Angiotensin stimulates respiration in spontaneously hypertensive rats

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Angiotensin stimulates respiration in spontaneously hypertensive rats. Am J Physiol Regulatory Integrative Comp Physiol 278: R1125–R1133, 2000.—Spontaneously hypertensive rats (SHR) have an activated brain angiotensin system. We hypothesized 1) that ventilation (V̇) would be greater in conscious SHR than in control Wistar-Kyoto (WKY) rats and 2) that intravenous infusion of the ANG II-receptor blocker saralasin would depress respiration in SHR, but not in WKY. Respiration and oxygen consumption (V̇O₂) were measured in conscious age-matched groups (n = 16) of adult female SHR and WKY. For protocol 1, rats were habituated to a plethysmograph and measurements obtained over 60–75 min. After installation of chronic intravenous catheters, protocol 2 consisted of 30 min of saline infusion (14 µl·kg⁻¹·min⁻¹) followed by 40 min of saralasin (1.3 µg·kg⁻¹·min⁻¹). V̇, tidal volume (VT), inspiratory flow [VT/inspiratory time (Tᵢ)], breath expiratory time, and V̇O₂ were higher, and breath Tᵢ was lower in “continuously quiet” SHR. In SHR, but not in WKY rats, ANG II-receptor block decreased V̇, VT, and VT/Tᵢ and increased breath Tᵢ. During ANG II-receptor block, an average decrease in V̇O₂ in SHR was not significant. About one-half of the higher V̇ in SHR appears to be accounted for by an ANG II mechanism acting either via peripheral arterial receptors or circumventricular organs.

The peripheral and brain angiotensin systems can play a role in the stimulation of ventilation and the lowering of PaCO₂ in dogs. In the dog, evidence indicates that neither peripheral baroreceptors (29) nor chemoreceptors (25) are stimulated by ANG II. However, in both the conscious and anesthetized dog, intravenous infusion of ANG II stimulates respiration, and there is evidence for a central mechanism (22, 25) that persists in the anesthetized dog after carotid sinus nerve denervation (25).

As well, in conscious dogs, there is an ANG II-mediated ventilatory stimulation in the presence of unchanged or decreased plasma ANG II: 1) acutely, after normoxic disinhibition of the angiotensin system by arginine vasopressin (AVP) V₁-receptor block (33) and 2) more chronically, during the secondary respiratory and acid-base adaptation to prolonged hypoxia (13). In both circumstances, systemic ANG II-receptor block with saralasin prevented the augmented ventilatory responses; by exclusion, we concluded that a brain ANG II system probably accounted for the stimulation of respiration and lowering of PaCO₂. Because intravenously administered saralasin does not cross the blood-brain barrier (BBB) (31), we also proposed (13, 33) that a brain angiotensin system acted on respiratory control via circumventricular organs (CVO). CVOs are specialized structures at the blood-brain interface, lack a BBB, have angiotensin receptors, and possess neural projections to respiratory centers (reviewed in Ref. 16).

To better study mechanisms for the stimulation of respiration by ANG II, we developed a conscious rat model (38). Sprague-Dawley (SD) rats have a high density of ANG II AT₁ receptors on carotid body glomus cells (1) as well as their CVOs (28, 40). Furthermore, there is a predominant stimulation of sinus nerve chemoreceptor activity by ANG II in superfused carotid bodies of SD rats (1). However, despite this and unlike dogs (22), intravenous infusion of ANG II into conscious SD rats did not stimulate respiration (35, 36), even when potential arterial respiratory depressor baroreflexes were abrogated by simultaneous infusions of sodium nitroprusside (36). Furthermore, unlike dogs (33, 34), disinhibition of ANG II by AVP V₁-receptor block did not stimulate respiration during either normoxia or normoxic hypercapnia in this rat strain (37). On the other hand, given our evidence of the possible importance of brain ANG II in stimulating respiration in dogs, we could not be certain that a brain ANG II system had been stimulated in any of our studies in SD rats.

The spontaneously hypertensive rat (SHR) is an interesting model for testing a potential brain ANG II mechanism for respiratory control. In SHR, there is a progressive decrease in both renal renin and liver angiotensinogen mRNA after birth, whereas brain renin and angiotensinogen mRNA increase with aging (18). Repeated studies indicate that SHR have an activated brain renin-angiotensin system compared with Wistar-Kyoto (WKY) rats and other normotensive controls (2, 11, 28, 40). In contrast, experimental observations indicate that the plasma level of plasma renin activity in SHR can be either normal or depressed (for example, see Refs. 24 and 30 and review in Ref. 40). The latter is consistent with the finding that increased brain ANG II inhibits the systemic renin-angiotensin system (reviewed in Ref. 28).
The literature also indicates that differences in respiration exist between SHR and other rat strains. For example, in some studies, anesthetized SHR have been reported to have a greater minute ventilation (V\text{\textsuperscript{\text{\textcircled{m}}}}) than normotensive control rats (15, 26). Furthermore, SHR have a compensated respiratory alkalosis, even before they develop hypertension, which suggests a chronic hyperventilation (15, 19). In the present study, we tested the hypotheses that 1) respiration would be greater in conscious SHR than in WKY control rats and 2) ANG II-receptor block with intravenous infusion of saralasin would depress V\text{\textsuperscript{\text{\textcircled{m}}}} in SHR, but not in WKY normotensive control rats. Because changes in metabolism affect respiration (reviewed in Ref. 21) and ANG II stimulates metabolism in conscious dogs (22), we measured oxygen consumption (V\text{\textsuperscript{o2}}). We also documented behavioral state during measurement periods for comparison between rat strains, because SHR are more active than WKY rats.

**METHODS**

**General**

All experimental procedures conformed to guidelines of the Canadian Council of Animal Care and were approved by the Queen's University Animal Care Committee. Groups of age-matched female SHR and WKY rats were obtained from Charles River Canada (St-Constant, Quebec) 4–7 wk before the beginning of experiments and adapted to the Animal Care Facility.

After the initial adaptive period, in the week before experiments, rats were separated into separate cages, brought to the laboratory daily, and were handled and weighed. On the Friday of this training week, when they were ~16 wk of age, a calibrated VM-FH temperature transmitter (Mini-Mitter, Sunriver, OR) was surgically implanted in their abdominal cavity for measurement of abdominal temperature (T\text{ab}). Surgery was carried out under ketamine (Rogarsetic, 70 mg/kg) in combination with xylazine (Rompun, 5 mg/kg) anesthesia, administered intraperitoneally, as previously described (38). At least 2 or more days of postsurgical recovery preceded a series of experiments (protocol 1) in which rats, in the conscious state, were habituated to a plethysmographic chamber while measurements were obtained over 60–75 min.

After protocol 1 was completed using the same anesthetic procedure, rats were chronically provided with two catheters implanted directly into the inferior vena cava and fixed in place with Super Glue (Lepage, Brampton, Ontario; see Ref. 38). After at least 48 h of recovery, a second series of experiments was performed with the rats conscious in the plethysmograph (protocol 2). Both sets of experiments in the conscious state were completed while rats were between 16 and 19 wk of age. On a subsequent day, after completion of both protocols, rats were anesthetized intraperitoneally with ketamine in combination with xylazine (same doses as previously outlined), and additional doses were administered to prevent a toe-pinch reflex response. A carotid artery was cannulated, and mean arterial pressure (MAP) was measured using a transducer/recorder system (BPA100, Micro-Med, Louisville, KY).

**Ventilatory and Metabolic Measurements**

A plethysmograph chamber (1.6 liter) was used to obtain both ventilatory and metabolic measurements. SHR are more active than WKY rats, and it required repeated training in the chamber (matched with the WKY rats) to obtain quiet resting measurements. Respiration, metabolism, and body temperature were measured when the animals were assessed by the experimenter as resting and "quiet."

For objective classification within protocols, we designated measurements obtained when rats were resting but still occasionally adjusting their position or looking around without complete relaxation, as "intermittently quiet"; when rats were continuously quiet with relaxed posture but open eyes and twitching ears, they were designated as "continuously quiet"; and when quietly resting rats had closed eyes and ears not twitching or were curled, they were classified as "sleeping. These criteria, assessed and documented at the time of experimental measurements, were applied retrospectively, without knowledge of the respiratory measurements, to group data.

Chamber relative humidity and temperature were measured using a Vaisala HMP 233 Transmitter (Helsinki, Finland). Inflow gas (bubbled through water at room temperature) and outflow gas were analyzed immediately before a ventilatory measurement period using carbon dioxide and oxygen analyzers (Beckman LB2 and OM-11 analyzers, respectively) for the calculation of V\text{\textsuperscript{o2}} (STPD; see Ref. 38). Average airflow through the chamber was 0.91 ± 0.02 l/min.

To make respiratory measurements, the inlet and outlet tubes of the chamber were clamped. Respiratory-related pressure differences between the plethysmograph chamber and a reference chamber, with a slow leak to atmosphere, were measured by a Validyne differential pressure transducer (model DP7) and recorded for 60–120 s with the computer software package CODAS (DATAQ, Akron, OH) at a frequency of 250 Hz. To calibrate the plethysmograph, 0.1 ml of gas was rapidly injected into the sealed plethysmograph chamber during expiration, as previously described (38). Volume calibration of the plethysmograph was flat to a frequency of 8 Hz.

Peaks and valleys of ventilatory waves were marked using computer analysis software and the corresponding tidal voltages determined. Tidal voltages and time points of breaths were imported into a spreadsheet containing the Drorbaugh and Fenn equation (7) for the calculation of tidal volume (VT; ml/kg), respiratory frequency (F; breaths/min), and V\text{\textsuperscript{o2}} (l·kg\textsuperscript{-1}·min\textsuperscript{-1}). Sequential breaths at each measurement period were analyzed for their individual total breath duration (TTOT), inspiratory time (TI), expiratory time (TE), inspiratory flow rate (VT/TI), and breath F (60 s/TTOT). Average minute values for F, VT, and V\text{\textsuperscript{o2}} over a given measurement period were also determined.

**Protocols**

**General**. A one-way mirror was placed between the rat and the experimenter. Rats were allowed to move freely within the plethysmograph chamber. When rats had adjusted to the chamber and exhibited quiet resting behavior, ventilatory and metabolic measurements were obtained.

**Protocol 1.** To train the SHR and WKY rats, habituate them to the chamber, and to obtain steady-state control data before the incorporation of venous catheters, two to three experiments were carried out on different days in each rat. Resting respiratory and metabolic measurements were made at 10- to 15-min intervals over 60–75 min.

**Protocol 2.** After protocol 1, catheters were surgically implanted in the abdominal vena cava of rats for the second protocol. After the recovery period from surgery, experiments consisted of an intravenous infusion of saline (30 min) followed by the infusion of the specific competitive ANG II antagonist saralasin ([Sar\textsuperscript{1},Val\textsuperscript{9},Ala\textsuperscript{11}]ANG II; Sigma Chemi-
cal, St Louis, MO) at a dose of 1.3 µg·kg^{-1}·min^{-1} (30-45 min). Beginning at time 0, measurements were obtained at 15-min intervals. In previous experiments in SD rats, we demonstrated that this infusion dose of saralasin blocks the pressor and heart rate responses to intravenous bolus injections of ANG II (10 ng), which increase MAP by ~45 mmHg.

For the infusion protocol, a glass syringe mounted on a digital infusion pump (model 22, Harvard Apparatus) was attached to a venous catheter extended outside the chamber. Either saline or the ANG II-receptor blocker saralasin was infused at a rate of 12–15 µl/min.

Data Analysis

Statistical analyses were carried out using the computer software package SYSTAT for Windows. Values presented are means ± SE unless otherwise indicated. For breath data, breath variables for each rat were binned by sequential breath F ranges of 25 breaths/min, and the mean respiratory values for each rat at each bin was calculated; a single mean value for each rat, therefore, contributed to the analysis of group data for each F bin. To determine differences within a protocol, either a paired t-test or, for repeated measures, an ANOVA was used and differences identified using a Tukey honestly significant difference post hoc test. To determine differences between protocols, absolute values for the control period of each protocol, or the values representing the "change" at each time period, were compared using an independent samples t-test.

RESULTS

General

At 16–17 wk of age, for protocol 1, the average weight of SHR was significantly less than that of age-matched WKY rats by ~25 g (Table 1). Despite depression of MAP by anesthesia, measurements confirmed that anesthetized SHR (mean MAP = 88 ± 3 mmHg; n = 13) had higher arterial pressures (P < 0.01) than anesthetized WKY rats (mean MAP = 75 ± 3 mmHg; n = 13).

 Conscious SHR were more restless than similarly trained WKY rats, requiring greater patience to obtain continuously quiet measurements. An example of differences between SHR and WKY in protocol 1 is exemplified by average Tab in SHR, Tab = −1°C higher than in WKY rats (Table 1). There were also differences between SHR and WKY rats in the way Tab varied over the time course for protocol 1 (Fig. 1). As exemplified by the last day's study in each rat, an elevated Tab was maintained in SHR, whereas, over 75 min, Tab progressively decreased in WKY rats by >1°C.

Behavioral State and Respiration

In protocol 1, measurements were obtained from seven WKY rats and from six SHR for all three behavioral states: intermittently quiet, continuously quiet, and sleep. Histograms of individual breath data from all rats, based on 2–3 measurement periods for each rat, are depicted in Fig. 2A for SHR and Fig. 2B for WKY rats. Distinct behavioral differences in the relative occurrence of values for individual breath F, VT, and V are apparent in SHR (Fig. 2A). The highest breath respiratory values were obtained during intermittently quiet behavior, and the lowest respiratory values were obtained during sleep (Fig. 2A). In SHR, intermittent quiet behavior and continuously quiet behavior. Similar differences in the distribution of individual breath respiratory parameters during the different behavioral states occurred in WKY rats with the exception of VT, where the distribution of percent occurrence of breath VT remained relatively fixed, independent of behavioral state (Fig. 2B). On the basis of this analysis, behavioral state became important to analysis, and we subsequently present only data, documented as continuously quiet, to compare experimental observations between SHR and WKY rats.

Respiration and Metabolism During Continuously Quiet Behavior in Conscious SHR and WKY Rats in Protocol 1

In the age-matched sets of 16 SHR and WKY rats, V̇ was significantly larger, in association with a higher VT, in SHR compared with WKY rats, but there was no significant difference in F between strains (Table 1). In

Table 1. Respiratory and metabolic data during "continuously quiet" behavior in protocol 1

<table>
<thead>
<tr>
<th>Variables</th>
<th>SHR</th>
<th>WKY</th>
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<tr>
<td>n</td>
<td>16</td>
<td>16</td>
</tr>
<tr>
<td>Wt, g</td>
<td>187 ± 2*</td>
<td>213 ± 3</td>
</tr>
<tr>
<td>V̇, l·kg^{-1}·min^{-1}</td>
<td>1.15 ± 0.42*</td>
<td>0.82 ± 0.20</td>
</tr>
<tr>
<td>F, breaths/min</td>
<td>85 ± 2</td>
<td>91 ± 2</td>
</tr>
<tr>
<td>VT, ml/kg</td>
<td>13.5 ± 0.4*</td>
<td>9.1 ± 0.3</td>
</tr>
<tr>
<td>V̇Ȯ2, ml·kg^{-1}·min^{-1}</td>
<td>41.9 ± 1.5*</td>
<td>29.7 ± 1.3</td>
</tr>
<tr>
<td>V̇Ȯ2, ml/ml</td>
<td>28.7 ± 0.7</td>
<td>31.5 ± 2.9</td>
</tr>
<tr>
<td>Tab, °C</td>
<td>38.9 ± 0.2*</td>
<td>37.9 ± 0.2</td>
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</table>

Values are means ± SE. SHR, spontaneously hypertensive rats; WKY, Wistar-Kyoto control rats; wt, body wt; V̇, minute ventilation; F, respiratory frequency; VT, tidal volume; V̇Ȯ2, oxygen consumption; V̇Ȯ2, ventilatory equivalent for oxygen; Tab, abdominal temperature; * P < 0.05 compared with WKY; n, number of rats; n = 12 rats/group for V̇Ȯ2 and V̇Ȯ2 measurements.
SHR, a higher $T_{ab}$ was associated with a significantly higher $V_{O2}$ compared with WKY rats (Table 1).

When individual breath timing of rats from protocol 1 was binned by breath F (bins of 25 breaths/min), $T_i$ was less and $T_T$ was greater in SHR compared with WKY rats between breath F of 50 and 125 breaths/min (Fig. 3A). Thus over this range of breath F, $T_i/TOT$ was significantly lower in SHR. In SHR, the shorter $T_i$ and the larger VT resulted in a greater breath VT/$T_i$ for bins of breath F between 50 and 124 breaths/min than in WKY rats (Fig. 4A).

Effects of ANG II-Receptor Block With Saralasin on Respiration and Metabolism During Continuously Quiet Behavior in Protocol 2

In 13 of the SHR and 12 of the WKY rats, continuously quiet data were obtained during both the saline infusion and the saralasin infusion components of protocol 2. After chronic surgical implantation of venous catheters, during saline infusion VT, $V$ (Table 2), and VT/$T_i$ (Fig. 4B) remained significantly greater, and $T_i/TOT$ lower (not shown), in conscious SHR compared with WKY rats, with no differences in F between strains (Table 2). However, after the surgical recovery period from catheter placements, an average tendency for a greater $V_{O2}$ in conscious SHR only approached significance ($P = 0.06$) compared with WKY rats; $T_{ab}$ was no longer statistically different between strains (Table 2).

Between 15 and 60 min of ANG II-receptor block, $V$ decreased in conscious SHR in association with decreased VT (Fig. 5) and VT/$T_i$ (Fig. 4B); there was no change in F. There was also a very small but significant effect of ANG II-receptor block in SHR to increase $T_i$ at some F bins (Fig. 3), which contributed to the decrease in breath VT/$T_i$. There were no effects of ANG II-receptor block on respiration or metabolism in WKY rats (Figs. 4B and 5). The percent decrease of VT in SHR during ANG II-receptor block was significantly different from the lack of effect in WKY rats (Fig. 5). An average tendency for $V_{O2}$ to be decreased in SHR during ANG II-receptor block was not significant (Fig. 5). As well, there was no effect of ANG II-receptor block on $T_{ab}$ in either rat strain.
DISCUSSION

The most important outcome of this study was to demonstrate, for the first time, that ANG II can play a role in modulating respiration in the conscious rat of the SHR strain as well as the conscious dog. Thus it would appear that an ANG II mechanism for respiratory control may be used with varying expression across species. This may be important for human subjects, given the common use of angiotensin converting enzyme inhibitors and angiotensin AT1-receptor blockers in the treatment of patients with hypertension.

Respiration in SHR Compared With WKY Rats

It is apparent from our analysis of gradations of behavior (Fig. 2) that it is important to rigorously document behavioral state for comparisons of respiratory parameters among conscious rat strains. This appears to be especially true for rats with spontaneous or engineered genetic differences. It is of interest that in SHR, behavioral decreases in breath V between intermittently quiet and sleep were attributable to both breath VT and F (Fig. 2A), whereas behavioral differences in breath V in WKY rats were attributable to changes in breath F only (Fig. 2B). Changes in respiratory pattern in sleeping WKY rats are typical of other rat strains, whereas changes in sleeping respiratory patterns of SHR are more typical of those in cats and rats.

Table 2. Respiratory and metabolic data during saline infusions in protocol 2

<table>
<thead>
<tr>
<th>Variables</th>
<th>SHR</th>
<th>WKY</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>13</td>
<td>12</td>
</tr>
<tr>
<td>Wt, g</td>
<td>191±2*</td>
<td>221±3</td>
</tr>
<tr>
<td>V, l·kg⁻¹·min⁻¹</td>
<td>1.28±0.05*</td>
<td>1.01±0.06</td>
</tr>
<tr>
<td>F, breaths/min</td>
<td>89±3</td>
<td>94±5</td>
</tr>
<tr>
<td>VT, ml/kg</td>
<td>14.5±0.5*</td>
<td>10.7±0.3</td>
</tr>
<tr>
<td>V0₂, ml·kg⁻¹·min⁻¹</td>
<td>39.2±2.6</td>
<td>32.0±2.6</td>
</tr>
<tr>
<td>VN0₂, ml·ml⁻¹</td>
<td>34.5±2.0</td>
<td>33.1±2.9</td>
</tr>
<tr>
<td>Tₐ, °C</td>
<td>39.0±0.3</td>
<td>38.4±0.1</td>
</tr>
</tbody>
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Values are means ± SE. *P < 0.05 between SHR and WKY.
who reported no significant difference in average 
agrees with the observations of Hayward et al. (12), 
but V˙ was similar between SHR and WKY and higher 
to be higher in WKY rats than in SHR and Wistar rats, 
chloralose-urethan anesthesia, Grisk et al. (10) found 
experiments in anesthetized rats. With the use of 
ings. 
measured by diaphragmatic electromyogram record-
among conscious SHR, WKY rats, and SD rats, as 
similar among all three strains. The latter finding for 
with WKY rats (Table 1 and 2 and Fig. 2). Both V˙ and 
were found to be greater in conscious SHR compared 
with WKY rats (5), which was not assessed in our studies; 
however, sleeping SHR had higher values for individual breath VT than WKY rats (Fig. 2, A and B), which might predispose to a greater central vagal inhibition of inspiration during sleep. 
Our data supported our hypothesis that an activated brain angiotensin system would be associated with an augmented respiration in conscious SHR. After criteria for continuously quiet behavior were applied, V and VT 
were found to be greater in conscious SHR compared 
with WKY rats (Table 1 and 2 and Fig. 2). Both V and VT in continuously quiet SHR and WKY rats, on a per-weight basis, are significantly higher than the same parameters in conscious SD rats as reported from our laboratory (38), using the same measurement techniques. In contrast, the distribution of breath F is similar among all three strains. The latter finding for F agrees with the observations of Hayward et al. (12), who reported no significant difference in average F among conscious SHR, WKY rats, and SD rats, as measured by diaphragmatic electromyogram recordings. 
The results from conscious rats differ, however, from experiments in anesthetized rats. With the use of chloralose-urethan anesthesia, Grisk et al. (10) found F to be higher in WKY rats than in SHR and Wistar rats, but V was similar between SHR and WKY and higher than in Wistar rats, similar to the observations of Przybiski et al. (26). Thus as we previously reported and discussed, the presence of anesthesia has important effects on respiratory control mechanisms, as measured in the conscious state (36). 

Breath Timing and Drive in SHR Compared With WKY Rats 

As evident from Fig. 3A, breath Ti was less at all but the highest range of breath F in SHR compared with WKY rats; thus breath Ti/TTOT was <0.4 in SHR compared with breath Ti/TTOT >0.4 in WKY rats. On average, Ti/TTOT for SD rats in our laboratory are intermediate to those of SHR and WKY (38). 
Individual breath VT was larger in SHR than in WKY rats (Fig. 2), in combination with the lower breath Ti (Fig. 3A), which resulted in a breath VT/Ti that was almost doubled in SHR compared with WKY rats (Fig. 4A). Boggs and Tenney (3) suggested that “VT/Ti must depend on an interaction between the mechanical properties of the respiratory system and central respiratory drive” and that it was tied to differences in metabolic rate, at least between species. In the present study, the elevated VT/Ti values in SHR were associated with a 40% higher metabolic rate compared with WKY rats (Table 1). 

ANG II Contributes to Respiratory Drive in SHR 

The present experiments support the hypothesis that an ANG II drive to breathe contributes to the higher V, VT, and VT/Ti in conscious SHR. For protocol 2, average V was ~21% higher in SHR compared with WKY rats during saline infusion (Table 2). During ANG II-receptor block, this difference in V was reduced (~9%; Fig. 5) so that about one-half of the increased ventilatory drive in SHR was ablated, primarily due to a decrease in VT and with a smaller and inconsistent decrease in F (Fig. 5). It is of interest that ANG II-receptor block increased Ti at some bins of breath F in SHR (Fig. 3B), which would also contribute to the decrease in VT/Ti (Fig. 4B). 

The present experiments did not definitively determine whether a central (the brain angiotensin system acting at a CVO) or a peripheral chemoreceptor ANG II mechanism played a role in stimulating respiration in conscious SHR. It will be important to compare the effects of intravenous ANG II-receptor block between intact and carotid denervated SHRs and to determine the effects of intracerebroventricular ANG II-receptor block on ventilation in SHRs. As described in the introduction, based on our previous negative experiments in conscious SD rats given intravenous infusions of ANG II and the positive evidence in the literature for an activated brain angiotensin system in SHRs, we speculated that an ANG II mechanism for respiratory control in SHRs would be central. In the present studies, if the brain ANG II system mediated the respiratory effect of ANG II in SHR, it would involve CVOs, because saralasin, administered intravenously, does not cross the BBB (31). 

Central Versus Peripheral Mechanisms for ANG II and Respiratory Control in SHR 

Our finding in conscious SD rats (35, 36) that ANG II did not stimulate respiration via a peripheral mechanism does not necessarily preclude a peripherally mediated ANG II mechanism in a different strain of rats, the SHR. There is evidence for differences in the carotid chemoreceptors in SHRs. Honig et al. (14) reported that the carotid bodies in SHR were enlarged and differed structurally from normotensive rats. Carotid body che-
moreceptor afferent activity is also greater in anesthetized SHR compared with normotensive controls after isocapnic hypoxic stimulation (8, 39). On the other hand, in anesthetized intact SHR, ventilatory responses to hypoxia were found to be similar (15), inconsistent with (9), or less than (26) ventilatory responses in normotensive control rats. In addition, in conscious SHR, stimulation of peripheral chemoreceptors with intravenous potassium cyanide did not evoke a different increase in F from that in WKY rats (12). Thus stimulation of carotid chemoreceptors in the intact animal fails to provide clear evidence for a different peripheral chemoreflex sensitivity in ventilatory response in SHRs; rather, the resting level of respiration is set at a higher level, which could theoretically involve either a central or a peripheral mechanism.

Recent studies continue to support a stimulated brain angiotensin system in the anterior hypothalamus of SHR with increased numbers of ANG II receptors (11) and an enhanced ANG II stimulation of transcription factor expression in a CVO, the subfornical organ, and the hypothalamic nuclei to which it has interconnections (2). There is increased neuronal activity in posterior hypothalamic brain slices from SHR compared with WKY rats (reviewed in Ref. 32). In SHR, ANG II could also act as a neurotransmitter at respiratory centers behind the BBB, including the nucleus of the solitary tract of the medulla (reviewed in Ref. 16), where it would be protected from intravenous infusions of the ANG II-receptor blocker saralasin. It is of interest that GABA administered centrally inhibits physiological responses to ANG II and that GABA is relatively reduced in selected areas of the brain in SHR (reviewed in Ref. 40), which might disinhibit the brain renin-angiotensin system.

Arterial Blood Pressure and Respiration in SHR

The finding that MAP was higher, albeit depressed, in anesthetized SHR compared with WKY rats supported the presumed presence of hypertension in the conscious state in our SHR. Measurements of MAP during anesthesia would not of course reflect absolute differences in MAP in the conscious state, because anesthesia is well known to depress arterial pressure (36). It has recently been demonstrated that the addition of intravenous xylazine to an intravenous ketamine anesthesia in rats can be very depressant to MAP (4). In retrospect, a different anesthetic, not as depressant for circulatory function, such as we previously used (36), would have been preferable for this final arterial pressure measurement when recovery of rats from anesthesia was not required.

The literature on the effects of peripheral and central block of ANG II in SHR on arterial pressure regulation is controversial. This controversy has potential implications as well for peripheral versus central mechanisms for ANG II in respiratory control that need to be resolved. Some reviews favor a major role for a brain renin-angiotensin system as a cause of hypertension in SHRs (for example, see Ref. 40). In some studies, intravenous infusion of saralasin had no effect on arterial pressure in conscious SHRs (for example, see Ref. 20), and in other studies, neither intravenous or intracerebroventricular administration of saralasin affected MAP in conscious SHR (for example, see Ref. 23). In contrast, other investigators (for example, see Ref. 17, in which the AT1-receptor blocker losartan was used) provided evidence that peripheral ANG II, rather than a brain ANG II, is the important causal factor for hypertension in conscious SHR. Differences in rat strains, experimental techniques and designs, blocking agents, and protocols appear to account for variable outcomes observed by different investigators.

The possibility exists that decreases in arterial blood pressure may have occurred in our SHR during intravenous saralasin infusion, which might have evoked a peripheral respiratory baroreceptor reflex (the latter reviewed in Ref. 16). However, if anything, a decreased arterial blood pressure in conscious SHR should induce a stimulation via the baroreceptor reflex, rather than a depression, of respiration (16). Such a respiratory baroreceptor reflex would, therefore, oppose the significant decrease in V observed in our SHR during ANG II-receptor block and could not account for the observed decreased respiration in SHR during intravenous saralasin infusion. As well, our studies of respiratory baroreflexes in conscious SD rats indicated that in rats, decreased arterial pressure has only a transient stimulatory effect on V, which disappears within <10 min (36). Thus measurements of respiration after 15 or more minutes of saralasin infusion in SHR in the present studies were unlikely to be affected by respiratory baroreceptor reflexes.

Relationship of Increased Respiratory Drive to Metabolism in SHR

Metabolism was higher in association with an elevated $\dot{V}_{O2}$ in SHR, compared with WKY rats, as indicated by the significantly higher $\dot{V}_{O2}$ in SHR before the surgical installation of catheters (Table 1). This difference between SHR and WKY rats has been documented by other workers (for example, see Ref. 6). The gradual progressive decrease of $\dot{V}_{O2}$ in WKY rats in the chamber, after the beginning of protocol 1 (Fig. 1), is typical of the temperature changes in conscious cats, dogs, and SD rats brought to our laboratory and maintained in a subsequent quiet state for an experiment. The maintained $\dot{V}_{O2}$ of SHR presumably reflects their relatively higher metabolic state, even when continuously quiet.

In SHR, the ratio of V to $\dot{V}_{O2}$ was not different from that of WKY rats (Table 1), which could be interpreted as indicating that the higher respiration in SHR was simply attributable to the higher metabolic rate (reviewed in Ref. 21). Alternatively, it might indicate that the increased $\dot{V}_{O2}$ in SHR was secondary to the increased V and an increased work of breathing.

ANG II, infused intravenously, increases $\dot{V}_{O2}$ in conscious dogs, but the associated ANG II-induced increase in respiration is, at least in part, independent of metabolism, as indicated by an increased alveolar ventilation and a decreased $P_{A_CO2}$ (22). After surgical implantation of venous catheters in the present experi-
ments, average V\textsubscript{O2} was not significantly different between SHR and WKY rats (Table 2), even though the absolute value of V\textsubscript{O2} was ~30% greater than that in WKY rats. As well, ANG II-receptor block with saralasin did not cause a “significant” decrease in V\textsubscript{O2} (P = 0.06). Thus a depressant effect of ANG II-receptor block on respiration in SHR was not definitively indirectly related to decreases in metabolic rate.

Perspectives

It is evident from the response differences between strains of conscious rats (SHR, WKY, and SD) and differences between conscious rats (SD) and dogs in our laboratory that an ANG II mechanism for respiratory control is subject to other modulating factors. AVP, by inhibiting the angiotensin system, modulates respiration in dogs (33, 34). Up- and downregulation of ANG II receptors, secondary to differences in NaCl dietary intake, also appear to be important for the effects of ANG II on respiratory control in the dog (Table 1 in Ref. 16). Thus genetics, expression of receptors, and physiological state of animals appear to be critical for the expression of an ANG II drive to breathe.

In conscious SHR, it will be important to determine the effects of intracerebroventricular administration of ANG II agonists and antagonists on respiration and metabolism and the effects of carotid sinus nerve denervation on respiration and the respiratory responses to ANG II-receptor block. A more comprehensive understanding of the modulatory factors affecting the ANG II drive to breathe among species may lead to a better diagnostic understanding and hence therapeutic strategy for patients with abnormalities of respiratory control.

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