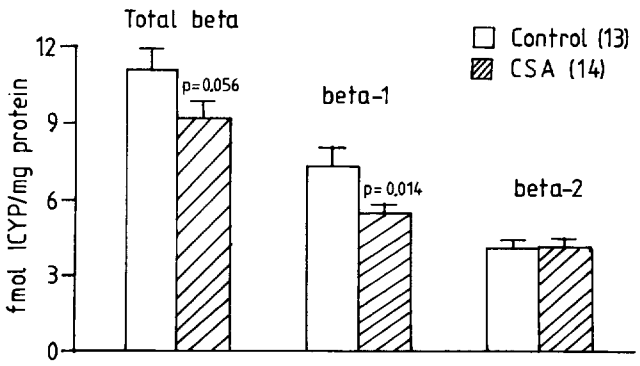


Figure 4. Effects of 6 weeks of treatment with cyclosporine A (CSA) (15 mg/kg per day orally) on cardiac beta-adrenoceptors in rats. **Ordinates** = total beta-, $beta_1$- and $beta_2$-adrenoceptor density in cardiac membranes in fmol of ICYP specifically bound/mg of protein. Data are mean value ± SEM (number of experiments); dissociation constant values for ICYP are 11.4 ± 2.1 pmol/liter (control) and 12.1 ± 1.9 pmol/liter (cyclosporine A treated). Other abbreviations as in Figure 1.

cyclosporine A, cardiac $beta_1$-adrenoceptor density was significantly reduced, whereas $beta_2$-adrenoceptor density was not affected (Fig. 4). Lisinopril alone did not affect cardiac beta-adrenoceptor density or subtype distribution (Fig. 5). However, lisinopril completely prevented the cyclosporine A–induced decrease in cardiac $beta_1$-adrenoceptor density (Fig. 5).

Discussion

Cyclosporine A causes selective downregulation of $beta_1$-adrenoceptors in the rat heart. In the present study, 6 weeks of treatment with cyclosporine A in normotensive Wistar rats caused a selective downregulation of cardiac $beta_1$-adrenoceptors but no significant alteration in cardiac $beta_2$-adrenoceptors. This alteration occurred not only with the high dose of 30 mg/kg per day of cyclosporine A, resulting in very high blood levels (3,000 to 5,000 ng/ml), but with the lower dose of 15 mg/kg per day as well, resulting in blood levels (~500 ng/ml) in the therapeutic range of cyclosporine A given the finding that in rats higher doses of cyclosporine A are needed to prevent rejection of transplanted organs than in humans [29]. Thus, the decrease in $beta_1$-adrenoceptor density was not an expression of toxic side effects of cyclosporine A and also occurred in therapeutically relevant doses.

We previously showed (11) in young spontaneously hypertensive rats that treatment with cyclosporine A (20 mg/kg per day for 7 weeks) augmented blood pressure and caused a decrease in cardiac $beta_1$-adrenoceptor density but no change in $beta_2$-adrenoceptor density. One could argue that this decrease in $beta_1$-adrenoceptor density is not caused by cyclosporine A but is due to the finding that in several forms of experimental hypertension, severe elevation of blood pressure is often associated with a selective downregulation of cardiac

Table 2. Laboratory Tests in Rats After 6 Weeks of Treatment With 15 mg/kg per Day of Cyclosporine A

	Control (n = 15)	CSA (n = 15)	Lisinopril (n = 15)	Lisinopril + CSA (n = 15)
Body weight (g)				
Before treatment	260 ± 11	279 ± 12	253 ± 7	309 ± 18
End of treatment	370 ± 14	381 ± 11	346 ± 8	366 ± 12
CSA blood levels (ng/ml)		506 ± 96		431 ± 50
Serum urea (mg/100 ml)	40.9 ± 1.5	45.0 ± 6.4	48.1 ± 1.6	94.9 ± 6.4*
Serum creatinine (mg/100 ml)	0.52 ± 0.02	0.56 ± 0.03	0.52 ± 0.04	0.59 ± 0.02

*$p < 0.01$ versus olive oil-treated rats (Control). Data presented are mean value ± SEM. CSA = cyclosporine A.

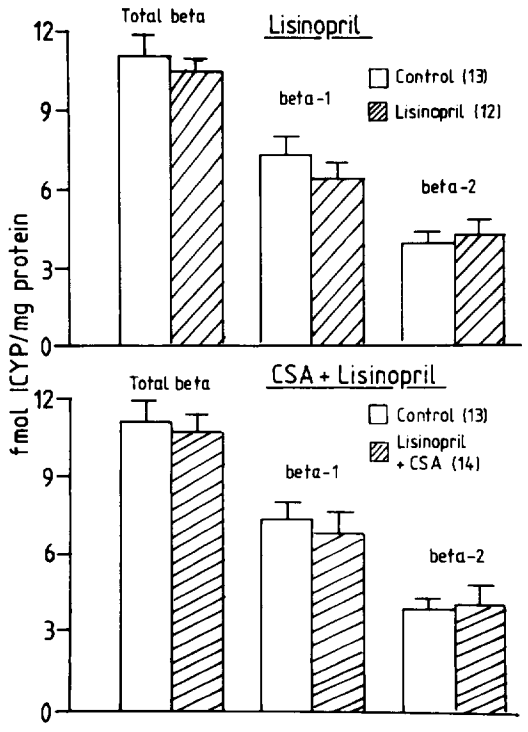


Figure 5. Effects of 6 weeks of treatment with lisinopril (10 mg/kg per day orally) **(top)** or cyclosporine A (CSA) (15 mg/kg per day) plus lisinopril (10 mg/kg per day orally) **(bottom)** on cardiac beta-adrenoceptors in rats. **Ordinates** = total beta-, $beta_1$- and $beta_2$-adrenoceptor density in cardiac membranes in fmol of ICYP specifically bound/mg of protein. Data are mean value ± SEM (number of experiments); dissociation constant values for ICYP are 10.9 ± 2.0 pmol/liter (lisinopril) and 12.2 ± 1.6 pmol/liter (lisinopril plus cyclosporine A). Other abbreviations as in Figure 1.

$beta_1$-adrenoceptors (for review, see ref. 30). However, in the present study, both doses of cyclosporine A also caused a similar decrease in $beta_1$-adrenoceptor density in normotensive rats, although long-term treatment with cyclosporine A does not increase blood pressure in normotensive rats (10) (Table 1). Thus, hypertension in itself is not the cause of the cyclosporine A–induced decrease in $beta_1$-adrenoceptor density.

However, in the present study we assessed only cardiac beta-adrenoceptor density, not cardiac G-protein(s) or adenylate cyclase activity. Thus, it remains to be elucidated whether the decrease in cardiac $beta_1$-adrenoceptor density after long-term cyclosporine A treatment is accompanied by functional changes. Moreover, the rat heart shows regional distribution of beta-adrenoceptors, and the total number of $beta_1$- and $beta_2$-adrenoceptors is higher in the atrioventricular conducting system than in myocardium (31–33). In the present study we assessed beta-adrenoceptors in the whole rat heart only. Thus, we cannot exclude the possibility that the effects of cyclosporine A on beta-adrenoceptor subtypes (i.e., downregulation of $beta_1$-adrenoceptors with no change in $beta_2$-adrenoceptors) might vary regionally.

The mechanism underlying the decrease in cardiac $beta_1$-adrenoceptor density after long-term treatment with cyclosporine A is not understood at present. However, it is well known that cyclosporine A in rats causes an increase in the activity of the sympathetic as well as the renin–angiotensin system (15). Both are closely interrelated. Thus, increases in sympathetic activity enhance renin release in the rat by means of $beta_1$-adrenoceptor stimulation (34), and angiotensin II can enhance noradrenaline release through activation of presynaptic angiotensin II receptors (35). Because an increase in sympathetic activity is accompanied by an increased release of noradrenaline, it is possible that in cyclosporine A–treated rats cardiac beta-adrenoceptors undergo long-term exposure to increased noradrenaline concentrations. However, noradrenaline is a selective $beta_1$-adrenoceptor agonist (36); for example, in rats harboring a noradrenaline-secreting pheochromocytoma, noradrenaline causes a selective downregulation of $beta_1$-adrenoceptors but no change in $beta_2$-adrenoceptors in various tissues (37,38). Moreover, long-term infusion of noradrenaline in rabbits has been shown (39) to induce selective downregulation of $beta_1$-adrenoceptors without affecting $beta_2$-adrenoceptors in various tissues. Thus, it is possible that in cyclosporine A–treated rats, increased sympathetic activity (by means of enhanced [locally?] released noradrenaline) causes the selective downregulation of cardiac $beta_1$-adrenoceptors.

Angiotensin-converting enzyme inhibitors prevent cyclosporine A–induced $beta_1$-adrenoceptor downregulation in the rat heart. In support of this hypothesis is the finding that in the present study, angiotensin-converting enzyme inhibitors prevented the cyclosporine A–induced downregulation of cardiac $beta_1$-adrenoceptors. As previously discussed, the sympathetic nervous and renin–angiotensin systems are closely interrelated. Thus, antagonization of one system should inhibit the other. For example, it has been shown (40–42) that angiotensin-converting enzyme inhibitors reduce angiotensin II–facilitated release of noradrenaline in animal models. Accordingly, the most likely explanation for the angiotensin-converting enzyme inhibitor–induced prevention of cardiac $beta_1$-adrenoceptor downregulation caused by cyclosporine A treatment is that enalapril and lisinopril reduce the cyclosporine A–induced activation of sympathetic activity, thereby preventing $beta_1$-adrenoceptor downregulation.

In favor of this assumption are results obtained in guinea pigs, where captopril partially prevented cardiac beta-adrenoceptor downregulation induced by long-term isoprenaline treatment (43), and in patients with chronic heart failure, where lisinopril restored the reduced cardiac beta-adrenoceptor density to pretreatment levels (28). In the latter study, lisinopril increased cardiac beta-adrenoceptor density in only those patients with high coronary sinus noradrenaline levels (i.e., expressing high sympathetic drive) but not in those exhibiting nearly normal noradrenaline levels. This strengthens the idea (see earlier) that angiotensin-converting enzyme inhibitors might prevent (or at least attenuate) beta-adrenoceptor downregulation by means of antagonizing sympathetic activity.

The calcium entry blocker diltiazem prevents cyclosporine A–induced beta$_1$-adrenoceptor downregulation in the rat heart. In the present study the calcium entry blocker diltiazem also prevented cyclosporine A–induced beta$_1$-adrenoceptor downregulation. This result was not brought about by the direct effect of diltiazem on cardiac beta-adrenoceptors because in the rat heart (present study) and in the nonfailing (44–46) and failing (46) human heart, calcium entry blockers had no effect on cardiac beta-adrenoceptor density. Thus, diltiazem must interfere with the effects of cyclosporine A; however, the mechanism of this interaction is still unknown. In this context, cyclosporine A induced an increase in renal sympathetic nerve activity in rats that was strongly associated with inhibition of calcineurin activity (47), the intracellular target of cyclosporine A, which binds the cyclosporine A–cyclophilin complex in a calcium ion–dependent manner (48). However, calcineurin, which is localized not only in lymphoid tissues, but has also been found in neural tissues (49), is involved in transcriptional processes (48). Furthermore, cyclosporine A has been shown to enhance basal calcium ion influx and to augment angiotension II–stimulated influx of calcium ions in vascular smooth muscle cells (50,51) and to increase angiotensin II–induced vasoconstriction in isolated rat aorta (21). Whether attenuation of these effects (cyclosporine A–induced calcium ion accumulation or inhibition of calcineurin-mediated transcriptional processes) by the calcium entry blocker diltiazem might be involved in its protective effect against cyclosporine A–induced downregulation of cardiac beta$_1$-adrenoceptors remains to be elucidated.

Conclusions. The results of the present study show that a therapeutically relevant dose of cyclosporine A caused selective downregulation of cardiac beta$_1$-adrenoceptors in both spontaneously hypertensive (11) and normotensive rats. Because this event can be prevented by the angiotensin-converting enzyme inhibitors enalapril and lisinopril, it might be due to the activating effects of cyclosporine A on the renin–angiotensin system or sympathetic nervous system, or both. The mechanism of the diltiazem-induced prevention of cyclosporine A–evoked beta$_1$-adrenoceptor downregulation is still unknown.

Clinical implications. The present findings of a selective downregulation of cardiac beta$_1$-adrenoceptors by cyclosporine A and its prevention by angiotensin-converting enzyme inhibitors or diltiazem could have clinical implications. Human heart transplant recipients with long-term cyclosporine A therapy show the same pattern of selective beta$_1$-adrenoceptor downregulation with no change in beta$_2$-adrenoceptor density with increasing time after transplantation (12–14). Moreover, it has recently been shown (52) that cyclosporine A also increases sympathetic activity in heart transplant recipients, and this might be enhanced because of the impaired cardiopulmonary baroreflex activity in such patients. The development of hypertension in heart transplant recipients is a common side effect of cyclosporine A that has been directly attributed to its administration (53). Thus, the present results suggest that treatment of this form of hypertension with calcium-entry blockers (and, under certain circumstances, possibly angiotensin-converting enzyme inhibitors) might be of benefit because they not only normalize blood pressure (8), but also may protect the transplanted heart against beta-adrenoceptor subtype redistribution.

We are grateful to Dr. R. J. Summers, University of Melbourne for giving us access to his data before publication.

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