Angiotensin II modulates respiratory and acid-base responses to prolonged hypoxia in conscious dogs

STEVEN J. HEITMAN AND DONALD B. JENNINGS
Department of Physiology, Queen's University, Kingston, Ontario, Canada K7L 3N6

Heitman, Steven J., and Donald B. Jennings. Angiotensin II modulates respiratory and acid-base responses to prolonged hypoxia in conscious dogs. Am. J. Physiol. 275 (Regulatory Integrative Comp. Physiol. 44): R390–R399, 1998.—We tested the hypothesis that angiotensin II (ANG II) contributes to ventilatory and acid-base adaptations during 3–4 h of hypoxia (partial pressure of O₂ in arterial blood = 43 Torr) in the conscious dog. Three protocols were carried out over 3–4 h in five dogs: 1) air control, 2) 12% O₂ breathing, and 3) 12% O₂ breathing with ANG II receptors blocked by infusion of saralasin (0.5 µg·kg⁻¹·min⁻¹). After 2 h of hypoxia, expired ventilation and alveolar ventilation progressively increased, and the partial pressure of CO₂ in arterial blood decreased. When the hypoxic chemoreceptor drive to breathe was abolished transiently for 30 s with 100% O₂, the resultant central apneic time decreased between 0.5 and 2.5 h of hypoxia. All these adaptive responses to hypoxia were abolished by ANG II receptor block. Because plasma ANG II levels were lower during hypoxia and hypoxic release of arginine vasopressin from the pituitary into the plasma was prevented by ANG II receptor block, the brain renin-angiotensin system was likely involved. It is possible that ANG II mediates ventilatory and acid-base adaptive responses to prolonged hypoxia via alterations in ion transport to decrease [SID] in brain extracellular fluid rather than acting by a direct neural mechanism.

adaptation to hypoxia; brain angiotensin II; angiotensin receptor block; arginine vasopressin; strong ion difference

During normoxia in the dog, angiotensin II (ANG II) stimulates respiration when infused intravenously if baroreceptor reflexes are accounted for (22, 26). However, any potential tonic stimulation of ventilation by ANG II levels in conscious dogs during normoxia (36) or normoxic hypercapnia (37) is eliminated by the inhibitory effects of arginine vasopressin (AVP). Thus there is no evidence for ventilatory depression after ANG II receptor block in resting dogs during normoxia (23, 24). In contrast, endogenous stimulation of the renin-angiotensin system (RAS) during normoxia by a modest acute hypotension (23) or by AVP V₁ receptor block (36, 37) results in an ANG II-mediated increase in ventilation. Because the RAS in conscious dogs is also stimulated by severe hypoxia (19), one might anticipate an ANG II drive to breathe during acute hypoxia. Despite this, we found that ANG II does not contribute to ventilatory drive within the first hour of moderate hypoxia in conscious dogs (24).

The “pathway” involved in the ANG II drive to breathe in dogs is central (26). Respiratory stimulation after AVP V₁ receptor block in normoxic dogs appears to involve disinhibition of a brain ANG II system (reviewed in Refs. 18 and 28) because plasma ANG II is unaltered (36). Central mechanisms through which ANG II may affect respiratory control include circumventricular organs (CVOs) of the brain. CVOs lack a blood-brain barrier (BBB) and are stimulated by ANG II in either the blood or brain fluids (reviewed in Refs. 18 and 28).

Respiratory adaptation to “short-term” hypoxia (1 h to months (9)] is characterized by a progressive secondary increase in alveolar ventilation (V̇A) in association with compensatory “metabolic” acid-base changes in the central nervous system; the time course and magnitude of this response vary among species (reviewed in Ref. 9). In human subjects with hypoxic hyperventilation, a transient hypoxic ventilatory decline within the first minutes to an hour (27) is followed by a subsequent secondary increase in hypoxic hyperventilation with the central acid-base compensation largely complete within 1–2 days (29). In the conscious dog, there is no evidence for an early hypoxic ventilatory decline (reviewed in Ref. 24) and the secondary respiratory stimulation during prolonged moderate hypoxia is complete within 3–6 h (3).

The central and peripheral mechanisms that contribute to the respiratory adaptation to prolonged hypoxia remain unclear (1, 9), and species variability occurs (14). Most recently, emphasis has been placed on the importance of peripheral chemoreceptors in mediating the secondary hypoxic respiratory adaptation (1, 14), including those in the dog (3). Previously, Severynhaus et al. (29) proposed that the secondary increase in ventilation during hypoxia in human subjects occurs in association with an “acidification” of the cerebrospinal fluid (CSF) and H⁺ stimulation of central chemoreceptors. However, several subsequent studies failed to provide evidence that H⁺ concentration ([H⁺]CSF) increases in the extracellular environment of the central chemoreceptors (3, 9) or within brain tissue (9, 12) to account for hypoxic ventilatory acclimatization.

During hypoxic respiratory adaptation, a decrease in CSF HCO₃⁻ concentration ([HCO₃⁻]CSF) is associated with an increase in the CSF concentration of the strong anions Cl⁻ and lactate (11, 29), which decreases the difference between the concentrations of strong cations and strong anions (strong ion difference, [SID]) (34). Decreases in [SID] of the CSF ([SID]CSF), which oppose alkalosis (34), are correlated with and predict increases in ventilation and decrease in the partial pressure of CO₂ in arterial blood (Paco₂) during different adaptive states, including chronic hypoxia (reviewed in Refs. 16 and 17). ANG II is known to affect strong ion transports, which could affect the [SID] and, thus, potentially, acid-base balance and respiratory control during hypoxia. ANG II also stimulates metabolic rate in the conscious dog (22), which is an important factor for...
interpreting ventilatory adaptations to hypoxia (reviewed in Ref. 9).

The hypothesis for the present study was that ANG II plays a role in the secondary hypoxic respiratory adaptation, either directly via neural mechanisms or indirectly via ion transport mechanisms and acid-base balance. Conscious dogs were studied over 3–4 h of hypoxia with and without ANG II receptor block. Hypoxic respiration was examined in relation to arterial acid-base balance, plasma strong ions, metabolism, and hormonal responses. The contribution of the direct hypoxic chemoreceptor stimulus on ventilatory drive was tested with a modified Dejours O₂ test (8). Our data indicate that ANG II not only mediates the secondary respiratory adaptation at 3 h of hypoxia in the dog but also contributes to the acid-base compensation.

METHODS

An animal preparation

Experiments were performed on five male mongrel dogs weighing 23.1–38.1 kg (average 28.9 ± 2.5 kg); the dogs were among those used previously for studies of ANG II receptor block during the first hour of hypoxia (24). Each dog was surgically prepared with a chronic tracheostoma and an exteriorized carotid artery. The dogs were trained and acclimatized to an ambient temperature of 20°C and a photoperiod between 0600 and 2100. All experimental procedures conformed to guidelines of the Canadian Council on Animal Care and were approved by the Queen's University Animal Care Committee.

For experiments, the exteriorized carotid artery and the right external jugular vein were catheterized under local lidocaine anesthesia (1% xylocaine). In addition, an endotracheal tube with inflatable cuff was placed in the tracheostoma and connected to a breathing circuit via a two-way breathing valve (model 1400; Hans Rudolph, Kansas City, MO).

For ANG II receptor block, the specific competitive antagonist saralasin ([Sar¹,Val⁵,Ala⁸]ANG II; Sigma, St. Louis, MO) was infused intravenously at a rate of 0.2 ml/min to administer a dose of 0.5 μg·kg⁻¹·min⁻¹ (use of this blocker is reviewed in Ref. 23). Completeness of ANG II receptor block was confirmed at the conclusion of each experiment by demonstrating that a 500-ng bolus injection of ANG II did not exert normal agonist effects on mean arterial pressure (MAP). When the ANG II receptors were not blocked, this dose of ANG II caused average MAP to increase 13.4 ± 1.9 mmHg, which compared with 0.3 ± 2.1 mmHg during saralasin infusion (P < 0.05).

Protocols

When the dogs were in a quiet steady state for 10–15 min, time 0 was established and one of three protocols was initiated. Each experiment lasted 3–4 h, during which the dogs breathed either room air or 12% O₂. Saline was infused intravenously at a rate of 0.2 ml/min with or without saralasin. Cardiorespiratory, arterial blood gas, electrolyte, osmolality, and hormone determinations were obtained at hourly intervals.

Protocol 1: Air control study. For determination of the respiratory, metabolic, and acid-base responses to 4 h of constraint in the Pavlov sling, the dogs were studied while breathing air during an intravenous infusion of saline.

Protocol 2: Unblocked hypoxia study. For determination of the respiratory, metabolic, and acid-base responses to 4 h of hypoxia, the dogs breathed 12% O₂ during an intravenous infusion of saline.

Protocol 3: Hypoxia, ANG II receptor block study. For determination of the effect of ANG II receptor block on the respiratory, metabolic, and acid-base responses to hypoxia, the dogs breathed 12% O₂ for 3–4 h during an intravenous infusion of the ANG II receptor blocker saralasin.

Cardiovascular, Respiratory, and Metabolic Measurements

MAP and heart rate (HR) were monitored with a pressure transducer (P23 Db, Statham). A 120-ml Tissot spirometer was used for the collection of expired gases for the calculation of expired minute ventilation (Ve) at body temperature, ambient pressure, saturated with water vapor. Ve was calculated by using the Bohr equation for dead space volume. O₂ consumption (VO₂), CO₂ production (VCO₂), and the respiratory exchange ratio (R) were calculated from Ve (expressed at standard temperature and pressure, dry) and the CO₂ and O₂ concentrations of the inspired and expired gases as previously described (22). Rectal temperature (Ṫrect) was continuously monitored with a rectal thermistor probe (no. 401, Yellow Springs Instruments, Yellow Springs, OH).

Modifications of the Dejours O₂ Test

During an interval of quiet breathing, the inspiratory circuit was switched to 100% O₂ for a 30-s period [a modified Dejours O₂ test (8)]; subsequently, the inspiratory circuit was switched back to 12% O₂. Inspiratory and expiratory flows were recorded using pneumotachographs (no. 1, Fleisch) and Validyne amplifiers (model CD15 carrier demodulator) connected to the computer data acquisition package CODAS (Dataq Instruments, Akron, OH). Apneas after 100% O₂ inhalation were defined, in conformity with other workers (5), as periods in which breathing ceased for a duration exceeding two normal breath cycles. Multiple apneas during and after each 30-s Dejours O₂ test were summed to give the total time spent in apnea.

Blood Gas and pH Measurements

Partial pressure of O₂ in arterial blood (PaO₂), PaCO₂, and arterial pH (pHₐ), converted to arterial H+ concentration ([H⁺]ₐ) were measured in anaerobic arterial blood samples with a Radiometer BMS3 MK2 blood gas analyzer. The gas calibrations of the analyzer were corrected each day by tonometry of the dog's blood. Blood gas measurements were corrected to Ṫrect, the time of sampling, and PaCO₂ was further corrected for the dilutional effect of heparin. Arterial HCO₃⁻ concentration ([HCO₃⁻]ₐ) was calculated from PaCO₂ and pHₐ using a dissociation constant and a solubility coefficient corrected for temperature and pHₐ.

Plasma Measurements

Plasma was separated anaerobically from arterial blood containing lithium heparin (Safely-Monovette, Sarstedt, Ville St. Laurent, Quebec, Canada). The blood was centrifuged at 4°C (400 g for 20 min). Plasma osmolality was measured by the freezing-point depression technique (model 4002, Os- mette S automatic osmometer; Precision Systems, Sudbury, MA). Arterial plasma was also stored at −70°C for electrolyte measurements. Arterial plasma concentrations of Na⁺ ([Na⁺]ₐ), K⁺ ([K⁺]ₐ), and Cl⁻ ([Cl⁻]ₐ) were determined with ion-specific electrodes (Baxter Paramax Autoanalyzer).Arterial concentrations of lactate ([lactate]ₐ) and glucose were...
determined by oxidation with L-lactate oxidase or glucose oxidase, respectively (model 2000 analyzer; Yellow Springs Instruments, Yellow Springs, NJ). Plasma protein concentration ([protein]) was measured with a serum protein refractometer (Atago no. 2730) and corrected to measurements using the biuret method (36).

\[
[protei_{biuret}(g/l) = 0.71[protein]_{refractometer}(g/l) + 15.21]
\]

To analyze acid-base using the physicochemical approach (34), the arterial plasma [SID] ([SID]a) was calculated as ([Na+][a] + [K+][a] - ([lactate][a] + [Cl-][a]). The total unidentified weak acid concentration in arterial plasma ([AT]a) was calculated as the arterial anionic weak acid concentration plus the arterial weak acid concentration (see Ref. 24).

For hormone measurements, arterial blood was collected in chilled Vacutainers (Becton Dickinson, Mississauga, Ontario, Canada) containing aprotinin (Trasylof, 200 KIU), EDTA (0.1 ml of a 15% solution), and potassium sorbate (0.016 mg). Samples were immediately centrifuged at 4°C (400 g for 20 min), and the plasma was stored at -70°C until assayed.

Plasma ANG II and AVP were measured with radioimmunoassay kits using 125I-labeled ANG II and AVP, respectively (Nichols Institute Diagnostics, San Juan Capistrano, CA); for each assay, all samples were analyzed together. The reported ANG II and AVP values were corrected for recovery during each assay, all samples were analyzed together. The reported percentage recovery was 69% for ANG II and AVP values were corrected for recovery during each assay, all samples were analyzed together.

The percentage recovery was 69% for ANG II and 63% for AVP, which compared well with that of the extraction procedure. The percentage recovery was 69% for ANG II and 63% for AVP, which compared well with that of the extraction procedure. The percentage recovery was 69% for ANG II and 63% for AVP, which compared well with that of the extraction procedure.

**Statistical Analysis**

All statistical analyses were carried out with the computer software package SYSTAT 5.0 for Windows. Unless otherwise indicated, values are presented as averages ± SE. Repeated measures for each of the five dogs were collected for up to 3 h in each protocol. However, during the hypoxia ANG II receptor block protocol, steady-state measurements were not obtained from two of the five dogs at 4 h. As such, for hours 1, 2, and 3, differences within and between protocols were determined using a multivariate ANOVA for repeated measures. A contrast matrix was designed to identify the differences between the unblocked hypoxia and the ANG II receptor block protocol. Differences in ANG II and AVP values were corrected for recovery during each assay, all samples were analyzed together. The reported percentage recovery was 69% for ANG II and 63% for AVP, which compared well with that of the extraction procedure. The percentage recovery was 69% for ANG II and 63% for AVP, which compared well with that of the extraction procedure. The percentage recovery was 69% for ANG II and 63% for AVP, which compared well with that of the extraction procedure.

During the unblocked hypoxia protocol, ANG II values were determined by oxidation with L-lactate oxidase or glucose oxidase, respectively (model 2000 analyzer; Yellow Springs Instruments, Yellow Springs, NJ). Plasma protein concentration ([protein]) was measured with a serum protein refractometer (Atago no. 2730) and corrected to measurements using the biuret method (36).

**RESULTS**

**PaO₂ During Protocols**

Average PaO₂ ranged from 87 to 88 Torr during the air control study (Fig. 1A). During the hypoxia protocols, average PaO₂ decreased to 43 Torr at 1 h (Fig. 1A). Average PaO₂ increased significantly at 2 and 3 h of hypoxia in the unblocked protocol compared with the 1-h level (45 ± 2 and 47 ± 2 Torr, respectively, vs. 43 ± 2 Torr; P < 0.05). There was no change in PaO₂ between 1 and 3 h when ANG II receptors were blocked during hypoxia.

**Effects of ANG II Receptor Block on Ve, f, and VT**

During the unblocked hypoxia protocol, Ve increased compared with air control (Table 1) and increased progressively by 47% between 1 and 4 h; Ve at both 3 and 4 h was significantly different from 1 h (Table 1). In contrast, although Ve at 1 h of hypoxia with ANG II receptor block was comparable to that in the unblocked protocol, there were no further changes in Ve over 3 h. In addition, when ANG II receptors were blocked during hypoxia, Ve did not differ significantly from the air control protocol at any time period. During the air control protocol, variability in Ve over 4 h was not different from 1-h measurements, although respiratory frequency (f) increased.

Increases in Ve during the unblocked hypoxia protocol were associated with decreases in tidal volume (VT) and increases in f by the fourth hour. There were no significant changes in Ve or VT over 3 h of hypoxia in the ANG II receptor block protocol; f and VT did not differ statistically from either the air control or unblocked hypoxia protocols.
Effects of ANG II Receptor Block on Gas Exchange and Metabolism During Hypoxia

Between 1 and 3 h, average \( \dot{V}_A \) increased progressively by 46% in the unblocked hypoxia protocol and was greater than air control at all time periods (Fig. 1B). At 3 h, \( \dot{V}_A \) in the unblocked hypoxia protocol was significantly greater than \( \dot{V}_A \) in the hypoxia ANG II receptor block protocol (Fig. 1B). In contrast, when ANG II receptors were blocked during hypoxia, \( \dot{V}_A \) was not significantly different from air control and \( \dot{V}_A \) did not change with time.

There was a significant increase in \( \dot{V}_{O_2} \) (and \( \dot{V}_{CO_2} \), not shown) between 1 and 3 h in the unblocked hypoxia protocol compared with the other two protocols (Fig. 1C). Stimulation of \( \dot{V}_A \) during the unblocked hypoxia protocol occurred independently of metabolism based on a significant increase in the ventilatory equivalent for \( O_2 \) between 1 and 3 h in the unblocked hypoxia protocol (not shown) and the progressive decrease in \( P_aO_2 \) (Fig. 2A). Neither of these changes occurred when ANG II receptors were blocked during hypoxia. There were no significant differences in the average R (range from 79 to 92%) within and among the three protocols (not shown). Average glucose levels did not differ between protocols (Table 2) or change during the protocols (not shown).

Effects of ANG II Receptor Block on Response to Dejours O2 Test

Figure 3 illustrates responses to the Dejours O2 test for all dogs (Fig. 4) is consistent with the above observations for one dog, shown in Fig. 3. At 0.5 h of both hypoxia protocols, average central apneic duration lasted 40–43 s, much longer than during the air control protocol (Fig. 4). However, during the unblocked hypoxia protocol, average apneic duration decreased progressively with time such that by 2.5 h there was no significant difference in apneic duration between the unblocked hypoxia and air control protocols (Fig. 4). Thus, after removal of the hypoxia stimulus to breathe. In contrast, when ANG II receptors were blocked during hypoxia, the duration of apneic periods did not change between 0.5 and 2.5 h and there was no enhancement of respiratory rhythm generation with time.

A quantitative analysis of apneic time after the Dejours \( O_2 \) test for all dogs (Fig. 4) is consistent with the above observations for one dog, shown in Fig. 3. At 0.5 h of both hypoxia protocols, average central apneic duration lasted 40–43 s, much longer than during the air control protocol (Fig. 4). However, during the unblocked hypoxia protocol, average apneic duration decreased progressively with time such that by 2.5 h there was no significant difference in apneic duration between the unblocked hypoxia and air control protocols (Fig. 4). Thus, central ventilatory drive, unrelated to an hypoxic stimulus, increased during prolonged hypoxia. This increased "central" ventilatory drive involved ANG II because a progressive decrease in apneic duration was not observed during hypoxia when ANG II receptors were blocked. At 2.5 h, apneic duration was significantly greater in the hypoxia ANG II receptor block protocol compared with the unblocked hypoxia protocol.

Table 1. Ventilatory and cardiovascular measurements

<table>
<thead>
<tr>
<th>Variable and Protocol</th>
<th>1 h</th>
<th>2 h</th>
<th>3 h</th>
<th>4 h</th>
</tr>
</thead>
<tbody>
<tr>
<td>( \dot{V}_E ), l·kg (^{-1} )·min (^{-1} )</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C</td>
<td>0.20 ± 0.02</td>
<td>0.23 ± 0.03</td>
<td>0.27 ± 0.05</td>
<td>0.28 ± 0.05</td>
</tr>
<tr>
<td>H</td>
<td>0.27 ± 0.03 (^{†} )</td>
<td>0.32 ± 0.04 (^{†} )</td>
<td>0.38 ± 0.05 (^{*} )</td>
<td>0.42 ± 0.07 (^{†} )</td>
</tr>
<tr>
<td>H/B</td>
<td>0.27 ± 0.04</td>
<td>0.29 ± 0.04</td>
<td>0.29 ± 0.05</td>
<td>0.29 ± 0.05</td>
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<tr>
<td>f, breaths/min</td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>C</td>
<td>176 ± 2.8</td>
<td>223 ± 4.4</td>
<td>271 ± 7.1</td>
<td>30.2 ± 7.0 (^{*} )</td>
</tr>
<tr>
<td>H</td>
<td>240 ± 1.9</td>
<td>321 ± 5.2</td>
<td>361 ± 6.7</td>
<td>54.1 ± 11.1 (^{†} )</td>
</tr>
<tr>
<td>H/B</td>
<td>306 ± 6.3</td>
<td>310 ± 5.1</td>
<td>306 ± 4.5</td>
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</tr>
<tr>
<td>( \dot{V}_T ), ml·kg (^{-1} )·min (^{-1} )</td>
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<td></td>
<td></td>
<td></td>
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<tr>
<td>C</td>
<td>12.3 ± 1.9</td>
<td>11.7 ± 2.0</td>
<td>11.5 ± 1.8</td>
<td>10.3 ± 1.9</td>
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<tr>
<td>H</td>
<td>11.8 ± 0.6</td>
<td>11.2 ± 0.7</td>
<td>11.4 ± 1.2</td>
<td>8.2 ± 0.7 (^* )</td>
</tr>
<tr>
<td>H/B</td>
<td>9.4 ± 0.6</td>
<td>9.8 ± 1.1</td>
<td>9.4 ± 0.7</td>
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<tr>
<td>MAP, mmHg</td>
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<td></td>
</tr>
<tr>
<td>C</td>
<td>110.3 ± 2.5</td>
<td>113.1 ± 2.5</td>
<td>113.1 ± 3.6</td>
<td>112.1 ± 3.4</td>
</tr>
<tr>
<td>H</td>
<td>110.2 ± 5.9</td>
<td>114.8 ± 5.8</td>
<td>114.0 ± 7.4</td>
<td>110.3 ± 6.2</td>
</tr>
<tr>
<td>H/B</td>
<td>113.4 ± 5.2</td>
<td>114.0 ± 5.6</td>
<td>114.9 ± 4.7</td>
<td></td>
</tr>
<tr>
<td>HR, beats/min</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>C</td>
<td>76.1 ± 8.4</td>
<td>76.2 ± 8.3</td>
<td>75.8 ± 7.1</td>
<td>79.4 ± 7.3</td>
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<tr>
<td>H</td>
<td>88.8 ± 6.6</td>
<td>93.3 ± 6.5 (^{†} )</td>
<td>96.3 ± 5.3 (^{†} )</td>
<td>99.4 ± 7.7 (^{†} )</td>
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<tr>
<td>H/B</td>
<td>91.3 ± 10.0</td>
<td>94.7 ± 10.1</td>
<td>97.6 ± 5.9 (^{†} )</td>
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</tbody>
</table>

Values are means ± SE. C, control protocol; H, unblocked hypoxia protocol; H/B, hypoxia with ANG II receptor block protocol; \( \dot{V}_E \), expired minute ventilation; f, respiratory frequency; \( \dot{V}_T \), tidal volume; MAP, mean arterial pressure; HR, heart rate. \(^{*} \) \( P < 0.05 \) compared with 1 h, same protocol; \(^{†} \) \( P < 0.05 \) compared with air control, same time period.
Effects of ANG II Receptor Block on Acid-Base Balance During Hypoxia

\( \text{PaCO}_2, [H^+]_a, [HCO_3^-]_a, \) and \([\text{SID}]_a\) did not change significantly during the air control protocol (Fig. 2). At 1 h in both hypoxic protocols, \( \text{PaCO}_2, [H^+]_a, \) and \([HCO_3^-]_a\) decreased relative to the air control protocol \((P < 0.05)\), but \([\text{SID}]_a\) remained unchanged (Table 2). By 3 h of the unblocked hypoxia protocol, \( \text{PaCO}_2 \) decreased from both the 1- and 2-h levels (Fig. 2A) in association with a decrease in \([HCO_3^-]_a\) (Fig. 2C). However, a progressive decrease in the dependent variable \([H^+]_a\) was prevented in the unblocked hypoxia protocol (Fig. 2B) by a gradual decrease in the independent variable \([\text{SID}]_a\) (Fig. 2D). In contrast, with ANG II receptor block during the hypoxia there were no progressive decreases in \( \text{PaCO}_2 \) or \([HCO_3^-]_a\) at 3 h from 2 h, and there was no change in \([\text{SID}]_a\) between 1 and 3 h (Fig. 2).

\([Na^+]_a\) and \([K^+]_a\) (Table 2) remained constant over time within each protocol. The significant decrease in \([\text{SID}]_a\) observed during the unblocked hypoxia study occurred via small and nonsignificant increases in the strong anions \([\text{Cl}^-]_a\) and \([\text{lactate}^-]_a\) (not shown). Average arterial protein concentration, \([\text{AT}]_a\), and osmolality were not significantly different among protocols and did not change over 3–4 h from their 1-h levels.

Effects of ANG II Receptor Block on Circulation During Hypoxia

There were no changes in MAP within protocols or differences among the three protocols (Table 1). With time, in both hypoxia protocols, HR became significantly greater than during air control; there were no time-related changes in HR within protocols (Table 1). There was also no effect of ANG II receptor block on HR during hypoxia.

Effects of Hypoxia on Plasma Levels of ANG II and AVP

During unblocked hypoxia, average plasma levels of ANG II in dogs were lower compared with air control levels \((P < 0.05)\) and, in particular, at 1 and 2 h (Fig. 5A). There were no time-related changes in plasma ANG II in either the air control or unblocked hypoxia protocols (Fig. 5).

Plasma levels of AVP were obtained in four of the five dogs studied; the plasma from one dog had a nonspecific cross-reaction which resulted in abnormal readings. Relative to plasma osmolality, AVP in the unblocked hypoxia protocol was higher than during the air control protocol (Fig. 5B). For a comparable range in osmolality, AVP during the ANG II receptor block hypoxia protocol was lower than in the unblocked

Table 2. Measurements at 1 h

<table>
<thead>
<tr>
<th></th>
<th>Air Control</th>
<th>Unblocked Hypoxia</th>
<th>Hypoxia ANG II Receptor Block</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blood gases and acid-base</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>( \text{PaCO}_2 )_a, Torr</td>
<td>36 ± 2</td>
<td>26 ± 1*</td>
<td>26 ± 1*</td>
</tr>
<tr>
<td>([\text{SID}]_a)_a, meq/l</td>
<td>35 ± 0</td>
<td>34 ± 1</td>
<td>36 ± 1</td>
</tr>
<tr>
<td>([\text{AT}]_a)_a, meq/l</td>
<td>16 ± 1</td>
<td>16 ± 1</td>
<td>19 ± 2</td>
</tr>
<tr>
<td>([HCO_3^-]_a)_a, meq/l</td>
<td>20.4 ± 0.6</td>
<td>17.4 ± 1.4*</td>
<td>18.2 ± 1.5*</td>
</tr>
<tr>
<td>([H^+]_a)_a, meq/l</td>
<td>43.1 ± 1.3</td>
<td>36.9 ± 1.0*</td>
<td>35.7 ± 1.0*</td>
</tr>
<tr>
<td>Electrolytes and glucose</td>
<td></td>
<td></td>
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<tr>
<td>([Na^+]_a)_a, meq/l</td>
<td>148 ± 0</td>
<td>146 ± 2</td>
<td>147 ± 1</td>
</tr>
<tr>
<td>([K^+]_a)_a, meq/l</td>
<td>3.9 ± 0.2</td>
<td>3.7 ± 0.1</td>
<td>3.6 ± 0.1</td>
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<td>([Cl^-]_a)_a, meq/l</td>
<td>115 ± 1.1</td>
<td>114 ± 1</td>
<td>113 ± 1</td>
</tr>
<tr>
<td>([\text{lactate}^-]_a)_a, meq/l</td>
<td>1.6 ± 0.2</td>
<td>1.8 ± 0.4</td>
<td>2.1 ± 0.5</td>
</tr>
<tr>
<td>([\text{glucose}]_a)_a, mmol/l</td>
<td>7.4 ± 0.6</td>
<td>7.1 ± 0.3</td>
<td>6.8 ± 0.2</td>
</tr>
<tr>
<td>Osmolality, hematocrit, and (T_r)</td>
<td></td>
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</tr>
<tr>
<td>Osmolality, mosmol/kg</td>
<td>302 ± 2</td>
<td>300 ± 2</td>
<td>299 ± 1</td>
</tr>
<tr>
<td>Hematocrit, %</td>
<td>42 ± 2</td>
<td>43 ± 2</td>
<td>43 ± 2</td>
</tr>
<tr>
<td>(T_r), °C</td>
<td>38.4 ± 0.2</td>
<td>38.1 ± 0.2</td>
<td>38.0 ± 0.1</td>
</tr>
</tbody>
</table>

Values are means ± SE. \( \text{PaCO}_2 \), arterial partial pressure of CO2; \([\text{SID}]_a\), difference between arterial concentrations of strong cations and strong anions; \([\text{AT}]_a\), arterial total weak acid concentration; \([HCO_3^-]_a\), arterial HCO3^- concentration; \([H^+]_a\), arterial H+ concentration; \([Na^+]_a\), arterial Na+ concentration; \([K^+]_a\), arterial K+ concentration; \([\text{Cl}^-]_a\), arterial Cl- concentration; \([\text{lactate}^-]_a\), arterial lactate ion concentration; \([\text{glucose}]_a\), arterial glucose concentration; \(T_r\), rectal temperature. *Different from air control, \(P < 0.05\).
hypoxia protocol (P < 0.05) and not different from the air control protocol (Fig. 5C).

DISCUSSION

ANG II Mediates Secondary Hypoxic Respiratory Adaptation in Dog

\( \dot{V}E \) increased progressively (47%) between 1 and 4 h of hypoxia in our dogs (Table 1). Others have shown that with a comparable degree of hypoxia in a hypobaric chamber (\( \text{PaO}_2 = 47-50 \) Torr), the secondary increase in ventilation and decrease in \( \text{PaCO}_2 \) is complete by 6 h in conscious dogs (3). The lack of hypoxic ventilatory decline in the dog makes this species similar to the goat (10) and unlike other animal models, including hypoxic human subjects (15), in which \( \dot{V}E \) becomes transiently depressed below the initial hypoxic hyperventilation.

As demonstrated previously (24), ANG II does not contribute to respiratory control in the dog during the first hour of hypoxia. In contrast, ANG II does contribute to the progressive increase in \( \dot{V}E \) and \( V_A \) after 2 h of hypoxia (Table 1 and Fig. 1). The progressive respiratory stimulation by ANG II in unblocked hypoxic dogs was due to increases in \( f \), and this was abolished by ANG II receptor block (Table 1). Stimulation of \( \dot{V}E \) by ANG II has always been associated with increased \( f \) in our conscious dogs (22, 36). However, an increased \( f \) during hypoxia differs from studies in which a constraining mask was placed around dogs’ muzzles for respiratory measurements; with a mask, hypoxic \( \dot{V}E \) increases via increased VT, not \( f \) (3).

Respiration in the dog is sensitive to relatively small changes in MAP (18, 23). Although ANG II can contribute to an increased MAP during severe hypoxia (\( \text{PaO}_2 \leq 31 \) Torr) (19), MAP did not increase during the more moderate hypoxia of the present studies. In contrast, HR increased during hypoxia in the present study, but an ANG II mechanism, blocked by saralasin, was not involved (Table 1).

The constancy of MAP and HR (Table 1) as well as R (see RESULTS) attests to the relative steady state of dogs when measurements were obtained. Our inability to obtain steady-state measurements at 4 h from two of the five dogs when ANG II receptors were blocked may have been related to an intolerance to prolonged hypoxia in the absence of an ANG II-mediated adaptive mechanism. However, respiratory, metabolic, blood gas, and circulatory measurements in the other three dogs at 4 h during hypoxia with ANG II receptor block were similar to the 3-h values with no indication of hypoxic intolerance. The lack of respiratory adaptation during
the hypoxic ANG II receptor block protocol was not related to a nonspecific effect of saralasin infusion. In five dogs (4 from this study), gas exchange, acid-base balance, and apneic time during a saralasin infusion over 4 h of air breathing were comparable to measurements during unblocked air control studies (data not shown).

ANG II Mechanism for Hypoxic Respiratory Adaptation is Central

When the hypoxic stimulus to breathe was removed at 0.5 h of hypoxia by inhalation of 100% O₂ (modified Dejours O₂ test), dogs had prolonged apneas whether or not ANG II receptors were blocked (Figs. 3 and 4). At this time period, the dogs were acutely hypocapnic and alkalotic and maintenance of breath initiation at the central respiratory rhythm generator appears to be highly dependent on hypoxic chemoreceptor input. With time, during apnea, secondary increases in PCO₂ and [H⁺] and a progressive decrease in PO₂ (plus other possible unknowns) ultimately reach a threshold level which triggers renewed respiratory efforts (8).

During the first seconds of 100% O₂ breathing, it is assumed that "hypoxic chemoreceptor drive" is suppressed (8) and the contribution of peripheral chemoreceptors to respiratory drive is "silenced" (reviewed in Ref. 33). However, neurons in the posterior hypothalamus that modulate respiration are also stimulated by hypoxia (reviewed in Ref. 31). It is possible that 100% O₂ breathing during hypoxia suppresses more than peripheral chemoreceptor activity in some species. In awake goats whose one intact carotid body is normoxic ("isolated brain normocapnic hypoxia"), respiration is still stimulated by hypoxia (32). However, hypoxia does not stimulate respiration in human subjects and other species after bilateral carotid body resection (14).

By 2.5 h of hypoxia in unblocked dogs, there was a dramatic reduction in apneic time during the Dejours O₂ test to within normoxic limits (Fig. 4) despite a progressively lower initial PaCO₂ (Fig. 2). The decreased apneic time after the inhalation of 100% O₂ at 2.5 h of hypoxia in unblocked dogs (Fig. 4) indicates an enhancement of central drive at the respiratory rhythm generator, which has become less dependent on the hypoxic stimuli from chemoreceptors. The greater residual ventilation by 2.5 h during the Dejours O₂ test lessens the apneic accumulation of other stimulating factors. Thus, at 2.5 h, the latter could not account for ventilatory stimulation and the decrease in apneic time. It is evident that this increased central drive to breathe during prolonged hypoxia is dependent on an ANG II-mediated mechanism because there was no reduction of apneic duration during the Dejours O₂ test when ANG II receptors were blocked (Fig. 4).

A central ANG II mechanism during hypoxic respiratory adaptation is consistent with other observations. ANG II does not stimulate carotid chemoreceptors in anesthetized dogs and stimulates respiration in carotid denervated and vagotomized dogs (26).

Stimulation of Brain ANG II System by Hypoxia

Plasma ANG II decreased in the dogs during hypoxia (Fig. 5A) so that activation of the renal RAS could not account for the secondary hypoxic respiratory adaptation. The decrease in plasma ANG II is consistent with the well-known inhibition of the renal RAS by the brain RAS (28). The conclusion that the brain RAS was activated during hypoxia was supported by a well-
established ANG II-mediated release of AVP from the pituitary into the plasma (reviewed in Ref. 28). The “classic” relation between plasma osmolality and AVP was shifted to the left by hypoxia, an effect prevented by ANG II receptor block during hypoxia (Fig. 5, B and C). ANG II stimulation of AVP release is only mediated centrally, not peripherally (28). It is of interest that carotid chemoreceptor stimulation induces an increase in plasma AVP in dogs (30). The probable activation of a brain RAS by hypoxia to stimulate respiration is comparable to what we observed after AVP V1 receptor block in normoxic conscious dogs (36).

Carotid Chemoreceptors and ANG II Mechanism During Hypoxic Adaptation

Normal hypoxic respiratory adaptation is attenuated by carotid chemoreceptor denervation in the dog (2) and other species (1, 9, 14). Thus it seems that activation of a brain RAS may be mediated, at least in part, by hypoxic stimulation of the carotid chemoreceptors. In conscious goats, isolated perfusion of the carotid chemoreceptors with hypoxic blood (brain remains normoxic) results in hypoxic respiratory adaptation, whereas hypoxia of the central nervous system, with the carotid chemoreceptors normoxic, does not (reviewed in Ref. 1). It is also of interest that experiments in human subjects were interpreted to indicate that carotid chemoreceptor activation is required for an hypoxic ventilatory decline of presumed central origin (6).

Central Site of Action of ANG II

In the present study, ANG II must have acted at a site in the central nervous system not protected by the BBB, because saralasin does not penetrate the BBB (35). Receptors for ANG II are localized on CVOs, including the subfornical organ, the organum vasculosum of the lamina terminalis, the area postrema, and the median eminence (reviewed in Refs. 18 and 28). If ANG II directly mediated hypoxic ventilatory adaptation through a neural mechanism by stimulating a CVO, changes in [HCO₃]CSF and [SID]CSF could occur secondary to the associated hyperventilation and hypocapnia (20).

On the other hand, ANG II could also affect ion transport at choroid plexuses where there are also ANG II receptors (28). ANG II in the central nervous system, but not in the blood, decreases the formation of CSF (4). The delayed nature of the respiratory adaptation to hypoxia in relation to acid-base changes supports the possibility that ANG II may act on respiratory control by affecting ion transport and brain acid-base balance.

ANG II and Acid-Base Balance During Respiratory Hypoxic Adaptation

ANG II mediated adaptive changes in [SID]a, (Fig. 2), as well as ventilation, in unblocked hypoxic dogs. Our data for plasma were in keeping with the literature of chronic hypoxia in which increases in [lactate] and [Cl⁻] decrease the [SID] (11, 29). Changes in the [SID] in both the arterial plasma and in the CSF predict changes in PaCO₂, including hypoxic respiratory adaptation in human subjects (see Ref. 17) when [H⁺] does not (16, 17). During hypoxic respiratory adaptation in dogs, [HCO₃]CSF, and hence [SID]CSF (17), decreases (3); presumably, this would be prevented by ANG II receptor block.

We previously hypothesized that the [SID] in brain fluids may act as a stimulus to central chemoreceptors, with a decrease in [SID] stimulating ventilation to lower PaCO₂ (16, 17). Under steady-state conditions, secondary changes in [SID]a parallel changes in [SID]CSF, and both are highly correlated with PaCO₂ (see Ref. 16). Relative to a given [SID]a, PaCO₂ decreased in unblocked dogs during hypoxia (Fig. 6A), which reflects the hypoxic chemoreceptor drive to breathe. As the [SID]a decreased in unblocked dogs during hypoxia, PaCO₂ also decreased (Fig. 6A). This relation between [SID]a and PaCO₂ in unblocked hypoxic dogs was not altered by ANG II receptor block (Fig. 6B); hypoxic stimulation of chemoreceptors to reduce PaCO₂ was maintained at a given [SID]a. However, during ANG II receptor block, there was no effect of hypoxia to progressively
decrease [SID] and PaCO2 (Figs. 2D and 6B). If [SID] is the chemoreceptor stimulus that regulates PaCO2, then ANG II may act indirectly by regulating [SID]. It remains to be resolved whether the ANG II modulation of hypoxic respiratory adaptation is common among species and whether it acts directly by a neural mechanism or indirectly by affecting other mechanisms such as ion transport and acid-base balance in the central nervous system.

Perspectives

By inhibiting the angiotensin system, abnormal stimulation of AVP at high altitude, as well as potentially contributing to the pulmonary edema of acute mountain sickness (13), might also delay respiratory and acid-base acclimatizations. Similar to hypoxic dogs with ANG II receptor block, acute mountain sickness is associated with a lower hypoxic ventilatory response (21). AVP inhibition of ANG II might also be important associated with an increase in the mammalian fetus, in which concentrations of AVP exceed those in the adult. There is a large release of AVP with the stress of birth (25), and this is potentiated by hypoxia (7). In the presence of an abnormal or inhibited brain RAS, the newborn infant might be dependent on an hypoxic chemoreceptor drive to breathe, and, as PaO2 increases with maturation, this could predispose to apnea. The prolonged apnea at 2.5 h in hypoxic dogs breathing O2, when ANG II receptors were blocked, was a striking observation (Fig. 4). Is it also possible that one of the etiologies for sudden infant death syndrome may be related to a dysfunction in the development of a brain ANG II system, or in the regulation of ANG II receptors in the brain, and that AVP inhibition of ANG II might be involved?

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Address for reprint requests: D. B. Jennings, Dept. of Physiology, Botterell Hall, 4th Floor, Queen’s University, Kingston, Ontario, Canada K7L 3N6.

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