PROCEEDINGS OF THE SOCIETY FOR EXPERIMENTAL BIOLOGY AND MEDICINE **181**, 169–172 (1986)

RAPID COMMUNICATIONS

ATRIAL NATRIURETIC FACTOR INHIBITS VASOTOCIN-INDUCED WATER REABSORPTION IN THE TOAD URINARY BLADDER

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Abstract. Peptides recently isolated from atrial extracts possess potent natriuretic and diuretic activities, which are thought to be due to hemodynamic actions, such as increased glomerular filtration or altered medullary blood flow. A direct tubular site of action cannot be ruled out; therefore we have examined the effect of one of these peptides, atriopeptin III on vasotocin-induced water absorption in the toad urinary bladder. Our results indicate that equimolar doses (10^{-12} to 10^{-11} M) of atriopeptin III can significantly inhibit vasotocin-induced water reabsorption in vitro and suggest a physiologic role for the cardiac peptides to alter water reabsorption directly at the level of the tubules or collecting ducts, independent of any hemodynamic effects they might also exert in vivo.

Introduction. Atrial myocytes contain secretory granules (1) suggesting that the heart, in addition to its muscular function, might be an endocrine organ. The observation that water deprivation resulted in increased atrial granularity led deBold (2) to speculate that the contents of these granules played a physiological role in the regulation of water and electrolyte balance. Later, his group (3) demonstrated that intravenous infusion of saline extracts of atrial tissues caused a rapid and potent increase in urinary sodium and chloride excretion and urine flow. They speculated that the extracts contained a substance which inhibited renal tubular sodium chloride reabsorption and that the atria, a tissue known to monitor intravascular volume (4), could be the source of hormonal agents which controlled fluid volume via renal mechanisms. A family of peptides possessing the natriuretic/diuretic and spasmolytic activity of atrial extracts has been isolated, purified, sequenced and synthesized by several groups (5-7). These peptides, called collectively atrial natriuretic factors (ANFs), have in common a central, 17-membered ring formed by an internal cystine disulfide.

The original description of the natriuretic/diuretic action of atrial extracts failed to have detected an alteration in glomerular filtration rate (GFR), thereby suggesting an action on NaCl transport in the ascending limb of Henle or medullary collecting duct (3). Subsequent micropuncture studies (8) also suggested a medullary collecting duct and tubule site of action, although high doses of extract did increase GFR (8). Later work with extracts (9) and synthetic ANF (6) corroborated the effect to increase GFR suggesting that the natriuretic/diuretic activity was due to hemodynamic and not tubular actions. Maack et al. (10) demonstrated increased GFR with ANF infusion in anesthetized and conscious dogs in the face of only transiently increased renal blood flow. These data suggest that the decrease in urine osmolality

0037-9727/86 $1.50

following ANF infusion is due to increased inner medullary blood flow and subsequent medullary wash out since free water clearance remained stable.

Although convincing data for the hemodynamic basis of the natriuretic effect of ANF exists, a direct tubular effect cannot be ruled out (10). ANF receptor binding is present, albeit in low levels, in the collecting tubules (11) and data from isolated rat kidneys (12) strongly suggest a dissociation of the hemodynamic and natriuretic /diuretic effects. We have approached this problem using a different experimental model, the urinary bladder of the toad. We reasoned that the diuretic affect of ANF might not be secondary to its natriuretic action, but instead be due to an inhibition of vasopressin stimulated water reabsorption.

Materials and Methods. Toads (Bufo marinus) of either sex, maintained in deionized water without food, were pithed and two hemibladder sacs were prepared from each toad using the technique described by Bentley (13). Bladders were emptied, and then washed twice and filled with 4-5ml, half-strength Ringer solution. These were incubated (22-23°C) in 30 ml Ringer solution (NaCl,110 mM; KCl, 3.0 mM; NaH_2PO_4,1.0 mM; Na_2HPO_4,4.0 mM; $CaCl_2$,0.9 mM; pH 7.25-7.35) aerated with humidified 0_2 (100%) in a covered, 50 ml beaker. The net flux of water was determined by weight differences with periods between weighing (approximately 40 min) being matched for the paired hemibladders. Water flow was calculated in terms of mg H_20 loss per hour per 50 mg wet weight of bladder (mg/hr x 50 mg).

After establishing that each of the matched pairs of hemibladders had similar and normal net water flux in the absence of exogenous peptides, the response of one hemibladder to the indicated dose of arginine vasotocin (AVT, Bachem) in the serosal bath was determined. If the response was inadequate, the dose was increased until a response occurred. The paired hemibladder was then treated with the combination of ANF (Atriopeptin III, Peninsula Labs) and AVT. The ANF:AVT molar dose ratio was 10 in series I, and equimolar in series II. In all of series I, and 7 of series II hemibladders exposed to both peptides the ANF preceded the addition of AVT by 8-10 minutes. In the remaining 5 experimental hemibladders of series II the two peptides were placed in the bath simultaneously. There were no differences in the observed effects due to the order of timing, therefore the data of all 12 pairs in series II were grouped. The dose of AVT was the same for both hemibladders (that exposed to AVT alone, as well as its matched pair receiving AVT and ANF). Fluxes were measured on the hemibladders for at least three, continuous, forty minute periods after placing AVT or ANF and AVT in the serosal bath (periods 1, 2 and 3). Since there was variability in the response to AVT, the mean differences between observed fluxes in hemibladder pairs were analyzed by the Student's t test. No attempt was made to determine whether or not the inhibitory effect of ANF was dose dependent.

Results. Series I. The effective dose of AVT in the 9 hemibladders varied from 5×10^{-12} to 10^{-11} M. The net water flux during the first 40 min period after exposure varied from 114 to 306 mg/hr X 50 mg bladder and averaged 197 ± 20 (mean ± SEM). The net water flux during the second period (41-80 minutes) averaged 145 ± 19. The effect on net water flux was inhibited in the matched paired hemibladders by the 10 fold molar excess of ANF. The mean net fluxes were: period 1, 139 ± 19; period 2, 128 ± 22. The mean difference in water absorption (Table 1) was significantly different during period 1 ($p/2 < 0.05$).

Series II. The effective dose of AVT in the 12 hemibladders ranged from 10^{-12} to 3×10^{-12} M. The net water flux during period 1 varied from 45 to 292 mg/hr X 50 mg bladder, with an average of 144 ± 26. For period 2, the average was 105 ± 25. The mean net water fluxes in paired

Table I Effect of ANF on AVT-induced water flux in the toad urinary bladder. One hemibladder was exposed to AVT alone, while its pair was exposed to a combination of AVT and ANF. The mean difference in flux was calculated as the absolute difference between each pair treated with AVT alone and its matched pair that was treated with AVT and ANF.

	AVT (dose) M	ANF:AVT (dose ratio in treated pair)	Mean Difference (± SEM) in Flux (mg/hr x 50mg bladder)	
			Period 1 * (0-40 min)	Period 2 * (41-80 min)
Series 1 (n=9)	$5x10^{-12}$ to 10^{-11}	10:1	58 ± 25 p/2<0.05	17 ± 21
Series 2 (n=12)	10^{-12} to 3×10^{-12}	1:1	72 ± 20 p/2<0.005	52 ± 16 p/2<0.005

* Time after AVT was placed in the serosal bath.

hemibladders treated with equimolar doses of ANF and AVT were: period 1, 72 ± 18; and period 2, 53 ± 13 mg/hr X 50. The mean difference in fluxes was significantly different during both periods (p/2<0.005). In six of the twelve pairs the inhibitory effect lasted into a third forty minute observation period.

Discussion. The urinary bladder of the toad is functionally analogous to the tubules and collecting ducts of the mammalian kidney with regard to water reabsorption. We selected this in vitro model because of its relative simplicity and because we would be able to ascertain a possible direct action of ANF on water reabsorption. Although the effect of ANF alone on net flux has yet to be determined, we have demonstrated the ability of this peptide to inhibit AVT-induced water absorption. These data suggest a physiological role for the cardiac peptides to alter water reabsorption directly at the level of the tubules or collecting ducts that is independent of any hemodynamic effects they might also exert in vivo.

This ability of ANF to reverse the action of another hormonal agent is quite analogous to its action to reverse norepinephrine (14,15), angiotensin II (15) and carbachol (14) stimulated smooth muscle contraction and experimentally induced vasopressin release (16). The antagonistic action of ANF on AVT-induced water flux in the toad bladder suggests yet another mechanism by which the cardiac peptides may interact with other hormonal agents in the physiologic control of fluid and electrolyte balance.

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Received July 29, 1985.
P.S.E.B.M. 1986, Vol. 181.
Accepted November 1, 1985.