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Cyclosporine Induces Progressive Attenuation of Baroreceptor Heart Rate Response and cumulative pressor response in Conscious Unrestrained Rats

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d) Nonstandard Abbreviations:

CsA, Cyclosporin A; SD, Sprague Dawley; T, testosterone; i.v., intravenous; BRS, baroreflex sensitivity; s.c., subcutaneous; PE, phenylephrine; bpm, beats per minute; BP, blood pressure; HR, heart rate; U, units; mmHg, millimeters of mercury; MAP, mean arterial pressure;

ANOVA, Analysis of variance; LSD, Fisher's Least Significant Difference;

RIA, radioimmunoassay; SEM, standard error measurements;

SE, standard error; vs., versus.

e) Cardiovascular

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 (i) dependent on treatment regimen, and (ii) causative of the pressor response. Further, 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 (HR), BRS, and body weight were investigated in conscious rats. CsA caused reproducible pressor responses (15.1±3.0 mmHg) starting after the 1st 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 days observation period following a single dose of CsA. Plasma testosterone levels were similar in all groups, while the body weight decreased approximately 10% in the CsA group on day 5. These findings suggest: (i) CsA attenuation of BRS is relatively rapid and cumulative, (ii) the attenuation of BRS may contribute to the delayed, but not to the acute, pressor elicited by CsA; and (iii) the cumulative reduction in BRS caused by short-term (5-day) CsA treatment is not testosterone related.

Introduction

The immunosuppressive drug cyclosporine A (CsA) has greatly improved long-term survival after organ transplantation and the treatment of autoimmune diseases (Cohen et al., 1984). However, CsA also causes serious cardiovascular disorders such as secondary hypertension (Schachter, 1988; Sander et al., 1996), which comprises an acute phase developing with the first dose (Sturrock et al., 1993) and chronic phase during long-term administration (Olivari et al., 1989). CsA induced hypertension is a major contributing factor to cardiovascular morbidity and mortality in the transplant population (Sander et al., 1996) and is, therefore, a major limiting factor for the use of CsA.

In rats, i.v. CsA produces a rapid elevation in blood pressure (BP) that resembles the acute CsA-induced hypertension in humans (Moeller et al., 1988; Lyson et al., 1994). While evidence suggests that the acute pressor response to CsA is sympathetically mediated (Moeller et al., 1988; Lyson et al., 1994), other findings argue against this possibility (Kaye et al., 1993; Stein et al., 1995). Nonetheless, whether the attenuation of BRS contributes to the acute pressor effect of CsA has not been investigated. Notably, CsA induced inhibition of BRS has been implicated in the chronic pressor effect of the drug (Gerhardt et al., 1999; Lucini et al., 2000). Other proposed mechanisms include: (i) endothelial dysfunction (Oriji and Keiser, 1999), direct vasoconstriction (Xue et al., 1987; Lo Russo et al., 1997), (ii) induction of endothelin release (Grieff et al., 1993), (iii) inhibition of endothelin inactivating peptidase (Janas et al., 1994) and (iv) activation of afferent nerve firing from subdiaphragmic region with a reflex sympathetic activation, which was shown to be related to calcineurin enzyme inhibition (Moss et al., 1985; Zhang et al., 2000; Zhang and Victor, 2000).

The mechanism by which CsA inhibits BRS is not fully understood. Recent evidence suggests that a reduction in serum testosterone level contributes to the inhibition of BRS caused by chronic CsA administration (El-Mas et al., 2002). This conclusion is based on the findings that chronic CsA causes a reduction in serum testosterone (Rajfer et al., 1987; Krueger et al., 1991) and the ability of testosterone to enhance BRS (El-Mas et al., 2001). Epidemiological studies 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 findings on the effects of a single dose (Ryuzaki et al., 1997) or multiple doses (>10 days) of CsA (El-Mas et al., 2002) on MAP were based on the use of doses, formulation, and route of administration that are not relevant to the clinical use of CsA.

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. Since 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 in 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 (SD) male rats before and after daily administration of CsA, vehicle, or saline for 5 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: (a) the acute (single dose) effects of CsA on BP, HR and body weight are fully reversible and (b) 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 (5^{th} day).

Materials and Methods

Preparation of the rats

Male Sprague-Dawley (SD) rats (300-350 g; Harlan Farms, 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, humidity of 50±10% and were maintained on a 12-12 h light-dark cycle with light off at 19:00 h. 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 s.c., 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 a s.c. injection of buprenorphine hydrochloride (Buprenex; 0.3 ng/rat) to control pain and an i.p. injection of 50,000 U/kg of 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 Gould-Statham pressure transducer (Oxnard, CA). Blood pressure and heart rate were recorded and analyzed using PolyView PRO/32 data acquisition and analysis system (Grass Instrument Division, Astro-Med, Inc. West Warwick, RI). BP and HR were also displayed on a Grass polygraph (model 7D, Grass Instruments Co., Quincy, MA). One of the venous catheters was used to infuse 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 (1800-2000 h) on the fifth 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 Corporation (Los Angeles, CA).

Protocols and experimental groups

Effect of cyclosporine 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 300-350 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 prior to 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 studies (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 following 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 (cremophor EL 195 mg/kg + ethanol 96.9 mg/kg) or saline through the intravenous catheter over 10 minutes 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 min after the end of infusion, another baroreflex curve, completed in approximately 1 hr, was constructed as described above. Each experiment lasted approximately 2.5h. These procedures were repeated for 5 consecutive days and on the 5^{th} 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 Chemical Co., St. Louis, MO), methohexital sodium (Eli Lilly and Company, Indianapolis, IN), buprenex (buprenorphine hydrochloride; Rickitt & Colman, Richmond, VA), Sandimmune injection (cyclosporine 50mg/ml; Novartis Pharmaceuticals Co, East Hanover, NJ), Cremophor EL (Sigma Chemical Co., St. Louis, MO), 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 ± SEM. 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 bpm/mmHg was taken as an index of BRS. Analysis of variance (ANOVA) followed by Fisher's Least Significant Difference (LSD) 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 .05 was considered significant.

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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±3.0 mmHg) in the CsA group (Fig.1A). On the second day, CsA also caused a significant increase in MAP ($p<0.05$) (21.6±3.7 mmHg); 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 minutes from the start of the infusion. These reproducible pressor responses caused by CsA were statistically significant ($p<0.05$) when compared with the vehicle or saline values (Figs. 1A, 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 if this elevation in MAP was reversible, an additional group of rats received a single dose of CsA on the first day followed by the vehicle over the consecutive days. In this group, the acute pressor effect of CsA (16.9 ± 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±10.8 beats/min) in the first day was observed (Fig 1B). This bradycardic response, which accompanied the CsA evoked pressor response, was gradually reduced over 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 five days of the experiment (see example in Fig 4A, 4B). 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,5D). Starting with a basal BRS value of -1.86 ±0.15 beats/min/mmHg, daily CsA administration resulted in a progressive reduction in BRS, which became -0.97±0.04 beats/min/mmHg (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 fifth 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).

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Discussion

In the present study we examined, for the first time, the temporal relationship between the attenuation in BRS and the pressor responses observed following short-term (5 days) 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: (i) the acute pressor response produced by the first dose of CsA occurred in the absence of any change in BRS; (ii) starting with the second dose, CsA caused a progressive reduction in BRS, which reached approximately 50% of control value by the 5^{th} day; (iii) CsA elicited, over the 5 day treatment period, a cumulative increase in MAP, (iv) the effect of CsA on MAP and BRS is reversible; and (v) 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, 11-28 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. Further, 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.

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Notably, the dose of CsA used in the present study was higher than the clinically used dose (15 vs. 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 1st 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, sino-aortic denervation failed to affect the pressor response elicited by CsA infusion (Lyson et al., 1994). Further, 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 vs. 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 in order 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 2nd 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. 1,5D) nor in BRS in the group that received a single dose of CsA on day 1 and the vehicle over the following 4 days. Further, The BRS values in the vehicle or saline treated rats did not change over the five-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 as compared to the group that received the cumulative dose regimen. This is unlikely for two reasons. First, CsA did not reduce BRS after the 1st 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).

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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 days) 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). Since we obtained a similar reduction in BRS at much earlier time (5 vs. 11 days), we decided to determine if 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 fifth 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, 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 kinase C pathway (Zhao et al., 1989). The chronic pressor response seen with the cumulative CsA treatment could be due to one or more of the CsA reported effects (Cartier et al., 1994), which include: (i) reduced release of NO mediated by muscarinic receptor activation, (ii) increased production of endothelium related constricting factors mediated by serotonin receptors, and (iii) increased vascular sensitivity to circulating catecholamines. Notably, the chronic pressor

effect of CsA, which seems to be masked somewhat by the depressor effect of the vehicle, must be distinguished from the acute pressor effect caused, at least partly, by enhancing sympathetic activity (Sander et al., 1996). It is also important to comment on the inverse relationship between baseline heart rate and BRS observed following cumulative administration of CsA. Interestingly, in an earlier study, we observed a similar relationship between baseline heart rate and BRS in normotensive humans when BRS was measured by the same method used in the present study (Abdel-Rahman et al., 1994). The mechanism by which CsA produces incremental increase in heart rate is not known. It is possible, however, that this response observed in the present study and following 13-day treatment with CsA (El-Mas et al., 2002) is secondary to the progressive attenuation of BRS. Nonetheless, since the inverse relationship between baseline heart rate and BRS observed in the present study and in our previous study (Abdel-Rahman et al., 1994) is based on association, it is also possible that the reduced BRS is secondary to the higher baseline heart rate. As discussed above, chronic CsA administration caused a reduction in vasodilation elicited by muscarinic receptor activation (Cartier et al., 1994). A similar interaction, whether direct or indirect, between chronic CsA and cardiac muscarinic receptor, if exists, may well explain the cumulative increase in baseline heart rate as well as the associated reduction in BRS. Further studies are needed to investigate this possibility. It is notable that CsA effects seem to involve the pharmacokinetic profile of CsA rather than the mere plasma level of the drug because the increases in heart rate and the associated reductions in BRS started to appear on the 3rd day of treatment. Neither response occurred following the acute administration of CsA or when the effects of a single dose CsA were followed over the 5-day observation period and compared with the appropriate control (vehicle) values.

Results of the present study demonstrated consistent bradycardic responses along with the reproducible and similar pressor responses elicited by CsA, which suggests these HR responses are baroreflex-mediated. In support of this notion is the gradual reduction in the bradycardic response that paralleled the progressive reduction in BRS over the 5-day observation period. It may be argued that the reduction in BRS in the CsA group was a secondary event to the pressor response because hypertension is associated with attenuated BRS (Goldstein, 1983). Our findings argue against this possibility because the acute (1^{st} dose) pressor response elicited by CsA was not associated with any change in BRS.

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 (0.1g/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, when 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 5^{th} 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 (1st dose) pressor response observed during CsA infusion, which was reproducible over the 5-day treatment period, was not associated with any alteration in BRS. Further, 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

Abdel-Rahman AA (1994) Differential effects of ethanol on baroreceptor heart rate responses of conscious spontaneously hypertensive and normotensive rats. *Alcohol Clin Exp Res* **18**:1515-1522.

Abdel-Rahman AA and Wooles WR (1987) Ethanol-induced hypertension involves impairment of baroreceptors. *Hypertension* **10**:67-73.

Abdel-Rahman AR, Merrill RH and Wooles WR (1994) Gender-related differences in the baroreceptor reflex control of heart rate in normotensive humans. *J Appl Physiol* **77**:606-613.

Cartier R, Dagenais F, Hollmann C, Cambron H and Buluran J (1994) Chronic exposure to cyclosporine affects endothelial and smooth muscle reactivity in the rat aorta. *Ann Thorac Surg* **58**:789-794.

Cohen DJ, Loertscher R, Rubin MF, Tilney NL, Carpenter CB and Strom TB (1984) Cyclosporine: a new immunosuppressive agent for organ transplantation. *Ann Intern Med* **101**:667-682.

El-Mas MM, Afify EA, Mohy El-Din MM, Omar AG and Sharabi FM (2001) Testosterone facilitates the baroreceptor control of reflex bradycardia: role of cardiac sympathetic and parasympathetic components. *J Cardiovasc Pharmacol* **38**:754-763.

El-Mas MM, Afify EA, Omar AG and Sharabi FM (2002) Cyclosporine adversely affects baroreflexes via inhibition of testosterone modulation of cardiac vagal control. *J Pharmacol Exp Ther* **301**:346-354.

Farthing MJ and Clark ML (1981) Nature of the toxicity of cyclosporin A in the rat. *Biochem Pharmacol* **30**:3311-3316.

Fernandez-Miranda C, Sanz M, De La Calle A, Loinaz C, Gomez R, Jimenez C, Garcia I, Gomez De La Camara A and Moreno E (2002) Cardiovascular risk factors in 116 patients 5 years or more after liver transplantation. *Transpl Int* **15**:556-562.

Gerhardt U, Riedasch M and Hohage H (1999) Cyclosporine A modulates baroreceptor function in kidney transplant recipients. *Int J Cardiol* **68**:203-208.

Goldstein DS (1983) Arterial baroreflex sensitivity, plasma catecholamines, and pressor responsiveness in essential hypertension. *Circulation* **68**:234-240.

Gordon FJ and Mark AL (1983) Impaired baroreflex control of vascular resistance in prehypertensive Dahl S rats. *Am J Physiol* **245**:H210-217.

Grieff M, Loertscher R, Shohaib SA and Stewart DJ (1993) Cyclosporine-induced elevation in circulating endothelin-1 in patients with solid-organ transplants. *Transplantation* **56**:880-884.

Janas J, Sitkiewicz D, Warnawin K and Janas RM (1994) Characterization of a novel, high-molecular weight, acidic, endothelin-1 inactivating metalloendopeptidase from the rat kidney. *J Hypertens* **12**:1155-1162.

Jaramillo-Juarez F, Rodriguez-Vazquez ML, Namorado MC, Martin D and Reyes JL (2000) Acidosis and weight loss are induced by cyclosporin A in uninephrectomized rats. *Pediatr Nephrol* **14**:122-127.

Kaye D, Thompson J, Jennings G and Esler M (1993) Cyclosporine therapy after cardiac transplantation causes hypertension and renal vasoconstriction without sympathetic activation. *Circulation* **88**:1101-1109.

Krueger BA, Trakshel GM, Sluss PM and Maines MD (1991) Cyclosporin-mediated depression of luteinizing hormone receptors and heme biosynthesis in rat testes: a possible mechanism for decrease in serum testosterone. *Endocrinology* **129**:2647-2654.

Leal AM and Moreira AC (1997) Daily variation of plasma testosterone, androstenedione, and corticosterone in rats under food restriction. *Horm Behav* **31**:97-100.

Lo Russo A, Passaquin AC and Ruegg UT (1997) Mechanism of enhanced vasoconstrictor hormone action in vascular smooth muscle cells by cyclosporin A. *Br J Pharmacol* **121**:248-252.

Lucini D, Milani RV, Ventura HO, Mehra MR, Messerli F and Pagani M (2000) Study of arterial and autonomic effects of cyclosporine in humans. *Hypertension* **35**:1258-1263.

Lyson T, McMullan DM, Ermel LD, Morgan BJ and Victor RG (1994) Mechanism of cyclosporine-induced sympathetic activation and acute hypertension in rats. *Hypertension* **23**:667-675.

Moeller H, Goecke B and Herter F (1988) Seasonal and diurnal changes of prostatic androgen receptor and circulating testosterone in young mature rats. *Res Exp Med (Berl)* **188**:451-462.

Morgan BJ, Lyson T, Scherrer U and Victor RG (1991) Cyclosporine causes sympathetically mediated elevations in arterial pressure in rats. *Hypertension* **18**:458-466.

Moss NG, Powell SL and Falk RJ (1985) Intravenous cyclosporine activates afferent and efferent renal nerves and causes sodium retention in innervated kidneys in rats. *Proc Natl Acad Sci U S A* **82**:8222-8226.

Olivari MT, Antolick A and Ring WS (1989) Arterial hypertension in heart transplant recipients treated with triple-drug immunosuppressive therapy. *J Heart Transplant* **8**:34-39.

Oriji GK and Keiser HR (1999) Nitric oxide in cyclosporine A-induced hypertension: role of protein kinase C. *Am J Hypertens* **12**:1091-1097.

Rajfer J, Sikka SC, Lemmi C and Koyle MA (1987) Cyclosporine inhibits testosterone biosynthesis in the rat testis. *Endocrinology* **121**:586-589.

Ryuzaki M, Stahl LK, Lyson T, Victor RG and Bishop VS (1997) Sympathoexcitatory response to cyclosporin A and baroreflex resetting. *Hypertension* **29**:576-582.

Sander M, Lyson T, Thomas GD and Victor RG (1996) Sympathetic neural mechanisms of cyclosporine-induced hypertension. *Am J Hypertens* **9**:121S-138S.

Schachter M (1988) Cyclosporine A and hypertension. *J Hypertens* **6**:511-516.

Stein CM, He H, Pincus T and Wood AJ (1995) Cyclosporine impairs vasodilation without increased sympathetic activity in humans. *Hypertension* **26**:705-710.

Sturrock ND, Lang CC and Struthers AD (1993) Cyclosporin-induced hypertension precedes renal dysfunction and sodium retention in man. *J Hypertens* **11**:1209-1216.

Xue H, Bukoski RD, McCarron DA and Bennett WM (1987) Induction of contraction in isolated rat aorta by cyclosporine. *Transplantation* **43**:715-718.

Zhang W, Li J-L, Hosaka M, Janz R, Shelton JM, Albright GM, Richardson JA, Sudhof TC and Victor RG (2000) Cyclosporine A-induced hypertension involves synapsin in renal sensory nerve endings. *PNAS* **97**:9765-9770.

Zhang W and Victor RG (2000) Calcineurin inhibitors cause renal afferent activation in rats: a novel mechanism of cyclosporine-induced hypertension. *Am J Hypertens* **13**:999-1004.

Zhao FK, Chuang LF, Israel M and Chuang RY (1989) Cremophor EL, a widely used parenteral vehicle, is a potent inhibitor of protein kinase C. *Biochem Biophys Res Commun* **159**:1359-1367.

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Fig.1. Changes in mean arterial pressure (MAP, mmHg, **A**) and heart rate (HR, beats/min, **B**) induced by cyclosporine A (CsA; 15 mg/kg), vehicle or saline in conscious unrestrained rats. CsA , vehicle or saline was infused over 10 minutes (0-10 min). Values are means ± SE. * P<0.05 vs. vehicle and saline.

Fig.2. Changes in mean arterial pressure (MAP, mmHg, **A**) and heart rate (HR, beats/min, **B**) induced by cyclosporine A (CsA; 15 mg/kg), vehicle or saline in conscious unrestrained rats. CsA, vehicle or saline was infused over 10 minutes (0-10 min). Values are means ± SE. * P<0.05 vs. vehicle and Saline.

Fig. 3 Effect of cyclosporine A (CsA; 15 mg/kg) on MAP **(A)** and bodyweight **(B)** presented as percent change from the baseline (day 1) values. Data are means ± SE. *, # and Ψ, P<0.05 compared to vehicle, saline and single dose CsA, respectively. Baseline body weight in the single dose CsA, cumulative CsA, vehicle and saline groups was 350± 10, 287± 12, 338±11 and 314±19 g respectively.

Fig. 4 Baroreflex curves relating decrements in HR to increments in MAP evoked by phenylephrine in conscious unrestrained rats before (baseline, ■) and after (▲) the infusion of saline **(A)** or vehicle **(B)** on the first day of study and before (baseline, ■) and after 1 day (▲) and 5 days (◊) CsA (15 mg/kg) treatments **(C)**. Values are means ± SE.

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Fig. 5 Effect of saline **(A)**, vehicle **(B)**, single dose cyclosporine A (CsA) **(C)** and cumulative CsA **(D)** on baroreflex sensitivity (BRS) in conscious unrestrained rats over the five days of the study before (▒) and after (▉) treatment. The single dose CsA group received CsA (15 mg/kg i.v.) on the first day and similar volume of vehicle on the 4 subsequent days of the study. Data are means ± SE. *, # $P<0.05$ compared to baseline BRS at day 1.

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Table. 1 Mean arterial pressure (MAP) and heart rate (HR) values obtained before the infusion of CsA, saline or vehicle and the change from the previous day.

		Saline		Vehicle		Single dose CsA		Cumulative CsA	
	Day	Before	Change	Before	Change	Before	Change	Before	Change
MAP	1	123.5±6.0	-------	118.0±7.0	-------	113.2±4.4	-------	114.8±2.6	-------
	2	117.7±3.5	-5.8±2.6	118.0±7.6	-5.93±3.5	116.7±5.3	3.5±2.2	115.6±3.2	0.8±1.1
	3	120.9±6.3	-2.6±2.0	112.0±7.6	-6.2±2.8	116.4±4.5	3.2±2.5	119.0±3.0	4.2±0.7
	4	118.7±6.2	-4.8±2.2	111.8±8.3	-4.9±1.9	115.0±5.5	1.8±1.8	121.7±2.7	6.3±0.8 #
	5	119.7±4.8	-3.8±1.5	109.5±4.9	-8.5±2.6	111.7±6.5	-1.5±2.8	125.5±3.5	10.7±1.1 *#Ψ
HR	1	426±20	-------	398±10	-------	415±10	-------	428±9	-------
	2	436±16	0.0±5	384±9	14±8	419±11	4±7.4	425±7	-3±7
	3	396±13	-40±9	365±3	-33±10	420±15	5±8.9	452±12	24±9 *#
	4	385±6	-50±16	360±10	-37±15	419±11	4±7.5 *	461±16	32±13 *#
	5	383±5	-52±16	377±13	-21±16	419±12	4±8.4 *	467±14	39±12 *#

*#Ψ represents significant difference compared with corresponding saline, vehicle and single dose CsA values, respectively. Data are means ± SE.

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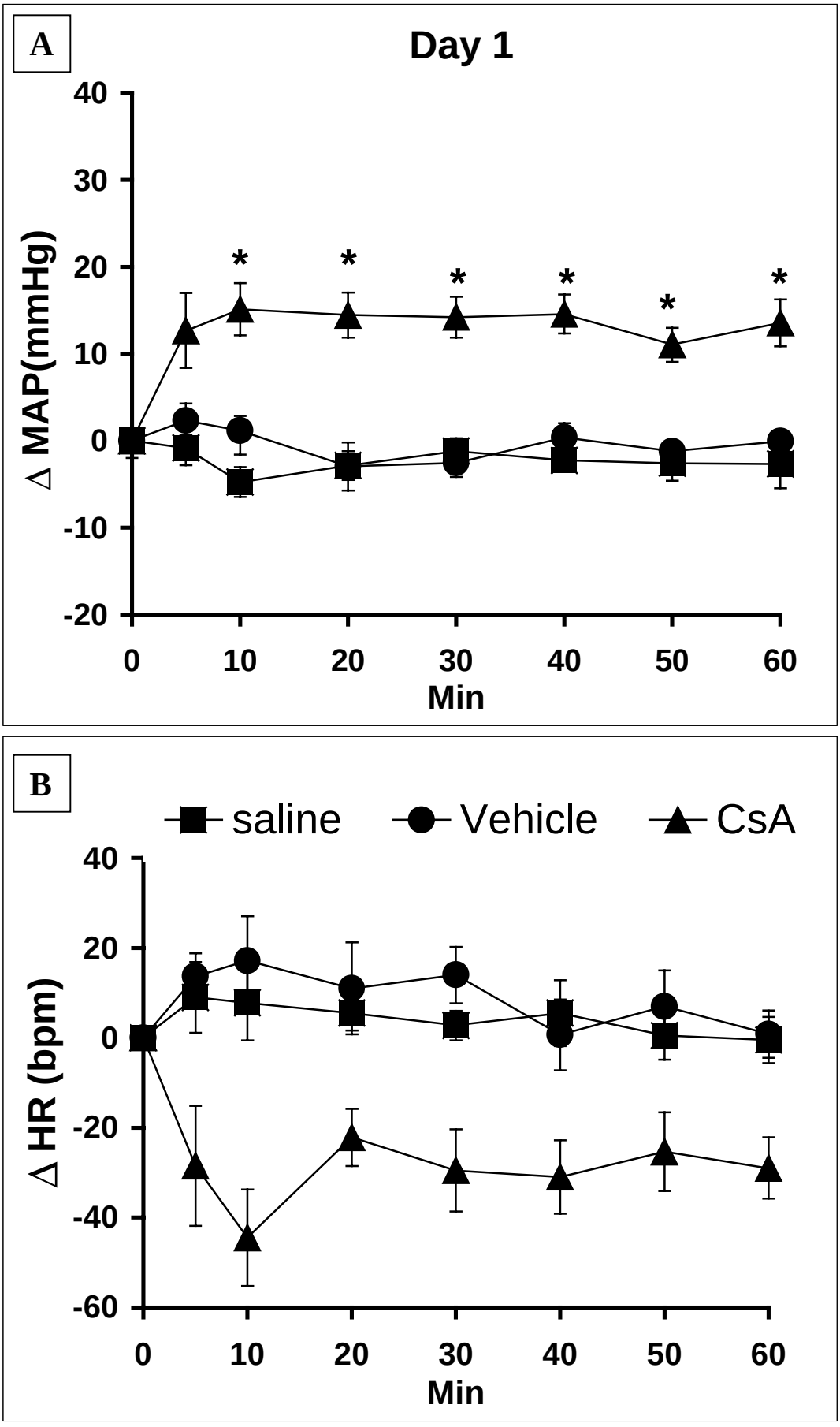

Fig. 1

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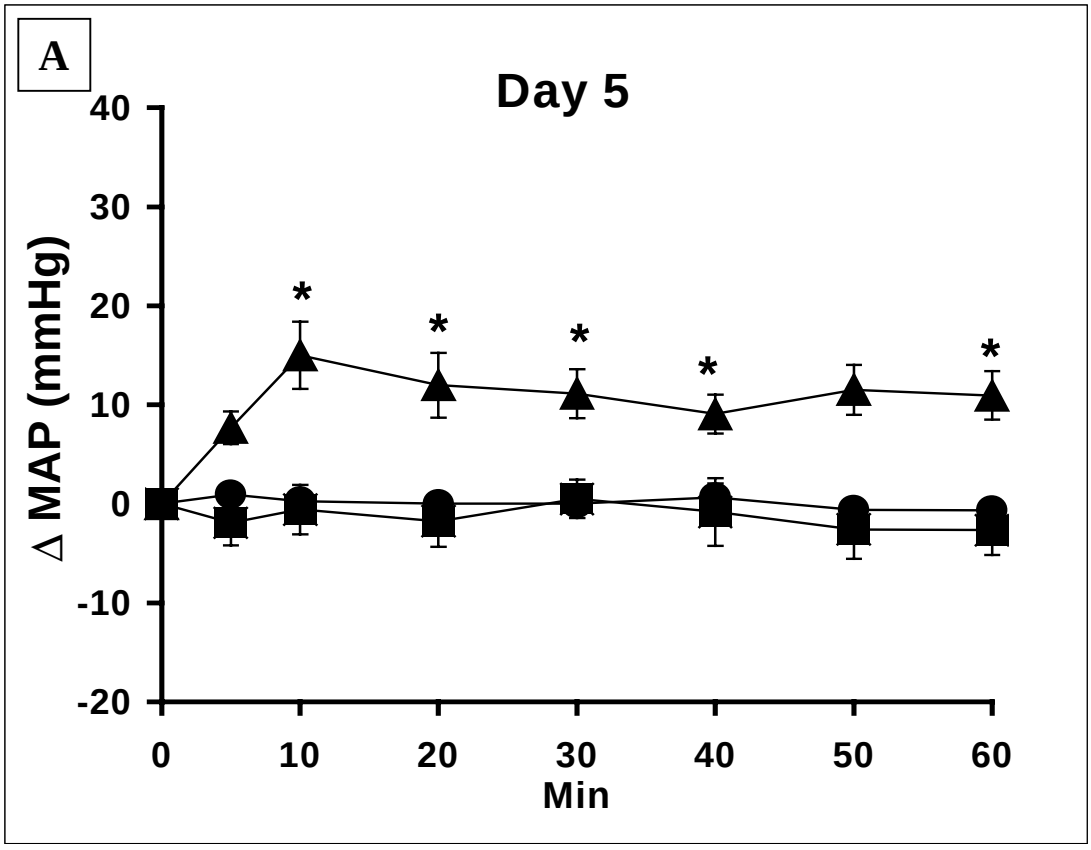

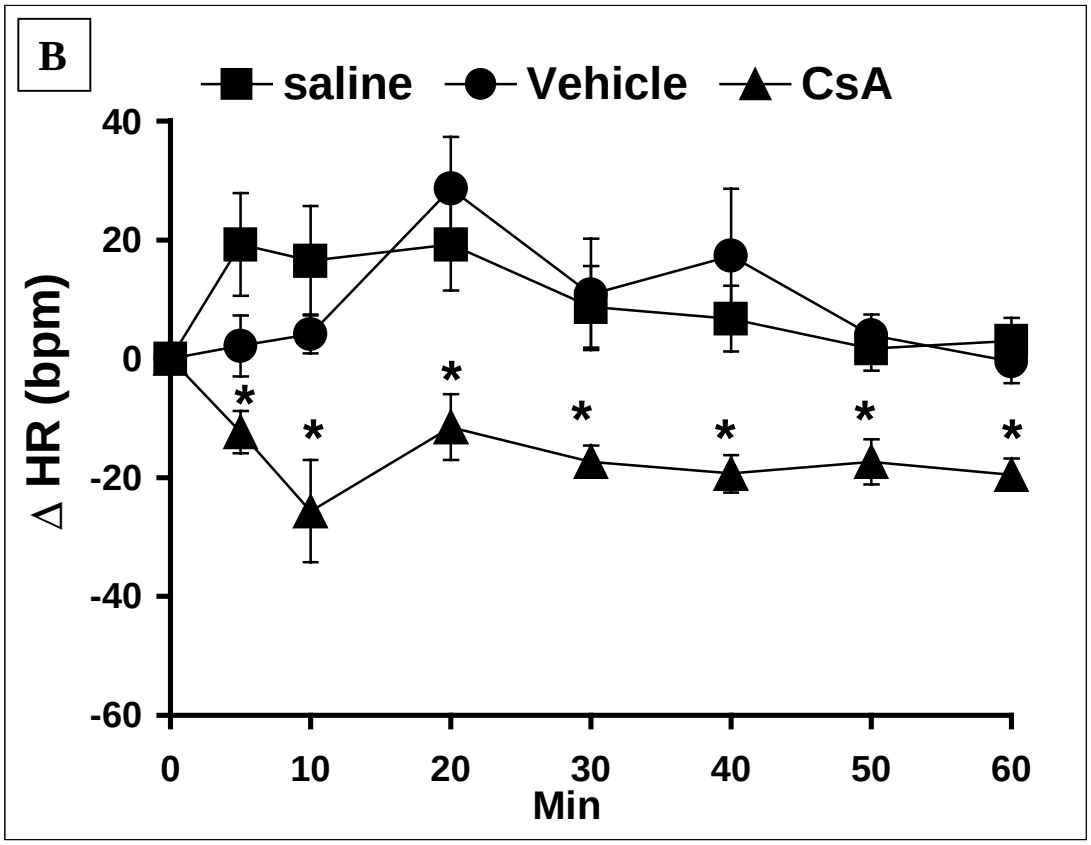

Fig. 2

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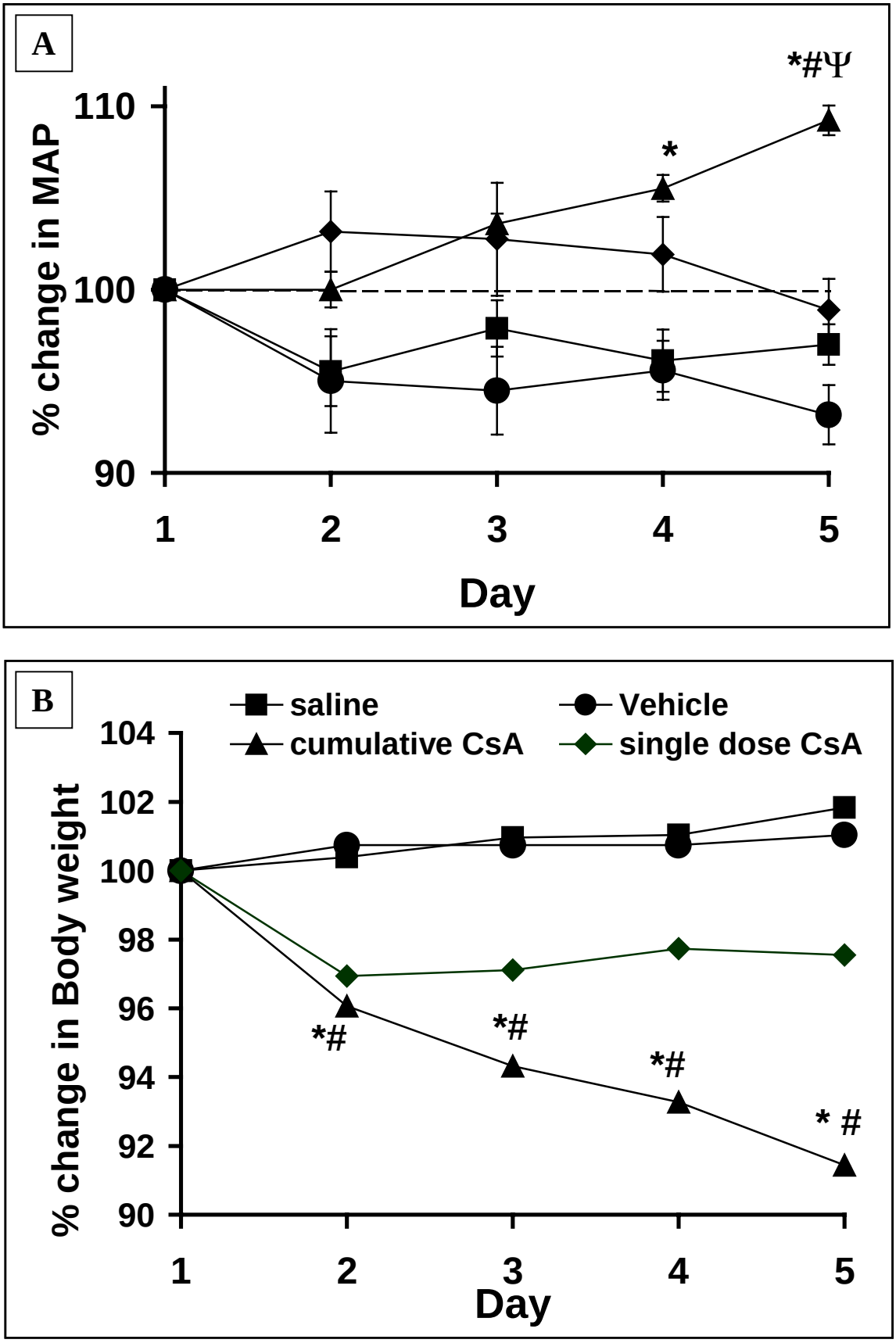

Fig. 3

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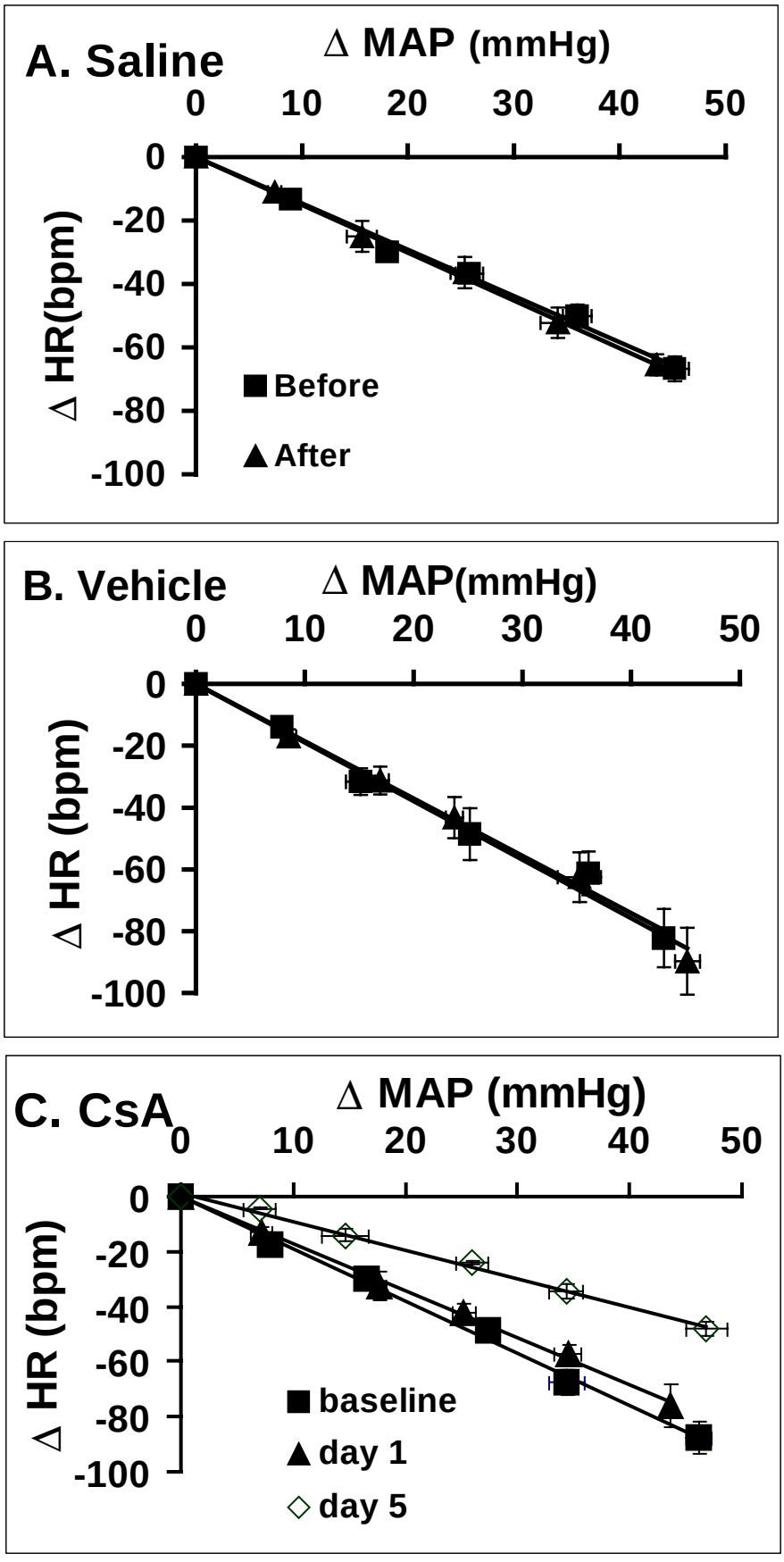

Fig. 4

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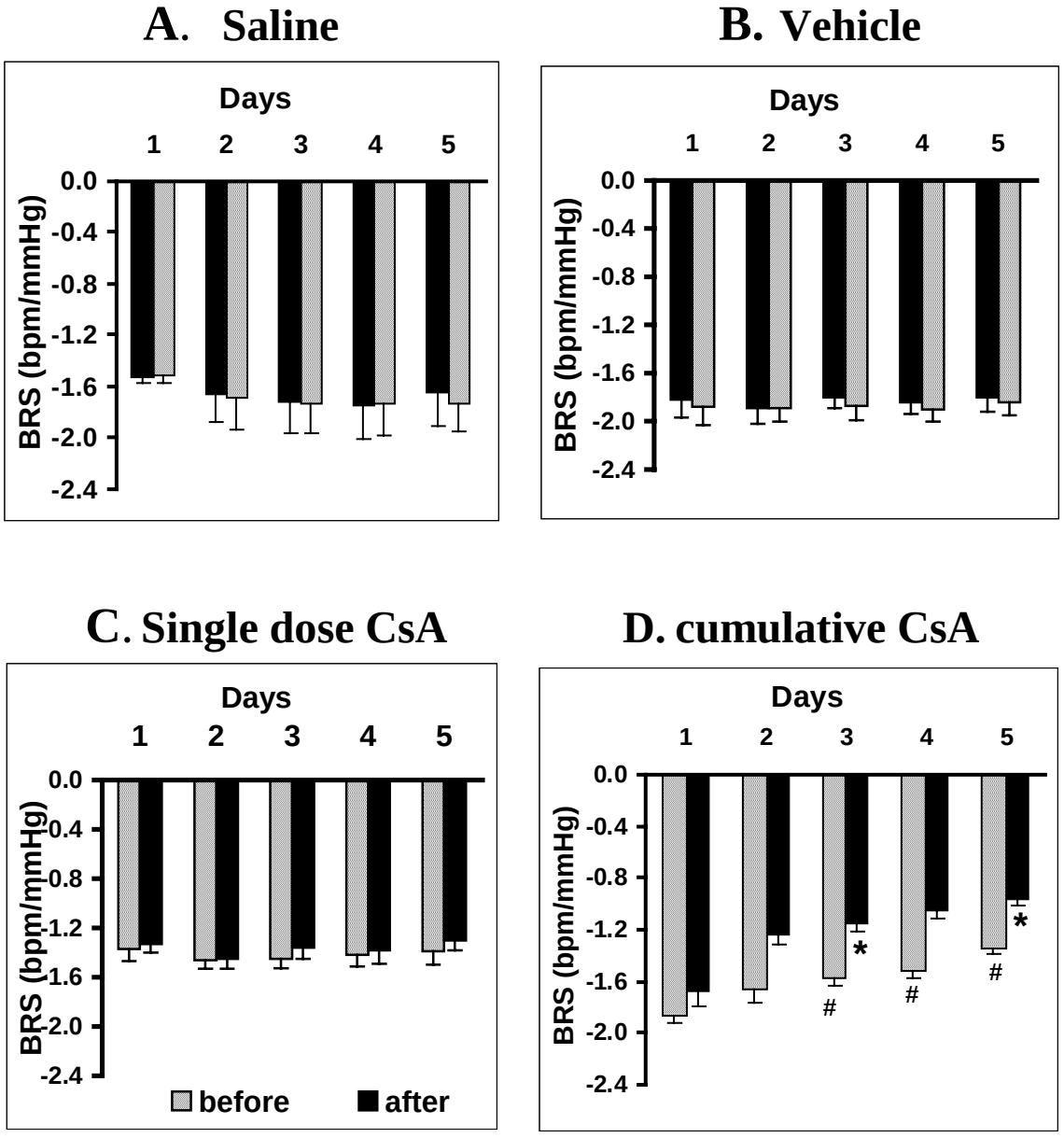

Fig. 5