for 2 to 4 hr, had no effect on basal or stimulated release of ANP (data not shown). However, long-term physiological effects of prolactin will also be tested in order to better elucidate the role of prolactin in ANP release.

The normal ANP secretion in pituitary-grafted animals raise several intriguing possibilities. It could be that hormones other than prolactin, GH, LH, and FSH secreted from the anterior pituitary gland act directly on the atrial myocyte to facilitate the physiological stimuli-induced release of ANP. The action could be receptor-mediated or mediated through membranal changes. Alternatively, hormones derived from the pituitary gland affect ANP release indirectly through the action on other endocrine organs such as the thyroid or adrenal cortex. The existence of "atriotrophic hormone" should also be considered. More work is needed to distinguish between these possibilities and to test them.

Further support for involvement of humoral factor(s) came from *in vitro* experiments. Addition of serum to the incubation media of primary culture cells prepared from adult rat atria stimulated the release of ANP fourfold within 10 min (data not shown).

In conclusion, we suggest that pituitary gland-atria interactions play a major role in the regulation of fluid and electrolyte homeostasis via ANP secretion.

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EFFECT OF ATRIAL NATRIURETIC PEPTIDE ON URINARY KALLIKREIN IN NORMAL HUMANS

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The mechanisms of the diuretic and natriuretic action of atrial natriuretic peptides (ANP) are not clearly understood (1). Renal kallikrein is an enzyme that promotes the release of kinins and is released at the level of the distal tubule (2,3). It has been shown that interventions associated with diuresis and natriuresis, such as acute administration of furosemide or renal vasodilatators, are associated with a marked increase in the renal release of kallikrein (2,3), suggesting that the kallikrein-kinin system may participate to the renal effects of these substances.

In the present studies we assessed the effect of ANP on urinary kallikrein in normal humans; the results suggest that the action of ANP resembles that of the distal diuretic amiloride.

SUBJECTS AND METHODS

Studies were conducted on eight normal volunteers, aged 23 to 28 years, maintained on a high-sodium diet (*ad libitum* sodium intake to which 5 g NaCl/day was added). Two doses (0.5 and 5 μg/min) of the synthetic 24-amino-acid ANP, Ile 12–(3–28) eicosahexapeptide (Merck Sharp and Dohme Research Laboratories, USA), and its vehicle were infused and compared at weekly intervals in a single blind three-way crossover study. In fact, five subjects were studied three times, and eight subjects only received the vehicle and the low dose of ANP. The study period consisted of a 2-hr baseline and a 4-hr infusion period followed by a 2-hr recovery phase. Throughout the study, subjects received an infusion of 0.9% saline at 2 ml/min and were asked to drink 200 ml water/hr; thus sodium intake was 18 mmol/hr and water intake was 320 ml/hr.

Blood was obtained prior to and 1 and 4 hr after the start of the infusion and 2 hr after discontinuation of the infusion for the measurement of electrolytes, plasma renin activity (PRA), and plasma aldosterone concentration (PAC). Urines were collected at hourly intervals for the determination of electrolytes and urinary kallikrein activity by the amidolytic assay using the chromogenic tripeptide D-Val-Leu-Arg-*p*-nitroanilide (S 2266, Kabi Diagnostic) as described by Amundsen et al. (4).

Data are expressed as mean ± SEM, and statistical significance of differences was evaluated by analysis of variance.

RESULTS

Urinary sodium excretion was 269 ± 25 during the 24-hr period prior to infusion of the vehicle, 254 ± 28 prior to the 0.5 μg/min (low dose) ANP infusion, and 207 ± 26 mmol prior to the 5 μg/min (high dose) ANP infusion.

Effect on Arterial Pressure

During infusion of the vehicle, mean arterial pressure (MAP) remained constant (85 ± 3 before infusion, 89 ± 2 mm Hg at the end of infusion). Administration of 0.5 μg/min ANP was not associated with a significant change in MAP, and during the high-dose ANP infusion, MAP decreased by 4.2 ± 1.8 mm Hg during the second hour and by 10.4 ± 2 and 9.2 ± 1.3 mm Hg during the third and fourth hours of infusion, respectively. Discontinuation of ANP was followed by a slow and progressive increase toward preinfusion values.

Effect on Urinary Water and Electrolytes

As summarized in Table 1, the cumulative (per 4 hr) urinary excretion of water and sodium was significantly increased when compared to the vehicle study only with the high-dose ANP infusion. In fact, calculation of water and sodium balance achieved during the 4-hr experimental period showed that they became highly negative during high-dose ANP infusion (−991 ± 273 ml and −109 ± 24 mmol for water and sodium, respectively) when compared to the balance achieved during infusion of the vehicle (−260 ± 166 ml and −12 ± 6 mmol, respectively).

During the 2-hr recovery period, water and sodium excretion was significantly lower in the high-dose ANP infusion (237 ± 41 ml and 19 ± 3 mmol, respectively) than in the vehicle study (744 ± 89 ml and 43 ± 4 mmol, respectively). These findings demonstrate that during recovery, renal water and sodium retention occurred as a physiological response to the marked negativation of water and sodium balance associated with infusion of ANP.

Effect on Urinary Excretion of Kallikrein

As shown in Table 1, urinary kallikrein excretion was significantly lower during the high-dose ANP than in the vehicle phase ($p < 0.05$).

Hormonal Changes

PRA and PAC were not significantly affected by infusion of high- and low-dose ANP by comparison with the vehicle study. However, following discontinuation of the high-dose ANP, the PRA and PAC were higher than the vehicle study [1.25 ± 0.18 versus 0.24 ± 0.06 ng/ml/hr ($p < 0.005$) for PRA; and 96 ± 18 versus 31 ± 2.4 ng/dl ($p < 0.005$) for PAC].

TABLE 1. *Effect of vehicle and ANP on urinary excretion of water, electrolytes, and kallikrein*[a]

Parameter	Vehicle ($n = 8$)	ANP, 0.5 μg/min ($n = 8$)	ANP, 5 μg/min ($n = 5$)
Urine volume (ml)	1540 ± 166	1643 ± 171	2271 ± 272[b]
Urinary sodium excretion (mmol)	84 ± 6	104.6 ± 8.7	181.5 ± 23[b]
Urinary potassium excretion (mmol)	18.4 ± 1.7	17.5 ± 2.6	13.6 ± 2.2[b]
Urinary kallikrein (milliunits)	156.4 ± 22.3	138.3 ± 20.7	118.7 ± 18[b]

[a] Mean values ± SEM represent cumulative excretion per 4 hr of infusion.
[b] $p < 0.05$ compared to vehicle study.

DISCUSSION

In the present studies it was found that the diuretic and natriuretic effects of ANP were not associated with kaliuresis and increase in urinary excretion of kallikrein, which actually decreased by comparison with the vehicle phase of the investigation. Such an observation was unexpected since acute administration of thiazide diuretics as well as furosemide is associated with a rise in urinary potassium and kallikrein excretion (2,3). These findings suggest that the effect of ANP resembles that of the potassium-sparing diuretic amiloride. In the rat, acute administration of amiloride is associated with a parallel decrease in urinary kallikrein and kinin excretion (5,6). Although it has been demonstrated that amiloride inhibits, *in vitro*, the kininogenase activity of kallikrein (7), this possibility was ruled out in both studies (5,6), thus suggesting that amiloride has an inhibitory action on kallikrein release, activation, and synthesis by renal cells. Since at the present time we do not know the intratubular fate of ANP, no attempt was made to study the influence of ANP or smaller peptides on *in vitro* kininogenase activity. Our results are, however, in favor of an inhibitory effect of ANP on the renal release of kallikrein.

The similarity of the renal action of ANP and amiloride, a diuretic acting at the luminal side of the distal tubule, is reinforced by the findings of Zeidel et al. (8), who reported that these substances had an identical effect on sodium entry-dependent oxygen consumption and inhibited, to the same extent, ^{22}Na uptake in suspensions of cultured rabbit inner medullary collecting duct cells.

In the present studies, PRA and PAC did not change during ANP infusion; however, the striking rise in these parameters following discontinuation of the infusion suggests that ANP actually inhibited the PRA and PAC responses to the fall in extracellular fluid volume and negativation of sodium and water balance (by 109 ± 24 mmol and 991 ± 273 ml, respectively) associated with ANP infusion, thus confirming the observations of other investigators (9–12). Once again it is interesting to note that the effects of amiloride and ANP on PRA may be similar, since in the rat amiloride inhibits the furosemide-induced increase in PRA (13).

Although the observed changes in urinary kallikrein may result from the lack of increase in PRA and aldosterone in response to natriuresis, the effect of ANP on urinary kallikrein suggests that among other intrarenal effects, ANP could act at the level of the distal tubule in humans.

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