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Effects of Atrial Natriuretic Peptides and Furosemide in Conscious Dogs (42590B)

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Abstract. Effects of ANF(8-33) and Auriculin A on renal variables were investigated in conscious water-diuretic dogs. The two substances were injected intravenously (1.08 μg/kg in 3 min) or ANF(8-33) was infused (0.2 μg/kg × min in 20 min). The effects were compared to those of an equinatriuretic dose of furosemide (1.0 μg/kg × min). Injections caused increases in sodium excretion, diuresis, and osmolar clearance. No significant change in exogenous creatinine clearance (C_{CREA}) occurred. Infusion of ANF(8-33) decreased blood pressure by 14% ($P < 0.01$) and increased sodium excretion by a factor of 10 ($P < 0.01$). The natriuresis was a function of increases in diuresis and urinary sodium concentration, the latter by a factor of 6 ($P < 0.01$). Diuresis and free-water clearance (C_{H_2O}) increased by 60% ($P < 0.01$), but urine osmolality did not change significantly. After the infusion a significant decrease in PAH clearance (C_{PAH}) ($P < 0.01$) was observed. Filtration fraction (FF) did not change. The furosemide natriuresis appeared later than that of ANF without significant deviations in diuresis, C_{H_2O}, C_{CREA}, C_{PAH}, and FF; urine osmolality increased by 35% ($P < 0.01$). The effects of ANF(8-33) differ from those of furosemide in several ways. First, the onset of natriuresis is faster; second, the natriuresis is associated by marked increases in diuresis and free-water clearance but not in urine osmolality; and third, natriuresis is followed by a reduction in renal blood flow. The rapid natriuresis of ANF can occur without changes in glomerular filtration rate.

Granula in cardiocytes were described for the first time in 1956 (1). More than two decades later it was reported (2) that the size of the granula varies with changes in extracellular volume: a load of sodium decreased the size of the granula, and sodium and water restrictions caused an increase. Recently the size of the granula was suggested to be dependent on the rate of synthesis and metabolism of atrial natriuretic factor (3). In a persuasive series of experiments deBold *et al.* demonstrated that injections of extracts of rat atria caused natriuresis and massive diuresis in contrast to identically prepared extracts of ventricular cardiac tissue (4). The subsequent studies led to isolation, characterization, and synthesis of a number of atrial natriuretic peptides. The atrial natriuretic factor, ANF(8-33), was synthetized in 1983 (5). The renal effects of atrial extracts have been compared with those of furosemide in several studies (6–8); the results suggest some similarities between the actions of the two compounds.

The conscious dog in servo-maintained water diuresis provides a model which allows precise studies of renal excretory patterns at 5-min time resolution as long as urine flow remains high. Under these conditions potentially misleading dead-space effects are virtually absent. In the present study we have used this model to compare the effects of injections of several atrial natriuretic peptides and to evaluate the changes elicited by infusion of ANF(8-33) on the basis of infusion of furosemide which caused a comparable increase in sodium excretion.

Materials and Methods. Experiments were performed on eight conscious dogs of both sexes (one castrated male and seven females) weighing 12 to 16 kg. They were fed once daily with commercial dog food, Latz Komplett mixed with Latz canned dog food, and had free access to tap water. The intakes of sodium and potassium were measured regularly and averaged 4.2 ± 0.7 and 3.6 ± 0.5 mmole/day × kg body wt, respectively. The dogs were trained to accept percutaneous catheterization and catheterization of the bladder and to stand quietly for several hours supported by a canvas sling. Each dog was used for all types of experiments with intervals of more than 1 week.

Prior to experiments the dogs were fasted for 18 hr without access to water. A sterile catheter was placed in the atrial area via an external jugular vein and another in a saphenous vein. A third catheter (angiocath) was placed in a carotid artery (the common carotid arteries had been placed in skin loops prior to

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the experiments). A modified silicone Foley catheter was inserted into the bladder. The dogs were hydrated by a gastric tube with a water load equivalent to 2% of body weight; thereafter the weight of the animal was kept constant by use of a servo-mechanism infusing a hypotonic solution of glucose and urea, 40 and 25 m*M*, respectively. After the hydration a bolus injection of creatinine and *para*-amino hippuric acid (PAH) was given, and a continuous infusion of both was started at a rate of 0.25 ml/min in order to allow measurements of creatinine and PAH clearances. The latter clearance was measured as described by Åsheim *et al.* (9). One hour later, when a steady-state water diuresis was achieved, sampling of urine was started. After four control periods of 5 min either an injection of a natriuretic peptide, lasting 3 min, or a continuous infusion of ANF(8-33) or furosemide lasting 20 min was carried out. Peptide or furosemide was dissolved in a hypotonic glucose–urea–Haemaccel solution, using the above-mentioned glucose–urea solution and Haemaccel (Polygelinum, Behringwerke AG, Marburg (Lahn), West Germany) in a ratio of 9 to 1. Haemaccel was added to avoid adsorption of peptide to tubing and glassware. Peptide (ANF(8-33) or Auriculin A) was injected at a dose of 1.08 μg/kg (n = 5). When infused, ANF(8-33) was given at a rate of 0.2 μg/kg × min (n = 8), while furosemide was infused at a rate of 1.0 μg/kg × min (n = 8). These doses were chosen in order to facilitate comparisons with previous results (10). Blood samples of 4.5 ml were obtained from the saphenous vein throughout the experiments. Mean arterial blood pressure was registered continuously. Urine was sampled at intervals of 5 min. The servo-mechanism made it possible to keep the body weight of the dogs within 102.0 ± 0.2% of the prehydration value (11).

Control experiments. The results were compared to time-control experiments performed by Baerwolff *et al.* (12), who in six animals used a protocol very similar to the present one except that urine was sampled at 10-min periods.

Analyses. Measurements of sodium and potassium were carried out by flame photometry (IL 243 LED flame photometer). Concentrations of PAH and creatinine in plasma and urine were measured by spectrophotometry (Spectronic 1001, Bausch & Lomb). The osmolalities of plasma and urine were estimated by freezing-point depression using an osmometer (Digimatic osmometer Model 3DII, Advanced Instruments).

Active substances. ANF(8-33) was synthetized and kindly provided by Merck & Co., New Jersey. Auriculin A was purchased from Peninsula Laboratories, California. Commercially available furosemide was used.

Statistics. Results are given as means ± SE. Data were subjected to one-way analysis of variance for repeated measures (13). Because of variance inhomogeneity, as indicated by Bartlett's test (13), the data for sodium excretion were transformed logarithmically prior to analysis of variance. In case of significantly large F values all possible differences were evaluated systematically by Newman–Keuls' test (13). The level of significance was 0.05 unless otherwise indicated. Values significantly different from all preinfusion periods are marked with filled symbols.

Results. *Injections.* Injection of natriuretic peptide was performed over the first 3 min of a 5-min urine sampling period. ANF(8-33) (Fig. 1) and Auriculin A (Fig. 2) elicited similar changes in sodium excretion, diuresis, and osmolar clearance. The increase in sodium excretion was observed despite no measurable change in C_{CREA}.

Infusion of ANF. Infusion of ANF(8-33) caused a decrease (−14%, $P < 0.01$) in mean arterial blood pressure, MABP (Table I). Sodium excretion in absolute figures (Fig. 3) as well as in fraction of filtered load increased 10-fold over that of control ($P < 0.01$). ANF(8-33) elicited a rise within 5 min after the start of the infusion and the rate of excretion was above control level until the termination of the infusion, although a statistically significant reversal toward control level was observed already during the infusion. The increase in sodium excretion was due to an increase in diuresis as well as an increase in urinary sodium concentration from an average of 1.0 ± 0.2 to 6.5 ± 1.6 mmole/liter ($P < 0.01$). Diuresis and free-water clearance (Fig. 4) increased by 60% ($P < 0.01$). The osmolality (Fig. 5) of the urine, however, did not change significantly during the experiment. C_{CREA} (Fig. 6) showed no significant changes compared to the time-control experiments. C_{PAH} (Fig. 6) showed a decrease

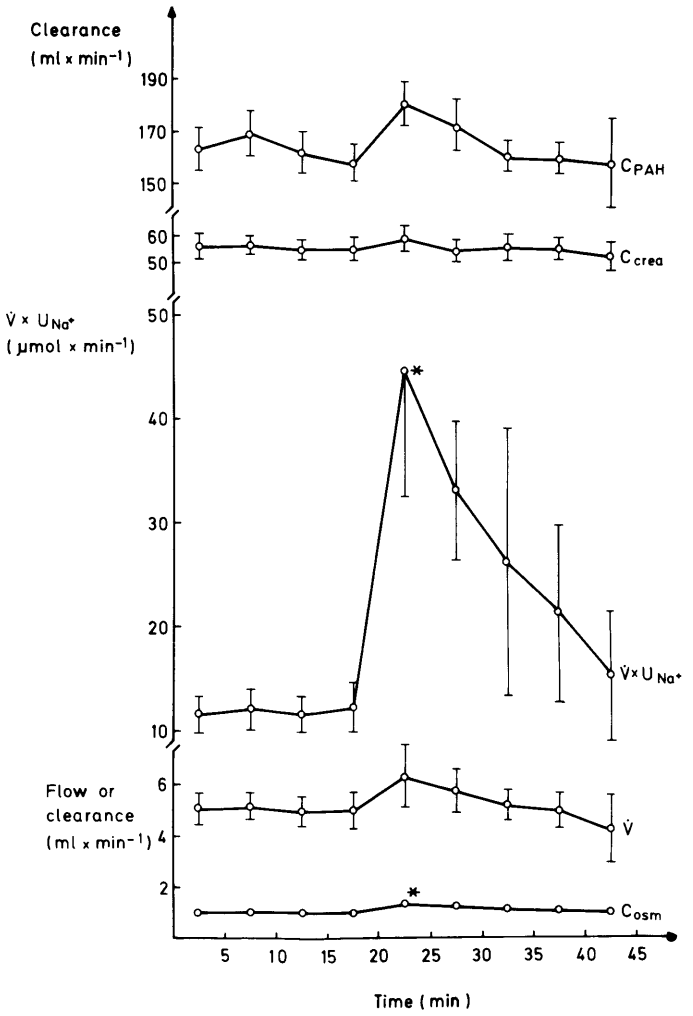

FIG. 1. Creatinine and PAH clearances, sodium excretion, diuresis, and osmolar clearance (means ± SE; $n = 5$). ANF(8-33) was injected over 3 min at a dose of 1.08 µg/kg beginning at the start of the fifth urine-sampling period. The injection period is marked with a black bar. Those values significantly different from the values of the preinfusion periods are marked with an asterisk (*).

after ANF(8-33) infusion but no significant changes during the infusion. There was no detectable change in filtration fraction.

Infusion of furosemide. Furosemide as ANF(8-33) caused a marked and significant increase in sodium excretion ($P < 0.01$) (Fig. 3); however, a delay of 5 min compared to ANF(8-33) was apparent. The effect of furosemide was present after the infusion, but sodium excretion did return to control level 15 min after termination of the infusion. The duration of the furosemide-induced natriuresis was thus similar to that of ANF(8-33). Diuresis and free-water clearance (Fig. 4) showed no statistically significant changes during infusion of furosemide. In contrast to the lack of change during ANF(8-33) infusion, urinary osmolality (Fig. 5) showed a marked increase (35%; $P < 0.01$) within the period of the infusion of furosemide. The increase in osmolality rather precisely reflected the increase in rate of sodium excretion. Furosemide did not cause any significant changes in C_{CREA} or C_{PAH} (Fig. 6).

Discussion. Our data elucidate several facets

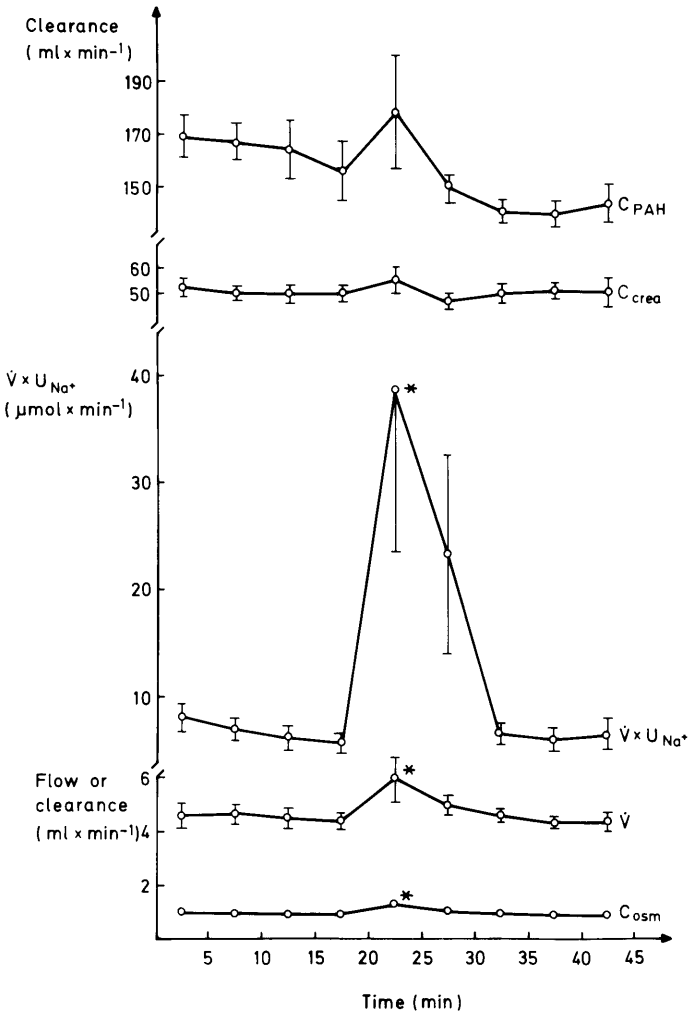

FIG. 2. Creatinine and PAH clearances, sodium excretion, diuresis, and osmolar clearance (means ± SE; $n = 5$). Auriculin A was injected over 3 min at a dose of 1.08 µg/kg beginning at the start of the fifth urine-sampling period. The injection period is marked with a black bar. Those values significantly different from the values of the preinfusion periods are marked with an asterisk (*).

of the actions of ANF(8-33): First, in contrast to furosemide, ANF(8-33) immediately increases free-water excretion in the water-diuretic dog. Second, the onset of the effect of ANF(8-33) on sodium excretion is more rapid than that of furosemide. Third, ANF(8-33) causes a decrease in C_{PAH} after termination of the infusion with no detectable change in filtration fraction.

In the present study in trained conscious dogs the cardiovascular response to ANF(8-33) infusion included a decrease in MABP. This is in line with the results appearing from *in vitro* studies, where atrial natriuretic substances were demonstrated to be smooth muscle relaxants (14, 15), and with the hypotensive effect observed in anesthetized rats (16) and in conscious dogs (17). The decrease is most likely a direct effect of ANF(8-33) on the cardiovascular system not related to the increase in sodium excretion (see below). The fall in MABP could have been elicited by the vasorelaxant effect of ANF(8-33) or by a decrease in cardiac output as described in the anesthe-

TABLE I. MEAN ARTERIAL BLOOD PRESSURE DURING ANF INFUSION

Period	Mean (mm Hg)	SE (mm Hg)
4	105.1	4.1
5	94.5*	3.6
6	95.3*	3.0
7	92.9*	3.0
8	90.8*	2.1
10	94.1*	2.5

Note. Data are expressed as means and SE; $n = 8$. ANF was infused at a rate of 0.2 μg/kg × min during the periods 5 to 8. The periods lasted 5 min. Values marked with an asterisk (*) are significantly different from those of the preinfusion level ($P < 0.01$).

tized rats (16) and in conscious dogs (17). In the latter, total peripheral resistance did not show any changes. However, whether the decrease in cardiac output is due to a reduced preload to the heart (18) or a decrease in cardiac contractility remains to be determined. It is notable that in the conscious rat significant changes in cardiac output could not be registered (19). These rats were, however, uninephrectomized and hypertensive due to a clip on the remaining renal artery.

Reports of the responses in renal perfusion and filtration rates to ANF are quite variable. In some studies it was not possible to register any changes in glomerular filtration rate, GFR (4, 20, 21), but others have described an increase in GFR (22–24). A dose-dependent increase has also been described in anesthetized animals or on isolated kidneys (25, 26). In conscious dogs it has been described that ANF was able to elicit an increase in GFR (17). The results were, however, achieved using doses considerably higher than those used in the present experiments and it is notable that a significant increase was measurable only in the first of three consecutive 10-min infusion periods.

During the infusion in the present experiments there was a tendency toward an increase in C_{PAH}, but in contrast to recent results in conscious one-clip, two-kidney hypertensive rats (27) and in conscious dogs (17), no significant change was observed. Neglecting a possible but small decrease in CVP (18) it can, however, be calculated that renal vascular resistance decreased from a preinfusion value of 0.80 to less than 0.67 mm Hg × min/ml during the infusion ($P < 0.01$). So we found a definite

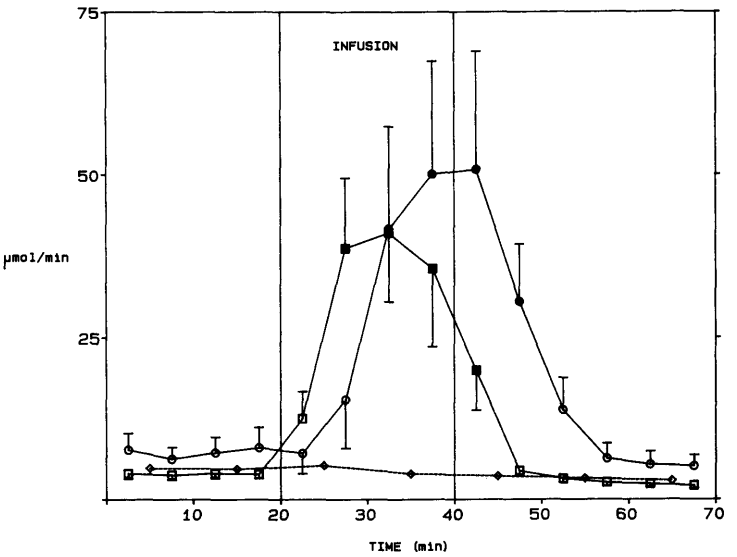

FIG. 3. Sodium excretion (means ± SE; $n = 8$). ANF(8-33) or furosemide was infused for 20 min at a rate of 0.2 and 1.0 μg/kg × min, respectively. ANF(8-33) is marked with a □, and furosemide is marked with a ○. Values obtained in control studies (12) are marked with a ◇ ($n = 6$). Those values significantly different from the values of all preinfusion periods are marked with filled symbols.

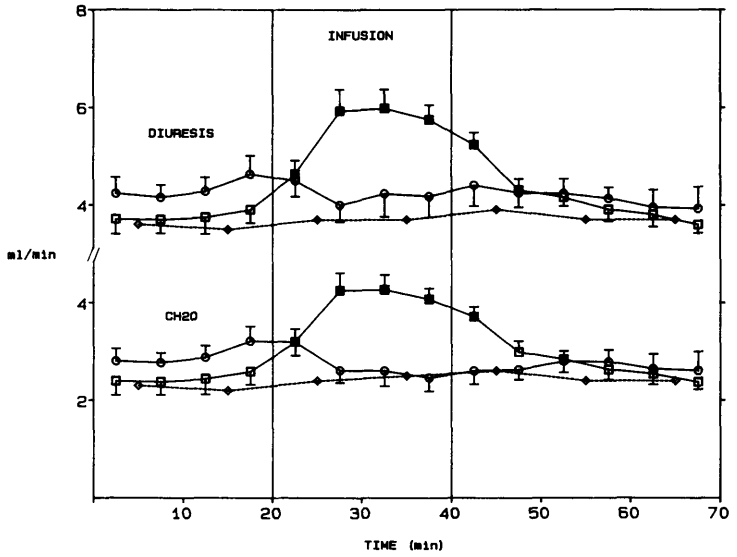

FIG. 4. Diuresis and free-water clearance (means ± SE; n = 8). ANF(8-33) or furosemide was infused for 20 min at a rate of 0.2 and 1.0 μg/kg × min, respectively. ANF(8-33) is marked with a □, and furosemide is marked with a ○. Values obtained in control studies (12) are marked with a ◇ (n = 6). Those values significantly different from the values of all preinfusion periods are marked with filled symbols.

fall in renal vascular resistance but no significant increase in C_{PAH}. Use of PAH clearance as a measure of renal plasma flow depends primarily on a constant extraction of PAH. The possibility must be considered that ANF(8-33) could influence the proximal tubular secretion of PAH and in that way change PAH clearance in a negative direction. This

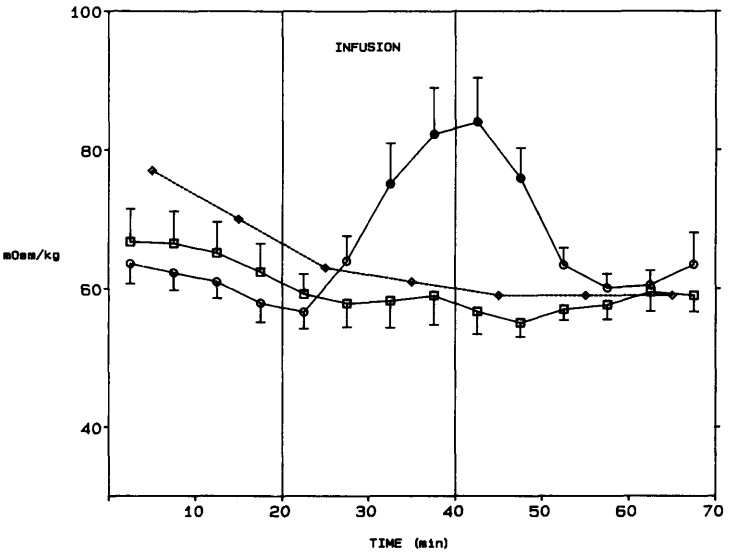

FIG. 5. Urinary osmolality (means ± SE; n = 8). ANF(8-33) or furosemide was infused for 20 min at a rate of 0.2 and 1.0 μg/kg × min, respectively. ANF(8-33) is marked with a □, and furosemide is marked with a ○. Values obtained in control studies (12) are marked with a ◇ (n = 6). Those values significantly different from the values of all preinfusion periods are marked with filled symbols.

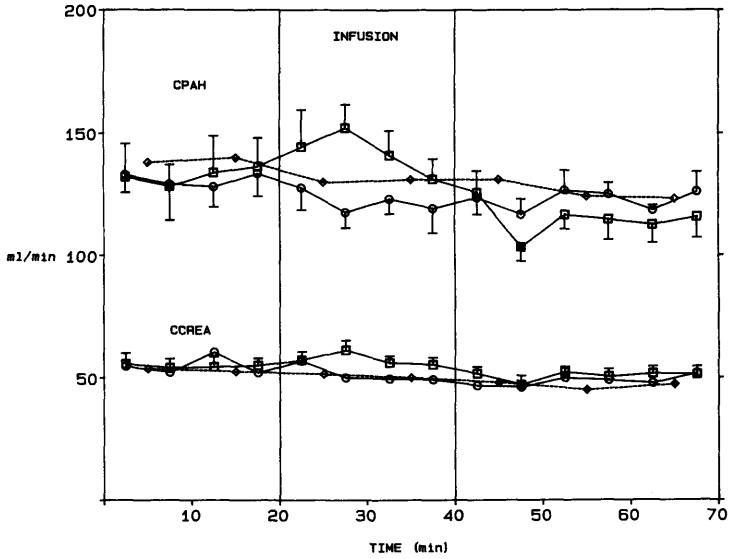

FIG. 6. Creatinine and PAH clearances (means ± SE; $n = 8$). ANF(8-33) or furosemide was infused for 20 min at a rate of 0.2 and 1.0 μg/kg × min, respectively. ANF(8-33) is marked with a □, and furosemide is marked with a ○. Values obtained in control studies (12) are marked with a ◇ ($n = 6$). Those values significantly different from the values of all preinfusion periods are marked with filled symbols.

could be the reason for the lack of significant increases during the infusion. Our average plasma concentration of PAH was about 125 μmole/liter, equivalent to 9 to 25% of the reported saturation threshold for secretion of PAH (28, 29). In addition, changes in PAH clearance did not occur during furosemide administration or during control experiments. As furosemide is secreted by the proximal tubule our results indicate that under the present circumstances renal handling of PAH is independent of this change in proximal tubular secretory activity. However, other factors influence the extraction of PAH in the kidney and may thus prevent variations in PAH clearance from reflecting changes in renal plasma flow. It is known (30) that renal PAH extraction varies due to individual factors, with the degree of diffusion into erythrocytes, and with changes in intrarenal blood flow distribution in addition to the variation caused by possible fluctuations in plasma concentrations and proximal tubular transport. Nevertheless, it appears most likely that changes in repeatedly measured PAH clearances in the same dog over a few hours at constant plasma concentrations represent changes in renal plasma flow.

In some aspects the decrease in C_{PAH} that we observed *after* ANF infusion (paralleled by an increase in renal vascular resistance from a preinfusional level of 0.80 to 0.93 mm Hg × min/ml ($P < 0.01$)) resembles the response to intrarenal infusion of larger amounts of ANF described by Burnett *et al.* (31). However, in the present experiments the evidence of renal vasoconstriction was not associated with measurable increases in C_{CREA} or filtration fraction. Decreases in renal plasma flow after infusion of ANF have also been described in anesthetized dogs by Maack *et al.* (32) using PAH clearance as a measure of renal plasma flow, but the decrease in renal plasma flow was associated in those experiments with an increase in filtration fraction. Our results could indicate that ANF(8-33) activates renal vasoconstrictor mechanisms as a reaction to the vasorelaxant effect of the hormone. In addition, it is conceivable that vasoconstriction could also be part of the response to the concomitant natriuresis via a decrease in extracellular volume, ECV. However, it can be calculated that the natriuresis in response to ANF(8-33) in the present experiments corresponds to a decrease in ECV of less than 0.1%. Therefore, the decrease in C_{PAH} after ANF in-

fusion is hardly a consequence of the preceding renal sodium loss.

The immediate increase in sodium excretion observed in the present experiments with injections or infusions of natriuretic peptides is not surprising. Our injection experiments showed that maximum natriureses in response to ANF(8-33) and Auriculin A were very similar, and it was not possible to detect any significant differences in the total amount of sodium excreted in response to the injections of the two peptides.

The increase in sodium excretion during the infusion of ANF(8-33) was caused by increases in urine flow and in urinary sodium concentration. The latter disagrees with results obtained in anesthetized dogs, by use of Auriculin A (32), or in conscious dogs, by use of Atriopeptin III (17). In these studies the increase in sodium excretion was due to an increase only in urine flow. This discrepancy is probably a reflection of differences between protocols, e.g., anesthesia, or differences in natriuretic peptides, sodium intake, or hydration levels.

The sensitivity of the dogs to natriuretic agents was about 10 times that reported in anesthetized dogs (13, 31). The ability of ANF(8-33) to increase free-water formation in the water-diuretic dog, where plasma vasopressin is believed to be very low (10), is compatible with the notion that ANF(8-33) has a complex way of action. It is notable that humans in response to a dose averaging half the dose used in the present study also increased free-water clearance (33). However, no further increase in C_{H_2O} could be elicited by augmentation of the rate of infusion. It is possible that modest amounts of ANF increase C_{H_2O}, and that increasing doses activate counteracting and eventually overriding mechanisms (e.g., vasopressin) and thereby influence C_{H_2O} in a negative direction. This would explain why other groups (17) in response to larger doses did see a decrease in C_{H_2O}.

Free-water clearance is a measure of the imbalance between solute and water reabsorption in the distal part of the nephron, i.e., beyond the level in the ascending limb of the loop of Henle at which the tubular fluid becomes hypotonic for the first time. Free-water formation thus is crucially dependent on the functional status of the loop of Henle and the collecting ducts. In water diuresis the reabsorbate of the collecting ducts is hypertonic to the tubular fluid and the ducts thus participate in the urinary dilution (34). This process is load dependent (34) and an increase in the inflow to the collecting ducts will, therefore, be followed by increases in the diuresis, sodium excretion, and free-water clearance. In the present study such changes were measured during one phase of the experiments as a part of the response to the infusion of ANF(8-33). However, the increases in diuresis and free-water clearance and sodium excretion occurred despite no detectable changes in C_{CREA} or C_{PAH} and could not, therefore, have been elicited by an increase in the filtered load. The increase in free-water clearance at unchanged GFR is difficult to explain on the basis of hemodynamic changes also because it is reasonable to assume that the medullary blood flow was high prior to the administration of ANF(8-33) due to the ongoing water diuresis (35). In addition, mean arterial blood pressure fell during ANF(8-33) infusion. A reasonable explanation of our results of ANF(8-33) infusion thus seems to require an inhibitory effect on tubular reabsorption somewhere between the glomeruli and the beginning of the collecting ducts or, alternatively, a combination of effects on the collecting ducts themselves. The finding that some tubular action of ANF must have taken place is compatible with results obtained in isolated perfused kidneys using a constant perfusion pressure. Here it was found that perfusates containing a concentration of atrial natriuretic factor incapable of increasing GFR were able to increase sodium excretion and, therefore, were assumed to indicate a tubular level of action (36). The explanation that ANF causes a decrease in the reabsorption of Na^+ and water in the collecting duct has been tested in a recently performed stop-flow study, where no changes in distal sodium handling could be detected in response to increasing doses of Atriopeptin III (37). This, however, is in contrast to another study using microcatherization technique, from which it was concluded that atrial natriuretic factor has a specific inhibitory effect on net sodium transport in the inner medullary collecting duct (38).

Given (i) the assumption that furosemide—in small doses as here applied—predominantly inhibits the reabsorption of NaCl in the thick

ascending loop of Henle and (ii) the marked differences between the patterns of responses to equinatriuretic doses of ANF(8-33) and furosemide, it is tempting to conclude that ANF(8-33) exerts its effects mainly outside the loop of Henle. Effects of ANF on the distal part of the nephron have been described in the mentioned microcatherization study (38). Further studies are necessary to elucidate these mechanisms.

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