Similarity of vasopressin receptors in seminal vesicles and renal medulla of pigs

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Summary. To test the hypothesis that the vasopressin receptors found in seminal vesicles are similar to those present in the renal tubules competition experiments were performed with vasopressin and several analogues with different specificities for the V1 and V2 subtypes of vasopressin receptor. Autoradiographic studies were carried out on sections from seminal vesicles and kidney to identify the cellular target of vasopressin. Vasopressin receptors in renal medulla and seminal vesicles of pigs shared the same rank order of potency for vasopressin and its analogues and were localized in the epithelium of the seminal vesicles and in collecting tubules of renal medulla. These results strongly suggest that the vasopressin receptors present in kidney and seminal vesicles belong to the same subtype, V2, of vasopressin receptor.

Keywords: arginine vasopressin; seminal vesicles; kidney; receptors; pigs

Introduction

Vasopressin receptors have been classified into two different subtypes, the calcium-dependent V1 receptors, present in blood vessels (Penit *et al.*, 1983) and liver (Cantau *et al.*, 1980), and the cyclic AMP-dependent V2 receptors, present in the medullary portion of the kidney (Bockaert *et al.*, 1973).

Arginine vasopressin (AVP) receptors have been identified in brain (Pearlmutter *et al.*, 1983), in the interstitial cells of the testis (Meidan & Hsueh, 1985), in several portions of the male genital tract (Maggi *et al.*, 1987) and in urinary bladder and spleen (Thibonnier *et al.*, 1987). All these sites seem to have the V1 subtype of AVP receptors.

We have identified and characterized a high concentration of AVP binding sites in the seminal vesicles of pigs (Maggi *et al.*, 1986). These receptors are linked to adenylate cyclase and display pharmacological characteristics different from those previously described for V1 AVP sites. These properties suggested to us that the AVP receptors in seminal vesicles were similar to the V2 receptors. However, the selective V2 agonist [1-deamino, 4-valine]D-Arg⁸-vasopressin (dVD⁸AVP) exhibited low affinity for this site and the V1 antagonist [1-deaminopenicillamine, 2-(0-methyl)tyrosine]Arg⁸-vasopressin (dPenTyrMeAVP) competed in nanomolar concentrations for [³H]AVP binding. Initially, we interpreted these results as indicating that vasopressin receptors in seminal vesicles were distinct from those of the V1 and V2 subtypes, and possibly related to those of the anterior pituitary (Antoni, 1984). However, the pig kidney differs from other species in terms of its pharmacological profile of affinities for several vasopressin analogues (Butlen *et al.*, 1978; S. Jard, personal communication). We have therefore reinvestigated the AVP receptors in seminal vesicle and kidney tissues of pigs under the same experimental conditions. Autoradiography was used to examine the target cells for vasopressin in the seminal vesicles and kidney.

Materials and Methods

Binding studies. [^3H]AVP (sp. act. 70 Ci/mmol) was purchased from New England Nuclear (Boston, MA, U.S.A.). To minimize degradation the tritiated ligand was placed in plastic tubes, sealed under nitrogen, and frozen at -80°C . AVP, dPenTyrMeAVP and [1-deamino]D-Arg⁸-vasopressin (dDVP) were from Sigma (St Louis, MO, U.S.A.). [1-(β -Mercapto- β , β -cyclopentamethylene propionic acid), 2-(0-methyl)tyrosine]Arg⁸-vasopressin (d(CH₂)₅TyrMeAVP) was from Peninsula Laboratories (San Carlos, CA, U.S.A.). [Thr⁴,Gly⁷]Oxytocin and [1-deamino,4-valine]D-Arg⁸-vasopressin (dVDVP) were synthesized and generously provided by Dr M. Manning (Medical College of Ohio, Toledo, OH, U.S.A.).

Domestic pigs, 1 month of age, were obtained from Cimex (Florence, Italy). Membrane fractions were prepared from seminal vesicles and from medullopapillary portions of kidneys according to the procedures previously described (Maggi *et al.*, 1986, 1987). Briefly, tissues were homogenized in Buffer 1 (10 mM-Tris-HCl, pH 7.4/1.5 mM-EDTA/0.5 mM-dithiothreitol/1 mM-benzamidine/0.01% bacitracin/0.002% soybean trypsin inhibitor) and crude membrane fractions were prepared by differential centrifugation between 1000 g (10 min) and 160 000 g (30 min). The pellets were washed once in Buffer 2 (50 mM-Tris maleate, pH 7.6/10 mM-MgSO₄/1 mM-benzamidine/0.01% bacitracin/0.002% soybean trypsin inhibitor). The membrane preparations were divided into small aliquants at a protein concentration of 2–4 mg/ml, frozen in solid CO₂ and stored at -80°C until assayed.

For receptor binding studies aliquants of membrane (0.2 mg/ml) were incubated with [^3H]AVP (0.075–0.6 nM) with or without increasing concentrations of unlabelled peptides. According to our previous findings, AVP is not selective for AVP receptors (Maggi *et al.*, 1986, 1987). AVP has a K_d in the nanomolar range for the AVP and oxytocin receptors. We therefore used 50 nM of the selective oxytocin agonist [Thr⁴,Gly⁷]oxytocin to block the oxytocin sites. [Thr⁴,Gly⁷]oxytocin at this concentration completely displaced [^3H]oxytocin from seminal vesicles membranes, but failed to affect [^3H]AVP binding (Maggi *et al.*, 1986). Binding reactions were carried out in Buffer 2 containing 0.1% bovine serum albumin (BSA), at 22°C for 60 min. Bound radioactivity was separated by rapid filtration through a Whatman GF/B filter using the M-48R Cell-Harvester (Brandel, Gaithersburg, MD, U.S.A.). Radioactivity retained on the filters was measured by liquid scintillation spectrometry 12 h after the addition of 10 ml Atomlight (Beckman). Protein content was measured using the Pierce protein assay reagent (Pierce Chemical Co., Rockford, IL, U.S.A.), based on the method of Bradford (1976). The results were analysed using the computer programs ALLFIT (De Lean *et al.*, 1978) and LIGAND (Munson & Rodbard, 1980).

Autoradiography. The autoradiographic study was carried out according to the protocol proposed by Young & Kuhar (1979). Tissue specimens were quickly frozen in isopentane cooled with liquid nitrogen. Serial sections (6–8 μm thick) of seminal vesicles and kidney cortex and medulla were obtained using a microtome cryostat and mounted on gelatin-coated microscope slides. The slides were air-dried at room temperature for 30 min and then preincubated at 4°C for 30 min in Buffer 1 containing 0.1% BSA. The slides were then washed twice at 4°C for 5 min in Buffer 2 containing 0.1% BSA and then incubated in the same buffer at 22°C for 60 min in the presence of 1 nM-[^3H]AVP and 50 nM-[Thr⁴,Gly⁷]oxytocin. Control sections were incubated in the same way but in the presence of 1 μM -AVP, to estimate the non-specific binding. At the end of the incubation, the slides were washed twice in ice cold 50-mM-Tris-HCl pH 7.4 for 5 min, air dried and fixed by exposure to formaldehyde vapour at 80°C for 60 min.

Coverslips coated with nuclear emulsion Ilford L4 (diluted 1:1, v/v, with distilled water) were applied to the sections which were kept in darkness at 4°C for 4–8 weeks. The slides were then developed in Kodak D-19, fixed, stained with toluidine blue or haematoxylin and eosin, and observed with dark- and bright-field microscopy. The sections stained with toluidine blue or haematoxylin and eosin were used for anatomical correlation. For further details on the autoradiographic procedures see Amenta (1986).

Results

Binding studies

Figure 1(a) shows a family of competition curves obtained in seminal vesicle membranes. AVP and the V1 antagonist dPenTyrMeAVP competed with high affinity for [^3H]AVP binding. The V2 agonists dVDVP and dDVP and the other V1 antagonist d(CH₂)₅TyrMeAVP tested showed lower potency. These results are in excellent agreement with our previous findings (Maggi *et al.*, 1986). Similar binding specificity was obtained in medullopapillary membranes (Fig. 1b).

Table 1 indicates the concentrations of receptors (binding capacity) and the affinity constant (K_d) in seminal vesicles and kidney medulla. These values were obtained by analysis with the program LIGAND (Munson & Rodbard, 1980). The affinities of the various peptides tested were virtually identical in both tissues.

Fig. 1. Displacement of specific [³H]AVP binding in (a) seminal vesicles and (b) the medullo-papillary portion of the kidney of pigs by AVP and several synthetic analogues. Membranes (0.2 mg/ml) were incubated for 60 min at 22°C with 0.6 nM-[³H]AVP in the presence or absence of increasing concentrations of AVP (▲), dPenTyrMeAVP (◆), d(CH₂)₅TyrMeAVP (△), ddAVP (●) or dVdAVP (○). A blocking concentration (50 nM) of the selective oxytocin agonist [Thr⁴,Gly⁷]oxytocin was present to mask the cross-reactivity of AVP to the oxytocin receptors.

Autoradiography

Figure 2 shows the main findings concerning the autoradiographic localization of [³H]AVP within seminal vesicles and renal medulla. In seminal vesicles specific [³H]AVP receptors were located almost exclusively in the epithelium (Fig. 2a-e). In the renal sections, [³H]AVP was bound to the epithelial cells of collecting tubules of the outer and inner medulla (Fig. 2f and g). In contrast, the renal cortex developed only non-specific binding (data not shown).

Table 1. Concentrations of receptors and affinities expressed as K_d for AVP, d(CH₂)₅TyrMeAVP, dPenTyrMeAVP, dVdAVP and ddAVP in membranes from seminal vesicles and renal medulla of pigs

	Seminal vesicles	Renal medulla
Binding capacity (fmol/mg protein)	246 ± 26	498 ± 29
K_d (nM)		
AVP	0.4 ± 0.07	0.3 ± 0.03
d(CH ₂) ₅ TyrMeAVP	28.3 ± 7.3	25.0 ± 3.5
dPenTyrMeAVP	0.8 ± 0.2	0.7 ± 0.1
dVdAVP	141.2 ± 36.7	89.7 ± 11.6
ddAVP	42.5 ± 10.6	38.8 ± 6.2

Values are mean ± s.e.m. of 3 experiments on pooled membranes derived from 4 prepubertal pigs.

Discussion

Our results support the presence of V2 (renal) vasopressin receptors in seminal vesicles of pigs. While V1 AVP receptors have been localized in several peripheral tissues, this study, together with our previous results (Maggi *et al.*, 1986), clearly indicates the presence of the V2 subtype of AVP receptors outside the kidney.

Binding competition studies performed in membranes prepared from pig seminal vesicles and kidney strongly suggest that the vasopressin receptors present in these tissues belong to the same subtype of vasopressin receptors. The finding that the two selective V2 (renal) vasopressin analogues tested in competition experiments exhibited low affinity for [³H]AVP binding in seminal vesicles and renal medulla of pigs is not surprising. Butlen *et al.* (1978) reported a different structural requirement for binding to pig or rat renal receptors. dVdAVP and ddAVP were potent ligands for the V2 receptors present in the rat kidney but had relatively low potency in the pig kidney in terms of binding or stimulation of adenylate cyclase.

The concentrations of AVP sites in seminal vesicles reported in the present study are 10-fold lower than those previously observed by us (Maggi *et al.*, 1986). However, there are differences in the age (1 month *versus* 3 months old) and the breed (domestic *versus* miniature pigs) of the animals used in these two studies. We are planning to perform further studies to clarify whether the density of AVP receptors in seminal vesicles is affected by age, breed or hormonal milieu.

Fig. 2. Autoradiographic demonstration of [³H]AVP binding sites within seminal vesicles and renal medulla of pigs. (a) Section of pig seminal vesicle stained with haematoxylin and eosin. Seminal vesicles have three tunics: an external areolar (a), a middle muscular (m) and an internal mucosa (arrow). × 40. (b) Higher magnification of (a). The mucous tunic surrounding the lumen (L), consists primarily of columnar epithelium cells (e). × 125. (c) Autoradiography of pig seminal vesicle incubated with 1 nM-[³H]AVP and 50 nM-[Thr⁴,Gly⁷]oxytocin in the presence of 1 μM-AVP showing a complete suppression of binding of labelled ligand. Dark field, × 125. (d) and (e) Autoradiography of sections of seminal vesicles after incubation with 1 nM-[³H]AVP and 50 nM-[Thr⁴,Gly⁷]oxytocin to block oxytocin receptors. Silver grains are located only within the epithelial cells (e) of the mucosa. L = lumen. Dark field: d, × 125; e, × 160. (f) Section of renal medulla stained with haematoxylin and eosin. Arrows indicate collecting tubules. × 80. (g) Section of pig renal medulla incubated with 1 nM-[³H]AVP and 50 nM-[Thr⁴,Gly⁷]oxytocin and processed for autoradiography. Specific silver grains are located at the level of the epithelial cells of the collecting tubules (t). Dark field, × 80.

The autoradiographic analysis of the anatomical localization of vasopressin receptors shows that in the kidney they are located, as expected, in the epithelial cells lining the medullary tubules, but not in the cortex (see Dorsa *et al.*, 1983). In the seminal vesicles AVP receptors were found in the epithelial cells facing the lumen of the glands. These findings, together with evidence that AVP receptors are coupled to adenylate cyclase in the kidney (Butlen *et al.*, 1978) and seminal vesicles (Maggi *et al.*, 1986), further indicate the similarity if not identity of AVP receptors in these two tissues. We therefore revise our previous interpretation that the seminal vesicle vasopressin receptors is of a subtype different from V1 or V2 receptors (Maggi *et al.*, 1986).

Renal vasopressin receptors regulate tubular fluid osmolarity, using cAMP as a second messenger. In contrast, the role of AVP receptors in the epithelial cells of the seminal vesicles is still obscure. Levine *et al.* (1975) showed that there is a cAMP-dependent active secretion of Cl^- in guinea-pig seminal vesicles and suggested that this active secretion may be a driving force for the initial secretion of electrolyte. Following this secretory phase, water and electrolytes are reabsorbed, resulting in an increased concentration of organic components. It is tempting to speculate that AVP affects one or several of the various steps involved in the regulation of seminal fluid osmolarity and perhaps other secretory activities of the epithelial cells of these glands. A large volume of fluid is produced by pig seminal vesicles, and the final volume of the ejaculate (200–500 ml) is two orders of magnitude higher than that of many other mammals. The presence of vasopressin receptors in the seminal vesicles of other animal species is under investigation.

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