Enhanced cardiac sympathetic afferent reflex involved in sympathetic overactivity in renovascular hypertensive rats

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Sympathetic outflow is increased in hypertension. The aim of the present study was to investigate whether the cardiac sympathetic afferent reflex (CSAR) is enhanced in two-kidney one-clip (2K1C) renovascular hypertensive rats, and whether the enhanced CSAR contributes, in part, to the increased sympathetic outflow. Furthermore, the role of central angiotensin II type 1 (AT<sub>1</sub>) receptors in mediating the CSAR was determined. Under urethane and α-chloralose anaesthesia, the renal sympathetic nerve activity (RSNA) and mean arterial pressure (MAP) were recorded in sinoaortic denervated and cervical vagotomized rats. The CSAR was evaluated by the response of RSNA and MAP to epicardial application of 1.0 nmol of capsaicin. Compared with sham-operated rats, the CSAR, baseline RSNA and plasma noradrenaline level were significantly enhanced in 2K1C rats. Intrapericardial administration of resiniferatoxin, which abolishes the CSAR because of the desensitization of transient receptor potential vanilloid 1-containing cardiac afferent fibres, decreased the RSNA and MAP. The enhanced CSAR in 2K1C rats was normalized by intracerebroventricular administration of the AT<sub>1</sub> receptor antagonist losartan. Intracerebroventricular administration of angiotensin II further potentiated the enhanced CSAR in 2K1C rats, a response which was abolished by pretreatment with losartan. These results indicate that the CSAR is enhanced in 2K1C rats and the enhanced CSAR contributes, in part, to the sympathetic activation and hypertension. Central AT<sub>1</sub> receptors are involved in the enhanced CSAR in 2K1C rats.

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It has been shown that sympathetic outflow is enhanced in patients with essential hypertension (Mary & Stoker, 2003; Hogarth et al. 2007), secondary hypertension in chronic kidney disease (Remuzzi, 1999; Neumann et al. 2007) or diabetes (Vecchione et al. 2000; Esler et al. 2001), or obesity-related hypertension (Lambert et al. 2007; Biaggioni, 2008). The enhanced sympathetic outflow is also well documented in a variety of experimental hypertensive models, such as spontaneously hypertensive rats (SHR; Li & Pan, 2007), salt-sensitive hypertensive rats (Fujita et al. 2007), two-kidney one-clip (2K1C) rats (Katholi et al. 1982) and obesity-induced hypertensive rats (Stocker et al. 2007). It is known that the elevated sympathetic tone plays an important role in the pathogenesis of hypertension and the progression of organ damage (Mancia et al. 1999; Rahn et al. 1999; Morise et al. 2000; Grisk, 2005). Inhibition of the enhanced sympathetic activity has been considered as an antihypertensive strategy (Del et al. 2007; Biaggioni, 2008; Kasparov & Teschemacher, 2008; Signolet et al. 2008).

Activation of the cardiac sympathetic afferent reflex (CSAR) is involved in the increases in sympathetic outflow and arterial pressure. The CSAR can be induced by directly stimulating the cardiac sympathetic afferent nerves or by stimulating the sympathetic afferent endings innervating the heart with epicardial application of capsaicin, bradykinin, adenosine or hydrogen peroxide (Malliani & Montano, 2002; Du & Chen, 2007). It has been found that the CSAR is enhanced in chronic heart failure (Wang & Ma, 2000; Malliani & Montano, 2002),
which is caused by both increased activity of cardiac sympathetic afferents (Wang et al. 1999; Longhurst et al. 2001; Pan & Chen, 2002) and increased central sensitivity of the CSAR (Wang & Ma, 2000; Zhu et al. 2004a,b). This excitatory sympathetic reflex can be initiated by myocardial ischaemia, since some substances released in the myocardium, such as adenosine, bradykinin and hydrogen peroxide, stimulate cardiac sympathetic afferents. The enhanced reflex then contributes to the increase in sympathetic outflow (Malliani & Montano, 2002; Zucker et al. 2004).

It is known that hypertension increases the workload of the left ventricle, which causes an increase in myocardial oxygen consumption and the development of hypertrophy and myocardial ischaemia (Cohn, 1977; Prys-Roberts, 1980). Some chemicals released from the myocardium in myocardial ischaemia may stimulate cardiac sympathetic afferents to increase sympathetic outflow (Malliani & Montano, 2002). The sympathetic overactivation in hypertension is deleterious to the organism over the long term (Mancia et al. 1999; Rahn et al. 1999; Morise et al. 2000; Grisk, 2005). We hypothesized that the CSAR may be enhanced in the hypertensive state and that the enhanced CSAR may be, in part, responsible for the increase in sympathetic outflow in hypertension. Therefore, the first aim of the present study was to determine whether the CSAR was enhanced in renovascular hypertensive rats and whether the enhanced CSAR partly contributed to the increased sympathetic outflow in hypertension. It is known that central angiotensin II (Ang II) is involved in the enhanced CSAR in chronic heart failure (Zhu et al. 2004b; Wang et al. 2005). We hypothesized that Ang II may be responsible for the enhancement of the CSAR in hypertension. Therefore, the second aim was to determine whether central Ang II and angiotensin II type 1 (AT\textsubscript{1}) receptors mediated the enhanced CSAR in renovascular hypertensive rats.

Methods

Experiments were carried out in male Sprague–Dawley rats weighing between 250 and 300 g. The procedures were approved by the Experimental Animal Care and Use Committee of Nanjing Medical University and complied with the Guide for the Care and Use of Laboratory Animals published by the US National Institutes of Health (NIH publication no. 85-23, revised 1996).

Renovascular hypertensive model

The renovascular hypertension was induced using the Goldblatt two-kidney one-clip (2K1C) method. Briefly, the rat was anaesthetized with pentobarbitone sodium (50 mg kg\textsuperscript{-1}, i.p.). A retroperitoneal flank incision was performed with sterile techniques. The right renal artery was exposed, and partly occluded by placing a silver clip with an internal diameter of 0.20 mm on the vessel to induce hypertension. The rats were kept in a temperature-controlled room on a 12 h–12 h light–dark cycle with free access to standard chow and tap water. The sham-operated (Sham) rats were treated in the same manner as the 2K1C rats except that their renal arteries were not occluded. The systemic arterial pressure (SBP) of the tail artery was monitored with a PowerLab tail-cuff system (8SP, ADInstruments, Bella Vista, Australia). The criterion of hypertension in the present study is set as SBP > 160 mmHg (Duke et al. 2005). In 73 2K1C rats and 58 sham-operated rats, seven 2K1C rats were excluded because the SBP in these rats was not high enough to meet the criterion mentioned above, and three 2K1C rats and two sham-operated rats were excluded because of unsuccessful animal surgical preparation.

Acute experiments

Each rat was anaesthetized with urethane (800 mg kg\textsuperscript{-1}, i.p.) and \(\alpha\)-chloralose (40 mg kg\textsuperscript{-1}, i.p.). Adequate depth of anaesthesia was assessed by the absence of corneal reflexes and paw withdrawal response to a noxious pinch. Supplemental doses of urethane and \(\alpha\)-chloralose were administered to maintain an adequate depth of anaesthesia. The trachea was cannulated for mechanical ventilation using a rodent ventilator (model 683, Harvard Apparatus Inc., Holliston, MA, USA). Arterial pressure was measured with a pressure transducer (MLT0380, ADInstruments) through a catheter placed into the right carotid artery. Baroreceptor denervation and vagotomy were carried out and identified as previously reported (Han et al. 2005).

The renal sympathetic nerve activity (RSNA) was recorded as previously described (Zhong et al. 2008a). Briefly, the left renal nerve at the side of non-clipped kidney was identified through a retroperitoneal incision with the aid of an operating microscope. After the renal nerve was cut distally to ensure that afferent activity was not recorded, the nerve was placed on a pair of silver recording electrodes and was immersed in mineral oil. The signals were amplified (\(\times\)1000) with an AC/DC differential amplifier (model 3000, A–M Systems Inc., Sequim, WA, USA) with low-frequency cut-off at 30 Hz and high-frequency cut-off at 3 kHz. The amplified and filtered signals were integrated with a time constant of 10 ms. The background noise was determined after section of the central end of the renal nerve at the end of the experiment and was subtracted from all the integrated values of the RSNA. The raw RSNA, integrated RSNA, arterial pressure and heart rate (HR) were simultaneously recorded on a PowerLab data acquisition system (8SP, ADInstruments).
A limited left lateral thoracotomy was performed to expose the heart, and the pericardium was removed. The CSAR was elicited by applying of a piece of filter paper (3 mm × 3 mm) containing capsaicin (1.0 nmol in 2.0 μl) to the epicardial surface of the anterior wall of the left ventricle. Each piece of paper was removed 1 min later. The epicardium was rinsed three times with 10 ml of warm normal saline (38°C). The CSAR was evaluated by the response of the RSNA to capsaicin. The capsaicin-induced percentage changes in the RSNA and mean arterial pressure (MAP) from control (the values before epicardial application of capsaicin) were used as an index of the sensitivity of the CSAR.

The rats were placed in a stereotaxic instrument (Stoelting, Chicago, IL, USA), and the skull was exposed through an incision on the mid-line of the scalp. After bregma was identified, an intracerebroventricular cannula (outer diameter 0.5 mm and inner diameter 0.1 mm) was positioned into the cerebral ventricle. The co-ordinates were 0.8 mm posterior and 1.4 mm lateral to the bregma and 3.8 mm ventral to the zero level according to the rat atlas of Paxinos & Watson (2005). The volume of the microinjection was 1.0 μl.

Finally, 1.0 μl of Evans Blue dye (2%) was injected into the microinjection site. The rat was killed with an overdose of sodium pentobarbitone (100 mg kg⁻¹, i.p.). The brain was removed from the skull, placed in 10% formalin, and sectioned for histological verification of the microinjection sites.

**Experimental protocols**

Acute experiments were carried out at the end of the fourth week following the 2K1C surgery or sham operation. The rats were subjected to sinoaortic denervation and vagotomy before intervention, except those rats used for determining the plasma noradrenaline and baseline RSNA. Experimental procedures started about 30 min after completion of all surgical preparation in order to allow haemodynamic and neural parameters to stabilize.

Firstly, plasma noradrenaline concentration and baseline RSNA were used as markers for neurohumoral activation. For measurement of the plasma noradrenaline, blood samples were collected in Sham (n = 6) and 2K1C rats (n = 6) from the carotid artery into a tube containing EDTA-K₂ at 4°C. Immediately after centrifugation, the plasma was frozen at −70°C until being assayed. The plasma noradrenaline (NA) level was determined with enzyme-linked immunosorbent assay (ELISA) as previously reported by Westermann et al. (2002). For determination of the baseline RSNA, sodium nitroprusside (SNP, 50 μg kg⁻¹) was intravenously administered in Sham (n = 6) and 2K1C rats (n = 6) to induce maximal RSNA, because SNP causes transient severe hypotension and thus unloads the baroreceptors. The baseline RSNA (Liu et al. 1999) was calculated as a percentage of maximal RSNA (the RSNA before SNP divided by the maximal RSNA after SNP) × 100%.

Secondly, the CSAR induced by epicardial application of three different doses of capsaicin (0.01, 0.1 and 1 nmol) was determined and compared in sinoaortic denervated and vagotomized Sham (n = 6) and 2K1C rats (n = 7).

Thirdly, resiniferatoxin (RTX) was used to desensitize transient receptor potential vanilloid 1 (TRPV1)-containing cardiac afferent fibres and therefore to abolish the CSAR. The effects of intrapericardial administration of RTX (60 pmol) or saline on both RSNA and MAP were determined in sinoaortic-denervated and vagotomized Sham rats and 2K1C rats (n = 7 for each group). To confirm the desensitizing effect of RTX on the cardiac afferent fibres, the pericardium was removed, and the CSAR evoked by epicardial application of capsaicin was determined at the 120th minute after administration of RTX.

Fourthly, the effects of intracerebroventricular injection of saline, Ang II (30 nmol), losartan (500 nmol) and the same dose of Ang II following pretreatment with losartan on the CSAR, RSNA and MAP were respectively determined in 24 2K1C rats and 24 Sham rats (n = 6 for each group). The losartan pretreatment was 10 min before administration of Ang II. The CSAR was determined 10 min after injection. In another group of 2K1C rats (n = 6), the effects of intravenous injection of the same dose of losartan were determined.

Lastly, to exclude the possibility that the increases in arterial pressure and RSNA may cause the enhancement in the CSAR, we investigated the CSAR change in rats with acute hypertension induced by aortic coarctation. The CSAR was determined before aortic coarctation and 10, 20 and 30 min after aortic coarctation in acute aortic coarctation hypertensive rats (n = 6). The acute hypertensive model of aortic coarctation used in this study was reported previously (VanNess et al. 1998). We did not use a simple pharmacological intervention to induce acute hypertension in the present study because aortic coarctation causes both hypertension and increased sympathetic activity. Most importantly, the model of aortic coarctation is better in imitating the enhanced sympathetic drive (Goodall & Sealy, 1969; Rocchini et al. 1976).

**Drugs**

Angiotensin II, capsaicin and sodium nitroprusside were obtained from Sigma Chemical Co. (St Louis, MO, USA). Resiniferatoxin was obtained from ALEXIS company (San Diego, CA, USA). Losartan was a gift from Merck & Co., Inc. (Darmstadt, Germany). All reagents used for the ELISA kit were provided by Adlitteram Diagnostic Laboratories (San Diego, CA, USA).
Table 1. Morphological variables and baseline MAP

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<th>Sham</th>
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<tr>
<td>n</td>
<td>56</td>
<td>63</td>
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<tr>
<td>Body weight (g)</td>
<td>345 ± 19</td>
<td>343 ± 23</td>
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<tr>
<td>Heart weight (mg)</td>
<td>990 ± 14</td>
<td>1066 ± 15*</td>
</tr>
<tr>
<td>Left ventricular weight (mg)</td>
<td>594 ± 6</td>
<td>718 ± 12*</td>
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<td>LVW/HW ratio (mg mg⁻¹)</td>
<td>0.61 ± 0.01</td>
<td>0.68 ± 0.01*</td>
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<tr>
<td>LVW/BW ratio (mg g⁻¹)</td>
<td>2.86 ± 0.04</td>
<td>3.11 ± 0.04*</td>
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<td>Baseline MAP (mmHg)</td>
<td>93.5 ± 1.4</td>
<td>141.7 ± 1.3*</td>
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Abbreviations: BW, body weight; HW, heart weight; LVW, left ventricular weight; and MAP, mean arterial pressure. *P < 0.05 compared with the Sham group.

Statistical analysis

A two-way repeated-measures ANOVA followed by the Newman–Keuls test for post hoc analysis was used when multiple comparisons were made. All data are expressed as means ± s.e.m. P < 0.05 was considered statistically significant.

Results

Morphological variables and MAP in 2K1C rats

There was no significant difference in body weight (BW) between Sham and 2K1C rats. However, the heart weight (HW), left ventricular weight (LVW), LVW/HW ratio and LVW/BW ratio were significantly increased in 2K1C rats compared with Sham rats. The baseline MAP in 2K1C rats was much higher than in Sham (Table 1). There was no significant difference in baseline MAP among each group of 2K1C rats or among each group of Sham rats.

Baseline RSNA and plasma NA level

The baseline RSNA in 2K1C rats was much higher than in Sham rats (75.4 ± 4.7 versus 46.7 ± 4.3%). The plasma NA level in 2K1C rats was significantly higher than in Sham rats (1827.3 ± 45.9 versus 760.7 ± 28.6 pg ml⁻¹).

Cardiac sympathetic afferent reflex induced by different doses of capsaicin

The representative recordings in Fig. 1 show that epicardial application of 1 nmol of capsaicin increased the RSNA and MAP in both 2K1C and Sham rats. Epicardial application of three doses of capsaicin (0.01, 0.1 and 1 nmol) resulted in dose-related increases in the RSNA and MAP responses. The CSAR was significantly enhanced in 2K1C rats compared with Sham rats (Fig. 2).

Effects of RTX on RSNA and MAP

Intrapericardial administration of RTX resulted in immediate and short-term increases in RSNA and MAP in both 2K1C and Sham rats followed by long-lasting decreases in RSNA and MAP in 2K1C rats but not in Sham rats. The RSNA-enhancing and pressor responses of RTX were significantly larger in 2K1C rats than in Sham rats. The capsaicin-induced CSAR was abolished at the 120th minute after RTX administration in both Sham and 2K1C rats (Fig. 3).

Effects of Ang II and losartan on baseline RSNA and MAP

Intracerebroventricular injection of Ang II increased the RSNA and MAP in both 2K1C and Sham rats (Fig. 4). The RSNA and MAP responses induced by Ang II were significantly greater in 2K1C rats than in Sham rats. Intracerebroventricular injection of losartan had no significant effect on RSNA and MAP in Sham rats, but decreased RSNA and MAP in 2K1C rats. Pretreatment with intracerebroventricular injection of losartan abolished the effects of Ang II in both 2K1C and Sham rats. However, intravenous injection of the same dose of losartan also caused significant decreases in baseline RSNA and MAP, which were similar to
those following intracerebroventricular administration of losartan in 2K1C rats (RSNA, $-10.7 \pm 2.1$ versus $-6.3 \pm 1.7\%$, $P > 0.05$; and MAP, $-16.5 \pm 2.8$ versus $-16.0 \pm 5.4$ mmHg, $P > 0.05$).

Effects of Ang II and losartan on CSAR

Intracerebroventricular injection of Ang II enhanced the CSAR in both 2K1C and Sham rats (Fig. 4). The Ang II-induced enhancement in CSAR was significantly greater in 2K1C rats than in Sham rats. Intracerebroventricular injection of losartan had no significant effect on CSAR in Sham rats, but decreased the CSAR in 2K1C rats. Pretreatment with intracerebroventricular injection of losartan abolished the effects of Ang II on CSAR in both 2K1C and Sham rats. Intravenous injection of the same dose of losartan had no significant effect on the CSAR in 2K1C rats (Table 2).

Figure 2 The CSAR induced by epicardial application of different doses of capsaicin (0.01, 0.1 and 1 nmol) in Sham ($n = 6$) and 2K1C rats ($n = 7$)

*$P < 0.05$ compared with Sham rats.

Figure 3 Effects of intrapericardial administration of resiniferatoxin (RTX, 60 pmol) on the baseline RSNA and MAP (upper panel) and CSAR (lower panel) in Sham and 2K1C rats ($n = 7$ for each group)

The CSAR was evaluated at the 120th minute after RTX. *$P < 0.05$ compared with 2K1C–Saline; †$P < 0.05$ compared with Sham–RTX; and #$P < 0.05$ compared with Sham–saline.
Cardiac sympathetic afferent reflex in acute hypertension induced by aortic coarctation

Although the baseline RSAR and MAP were significantly increased after aortic coarctation, the CSAR was not significantly enhanced at 10, 20 and 30 min after aortic coarctation compared with control values (Fig. 5).

Discussion

Cardiac sympathetic afferent fibres have been accepted as part of the neural afferent pathway that plays a role in regulating sympathetic outflow and cardiovascular functions. These sympathetic afferent fibres mediate the CSAR that exhibits positive feedback characteristics. Activation of the CSAR enhances the central sympathetic drive, which is demonstrated by representative cardiac or renal sympathetic activity recordings, producing increases in arterial blood pressure, myocardial contractility and heart rate. (Zucker & Pliquett, 2002; Malliani & Montano, 2002). This excitatory sympathetic afferent reflex is activated by various substances that may be released from the myocardium or by an increase in cardiac pressure and dimensions during ischaemia or chronic heart failure (Inoue et al. 1988). The primary new findings in the present study were that the CSAR was enhanced in 2K1C rats. Desensitization of TRPV1-containing cardiac afferent fibres with intrapericardial administration of RTX caused decreases in the RSNA and MAP. The enhanced CSAR in 2K1C rats was normalized by intracerebroventricular administration of the AT1 receptor antagonist losartan. Intracerebroventricular administration of angiotensin II

Table 2. Effects of intracerebroventricular and intravenous injection of losartan on the CSAR in 2K1C rats

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<th>Losartan, i.C.V.</th>
<th>Losartan, i.V.</th>
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<tr>
<td></td>
<td>Before</td>
<td>After</td>
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<tr>
<td>RSNA change (%)</td>
<td>23.8 ± 1.9</td>
<td>9.2 ± 2.0*</td>
</tr>
<tr>
<td>MAP change (mmHg)</td>
<td>8.3 ± 0.8</td>
<td>4.0 ± 1.2*</td>
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*P < 0.05 compared with the values before losartan; n = 6 for each group.

Figure 4 Effects of intracerebroventricular injection of angiotensin II (Ang II, 30 nmol) and losartan (Los, 500 nmol) on the baseline RSNA and MAP (upper panel) and CSAR (lower panel) in Sham and 2K1C rats (n = 6 for each group)

*P < 0.05 compared with Sham; †P < 0.05 compared with Saline; and #P < 0.05 compared with Ang II in the absence of losartan pretreatment.
further potentiated the enhanced CSAR in 2K1C rats, a response which was abolished by pretreatment with losartan. These results indicate that the CSAR is enhanced in 2K1C rats and the enhanced CSAR contributes, in part, to the sympathetic activation and hypertension. Central AT1 receptors are involved in the enhanced CSAR in 2K1C rats.

The sympathetic outflow is increased in 2K1C hypertensive models (Katholi et al. 1982; Tuncel et al. 2002; Head & Burke, 2003; Peotta et al. 2007). However, MAP in 2K1C rats was almost normalized by Ang II receptor blockade but not significantly affected by the sympathetic blockade (Nystrom et al. 2002). Recently, a threefold increase in plasma noradrenaline level has been found in 2K1C hypertensive rats (Cohn et al. 2008). There is a highly significant positive correlation between mean arterial pressure and the plasma noradrenaline, and the increased sympathetic nervous system activity is important in the maintenance of hypertension in the 2K1C model (Katholi et al. 1982). The baseline RSNA and plasma NA levels were higher in 2K1C rats than in Sham rats, indicating that the 2K1C hypertensive rats used in the present study showed similar sympathetic overactivity. It is known that the plasma noradrenaline is principally derived from neurotransmitter released from noradrenergic nerve endings. The elevation of plasma noradrenaline observed in the 2K1C rats is interpreted as a consequence of enhanced neurotransmitter release secondary to increased sympathetic neuronal activity. The sympathetic overactivation is involved in the pathogenesis of hypertension and progression of organ damage (Mancia et al. 1999; Rahn et al. 1999; Coote, 2007; Joyner et al. 2008). In the present study, we found that epicardial application of capsaicin caused dose-related increases in RSNA and MAP in either 2K1C or Sham rats. Importantly, the CSAR induced by capsaicin was significantly enhanced in 2K1C rats compared with Sham rats. It is probable that the enhanced CSAR contributes, in part, to the hypertension and the overexcitation of the sympathetic system which is deleterious in hypertension over the long term. To investigate this possibility, we studied the effects of intrapericardial administration of RTX on the RSNA and MAP in both 2K1C and Sham rats.

Resiniferatoxin is an ultrapotent analogue of capsaicin that binds TRPV1. Transient receptor potential vanilloid 1 is known as a ligand-gated, non-selective cation channel preferentially expressed in small-diameter, primary afferent neurons, including nociceptive sensory nerves. Resiniferatoxin has been demonstrated to cause degeneration of capsaicin-sensitive afferent neurons in adult rats upon systemic administration, and is used to deplete capsaicin-sensitive afferent fibres (Zahner et al. 2003; Pan & Chen, 2004; Qin et al. 2006). The TRPV1-containing afferent nerve fibres are present...
on the epicardial surface of the rat heart, and single intraperitoneal injection of RTX abolished the CSAR, as well as TRPV1 immunoreactivity in the epicardium and dorsal root ganglia, suggesting that the TRPV1-expressing afferent nerves are essential for the CSAR (Zahner et al. 2003). In the present study, intrapericardial administration of RTX caused immediate and short-term increases in both RSNA and MAP. These responses of RTX were significantly larger in 2K1C rats than in Sham rats. The excitatory effects of RTX can be explained as stimulating the TRPV1 on the epicardial surface of the heart and then activating the TRPV1-containing cardiac afferents. The augmented excitatory effects of RTX mean that the CSAR induced by RTX is enhanced in 2K1C rats, which is similar to the enhanced CSAR induced by capsaicin in 2K1C rats. More importantly, RTX caused long-lasting decreases in both RSNA and MAP in 2K1C rats but not in Sham rats. The inhibitory effects of RTX are the result of blockade of the TRPV1-containing cardiac afferents, which is confirmed by the finding that the CSAR cannot be induced by capsaicin after RTX intervention. The results indicate that the enhanced CSAR contributes, in part, to the overexcitation of the sympathetic system and to hypertension in 2K1C rats. Since the sympathetic overactivation plays an important role in the pathogenesis of hypertension and the progression of organ damage (Mancia et al. 1999; Rahn et al. 1999; Morise et al. 2000; Brook & Julius, 2000), abolishing the CSAR by blockade of the cardiac afferents to inhibit the sympathetic activity may be a possible strategy for antihypertensive therapy.

In order to determine whether the enhanced CSAR is directly secondary to the increased baseline MAP and RSNA in 2K1C rats, we examined the CSAR change in rats with acute hypertension induced by aortic coarctation. It has been reported that aortic coarctation causes increases in both arterial pressure and sympathetic outflow (Goodall & Sealy, 1969; Rocchini et al. 1976). In the present study, the CSAR was not enhanced in this acute hypertensive model, although aortic coarctation caused a great increase in the baseline RSNA and MAP. The results indicate that the acute increases in baseline RSNA and MAP do not cause the enhancement of the CSAR, which is further supported by our previous findings that microinjection of the GABA_A receptor antagonist gabazine into the paraventricular nucleus increases the baseline RSNA and MAP, but fails to enhance the CSAR (Zhong et al. 2008b). Therefore, it is probable that the enhanced CSAR in 2K1C rats is not directly secondary to the increased baseline RSNA and MAP and it might be involved in a complicated pathological process of hypertension.

It is well accepted that Ang II in the central nervous system regulates sympathetic outflow and cardiovascular function. Previous studies in our laboratory have shown that central AT_1 receptors are involved in the enhanced CSAR in rats with chronic heart failure (Wang et al. 2005). In the present study, we found that the CSAR was significantly augmented by intracerebroventricular injection of Ang II. Although the CSAR was enhanced in 2K1C rats, Ang II further potentiated the CSAR. The effects of Ang II were abolished by pretreatment with the AT_1 receptor antagonist losartan. Intracerebroventricular injection of losartan alone had no significant effect on the CSAR in Sham rats, but it normalized the enhanced CSAR in 2K1C rats. These results suggest that Ang II in the central nervous system enhances the CSAR in 2K1C rats, and the enhanced central gain of the CSAR in 2K1C rats involves the AT_1 receptors in the brain. Although intracerebroventricular or intravenous injection of the same dose of losartan caused a similar decrease in baseline MAP in 2K1C rats, the CSAR was inhibited by intracerebroventricular losartan but not by intravenous losartan. Therefore, it is unlikely that the effect of intracerebroventricular administration of losartan on the CSAR was due to a peripheral action of losartan that may have leaked from the cerebrospinal fluid through the blood–brain barrier. It was demonstrated that intravenous administration of losartan (5 mg kg\(^{-1}\)) normalized the enhanced CSAR in dogs (Ma et al. 1997). However, in the present study the intravenous administration of losartan (500 nmol) had no significant effects on the CSAR in 2K1C rats, since the dose of losartan used in the rats was much lower than that used in the dog study.

The reasons for the enhancement of the CSAR in 2K1C rats are complicated. One possibility is that the cardiac sympathetic afferent inputs increase in hypertension, which may be caused by more substances released in the myocardium stimulating the cardiac sympathetic afferents because of myocardial ischaemia in hypertension and by the increased sensitivity of cardiac sympathetic afferents to the chemical stimulus. The former suggestion is supported by the fact that hypertension increases the workload of the left ventricle and myocardial oxygen consumption, and then persistent hypertension causes myocardial hypertrophy and ischaemia (Cohn, 1977; Prys-Roberts, 1980). The latter suggestion is supported by the present findings that depletion of capsaicin-sensitive cardiac afferents with RTX decreases the baseline RSNA and MAP in 2K1C rats but not in Sham rats. Another possibility is that the central gain of the CSAR is increased in hypertension, which is supported by the present findings that the CSAR responses induced by Ang II in the brain were greater in 2K1C rats than in Sham rats, and that losartan in the brain abolished the enhanced CSAR in 2K1C rats.

In conclusion, the present study demonstrates that the CSAR evoked by epicardial application of capsaicin is enhanced in 2K1C rats, and intrapericardial administration of RTX to block the TRPV1-containing cardiac afferents of the CSAR causes long-lasting decreases in RSNA and MAP in 2K1C rats but not in Sham rats.
Intracerebroventricular injection of Ang II potentiates the enhanced CSAR and losartan normalizes the enhanced CSAR in 2K1C rats. These results indicate that the enhanced CSAR in 2K1C rats contributes, in part, to the increased sympathetic outflow and hypertension, and that central Ang II and AT\textsubscript{1} receptors play an important role in the enhanced central gain of the CSAR in 2K1C rats.

**References**


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