Blockade of AT₁ receptors in the rostral ventrolateral medulla increases sympathetic activity under hypoxic conditions

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The rostral part of the ventrolateral medulla (RVLM) contains a group of sympathoexcitatory neurons that play a major role in the tonic and phasic regulation of sympathetic activity and arterial blood pressure (8, 16). These neurons project directly to sympathetically preganglionic neurons in the spinal cord and are an essential component of the central pathways mediating cardiovascular reflex responses to stimulation of baroreceptors, chemoreceptors, and many other peripheral receptors (8, 16).

There is considerable evidence suggesting a role for endogenous ANG peptides in the regulation of RVLM neurons. Studies using in vitro autoradiography have demonstrated a high density of ANG II receptor binding sites in the RVLM of most species studied, including humans (2). These receptors are primarily of the ANG type 1 (AT₁) subtype (2), and recent studies using an antibody against the AT₁ receptor has confirmed the existence of these receptors on neurons in the RVLM of the rat, including catecholamine neurons of the C1 group (19). Microinjection of ANG II in the RVLM results in an increase in arterial pressure and sympathetic activity, an effect that is mediated by AT₁ receptors (3, 14, 17).

Blockade of AT₁ receptors by microinjection of selective AT₁ receptor antagonists in the RVLM in anesthetized normotensive rats or rabbits has little effect on resting arterial pressure and sympathetic nerve activity (14, 22, 37), suggesting that under normal conditions in anesthetized animals endogenous ANG II does not make a significant contribution to the firing rate of RVLM presympathetic neurons. On the other hand, bilateral blockade of AT₁ receptors in the RVLM does result in a decrease in arterial pressure and sympathetic activity in spontaneously hypertensive rats (SHR) and in Dahl salt-sensitive hypertensive rats (1, 21, 22). A similar effect has also been observed in rats on a low-salt diet in which there is activation of the renin-angiotensin system (9). Thus these studies indicate that RVLM sympathoexcitatory neurons are tonically activated by endogenous ANG II in these two different hypertensive rat models and in rats on a low-salt diet.

Although blockade of AT₁ receptors in the RVLM of normotensive rats does not alter arterial pressure under normal conditions, we have demonstrated that after bilateral injections of the GABAₐ receptor antagonist bicuculline in the RVLM of normotensive anesthetized rats, subsequent bilateral microinjections of the selective AT₁ receptor antagonists losartan or L-158,809 in the RVLM reduced both arterial pressure and renal sympathetic nerve activity (RSNA; see Ref. 41). This indicates that endogenous ANG II does have a tonic excitatory effect on RVLM sympathoexcitatory neurons in normotensive anesthetized rats that is unmasked when tonic GABAergic synaptic inputs are blocked. It is conceivable, therefore, that under normal conditions in anesthetized rats, this endogenous tonic excitatory effect of ANG II on RVLM sympathoexcitatory neurons is balanced by a tonic inhibitory action that is dependent on GABAergic synaptic transmission. In conscious rats, microinjection of an AT₁ receptor antagonist in the RVLM results in an increase in blood pressure (12, 13), indicating that in this case also there is a tonic AT₁ receptor-mediated inhibitory action on RVLM sympathoexcitatory neurons that is not balanced by a tonic excitatory action.

If endogenous ANG II in the RVLM has both tonic excitatory and inhibitory effects on sympathoexcitatory neurons, mediated via separate mechanisms, then its contribution to sympathetic
vasomotor tone may differ according to the level of activity of these excitatory or inhibitory inputs. In this study, we have tested this hypothesis, by determining the effect of selective bilateral blockade of AT1 receptors in the RVLM on arterial pressure and sympathetic vasomotor tone under conditions of moderate systemic hypoxia, which activates peripheral chemoreceptors and greatly increases the activity of RVLM sympathoexcitatory neurons, via glutamatergic excitatory synaptic inputs (29). As a control, we have also determined whether hypoxia alters the cardiovascular response to microinjection in the RVLM of the compound [Sar1,Thr8]ANG II (sarthran), which has been shown to decrease sympathetic activity via a mechanism that is independent of AT1, glutamate, or GABA receptors (23, 37, 42).

**MATERIALS AND METHODS**

**General procedures.** Experiments were performed on male Sprague-Dawley rats (350–500 g body wt), supplied by the University of Sydney Laboratory Animal Services. All experimental procedures were approved by the Animal Ethics Committee of the University of Sydney and were carried out in accordance with the Guidelines for Animal Experimentation of the National Health and Medical Research Council of Australia. Anesthesia was initially induced by inhalation of isoflurane (2% in oxygen). The trachea was cannulated, and catheters were placed in the femoral artery and femoral vein for the recording of pulsatile arterial pressure and drug application, respectively. Isoflurane was gradually withdrawn over period of 3–5 min while being replaced by urethane (1.3 g/kg iv with supplementary doses of 0.1 g/kg iv, if required). No supplementary doses of urethane were required during the period in which the experimental protocol was performed. Body temperature was monitored with a rectal probe and maintained in the range 37–38°C with a thermoregulated heating pad. The head was placed in a stereotaxic frame with the tooth bar fixed 19 mm below the interaural line, and the dorsal surface of the medulla was exposed. The mean arterial pressure (MAP) and heart rate (HR) were derived from the pulsatile signal of arterial pressure by means of a low-pass filter and rate meter, respectively. RSNA was recorded according to the procedures previously described (18). After the completion of all surgical procedures, neuromuscular blockade was induced with alcuronium chloride (0.2 mg/kg iv every 1–2 h), and all animals were artificially ventilated via a respiratory pump with oxygen-enriched air (the level of O2 in the inspired gas was ~40%). The ventilation was adjusted so that the end-tidal CO2 was maintained in the range 4.0–4.5%. The adequacy of anesthesia without neuromuscular blockade was verified by the absence of a withdrawal response to nociceptive stimulation of a hind paw and during neuromuscular blockade by a stable baseline MAP, HR, and RSNA. The MAP, HR, and RSNA were recorded continuously on a computer (PowerLab system; AD Instruments).

Microinjections of drugs were made in the RVLM from micropipettes according to the procedure described previously (18). The micropipettes were held in a micromanipulator at an angle of 20° to the vertical (tip rostral). The compounds injected were sodium glutamate (40–50 nl of 50 mM solution; Sigma), candesartan (100 nl of 1 mM solution; gift of AstraZeneca), L-158,809 (100 nl of 10 mM solution; gift of Merck Sharp & Dohme, Rahway, NJ), or the broad-spectrum ANG II receptor antagonist sarthran (100 nl of 10 mM solution; Sigma), ANG II (100 nl of 1 mM solution; Bachem), and bicuculline methochloride (100 nl of 1 mM solution; Tocris). The vehicle solution was artificial cerebrospinal fluid (pH 7.4).

The pressor region within the RVLM on each side was functionally identified as the site at which a microinjection of sodium glutamate evoked a pressor response of at least 20 mmHg with short-onset latency (<5 s) and rapid rise (latency from onset to peak response of <10 s), together with an increase in RSNA of at least 40% with respect to the preinjection level. The coordinates of the pressor site in the RVLM were 0.8–1.2 mm rostral to the calamus scriptorius (at the point of entry of the pipette at the dorsal surface), 2.0 mm lateral to the midline, and 3.0–3.4 mm below the dorsal surface. Usually, less than three penetrations of the medulla were required to identify the pressor region on each side.

**Experimental procedures.** The cardiovascular effects produced by microinjections of candesartan, L-158,809, bicuculline methochloride and candesartan, sarthran, ANG II, or vehicle in the RVLM during hypoxia were examined. Baseline cardiovascular variables (MAP, HR, and RSNA) were recorded for a 30-min period. After this period, hypoxia was induced by passing a gas mixture of 10% oxygen in nitrogen in the input gas of the respirator such that the measured O2 concentration in the inspired gas was reduced to 10–12%. When this concentration was achieved, there was then a further waiting period of 2–5 min, after which microinjections of candesartan, L-158,809, sarthran, or vehicle solution were made bilaterally in the RVLM (~2 min between injections). In some experiments, a unilateral microinjection of ANG II was made in the RVLM. In another series of experiments, a unilateral microinjection of bicuculline methochloride was made in the RVLM. Once the increase in MAP, HR, and RSNA stabilized after the bicuculline microinjection, a subsequent unilateral microinjection of candesartan was made in the same site in the RVLM. After a further 10 min, the O2 concentration of the inspired gas was restored to the control level. In other series of control experiments, we also tested the effects of bilateral microinjections of candesartan in the RVLM during normoxia and also during a period of hypotension induced by continuous intravenous infusion of sodium nitroprusside (4.2 µg·kg⁻¹·min⁻¹). The purpose of the latter group of experiments was to determine whether the effects of candesartan microinjection during hypoxia may be a consequence of the hypotension associated with the hypoxia, rather than the hypoxia itself.

**Histology.** At the end of each experiment, a microinjection of the vehicle solution containing fast blue dye (100 nl) was made in the RVLM injection sites, using the same coordinates as used for injections of drugs. The animal was then euthanized by an overdose of pentobarbitone sodium, the brain stem was removed, and histological and microscopic procedures were performed as previously described (18), to determine the locations of the centers of the injection sites within the RVLM.

**Data analysis.** The changes in MAP and RSNA induced by hypoxia were measured as the difference between the values of these variables at the time when they had stabilized, 3–4 min after the induction of hypoxia, and their average values during the 1-min period immediately preceding the induction of hypoxia. The time course and magnitude of these changes in MAP and RSNA evoked by microinjections of candesartan, L-158,809, sarthran, ANG II, or vehicle in the RVLM during the period of hypoxia were measured by taking their average values over successive 5-s periods before and after the microinjections. The change in MAP or RSNA evoked by microinjection of candesartan, sarthran, or the vehicle solution was measured by determining the difference between the average value of MAP or RSNA over the period 20–40 s after the second injection and the average value of each variable during the 20-s period immediately preceding this injection. Similarly, in the experiments in which unilateral injections of bicuculline followed by candesartan were made in the same site in the RVLM during hypoxia, the change in MAP or RSNA evoked by candesartan was measured as the difference between the average value of MAP or RSNA over the period 20–40 s after injection of candesartan and the average value of each variable during the 20-s period immediately preceding this injection. A paired t-test was used to determine whether these changes were statistically significant, with application of the Holm step-down procedure for repeated measures as appropriate (39). A value of P < 0.05 was taken to indicate a statistically significant difference. All values are presented as means ± SE.

**RESULTS**

Table 1 shows the baseline MAP and HR for all groups of experiments, and Table 2 shows the changes in MAP, HR, and RSNA evoked by hypoxia in the four groups of experiments in which rats were subjected to hypoxia. After induction of
hypoxia, the MAP decreased (sometimes preceded by an initial rise), and the RSNA increased, in both cases stabilizing at a new level by 3–4 min after the induction of hypoxia (Fig. 1 and Table 2). The HR also increased during the period of hypoxia (Fig. 1 and Table 2).

**Candesartan.** When the MAP and RSNA had stabilized after the induction of hypoxia, a microinjection of candesartan (100 pmol) was made in the pressor region on one side of the RVLM. This was followed by immediate and rapid increases in MAP and RSNA (Figs. 1 and 2), although there was little change in HR. The lack of increase in HR presumably reflects the fact that the HR was at or close to maximum levels as a result of the hypoxia.

The MAP and RSNA remained at elevated levels until the second injection of candesartan in the contralateral RVLM pressor region. After this second microinjection, there was a further rapid but transient increase in RSNA, such that it rose to a maximum value by 10–40 s postinjection, before decreasing back over ~2 min toward its level just before the second microinjection (Figs. 1 and 2). The RSNA then remained at an elevated level compared with the baseline level just before the first injection of candesartan in the RVLM during the period of hypoxia (Fig. 1). The MAP showed a similar pattern of changes after the second microinjection of candesartan. After this injection, the MAP increased transiently, before decreasing back over ~2 min toward the level just before the second microinjection (Figs. 1 and 2). As shown in Table 3, the MAP and RSNA (averaged during the period 20–40 s after the second of the bilateral microinjections of candesartan in the RVLM, when the changes were maximal) were significantly increased (by 23 ± 6 mmHg and 54 ± 8%, respectively) compared with their respective preinjection baseline levels. At the end of the period of hypoxia, the MAP had returned to a level not significantly different from its baseline preinjection level ($P > 0.25$), but the RSNA was still significantly increased (by 15 ± 6%, $P < 0.05$) compared with its baseline level.

In four experiments, another AT$_1$ receptor antagonist, L-158,809 (1 nmol), was injected instead of candesartan. In these experiments, bilateral microinjections of L-158,809 in the RVLM during hypoxia also resulted in increases in MAP and RSNA of 17 ± 5 mmHg and 81 ± 31%, respectively, with respect to the preinjection baseline levels.

In contrast to hypoxia, under normoxic conditions microinjections of candesartan in the RVLM had no significant effect on either MAP or RSNA (Fig. 2 and Table 3). In addition, microinjections of the vehicle solution in the RVLM during hypoxia also had no significant effect on either MAP or RSNA (Table 3).

A further series of experiments was performed to test the possibility that the pressor and sympathoexcitatory effects of candesartan microinjection may be related to the hypotension associated with the hypoxia, rather than the hypoxia itself. In this series of three rats, hypotension (decrease in MAP of 27 ± 6 mmHg) was induced by intravenous infusion of sodium nitroprusside solution. This hypotension was associated with a reflex increase in RSNA of 35 ± 7%. The magnitudes of the decrease in MAP and increase in RSNA were not significantly different ($P > 0.2$ and 0.6, respectively) from the corresponding hypoxia-evoked changes in these variables in the series of experiments in which candesartan was injected under hypoxic

### Table 1. Baseline levels of MAP and HR in the different series of experiments

<table>
<thead>
<tr>
<th></th>
<th>ACSF</th>
<th>Cand</th>
<th>Sarthran</th>
<th>ANG II</th>
<th>Bic + Cand</th>
<th>Normoxia Cand</th>
<th>Hypotension Cand</th>
</tr>
</thead>
<tbody>
<tr>
<td>MAP, mmHg</td>
<td>95 ± 5</td>
<td>82 ± 3</td>
<td>83 ± 5</td>
<td>86 ± 5</td>
<td>101 ± 4</td>
<td>83 ± 3</td>
<td>92 ± 3</td>
</tr>
<tr>
<td>HR, beats/min</td>
<td>438 ± 8</td>
<td>431 ± 13</td>
<td>366 ± 12</td>
<td>398 ± 6</td>
<td>379 ± 8</td>
<td>431 ± 7</td>
<td>345 ± 8</td>
</tr>
<tr>
<td>n</td>
<td>8</td>
<td>8</td>
<td>5</td>
<td>4</td>
<td>4</td>
<td>4</td>
<td>5</td>
</tr>
</tbody>
</table>

Values are means ± SE; $n$, number of rats. The headings refer to different series of experiments in which artificial cerebrospinal fluid (ACSF), candesartan (Cand), sarthran, ANG II, or bicuculline (Bic) was injected in the rostral ventrolateral medulla (RVLM) under conditions of hypoxia, normoxia, or hypotension induced by sodium nitroprusside infusion.

### Table 2. Changes in MAP, HR, and RSNA induced by hypoxia in the different series of experiments

<table>
<thead>
<tr>
<th></th>
<th>ACSF</th>
<th>Cand</th>
<th>Sarthran</th>
<th>ANG II</th>
<th>Bic + Cand</th>
</tr>
</thead>
<tbody>
<tr>
<td>ΔMAP, mmHg</td>
<td>−47 ± 6</td>
<td>−39 ± 5</td>
<td>−36 ± 5</td>
<td>−48 ± 2</td>
<td>−38 ± 12</td>
</tr>
<tr>
<td>ΔHR, beats/min</td>
<td>20 ± 15</td>
<td>41 ± 8</td>
<td>55 ± 12</td>
<td>36 ± 11</td>
<td>53 ± 27</td>
</tr>
<tr>
<td>ΔRSNA, % baseline</td>
<td>64 ± 10</td>
<td>42 ± 10</td>
<td>37 ± 7</td>
<td>69 ± 10</td>
<td>35 ± 4</td>
</tr>
<tr>
<td>n</td>
<td>8</td>
<td>8</td>
<td>5</td>
<td>4</td>
<td>5</td>
</tr>
</tbody>
</table>

Values are means ± SE; $n$, number of rats. The headings refer to different series of experiments in which ACSF, candesartan, sarthran, bicuculline, or ANG II was injected in the RVLM under conditions of hypoxia. The changes (Δ) in MAP, HR, and renal sympathetic nerve activity (RSNA) were calculated as the difference, for each of these variables, between the value during hypoxia just before injections of the different compounds in the RVLM and the baseline value before induction of hypoxia.

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Fig. 1. Chart recording showing the cardiovascular response evoked by bilateral microinjections of candesartan (Cand, 100 pmol) in the rostral ventrolateral medulla (RVLM) during hypoxia. The arrows and dashed lines indicate the time of injection in the left (L) and right (R) side, respectively. The duration of the period of hypoxia is indicated by the filled horizontal bar. RSNA, renal sympathetic nerve activity.
changes in MAP or RSNA (Table 3).

**Table 3. Changes in MAP and RSNA evoked by microinjection of different compounds during hypoxia and normoxia**

<table>
<thead>
<tr>
<th>Condition</th>
<th>Injectate</th>
<th>ACSF</th>
<th>Cand</th>
<th>Sarthran</th>
<th>ANG II</th>
<th>Normoxia Cand</th>
<th>Hypotension Cand</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>MAP</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hypoxia</td>
<td>1 ± 1</td>
<td>23 ± 6*</td>
<td>-14 ± 3*</td>
<td>0 ± 2</td>
<td>0 ± 2</td>
<td>9 ± 3</td>
<td></td>
</tr>
<tr>
<td>Normoxia</td>
<td>1 ± 1</td>
<td>54 ± 8*</td>
<td>-31 ± 9*</td>
<td>-1 ± 2</td>
<td>4 ± 3</td>
<td>9 ± 4</td>
<td></td>
</tr>
<tr>
<td>n</td>
<td>8</td>
<td>8</td>
<td>5</td>
<td>4</td>
<td>4</td>
<td>3</td>
<td></td>
</tr>
</tbody>
</table>

Values are means ± SE; n, number of rats. The headings refer to different series of experiments in which ACSF, candesartan, sarthran, or ANG II was injected bilaterally in the RVLM under conditions of hypoxia, normoxia, or hypotension induced by sodium nitroprusside infusion. In each case, the change represents the difference between the average value 20–40 s after the second of the bilateral injections in the RVLM and the average baseline value during the 20 s preceding the first injection. *P < 0.01 or 0.001 and †P < 0.05 compared with 0 change.

**DISCUSSION**

The main new finding of this study is that under conditions of hypoxia, but not normoxia, bilateral microinjections of the AT₁ receptor antagonist candesartan in the RVLM resulted in significant increases in arterial pressure and RSNA. This observation suggests that under conditions of hypoxia, endogenous ANG II in the RVLM has a sympatoexcitatory effect, presumably as a consequence of inhibiting the activity of RVLM presympathetic neurons. This finding is surprising, because it has been shown that exogenous ANG II has a sympatoexcitatory effect when microinjected in the RVLM (3, 7, 14, 17). Furthermore, recent studies have indicated that, in the SHR and Dahl salt-sensitive hypertensive rat, endogenous ANG II in the RVLM has a tonic sympatoexcitatory effect (1, 21, 22), whereas in the normotensive rat an excitatory input to RVLM neurons, mediated via AT₁ receptors, is activated by disinhibition of the paraventricular nucleus in the hypothalamus (40). Thus the present finding, together with these previous findings, indicate that the effect of endogenous ANG II in the RVLM on the firing rate of sympatoexcitatory neurons depends on the physiological or pathophysiological state. We discuss below the significance of our results with regard to the role of AT₁ receptors in the RVLM in the regulation of sympathetich vasomotor activity in normal and abnormal conditions, after first considering some methodological issues.

Candesartan has been shown to be a highly potent and selective antagonist of AT₁ receptors (27, 31, 43). The dose injected (100 pmol) is within the range (10–1,000 pmol) that, injection of bicuculline (100 pmol) was made in the RVLM. This was followed by immediate and significant increases in MAP and RSNA (16 ± 3 mmHg and 49 ± 9%, respectively, P < 0.01 in both cases). Once the MAP and RSNA had stabilized at these increased levels, a unilateral microinjection of candesartan (100 pmol) was made in the same site in the RVLM. In contrast to the marked increases in MAP and RSNA evoked by candesartan under hypoxic conditions (without blockade of GABA receptors), in these experiments candesartan microinjection resulted in a modest but significant decrease in RSNA (15 ± 4%, P < 0.05), but had little effect on MAP (decrease of 1 ± 1 mmHg), as shown in Fig. 4.

**Histology.** The histological analysis confirmed that the centers of the injection sites for all microinjections were located within the RVLM, between the level of the caudal pole of the facial nucleus and the level 0.5 mm more caudal [close to the level 11.96 mm caudal to bregma, according to the atlas of Paxinos and Watson (36); Fig. 5].

![Fig. 2. Grouped results showing the averaged changes in mean arterial pressure (MAP) and RSNA (with respect to the preinjection baseline) produced by bilateral microinjections of candesartan or sarthran in the RVLM during normoxia or hypoxia. Because the time interval between the first injection in one side and the second injection in the contralateral side varied slightly between experiments, the values of the variables were averaged at the same time points with respect to the time of the first injection (left) and the time of the second injection (right). The arrows and dashed lines indicate the times of the injections.](image-url)
when injected in the RVLM, produces blockade of AT₁ receptors (1, 9, 12, 27) and is less than the dose (200 pmol) that has been shown to have no effect on glutamate receptors in the RVLM (27). Thus the effect of candesartan in evoking increases in MAP and RSNA is likely to be a consequence of specific blockade of AT₁ receptors rather than a nonspecific effect of the drug. This is further supported by the finding in the present study that microinjections in the RVLM of L-158,809, another highly specific antagonist of AT₁ receptors (6), also resulted in increases in MAP and RSNA under hypoxic conditions, although it also, like candesartan, has no sympathoexcitatory effect when injected in the RVLM under normoxic conditions (40). With regard to the degree of blockade of AT₁ receptors, candesartan is known to have an affinity for these receptors that is greater than that of losartan (10, 25). Averill et al. (3) showed that a dose of 100 pmol losartan produces a very substantial (~75%) attenuation of the pressor and sympathoexcitatory response evoked by ANG II in the RVLM, so that the same dose of candesartan, as was used in the present study, is likely to have produced an even greater degree of blockade of AT₁ receptors.

It could be argued that the sympathoexcitatory effect of candesartan in the RVLM under hypoxic conditions is a consequence of the hypotension associated with hypoxia, rather than the hypoxic stimulus itself. This seems unlikely, however, because after nitroprusside-induced hypotension, microinjection of candesartan in the RVLM had no significant effect on MAP and RSNA. Thus this effect of candesartan appears to be a specific consequence of the hypoxia and not the associated hypotension.

In contrast to candesartan, microinjection of the ANG II receptor antagonist sarthran in the RVLM under hypoxic conditions resulted in significant decreases in MAP and RSNA, as has been observed previously in anesthetized rats and rabbits under normoxic conditions (23, 37, 42). This is an important observation, because it has been demonstrated previously that the depressor and sympathoinhibitory effect of sarthran is independent of its effects on AT₁, glutamate, or GABA receptors (23, 37, 42). Therefore, the fact that microinjections of sarthran in the RVLM have similar depressor and sympathoinhibitory effects under both normoxic and hypoxic conditions indicates that the effect of hypoxia in profoundly altering the response of RVLM neurons to candesartan is not simply because of a nonspecific effect of hypoxia on these neurons. Thus the available evidence indicates that the effects of micro-
injection of this dose of candesartan in the RVLM can be attributed to its blockade of AT₁ receptors rather than to a nonspecific sympathoexcitatory action.

The sympathoexcitatory response evoked by candesartan in the RVLM was more sustained than the pressor response. By the end of the period of hypoxia, the RSNA was still significantly increased compared with its baseline preinjection level, whereas the MAP had returned to a level not significantly different from its baseline preinjection level. This may reflect the fact that the MAP is dependent on several factors other than the level of sympathetic activity, including the direct vasodilator effect of hypoxia on vascular smooth muscle and the cardiac output. Thus changes in MAP do not necessarily reflect changes in sympathetic activity during the period of hypoxia.

As mentioned in the introduction, we have previously demonstrated in anesthetized rats that, after bilateral injections of the GABA receptor antagonist bicuculline in the RVLM, subsequent bilateral microinjections of losartan or L-158,809 in the RVLM reduced both MAP and RSNA (41). This indicates that endogenous ANG II does have a tonic excitatory effect on RVLM sympathoexcitatory neurons that is unmasked when tonic GABAergic synaptic inputs are blocked. Thus we have previously proposed the hypothesis that the effect of endogenous ANG II in the RVLM on sympathetic vasomotor activity depends on the balance between tonic excitatory inputs and tonic GABAergic inhibitory inputs to RVLM sympathoexcitatory neurons (41). In the present study, we found that under hypoxic conditions the increase in RSNA evoked by candesartan in the RVLM was reversed to a decrease, whereas the pressor response was abolished, after bicuculline microinjection in the RVLM. The fact that the decrease in RSNA was not accompanied by a decrease in MAP may reflect the fact, as mentioned above, that the MAP is dependent on several factors other than the level of sympathetic activity. Nevertheless, the results of these experiments clearly indicate that under hypoxic conditions endogenous ANG II has a sympathoinhibitory effect in the RVLM that is dependent on GABA_A receptors and are consistent with the hypothesis that under these conditions the balance between tonic excitatory and tonic GABAergic inhibitory inputs is shifted such that the sympathoinhibitory effects of endogenous ANG II predominate. Such a shift could also explain why during hypoxia exogenously applied ANG II had little effect on RSNA, because under these conditions the excitatory actions of ANG II on RVLM sympathoexcitatory neurons may be balanced by its inhibitory actions, leading to little net change in RSNA.

Recent studies support the view that endogenous ANG II in the RVLM may have both inhibitory and excitatory effects. In particular, a study by Hu et al. (19) found that AT₁ receptors are located on the majority of both glutamatergic and GABAergic neurons in the RVLM. The latter group of neurons may well include tonically active GABAergic interneurons, which are known to project to and inhibit RVLM sympathoexcitatory neurons (8).

Quite apart from direct effects on glutamatergic and GABAergic neurons, it is also possible that endogenous ANG II in the RVLM may also modulate excitatory or inhibitory synaptic transmission to RVLM sympathoexcitatory neurons. ANG II appears to have such effects in the nucleus tractus solitarius (NTS), where it has been shown that ANG II can potentiate both inhibitory (GABAergic) and excitatory synaptic transmission to different subpopulations of NTS neurons (4, 26, 35). Similarly, in the hypothalamic paraventricular nucleus, ANG II also facilitates GABA release (11). Consistent with this possibility, Bertram and Coote (5) have reported that iontophoretic application of ANG II can inhibit the activity of barosensitive RVLM neurons, which they suggested may be the result of a presynaptic facilitation of GABA release from nerve terminals.

Although our findings indicate that the sympathoinhibitory effects of endogenous ANG II under hypoxic conditions are dependent on GABA_A receptors, they do not demonstrate the precise mechanisms by which under these conditions this sympathoinhibitory effect predominates over the sympathoexcitatory effect of endogenous ANG II in the RVLM. One possibility, however, is that nitric oxide may play a role. In both the NTS and the paraventricular nucleus, there is evidence that the ANG II-induced facilitation of GABA release is mediated via nitric oxide, i.e., ANG II induces nitric oxide release, which then acts to increase GABA release (11, 35). In this regard, it is interesting to note that Kishi et al. (28) have recently reported that increased production of nitric oxide in the RVLM (by overexpression of endothelial nitric oxide synthase) results in decreased sympathetic activity mediated via increased release of GABA. Thus these observations could suggest that during hypoxia there is increased activation of AT₁ receptors, which then leads to release of nitric oxide, which via increased release of GABA limits the hypoxia-induced activation of RVLM sympathoexcitatory neurons.

Consistent with this hypothesis, Zanzinger et al. (46) found that blockade of nitric oxide synthesis in the ventrolateral medulla resulted in a marked increase in the sympathoexcitatory response to systemic hypoxia, very similar to the effects of AT₁ receptor blockade as observed in the present study. Thus the present study together with the previous study by Zanzinger et al. (46) indicate that both activation of AT₁ receptors and release of nitric oxide in the RVLM might have critical roles in limiting the sympathoexcitatory response to hypoxia.

There are also several other potential mechanisms by which hypoxia could alter the actions of endogenous ANG II in the RVLM. For example, hypoxia causes powerful chemoreflex-mediated excitation of RVLM sympathoexcitatory neurons, via glutamate receptors (29). It is possible that this may limit the extent to which endogenous ANG II can further excite these neurons, either by a presynaptic or postsynaptic action. It is also possible that hypoxia may directly affect synaptic transmission, again by either a presynaptic or postsynaptic action. For example, studies in vitro have shown that severe hypoxia or anoxia can result in membrane depolarization and inhibition of synaptic transmission (34). It is not clear whether such effects also occur under conditions of more moderate hypoxia, as in the present study, but it is conceivable that such alterations in neuronal function or transmitter release could be directly induced by hypoxia in the RVLM, leading to a shift in the balance between the sympathoexcitatory and sympathoinhibitory effects of endogenous ANG II in this region. Further studies are needed to determine the precise cellular mechanisms involved.

The hypothesis that endogenous ANG II has both sympathoexcitatory and sympathoinhibitory actions in the RVLM is consistent with previous observations that have reported quite different effects of blockade of AT₁ receptors in the RVLM,
depending on the experimental conditions. For example, Lima et al. (32) found that in anesthetized rats bilateral microinjections of losartan in the RVLM pressor region reduced the fall in blood pressure observed after hemorrhage, implying that under these conditions endogenous ANG II has a net inhibitory effect on RVLM sympathoexcitatory neurons. Similarly, microinjection of various specific AT1 receptor antagonists (lo-}

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