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Renal Nerves Are Not Involved in Sodium and Water Retention during Mechanical Ventilation in Awake Dogs

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Background: **The role of renal nerves during positive end-expiratory pressure ventilation (PEEP) has only been investigated in surgically stressed, anesthetized, unilaterally denervated dogs. Anesthesia, sedation, and surgical stress, however, decrease urine volume and sodium excretion and increase renal sympathetic nerve activity independent of PEEP. This study investigated in awake dogs the participation of renal nerves in mediating volume and water retention during PEEP.**

Methods: **Eight tracheotomized, trained, awake dogs were used. The protocol consisted of 60 min of spontaneous breathing at a continuous positive airway pressure of 4 cm H_2O, followed by 120 min of controlled mechanical ventilation with a mean PEEP of 15–17 cm H_2O (PEEP), and 60 min of continuous positive airway pressure. Two protocols were performed on intact dogs, in which volume expansion had (hypervolemic; electrolyte solution, 0.5 $ml \cdot kg^{-1} \cdot min^{-1}$) and had not (normovolemic) been instituted. This was repeated on the same dogs 2 or 3 weeks after bilateral renal denervation.**

Results: **Hypervolemic dogs excreted more sodium and water than did normovolemic dogs. There was no difference between intact and renal-denervated dogs. Arterial pressure did not decrease when continuous positive airway pressure was switched to PEEP. Plasma renin activity, aldosterone, and antidiuretic hormone concentrations were greater in normovolemic dogs. The PEEP increased aldosterone and antidiuretic hormone concentrations only in normovolemic dogs.**

Conclusions: **In conscious dogs, renal nerves have no appreciable contribution to sodium and water retention during PEEP. Retention in normovolemic dogs seems to be primarily caused by an activation of the renin–angiotensin system and an increase in the antidiuretic hormone. Excretion rates depended on the volume status of the dogs. (Key words: Conscious; continuous positive-pressure ventilation; hormones; kidney; sympathetic nervous system.)**

SODIUM and water retention frequently is observed during controlled mechanical ventilation (CMV), especially when positive end-expiratory pressure (PEEP) is applied. The current study was conducted to determine whether the renal sympathetic nerves participated in this process.

In contrast to the few studies performed to describe the role of the renal nerves for sodium and water retention during CMV with PEEP, there is an abundance of data that describe hemodynamic and hormonal changes.[1] The hemodynamic changes (*e.g.*, decreases in venous return, transmural atrial pressures, cardiac output, and mean arterial pressure [MAP]) may *per se* account for decreased excretion rates but may also stimulate the secretion of sodium- and water-retaining hormones, such as antidiuretic hormone (ADH), angiotensin II, and aldosterone.[2,3] The hemodynamic and hormonal responses to CMV and PEEP depend on the extracellular volume status (*e.g.*, in awake hypervolemic dogs it has been shown that at the same level of PEEP, plasma ADH, aldosterone, and renin activity do not increase[2–4]). Furthermore, in hypervolemic dogs, it has been shown that increasing inferior vena caval pressure, by means of a vena caval constrictor cuff, to values equal to those observed during positive-pressure ventilation, results in a decrease in urinary output without measurable changes in ADH, aldosterone, and atrial natriuretic peptide concentrations.[5]

The neural effects that may induce sodium and water retention during CMV with PEEP primarily are mediated *via* low-pressure cardiopulmonary and high-pressure baroreceptor mechanisms. An increase in efferent renal

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sympathetic traffic may stimulate renin release, induce renal vasoconstriction, and augment tubular sodium and water retention.[6] In anesthetized dogs, PEEP has been shown to increase plasma catecholamine levels and general sympathetic activity.[7] Studies in anesthetized animals must be looked at with caution, however, because side effects of anesthesia, sedation, and surgical stress are known to decrease urine volume (UV) and sodium excretion (*via* stimulation of the renal sympathetic nervous system[6,8,9]). Therefore, the dogs in the current study were long-term tracheotomized weeks before the experiments, and renal function during CMV with PEEP was then evaluated in the trained, awake animals, both before and after bilateral renal denervation during normovolemic and hypervolemic conditions. All experiments were performed during a standardized diet to guarantee a reproducible preexperimental stimulation of the renin–angiotensin–aldosterone system.

Materials and Methods

The study was approved officially in accordance with the German animal protection law (Sen Ges Soz, AZ 58/89).

Animals and Maintenance

Eight pure-bred female beagle dogs, 1–2 yr old, 15.2 ± 2.6 kg body weight, were selected from the central animal laboratories of the Free University of Berlin. The dogs were vaccinated, dewormed, and tested for social behavior and tolerance to urinary bladder catheterization. They were kept under highly standardized conditions: air-conditioned environment (21°C; humidity, 55–60%), animal quarters during the day, and individual compartments during the night. Physical health and well-being was controlled by daily check-ups, including measurements of body temperature and body weight.

Anesthesia and Surgery

Each dog underwent three operations in preparation for the experiments. All operations were conducted during aseptic conditions. Only for the operations, anesthesia was induced with methohexital sodium (~8 mg/kg body weight intravenously) and, after tracheal intubation, maintained with halothane (0.5 to 1.5%) in nitrous oxide and oxygen (2:1).

First Operation. The left common carotid artery was exteriorized long-term (a "carotid loop") to facilitate continuous arterial blood pressure monitoring during the experiments.

Second Operation. Approximately 3 weeks later, a permanent tracheotomy was performed using a technique described by Dalgard *et al.*[10] with minor modifications. Antibiotic prophylaxis that consisted of 1.5 g flucloxacillin intramuscularly and 240 mg gentamycin intramuscularly per day was administered before operation and was continued for 3 days. When surgery was complete, the dogs were given at least 3 weeks to recover. Barking and breathing were not impaired by the tracheotomy.

When the tracheotomy was complete, the dogs were accustomed to the endotracheal tube (size, 7–8 mm inner diameter; Ultra Tracheoflex, Rüsch, Kernen, Germany) and to the experimental conditions. In daily training sessions lasting as many as 4 h, the dogs were placed on an animal table and were connected to a ventilator (Servo Ventilator 900 C; Siemens-Elema, Erlangen, Germany) set to continuous positive airway pressure (CPAP) and CMV mode.

Third Operation. After the dogs finished the first two protocols (see experimental protocols), they were surgically renally denervated. *Via* an abdominal approach, the renal artery, renal vein, and ureter of both kidneys were exposed and stripped of all visible nerves and adventitia for a distance of approximately 2 cm. The stripped areas then were painted with 10% phenol in alcohol. In addition, all connective tissue were cut around the hilus and down to the retroperitoneal muscles. During the operation, the dogs were treated using antibiotics as described before. After 14–21 days of recovery, the experiments were resumed.

At the end of the study, five of the renally denervated dogs were anesthetized, and pieces of tissue were taken from the renal cortex to determine kidney tissue catecholamines. The results were compared with specimens taken from the renal cortex of four healthy beagle dogs that underwent the same dietary regimen and environmental conditions as the dogs in the current study.

Dietary Regimen

For the purpose of equilibration, the dogs were fed a standardized diet beginning at least 5 days before the studies. Each dog received 91 ml water, 2.5 mmol sodium, and 3.5 mmol potassium per kilogram of body weight and day (contained in a mixture of minced meat and rice with an energy content of 227 kJ). The food mash was offered once a day at 2 PM. All dogs finished their meals within 1 h. The dogs were not allowed any other food or water.

Experimental Protocols

Four experimental protocols lasting 240 min each were performed on each of the eight dogs (32 experimental days).

Every experimental day began at 8 AM. A double-lumen central venous catheter (Arrow, Reading, PA) was inserted *via* the external jugular vein during local anesthesia. The position of the catheter tip was determined to be correct when typical atrial pressure tracings were recorded. For continuous arterial blood pressure monitoring and blood sampling, a Teflon cannula (Medicut Argyle, 20 gauge; Sherwood, Tullamore, Ireland) was inserted into the carotid loop. Next, a tracheotomy tube was inserted and the dogs were allowed to breathe spontaneously *via* a ventilator circuit (CPAP). The dogs were placed on their right sides and remained in this position throughout the experiment. The pressure transducers were positioned at the level of the right atrium, and the dogs were given about 30 min to adjust to the experimental situation before the actual protocol began.

During each protocol, the dogs began breathing spontaneously at a CPAP of 4 cm H_2O for 60 min, followed by 120 min of CMV with a PEEP chosen to be 15–17 cm H_2O (mean airway pressure, 20 cm H_2O) (PEEP), followed by 60 min of spontaneous ventilation (CPAP). Respiratory rates during CMV with PEEP were adjusted to each dog's values registered during the first hour of spontaneous ventilation. The trigger sensitivity was set at -0.5 cm H_2O. The inspiratory fraction of oxygen was always 0.21.

This regimen was performed on separate study days during four different conditions (1 and 2 at random; after renal denervation, 3 and 4 at random). The interval between the study days was at least 1 week: (1) normovolemic, kidneys innervated; (2) hypervolemic, kidneys innervated; (3) normovolemic, kidneys denervated; and (4) hypervolemic, kidneys denervated.

Hypervolemia in protocols 2 and 4 was achieved by infusion of a balanced electrolyte solution (Ionosteril; Braun Melsungen, Melsungen, Germany; composed of 137 mM sodium, 4 mM potassium, 110 mM chloride, and 36.8 mM acetate) administered at a rate of 0.5 ml/kg body weight^{-1}·min^{-1} starting at the beginning of the first CPAP period (~ 450 ml/h).

The term *normovolemic* is used for dogs without infusion of an electrolyte solution. Because the dogs are examined 18 h after last food and water intake (see the dietary regimen already described), they should have been at the verge of volume depletion with prestimulated sodium- and water-retaining systems.

Measurements and Calculations

The mean airway pressure, measured *via* a catheter positioned at the distal end of the endotracheal tube, central venous pressure, MAP, and heart rate were recorded continuously. Urine volume was collected at 60 min intervals using a Foley catheter (air washout). and potassium (U_KV) (flame photometry, Eppendorf, Hamburg, Germany), urine osmolality (freezing point depression; osmometer, Roebling, Berlin, Germany), and urine creatinine concentration (modified Jaffé reaction; Creatinine Analyzer 2, Beckman Instruments, Brea, CA) were determined. The glomerular filtration rate (GFR) was assessed by exogenous creatinine clearance, dissolving 1.4 g creatinine in 50 ml D5W, which was infused 30 min before the start of the experiments, followed by a maintenance infusion of 280 mg/h (10 ml/h). Creatinine clearance was calculated using the standard formula. Plasma samples (30 ml) were taken at the end of each hour to measure plasma sodium, potassium, and creatinine concentrations; osmolality; hematocrit level (Hawksley centrifuge); and hormones. Plasma and urinary values given for the PEEP period are the mean of two samples taken after hours 2 and 3. Blood gas analyses were performed at 30-min intervals (ABL 3; Radiometer, Copenhagen, Denmark). Blood samples for plasma hormone determinations were placed into precooled Na-ethylenediaminetetraacetic acid vacutainers and centrifuged at 4°C. The separated plasma was stored at −22°C until analysis. Commercially available radioimmunoassays were used to measure plasma concentrations of antidiuretic hormone (Biermann Company, Bad Nauheim, Germany; intraassay coefficient of variation (CV), 8%; interassay CV, 6%), atrial natriuretic peptide (Henning, Berlin, Germany; intraassay CV, 11.5%; interassay CV, 14%), aldosterone (AldoCtk-2, Sorin, Sallugia, Italy; intraassay CV, 12.7%; interassay CV, 12.4%) and plasma renin activity (PRA), expressed as nanograms of angiotensin I generated per milliliter of plasma per hour of incubation (ng Angiotensin I· ml^{-1}·h^{-1}; New England Nuclear, North Billerica, MA; intraassay CV, 11%; interassay CV, 8.4%). Plasma epinephrine and norepinephrine concentrations were analyzed by high-performance liquid chromatography with electrochemical detection according to the method described by Weicker *et al.*[11] With a 50-μl injection volume and a baseline noise of 1 pA, the detection limits were 0.6 pg/ml norepinephrine and 0.7 pg/ml epinephrine. The CV ranged from 3% to 6%. In evaluating tissue catecholamines, the specimens were weighed, homogenized, and centrifuged at 10,000*g* for 20 min at 4°C. The supernatants were stored

at −80°C until analysis. The catecholamines were then purified and enriched by solid-phase extraction, as described by Smedes *et al.*[12] and Maycock *et al.*[13] High-performance liquid chromatography with electrochemical detection was used for catecholamine analysis.[11]

Any blood that was withdrawn in the experiments was replaced instantly with the same amount of the dog's own blood, which had been collected approximately 1 week before the experiments and stored at 4°C (Biopack CPDA-1; Biotrans, Dreieich, Germany).

Statistical Analysis

Differences between the first CPAP period, PEEP, the final CPAP period, and the time course of different protocols were evaluated by analysis of variance for subsequent measures (Number Cruncher Statistical Systems 97, Jerry Hintze, Kaysville, Utah). *Post hoc* testing of the mean was performed using the Student's *t* test with Bonferroni correction for multiple comparisons. Statistical significance was considered at $P < 0.05$. Values in the text and the tables are mean ± SD.

Results

Completeness of renal denervation was determined in five dogs by measuring renal tissue catecholamines 3 or 4 weeks after denervation, that is, immediately after the dogs finished the last protocol. The wet-tissue norepinephrine concentration in these kidneys averaged 6.6 ± 4.25 ng/g. These concentrations were 2% of those measured in neurally intact dogs, which averaged 322 ± 24 ng/g ($P < 0.05$). Wet-tissue epinephrine concentrations were 8.5 ± 5.8 ng/g in innervated and 0.5 ± 0.4 ng/g in renal denervated dogs ($P < 0.05$).

Renal Function Data

Urine Volume. Renal denervation did not increase UV. Figure 1 shows each dog's urinary excretion rates during the experiments. Among the eight normovolemic dogs, four had relatively great baseline excretion rates of approximately 40–90 μl·kg body weight^{-1}·min^{-1} during the first CPAP period. In these four dogs, UV decreased to approximately 20 μl·kg body weight^{-1}·min^{-1} during PEEP. The other four dogs started with low UV values of 10–20 μl·kg body weight^{-1}·min^{-1}, but these dogs did not have further decreases in UV during PEEP. When PEEP was switched to CPAP again for the last 60 min of the experiment, UV increased markedly in three of the eight dogs, it increased slightly in three, and the same excretion rate was maintained as during PEEP in two dogs ($P < 0.05$; fig. 1).

In hypervolemic dogs, the mean UV during CPAP was approximately 200 μl·kg body weight^{-1}·min^{-1}. This is approximately five times the average UV of normovolemic dogs ($P < 0.05$; fig. 1). Despite continuous volume expansion, UV did not change when CPAP was switched to PEEP (mean UV 220 μl·kg body weight^{-1}·min^{-1}). The UV increased in all hypervolemic dogs when PEEP was switched to CPAP during the last 60 min (mean UV, 410 μl·kg body weight^{-1}·min^{-1}; $P < 0.05$).

Sodium Excretion. In normovolemic dogs, the urinary excretion rate of sodium was low before, during, and after PEEP (values approximately 1 μmol Na·kg body weight^{-1}·min^{-1}). There was no difference between renally innervated and denervated dogs (fig. 2).

In hypervolemic dogs, the urinary excretion rate of sodium was always greater than in normovolemic dogs ($P < 0.05$). It increased continuously from approximately 8 μmol Na·kg body weight^{-1}·min^{-1} during the first CPAP period to approximately 20 μmol Na·kg body weight^{-1}·min^{-1} during PEEP ($P < 0.05$), to 40 μmol Na·kg body weight^{-1}·min^{-1} during the last CPAP period ($P < 0.05$). There was no difference between renally innervated and denervated dogs.

The pattern of fractional sodium excretion resembles that of the urinary excretion rate of sodium because GFR (fig. 2) was relatively stable during all protocols.

Cumulative Sodium and Water Balance. The hypervolemic dogs retained great amounts of sodium and water during the 4-h study period ($P < 0.05$), as determined by the excretion rates of sodium (fig. 2) and urine (fig. 1), which were less than the continuous infusion rates of sodium (68 μmol·kg body weight^{-1}·min^{-1}) and water (500 μl·kg body weight^{-1}·min^{-1}; fig. 3). Renal denervation did not reduce the amounts of sodium and water retained (fig. 3).

Potassium Excretion. In hypervolemic dogs, the urinary excretion rate of potassium was approximately three times more than in normovolemic dogs ($P < 0.05$) and continued to increase even throughout PEEP ($P < 0.05$). There was no difference in the time course of the urinary excretion of potassium between intact and renally denervated dogs (fig. 2).

Plasma Values

Table 1 shows values of plasma sodium, potassium, hematocrit, and osmolality.

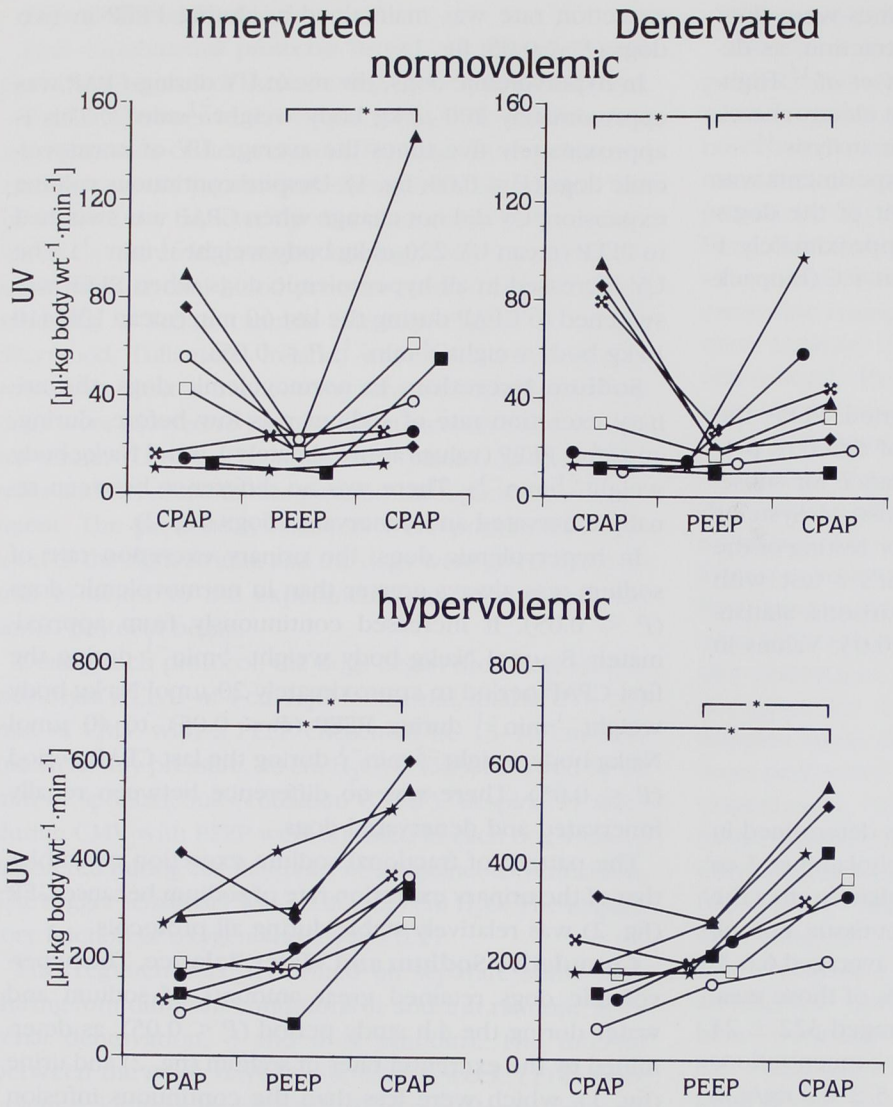

Fig. 1. Individual time courses of mean urinary volume (UV) excretion rates in normovolemic and hypervolemic renal innervated and denervated dogs during 60-min spontaneous respiration with continuous positive-pressure airway pressure of 4 cm H_20 (CPAP), 120-min positive end-expiratory pressure (PEEP), and 60-min CPAP (n = 8). Individual dogs are characterized by identical symbols. *Significant difference ($P < 0.05$).

Plasma Hormones

Plasma Renin Activity. Plasma renin activity was always higher in normovolemic than in hypervolemic dogs ($P < 0.05$), regardless of whether the dogs were renally denervated or intact (fig. 4). At the time of the experiment (2 to 3 weeks after renal denervation), no difference in PRA values were found between renally denervated and intact dogs (hypervolemic: 1.5 ± 1.9 intact *vs.* 1.4 ± 2.4 denervated; normovolemic: 4.3 ± 2.4 *vs.* 3.6 ± 3.6 ng Angiotensin I·ml^{-1}·h^{-1}). In hypervolemic dogs, the already low PRA values decreased even further with ongoing volume expansion ($P < 0.05$). During PEEP, the PRA increase was significant in renally denervated normovolemic dogs only ($P < 0.05$).

Aldosterone. Mean aldosterone in normovolemic dogs was considerably greater than in hypervolemic dogs ($P < 0.05$). During PEEP, aldosterone increased by approximately 125 pg/ml in normovolemic dogs, regardless of whether the dogs were intact or renally denervated ($P < 0.05$) (fig. 4).

Antidiuretic Hormone. In intact and renally denervated normovolemic dogs both, ADH increased markedly during PEEP ($P < 0.05$), whereas in hypervolemic dogs ADH did not change during PEEP (fig. 4).

Atrial Natriuretic Hormone. Neither in intact nor in renally denervated dogs were atrial natriuretic peptide concentrations changed because of PEEP. In summary, the average values for PRA, aldosterone, and ADH were

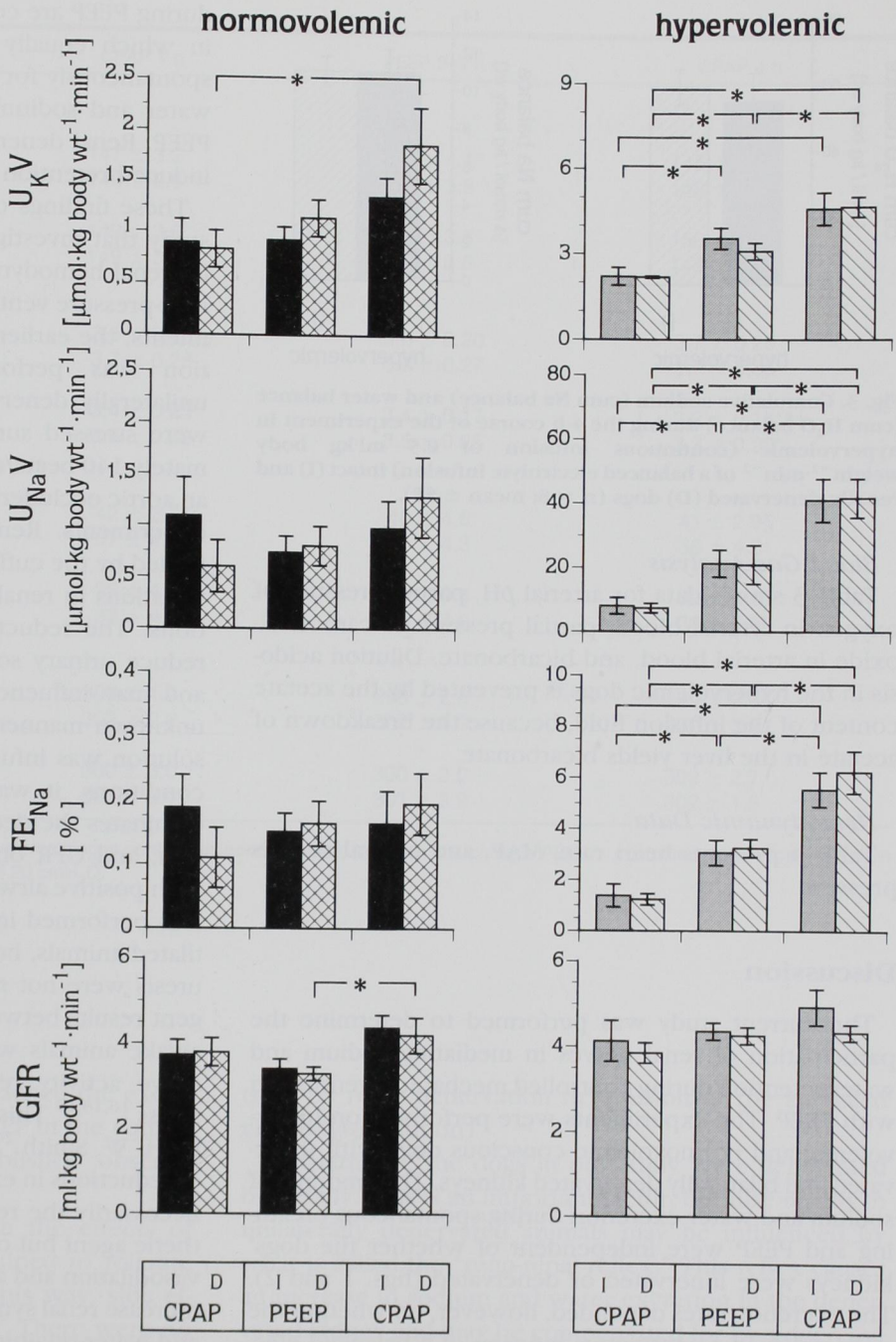

Fig. 2. Potassium excretion ($U_K V$), sodium excretion ($U_{Na} V$), fractional sodium excretion (FE_{Na}), and glomerular filtration rate (GFR) in normovolemic and hypervolemic intact (I) and denervated (D) dogs during 60 min spontaneous respiration with continuous positive-pressure airway pressure (CPAP) of 4 cm H_20 (CPAP), 120-min positive end-expiratory pressure, and again at 60-min CPAP (n = 8). Values are mean ± SE. *Significantly different ($P < 0.05$).

affected by the extracellular volume status of the dogs but were unaffected by renal denervation.

Plasma Catecholamines. There was considerable interindividual variability in the baseline concentrations of norepinephrine and epinephrine (table 2). Concentrations of norepinephrine in renal denervated hypervolemic dogs were increased compared with intact dogs ($P < 0.05$), whereas in renally denervated normovolemic dogs the somewhat greater norepinephrine concentrations did not reach significance compared with the intact dogs. When CPAP was switched to PEEP, epinephrine increased by approximately three times in normovolemic denervated dogs only ($P < 0.05$).

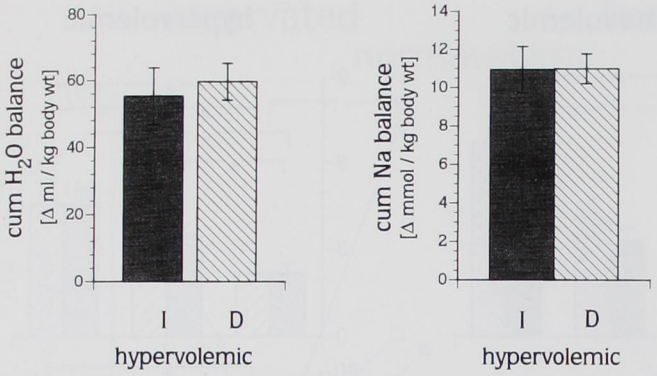

Fig. 3. Cumulative sodium (cum Na balance) and water balance (cum H_2O balance) during the 4-h course of the experiment in hypervolemic (continuous infusion of 0.5 ml·kg body weight^{-1}·min^{-1} of a balanced electrolyte infusion) intact (I) and renally denervated (D) dogs (n = 8; mean ± SE).

Blood Gas Analysis

Table 3 shows data for arterial *p*H, partial pressure of oxygen in arterial blood, partial pressure of carbon dioxide in arterial blood, and bicarbonate. Dilution acidosis in the hypervolemic dogs is prevented by the acetate content of the infusion fluid, because the breakdown of acetate in the liver yields bicarbonate.

Hemodynamic Data

Table 4 presents heart rate, MAP, and central venous pressure.

Discussion

The current study was performed to determine the participation of renal nerves in mediating sodium and water retention during controlled mechanical ventilation with PEEP. The experiments were performed on hypervolemic and normovolemic conscious dogs with innervated and bilaterally denervated kidneys. The amounts of sodium and water excretion during spontaneous breathing and PEEP were independent of whether the dogs' kidneys were innervated or denervated (figs. 1 and 2). The excretion rates depended, however, on whether the dogs received a continuous infusion of a balanced electrolyte infusion during the experiments. Conscious hypervolemic dogs excreted more sodium and water than did normovolemic dogs. Nevertheless, hypervolemic dogs retained considerable amounts of sodium and water because the continuous infusion rates were greater than the simultaneous excretion rates (fig. 3). The water- and sodium-retaining capacity of PEEP ventilation becomes even more obvious when the excretion rates during PEEP are compared with those of a former study in which equally hypervolemic dogs were breathing spontaneously for 4 h.[4] In that study, excretion rates of water and sodium were twice those observed during PEEP. Renal denervation could not overcome the PEEP-induced retention of sodium and water.

These findings contrast with the findings of the only study that investigated the effects of renal denervation on renal hemodynamics and urinary output during positive-pressure ventilation.[14] But unlike the current experiments, the earlier study of the effects of renal denervation was performed in pentobarbital-anesthetized unilaterally denervated dogs. Furthermore, these dogs were stressed surgically (heart rate averaged approximately 140 beats/min and MAP was 130 mmHg) because an aortic occluder cuff was implanted directly before the experiments. Renal perfusion pressure was servocontrolled by the cuff at 100 mmHg to reduce the effects of variations in renal perfusion pressure on urinary excretions. The reduction of renal perfusion pressure may reduce urinary sodium and water excretion, however, and may influence regulation of urinary output in an unknown manner. In addition, an isoosmotic mannitol solution was infused to increase diuresis. During these conditions, it was determined that renal denervation eliminates the decrease in urine volume, sodium excretion, and GFR observed during mechanical ventilation with positive airway pressure.[14] When renal denervation was performed in conscious but not mechanically ventilated animals, however, denervation diuresis and natriuresis were not reproducibly observed.[15–17] The divergent results between renal denervated anesthetized and awake animals were ascribed to increased basal renal nerve activity resulting from anesthesia and surgical stress.[16,18,19] This notion was already suggested in 1937 by H. W. Smith.[20]

Reductions in excretion rates during anesthesia are not necessarily the result of direct drug effects of the anesthetic agent but often are caused by side effects, such as vasodilation and a decrease in arterial pressure, that may increase renal sympathetic activity and stimulate sodium- and water-retaining hormones. For instance, when three of the volume-expanded dogs of the current study were ventilated at a PEEP of 15 cm H_2O during isoflurane–nitric oxide anesthesia (1.1 minimum alveolar concentration), a profound decrease in sodium and urine excretion occurred, together with slight reductions in MAP, renal perfusion pressure, and GFR, and an exorbitantly stimulated renin-angiotensin-aldosterone system. During a 2-h observation period, sodium and water excretion

Table 1. Plasma Values and Hormones

	CPAP 1 h	PEEP 2–3 h	CPAP 4 h
Sodium (mM)			
Normovolemic			
I	147 ± 3.2	146 ± 4.1	145 ± 5.3
D	149 ± 2.6	147 ± 3.8	148 ± 3.9
Hypervolemic			
I	149 ± 3.6	149 ± 3.5	150 ± 3.8*
D	149 ± 3.8	149 ± 3.5	149 ± 5.4
Potassium (mM)			
Normovolemic			
I	3.5 ± 0.23	3.6 ± 0.20	3.7 ± 0.25
D	3.7 ± 0.28	3.7 ± 0.27	3.7 ± 0.35
Hypervolemic			
I	3.4 ± 0.23	3.4 ± 0.17	3.2 ± 0.13*†‡
D	3.6 ± 0.32	3.5 ± 0.25	3.3 ± 0.22*
Hematocrit (%)			
Normovolemic			
I	42 ± 2.7	40 ± 4.0	41 ± 2.9§
D	37 ± 4.7	37 ± 4.3	36 ± 4.2
Hypervolemic			
I	35 ± 4.5	34 ± 3.4	32 ± 4.8*
D	32 ± 4.6	30 ± 4.8	30 ± 5.0*
Osmolality (mosm/kg H_2O)			
Normovolemic			
I	300 ± 1.9	298 ± 2.8	299 ± 2.6§
D	302 ± 2.0	302 ± 4.0	302 ± 3.8
Hypervolemic			
I	300 ± 3.0	300 ± 3.0	302 ± 2.7
D	301 ± 3.1	301 ± 3.9	302 ± 1.6

I = intact; D = renal denervated; CPAP = spontaneous breathing at continuous positive airway pressure of 4 cmH_2O; PEEP = controlled mechanical ventilation with 15 cmH_2O PEEP and a mean airway pressure of 20 cmH_2O.

Values are mean ± SD; n = 8.

* Normovolemic *versus* hypervolemic ($P < 0.05$).

† PEEP *versus* CPAP 4 h ($P < 0.05$).

‡ CPAP 1 h *versus* CPAP 4 h ($P < 0.05$).

§ Innervated *versus* renal denervated ($P < 0.05$).

rates in the anesthetized dogs never reached the excretion rates found at the same level of PEEP in the equally hypervolemic conscious dogs (unpublished observations).

Therefore, we decided to determine the effects of PEEP ventilation in conscious dogs trained to tolerate controlled mechanical ventilation. In this way, side effects of anesthesia were avoided.[16,19,21] There were no apparent signs of excitation or stress in the animals in this study; for example, heart rates were approximately 95 beats/min and MAP was approximately 108 mmHg (table 4).

Because we fed the animals a strict diet for 5 days before each protocol, any increase in PRA, aldosterone, ADH, or atrial natriuretic peptide had to be caused by factors other than differences in sodium or water intake (*e.g.*, by renal denervation or infusion of the balanced electrolyte solution).

Furthermore, the dogs in our study were denervated bilaterally. This is an important factor, because results in unilaterally denervated animals may be influenced by what is called the "reno-renal reflex." This reflex causes an increase in sodium and water excretion in the denervated kidney and may be compensated for by a decrease in sodium and water excretion in the innervated kidney.[22] The extent of interdependence between the innervated and denervated kidney cannot be foreseen, however. This shortcoming makes interpretation of data obtained in unilaterally denervated animals difficult.

Interpretation of the effects of acute and chronic renal denervation necessitates that special consideration be given to verification of renal denervation and renal rein-

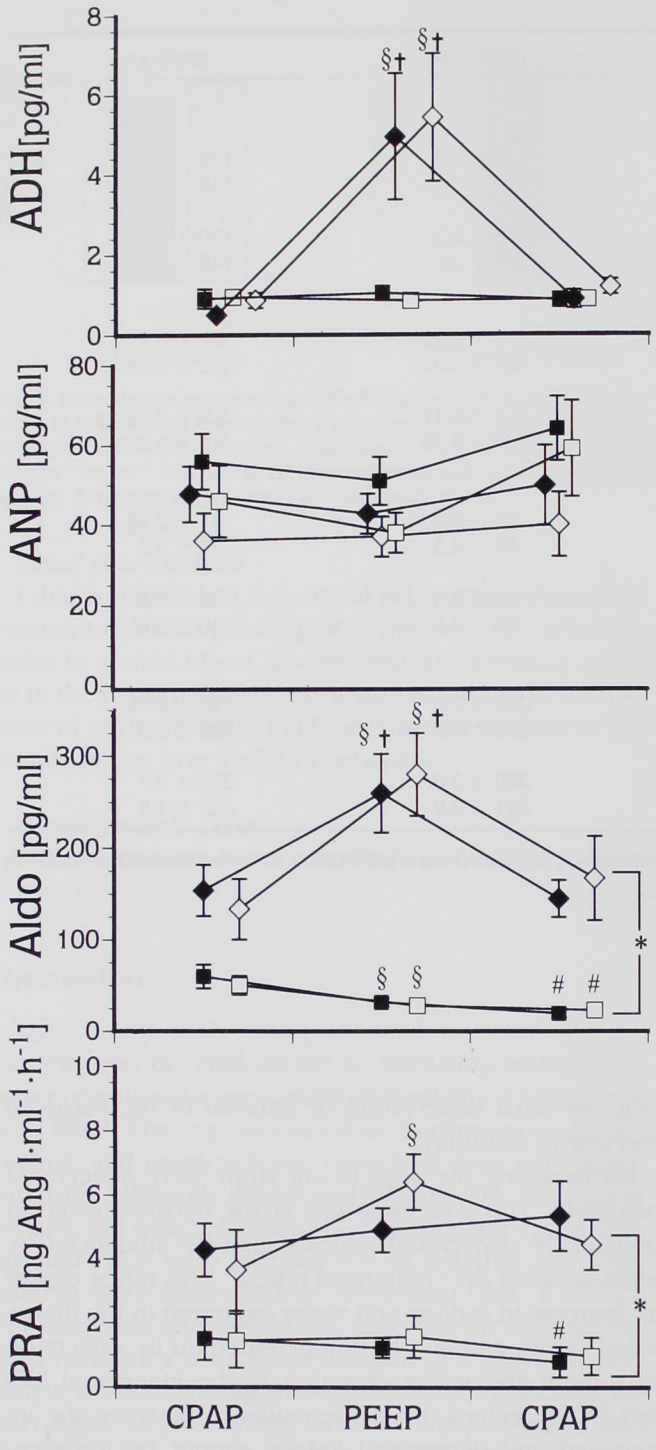

Fig. 4. Plasma values of antidiuretic hormone (ADH), atrial natriuretic hormone (ANP), aldosterone (Aldo), plasma renin activity (PRA) during spontaneous respiration with continuous positive-pressure aiway pressure (CPAP) of 4 cm H_20, positive end-expiratory pressure (PEEP), and again CPAP. ♦ Normovolemic, intact; ♡ normovolemic, renally denervated; hypervolemic, intact; □ hypervolemic, renally denervated (n = 8; means ± SE). Significant differences ($P < 0.05$): #CPAP first hour *vs.* CPAP fourth hour; §CPAP first hour *vs.* PEEP; † PEEP *vs.* CPAP fourth hour; * normovolemic *vs.* hypervolemic.

nervation. Low renal tissue norepinephrine content generally is taken as evidence of complete renal denervation and was also determined in the current study. The values obtained were similar to those found by other investigators.[23] Therefore, the failure to detect any difference in sodium and water excretion among mechanically ventilated conscious dogs with innervated and denervated kidneys cannot be attributed to incomplete renal denervation.

Denervation supersensitivity toward circulating catecholamines could be one of the factors that obscured differences between intact and renally denervated conscious dogs, such as by affecting renal hemodynamics, by tubular adrenergic receptors remaining within the denervated kidney, or by both.[6,24] Plasma catecholamines also were measured in this study. Norepinephrine concentrations in renally denervated hypervolemic dogs were found increased above those of intact dogs. However, dissimilar to the results in studies in unilaterally denervated anesthetized animals,[14] there were apparently no major differences in the overall renal hemodynamics among our intact and bilaterally denervated dogs, because GFR was maintained at almost constant values. This does not exclude differences in the

Table 2. Plasma Catecholamines

	CPAP 1 h	PEEP 2–3 h	CPAP 4 h
Norepinephrine (pg/ml)			
Normovolemic			
I	275 ± 224	254 ± 185	208 ± 180
D	303 ± 376	351 ± 345	276 ± 407
Hypervolemic			
I	188 ± 157	220 ± 194	190 ± 185
D	367 ± 398	426 ± 299	433 ± 455*
Epinephrine (pg/ml)			
Normovolemic			
I	194 ± 164	237 ± 135	234 ± 189
D	107 ± 57	343 ± 232†	150 ± 76
Hypervolemic			
I	135 ± 74	178 ± 68	158 ± 114
D	151 ± 139	156 ± 105	121 ± 136

I = intact; D = renal denervated; CPAP = spontaneous breathing at continuous positive airway pressure of 4 cmH_2O; PEEP = controlled mechanical ventilation with 15 cmH_2O PEEP so that mean airway pressure is 20 cmH_2O.

Values are mean ± SD; n = 8.

* Intact *versus* renal denervated ($P < 0.05$).

† CPAP 1 h *versus* PEEP ($P < 0.05$).

Table 3. Airway Pressure and Arterial Blood Gases

	CPAP 1 h	PEEP 2–3 h	CPAP 4 h
P_{AW} (cmH_2O)			
Normovolemic			
I	4.1 ± 0.4	19.1 ± 1.7*†	4.8 ± 1.1
D	4.2 ± 0.9	18.1 ± 2.1*†	4.3 ± 0.5
Hypervolemic			
I	4.0 ± 0.8	19.2 ± 1.3*†	4.8 ± 1.2
D	5.1 ± 1.4	20.1 ± 0.9*†	5.8 ± 1.8
Pa_{O_2} (mmHg)			
Normovolemic			
I	103 ± 14	106 ± 12	101 ± 16‡
D	94 ± 5	99 ± 6	93 ± 9
Hypervolemic			
I	98 ± 11	101 ± 7	98 ± 8
D	101 ± 13	100 ± 10	101 ± 17
Pa_{CO_2} (mmHg)			
Normovolemic			
I	33 ± 7.2	33 ± 4.1	34 ± 5.8
D	36 ± 4.3	34 ± 5.2	32 ± 4.4
Hypervolemic			
I	34 ± 4.3	36 ± 3.7	34 ± 1.9
D	34 ± 6.4	35 ± 2.5	36 ± 5.5
pH			
Normovolemic			
I	7.41 ± 0.08	7.40 ± 0.04	7.41 ± 0.04
D	7.39 ± 0.04	7.40 ± 0.03	7.44 ± 0.06†¶
Hypervolemic			
I	7.43 ± 0.03	7.43 ± 0.02	7.46 ± 0.04
D	7.44 ± 0.08	7.44 ± 0.04	7.43 ± 0.03
Arterial HCO_3^- (mM)			
Normovolemic			
I	21 ± 1.9	21 ± 1.6	22 ± 2.5
D	22 ± 2.9	22 ± 2.7	23 ± 2.2
Hypervolemic			
I	23 ± 2.2	24 ± 2.1	25 ± 1.6§
D	23 ± 2.2	25 ± 1.6	25 ± 4.6§

I = renal innervated; D = renal denervated; CPAP = spontaneous breathing at continuous positive airway pressure of 4 cmH_20; PEEP = controlled mechanical ventilation with 15 cmH_2O PEEP so that mean airway pressure is 20 cmH_2O.

Values are means ± SD; n = 8 dogs.

* CPAP 1 h *versus* PEEP ($P < 0.05$).

† PEEP *versus* CPAP 4 h ($P < 0.05$).

‡ Intact *versus* renal denervated ($P < 0.05$).

§ CPAP 1 h *versus* CPAP 4 h ($P < 0.05$).

¶ CPAP 1 h *versus* CPAP 4 h ($P < 0.05$).

intrarenal corticomedullary perfusion ratio, however, which may not be visible in changes of GFR.

In evaluating the results of this study, it is reasonable to conclude that in bilaterally denervated conscious dogs the renal nerves have no appreciable contribution to the regulation of renal sodium and water excretion during spontaneous breathing and CMV with PEEP. Therefore, the question remains, "What, if not the renal nerves, are the factors that decrease sodium and water excretion in conscious dogs during CMV with PEEP?"

The Renin-Angiotensin-Aldosterone System. Plasma renin activity and angiotensin II have been shown to increase during CMV with PEEP in anesthetized animals and patients.[25–28] During anesthesia, a decrease in MAP often increases renal sympathetic nerve activity *via* a decrease in carotid sinus and aortic arch baroreceptor activity.[29] Furthermore, the renal baroreflex mechanism becomes activated when renal perfusion pressure decreases. This also augments renin secretion. Finally, the PEEP-induced decrease in atrial transmural pressure may increase renin release by reducing vagal inhibition on atrial or cardiopulmonary receptors.[30]

In the conscious dogs, MAP did not decrease when the dogs were switched from spontaneous breathing to CMV with PEEP. Conversely, MAP even increased in the hypervolemic dogs because of the continuous infusion of the electrolyte solution (table 4). The expansion of the extracellular volume in these dogs was combined

Table 4. Hemodynamic Data

	CPAP 1 h	PEEP 2–3 h	CPAP 4 h
HR (beats/min)			
Normovolemic			
I	85 ± 25	97 ± 18	82 ± 18
D	86 ± 22	105 ± 20*†	86 ± 18
Hypervolemic			
I	92 ± 21	95 ± 32	99 ± 23
D	100 ± 21	100 ± 16	104 ± 23
MAP (mmHg)			
Normovolemic			
I	108 ± 15	103 ± 11	107 ± 16
D	100 ± 13	104 ± 14	105 ± 12
Hypervolemic			
I	106 ± 11	116 ± 10*	119 ± 10‡§
D	106 ± 12	114 ± 12*	119 ± 10‡§
CVP (cmH_2O)			
Normovolemic			
I	3.5 ± 1.9	7.7 ± 2.6*†	4.4 ± 1.6
D	4.6 ± 3.3	9.6 ± 2.9*†	6.0 ± 1.5
Hypervolemic			
I	4.5 ± 2.6	9.4 ± 2.6*†	6.1 ± 2.2
D	5.9 ± 2.6	10.4 ± 3.4*†	8.1 ± 4.2‡

HR = heart rate; MAP = mean arterial blood pressure; CVP = central venous pressure; I = renal innervated; D = renal denervated; CPAP = spontaneous breathing at continuous positive airway pressure of 4 cmH_2O; PEEP = controlled mechanical ventilation with 15 cmH_2O PEEP so that mean airway pressure is 20 cmH_2O.

Values are mean ± SE; n = 8 dogs.

* CPAP 1 h *versus* PEEP ($P < 0.05$).

† PEEP *versus* CPAP 4 h ($P < 0.05$).

‡ CPAP 1 h *versus* CPAP 4 h ($P < 0.05$).

§ Normovolemic *versus* hypervolemic ($P < 0.05$).

with constant PRA and slightly decreasing aldosterone values during CMV with PEEP. In contrast, the larger overall PRA and aldosterone values in the normovolemic—or borderline hypovolemic—dogs reflect the prestimulation of sodium- and water-retaining hormonal systems in these dogs.[3,6] With regard to PRA, the additional stimulatory effect of PEEP ventilation becomes visible in normovolemic renally denervated dogs only, whereas aldosterone concentrations were increased during PEEP in intact and renally denervated normovolemic dogs both (fig. 4). Because not only PRA but also plasma potassium and sodium concentrations remained unchanged in normovolemic intact dogs, aldosterone release must have been stimulated by other mechanisms, such as the influence of ADH on pituitary adrenocorticotropic hormone secretion.[31] A similar change in the PRA:aldosterone ratio during PEEP was also observed in one of our previous studies.[2]

The increase in PRA during PEEP ventilation in the normovolemic denervated dogs could have been prompted by the simultaneous increase in plasma epinephrine concentration. Epinephrine has been shown to increase renin secretion when infused intravenously in chronically renally denervated dogs.[32]

The reasons overall PRA values were not less in renally denervated compared with intact dogs are manifold and include denervation-induced alterations in intrarenal blood pressure distribution and blood flow, activation of the extrarenal vascular renin-angiotensin system, and supersensitivity of the renin-secreting mechanisms toward catecholamines.[6,33]

Antidiuretic Hormone. Antidiuretic hormone concentration often has been found to be increased during PEEP ventilation in anesthetized and conscious normovolemic subjects, but it has also been shown that the increase in ADH can be prevented completely by extracellular volume expansion.[2,34,35] These findings correspond with the results of the current study and were true for intact and renally denervated dogs both. In the current study, ADH release may have been augmented by a decrease in atrial or ventricular transmural pressure, or both, as a result of the application of PEEP, because MAP, plasma osmolality, and blood gas partial pressures remained at nearly constant values during PEEP.[36] There is evidence that transmural pressures in hypervolemic subjects are relatively higher than during CMV with PEEP, transmural pressures in normo- or hypovolemic animals.[37] In the current study, central venous pressures in the normovolemic dogs were not significantly less compared with the hypervolemic dogs ($P = 0.056$). It is possible, however, that the high intrathoracic pressure during PEEP, which causes the central venous pressure to increase by 4 or 5 cm H_2O in normovolemic and in hypervolemic dogs, masked the small differences present. Nevertheless, it may be assumed that relatively lower transmural pressures in the normovolemic dogs stimulated ADH release during the PEEP period.

Atrial Natriuretic Peptide. Atrial natriuretic peptide in some studies has been shown to decrease during PEEP,[38] but we and others,[39] during different conditions, could not demonstrate this relation.

Renal Venous Pressure. Furthermore, CMV with PEEP causes an increase in renal venous pressure because of the increase in intrathoracic pressure. In a previous study from our laboratory, it was shown that this increase in inferior vena caval pressure may contribute remarkably to the water- and sodium-retaining effects of CMV with PEEP in hypervolemic dogs,[5] without any stimulation of sodium- and water-retaining hormones.

In conclusion, the current experiments with hypervolemic and normovolemic conscious dogs show that the renal nerves *per se* have no substantial role in water and sodium retention during CMV with PEEP.

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