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CHARACTERIZATION OF SPECIFIC RECEPTORS FOR VASOPRESSIN IN THE PITUITARY GLAND

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SUMMARY: The present paper reports new findings concerning interaction of [^{3}H]-Arginine-vasopressin with putative receptors in rat anterior pituitary gland.It shows the presence of a single type of receptor sites,with a limited binding capacity and a dissociation constant of nearly 1nM.The parent neurohormone oxytocin revealed weak affinity as compared with vasopressin [Ki=100nM and Ki=1nM, respectively].None of the various peptides tested and,especially corticotropin-releasing factor CRF,competed for binding.Receptor characteristics appeared to be unaffected by lack of circulating vasopressin in Brattleboro rats presenting complete deficiency in synthesis of that peptide.

Several lines of evidence show that vasopressin, besides its effects on the kidney and the cardiovascular system,seems to play a role as a central neuromodulator in a variety of processes [1,2] and, in addition,appears to participate in the overall regulation of pituitary corticotropic activity [3].Indeed, arginine-vasopressin not only exhibits intrinsic corticotropin-releasing-factor-like properties, but also dramatically potentiates the effect of synthetic CRF on ACTH secretion [4]. Interestingly, although AVP failed to stimulate pituitary adenylate cyclase activity, it was able to enhance accumulation of cyclic AMP as triggered by CRF [5].

The aim of the present study was to examine the pituitary gland for the presence of putative vasopressin receptors, which were further characterized in terms of tissue concentration,binding affinity and specificity, especially with regard to other CRF-like components. Also, in order to evaluate the possible effect of complete lack of circulating vasopres-

ABBREVIATIONS: AVP,arginine-vasopressin; dDAVP,1-deamino-[8-D-arginine]VP; dPVDAVP, 1-deaminopencillamine-[Val^4-D-Arg^8]-VP; CRF, corticotropin-releasing factor.

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sin on binding parameters of those receptors, we used pituitary tissues from Brattleboro rats, which show a genetically deficiency in synthesis of that peptide (6).

MATERIAL AND METHODS

Chemicals: Tritiated 8-L-arginine[phenylalanine-3,4,5-^{3}H]-VP, with a specific activity of 55.4 Ci/mmol, was purchased from NEN.Unlabeled AVP and dOAVP were from Ferring and all other peptides, including dPVOAVP, CRF, angiotensine II, VIP, TRH, LHRH and somatostatine were from Bachem. (+)Butaclamol, a dopamine receptor antagonist, was a gift from Ayerst Laboratories.

Tissue processing: We used pituitary tissues from female Wistar rats, as well as from Brattleboro homozygotes and heterozygote controls. Animals were killed under resting conditions to minimize the effect of stress on release of CRF-like peptides. The anterior part of the pituitary was dissected out and homogenized in ice-cold 0.25M sucrose-5mM $MgCl_2$ by means of a Dounce homogenizer and the homogenate centrifuged at 1,000xg for 5min at 4°C. The supernatant was then spun at 11,000xg for 20min and the resulting pellet resuspended in incubation buffer (50mM Tris-HCl, 5mM $MgCl_2$, 1mM EGTA and 0.1% bovine serum albumin, pH 7.3 at 20°C. The suspension was adjusted to contain 4 pituitary-equivalents of membranes and was referred to as the crude pituitary membrane fraction.

Binding assays: Aliquots of 0.25 ml of membrane suspension corresponding to 0.8-1.0 pituitary-equivalent or 250-300 µg of protein, were incubated at 20°C in presence of varying concentrations of [^{3}H]-AVP, either alone or with a 1,000-fold excess of unlabeled AVP. Competitive inhibition of binding by various components was measured in presence of 2nM [^{3}H]-AVP during a 30min incubation period.Separation of bound and unbound moieties was achieved by centrifugating membrane suspensions in conical tubes at 10,000xg for 2min and rinsing the tubes with 0.32M sucrose in incubation buffer. The tips of the tubes were cut off and the pellets solubilized with 100 µl 80% formic acid and counted in a Triton X100-supplemented counting mixture, with an efficiency of 31%. A preliminary study showed that filtration and washing of labeled membrane fractions over GFC Whatman filters (coated overnight with bovine serum albumin in incubation buffer) gave less satisfactory results, as a substantial loss of specific binding occured compared with that measured by centrifugation.

RESULTS AND DISCUSSION

Data displayed in Fig.1 show total and nonspecific binding of [^{3}H]-AVP to pituitary membranes and, by difference, specific binding of the peptide. It clearly appears that vasopressin interacted with a limited number of receptor sites, which were saturated at a ligand concentration of about 5nM. Scatchard plot of these data generated a linear curve indicative of a single class of high affinity sites, with an apparent dissociation constant of K_d=0.7nM and a maximum binding capacity

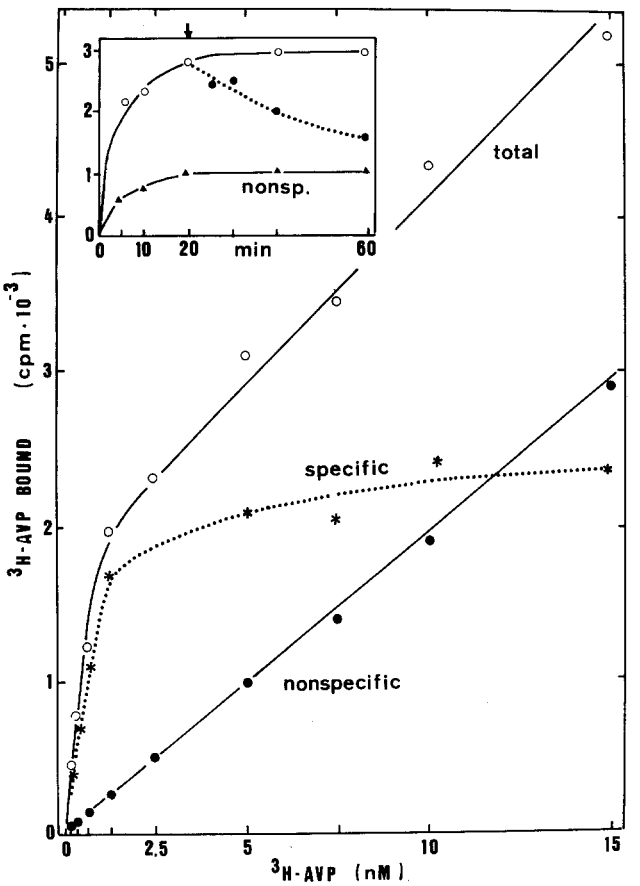

Fig. 1: Binding of [^{3}H]-AVP, at doses of 0.15 to 15nM, to pituitary membranes in the absence [total binding] and presence [nonspecific binding] of 15 μM unlabeled AVP. Incubation time was 30min at 20°C. Protein concentration was 300 μg/assay tube and binding was found to be a linear function of protein concentrations of 100-400 μg/tube. Inset depicts reversibility of binding of 2nM [^{3}H]-AVP after addition of 2 μM unlabeled peptide [arrow]. Each point is the mean of duplicates from 2 pituitary-equivalents of membranes.

of B_{max}=220 fmol/mg membrane protein [Fig.3A]. As recently reviewed [7], plasma levels of AVP are lower by several orders of magnitude than the dissociation constant of specific receptors in hepatocytes and aortic smooth muscle cells. By contrast, we show here that in the case of the pituitary gland the K_d value is in good keeping with the concentration of AVP found in hypophyseal portal blood, which amounts 2-3ng/ml plasma in the rat [8]. However, one must emphasize that this amount probably represents maximum level, resulting from the stressful conditions inherent to the procedure employed for blood collection. Moreover, the biological response of pituitary cells

[i.e. enhanced secretion of ACTH] also occured with doses of AVP within the nM range [4,9]. That effect,being uncoupled to cyclic AMP formation, suggests the pituitary vasopressin receptor be of the V1-type,or, as it is most likely, of a sub-population of that type of receptor since AVP dramatically increases cAMP accumulation in presence of synthetic CRF [5].

In order to analyse the binding specificity of the vasopressin receptor, competitive displacement of [^{3}H]-AVP binding to pituitary membranes was determined in presence of a variety of peptides and, especially, those which display CRF-like activities: oxytocin [10], angiotensin II [11], VIP [12] and synthetic bovine CRF. As shown in Fig.2, the following compo-

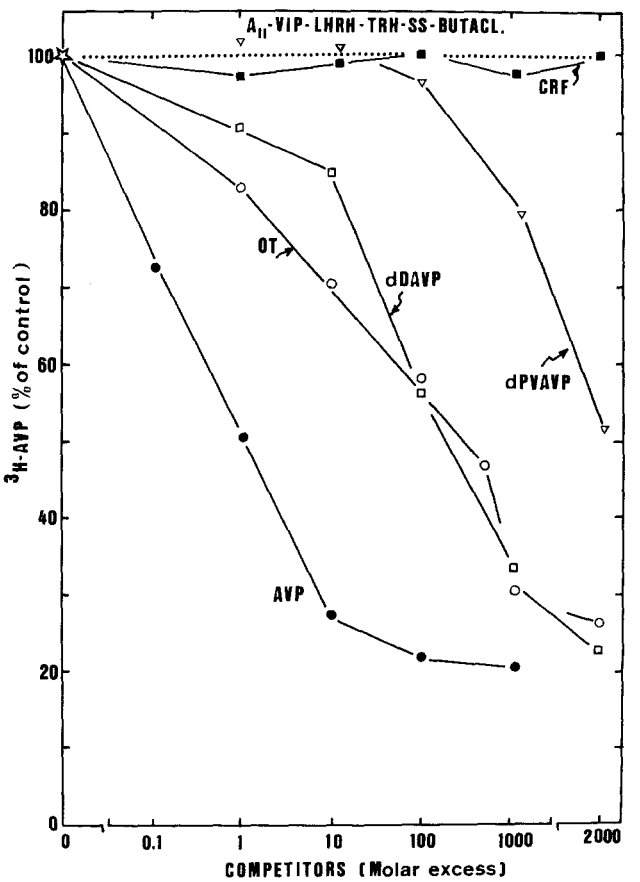

Fig.2: Competitive displacement of 2nM [^{3}H]-AVP binding to pituitary membranes by increasing concentrations of unlabeled AVP, dDAVP, dPVAVP, oxytocin [OT], angiotensin II [AII], VIP, LHRH, somatostatine [SS] and [+]butaclamol [BUTACL.]. Each substance was tested in duplicates, except for AVP, OT and CRFwhich were tested in 3 independent experiments with 2 assays in each.

nents failed to significantly compete for AVP binding sites: angiotensin II, VIP, LHRH, TRH, somatostatine and (+)Butaclamol and, importantly, CRF. The latter observation thus suggests the presence of two distinct populations of receptors for CRF and AVP-CRF; an issue which also arose from data based on measurement of biological effects of the peptides [13], as well as from binding studies performed using [^{125}I]radiolabeled CRF [14,15]. Interestingly, the parent hormone oxytocin appeared to be a weak competitor compared with vasopressin for [^{3}H]-AVP binding: K_i=110nM and K_i=1nM, respectively. Also, dDAVP exhibited displacement capacity similar to that of oxytocin,

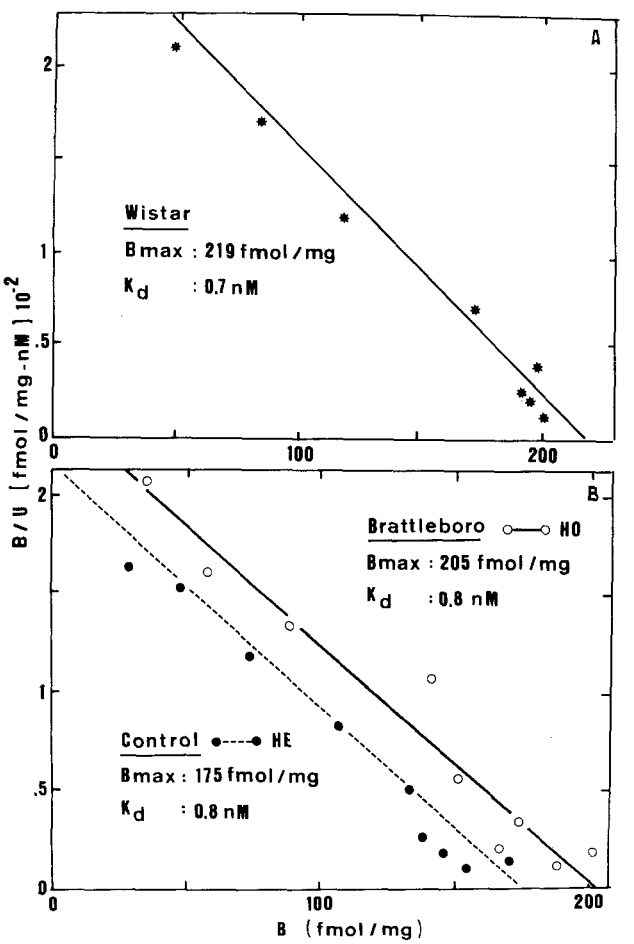

Fig.3: Scatchard plot of specific binding of [^{3}H]-AVP to pituitary membranes from normal Wistar rats (A), as well as from Brattleboro homozygotes (HO) and heterozygote (HE) controls (B). Each point is derived from 4 pituitary-equivalents of membranes.

a finding which correlates well with weak CRF-like properties of both peptides [16,17].

It is well recognized that receptors for peptidic hormones may be up- or down-regulated by their proper ligands [18]. We tested whether complete lack of vasopressin in homozygous Brattleboro rats would affect binding characteristics of putative pituitary receptors. Results in Fig.3B show not only that AVP receptors were present in pituitary tissue of these animals, but also that maximum binding capacity and dissociation constant were similar to those of the heterozygote controls. Studies are in progress to get a better insight into the mechanisms underlying receptor regulation and the apparent lack of effect of vasopressin. The receptor system of these vasopressin-deficient rats seems to be functionally relevant, since AVP was able to exert CRF-like effects at the level of the pituitary gland [17] and alter firing rate of hippocampal pyramidal cells [1].

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