Acute hypoxic pulmonary vasoconstriction in conscious dogs decreases renin and is unaffected by losartan

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Krebs, Martin O., Willehad Boemke, Stephan Simon, Maieli Wenz, and Gabriele Kaczmarczyk. Acute hypoxic pulmonary vasoconstriction in conscious dogs decreases renin and is unaffected by losartan. J. Appl. Physiol. 86(6): 1914–1919, 1999.—Acute hypoxic pulmonary vasoconstriction (HPV) may be mediated by vasoactive peptides. We studied eight conscious, chronically tracheostomized dogs kept on a standardized dietary sodium intake. Normoxia (40 min) was followed by hypoxia (40 min, breathing 10% oxygen, arterial oxygen pressures 36 ± 1 Torr) during both control (Con) and losartan experiments (Los; iv infusion of 100 µg·min⁻¹·kg⁻¹ losartan). During hypoxia, minute ventilation (by 0.9 l/min in Con, by 1.3 l/min in Los), cardiac output (by 0.36 l/min in Con, by 0.30 l/min in Los), heart rate (by 11 beats/min in Con, by 30 beats/min in Los), pulmonary artery pressure (by 9 mmHg in both protocols), and pulmonary vascular resistance (by 280 and 254 dyn·s·cm⁻⁵ in Con and Los, respectively) increased. Mean arterial pressure and systemic vascular resistance did not change. In Con, PRA decreased from 4.2 ± 0.7 to 2.5 ± 0.5 ng ANG I·ml⁻¹·h⁻¹, and plasma ANG II decreased from 11.9 ± 3.0 to 8.2 ± 2.1 pg/ml. The renin-angiotensin system is inhibited during acute hypoxia despite sympathetic activation. Under these conditions, ANG II AT₁-receptor antagonism does not attenuate HPV.

In vitro studies demonstrated the sensitivity of the pulmonary vascular bed toward angiotensin II. Angiotensin II contracted isolated canine pulmonary artery rings (24) and pulmonary arteries of isolated rat lungs (1). However, early studies called into question the involvement of angiotensin II in acute HPV in anesthetized dogs (8). This study (8), however, was performed by using a competitive inhibitor of angiotensin II that may itself have agonistic properties, in contrast to the now-available specific angiotensin II-receptor-blocking agents.

We studied pulmonary hemodynamics in conscious dogs, which demonstrated an integrated vascular response with all regulation mechanisms left intact. We applied alveolar hypoxia both with and without the application of the specific angiotensin II-receptor antagonist losartan, which selectively blocks the angiotensin II AT₁ receptor. The use of a selective angiotensin II-receptor antagonist avoids possible bradycinin effects that may result from an ACE inhibition performed in another study (2).

MATERIALS AND METHODS
Animals, Maintenance, and Diet

Eight purebred female beagle dogs (body wt, 13.2 ± 0.8 kg) were used. The dogs were obtained from the Central Animal Facilities of the Humboldt-University in Berlin, Medical Faculty Charité. The dogs were selected for their social behavior and tolerance to intravascular cannulas and to minor experimental procedures. Permission to perform the experiments was obtained from the Animal Care Committee of the Government of Berlin (AZ IV A4–5855/17–114/95).

The dogs were kept under standardized conditions in air-conditioned rooms (55% humidity) and were checked daily for general status, body weight, and temperature. Next, they were admitted to the laboratory, where they were trained to lie quietly on a padded animal table for a period of at least 4 h. The dogs were fed once daily at 2 PM. Food intake was always complete after 1 h. The amount of food and the ingredients were calculated for each individual dog and consisted (all values per kilogram body weight per day) of boiled rice (58 g), minced beef (12 g), potassium chloride (3.5 mmol), sodium (2.5 mmol), and water (91 ml). The caloric content of the diet (277 kJ·kg⁻¹·body wt⁻¹·day⁻¹) was sufficient to keep body weight constant. One week before an experiment was performed in an individual dog, 150 ml of blood were taken (puncture of a foreleg vein) into a sterile and pyrogen-free plastic bag containing 20 ml CPDA-1 stabilizer (Biotap, Biotrans, Dreieich, Germany) and stored at 4°C. Intervals between experiments in the same dog were at least 10 days.

Surgical Procedure

General anesthesia was induced with methohexital sodium (7–9 mg/kg iv) and, after endotracheal intubation, was main-
tained with isoflurane (1.0–1.5%) and nitrous oxide-O2 (2:1). A permanent tracheostoma was produced by using a technique described by others (5) with minor modifications. Postoperative analgesia was provided by 2–4 mg xylazine hydrochloride (Rompun, 2%, Bayer, Leverkusen, Germany). Wound healing was complete after a period of ~3 wk. Thereafter, the dogs were trained in daily sessions of increasing length to tolerate a tracheal tube (size 7–8, Ultra Tracheoflex, Rüsch) and to breathe via a ventilator (Servo ventilator 900 C, Siemens). A small continuous positive airway pressure of 4 cmH2O (measured at the distal end of the tracheal tube) was used to overcome mechanical resistance of ventilator tubes.

**Experimental Protocols**

Preparation of the dogs started at 8 AM. Body temperature and body weight were recorded. Under aseptic conditions and by use of local anesthesia (5 ml 1% lidocaine), a 5-Fr thermodilution pulmonary artery catheter (Baxter Edwards) was introduced via the superficial jugular vein to measure central venous pressure (CVP), cardiac output (CO), pulmonary artery pressure (PAP), and pulmonary capillary wedge pressure. Local anesthesia was also administered, and an arterial catheter (Baxter) was introduced in one of the femoral arteries to measure mean arterial blood pressure (MAP) and to obtain arterial blood samples. The catheters were connected to pressure transducers. The tracheal tube was inserted, blocked, and connected to the respirator. After a resting period of 30–45 min, the experiments were started. Each of eight dogs underwent two different experiments [1 control (Con), 1 losartan (Los)] in randomized order. In six dogs, time control experiments were performed.

Con. The dogs breathed room air for 40 min (21% O2, 79% N2, normoxia); thereafter, they breathed a gas mixture containing 10% O2, 90% N2 (hypoxia) for another 40 min. Los. Los were the same as Con, except that losartan (100 µg·kg\(^{-1}\)·min\(^{-1}\)) was started at the beginning of the resting period. As intravenous single doses were rapidly eliminated (3), a continuous intravenous infusion of losartan was applied. An intravenous bolus of 1,000 ng angiotensin II (Sigma, Germany) was administered. An intravenous bolus of 1,000 ng angiotensin II (Sigma, Germany) was administered immediately after the experiment. The lack of the typical blood pressure increase after angiotensin II indicated angiotensin II AT\(_1\)-receptor antagonism.

Time control experiments. The dogs breathed room air for two periods of 40 min each.

CO was measured by thermodilution. Five consecutive determinations were performed; the highest and the lowest values were omitted. The mean was calculated from the remaining three determinations and taken for calculation of pulmonary and systemic vascular resistance (PVR and SVR, respectively) by conventional formulas. Heart rate, MAP, CVP, and PAP rate were stored in a computer (Commodore PC 40-111). Measurements were performed continuously, and values presented were taken over a period of 5 min at the end of both the normoxia and hypoxia periods.

Two-milliliter blood samples were taken every 20 min for arterial blood-gas analysis. Thirty-milliliter blood samples were taken at the end of the normoxia and hypoxia periods. The 30-ml samples were replaced immediately by 30 ml of the dog's own blood taken at least 1 wk before the experiment by using a blood filter (Pall-Ultrapor, Pall Biomedizin, Dreieich, Germany).

Sodium and potassium were measured by flame photometry (Photometer Eppendorf, Hamburg, Germany). For immunologic determination of aldosterone, arginine vasopressin (AVP), PRA, and angiotensin II, blood was collected in precooled Na-EDTA vacutainers, centrifuged at 4°C, and the plasma was stored at −22°C until analysis. Commercially available radioimmunoassay kits were used to measure hormones. Data on interassay and intra-assay variations that were provided by the manufacturer were checked regularly and proved to be in the same range. We measured aldosterone (Alodock-2, Sorin, intra-assay variation 12.7%, interassay variation 12.4%), angiotensin II (Euro-Diagnostica, Arnhem, The Netherlands; intra-assay variation 4%, interassay variation 8%), and PRA (New England Nuclear, North Billerica, ME; intra-assay variation 11%, interassay variation 8%), expressed as nanograms of angiotensin I generated per hour of incubation per milliliter of plasma. AVP was determined by a radioimmunoassay kit (Biermann, Bad Nauheim, Germany) by using a double-antibody separation technique after extraction. The standard range of the assay was 1.2–80.0 pg/ml, sensitivity was 0.6 pg/ml, and the intra-assay variability was 8% at the middle sensitivity range.

**End of Study**

On completion of the study, the dogs were anesthetized again and the tracheostoma was repaired surgically. After an observation and healing time of ~6 wk, the dogs were given to private individuals with the assistance of university veterinarians.

**Statistical Analysis**

All values are given as means ± SE. We compared normoxia and time controls as well as normoxia and hypoxia values during Con and Los by using paired Student's t-test or the U-test (Mann-Whitney) for paired data. A general linear model ANOVA for repeated measures (SPSS 7.5) was used when appropriate. A P < 0.05 was considered statistically significant.

**RESULTS**

**Arterial Blood Gases, Minute Ventilation, pH, and Bicarbonate Concentrations**

During hypoxia, arterial O\(_2\) and CO\(_2\) pressure (Pao\(_2\) and PaCO\(_2\), respectively) decreased, and pH increased similarly in Con and Los. Minute ventilation increased during hypoxia, resulting in a moderate, uncompensated respiratory alkalosis in both protocols (Table 1).

**Hemodynamics**

Mean PAP was similar during normoxia in Con (14 ± 1 mmHg) and Los (15 ± 1 mmHg). During hypoxia, PAP increased by an average of 9 ± 1 mmHg in both protocols (Fig. 1).

Heart rate was similar during normoxia in Con (92 ± 4 beats/min) and Los (84 ± 6 beats/min). During hypoxia, heart rate increased in Con to 103 ± 5 beats/min and in Los to 114 ± 9 beats/min (Fig. 2).

MAP was similar during normoxia in Con (105 ± 5 mmHg) and Los (108 ± 5 mmHg). During hypoxia, MAP did not change in either protocol (Fig. 2).

PVR was similar during normoxia in Con (361 ± 60 dyn·s·cm\(^{-5}\)) and Los (434 ± 19 dyn·s·cm\(^{-5}\)). During hypoxia, PVR increased to 641 ± 81 dyn·s·cm\(^{-5}\) (Con) and to 688 ± 61 dyn·s·cm\(^{-5}\) (Los) (Fig. 2).
Table 1. Arterial blood gases and minute ventilation

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Con</th>
<th>Los</th>
</tr>
</thead>
<tbody>
<tr>
<td>PaO₂, Torr</td>
<td>98 ± 3</td>
<td>96 ± 3</td>
</tr>
<tr>
<td>Hypoxia</td>
<td>36 ± 1*</td>
<td>35 ± 1*</td>
</tr>
<tr>
<td>PaCO₂, Torr</td>
<td>36 ± 1</td>
<td>34 ± 1</td>
</tr>
<tr>
<td>Normoxia</td>
<td>27 ± 0.5*</td>
<td>28 ± 1*</td>
</tr>
<tr>
<td>Hypoxia</td>
<td>7.38 ± 0.01</td>
<td>7.38 ± 0.01</td>
</tr>
<tr>
<td>pH</td>
<td>7.45 ± 0.003*</td>
<td>7.45 ± 0.01*</td>
</tr>
<tr>
<td>Normoxia</td>
<td>21.4 ± 0.04</td>
<td>20.8 ± 0.3</td>
</tr>
<tr>
<td>Hypoxia</td>
<td>20.7 ± 0.03</td>
<td>21.1 ± 0.5</td>
</tr>
<tr>
<td>Ve, l/min</td>
<td>4.5 ± 0.4</td>
<td>4.4 ± 0.3</td>
</tr>
<tr>
<td>Hypoxia</td>
<td>5.4 ± 0.4*</td>
<td>5.7 ± 0.3*</td>
</tr>
</tbody>
</table>

Values are means ± SE; n = 8 days. Concentration is denoted by brackets. PaO₂, arterial oxygen pressure; PaCO₂, arterial carbon dioxide pressure; HCO₃⁻, arterial pH; Ve, minute ventilation. Values are during normoxia (21% inspiratory oxygen concentration) and during hypoxia (10% inspiratory oxygen concentration) in conscious dogs without control (Con), and with losartan application (Los; 100 µg·min⁻¹·kg⁻¹). *P < 0.05 vs. values during normoxia.

SVR was 3,682 ± 199 dyn·s·cm⁻² (Con experiments) and 4,009 ± 338 dyn·s·cm⁻² (Los experiments). SVR did not change during hypoxia in either group (Fig. 2).

CO was similar during normoxia in Con (2.06 ± 0.15 l/min) and in Los (2.07 ± 0.14 l/min). During hypoxia, CO increased significantly in Con to 2.42 ± 0.14 l/min as well as in Los (to 2.37 ± 0.10 l/min). The increase in CO was due to the increase in heart rate in both protocols; calculated stroke volume did not change.

Pulmonary capillary wedge pressure was similar during normoxia in Con (4.5 ± 0.5 mmHg) and Los (4.1 ± 1 mmHg). During hypoxia, it did not change in either protocol.

CVP was similar during normoxia in Con (3.0 ± 0.6 mmHg) and Los (3.3 ± 0.6 mmHg). During hypoxia, CVP did not change in Con; it decreased in Los to 2.4 ± 0.7 mmHg (P < 0.05).

Plasma Values

During hypoxia, PRA and angiotensin II decreased in Con (Fig. 3, Table 2). In Los, PRA and angiotensin II were increased during normoxia as well as during hypoxia compared with Con (Fig. 3, Table 2).

Plasma aldosterone was decreased during hypoxia in Los compared with Con and did not change in either group during hypoxia.

AVP, plasma sodium, and plasma potassium were similar in Con and Los and did not change during hypoxia (Table 2).

Time Control Experiments

All variables measured in time control experiments were not statistically different from the values obtained in Con during the first 40-min period of normoxia. Values did also not change during the following 40-min period of normoxia.

DISCUSSION

The purpose of the present study was to find out whether, in a situation of sympathetic stimulation due to acute hypoxia, the renin-angiotensin system is involved in HPV. We investigated conscious dogs under both normoxic and hypoxic conditions and either blocked their angiotensin II AT₁ receptors or left them intact.

The results of our experiments demonstrate that the renin-angiotensin system does not contribute to HPV but, in contrast, decreases its activity during hypoxia. In accordance with this result, blocking of angiotensin II AT₁ receptors with the specific antagonist losartan did not decrease pulmonary vasoconstriction during hypoxia. Losartan has been given by continuous intravenous infusion to eliminate possible species-specific lack of generation of the active metabolite EXP3174 in dogs (15). The effectiveness of the angiotensin II receptor antagonism by losartan is demonstrated by the following results: MAP did not change after intravenous bolus injection of angiotensin II, and PRA as well as plasma angiotensin II were increased, whereas plasma aldosterone concentration was decreased in Los compared with Con (Table 2).

As expected, acute hypoxia was followed by an increase in ventilatory drive because of sympathetic stimulation, which also increased heart rate and CO. Sympathetic activity has been shown to be tremendously augmented in dogs with PaO₂ in the range of our values (10). It is well known that sympathetic activation coincides with an increase in PRA in dogs, mainly via renal β-adrenergic receptors (9). If the angiotensin II generation is not blocked, the hormone should then increase SVR and PVR. Angiotensin II receptors from the AT₁ type were found in isolated canine pulmonary arteries (24). Furthermore, it has been shown that exogenously applied angiotensin II in borderline physiological concentrations is a pulmonary vasoconstrictor in conscious dogs (7). In the present study, the con-
scious dogs demonstrated sympathetic activation (increase in heart rate, CO, and ventilation) during hypoxia; however, at the same time, all but one dog decreased their PRA (Fig. 3) and all but one dog decreased their angiotensin II levels as well. In the face of decreased PRA and angiotensin II values during hypoxia, it was almost mandatory that the specific angiotensin II antagonist would not diminish HPV, as

Fig. 2. Heart rate (A), mean arterial pressure (B), systemic vascular resistance (C), and pulmonary vascular resistance (D) during normoxia (N; 21% oxygen) and during hypoxia (H; 10% oxygen) in conscious dogs. Values are means ± SE; n = 8. Symbols are defined as in Fig. 1. *P < 0.05 vs. N values.

Fig. 3. Plasma renin activity (A) and plasma angiotensin II (B) concentrations during N (21% oxygen) and H (10% oxygen) in conscious dogs. Values are means ± SE; n = 8. Symbols are defined as in Fig. 1. *P < 0.05 vs. N values.
Table 2. Plasma hormones and electrolytes

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Con</th>
<th>Los</th>
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</thead>
<tbody>
<tr>
<td>PRA, ng ANG I·ml⁻¹·h⁻¹</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Normoxia</td>
<td>4.2 ± 0.7</td>
<td>19.0 ± 7.8†</td>
</tr>
<tr>
<td>Hypoxia</td>
<td>2.5 ± 0.5*</td>
<td>9.3 ± 5.1†</td>
</tr>
<tr>
<td>[Angiotensin II], pg/ml</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Normoxia</td>
<td>11.9 ± 3.0</td>
<td>23.1 ± 5.5†</td>
</tr>
<tr>
<td>Hypoxia</td>
<td>8.2 ± 2.1*</td>
<td>23.4 ± 2.1†</td>
</tr>
<tr>
<td>[Aldosterone], pg/ml</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Normoxia</td>
<td>72 ± 9</td>
<td>42 ± 8†</td>
</tr>
<tr>
<td>Hypoxia</td>
<td>56 ± 3</td>
<td>36 ± 6</td>
</tr>
<tr>
<td>[AVP], pg/ml</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Normoxia</td>
<td>1.4 ± 0.2</td>
<td>1.7 ± 0.2</td>
</tr>
<tr>
<td>Hypoxia</td>
<td>1.3 ± 0.3</td>
<td>1.4 ± 0.2</td>
</tr>
<tr>
<td>[Sodium], mmol/l</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Normoxia</td>
<td>145 ± 1</td>
<td>144 ± 1</td>
</tr>
<tr>
<td>Hypoxia</td>
<td>144 ± 1</td>
<td>143 ± 2</td>
</tr>
<tr>
<td>[Potassium], mmol/l</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Normoxia</td>
<td>3.9 ± 0.2</td>
<td>3.5 ± 0.1</td>
</tr>
<tr>
<td>Hypoxia</td>
<td>3.7 ± 0.3</td>
<td>3.6 ± 0.2</td>
</tr>
</tbody>
</table>

Values are means ± SE; n = 8 dogs. PRA, plasma renin activity; AVP, arginine vasopressin. Values are during normoxia (21% inspiratory oxygen concentration) and during hypoxia (10% inspiratory oxygen concentration) in conscious dogs. *P < 0.05 vs. values during normoxia. †P < 0.05 vs. Con values.

was indeed demonstrated in the present study. Because of the experimental conditions, the results from our study cannot be compared with studies in which the renin-angiotensin system is activated to an unpredictable extent, e.g., by anesthesia and acute preparation (16).

In contrast to results obtained in humans (6), the increase in PVR in our dogs was already complete within 20 min (Fig. 1). Thus it is unlikely that angiotensin II, a fast-acting hormone, may become involved in HPV at a later point.

In contrast to our results, in other studies PRA was reported unchanged (4) or increased (22) during acute hypoxia in conscious dogs. One possible reason for this discrepancy is that the concomitant increase in PaCO₂ in the other experiments (4, 22) and/or extensive acute preparations with several intravascular catheters introduced under light sedation only (13) had stimulated the sympathetic nervous system to a greater extent than in the present study. A brisk activation of the renin-angiotensin system during hypoxemia in conscious dogs indicates that blood O₂ saturation levels of between 75 and 80% did not change PRA. When ACE was inhibited, however, acute HPV was decreased in humans (2) as well as in dogs (7). This could indicate a contribution of vasodilatory peptides, e.g., bradykinin, generated by ACE inhibition. In the other study from this group (12), direct AT₁-receptor blockade with losartan also attenuated acute HPV. These results are surprising, because the renin-angiotensin system was apparently not stimulated by hypoxia, and measured PRA levels were unchanged (and presumably angiotensin II plasma concentrations as well) (12). Among other causes, species differences play a role. Our results in dogs are conclusive insofar as a decrease in the activity of the renin-angiotensin system during hypoxia coincides with the ineffectiveness of angiotensin II-receptor antagonism on the pulmonary vasculature. Both results were obtained simultaneously in the same dogs and support the assumption that the renin-angiotensin system is not involved in HPV under these experimental conditions.

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