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SYNTHETIC ATRIAL NATRIURETIC FACTOR INDUCES RELEASE (POSSIBLY RECEPTOR-MEDIATED) OF VASOPRESSIN FROM RAT POSTERIOR PITUITARY

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Abstract. The synthetic fragment (Arg 101-Tyr 126) of atrial natriuretic factor (ANF) induces release of arginine vasopressin from the isolated posterior lobe of the rat hypophysis in vitro. At a physiological concentration (3×10^{-10}M) ANF was three times more effective than 61 mM KCL. In vitro binding studies with ^{125}I-ANF revealed the presence of high affinity receptor sites displaying a pK = 9.9, a Kd = 0.14 nM, a Bmax = 20 fmol/posterior lobe and and IC_{50} = 200 pM. These results suggest that arginine vasopressin release by synthetic atrial natriuretic factor may be receptor mediated.

The cardiocytes of the atria in mammals (1,2,3) and of both atria and ventricles of non mammalian vertebrates (4) harbour, apart from their contractile apparatus, a large Golgi complex and secretory-like granules. These granules have been shown to be made up of proteins (5) and to contain a peptide (atrial natriuretic factor (ANF)) which induces diuresis and natriuresis of rapid onset and short duration (6,7). The active (C-terminal) portion of the peptide has been isolated, sequenced and synthetized (8). Isolation of larger molecular weight forms has revealed extension in the N-terminal portion of the molecule (9,10) and, finally, cloning of the rat cDNA has delineated a long peptide of 152 amino acids (14,15) the C-terminus of which is identical with the portion which had previously been synthetized. The signal peptide being made up of 24 amino acids, the remaining ones have been numbered from 1 to 128 from the N- to C-terminus according to standard practice. We have shown that the synthetic fragment (Arg 101 - Tyr 126) possesses the same biological activity as its native counterpart as regards induction of diuresis, natriuresis, vasodilation and inhibition of arterial contraction produced by catecholamines or angiotensin II (8, 11). ANF is also a potent inhibitor of both aldosterone and cortisol secretion from beef adrenal cortex and acts on these target cells through receptors (12,13). We now report that ANF is a potent stimulant of arginine vasopressin (AVP) secretion from the isolated posterior lobe of the hypophysis and that it may act through specific receptors.

MATERIALS AND METHODS

Preparation of neural lobes: Female Sprague-Dawley rats (190-210 g b.w.) were maintained with free access to food and water for one week prior to experiments. The rats were decapitated, the brains quickly removed and the neurohypophysis isolated from the anterior lobe with the aid of a magnifying glass. Neural lobes (including pars intermedia) were cut in four and placed in 1 ml medium saturated with 95% O_2 5% CO_2 (Minimum Essential Medium, Dulbecco's Modification, 0.05% bovine serum albumin added (Flow Laboratories)) at 37°C for a 40 min equilibration period. After discarding the first incubation medium, the samples were exposed to fresh bath fluid at 10 min intervals over periods of up to 30 min. After each period, the media were immediately assayed by radioimmunoassay (RIA) for their content of AVP. In each experiment, isolated rat neural lo-

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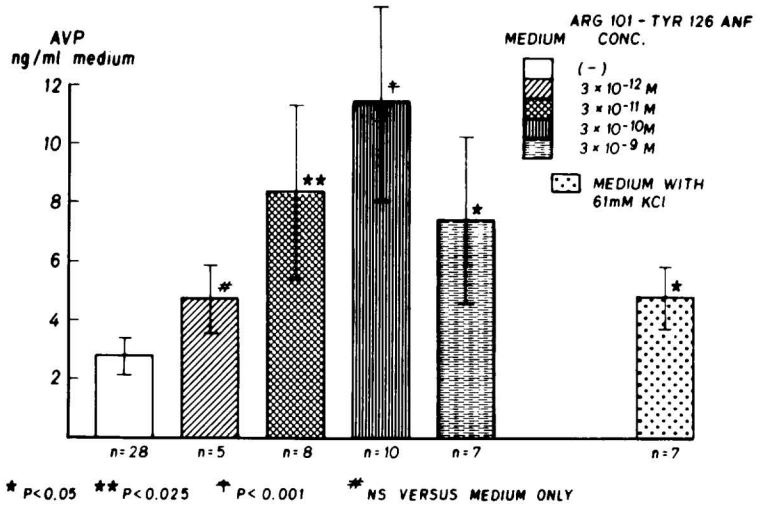

Fig. 1. Effect of ANF and KCl depolarization on arginine vasopressin release from isolated rat neurohypophyses.

bes were randomly allocated to one of six groups: Group 1, medium only: Group 2, medium plus $3x10^{-12}$M ANF; Group 3, medium plus $3x10^{-11}$M ANF; Group 4, medium plus $3x10^{-10}$M ANF; Group 5, medium plus $3x10^{-9}$M ANF; Group 6, medium containing 61 mM KCl. Isolated neural lobes of Brattleboro rats and isolated anterior hypophyses of Sprague-Dawley rats processed as described above served as absolute controls.

RIA of AVP: AVP content in the incubation medium was measured by RIA (16). White New Zealand rabbits were immunized with arginine vasopressin conjugated to thyroglobulin giving on antiserum with a cross-reactivity of 0% with oxytocin and of less than 0.02% with lysine-vasopressin and arginine-vasotocin. Scatchard analysis showed the presence of a single antibody population with a binding capacity of 5.7 pmol/L and an affinity constant (K_1) of 0.122 L/pmol. The statistical analysis of standard curves showed a detection limit of 1.5 pg/tube. In analytical recovery studies with different quantities of AVP standard added to the medium (50-125 ng/L) the mean recovery was 97.2% (n=3), the within-assay coefficient of variation was 5.2% (n=8) and linearity of dilution of the samples was observed with a correlation coefficient of 0.94%. Statistical analysis was performed using Student's t-test. Results are expressed as mean ± standard error of the mean. A $p<0.05$ was considered as significant. (Fig. 1).

Receptor study: ANF was iodinated to a specific activity of 500Ci/mmol as previously described (17). Rat pituitary posterior lobes were rinsed in 0.9% NaCl and homogenized in 50mM Tris-HCl pH 7.4, 150 mM NaCl, 1mM $MgCl_2$, 0.1 mM EDTA at 4°C (10 lobes/10 ml buffer). Following centrifugation at 40,000 g for 10 min, the membrane pellet was resuspended at 25°C in 50mM Tris-HCl pH 7.4, 150 mM NaCl, 5 mM $MnCl_2$, 0.1 mM EDTA, 0.5% BSA and aliquots (0.8 lobe/tube) were incubated for 90 min in 0.5 ml of the same buffer containing 20,000 CPM (40 pM) ^{125}I-ANF and varying concentrations of unlabeled ANF. Membrane-bound radioligand was separated by filtration on GF/C glass fiber filters and washed 5 times with 5 ml of ice-cold buffer. The competition curves were analyzed by non-linear regression using a four-parametric logistic equation (12, 13) or a mass action model (12, 13).

RESULTS

AVP release: As can be seen in Fig. 1, ANF produced a massive release of AVP from isolated rat neurohypophyses during the first 10 min of incubation. The

release was more important at a concentration of $3x10^{-10}M$ than that induced by KCL depolarization. Exposure of the lobes to higher concentration ($3x10^{-9}M$), of ANF induced a lesser increase than that observed at a concentration of $3x10^{-10}M$ while the effect of $3x10^{-12}M$ was not significantly different from the release observed in medium alone. While in the medium alone AVP release could be observed after 20 min. (0.19 ± 0.02ng/ml medium) and 30 min. (0.12 ± 0.03ng/ml medium) and statistically significant increase of AVP release was still present after 20 min. exposure of neurohypophyses to $3x10^{-10}M$ ANF (0.33 ± 0.06ng/ml medium, $p<0.025$), no further stimulation of AVP release could be observed from the neural lobes exposed to ANF after 30 min. Potassium depolarization evoked significantly greater AVP release during the whole experiment when compared to the controls (20 min: 0.56 ± 0.05ng/ml medium, $p<0.001$; 30 min: 0.33 ± 0.08ng/ml medium, $p<0.025$). Interestingly, when intact neural lobes were used in a separate series of experiments, a much smaller basal AVP release was noted (9.7% of the amount released by cut lobes) and no increase in AVP release was observed when different amounts of ANF were present in the media: only 7.6% of the basal AVP release when $3x10^{-10}M$ of ANF, and 13.2% when $3x10^{-9}M$ of ANF was added.

Radioligand binding of ^{125}I iodo ANF: Radioligand binding of ^{125}I-ANF was specific and displayed and IC_{50} (inhibitory concentration at 50% maximal effect) of 200 pM for ANF as a competing ligand. Computerized analysis of the competition curve indicates that ANF predominantly binds to high affinity receptor sites with a pK of 9.9 (Kd= 0.14 nM) and a density of 20fmol/posterior lobe.

Discussion. The present results indicate that synthetic ANF has an extremely potent effect on the release of AVP from the isolated posterior lobe of the rat hypophysis. Receptors for ANF have now been described and characterized in the zona glomerulosa of the adrenal cortex (12, 13), in arterial walls (24) in renal cortical membranes (24) and in cultured renal tubular cells of the $LLC-PK_1$ line (24). Binding sites have been localized in a variety of tissues by radioautography following injection of ^{125}I-ANF (25). The range of stimulatory activity of ANF on AVP release ($\sim$ 300pM) correlates well with the IC_{50} (200 pM) of the ligand for receptor sites suggesting that the effects of ANF on the posterior lobe may be receptor-mediated.

Separate studies have shown that ANF inhibits basal adenylate cyclase activity of rat posterior pituitary homogenates in a dose-dependent manner (maximum inhibition of 25% with an apparent Ki of $10^{-11}M$). ANF also inhibits the stimulatory effect on adenylate cyclase activity of various agents such as NECA (N-Ethylcarboxamide adenosine), sodium fluoride and forskolin whose action on adenylate cyclase is not receptor mediated (18).

The enzymes necessary for the formation and hydrolysis of cyclic nucleotides have been shown to be present in the neural lobe of the pituitary gland and release of AVP by KCl depolarization is preceded by a rise of both cAMP and cGMP (19). Whether the effects of ANF are also mediated by cyclic nucleotides remains to be determined.

The recent observations of the presence of immunoreactive ANF in rat plasma as determined by radioimmunoassay (20) provides some evidence for a role of the peptide as a hormone. Our results suggest that increased plasma concentration of ANF induced by various hemodynamic stimuli might lead to increased AVP secretion. While distension of the right atrium does not produce any change in the plasmatic levels of AVP (22), distension of the left atrium induces a decrease in plasmatic AVP concentration (21). When the vagi are cooled, however, plasma AVP rises and simultaneous distention of the left atrium then produces a further rise of plasmatic AVP (23). Although the present results indicate that ANF has a direct releasing effect for AVP on the isolated posterior lobe, the overall effect of ANF on vasopressin release by the intact hypothalamic-hypophyseal system as modulated by a host of nervous reflexes may be quite different. In fact the above cited results may indicate that the overall effect of atrial distension, as possibly mediated both by ANF release and by nervous reflexes, may well be a decrease in AVP release.

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