



## Facilitation by the renin-angiotensin system of cyclosporine-evoked hypertension in rats: Role of arterial baroreflexes and vasoreactivity

Suzanne A. Nasser<sup>a</sup>, Ramzi Sabra<sup>b</sup>, Ahmed I. Elmallah<sup>c</sup>, Mahmoud M. Mohy El-Din<sup>c</sup>, Mohamed M. Khedr<sup>d</sup>, Mahmoud M. El-Mas<sup>c,\*</sup>

<sup>a</sup> Department of Pharmacology, Faculty of Pharmacy, Beirut Arab University, Lebanon

<sup>b</sup> Department of Pharmacology, Faculty of Medicine, American University of Beirut, Lebanon

<sup>c</sup> Department of Pharmacology, Faculty of Pharmacy, Alexandria University, Egypt

<sup>d</sup> Department of Clinical Pharmacology, Faculty of Medicine, Alexandria University, Egypt

### ARTICLE INFO

#### Article history:

Received 1 July 2016

Received in revised form 16 August 2016

Accepted 25 August 2016

Available online 26 August 2016

#### Keywords:

Cyclosporine

Renin-angiotensin system

Endothelin receptors

Hypertension

Arterial baroreceptors

Endothelium-dependent relaxations

### ABSTRACT

**Aims:** Cyclosporine (CSA) elevates blood pressure (BP) and alters arterial baroreflex sensitivity (BRS) and vasoreactivity. In this study we determined whether the renin-angiotensin system (RAS) interplays with other vasopressor pathways in mediating the CSA actions.

**Materials and methods:** Whole animal and isolated vascular preparations were employed to determine the effects of pharmacologic interruption of angiotensin II (Ang II), endothelin (ET), or thromboxane (TXA<sub>2</sub>) signaling on the adverse cardiovascular effects of CSA.

**Key findings:** CSA (25 mg/kg/day i.p. for 7 days) caused significant increases in BP that were paralleled with (i) reduced BRS measured by phenylephrine (BRS<sub>PE</sub>) or sodium nitroprusside (BRS<sub>SNP</sub>), (ii) enhanced aortic contractile responses to Ang II and U-46619 (thromboxane analogue), and (iii) reduced aortic eNOS expression and acetylcholine, but not SNP, vasorelaxations. Except for the reduced BRS<sub>SNP</sub>, the CSA effects disappeared upon concurrent administration of losartan (angiotensin AT<sub>1</sub> receptor antagonist), captopril (angiotensin converting enzyme inhibitor), or their combination. Moreover, CSA augmentation of Ang II contractions was abolished after cyclooxygenase inhibition (indomethacin) or endothelin ET<sub>A</sub>/ET<sub>B</sub> receptor blockade (atrasentan/BQ788). By contrast, the blockade of thromboxane receptors (terutroban) failed to alter the CSA-evoked facilitation of Ang II responsiveness.

**Significance:** The facilitation of baroreflex control and inhibition of vascular responsiveness to Ang II and thromboxane contribute to the BP lowering effect of RAS inhibitors in CSA-treated rats. Further, endothelin receptors and vasoconstrictor prostanoids contribute to the CSA-evoked exaggeration of Ang II vascular responsiveness and hypertension.

© 2016 Elsevier Inc. All rights reserved.

### 1. Introduction

The renin-angiotensin system (RAS) is an important regulator of BP and fluid homeostasis. Ang II, a key product of RAS, causes vasoconstriction and consequent rises in systemic and local BP, stimulates aldosterone release from the adrenal gland, and enhances renal sodium and water retention [1,2]. Pathophysiologically, Ang II is a fundamental etiology factor of hypertension as well as several other cardiovascular disorders [3]. Ang II exerts AT<sub>1</sub> receptor-mediated inhibitory influences on baroreceptor reflex control of HR [4]. AT<sub>1</sub> receptors are expressed at

multiple sites throughout the baroreceptor reflex pathway including the sensory afferent neurons and their terminals in the nucleus of the solitary tract [5,6] and play a critical role in the processing of the cardiac baroreflex depressant effect of circulating Ang II [4].

The immunosuppressant drug CSA is a calcineurin/protein phosphatase 2B inhibitor that is used in organ transplantation to reduce the incidence and severity of graft rejection [7,8]. Moreover, CSA produces favorable outcomes in autoimmune conditions such as psoriasis, atopic dermatitis, rheumatoid arthritis, and glomerular disorders [9]. Clinical practice showed that CSA use is associated with serious cardiovascular events such as hypertension [10]. Among several other mechanisms, vascular abnormalities [11,12] and upregulation of RAS activity [13,14] are possible mechanisms for the hypertensive effect of CSA. Ang II levels [15], angiotensin converting enzyme activity [16] and angiotensin AT<sub>1</sub> receptor expression [17] are all elevated by CSA. CSA also augments angiotensin-induced vasoconstriction in isolated arterioles [13]. Clinical studies showed that treatment with ACE inhibitors or AT<sub>1</sub> receptor

*Abbreviations:* CSA, cyclosporine; PE, phenylephrine; SNP, sodium nitroprusside; Ang II, angiotensin II; RAS, renin-angiotensin system; BP, blood pressure; MAP, mean arterial pressure; HR, heart rate; BRS, baroreflex sensitivity; eNOS, endothelial nitric oxide synthase.

\* Corresponding author at: Department of Pharmacology and Toxicology, Faculty of Pharmacy, Alexandria University, Alexandria, Egypt.

E-mail address: [mahelm@hotmail.com](mailto:mahelm@hotmail.com) (M.M. El-Mas).

antagonists reduce BP in hypertensive CSA-treated renal transplant recipients [14,18].

The current experimental study addresses several questions related to the mechanism by which RAS facilitates the hypertensive effect of CSA. First, given the inverse relationship between BP and arterial baroreflex gain [19,20] and the inhibitory effect of CSA on baroreflex activity [21,22], we tested the hypothesis that the counteraction of CSA hypertension evoked by individual or combined administration of captopril (angiotensin converting enzyme inhibitor) and losartan (angiotensin AT<sub>1</sub> receptor antagonist) is coupled with normalization of arterial baroreflex activity. Second, tension studies in isolated aortas were employed to investigate (i) whether vasopressor pathways of endothelin, thromboxane, and prostanoids contribute to the enhancing effect of CSA on Ang II vasoreactivity, and (ii) modulatory effects of vasopressor and vasodepressor pathways of thromboxane and acetylcholine, respectively, on the CSA-RAS interaction.

## 2. Materials and methods

Male Sprague–Dawley rats (240–280 g; Animal Facility, American University of Beirut Medical Center) were employed in the present study. Rats were housed in standard plastic cages and allowed free access to water and rat chow. All experiments were approved by the institutional animal care and use committee.

### 2.1. Intravascular cannulations

As described in our previous studies [23,24], rats were anesthetized with thiopental (50 mg/kg, i.p.) and catheters were placed in the abdominal aorta and vena cava via the femoral artery and vein for measurement of BP and intravenous administration of drugs, respectively. The arterial catheter was connected to a pressure transducer (Model PT300, Grass Technologies, Warwick, RI, USA) with an amplifier (Model P11T, Grass Technologies, Warwick, RI, USA), and arterial BP was displayed and analyzed by Grass Polyview16 data acquisition and analysis system (Version 1.0, Grass Technologies, Warwick, RI, USA). HR was computed from BP waveforms by a Grass tachograph. Catheters were tunneled subcutaneously, exteriorized at the back of the neck between the scapulae, flushed with heparin, and plugged by stainless steel pins. Each rat received intramuscular injection of 60,000 IU of benzathine benzyl penicillin (Retarpen®) and 2 mg/kg ketorolac tromethamine and was housed individually. The measurements of BP, HR, and baroreflex function were performed 48 h later in conscious freely moving rats.

### 2.2. Isolated aortic ring preparations

Rats were euthanized by exposure to 100% CO<sub>2</sub> to produce deep anesthesia [25], then rapidly decapitated. The aorta was isolated and aortic ring segments (3–4 mm long) were mounted in an individual 15 ml organ bath filled with Krebs' solution (119 mM NaCl, 4.7 mM KCl, 2.5 mM CaCl<sub>2</sub>·H<sub>2</sub>O, 1.17 mM MgSO<sub>4</sub>·7H<sub>2</sub>O, 1.18 mM KH<sub>2</sub>PO<sub>4</sub>, 25 mM NaHCO<sub>3</sub>, 11.1 mM glucose) by means of two stainless steel wire hooks [26–28]. One wire was attached to a fixed support at the bottom of the organ bath, and the second wire was connected to a movable holder supporting an isometric force displacement transducer (FORT 10, World Precision Instruments, Inc., USA) that was connected to a multichannel polygraph (Transbridge 4M, World Precision Instruments, Inc., USA) for recording isometric contractions. Tissues were continuously bubbled with a mixture of 95% O<sub>2</sub> and 5% CO<sub>2</sub> and kept at 37 °C with an outer water jacket and a circulating heat pump. A resting tension of 2 g was placed on the tissue and an equilibrium period of 1 h was allowed before the start of the experiment. A dose of PE (1 μM), which was found in preliminary experiments to produce ~60% of the maximum response [28], was added to the organ bath on two separate occasions during the 1 h equilibration to acclimatize the preparation to the PE effect.

Data were analyzed using data acquisition software, AcqKnowledge 3.9.1., Biopac Systems, Inc., USA for Windows XP.

### 2.3. Western blotting

Snap-frozen aortas were pulverized with mortar and pestle under liquid nitrogen. The powder was dissolved in 150 μl of RIPA buffer (50 mM TrisHCl, 150 mM NaCl, 1% IGEPAL CA-630, 0.1% SDS, 1% Na deoxycholate) supplemented with a proteinase inhibitor cocktail. The samples were vortexed and sonicated for 5 s for five cycles. Samples were incubated for 10 min at room temperature and then centrifuged for 12 min at 18,000 ×g at 4 °C. The protein concentration in the supernatants was measured using Bradford method. A total of 100 μg protein samples were run on a 10% acrylamide gel and electroblotted to nitrocellulose membranes. Blots were blocked for 1 h at room temperature in 5% bovine serum albumin in TBS/Tween. They were then incubated overnight at 4 °C with rabbit polyclonal eNOS antibody (1:1000 Abcam, UK). After 3 washes with TBS/Tween buffer, the blots were incubated for 1 h at room temperature with the secondary antibody (Donkey Anti-Rabbit Polyclonal IgG-HRP, 1:5000, Jackson ImmunoResearch Labs Inc., USA). After 3 washes with the TBS/Tween buffer, immunoreactive sites were detected by enhanced chemiluminescence system and exposed to an X-ray film, which were processed by Kodak RP X-omat processor (Model M6B). Equal loading and transfer was achieved by probing the corresponding slice of the membrane for β-actin primary antibody (Sigma Chemical Co., USA). Densitometric analysis of the Western bands was performed using the Image J software (Version 1.42). Data were normalized in relation to β-actin, and expressed as a percent of control values as in our previous studies [28,29].

### 2.4. Arterial baroreflex testing

BRS was assessed by the vasoactive (Oxford) method [30–32], which measures HR responses to increments or decrements in BP evoked by random bolus injections of i.v. doses (1–16 μg/kg) of PE and SNP at 5-min intervals. PE or SNP was dissolved in saline, and the injection volume was kept constant at 0.05 ml/100 g of body weight with a flush volume of approximately 0.1 ml of saline. The mean arterial pressure (MAP) and HR values before and after PE and SNP administration were measured, and the peak changes in both variables (ΔMAP and ΔHR) were used for construction of the baroreflex curves. The regression coefficient (slope of the regression line) expressed as beats/min/mmHg was taken as an index of BRS.

### 2.5. Experimental groups and protocols

#### 2.5.1. Effect of RAS inhibitors on CSA-evoked hypertension and baroreflex dysfunction

Eight groups of male rats ( $n = 6$ ) were allocated to receive one of the following i.p. drug regimens for 7 consecutive days: (i) vehicle (cremophor EL, 1 ml/kg/day), (ii) CSA (25 mg/kg/day) [28], (iii) captopril (ACE inhibitor, 10 mg/kg/day, [33]), (iv) captopril + losartan, (v) losartan (selective AT<sub>1</sub> receptor antagonist, 10 mg/kg/day, [33]), (vi) CSA + captopril, (vii) CSA + losartan, (viii) CSA + captopril + losartan. Intravascular cannulation was performed on day 5 of drug administration. Two hours after drug administration on day 7, the arterial catheter was connected to a pressure transducer for hemodynamic measurements. A period of 30 min was allowed for hemodynamic stabilization, after which baseline BP and HR were recorded and baroreflex testing was performed as described above by the vasoactive method.

#### 2.5.2. Role of vasopressor pathways in CSA interaction with Ang II aortic contractions

The first part of this experiment investigated the effect of chronic CSA treatment on Ang II contractions in isolated aortas and the modulation of these responses by concurrent administration of RAS inhibitors.

Eight groups of male rats ( $n = 7-9$  each) were employed and received the same drug treatments employed in the preceding experiment. Rats were sacrificed on day 7 and their thoracic aortas were isolated and prepared for tension studies as described earlier. Aortic segments were mounted in a 15-ml organ bath filled with Krebs' solution. After 1 h acclimatization, dose-contractile response curves to cumulative concentrations of Ang II ( $1 \times 10^{-10}$ – $3 \times 10^{-7}$  M) were established. Each concentration of Ang II was added after the response to the previous concentration had plateaued. Contractile responses to cumulative doses of Ang II were measured as the maximum increase in the isometric force above the baseline and were expressed in grams.

Because the results showed that Ang II contractions were enhanced by CSA, 5 more groups of rats ( $n = 6$  each) were used to determine the role of vasoconstrictor pathways of endothelin, prostanoids, or thromboxane in the CSA-angiotensin interaction. The rats in these groups received i.p. CSA together with one of the following treatments for 7 consecutive days: (i) atrasentan ( $ET_A$  receptor blocker, 10 mg/kg/day [34]), (ii) BQ 788 ( $ET_B$  receptor blocker, 0.1 mg/kg/day [35]), (iii) atrasentan + BQ788 (iv) indomethacin (cyclooxygenase inhibitor, 5 mg/kg/day [36]), or (v) terutroban (selective thromboxane receptor antagonist, 10 mg/kg/day [28]). At the conclusion of treatment period, tissue bath studies were conducted in aortic rings to measure the cumulative contractile responses to Ang II as detailed above.

#### 2.5.3. Effect of RAS inhibitors on CSA interaction with vasoreactivity to thromboxane, acetylcholine, sodium nitroprusside

Some aortic rings obtained from rats employed in the preceding experiments were used to determine the effects CSA on cumulative aortic vasoconstrictions caused by U-46619 (thromboxane analogue,  $1 \times 10^{-10}$ – $3 \times 10^{-6}$  M) or vasorelaxations induced by acetylcholine ( $1 \times 10^{-9}$ – $1 \times 10^{-5}$  M) or sodium nitroprusside ( $1 \times 10^{-10}$ – $3 \times 10^{-6}$  M) in the absence and presence of captopril, losartan, or both. For the assessment of vasorelaxations, aortic rings were precontracted with a submaximal concentration of phenylephrine (1  $\mu$ M). Once the steady state tonic response was attained, acetylcholine or nitroprusside concentrations were added cumulatively; one concentration was added when the relaxant response to the previous concentration reached its peak response. Vasorelaxant responses to individual concentrations of acetylcholine or nitroprusside were expressed as a percentage of the phenylephrine contractile response [28].

#### 2.5.4. Drugs

Losartan potassium (Pharmaline S.A.L., Lebanon), phenylephrine hydrochloride (Pharmadex, S.A.L., Lebanon), Sandimmune® ampoules (50 mg/ml, Novartis Pharma, AG, Basel, Switzerland), atrasentan (Abbott Laboratories, Illinois, USA), terutroban (Servier, Paris, France), U-46619 (Cayman chemical Co, USA), angiotensin II, captopril, cremophor EL (Sigma Chemical Co., U.S.A.), acetylcholine chloride, indomethacin (Fluka Biochemika, Italy), BQ 788 sodium (Peptides International, Inc., USA), Retarpen® (October Pharma S.A.E., Egypt), Heparin® ampoules (5000 IU/ml, Nile pharmaceutical Co. Egypt), Ketorolac® (Amriya Pharm. Ind. Co., Alex., Egypt), sodium nitroprusside (Nipruss®, Schwarz pharma, Germany) and thiopental® (Rotexmedica, Germany). Captopril, losartan, indomethacin, acetylcholine, and Ang II were dissolved in saline, Terutroban and BQ788 were dissolved in 0.055 N NaOH and 0.001 N NaOH, respectively. U-46619 was supplied as a 1% solution in methyl acetate; a stock solution of  $1 \times 10^{-2}$  M U-46619 was prepared by simply evaporating 0.1 ml U-46619 solution, equivalent to 1 mg U-46619, under a gentle stream of nitrogen and immediately adding 92.72  $\mu$ l dimethylsulfoxide and 192.58  $\mu$ l saline.

#### 2.5.5. Statistical analysis

Values are expressed as means  $\pm$  S.E.M. Nonlinear regression analysis was used to fit sigmoidal curves to individual dose-response curves to determine the agonist maximum effect ( $E_{max}$ ) and potency (the

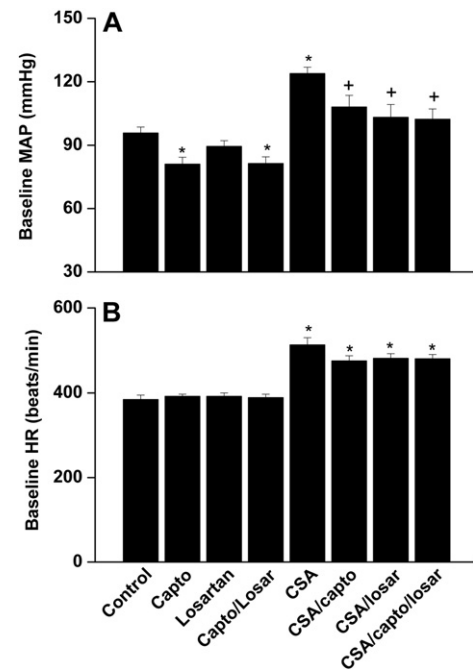
dose giving half the maximum vasoconstrictor or vasorelaxant response,  $EC_{50}$ ) [37]. Analysis of variance (ANOVA) followed by the Tukey's post-hoc analysis was used for multiple comparisons with the level of significance set at  $P < 0.05$ .

### 3. Results

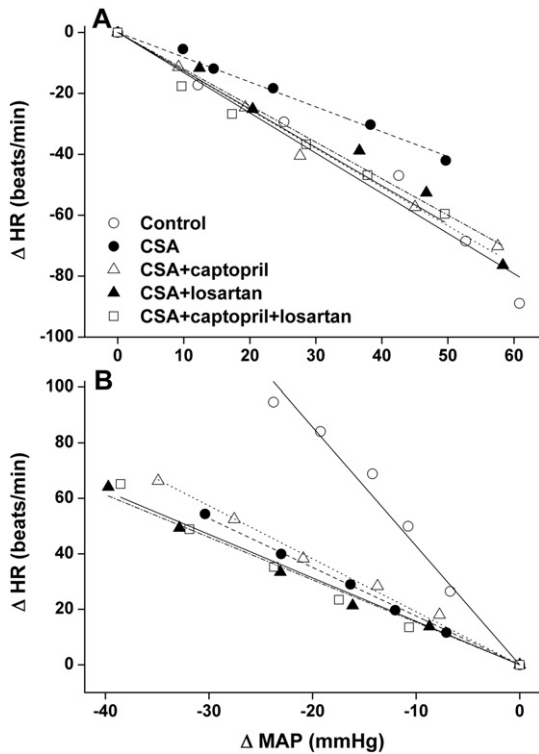
#### 3.1. RAS inhibitors blunt the hypertensive and baroreflex depressant effects of CSA

Figs. 1–3 depict the effect of ACE inhibition and/or angiotensin  $AT_1$  receptor antagonism on hemodynamic and baroreflex responses elicited by chronic CSA administration in conscious freely moving rats. Hemodynamic measurements in rats fitted with femoral indwelling catheters showed that compared with control (cremophor-treated) rats, treatment with CSA (25 mg/kg/day i.p.) for 7 days caused significant increases in MAP and HR by approximately 25 mm Hg and 125 beats/min, respectively (Fig. 1). The hypertensive, but not the tachycardic, effect of CSA was significantly ameliorated in rats treated concurrently with captopril (ACE inhibitor, 10 mg/kg/day), losartan ( $AT_1$  receptor antagonist, 10 mg/kg/day), or their combination. While the use of losartan alone had no effect on BP, the treatment with captopril alone or combined with losartan caused modest but significant reductions in MAP (Fig. 1A).

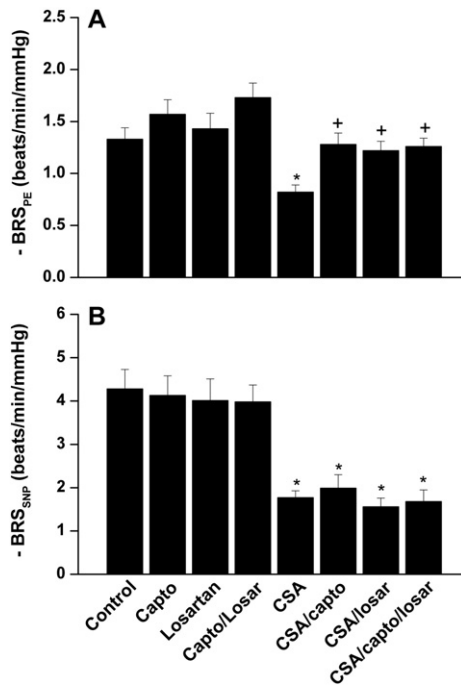
Analysis of the baroreflex curves relating MAP changes elicited by PE or SNP to the associated reciprocal reflex changes in HR showed that CSA produced upward and downward shifts in the PE and SNP curves, respectively (Fig. 2), suggesting reduced reflex HR responsiveness. The slopes of the linear regression lines, which represented the  $BRS_{PE}$  and  $BRS_{SNP}$ , were significantly ( $P < 0.05$ ) smaller in CSA compared with control values (Fig. 3). These effects of CSA were variably affected after concurrent administration of RAS inhibitors. The CSA-evoked upward shifts in the baroreflex curves generated by PE (Fig. 2A) and the associated reductions in  $BRS_{PE}$  (Fig. 3A) were similarly abolished in rats co-treated with the captopril, losartan, or both. By contrast, none of these regimens affected the shifts in the SNP baroreflex curves caused by CSA (Fig. 2B)



**Fig. 1.** Effects of 7-day treatment with CSA (25 mg/kg/day), captopril (ACE inhibitor, 10 mg/kg/day), losartan ( $AT_1$  receptor blocker, 10 mg/kg/day) or their combination on mean arterial pressure (MAP, panel A) and heart rate (HR, panel B) in conscious rats. Values are means  $\pm$  S.E.M. of 6 observations. \* $P < 0.05$  vs. control values, +  $P < 0.05$  vs. CSA values.



**Fig. 2.** Baroreflex curves relating increases and decreases in mean arterial pressure (MAP) caused by phenylephrine (panel A) and sodium nitroprusside (panel B), respectively, and associated reciprocal changes in heart rate (HR) in conscious rats treated with CSA (25 mg/kg/day), captopril (ACE inhibitor, 10 mg/kg/day), losartan (AT<sub>1</sub> receptor blocker, 10 mg/kg/day) or their combination for 7 consecutive days. Values are means  $\pm$  S.E.M. of 6 observations.



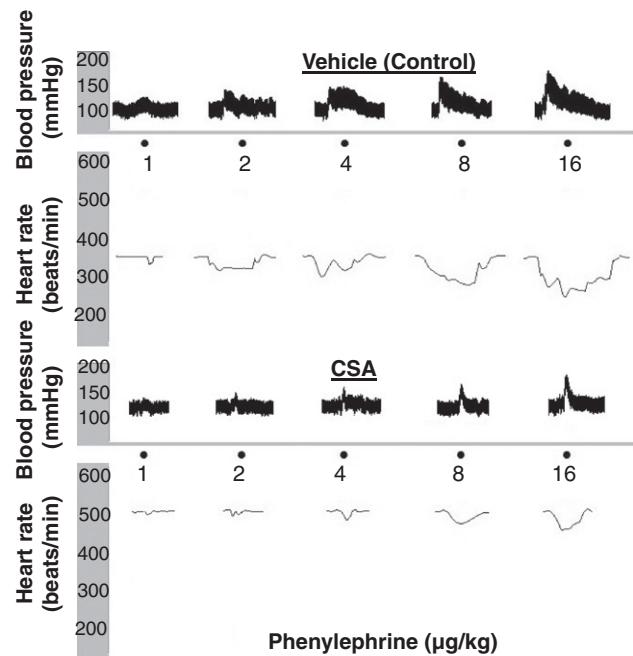
**Fig. 3.** Effects of 7-day treatment with CSA (25 mg/kg/day), captopril (ACE inhibitor, 10 mg/kg/day), losartan (AT<sub>1</sub> receptor blocker, 10 mg/kg/day) or their combination on the baroreflex sensitivity measured by phenylephrine (BRS<sub>PE</sub>, panel A) and sodium nitroprusside (BRS<sub>SNP</sub>, panel B) in conscious rats. Values are means  $\pm$  S.E.M. of 6 observations. \*  $P < 0.05$  vs. control values, +  $P < 0.05$  vs. CSA values.

or the concomitant reductions in reflex tachycardic responses (Fig. 3B). BRS<sub>PE</sub> was slightly increased in rats receiving the captopril plus losartan regimen, but this increase was not statistically different from respective control values. Representative tracings of the BP and HR responses evoked by bolus i.v. injections of PE or SNP in rats treated with CSA or the vehicle are shown in Figs. 4 and 5, respectively.

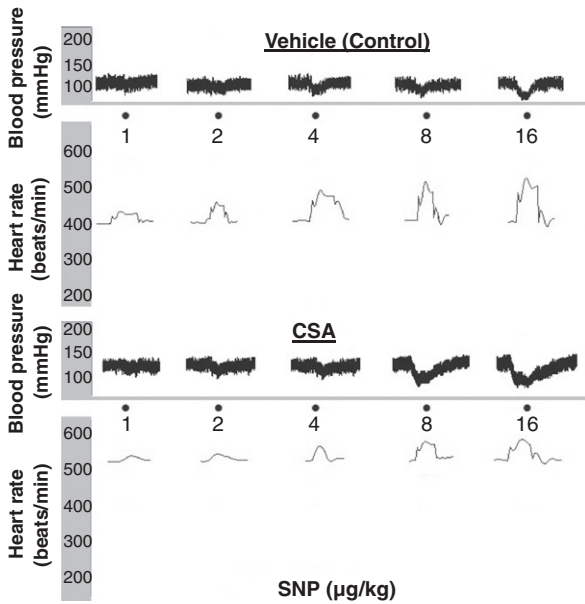
### 3.2. Role of vasopressor pathways in CSA interaction with angiotensin vasoreactivity

This experiment investigated the effect of chronic CSA on aortic contractions induced by Ang II and the modulation of this interaction by vasoconstrictor pathways of RAS, endothelin, prostanoids, or thromboxane. Cumulative additions of Ang II ( $1 \times 10^{-10}$ – $3 \times 10^{-7}$  M) elicited concentration-dependent contractions of aortic smooth muscles (Figs. 6 and 7). While the contractile responses elicited by Ang II were not modified in aortas isolated from rats treated chronically with captopril (ACE inhibitor, 10 mg/kg/day, i.p., 7 days) compared with respective control values, they were significantly reduced in aortas isolated from rats treated with the AT<sub>1</sub> receptor antagonist losartan (10 mg/kg/day, i.p., 7 days) or the combined losartan plus captopril regimen (Fig. 7A). The reduced responsiveness to Ang II in the presence of losartan was associated with decreases and increases in the  $E_{max}$  and  $EC_{50}$  of Ang II, respectively, while those observed with losartan plus captopril treatment were associated with decreases in the  $E_{max}$  only (Fig. 7B–D).

Compared with control (vehicle-treated) values, aortic contractile responses to Ang II were significantly augmented in aortas obtained from CSA-treated rats (Fig. 7C). The  $E_{max}$  and  $EC_{50}$  values of Ang II curves were increased and decreased, respectively, in CSA-treated preparations (Fig. 7B–D). The enhancing effect of CSA on Ang II responses (Fig. 7B) and the associated alterations in  $E_{max}$  and  $EC_{50}$  (Fig. 7B–D) were eliminated in aortas obtained from rats concurrently treated with captopril, losartan or their combination. Likewise, Fig. 7E shows that cyclooxygenase inhibition by indomethacin (5 mg/kg/day) or combined endothelin ET<sub>A</sub>/ET<sub>B</sub> receptor blockade by atrasentan (10 mg/kg/day) plus BQ788 (0.1 mg/kg/day) abolished the augmented Ang II contraction in CSA-treated rats. By contrast, the individual blockade of ET<sub>A</sub> (atrasentan) or ET<sub>B</sub> (BQ788) receptors (data not shown) or the blockade of



**Fig. 4.** Representative tracings showing the effects of i.v. bolus injections of phenylephrine on blood pressure and heart rate in conscious freely moving rats treated with CSA (25 mg/kg/day, i.p., 7 days) or the vehicle.

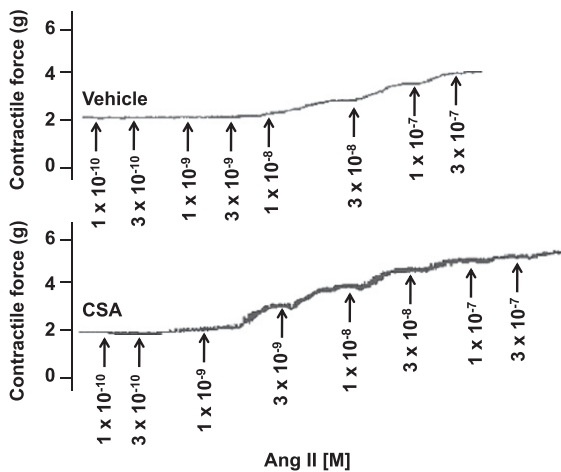


**Fig. 5.** Representative tracings showing the effects of i.v. bolus injections of sodium nitroprusside on blood pressure and heart rate in conscious freely moving rats treated with CSA (25 mg/kg/day, i.p., 7 days) or the vehicle.

thromboxane receptors by terutroban (10 mg/kg/day; Fig. 7E) failed to alter the augmented Ang II responses in CSA-treated preparations.

### 3.3. Vasoreactivity to thromboxane modulates the CSA-RAS interaction

The effects of CSA in the absence and presence of RAS inhibitors or thromboxane receptor antagonist terutroban on aortic contractile responses caused by the thromboxane analogue U-46619 are illustrated in Fig. 8. Cumulative additions of U-46619 ( $1 \times 10^{-10}$ – $3 \times 10^{-6}$  M) elicited concentration-dependent contractions of aortic smooth muscle that were significantly reduced in rats treated concurrently with terutroban (Fig. 8A). Compared with control values, CSA caused no changes in the maximum contraction ( $E_{\max}$ ) of U-46619 (Fig. 8B) but resulted in leftward shifts of the dose-response curves and significant decreases in  $EC_{50}$  (Fig. 8D). The decreases in  $EC_{50}$  values caused by CSA were abolished in preparations obtained from rats treated concurrently with captopril or losartan plus captopril, suggesting a reduced sensitivity to U-46619 in these preparations (Fig. 8D). Further,  $E_{\max}$



**Fig. 6.** Representative tracings of the contractile responses caused by cumulative additions of Ang II in aortas obtained from male rats treated with CSA (25 mg/kg/day, i.p., 7 days) or the vehicle for 7 days.

values of the U-46619 dose-response curves generated in rats receiving the combined CSA + losartan, and CSA + losartan + captopril regimens were significantly smaller than respective CSA-treated values (Fig. 8B). On the other hand, losartan alone or combined with captopril significantly attenuated U-46619 contractions and  $E_{\max}$  of the U-46619 response (Fig. 8A–B). However,  $EC_{50}$  was significantly increased in preparations isolated from losartan- or captopril-treated rats (Fig. 8D).

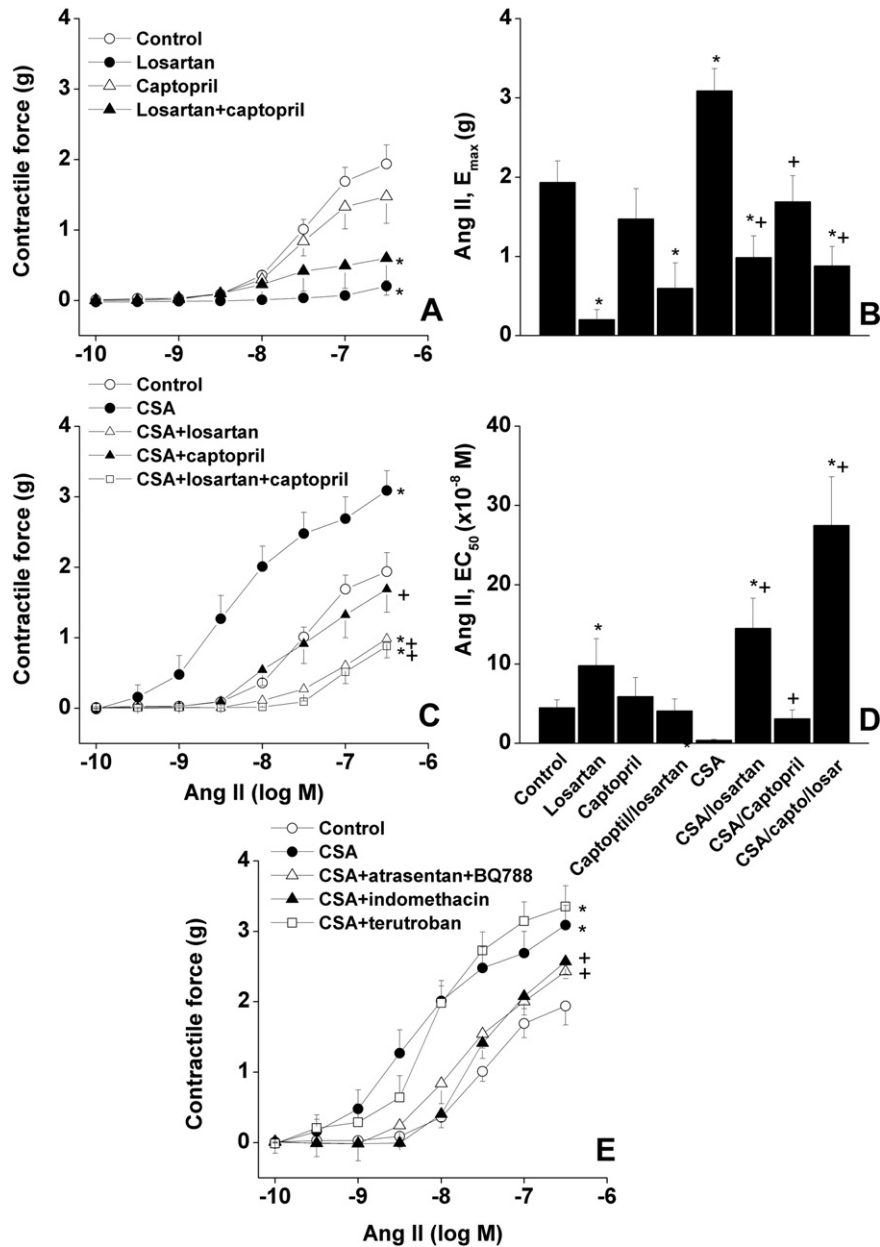
### 3.4. RAS inhibitors fail to normalize CSA inhibition of endothelium-dependent relaxations

The individual or combined effects of captopril and losartan on the interaction of CSA with endothelium-dependent (acetylcholine) and -independent (SNP) relaxations in rat aortas were investigated. Under conditions of sustained elevations in vascular tone induced by PE (1  $\mu$ M), cumulative additions of acetylcholine ( $1 \times 10^{-9}$ – $1 \times 10^{-5}$  M) elicited concentration-dependent relaxations that were significantly smaller in aortas obtained from CSA-treated compared with control values (Fig. 9A–C). Further,  $E_{\max}$  and  $EC_{50}$  values of the acetylcholine dose-response curves were decreased and increased, respectively, by CSA (Figs. 10A–C). Western analysis showed that CSA significantly reduced the protein expression of eNOS in aortic tissues compared with vehicle-treated rats (Fig. 11). The CSA-induced reductions in acetylcholine responses were not affected by the co-administration of losartan, captopril, or their combination (Figs. 9 and 10). The reduction in aortic eNOS expression caused by CSA was maintained in rats treated simultaneously with captopril (Fig. 11). In rats treated with losartan or captopril alone, the relaxant responses elicited by acetylcholine (Fig. 9A) and the  $E_{\max}$  of the acetylcholine dose-response curve (Fig. 10A) were augmented compared with control values. On the other hand, aortic vasorelaxations caused by SNP ( $1 \times 10^{-10}$ – $3 \times 10^{-6}$  M) were not affected by CSA or individual RAS inhibitors (Fig. 9A–B). The combined regimens of CSA + captopril, CSA + losartan, and CSA + captopril + losartan resulted in rightward shifts in the SNP curve (Fig. 9D) with subsequent significant increases in  $EC_{50}$  values compared to that of CSA-treated preparations, suggesting reduced SNP potency (Fig. 10D).

## 4. Discussion

The present study provides several novel observations regarding the roles of arterial baroreceptors and vasopressor and vasodepressor pathways in the protective effect of RAS inhibitors against CSA hypertension. First, the counteraction of CSA hypertension seen after concurrent treatment with captopril, losartan or their combination was coupled with preferential facilitation of reflex bradycardia but not tachycardia. Second, the CSA-evoked enhancement of aortic Ang II contractility disappeared in rats co-treated with RAS inhibitors or blockers of vasoconstrictor prostanoids synthesis or endothelin receptors. Third, CSA augmentation of Ang II contractility was preserved in terutroban-treated rats, implying no role for thromboxane receptors in CSA-Ang II interaction. Nonetheless, RAS inhibitors reduced the vasoconstrictor activity of the thromboxane analogue U-46619 in CSA-treated rats, highlighting the potential contribution of reduced thromboxane responsiveness in the vasculoprotective and antihypertensive effects of RAS inhibitors in the CSA model. Fourth, vascular endothelium does not seem to contribute to the protective effect of RAS inhibitors because the latter failed to reverse the reduced aortic acetylcholine relaxation or eNOS expression induced by CSA.

The current study is the first to report on the individual and combined effects of ACE inhibition and  $AT_1$  receptor blockade by captopril and losartan, respectively, on baroreflex dysfunction caused by chronic CSA in conscious rats. The vasoactive method of baroreflex measurement revealed that RAS inhibitors abolished the CSA-evoked downward shifts in the PE baroreflex curves and decreases in the slopes of the regression lines ( $BRS_{PE}$ ), suggesting a favorable effect for RAS inhibition on reflex bradycardia. These findings together with the lack of effect of

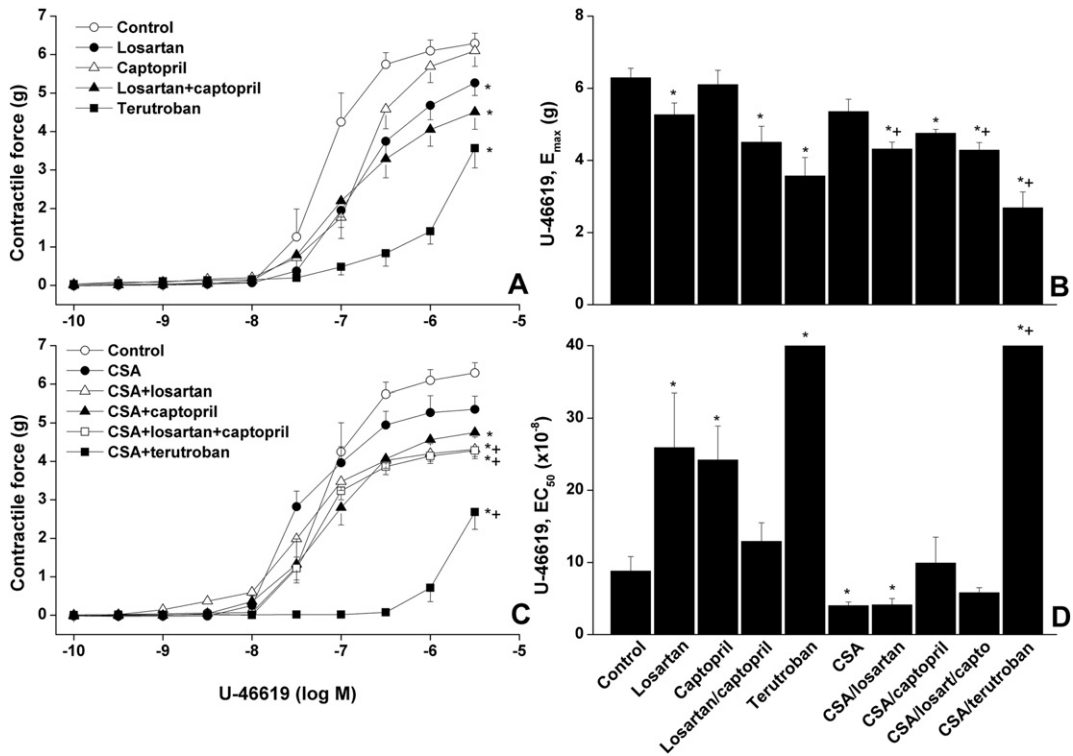


**Fig. 7.** Effects of 7-day treatment with CSA (25 mg/kg/day), captopril (ACE inhibitor, 10 mg/kg/day), losartan (AT<sub>1</sub> receptor blocker, 10 mg/kg/day), losartan plus captopril, or their combination on the contractile responses to cumulative doses of Ang II ( $1 \times 10^{-10}$ – $3 \times 10^{-7}$  M) in rat thoracic aortic rings (panels A–D). Panel E illustrates the effect of CSA on Ang II responses in rats treated concurrently with (i) atrasentan (ET<sub>A</sub> receptor blocker, 10 mg/kg/day) plus BQ788 (ET<sub>B</sub> receptor blocker, 0.1 mg/kg/day), (ii) indomethacin (cyclooxygenase inhibitor, 5 mg/kg/day), or (iii) terutroban (thromboxane receptor blocker, 10 mg/kg/day). Values are means  $\pm$  S.E.M. of 6 observations. \*  $P < 0.05$  vs. control values, +  $P < 0.05$  vs. CSA values.

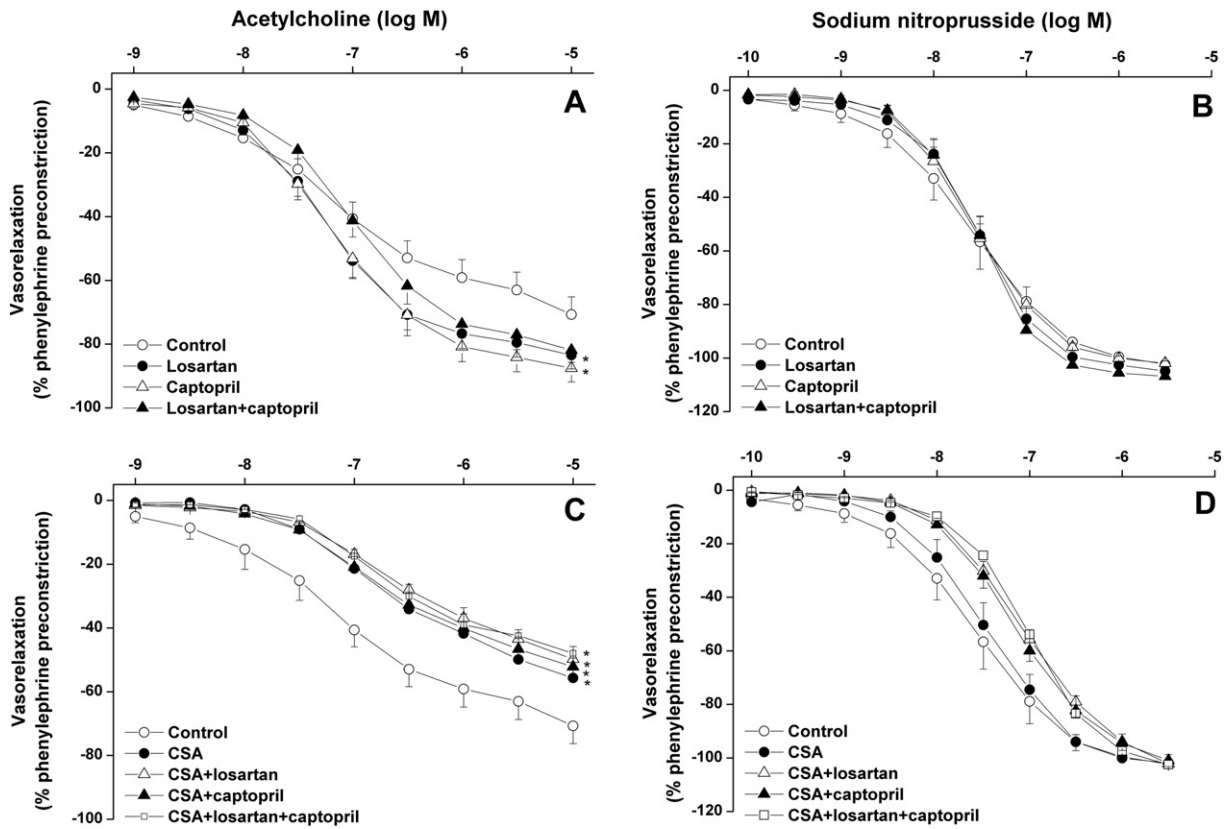
RAS inhibitors on reflex increases in HR response to baroreceptor unloading (by SNP) reflect the preferential ability of RAS inhibitors to rectify the CSA-evoked attenuation of reflex bradycardic, but not tachycardic, responses. Given that Ang II correlates negatively and positively with baroreflex and BP control [1,4], respectively, it is conceivable that the inhibition of Ang II signaling by captopril or losartan might underlie the capacity of these drugs to blunt the hypertensive and baroreflex depressant effects of CSA. Notably, one limitation of the current study was the use of single daily doses of individual RAS inhibitors and measuring BP and baroreflexes 2 h after the last dosing. That said, it is not clear whether the favorable effect of these drugs would be maintained for a 24 h period. Morgan et al. [33] reported that captopril at a dose similar to that used here caused significant BP reductions in spontaneously hypertensive rats for 8 h and this effect disappeared after 24 h. Therefore, twice a day dosing

might have been necessary to ensure adequate full-day BP lowering effect.

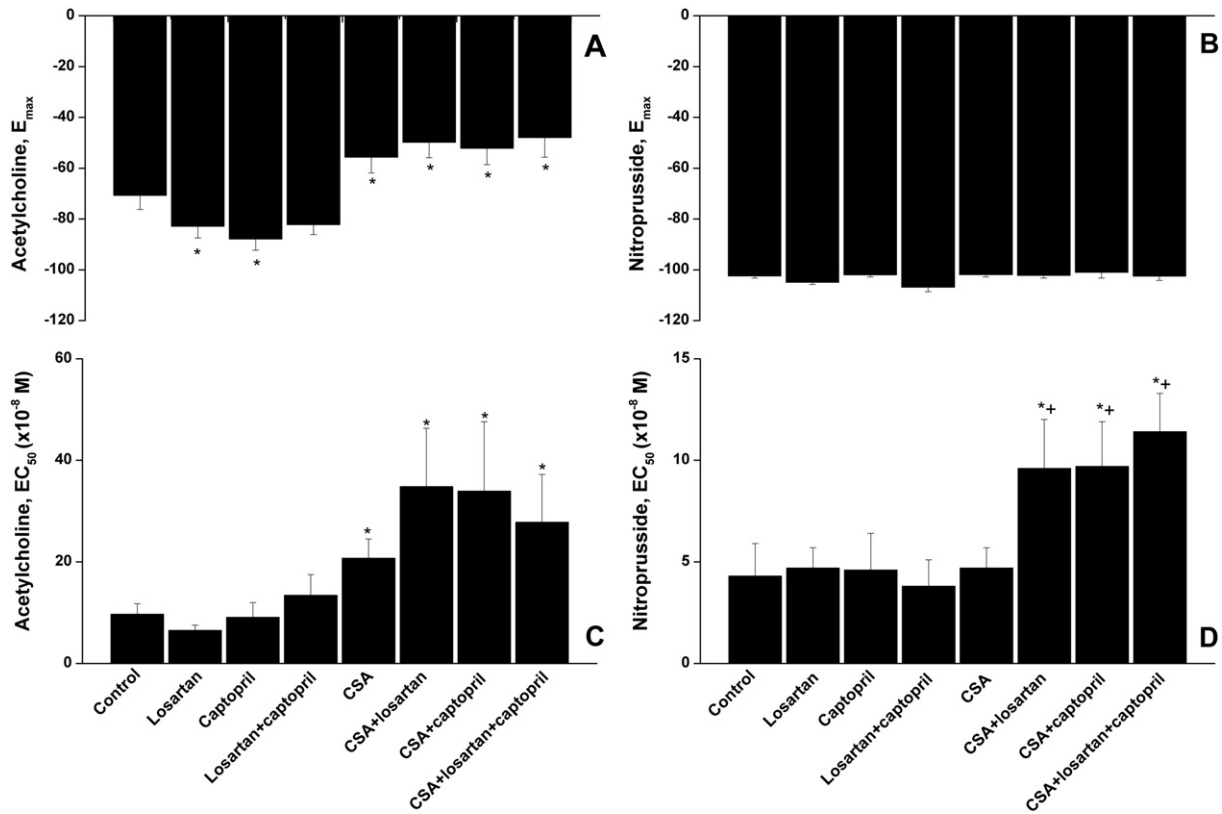
Other mechanisms, however, may contribute to the advantageous hemodynamic action of RAS inhibitors. For example, the elevations in angiotensin-(1–7) and bradykinin levels that follow ACE inhibition might contribute to the antihypertensive [38,39] and baroreflex facilitatory actions of ACE inhibitors [40]. Moreover, under conditions of AT<sub>1</sub> receptor blockade, Ang II activates AT<sub>2</sub> receptors that lower BP [41] and augment baroreflexes [42]. Based on the current findings, it is likely that enhanced reflex bradycardia, which reflects increased cardiac vagomotor activity and parasympathetic dominance [21,22,43], may explain the BP lowering effect caused by RAS inhibition. The concept that baroreflex dysfunction predisposes to hypertension has been established both clinically [44] and experimentally [45]. The impairment of baroreceptor function precedes the development of



**Fig. 8.** Effects of 7-day treatment with CSA (25 mg/kg/day), captopril (ACE inhibitor, 10 mg/kg/day), losartan (AT<sub>1</sub> receptor blocker, 10 mg/kg/day), losartan plus captopril, or their combination on the contractile responses to cumulative doses of U-46619 (thromboxane analogue,  $1 \times 10^{-10}$ – $3 \times 10^{-6}$  M) in rat thoracic aortic rings. Values are means  $\pm$  S.E.M. of 6 observations. \*  $P < 0.05$  vs. control values, +  $P < 0.05$  vs. CSA values.

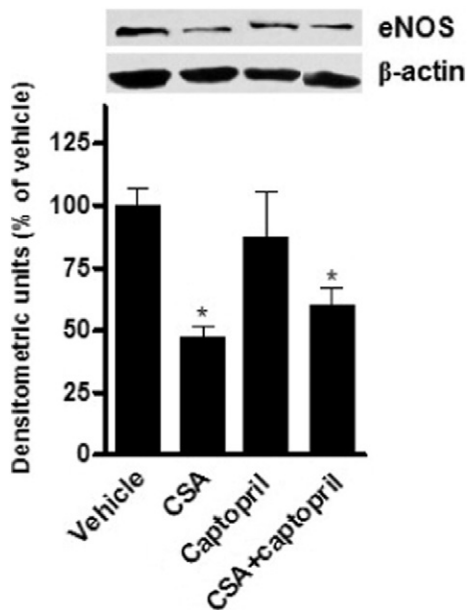


**Fig. 9.** Effects of 7-day treatment with CSA (25 mg/kg/day), captopril (ACE inhibitor, 10 mg/kg/day), losartan (AT<sub>1</sub> receptor blocker, 10 mg/kg/day), losartan plus captopril, or their combination on vasorelaxant responses to cumulative doses of acetylcholine ( $1 \times 10^{-9}$ – $1 \times 10^{-5}$  M) or sodium nitroprusside ( $1 \times 10^{-10}$ – $3 \times 10^{-6}$  M) in rat thoracic aortic rings. Values are means  $\pm$  S.E.M. of 6 observations. \*  $P < 0.05$  vs. control values, +  $P < 0.05$  vs. CSA values.



**Fig. 10.**  $E_{max}$  (panels A–B) and  $EC_{50}$  (panels C–D) of the vasorelaxant effects of acetylcholine ( $1 \times 10^{-9}$ – $1 \times 10^{-5}$  M) or sodium nitroprusside ( $1 \times 10^{-10}$ – $3 \times 10^{-6}$  M) in rat thoracic aortic rings of rats treated for 7 days with CSA (25 mg/kg/day), captopril (ACE inhibitor, 10 mg/kg/day), losartan (AT<sub>1</sub> receptor blocker, 10 mg/kg/day), losartan plus captopril, or their combination. Values are means  $\pm$  S.E.M. of 6 observations. \*  $P < 0.05$  vs. control values, +  $P < 0.05$  vs. CSA values.

hypertension in Dahl salt-sensitive rats [46] and high-fat-fed rats [45]. Therefore, it is conceivable that the counteraction by RAS inhibitors of CSA hypertension is mediated, in part, by the enhanced reflex chronotropism.



**Fig. 11.** Aortic eNOS protein expression in rats treated for 7 days with CSA (25 mg/kg/day), captopril (10 mg/kg/day), or their combination. Illustrative gels depicting aortic eNOS expression are shown. Values are means  $\pm$  S.E.M. of 5 observations. \*  $P < 0.05$  vs. vehicle values.

It is also important to comment on the role of calcineurin in the CSA-RAS hemodynamic interaction. The contention that calcineurin inhibition and hypertension are causally related is supported by the observations that: (i) calcineurin inhibition in extra-lymphoid tissues mediates the hypertensive effects of CSA and tacrolimus [47], (ii) hypertension is less common in organ transplant patients receiving sirolimus, a calcineurin-independent immunosuppressant drug [48], and (iii) the hypertension induced by CSA or tacrolimus in rats with liver fibrosis is improved upon switching to the calcineurin-independent immunosuppressants such as sirolimus and everolimus [49]. Paradoxically, other studies have shown that the antihypertensive action of AT<sub>1</sub> receptor blockade or ACE inhibition is coupled with reduced calcineurin activity [50,51]. In view of these contradictory reports, more studies are apparently required to precisely define the role of calcineurin in BP control and in the CSA-RAS interaction.

Data of the current tension studies performed in isolated aortas suggest key roles for the interplay of Ang II and other vasopressor pathways in the hypertensive action of CSA. Consistent with previous reports [13], Ang II vasoconstriction was augmented by CSA as indicated by increases in  $E_{max}$  and decreases in  $EC_{50}$  of Ang II dose-response curves in isolated aortas. This has been attributed to CSA-evoked upregulation of AT<sub>1</sub> receptor mRNA synthesis or stability or rise in intracellular  $Ca^{2+}$  [17]. The CSA-induced increases in Ang II contractions were abrogated after concomitant treatment with losartan, captopril, or their combination. While the effect of losartan as an antagonist at AT<sub>1</sub> receptors was anticipated, the ability of ACE inhibition by captopril to offset the augmented Ang II contractions might probably relate to increases in the vasodilatory actions of Ang-(1–7) and bradykinin known to be produced as a consequence of ACE inhibition [52].

Considering the importance of vasopressor pathways of ET-1, thromboxane, and prostaglandins in the vascular effects of Ang II [53, 54], we hypothesized that these vasoactive molecules might be

involved in the CSA-Ang II interaction. This postulate receives support from the observation that the augmented Ang II responsiveness in aortas of CSA-treated rats disappeared after simultaneous treatment with indomethacin, thereby highlighting the importance of vasoconstrictor prostanoids in mediating the CSA/Ang II interaction. Alternatively, the CSA-evoked enhancement of Ang II aortic contractions were maintained after concurrent treatment with atrasentan, BQ788, or terutroban, precluding the involvement of their respective receptor sites ( $ET_A$ ,  $ET_B$ , or thromboxane) in the CSA effect. Unlike the individual targeting of these receptors, the combined blockade of endothelin  $ET_A/ET_B$  receptors by atrasentan/BQ788 regimen virtually abolished the CSA augmentation of Ang II responsiveness. These data corroborate that the co-existence of functional  $ET_A$  and  $ET_B$  receptors is pivotal for the CSA-Ang II interaction to manifest. Notably, whereas  $ET_A$  receptors exist in smooth muscle cells and mediate vasoconstriction,  $ET_B$  receptors locate in endothelial cells and cause vasodilation via releasing NO and prostacyclin [55]. Contrary to the vasodilator capability of  $ET_B$  receptors, evidence is also available that the activation of  $ET_B$  receptors, like  $ET_A$ , might elicit vasoconstriction depending on the animal species and vascular bed [56]. It is tempting, therefore, to speculate that both  $ET_A$  and  $ET_B$  receptors evoke vasoconstriction upon activation and mediate the facilitated Ang II vasoreactivity in our model system. More studies are needed to resolve this possibility.

We demonstrated that CSA caused leftward shifts of the U-46619 aortic dose-response curves and significant decreases in  $EC_{50}$ , indicating increased sensitivity of thromboxane receptors to U-46619. Nonetheless, the maximum contractile response to U-46619 remained unaltered after CSA treatment compared with control preparations, which may explain the inability of terutroban to offset the hypertensive response elicited by CSA in rats in this study or others [57]. That said, the remarkable capability of terutroban to reduce  $E_{max}$  of the U-46619 dose-response curves and U-46619 potency (greater  $EC_{50}$ ) confirm the adequate blocking of thromboxane receptors by terutroban. This finding together with the observation that RAS inhibition (by losartan, captopril, or their combination) negatively influenced the contraction characteristics of U-46619 (decreasing  $E_{max}$  and/or increasing  $EC_{50}$ , Fig. 8) indicate that Ang II acts downstream of thromboxane signaling to provoke CSA vasculotoxicity.

The ability of CSA to reduce aortic acetylcholine relaxations and eNOS expression are consistent with earlier studies, which suggested a causal relationship between impaired endothelial activity and CSA hypertension [29,54]. The lack of effect of CSA on endothelium-independent relaxation induced by sodium nitroprusside argues against a direct interaction of CSA with vascular guanylate cyclase and downstream substrates. Unexpectedly, we found that the use of CSA along with captopril (ACE inhibitor) or losartan ( $AT_1$  receptor antagonist), interventions that attenuated the hypertension and baroreflex dysfunction caused by CSA as discussed earlier, failed to improve the CSA-induced decreases in aortic acetylcholine vasorelaxation or eNOS expression. In fact, the increases in acetylcholine relaxations observed in aortas of rats treated with captopril or losartan alone (Fig. 9A) disappeared after simultaneous administration of CSA (Fig. 9C). These results are not in line with the observation that valsartan or enalapril improves CSA impairment of endothelium-dependent relaxations [58]. This discrepancy between current and reported studies may relate to differences in the dose of the protective drugs (10 mg/kg/day vs. 30 mg/kg/day), regimen duration (7 vs. 42 days), hypertension model (CSA vs. spontaneously hypertensive rats).

The current findings that the co-administration of CSA and RAS inhibitors caused rightward shifts in the aortic dose-relaxant response curves of Ach and SNP and increased the  $EC_{50}$  value of either relaxant (Figs. 9 and 10) indicate impaired signaling pathways downstream of NO (e.g. cGMP). One possible underlying mechanism might relate to the exaggerated smooth muscle oxidative injury that might be evoked by this combined regimen. Indeed, the increased generation of bradykinin that follows ACE inhibition [52] or  $AT_1$  receptor blockade [1] is

believed to boost reactive oxygen species generation [59]. Such oxidative effect of RAS inhibitors could be accentuated by the oxidative damage induced by CSA [29] and results in enhanced scavenging of NO released from endogenous (e.g. vascular endothelial) or exogenous (e.g. SNP) sources. More studies are needed to ascertain the role of vascular oxidative stress and perhaps endothelial biomarkers (e.g. phosphorylated eNOS expression, NO metabolites) in the RAS modulation of CSA vasculotoxicity.

In summary, the parallelism between the hypertensive and impaired reflex bradycardic effects of CSA and the alleviation of both effects upon simultaneous administration of RAS inhibitors suggest a predisposing role for RAS in CSA effects. The enhancement of Ang II vasoreactivity induced by the upregulation of endothelin and vasoconstrictor prostanoid signaling pathways is another mechanism that might contribute to the RAS-dependent hypertensive effect of CSA. More mechanistic studies are required, however, to investigate the ways by which these vasoconstrictor pathways crosstalk in modulating CSA hypertension and vasculopathy.

#### Conflict of interest statement

None.

#### Acknowledgements

We thank Rowayda Khattab, Rana Ghali-Ghoul, and Nahed Mougharbel (Faculty of Medicine, American University of Beirut, Lebanon) for technical assistance, Pharmadex S.A.L. (Lebanon) for providing phenylephrine, Pharmaline (Lebanon) for providing losartan, Novartis Pharma, AG (Basel, Switzerland) for providing Sandimmune® ampoules (50 mg/ml, Abbott Laboratories (Illinois, USA) for providing atrasentan, and Servier (Paris, France) for providing terutroban.

#### References

- [1] H. Kobori, M. Nangaku, L.G. Navar, A. Nishiyama, The intrarenal renin-angiotensin system: from physiology to the pathobiology of hypertension and kidney disease, *Pharmacol. Rev.* 59 (2007) 251–287.
- [2] R. Satou, W. Shao, L.G. Navar, Role of stimulated intrarenal angiotensinogen in hypertension, *Ther. Adv. Cardiovasc. Dis.* 9 (2015) 181–190.
- [3] L. Te Riet, J.H. van Esch, A.J. Roks, A.H. van den Meiracker, A.H. Danser, Hypertension: renin-angiotensin-aldosterone system alterations, *Circ. Res.* 116 (2015) 960–975.
- [4] P.S. Tan, S. Killinger, J. Horiuchi, R.A. Dampney, Baroreceptor reflex modulation by circulating angiotensin II is mediated by  $AT_1$  receptors in the nucleus tractus solitarius, *Am. J. Physiol. Regul. Integr. Comp. Physiol.* 293 (2007) R2267–R2278.
- [5] S.J. Lewis, A.M. Allen, A.J. Verberne, R. Figdor, B. Jarrott, F.A. Mendelsohn, Angiotensin II receptor binding in the rat nucleus tractus solitarius is reduced after unilateral nodose ganglionectomy or vagotomy, *Eur. J. Pharmacol.* 125 (1986) 305–307.
- [6] A.M. Allen, I. Moeller, T.A. Jenkins, J. Zhuo, G.P. Aldred, S.Y. Chai, F.A. Mendelsohn, Angiotensin receptors in the nervous system, *Brain Res. Bull.* 47 (1998) 17–28.
- [7] S. Matsuda, S. Koyasu, Mechanisms of action of cyclosporine, *Immunopharmacology* 47 (2000) 119–125.
- [8] M. Kockx, D.L. Guo, M. Traini, K. Gaus, J. Kay, S. Wimmer-Kleikamp, C. Rentero, J.R. Burnett, W. Le Goff, M. Van Eck, J.L. Stow, W. Jessup, L. Kritharides, Cyclosporin A decreases apolipoprotein E secretion from human macrophages via a protein phosphatase 2B-dependent and ATP-binding cassette transporter A1 (ABCA1)-independent pathway, *J. Biol. Chem.* 284 (2009) 24144–24154.
- [9] M.H. Kapturczak, H.U. Meier-Kriesche, B. Kaplan, Pharmacology of calcineurin antagonists, *Transplant. Proc.* 36 (2004) 255–325.
- [10] H.M. El-Gowell, M.M. El-Mas, Central modulation of cyclosporine-induced hypertension, *Naunyn Schmiedeberg's Arch. Pharmacol.* 388 (2015) 351–361.
- [11] M.M. El-Mas, M.M. Mohy El-Din, S.M. El-gowilly, F.M. Sharabi, Regional and endothelial differences in the cyclosporine attenuation of adenosine receptor-mediated vasorelaxations, *J. Cardiovasc. Pharmacol.* 43 (2004) 562–573.
- [12] M.M. El-Mas, M.M. Mohy El-Din, S.M. El-gowilly, F.M. Sharabi, Relative roles of endothelial relaxing factors in cyclosporine-induced impairment of cholinergic and  $\beta$ -adrenergic renal vasodilations, *Eur. J. Pharmacol.* 487 (2004) 149–158.
- [13] Y. Takeda, I. Miyamori, P. Wu, T. Yoneda, K. Furukawa, R. Takeda, Effects of an endothelin receptor antagonist in rats with cyclosporine-induced hypertension, *Hypertension* 26 (1995) 932–936.
- [14] L.A. Calò, P.A. Davis, B. Giacoin, E. Pagnin, M. Sartori, P. Riegler, A. Antonello, W. Huber, A. Semplicini, Oxidative stress in kidney transplant patients with calcineurin inhibitor-induced hypertension: effect of ramipril, *J. Cardiovasc. Pharmacol.* 40 (2002) 625–631.

- [15] M.H. Shang, W.J. Yuan, S.J. Zhang, Y. Fan, Z. Zhang, Intrarenal activation of renin-angiotensin system in the development of cyclosporine A induced chronic nephrotoxicity, *Chin. Med. J.* 121 (2008) 983–988.
- [16] C. Letizia, C. d'Ambrosio, A. De Ciochis, D. Scavo, P. Pozzilli, Serum angiotensin-converting enzyme levels in patients with recent-onset insulin-dependent diabetes after one year of low-dose cyclosporin therapy, IMDIAB Study Group, *Int. J. Clin. Pharmacol. Res.* 15 (1995) 209–213.
- [17] P.V. Avdonin, F. Cottet-Maire, G.V. Afanasjeva, S.A. Loktionova, P. Lhote, U.T. Ruegg, Cyclosporine A upregulates angiotensin II receptors and calcium responses in human vascular smooth muscle cells, *Kidney Int.* 55 (1999) 2407–2414.
- [18] A. Schmidt, U. Gruber, G. Bohmig, E. Koller, G. Mayer, The effect of ACE inhibitor and angiotensin II receptor antagonist therapy on serum uric acid levels and potassium homeostasis in hypertensive renal transplant recipients treated with CsA, *Nephrol. Dial. Transplant.* 16 (2001) 1034–1037.
- [19] S.E. DiCarlo, V.S. Bishop, Central baroreflex resetting as a means of increasing and decreasing sympathetic outflow and arterial pressure, *Ann. N. Y. Acad. Sci.* 940 (2001) 324–337.
- [20] E.B. Oliveira-Sales, M.A. Toward, R.R. Campos, J.F. Paton, Revealing the role of the autonomic nervous system in the development and maintenance of Goldblatt hypertension in rats, *Auton. Neurosci.* 183 (2014) 23–29.
- [21] M.M. El-Mas, E.A. Afify, A.G. Omar, F.M. Sharabi, Cyclosporine adversely affects baroreflexes via inhibition of testosterone modulation of cardiac vagal control, *J. Pharmacol. Exp. Ther.* 301 (2002) 346–354.
- [22] M.M. El-Mas, E.A. Afify, A.G. Omar, F.M. Sharabi, Cyclosporine attenuates the autonomic modulation of reflex chronotropic responses in conscious rats, *Can. J. Physiol. Pharmacol.* 80 (2002) 766–776.
- [23] M.M. El-Mas, A.A. Abdel-Rahman, Contrasting effects of urethane, ketamine and thiopental anesthesia on ethanol-clonidine hemodynamic interaction, *Alcohol. Clin. Exp. Res.* 21 (1997) 19–27.
- [24] M.M. El-Mas, A.A. Abdel-Rahman, Ethanol counteraction of I<sub>1</sub>-imidazoline but not alpha-2 adrenergic receptor-mediated reduction in vascular resistance in conscious spontaneously hypertensive rats, *J. Pharmacol. Exp. Ther.* 288 (1999) 455–462.
- [25] J.B. Glen, W.N. Scott, Carbon dioxide euthanasia of cats, *Br. Vet. J.* 129 (1973) 471–479.
- [26] M.M. El-Mas, A.A. Abdel-Galil, H.M. El-Gowelli, T.T. Daabees, Short-term aortic barodenervation diminishes  $\alpha_1$ -adrenoceptor reactivity in rat aortic smooth muscle, *Eur. J. Pharmacol.* 322 (1997) 201–210.
- [27] M. Rezik, M.M. El-Mas, S.J. Mustafa, A.A. Abdel-Rahman, Role of endothelial adenosine receptor-mediated vasorelaxation in ethanol-induced hypotension in hypertensive rats, *Eur. J. Pharmacol.* 452 (2002) 205–214.
- [28] S.A. Nasser, A.I. Elmallah, R. Sabra, M.M. Khedr, M.M.M. El-Din, M.M. El-Mas, Blockade of endothelin ET<sub>A</sub>, but not thromboxane, receptors offsets the cyclosporine-evoked hypertension and interrelated baroreflex and vascular dysfunctions, *Eur. J. Pharmacol.* 727 (2014) 52–59.
- [29] M.M. El-Mas, H.M. El-Gowelli, K.S. Abd-Elrahman, E.I. Saad, A.G. Abdel-Galil, A.A. Abdel-Rahman, Pioglitazone abrogates cyclosporine-evoked hypertension via rectifying abnormalities in vascular endothelial function, *Biochem. Pharmacol.* 81 (2011) 526–533.
- [30] H.S. Smyth, P. Sleight, G.W. Pickering, Reflex regulation of arterial pressure during sleep in man. A quantitative method for assessing baroreflex sensitivity, *Circ. Res.* 24 (1969) 109–121.
- [31] M.M. El-Mas, A.A. Abdel-Rahman, Direct evidence for selective involvement of aortic baroreceptors in ethanol-induced impairment of baroreflex control of heart rate, *J. Pharmacol. Exp. Ther.* 264 (1993) 1198–1205.
- [32] M.M. El-Mas, M.A. Fouda, S.M. El-Gowilly, E.I. Saad, Central estrogenic pathways protect against the depressant action of acute nicotine on reflex tachycardia in female rats, *Toxicol. Appl. Pharmacol.* 258 (2012) 410–417.
- [33] T. Morgan, C. Griffiths, L. Delbridge, Interaction of ACE inhibitors and AT(1)-receptor blockers on maximum blood pressure response in spontaneous hypertensive rats, *J. Renin-Angiotensin-Aldosterone Syst.* 3 (2002) 16–18.
- [34] M.F. Jarvis, J.L. Wessale, C.Z. Zhu, J.J. Lynch, B.D. Dayton, S.V. Calzadilla, R.J. Padley, T.J. Opgenorth, E.A. Kowaluk, ABT-627, an endothelin ET(A) receptor-selective antagonist, attenuates tactile allodynia in a diabetic rat model of neuropathic pain, *Eur. J. Pharmacol.* 388 (2000) 29–35.
- [35] P.R. Câmara, G.J. Ferraz, L.A. Velloso, J.M. Zeitune, F.A. Suassuna, J.G. Ferraz, Endothelin and neonatal capsaicin regulate gastric resistance to injury in BDL rats, *World J. Gastrointest. Pathophysiol.* 3 (2012) 85–91.
- [36] A. Bischoff, V. Limmroth, M.C. Michel, Indomethacin inhibits the natriuretic effects of neuropeptide Y in anesthetized rats, *J. Pharmacol. Exp. Ther.* 286 (1998) 704–708.
- [37] S.M. El-gowilly, A.M. Ghazal, E.Y. Gohar, M.M. El-Mas, Exacerbation by nicotine of the cyclosporine A-induced impairment of  $\beta$ -adrenoceptor-mediated renal vasodilation in rats, *Clin. Exp. Pharmacol. Physiol.* 35 (2008) 1164–1171.
- [38] C. Höcht, M.M. Gironacci, M.A. Mayer, M. Schuman, F.M. Bertera, C.A. Taira, Involvement of angiotensin-(1–7) in the hypothalamic hypotensive effect of captopril in sinoaortic denervated rats, *Regul. Pept.* 146 (2008) 58–66.
- [39] M.M. Gironacci, Angiotensin-(1–7). Beyond its central effects on blood pressure, *Ther. Adv. Cardiovasc. Dis.* 9 (2015) 209–216.
- [40] S. Heringer-Walther, E.N. Batista, T. Walther, M.C. Khosla, R.A. Santos, M.J. Campagnole-Santos, Baroreflex improvement in SHR after ACE inhibition involves angiotensin-(1–7), *Hypertension* 37 (2001) 1309–1314.
- [41] H. Li, Y. Gao, J.L. Grobe, M.K. Raizada, M.J. Katovich, C. Sumners, Potentiation of the antihypertensive action of losartan by peripheral overexpression of the ANG II type 2 receptor, *Am. J. Physiol. Heart Circ. Physiol.* 292 (2007) H727–H735.
- [42] M.H. Abdulla, E.J. Johns, Nitric oxide impacts on angiotensin AT2 receptor modulation of high-pressure baroreflex control of renal sympathetic nerve activity in anaesthetized rats, *Acta Physiol. (Oxf.)* 210 (2014) 832–844.
- [43] V.J. Dias da Silva, N. Montano, H.C. Salgado, R. Fazan Júnior, Effects of long-term angiotensin converting enzyme inhibition on cardiovascular variability in aging rats, *Auton. Neurosci.* 124 (2006) 49–55.
- [44] M. Gollasch, J. Tank, F.C. Luft, J. Jordan, P. Maass, C. Krasko, A.M. Sharma, A. Busjahn, S. Bähring, The BK channel beta1 subunit gene is associated with human baroreflex and blood pressure regulation, *J. Hypertens.* 20 (2002) 927–933.
- [45] N.M. Fardin, L.M. Oyama, R.R. Campos, Changes in baroreflex control of renal sympathetic nerve activity in high-fat-fed rats as a predictor of hypertension, *Obesity (Silver Spring)* 20 (2012) 1591–1597.
- [46] F.J. Gordon, A.L. Mark, Mechanisms of impaired baroreflex control in prehypertensive Dahl salt-sensitive rats, *Circ. Res.* 54 (1984) 378–387.
- [47] W. Zhang, R.G. Victor, Calcineurin inhibitors cause renal afferent activation in rats: a novel mechanism of cyclosporine-induced hypertension, *Am. J. Hypertens.* 13 (2000) 999–1004.
- [48] M. Zeier, M. Van Der Giet, Calcineurin inhibitor sparing regimens using m-target of rapamycin inhibitors: an opportunity to improve cardiovascular risk following kidney transplantation? *Transpl. Int.* 24 (2011) 30–42.
- [49] E. Patsenker, V. Schneider, M. Ledermann, H. Saegesser, C. Dorn, C. Hellerbrand, F. Stickel, Potent antifibrotic activity of mTOR inhibitors sirolimus and everolimus but not of cyclosporine a and tacrolimus in experimental liver fibrosis, *J. Hepatol.* 55 (2011) 388–398.
- [50] G.Q. Zhang, Z. Zhu, W. Zhang, Inhibitory effect of antihypertensive drugs on calcineurin in cardiomyocytes, *Am. J. Hypertens.* 22 (2009) 132–136.
- [51] M. Fu, J. Zhou, J. Xu, H. Zhu, J. Liao, X. Cui, A. Sun, M. Fu, Y. Zou, K. Hu, J. Ge, Olmesartan attenuates cardiac hypertrophy and improves cardiac diastolic function in spontaneously hypertensive rats through inhibition of calcineurin pathway, *J. Cardiovasc. Pharmacol.* 63 (2014) 218–226.
- [52] R.A. Santos, A.J. Ferreira, A.C. Simões, E. Silva, Recent advances in the angiotensin-converting enzyme 2-angiotensin(1–7)-Mas axis, *Exp. Physiol.* 93 (2008) 519–527.
- [53] B. Ponnuchamy, R.A. Khalil, Cellular mediators of renal vascular dysfunction in hypertension, *Am. J. Physiol. Regul. Integr. Comp. Physiol.* 296 (2009) R1001–R1018.
- [54] E.A. Guzmán-Hernández, R. Villalobos-Molina, M.A. Sánchez-Mendoza, L. Del Valle-Mondragón, G. Pastelín-Hernández, M. Ibarra-Barajas, Early co-expression of cyclooxygenase-2 and renin in the rat kidney cortex contributes to the development of N(G)-nitro-L-arginine methyl ester induced hypertension, *Can. J. Physiol. Pharmacol.* 93 (2015) 299–308.
- [55] S.L. Bourque, S.T. Davidge, M.A. Adams, The interaction between endothelin-1 and nitric oxide in the vasculature: new perspectives, *Am. J. Physiol. Regul. Integr. Comp. Physiol.* 300 (2011) R1288–R1295.
- [56] A.P. Davenport, International union of pharmacology. XXIX. Update on endothelin receptor nomenclature, *Pharmacol. Rev.* 54 (2002) 219–226.
- [57] J.J. Tresham, J.A. Whitworth, B.A. Scoggins, W.M. Bennett, Cyclosporine-induced hypertension in sheep. The role of thromboxanes, *Transplantation* 49 (1990) 144–148.
- [58] M. Lassila, P. Finckenberg, A.K. Pere, H. Vapaatalo, M.L. Nurminen, Enalapril and valsartan improve cyclosporine A-induced vascular dysfunction in spontaneously hypertensive rats, *Eur. J. Pharmacol.* 398 (2000) 99–106.
- [59] S. Sesti, G. Martino, S. Mazzulla, R. Chimenti, Effect of bradykinin on nitric oxide production, urea synthesis and viability of rat hepatocyte cultures, *BMC Physiol.* 5 (2005) 2.