ABSTRACT

Cyclosporine A (CsA) use is associated with hypertension and reduced baroreceptor sensitivity (BRS), but the underlying mechanisms remain unresolved. In this study, we investigated whether CsA attenuation of BRS is 1) dependent on treatment regimen, and 2) causative of the pressor response. Furthermore, we investigated whether a reduction in plasma testosterone contributes to BRS attenuation caused by short-term CsA administration. The effects of the clinically used CsA formulation (15 mg/kg/day i.v. for 5 days) on mean arterial pressure (MAP), heart rate, BRS, and body weight were investigated in conscious rats. CsA caused reproducible pressor responses (15.1 ± 3.0 mm Hg) starting after the first dose and continuing through the 5 days of the study. BRS and baseline MAP were inversely related in the CsA group because of a progressive reduction in BRS, which started on day 2 and reached ~50% of baseline on day 5 and a cumulative elevation in MAP. The inverse BRS and MAP responses required daily administration of CsA because neither response was evident throughout the 5-day observation period after a single dose of CsA. Plasma testosterone levels were similar in all groups, whereas the body weight decreased approximately 10% in the CsA group on day 5. These findings suggest 1) CsA attenuation of BRS is relatively rapid and cumulative; 2) the attenuation of BRS may contribute to the delayed, but not to the acute, pressor elicited by CsA; and 3) the cumulative reduction in BRS caused by short-term (5-day) CsA treatment is not testosterone-related.
have revealed similar incidence of CsA-evoked hypertension in female and male patients (Fernandez-Miranda et al., 2002), but it is not known whether the magnitude of hypertension is influenced by gender. Nonetheless, whether a rapid inhibitory effect of CsA on BRS occurs in rodents and whether such effect is secondary to a reduction in serum testosterone has not been investigated. Furthermore, the reported effects on the efficacy of a single dose (Ryuzyaki et al., 1997) or multiple doses (50 mg/kg, 200 mg/kg) have revealed similar incidence of systemic CsA-evoked hypertension. Our previous studies (Abdel-Rahman, 1994) have revealed similar incidence of systemic CsA-evoked hypertension. The main objective of the present study was to investigate the acute and short-term effects of CsA on MAP and BRS using the clinically prescribed formulation and route of administration (i.v. infusion). Because measurements of MAP and BRS were made daily in the same animal over the 5-day course of the study, we determined the temporal relationship between CsA-induced inhibition of BRS and the acute and chronic pressor responses. Because our experimental design and the use of the clinical formulation of CsA lead to a relatively rapid and cumulative inhibition of BRS, a second objective of the study was to determine whether the substantial (50%) inhibition of BRS was a consequence of CsA-induced reduction in serum testosterone levels. To achieve these goals, MAP and BRS (Oxford method) were measured in conscious freely moving Sprague-Dawley male rats before and after daily administration of CsA, vehicle, or saline for five consecutive days. Finally, an additional group of rats received a single dose of CsA followed by vehicle for the remaining 4 days to determine whether the acute (single-dose) effects of CsA on BP, HR, and body weight are fully reversible, and 2) the sustained reduction in BRS and increase in MAP require daily treatment with CsA. Body weights of the rats were recorded daily, and blood samples were collected for measurement of plasma testosterone levels at the conclusion of the study (5th day).

Materials and Methods

Preparation of Rats

Male Sprague-Dawley rats (300–350 g; Harlan, Indianapolis, IN) were used in this study. Upon arrival, the rats were housed in a room with controlled environment at constant temperature of 23 ± 1°C and humidity of 50 ± 10% and were maintained on a 12/12-h light/dark cycle with light off at 7:00 PM. The rats were housed individually in standard plastic cages and allowed free access to water and food. Arterial blood pressure was measured according to the method used in our previous studies (Abdel-Rahman, 1994). Briefly, the rats were anesthetized with methohexital sodium (50 mg/kg, i.p.). Catheters (polyethylene-10 connected to polyethylene-50), filled with heparinized saline (100 U/ml), were placed in the abdominal aorta and vena cava via the femoral artery and vein for measurement of BP and i.v. administration of drugs, respectively. The catheters were inserted about 5 cm into the femoral vessels and secured in place with sutures. Finally, the catheters were tunneled a.c.e., exteriorized at the back of the neck between the scapulae, and plugged by stainless-steel pins. Incisions were closed by surgical staples and swabbed with povidone-iodine solution. Each rat received an s.c. injection of buprenorphine hydrochloride (Buprenex; 0.3 mg/rat) to control pain and an i.p. injection of 50,000 U/kg penicillin G benzathine and penicillin G procaine in an aqueous suspension (Durapen) and was housed in a separate cage. Each experiment started 2 days later, by connecting the arterial catheter to a pressure transducer (Gould-Statham, Oxnard, CA). Blood pressure and heart rate (HR) were recorded and analyzed using PolyView PRO/62 data acquisition and analysis system (Grass Instrument Division, Astro-Med, Inc., West Warwick, RI). BP and HR were also displayed on a polygraph (model 7D; Grass Instruments, Quincy, MA). One of the venous catheters was used to inject CsA, vehicle, or saline. The other venous catheter was used to inject phenylephrine for the measurement of BRS. Experiments were performed in strict accordance with institutional animal care and use guidelines, and in accordance with the principles and guidelines of the National Institutes of Health Guide for the Care and Use of Laboratory Animals.

Measurement of Plasma Testosterone

A blood sample (0.6 ml) was withdrawn from the arterial catheter of each rat at specified hour (6:00–8:00 PM) on the 5th day (last day) of the experiment after baroreflex testing. Plasma testosterone level was measured by a commercially available radioimmunoassay Coat-A-Count total testosterone kit purchased from Diagnostic Products (Los Angeles, CA).

Protocols and Experimental Groups

Effect of Cyclosporin on Baroreceptor Reflex Control of HR.

Four groups of rats (cumulative cyclosporine, n = 6; single-dose cyclosporine, n = 6; vehicle, n = 6; and saline, n = 4) weighing 300 to 350 g were used in this study to investigate the effect of cyclosporine on BP and baroreflex HR response in conscious unrestrained rats. On the day of the experiment, the rats were allowed to acclimatize to laboratory conditions for at least 2 h before experimentation. Subsequently, the arterial catheter was connected to a pressure transducer for measurement of BP and HR. A period of 30 min was then allowed for further stabilization of BP and HR. Baroreflex curves were constructed in all rats by the i.v. bolus injection of randomized doses of PE (1–16 µg/kg) at 5-min intervals as in our previous study (Abdel-Rahman, 1994). Phenylephrine was dissolved in saline and administered in varying volumes of a stock concentration (36 µg/ml) of PE to achieve the desired doses. The peak changes in MAP and HR, obtained after PE injections, were used for the construction of the baroreflex curves. Then the rat in a particular group received i.v. infusion of cyclosporine (15 mg/kg), vehicle (195 mg/kg Cremophor EL + 96.9 mg/kg ethanol) or saline through the intravenous catheter over 10 min using CMA/100 microinfusion pump. The group assigned a single-dose regimen, received 15 mg/kg CsA on day 1 and vehicle over the consecutive 4 days. Twenty minutes after the end of infusion, another baroreflex curve, completed in approximately 1 h, was constructed as described above. Each experiment lasted approximately 2.5 h. These procedures were repeated for five consecutive days and on the 5th day blood samples (0.6 ml) were collected from the femoral artery, for measurements of serum testosterone level at specified time, to avoid the daily variation of plasma testosterone (Moeller et al., 1988; Leal and Moreira, 1997).

Drugs

Phenylephrine hydrochloride (Sigma-Aldrich, St. Louis, MO), methohexital sodium (Eli Lilly & Co., Indianapolis, IN), Buprenex (buprenorphine hydrochloride; Rickitt & Colman, Richmond, VA), Sandimmune injection (50 mg/ml cyclosporine; Novartis Pharmaceuticals Co., East Hanover, NJ), Cremophor EL (Sigma-Aldrich), povidone-iodine solution (Norton Co., Rockford, IL), and Durapen (penicillin G benzathine and penicillin G procaine; Vedco, Overland Park, KS) were purchased from commercial vendors.

Statistical Analysis

Values are expressed as means ± S.E.M. The relationship between increases in MAP [MAP = diastolic pressure + one-third (systolic – diastolic pressures)] and associated decreases in HR was assessed by regression analysis for individual animals, as described
in our previous studies (Abdel-Rahman, 1994). The regression coefficient (slope of the regression line) expressed as beats per minute per millimeter of Hg was taken as an index of BRS. Analysis of variance followed by Fisher’s least significant difference post hoc analysis was used for multiple comparisons. This test was used to analyze the effects of cyclosporine administration on MAP, HR, baroreflex, body weight, and plasma testosterone levels. The Student’s t test was used in the analysis of unpaired data. Probability levels less than 0.05 was considered significant.

### Results

**Effect of Cyclosporine on Mean Arterial Pressure and Heart Rate.** The baseline values of MAP measured in freely moving conscious rats, before treatment, on the first day of the experiment were similar in all groups of rats (Table 1). MAP remained unchanged after the infusion of vehicle or saline but was significantly \((p < 0.05)\) increased \((15.1 \pm 3.0 \text{ mm Hg})\) in the CsA group (Fig. 1A). On the 2nd day, CsA also caused a significant increase in MAP \((p < 0.05)\) \((21.6 \pm 3.7 \text{ mm Hg})\); MAP remained unchanged in the vehicle and saline groups. Similar rises in MAP were observed over the next 3 days of the study with a maximum increase in MAP after 10 min from the start of the infusion. These reproducible pressor responses caused by CsA were statistically significant \((p < 0.05)\) compared with the vehicle or saline values (Figs. 1A and 2A). In addition to the pressor response observed during the infusion, CsA produced a chronic cumulative elevation in MAP that started on the 3rd day and reached significance \((p < 0.05)\) on the 4th (compared with vehicle) and 5th (compared with vehicle and saline) day of CsA treatment (Table 1; Fig. 3A). To test whether this elevation in MAP was reversible, an additional group of rats received a single dose of CsA on the 1st day followed by the vehicle over the consecutive days. In this group, the acute pressor effect of CsA \((16.9 \pm 3.9, p < 0.05)\) was evident and the baseline MAP was slightly elevated on the 2nd day but decreased gradually and subsided to the baseline level by the 5th day (Table 1). The baseline heart rate values for the four groups were similar (Table 1). Daily CsA infusion caused a significant \((p < 0.05)\) increase in HR by the 3rd day (Table 1). However, during CsA infusion a significant \((p < 0.05)\) reduction of HR \((44 \pm 10.8 \text{ beats/min})\) in the 1st day was observed (Fig. 1B). This bradycardic response, which accompanied the CsA-evoked pressor response, was gradually reduced over

### Table 1

MAP and HR values obtained before the infusion of CsA, saline, or vehicle and the change from the previous day.

Data are means ± SE.

| Day | Saline | | Vehicle | | | Single-Dose CsA | | | Cumulative CsA | |
|-----|--------|-----------------|--------|-----------------|--------|-----------------|--------|-----------------|--------|
|     | Before | Change          | Before | Change          | Before | Change          | Before | Change          | Before | Change          |
| MAP | 1  | 123.5 ± 5.0  | 118.0 ± 7.0  | 113.2 ± 4.4  | 114.8 ± 2.8  | 2  | 117.7 ± 3.5  | 118.0 ± 7.6  | 116.7 ± 5.3  | 115.6 ± 3.2  | 3  | 120.9 ± 6.3  | 112.0 ± 7.6  | 116.4 ± 4.5  | 119.0 ± 3.0  | 4  | 118.7 ± 6.2  | 111.8 ± 8.3  | 115.0 ± 5.5  | 121.7 ± 2.7  | 5  | 119.7 ± 4.8  | 109.5 ± 4.9  | 111.7 ± 6.5  | 125.5 ± 3.5  | |
| HR  | 1  | 426 ± 20    | 398 ± 10   | 415 ± 10     | 428 ± 9     | 2  | 436 ± 16    | 384 ± 9    | 419 ± 11     | 425 ± 7     | 3  | 396 ± 13    | 365 ± 3     | 420 ± 15     | 452 ± 12     | 4  | 385 ± 6     | 360 ± 10   | 419 ± 11     | 461 ± 16     | 5  | 383 ± 5     | 377 ± 13  | 419 ± 12     | 467 ± 14     | |
|     | 1  | 426 ± 20    | 398 ± 10   | 415 ± 10     | 428 ± 9     | 2  | 436 ± 16    | 384 ± 9    | 419 ± 11     | 425 ± 7     | 3  | 396 ± 13    | 365 ± 3     | 420 ± 15     | 452 ± 12     | 4  | 385 ± 6     | 360 ± 10   | 419 ± 11     | 461 ± 16     | 5  | 383 ± 5     | 377 ± 13  | 419 ± 12     | 467 ± 14     | |

\*\# Significant difference compared with corresponding saline, vehicle, and single-dose CsA values, respectively.
the following days (26 ± 8.6 beats/min by the 5th day) in spite of similar pressor responses (Fig. 2B).

**Effect of Cyclosporine on Baroreceptor Reflex Control of HR (BRS).** Baroreflex curves, relating decrease in HR to phenylephrine-induced increase in MAP, were constructed (Fig. 4), and the slopes of the linear regression line represented the BRS (Fig. 5). At any given rise in MAP, the reflex bradycardic response remained unchanged after the infusion of vehicle or saline throughout the 5 days of the experiment (see example in Fig. 4, A and B). However, in the CsA-treated group at any given rise in MAP, the reflex bradycardia was significantly (p < 0.05) reduced starting with the 2nd dose (Fig. 4C); there was no significant change in the BRS after the 1st dose (day 1; Fig. 4C). The CsA induced inhibition of BRS was cumulative (Figs. 4C and 5D). Starting with a basal BRS value of −1.86 ± 0.15 beats/min/mm Hg, daily CsA administration resulted in a progressive reduction in BRS, which became −0.97 ± 0.04 beats/min/mm Hg (47% reduction) by the 5th day (Fig. 5D). In the group that received single-dose CsA, BRS value did not change during the 5-day observation period (Fig. 5C).

The correlation coefficients of the regression lines were highly significant and ranged from 0.935 to 0.995.

As shown in Fig. 3B, CsA caused a significant (p < 0.05) reduction in body weight amounting to 10% by the 5th day. Again, this reduction was cumulative. In the single-dose CsA group, there was a slight but nonsignificant reduction in body weight (Fig. 3B). The body weights of the saline and vehicle groups showed a slight increase over the same time period (Fig. 3B).

**Effect of Cyclosporine on Plasma Testosterone Level.** Analysis of the blood samples collected on the 5th day of the experiment revealed no significant differences in plasma testosterone levels between the rats that received CsA, vehicle, or saline (32.2 ± 14.4, 45.4 ± 19.6, and 35.7 ± 9.0 ng/dl, respectively).

**Discussion**

In the present study, we examined, for the first time, the temporal relationship between the attenuation in BRS and the pressor responses observed after short-term (5-day) CsA
treatment regimen using the clinically prescribed formulation. The study was extended to test the hypothesis that the early reduction in BRS caused by CsA precedes, and hence might not be attributed to, a reduction in serum testosterone; the latter is a consequence of long-term CsA therapy. The most important findings of the present study are 1) the acute pressor response produced by the first dose of CsA occurred in the absence of any change in BRS, 2) starting with the second dose, CsA caused a progressive reduction in BRS, which reached approximately 50% of control value by the 5th day, 3) CsA elicited, over the 5-day treatment period, a cumulative increase in MAP, 4) the effect of CsA on MAP and BRS is reversible, and 5) the alterations in BRS caused by CsA occurred in the absence of any change in serum testosterone.

In most of the reported studies, CsA was administered s.c. in oil and in high doses (>15 mg/kg/day) for extended periods, 11 to 28 days. MAP, HR, and BRS were measured only at the conclusion of the study (El-Mas et al., 2002), and in some instances, blood pressure was measured by the tail cuff method (Oriji and Keiser, 1999). Even when the i.v. route was used, a single dose of CsA was administered (Ryuzaki et al., 1997). To establish clinical relevance, we investigated the daily effects of CsA on blood pressure and BRS in conscious unrestrained rats. Furthermore, we used the clinical CsA formulation (Sandimmune) and administered the drug by i.v. infusion to establish a model system that permits generation of information that might bear scientific and clinical implications. We measured MAP and constructed baroreflex curves before and after daily infusion of CsA or its vehicle for 5 days. Because the vehicle contained Cremophor EL and alcohol, it was important to include another control (saline) group in the study.

Notably, the dose of CsA used in the present study was higher than the clinically used dose (15 versus 5 mg/kg), which may limit the clinical relevance of the present findings. It must be remembered, however, that because the dose used in the present study is equal to (Krueger et al., 1991; Jaramillo-Juarez et al., 2000) or slightly higher (Moss et al., 1985; Zhang et al., 2000) and even smaller (Ryuzaki et al., 1997) than the doses of CsA administered to experimental animals in reported studies, the present findings may have scientific implications. We observed an acute pressor response caused by the first dose of CsA that was not associated with any change in BRS, which rules out a role for BRS in the acute pressor effect of CsA. Our findings are consistent with reported acute increases in MAP produced by CsA infusion in rats (Morgan et al., 1991; Lyson et al., 1994), although in these studies BRS was not measured. It is unlikely that CsA-induced reduction in BRS contributes to the acute pressor response caused by CsA. In support of this notion, sinoaortic denervation failed to affect the pressor response elicited by CsA infusion (Lyson et al., 1994). Furthermore, CsA-induced hypertension seems to be mediated by synapsin, stored in the renal afferent nerve endings, because in the synapsin knockout mice CsA failed to induce hypertension (Zhang et al., 2000). On the other hand, our findings are not consistent with reported findings where an acute CsA infusion (20 mg/kg over 30 min) caused attenuation in baroreflex control of sympathetic discharge and no change in BP in rabbits (Ryuzaki et al., 1997). The difference in the BP and BRS responses could be attributed to species differences and the reflex response, HR in the present study versus sympathetic discharge in the reported study (Ryuzaki et al., 1997). Notably, the acute pressor effect obtained in the present study resembles a similar effect in humans (Schachter, 1988; Sturrock et al., 1993; Sander et al., 1996). It remains to be investigated, however, whether the clinically used dose of CsA produces similar effects on blood pressure and BRS in our model system to support the clinical relevance of our findings.

The present study is the first to report that CsA attenuates BRS in a progressive manner starting with the second dose,
and reaching approximately 50% reduction by the 5th day. The possibility must be considered that the increase in blood pressure and the decrease in heart rate caused by acute CsA administration might have contributed to the observed reduction in BRS in the present study. This possibility is unlikely because BRS did not change after the first dose of CsA in the presence of elevated blood pressure (Figs. 1 and 5D) nor in BRS in the group that received a single dose of CsA on day 1 and the vehicle over the following 4 days. Furthermore, the BRS values in the vehicle- or saline-treated rats did not change over the 5-day period. Notably, the progressive reductions in BRS in CsA-treated rats were associated with cumulative increases in baseline MAP. Whether this relationship is causal remains to be investigated. It is noteworthy that in the absence of any change in the BRS of the rats that received a single dose of CsA, MAP also remained unchanged compared with control (saline or vehicle) values on the 2nd through the 5th day of the study. It may be argued that the lack of inhibition of BRS in the group that received the single dose of CsA was due to a lower baseline value compared with the group that received the cumulative dose regimen. This is unlikely for two reasons. First, CsA did not reduce BRS after the first dose in the group that received the cumulative regimen in spite of a significantly higher baseline BRS (Fig. 5). Second, by the 5th day, the BRS in the group that received cumulative CsA was significantly lower than the BRS value in the group that received the single dose, indicating that the starting low baseline value in the latter group was not a limiting factor. Furthermore, in some models of hypertension, a reduction in BRS contributes to the consequent elevation in blood pressure (Gordon and Mark, 1983; Abdel-Rahman and Wooles, 1987). Together, it is likely that the chronic pressor response caused by CsA is a consequence of the attenuation of BRS.

The mechanism of a relatively rapid (2-day) CsA-induced attenuation of BRS is not known. A recent study attributed a 50% reduction in BRS caused by CsA to a reduction in serum testosterone (El-Mas et al., 2002). Because we obtained a similar reduction in BRS in rats at much earlier time (5 versus 11 days), we decided to determine whether the testosterone link also applies to our findings. We started with the measurements of plasma testosterone levels after 5 days of CsA administration. The lack of a change in serum testosterone level precludes a role for this hormone in the early (<5 days) attenuation of BRS caused by CsA in our study. Notably, to rule out any effect of diurnal variation in plasma testosterone levels, blood samples were withdrawn from all rats at a specified time on the 5th day (Moeller et al., 1988; Leal and Moreira, 1997).

Results of the present study demonstrated, for the first time, a temporal relationship between the time-dependent increases in baseline heart rate and blood pressure and the decline in BRS observed in the rats that received cumulative CsA treatment (Table 1; Figs. 3 and 5). The progressive increases in baseline blood pressure and heart rate become more apparent when comparisons are made with the appropriate control (vehicle) values. This is particularly important given the present findings that the vehicle, Cremophor EL, elicited a depressor response (Table 1; Fig. 3A), which has been attributed to inhibition of the diacylglycerol-protein

Fig. 5. Effect of saline (A), vehicle (B), single-dose CsA (C), and cumulative CsA (D) on BRS in conscious unrestrained rats over the 5 days of the study before (square) and after (circle) treatment. The single-dose CsA group received CsA (15 mg/kg i.v.) on the 1st day and similar volume of vehicle on the four subsequent days of the study. Data are means ± S.E. *, #, p < 0.05 compared with baseline BRS at day 1.
In the present study, we used CsA in the clinically available formulation, which contains Cremophor EL and ethanol. It is noteworthy that in reported studies, including our own, ethanol elicits dose-related attenuation of BRS. It is unlikely, however, that the amount of ethanol (0.1 g/kg/day) administered along with CsA, in our study, contributed to the attenuation of BRS for two reasons. First, we have shown in previous studies that ethanol in doses less than 0.25 g/kg has no effect on BRS (Abdel-Rahman, 1994). Second, the vehicle used in the present study, which contains the same amount of ethanol had no effect, compared with saline, on BRS over the 5-day treatment period. Finally, results of the present study showed a gradual reduction in body weight, which reached 10% by the 5th day. The reasons for the reduction in body weight in CsA-treated animals are not fully understood. However, our findings agree with the reported findings, which demonstrated similar loss in body weight that was attributed to anorexia and increased catabolism (Farthing and Clark, 1981) or to acid-base imbalance induced by CsA (Jaramillo-Juarez et al., 2000).

In summary, CsA induced progressive attenuation in BRS along with cumulative elevation of MAP over the 5-day course of the study in conscious unrestrained rats. On the other hand, the acute (first dose) pressor response observed during CsA infusion, which was reproducible over the 5-day treatment period, was not associated with any alteration in BRS. Furthermore, CsA had no effect on plasma testosterone levels after 5 days of daily treatment, which rules out a role for serum testosterone in the progressive attenuation of BRS. The present findings suggest that CsA-evoked reduction of BRS, which requires at least two daily doses of the drug, seems to contribute to the cumulative (chronic) but not to the acute elevation in blood pressure.

References


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