Renal and hemodynamic effects of losartan in conscious dogs during controlled mechanical ventilation

MARTIN O. KREBS, THORSTEN KRÖHN, WILLEHAD BOEMKE, RAINER MOHNHAUPT, AND GABRIELE KACZMARCZYK

Klinik für Anästhesiologie und Operative Intensivmedizin, Medizinische Fakultät Charité der Humboldt Universität zu Berlin, Campus Virchow-Klinikum, D-13353 Berlin, Germany

Krebs, Martin O., Thorsten Kröhn, Willehad Boemke, Rainer Mohnhaupt, and Gabriele Kaczmarczyk. Renal and hemodynamic effects of losartan in conscious dogs during controlled mechanical ventilation. Am. J. Physiol. 276 (Renal Physiol. 45): F425–F432, 1999.—In 12 conscious dogs, we investigated whether the angiotensin II-receptor antagonist losartan increases renal sodium excretion and urine volume during controlled mechanical ventilation (CMV) with positive end-expiratory pressure. In four experimental protocols, the dogs were extracellular volume (ECV) expanded (electrolyte solution, 0.5 ml·kg⁻¹·min⁻¹ iv) or not and received losartan (100 µg·kg⁻¹·min⁻¹ iv) or not. They breathed spontaneously during the 1st and 4th hour and received CMV with positive end-expiratory pressure (mean airway pressure 20 cmH₂O) during the 2nd and 3rd hours. In the expansion group, dogs with losartan excreted ~18% more sodium (69.7 vs. 38.5 mmol·min⁻¹·kg⁻¹) and 15% more urine during the 2h of CMV because of a higher glomerular filtration rate (5.3 vs. 0.7 mmol·min⁻¹·kg⁻¹) and the tubular effects of losartan. In the group without expansion, sodium excretion (2.0 vs. 0.6 mmol·min⁻¹·kg⁻¹) and glomerular filtration rate (3.8 vs. 0.3 mmol·min⁻¹·kg⁻¹) did not change, and urine volume decreased similarly in both groups during CMV. Plasma vasopressin and aldosterone increased in both groups, and plasma renin activity increased from 4.9 ± 0.7 to 7.8 ± 1.3 ng ANG I·ml⁻¹·h⁻¹ during CMV in nonexpanded dogs without losartan. Mean arterial pressure decreased by 10 mmHg in nonexpanded dogs with losartan. In conclusion, losartan increases sodium excretion and urine volume during CMV if the ECV is expanded. If the ECV is not expanded, a decrease in mean arterial blood pressure and/or an increase in aldosterone and vasopressin during CMV attenuates the renal effects of losartan.

Renin; vasopressin; atrial natriuretic peptide; intrathoracic pressure; extracellular volume

STIMULATION of the renin-angiotensin system (RAS) has frequently been demonstrated during controlled mechanical ventilation (CMV) with positive end-expiratory pressure (PEEP) in humans (1, 23, 25) and anesthetized animals (2, 3, 24). The increase in plasma renin activity (PRA) during an increase in intrathoracic pressure is, among others, brought about by a decrease in renal perfusion pressure due to alterations in venous return and a fall in cardiac output (24). In addition, sympathetic activation occurs because of unloading of low- and high-pressure baroreceptors (10). Angiotensin II (ANG II) may not only contribute to the maintenance of arterial pressure during CMV but also increases renal tubular reabsorption of sodium and water immediately, independently of aldosterone (17). Sodium and water retention during CMV with PEEP has been repeatedly demonstrated in clinical (15) as well as in experimental (18) studies. Volume-expanded conscious dogs excrete less urine and sodium when mechanically ventilated compared with those with spontaneous breathing (18). CMV with PEEP is a routine procedure in the intensive care unit; however, its side effects are not well tolerated. Inhibition of the RAS may become a valuable alternative against the extremely high dosages of diuretics commonly used.

Nonpeptide molecules such as losartan (DuP-753) are known to block specifically and competitively the ANG II receptor subtype 1 (AT₁) (13). The AT₁-receptor subtype mediates the ANG II-stimulated decrease in renal sodium and water excretion by affecting renal performance on different functional levels (9, 26). In addition, part of the adrenal aldosterone release is AT₂-receptor mediated (27).

The present study was performed to determine whether AT₁-receptor blockade with losartan, which lacks the prostaglandin-kinin-inducing activities of converting enzyme inhibitors, increases urine volume and sodium excretion during CMV with PEEP in conscious dogs. To demonstrate the renal effects of losartan, we established adequate sodium and water excretion during CMV and investigated dogs with extracellular volume (ECV) expansion. We also investigated dogs without ECV expansion because these dogs have been shown to stimulate their RAS during PEEP to maintain mean arterial blood pressure (MAP) and glomerular filtration rate (GFR) during PEEP (19) and may not be able to maintain their MAP when given losartan. In addition, it can be presumed that in these dogs, losartan may be without an effect on renal excretions as other hormonal and neural factors activated during CMV may influence renal function.

Because anesthesia and surgical stress stimulate the RAS to an unpredictable extent, we used conscious, well-trained dogs to overcome this problem. In addition, we provided a standardized dietary sodium and water intake to establish comparable responses to volume expansion as well as a reproducible activation of the RAS.
MATERIAL AND METHODS

Animals, Maintenance, and Diet

Twelve pure-bred female beagle dogs (body wt 13.2 ± 1.8 kg) were randomly allocated to two groups. Six dogs were used in 12 experiments with EVC expansion, and six dogs were used in 12 experiments without ECV expansion; each dog served as its own control with and without the application of losartan (Dup-753/MK-954). The dogs were obtained from the Central Animal Facilities of the Free University of Berlin. Permission to perform the experiments was obtained from the Berlin Animal Care Committee (AZ-114/95).

The dogs were kept under standardized conditions in air-conditioned rooms (55% humidity). They were trained to lie quietly on a padded animal table for a period of at least 5 h. Food was given once daily at 2 PM. The amount of food and the food constituents were calculated for each individual dog and consisted of (all values per kg of body wt per day) 58 g boiled rice, 12 g minced beef meat, 3.5 mmol potassium chloride, and 2.5 mmol sodium. Water was added to provide a total water content of 91 ml. The caloric content of the diet was 277 kJ and was sufficient to maintain a constant body weight. One week before an experiment was performed, 150 ml of blood were taken from each dog by puncture of a foreleg vein and stored at 4°C. Intervals between experiments in the same dog were at least 7 days. After termination of the study, the tracheostomy was surgically closed, and the dogs were discharged to private persons with the assistance of our veterinarians.

Surgical Procedures

Under aseptic conditions, two separate operations were performed in each dog. General anesthesia was induced with methohexital sodium (7–9 mg/kg iv) and was maintained after endotracheal intubation with halothane (0.5–1.5%) and nitrous oxide and oxygen (2:1). The left carotid artery was exteriorized (carotid loop). About 3–4 wk later, a permanent tracheostoma was produced using a technique described by others (8) with some modifications. After another 3–4 wk, the dogs were trained in daily sessions of increasing duration to tolerate a tracheal tube (size 7–8, Ultra Tracheoflex, Rüsch, Germany) and mechanical ventilation with PEEP (Servo ventilator 900 C, Siemens) using a trigger sensitivity of 0.35 g/h; creatinine concentration 28 mg/ml) was started. By use of local anesthesia, a double-lumen catheter (Arrow Howes, Dahlhausen) was introduced into the right atrium, and the carotid loop was punctured. The tracheal tube was inserted, blocked, and connected to the respirator. Thereafter, an intravenous infusion of losartan was started in both expanded and nonexpanded losartan protocols (protocols 2 and 4). Thirty minutes later, a bolus of 1,000 ng of ANG II was injected intravenously. After we confirmed that no changes in blood pressure had occurred after this maneuver, the experiment was started with CPAP, and, in expanded control and expanded losartan protocols, volume expansion was begun. The respiratory frequencies and tidal volumes that were recorded during spontaneous respiration (with CPAP) were also applied during the 2 h of CMV (with PEEP).

Pressures were measured continuously and averaged over 20-min periods. Airway pressure (Paw) was measured at the distal end of the tracheal tube. All data were stored on a Commodore PC 40-111.

At hourly intervals, renal sodium, water, potassium, and creatinine excretions were measured after complete evacuation of the urinary bladder (air washout). GFR was calculated from renal creatinine excretion rate and the average of two creatinine plasma samples taken at 30-min intervals.

Five-milliliter blood samples were taken at 30-min intervals (creatine and arterial blood gas analysis), and we performed one additional arterial blood gas analysis during the first minutes after having changed the ventilation from CPAP to CMV, to adjust the respirator settings, if necessary. Thirty-milliliter blood samples were taken at the end of each hour [sodium, potassium, plasma aldosterone, PRA, atrial
natriuretic peptide (ANP), arginine vasopressin (AVP), and hematocrit). Each 30-ml sample was replaced immediately by 30 ml of the dog's own blood that was taken and stored 1 wk before (Blood Filter Pall-Ultipor, Pall Biomedizin, Dreieich, Germany).

Sodium and potassium were measured by flame photometry (Photometer Eppendorf), and creatinine was measured with a creatinine analyzer (Hoffmann Instruments). Fractional sodium excretion (FE\textsubscript{Na}, %) was calculated by a standard formula. For radioimmunologic determination of plasma aldosterone, PRA, ANP, and AVP, blood was collected in precooled sodium-EDTA vacutainers, and centrifuged at 4°C, and the plasma was stored at −22°C until analysis. Interassay and intra-assay variations as given by the manufacturers were proven to be in the same range: we measured ANP (14 and 11%, respectively; Henning, Berlin, Germany), plasma aldosterone (12.4 and 12.7%, respectively; Aldoctk-2, Sorin) and PRA (8.4 and 11%, respectively; New England Nucleus, North Billerica, ME). AVP was also determined radioimmunologically (Biermann, Bad Nauheim, Germany) with a double-antibody separation technique after extraction. The standard range of the assay was 1.2–80.0 pg/ml, the sensitivity was 0.6 pg/ml, and the intra-assay variability was 8% at the middle of the sensitivity range.

Statistical analysis

Values are given as means ± SE. CPAP (1st hour), CMV (2nd and 3rd hour), and CPAP (4th hour) values of each experimental protocol as well as hourly values between expanded control vs. expanded losartan groups and nonexpanded control vs. nonexpanded losartan groups were compared by ANOVA for repeated measurements, followed by a Newman-Keuls test (5.2 NCSS Statistical Package, J erry Hintze, Kaysville, UT). Student's unpaired t-test was used to compare 1st hour values of expanded and nonexpanded dogs. P < 0.05 was considered significant.

RESULTS

Airway Pressure

Mean airway pressure was the same in all protocols: 4 ± 0 cmH\textsubscript{2}O during the 1st and 4th hour (CPAP), and between 19 ± 1 and 21 ± 1 cmH\textsubscript{2}O during CMV with PEEP (2nd and 3rd hour).

Renal Function Data

Expanded. Sodium excretion, FE\textsubscript{Na}, urine volume, and potassium excretion were increased during CMV above the 1-h CPAP values, because of the ongoing ECV expansion (Fig. 2). However, these variables as well as GFR were significantly greater during CMV in the expanded losartan group than in the expanded control group. Altogether, during CMV, sodium excretion was increased by an average of 18% and urine volume was increased by an average of 15% in the expanded losartan group compared with the expanded control group. All variables remained increased in the expanded losartan group compared with the expanded control groups during the 4th hour.

Calculated retention of water and sodium. From the total amount of water infused over 4 h (120 ml/kg body wt), 48 ± 4% was retained in the expanded control group and 30 ± 2% was retained in the expanded losartan group (P < 0.05). From the total amount of
sodium infused over 4 h (16.44 mmol/kg body wt), 62 ± 6% was retained in the expanded control group and 47 ± 4% was retained in the expanded losartan group (P < 0.05).

Nonexpanded. Sodium excretion, FE\textsubscript{Na}, potassium excretion, and GFR were similar during the 1st hour of CPAP in the nonexpanded control and nonexpanded losartan groups and did not change during CMV in either group (Fig. 3). Urine volume was increased in the nonexpanded losartan group, and it decreased similarly in both groups during CMV and reached 1-h CPAP values again during the 4th hour of CPAP.

**Hemodynamics**

Expanded. Heart rate (HR) was at 96 beats/min in the expanded control group and did not change (Fig. 4, left). HR was increased in the expanded losartan group compared with the expanded control group most of the time. MAP increased during CMV in the expanded control group and remained increased, whereas MAP did not change throughout the experiment in the expanded losartan group. In both groups, right atrial pressure increased during CMV; in the expanded losartan group, it increased 40–60 min later and by 3–4 mmHg less.

Nonexpanded. HR did not change in the nonexpanded control group (Fig. 4, right). HR increased transiently in the nonexpanded losartan group compared with the nonexpanded control group. MAP was similar in both groups during CPAP; it decreased by an average of ~10 mmHg during CMV in the nonexpanded losartan group and remained decreased during the 4th hour (CPAP). Right atrial pressure increased almost similarly during CMV in both groups.

**Plasma Hormones**

Expanded. Vasopressin did not change in either group (Fig. 5, left). ANP did not change in either group during CMV, but increased during the 4th hour (CPAP) in the expanded control group. The plasma aldosterone concentration decreased in both groups during CMV. PRA did not change in the expanded control group. In the expanded losartan group, PRA was increased during CMV and decreased during CPAP.

Nonexpanded. Vasopressin increased during CMV in both groups (Fig. 5, right) and remained increased during the 4th hour (CPAP) in the nonexpanded losartan group. ANP did not change. The plasma aldosterone concentration was decreased in the nonexpanded losartan group compared with the nonexpanded control group all the time and increased during CMV in both groups. PRA increased during CMV in the nonexpanded losartan group compared with the nonexpanded control group. PRA was remarkably increased in the nonexpanded losartan group compared with the nonexpanded control group all the time and did not change during CMV.

**Plasma Values and Arterial Blood Gases**

Values during the 1st hour (CPAP) are presented in Table 1. None of these values changed in either group throughout the experiments.
Hematocrit was 35 ± 1% (expanded control) and 37 ± 1% (expanded losartan) and decreased with ongoing volume expansion to significantly lower 4-h values of 31 ± 1 and 34 ± 2%, respectively. Hematocrit was 39 ± 1% (nonexpanded control) and 40 ± 1% (nonexpanded losartan) and did not change throughout the experiments.

Baseline data (CPAP, 1st hour) of Expanded and Nonexpanded Dogs

Hemodynamics. Expanded and nonexpanded control groups had the same MAP and HR as did expanded and nonexpanded losartan groups.

Renal function data. Renal excretions were significantly higher (P < 0.05) in the expanded control group compared with the nonexpanded control group: sodium excretion was 10.1 ± 1.0 vs. 2.0 ± 0.6 µmol·min⁻¹·kg⁻¹, FE Na was 1.5 ± 0.2 vs. 0.3 ± 0.1%, urine volume was 172 ± 11 vs. 36 ± 4 µl·min⁻¹·kg⁻¹, and potassium excretion was 2.2 ± 0.2 vs. 1.1 ± 0.26 µmol·min⁻¹·kg⁻¹, and GFR was 6.1 ± 0.3 vs. 3.8 ± 0.4 ml·min⁻¹·kg⁻¹.

Plasma hormones. PRA was increased in the nonexpanded control group compared with the expanded control group (4.9 ± 0.7 vs. 0.7 ± 0.1 ng ANG I·ml⁻¹·h⁻¹) and in the nonexpanded losartan group compared with the expanded losartan group (20.8 ± 6.6 vs. 4.7 ± 1.5 ng ANG I·ml⁻¹·h⁻¹). Aldosterone concentration was increased in the nonexpanded control compared with the expanded control group (109 ± 15 vs. 37 ± 7 pg/ml).

Plasma values and arterial blood gases. There were no differences in baseline plasma values and arterial blood gases (Table 1).

DISCUSSION

The present study was performed to find out whether the application of the specific ANG II-receptor antagonist losartan increases sodium and water excretion during mechanical ventilation with PEEP. Conscious, chronically instrumented dogs were used in a 4-h
protocol that included a 2-h period of CMV. We compared their renal, hormonal, and hemodynamic responses to mechanical ventilation after their ANG II receptors had either been blocked effectively or had been left intact. The dogs were either ECV expanded or not. The results demonstrate that, in this animal model, sodium and water excretion is increased during CMV with PEEP when the ANG II receptors are blocked. This renal effect could be demonstrated only in the ECV-expanded dogs. In the nonexpanded dogs, urine volume and sodium excretion were low from the beginning, and distinct effects of the ANG II-receptor antagonist on renal excretions during CMV could not be demonstrated. The failure of the ANG II-receptor antagonist to increase GFR, urine volume, and sodium excretion in these nonexpanded animals may be due to the simultaneous fall in blood pressure. The critical role of MAP in the renal response to ANG II antagonism has been demonstrated recently (5). The increase in plasma vasopressin and aldosterone concentrations

Fig. 5. Vasopressin, atrial natriuretic peptide (ANP), aldosterone concentration, and plasma renin activity (PRA) during spontaneous breathing (CPAP) and during CMV with a mean airway pressure of 20 cmH\textsubscript{2}O. Data are means ± SE, n = 6 dogs. ○, Control; ●, intravenous infusion of 100 µg·kg\textsuperscript{-1}·min\textsuperscript{-1} losartan. *P < 0.05 vs. respective 1-h protocol; †P < 0.05 vs. respective control.

Table 1. First-hour plasma values and arterial blood gases in expanded and nonexpanded conscious dogs with or without losartan.

<table>
<thead>
<tr>
<th></th>
<th>Expanded</th>
<th>Nonexpanded</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
<td>Losartan</td>
</tr>
<tr>
<td>P\textsubscript{Na}, mmol/l</td>
<td>149 ± 1</td>
<td>151 ± 1</td>
</tr>
<tr>
<td>P\textsubscript{K}, mmol/l</td>
<td>3.5 ± 0.1</td>
<td>3.5 ± 0.1</td>
</tr>
<tr>
<td>P\textsubscript{oem}, mosmol/l</td>
<td>301 ± 1</td>
<td>302 ± 1</td>
</tr>
<tr>
<td>P\textsubscript{A}O\textsubscript{2}, mmHg</td>
<td>105 ± 2</td>
<td>102 ± 4</td>
</tr>
<tr>
<td>P\textsubscript{A}CO\textsubscript{2}, mmHg</td>
<td>35 ± 1</td>
<td>35 ± 2</td>
</tr>
<tr>
<td>pH</td>
<td>7.43 ± 0.01</td>
<td>7.47 ± 0.02</td>
</tr>
<tr>
<td>HCO\textsubscript{3}, mmol/l</td>
<td>23 ± 1</td>
<td>26 ± 1</td>
</tr>
</tbody>
</table>

Values are means ± SE; n = 6 dogs/group. Plasma sodium concentration (P\textsubscript{Na}), plasma potassium concentration (P\textsubscript{K}), plasma osmolarity (P\textsubscript{oem}), arterial PO\textsubscript{2} (P\textsubscript{A}O\textsubscript{2}), arterial PCO\textsubscript{2} (P\textsubscript{A}CO\textsubscript{2}), arterial pH, and arterial bicarbonate concentration (HCO\textsubscript{3}) in expanded and nonexpanded dogs with losartan (100 µg·kg\textsuperscript{-1}·min\textsuperscript{-1} iv) and without losartan (control). Breathing was spontaneous with continuous positive airway pressure (CPAP) of 4 cmH\textsubscript{2}O during first experimental hour.
excretion increased in all dogs with ECV expansion. It increased more, however, in the dogs receiving losartan (Fig. 2). It remains uncertain whether this is due to a specific effect of losartan (4) or whether, in this group, the increase in potassium excretion is related to the increase in urine volume. It is interesting to note, however, that potassium excretion increased despite a decrease in plasma aldosterone concentration in both expanded groups. This finding was also demonstrated in another study (6).

In the nonexpanded dogs, GFR was unchanged by losartan. It may be that the simultaneous MAP decrease induced by CMV attenuated the effect of losartan on GFR. In anesthetized dogs, ANG II antagonism increased GFR; however, in these animals MAP was constant (6). In anesthetized rats, a simultaneous decrease in MAP attenuated the increase of GFR by ANG II antagonism (5).

The plasma aldosterone concentration was lower in the nonexpanded dogs given losartan because of the inhibition of ANG II-mediated adrenal aldosterone release. However, during CMV, the plasma aldosterone concentration increased also in these dogs, although their AT1 receptors were blocked. It is uncertain whether unblocked AT2 receptors may have played a role or whether stimuli other than ANG II must be responsible for the increase of aldosterone during CMV. Because neither plasma sodium nor plasma potassium changed, central release of corticotropin due to sympathetic stimulation, e.g., induced by the decrease in MAP, may have occurred.

PRA increased during CMV in the nonexpanded, unblocked dogs. This increase in PRA seems to be necessary for the maintenance of MAP because MAP decreased by −10 mmHg in the nonexpanded dogs given losartan (Fig. 4). As losartan lessens central vasopressin release (16), the observed increase in plasma vasopressin concentration may be insufficient to prevent a decrease in MAP during CMV in the nonexpanded dogs given losartan.

PRA was increased in all dogs receiving losartan (Fig. 5). The increase in PRA is due to the interruption of the negative feedback loop between ANG II and renal renin release. These high PRA values demonstrate the presence of ANG II-receptor antagonism and should be physiologically ineffectve. Healthy volunteers have been shown to increase their PRA eightfold when taking losartan (12). It is interesting to note that PRA was increased to much higher values (20.8 ± 6.6 vs. 4.7 ± 1.5 ng ANG I · ml−1 · h−1 during the 1st hour; P < 0.05; Fig. 5) in the nonexpanded dogs than in the expanded dogs. This finding indicates that a smaller ECV augments the feedback response of renal renin release during ANG II-receptor antagonism. From the present study, it cannot be concluded to what extent neurogenic renin release is involved in this process (22).

ANP did not change in any of the groups during CMV, although a decrease in ANP might be expected because of the decrease in atrial transmural pressures during CMV. In the expanded dogs, a decrease in ANP may
have been prevented by the expansion. This assumption is supported by the increase in ANP in the expanded control group after termination of CMV (Fig. 4). In the nonexpanded dogs, the decrease in ANP during CMV may have been too small to achieve significance.

In summary, we have found that the effectiveness of losartan does not depend on the preexisting activation of the RAS, with angiotensin being high in the nonexpanded dogs. However, a positive sodium and water balance of the expanded dogs may promote the renal effects of losartan, although the RAS activation was small under this condition. The background situation has to be taken into account when renal action of ANG II antagonism is expected.

We thank DuPont Merck Pharmaceutical (Wilmington, DE) for the generous supply of losartan, and Prof. Marta Weinstock for language assistance.

This study was supported by a grant from the Deutsche Forschungsgemeinschaft to G. Kaczmarczyk (Ka 526/5-3).

Portions of this work were presented in abstract form at Experimental Biology '98, San Francisco, CA.

REFERENCES


