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Effects of changes in extracellular fluid volume on the release of atrial natriuretic peptide

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Introduction

Homeostatic regulation of sodium and body fluid volumes are under the influence of a variety of hormonal control systems such as the renin angiotensin system [1, 2] aldosterone [2, 3], prostaglandins [4, 5] etc. Recently, it has been reported that other peptides known as atrial natriuretic peptides (ANP), have potent natriuretic and diuretic effects [8-10] that are accompanied by significant decreases of both renin [9, 11] and aldosterone [12] release and by an increase of prostaglandin secretion [9]. The renal effects of these peptides have suggested that ANP could be involved in the regulation of extracellular fluid volume. At the present time, however, it is uncertain what stimuli induce the release of ANP, nor are the chronic effects of ANP known since the actions of this peptide have been only analyzed during acute experiments. Therefore, in the present study we describe the reponse of ANP release during different experimental maneuvers that induce acute and chronic changes in extracellular fluid volume. Since atrial pressure has been implicated in the release of ANP [6, 7], changes in right atrial pressure have been correlated with changes in plasma concentrations of ANP.

Furthermore, the effects of chronic infusions of ANP on renal hemodynamics and arterial pressure are analyzed in conscious dogs.

Methods

Animal Preparation. Female mongrel dogs (19-23 kg.) were anesthetized with pentobarbital sodium (30 mg/kg., i.v.) for implantation of Tygon catheters into the femoral artery and vein under aseptic conditions. The tips of both catheters were placed in the aorta, distal to the origins of the renal arteries, and in the vena cava, respectively. The catheters were tunneled subcutaneously and exited near the neck of the animal. The dogs were allowed to recover from surgery for at least two weeks before any experiments were performed.

Experimental Protocols

a) *Acute Saline Volume Expansion.* Isotonic saline was infused during 30 minutes in dogs trained to lie quietly on a table (n=7). Plasma concentrations of ANP (pANP) and plasma renin activity were analyzed before, during and after the acute volume expansion.

b) *Hypertonic Saline and Water Drinking.* In this experimental group (n=5), a catheter was implanted in the right atrium to measure right atrial pressure (RAP). Hypertonic saline (6 % NaCl, 1.4 ml/min) was infused during 4 hours of the experiment. Mean arterial pressure (MAP), RAP, plasma osmolality (pOsm), hematocrit and plasma levels of vasopressin (pAVP) and ANP (pANP) were analyzed during control period and hypertonic saline infusion before and after water drinking was allowed. After an interval of three days of resting, a similar protocol was undertaken in the same group of animals with the exception that an antagonist to the pressor effect of AVP (Peninsula Labs) was infused intravenously at a dose of 10 μg/kg.

c) *Chronic Saline Loading.* Seven days before the experiments were started, the dogs (n=7) were housed in individual metabolic cages and fitted with harnesses that contained blood pressure transducers mounted at heart level and connected to a graph recorder. The same day, furosemide was injected (30 mg, i.v.) and then all dogs were fed a sodium-deficient diet (H/D, Hill Pet Products, Inc.) that provided approximately 5 mEq of sodium and 65 mEq of potassium per day. Water was allowed ad libitum. Total sodium intake during the first, second and third week of study was 5, 75 and 300 mEq/day,

respectively. Isotonic saline was continuously infused i.v. to maintain the total sodium intake at 75 and 300 mEq/day during the second and third week of study, respectively. After the third week, total sodium was reduced to 75 mEq/day for 3 additional days. Twenty-four hour urine samples, infusion volume and water intake were measured between 7:30 and 8:30 a.m. each day. Arterial pressures were continuously monitored 24 hr/day, on a graph recorder [8, 13]. Samples for the measurement of glomerular filtration rate (GFR) (24 hr creatinine clearance), plasma sodium and potassium, osmolality, plasma renin activity (PRA), plasma aldosterone concentration (PAC) and pANP were drawn daily, 20-22 hours after the last feeding.

d) *Chronic Effects of ANP Infusion.* Dogs (n=6) were housed in metabolic cages similar to protocol c. Isotonic saline was infused i.v. to maintain the total sodium intake constant at approximately 45 mEq/day, including the sodium provided in the food. Following a four day control period, ANP was infused i.v. for five days at a rate of 50 ng/kg/min., 24 hours a day, in the isotonic saline vehicle solution. Measurement of arterial pressure, 24-hour urine excretion of sodium, potassium, urine osmolality and 24-hour water intake were obtained daily. In addition, GFR, 24-hour creatinine clearance, pANP, PAC, PRA and plasma sodium, potassium and osmolality were measured daily.

Analytical Methods

Sodium and potassium concentrations in the urine and plasma were measured using a Beckman E2A Electrolyte Analyzer. Plasma osmolality and plasma creatinine were measured using a Wescor 5100 C vapor pressure osmometer and a Beckman Creatinine Analyzer, respectively. PRA, PAC, pAVP and pANP were assayed by radioimmunoassay according to methods published elsewhere [16-19].

Statistical comparisons were made using the Dunnett's-test for multiple comparisons with a control [20]. Results are presented as means ± SE of means. Correlation coefficient between RAP and PANP was performed by a standard correlation test.

esults

Effects of Acute Sodium Loading

Figure 1 shows changes in pANP and PRA during the acute saline expansion (5 % b.w.). It can be seen that pANP increased significantly from 48 ± 5 to 119 ± 24 pg/ml ($p<0.01$) at 30 minutes of acute loading and then decreased gradually toward control levels (71 ± 9.7 pg/ml, $p<0.01$) 30 minutes after saline infusion was stopped. PRA decreased from 3.8 ± 1.1 to 1.0 ± 0.2 ngAI/ml/hr ($p<0.01$) during the acute saline infusion.

Effects of Chronic Changes in Sodium Loading

The effects of chronic changes in sodium intake on pANP are illustrated in figure 2. Plasma concentration of ANP did not change significantly when sodium intake was increased from 5 to 75 to 300 mEq/day and then decreased from 300 to 75 mEq/day. Plasma ANP averaged 37 ± 7, 39 ± 8 and 33 ± 5 pg/ml on sodium intakes of 5, 75, and 300 mEq/day, respectively. The increment of sodium intake from 5 to 75 mEq/day induced significant

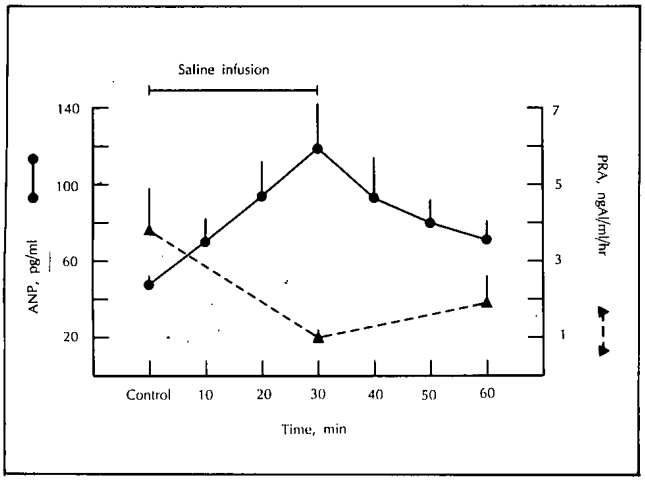

Fig. 1.—Effects of acute saline infusion (5 % BW over 30 mins.) on plasma levels of atrial natriuretic peptide (ANP) and plasma renin activity (PRA) inconscious dogs. Values are means ± SE. Reproduced from [13] with permission from American Physiological Society.

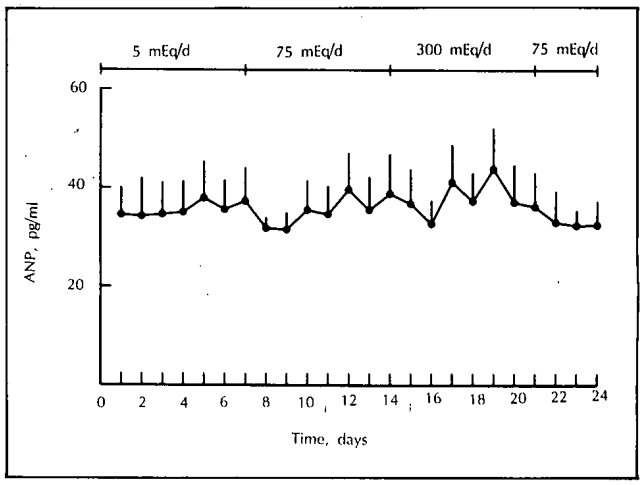

Fig. 2.—Effects of progressive increments of sodium intake by continuous intravenous saline infusion on plasma levels of atrial natriuretic peptide (ANP) in conscious dogs. Values are means ± SE. Reproduced from [13] with permission from American Physiological Society.

decreases of both PRA (2.5 ± 0.5 to 1.5 ± 0.4 ngAI/ml/hr, $p<0.05$) and PAC (19.3 ±5.4 to 2.9 ± 0.4 pg/dl, $p<0.05$).

Both, PRA and PAC decreased to undetectable levels as sodium intake was elevated from 75 to 300 mEq/day. It should be noted that mean arterial pressure (MAP) and glomerular filtration rate (GFR) did not change significantly throughout the study. Urinary sodium excretion ($U_{Na}V$) increased from 7 ± 0.7 to 74 ± 7 to 289 ± 20 mEq/day when sodium intake increased from 5 to 75 to 300 mEq/day, respectively. Sodium balance was reached within one to two days after each change in sodium intake.

Effects of Hypertonic Saline Infusion and Drinking Water

Changes in MAP, right atrial pressure, and pANP during hypertonic saline infusion (4 hours) are shown in figure 3. Drinking water was only allowed during the last two hours. Hypertonic saline infusion induced a significant increase of MAP (107 ± 4 to 115 ± 6 mmHg, $p<0.05$), RAP (1.9 ± 0.6 to 3.1 ± 0.7 mmHg, $p<0.05$), and pANP (68 ± 14 to 120 ± 33 pg/ml, $p<0.05$). When drinking water was allowed, higher increases of RAP (7.9 ± 1.0 mmHg, $p<0.05$) and pANP (228 ± 61 pg/ml, $p<0.05$) were obtained.

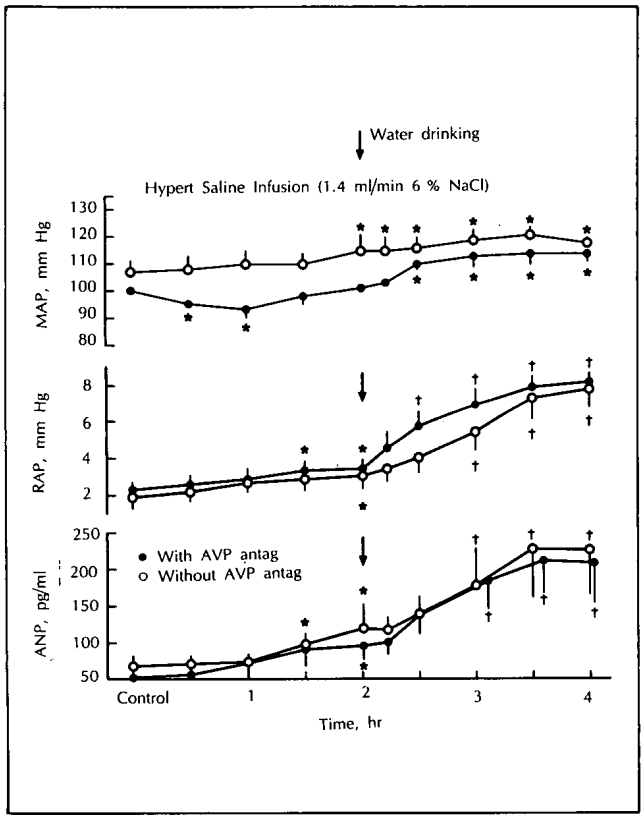

*Fig. 3.—Changes in mean arterial pressure (MAP), right atrial pressure (RAP) and plasma concentration of ANP during 4 hours of hypertonic saline infusion in dogs treated and not treated with an antagonist of the pressor effect of vasopressin (AVP). Water intake was only allowed during the last two hours * $p<0.05$ vs. control; + $p<0.05$ vs. 2 hours. Values are means ± SE. Reproduced from [36] with permission from the American Physiological Society.*

However, MAP did not change during the water drinking phase. As shown in figure 3, these changes in RAP and pANP during the hypertonic saline infusion and drinking water were not modified by the previous administration of an AVP pressor antagonist. However, MAP decreased transiently during the hypertonic saline infusion and increased significantly ($p<0.05$) when drinking water was allowed (114 ± 3 mmHg) as compared with the control period (103 mmHg). Figure 4 illustrates the significant ($p<0.001$) and positive linear correlation that was found between increments of RAP and pANP. The hypertonic saline infusion induced significant increases of plasma levels of vasopressin (pAVP) (1.7 ± 1 to 11.3 ± 4.3 pg/ml, $p<0.05$) and plasma osmolality (pOsm) 300 ± 2 to 324 ± 4 mOsm/kg., $p<0.005$) and a decrease of hematocrit (38 ± 2 to 34 ± 1 %, $p<0.05$). Water drinking induced significant ($p<0.05$) decreases of pAVP (2.1 ± 0.7 pg/ml.), pOsm (305 ± 3 mOsm/kg.) and hematocrit (30 ± 1 %).

Chronic Effects of ANP Infusion

A summary of the changes in MAP, GFR and $U_{Na}V$ before, during and after a continuous infusion of ANP (50 ng/kg/min.) for 5 days are shown in figure 5. The intravenous administration of ANP induced a significant decrease of MAP from an average control level of 90 ± 3 mmHg to 74 ± 3 and 75 ± 4 mmHg

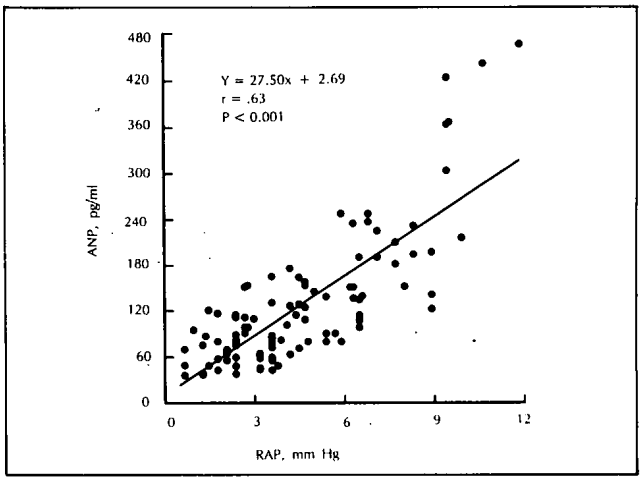

Fig. 4.—Relationship between right atrial pressure (RAP) and plasma concentration of ANP during 4 hours of hypertonic saline infusion in conscious dogs that were allowed to drink water the last two hours only. Reproduced from [36] with permission from American Physiological Society.

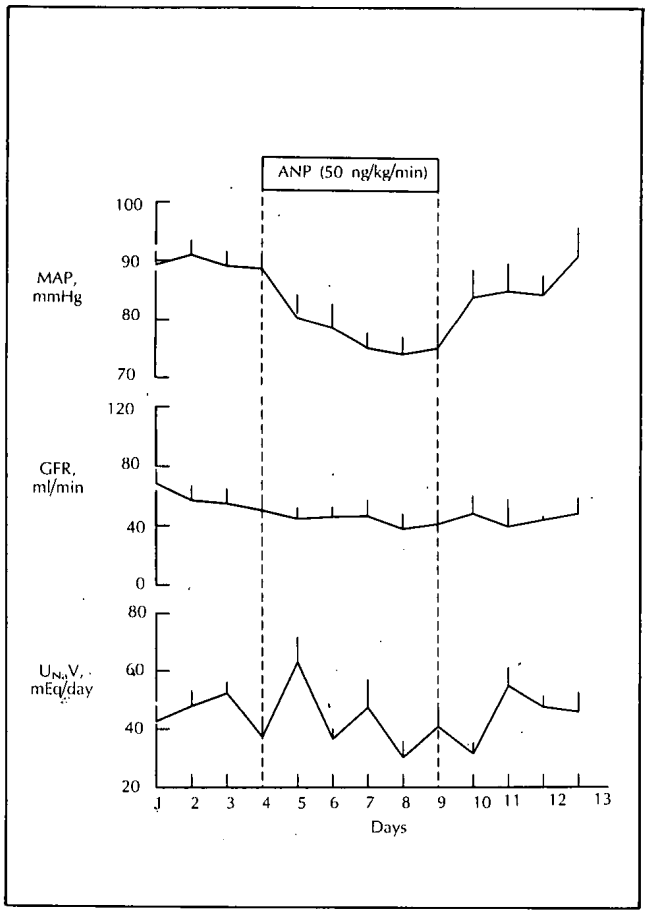

Fig. 5.—Effects of intravenous infusion of atrial natriuretic peptide (50 ng/kg/min.) for 5 days on mean arterial pressure (MAP), glomerular filtration rate (GFR), and urinary sodium excretion ($U_{Na}V$) in six conscious dogs. Values are means ± SE. Reproduced from [8] with permission from American Physiological Society.

($p<0.05$) by days 4 and 5 of this infusion. During the recovery period, MAP increased to control levels. Glomerular filtration rate did not change throughout the experiment. There was no significant long-term effect on $U_{Na}V$, although a slight increase of sodium excretion was found on the first day of ANP infusion (45 ± 5 to 63 ± 10 mEq/day). The acute intravenous infusion of the same dose of ANP induced a significant increase of $U_{Na}V$ (74 ± 33 to 147 ± 37 Eq/min, $p<0.05$).

Discussion

The results of these studies show that plasma levels of atrial natriuretic peptide (pANP) are increased during stimuli that produce acute changes in extracellular volume, such as isotonic saline infusion or water drinking, but does not change during chronic increments in sodium intake. Acute increases in plasma osmolality during hypertonic saline infusion induced a potent stimulus of ANP release. This change in pANP was not induced by the increase of plasma levels of vasopressin (pAVP). A significant and positive linear correlation between right atrial pressure (RAP) and pANP suggests that the release of atrial peptides is regulated mainly by changes in atrial pressure. Furthermore, the results of this study demonstrate that chronic elevations in pANP result in a significant reduction in mean arterial pressure (MAP) without having long-term effects on sodium excretion.

Atrial natriuretic peptide has been suggested to play an important role in the acute and chronic regulation of sodium excretion and extracellular fluid volume [7]. A variety of experimental maneuvers which lead to acute central volume expansion such as water immersion, and postural change, result in significant increases in circulating levels of ANP [14, 21]. In the present study, other acute changes of extracellular volume in conscious dogs such as acute saline loading, water drinking and acute changes in plasma osmolality resulted in elevations of 15 %, 230 % and 75 %, respectively, in plasma circulating levels of ANP. These changes were positively correlated with increases of RAP which support the notion suggested in previous studies [15, 22] that small increments in RAP provide a potent stimulus to induce the release of ANP. It has been suggested that AVP also promotes the secretion of ANP [23, 24]. However, such an assumption is not supported by our study, since the increases of pANP during hypertonic saline infusion are not altered when a specific antagonist of AVP is previously administered. One reason for the discrepancy between our results with previous studies is that they were performed in vitro [24] and infusing pharmacological doses of AVP [23]; whereas, in our study, the exogenous release of AVP was stimulated by an 11 % increase in plasma osmolality which constitutes a supramaximal stimulation for AVP release [25]. The significant increase of pANP during hypertonic saline infusion could be produced by an increase in blood volume because RAP increased, and hematocrit decreased significantly. Spontaneous water drinking resulted in a significant increase of RAP and pANP. According to the results of Ledsome et al [26] and Katsube et al [27], the increase of RAP observed in our study after the water intake appears to be the major stimulus responsible for inducing the observed increase in pANP. These studies have shown that the release of ANP depends on direct stretch of the atrium, and is correlated with changes in atrial pressure.

Chronic changes in sodium intake from 5 to 75 to 300 mEq/day by continuous intravenous infusion of saline, did not result in any significant changes in circulating levels of ANP. These results are supported

by other studies in humans [28] which have reported no significant changes of pANP during increments in dietairy sodium intake. In contrast, Shenker et al [29] and Sagnella et al [14] reported that increases in dietary sodium intake were associated with increases in circulating levels of ANP. The reasons for these discrepancies are not clear, but may be related to experimental procedures such as sampling time after a meal, a condition which increases atrial pressure [30]. In the present study, the dogs were maintained on a sodium-free diet and sodium intake was progressively increased by continuous intravenous saline infusion. In addition, blood samples for ANP determinations were collected 20 to 22 hours after the last meal and chronic sodium loading, to a similar extent as in the present study, has been shown to increase significantly the extracellular fluid volume [31]. Patients wit congestive heart failure or chronic renal failure who have severe extracellular fluid volume expansion are reported to have marked elevations in circulating ANP [6, 19]. The significant increase in pANP under these pathophysiological conditions and not under chronic saline loading may be due to the fact that, in or study, volume and/or cardiac filling pressures are not sufficiently increased to trigger the release of ANP. Although chronic changes in sodium intake had no significant effect on circulating pANP, it induced important changes in the activity of the renin-angiotensin system and aldosterone as shown in previous studies [1, 2]. These results suggest that these systems are probably more important in the maintenance of sodium balance during chronic changes in sodium intake than atrial natriuretic peptide.

Chronic elevation in the plasma levels of ANP resulted in a significant reduction in MAP, but only induced a transient increase in sodium excretion on the first day of infusion and then returned to normal levels. A lack of a sustained effect on sodium excretion by ANP does not necessarily imply that this substance does not have a long-term effect on the capability of the kidney to excrete sodium and water. On the contrary, ANP appears to increase the capability of the kidney to excrete sodium and water by allowing the animal to achieve sodium and water balance at a lower renal perfusion pressure. Consistent with our results, Takezawa et al [32] reported that ANP infusion produced a downward shift in the pressure-natriuresis curve. The mechanism involved in the hypotensive response during the chronic administration of ANP is unknown. Acute infusion of ANP is associated with a reduction in cardiac output while having no effect on total peripheral resistance [33].

Chronic studies in hypertensive rat models have consistently found a larger reduction in MAP when ANP is infused chronically as compared to acute administration [34, 35]. Further studies will be needed to clarify the hemodynamic mechanism of this response.

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