Vasopressin Receptors in Human Seminal Vesicles: Identification, Pharmacologic Characterization, and Comparison with the Vasopressin Receptors Present in the Human Kidney

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Because of the presence of a high density of vasopressin receptors in the epithelial cells of porcine seminal vesicles similar to the V2 vasopressin receptors of renal tubules, human seminal vesicles and kidney were investigated using quantitative binding and adenylate cyclase studies. Tissues were obtained at surgery from 17 patients with urologic diseases. A homogeneous class of vasopressin binding sites have been found in both seminal vesicles and renal medulla. However, the vasopressin receptors present in these tissues are different in terms of ligand specificity and adenylate cyclase activation. In seminal vesicles, the V1 vasopressin antagonist d(CH$_2$)$_3$TyrMeAVP is 36-fold, more potent than the V2 agonist dVDAVP in displacing [H]AVP binding, while in the medullolapillary portion of kidney dVDAVP is 24-fold, more selective than d(CH$_2$)$_3$TyrMeAVP for the arginine vasopressin binding site. Furthermore, arginine vasopressin induces a dose-dependent increase in adenylate cyclase activity in renal membranes, while it was ineffective in seminal vesicle membranes. These results indicate that a very high affinity (0.2 nM), low capacity (14 fmoles/mg protein) class of vasopressin receptors is present in human seminal vesicles, having pharmacologic characteristics similar to the V1 subtype of vasopressin receptors. The presence of a high affinity (1.6 nM), high capacity (350 fmoles/mg protein) V2 subtype of vasopressin receptors in human renal membranes is also confirmed. The density of the vasopressin receptors present in human seminal vesicles is inversely correlated with patient age, consistent with a physiologic role for vasopressin in the regulation of accessory sex gland activity.

Key words: arginine vasopressin, oxytocin, seminal vesicles, kidney.

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Oxytocin and vasopressin (VP) are nonapeptide hormones synthesized by mammalian hypothalamus as well as by several peripheral tissues, including the gonads (Clements and Funder, 1986). Oxytocin is mainly involved in the control of parturition and
the expression of milk, whereas vasopressin plays a role in the regulation of plasma osmolality and the contractility of blood vessels. A single class of oxytocin receptors has been described in uterus and mammary gland (Soloff and Swartz, 1973, 1974), while two different subtypes of vasopressin receptors have been identified (Jard, 1983); (1) the V2 vasopressin receptors present in renal tubules, which are coupled to adenylate cyclase, and (2) the V1 vasopressin receptors present in the smooth muscle cells of blood vessels, functionally linked to intracellular calcium mobilization.

In addition to these hormonal functions, several lines of evidence suggest a role for oxytocin and vasopressin in stimulating the motility of the male genital tract (Cross, 1955; Melin, 1970; Knight, 1972; Wales, 1972; Hib, 1974a, 1974b, 1977; Ohanian et al, 1979; Jaakkola and Talo, 1981) and ejaculation (Kihlstrom and Melin, 1963; Fjellstrom et al, 1968; Knight and Lindsay, 1970; Kihlstrom and Agmo, 1974; Agmo, 1975). Receptors for the two neurohypophyseal hormones were identified throughout the porcine male genital tract (Maggi et al, 1986, 1987). Both oxytocin and the V1 subtype of vasopressin receptors were present in the tunica albuginea, epididymis and vas deferens (Maggi et al, 1987). In the seminal vesicles, a high density of vasopressin binding sites have been identified that are similar, if not identical, to those present in renal medulla (Maggi et al, 1986, 1988a). Autoradiographic studies performed on porcine seminal vesicles demonstrated that these receptors are localized in the epithelial cells facing the lumen of the gland (Maggi et al, 1988a). We have suggested that the vasopressins sites present in the seminal vesicles are probably involved in the regulation of the permeability of the epithelium and therefore, seminal fluid osmolality. At least in the pig, the V1 vasopressin receptors and the oxytocin receptors present in vas deferens, epididymis, and tunica albuginea may modulate the contractility of these structures.

To examine whether oxytocin and vasopressin play a role in the regulation of male accessory sex gland activity in the human, we performed quantitative binding studies using [3H]oxytocin, [3H]arginine vasopressin and [3H]1-(β-mercaptopo-β-cyclopentamethylene propionic acid), 2-(O-methyl)tyrosine[Arg⁸]-vasopressin (d(CH₃)₂TyrMeAVP) and measurements of adenylate cyclase activity in membranes prepared from human seminal vesicles. The medullopapillary portion of human kidney was also investigated as a control.

Materials and Methods

Chemicals

[3H]arginine vasopressin (AVP) (70 Ci/mmol), [3H]oxytocin (36 Ci/mmol) and [3H]d(CH₃)₂TyrMeAVP (56 Ci/mmol) were purchased from New England Nuclear (Boston, MA). The tritiated ligands were aliquoted in plastic tubes, sealed under nitrogen and frozen at −80 C. The purity of the tracers was assessed by HPLC as previously described (Maggi et al, 1988b). [3C]ATP (58 mCi/mmol) and [3H]cAMP (26.1 Ci/mmol) were purchased from Amersham (Buckinghamshire, UK). Arginine vasopressin, oxytocin, arginine vasotocin (AVT), lysine VP (LVP), [1-deamino-penicillamine, 2-(O-methyl)-tyrosine]Arg⁸-vasopressin (dPenTyrMeAVP), [1-deamino-penicillamine, 4-valine]D-Arg⁸-vasopressin (dPenVDAVP) and 1-deamino-8-D-AVP (dDAVP) were from Sigma (St. Louis, MO). [1-(β-mercaptopo-β-cyclopentamethylene propionic acid), 2-(O-methyl)-tyrosine]Arg⁸-vasopressin (d(CH₃)₂TyrMeAVP), [Phe⁴, Ile⁸, Orn⁴]-vasopressin ([Phe⁴, Orn⁴]AVP) and [1-(β-mercaptopo-β-cyclopentamethylene propionic acid)]D-Ile⁸, Ile⁴]AVP were from Peninsula Laboratories (San Carlos, CA). [1-deamino, 4-valine]D-Arg⁸-vasopressin (dVDAVP) and [threonine⁴, glycine⁷] oxytocin ([Thr⁴, Gly⁷]oxytocin) were synthesized and generously provided by Dr. M. Manning (Medical College of Ohio, Toledo, OH).

Tissues

Seminal vesicles were obtained from 10 patients (age 67 ± 3 years, mean ± SE, range 49–79) undergoing abdominal surgery for carcinoma of the bladder. The seminal vesicles were immediately plunged into ice-cold 0.9% NaCl, slit open longitudinally with a pair of sharp, fine scissors, and swirled in the saline to dislodge the seminal plasma. A large segment of kidney was obtained from seven patients (age 62 ± 3 years, mean ± SE, range 49–75) undergoing nephrectomy for neoplasia that had left a substantial portion of the kidney intact. The renal tissue was immediately chilled in ice-cold 0.9% NaCl and the medullopapillary portion of the kidney was dissected from cortex and connective tissue. Membranes were then prepared from seminal vesicles and renal medulla at 0–4 C according to the protocols for binding studies or adenylate cyclase assays, described below.

Binding Studies

Membrane fractions were obtained from seminal vesicles and the medullopapillary portion of kidneys as previously described (Maggi et al, 1986). Briefly, the tissues were homogenized in 5 vol of buffer I (10 mM Tris-HCl, pH 7.4, containing 1.5 mM EDTA, 0.5 mM dithiothreitol, 1 mM benzamidine, 0.01% bacitracin, and 0.002% soybean trypsin inhibitor) using an Ultra-Turrax homogenizer (Janke and Kunkel KG, Staufen, FRG), filtered through cheesecloth, and rehomogenized with a glass Teflon homogenizer (Thomas Scientific, Swedesboro, NJ). A particulate fraction was obtained through differential centrifugation between 1,000 × g (10 min) and 160,000 × g (30 min). Pellets were washed once in buffer II (50
mM Tris-maleate, pH 7.6, containing 10 mM MgSO₄, 1 mM benzamidine, 0.01% bacitracin, 0.002% soybean trypsin inhibitor) and finally resuspended at a protein concentration of 2.5–4.5 mg/ml. The membrane preparations were divided into 2 ml aliquots, frozen on dry ice and stored at −80°C until assayed. Binding studies were performed in buffer II in the presence of 0.1% BSA. Aliquots of membranes (final protein concentration: 0.3 mg/ml) were incubated at 22°C for 1 hour either with increasing concentrations of labeled ligands (0.06–0.7 nM) without unlabeled ligands or with a fixed concentration (0.7 nM) of labeled ligand in the presence of increasing concentrations (10⁻¹⁰ to 10⁻⁵ M) of unlabeled peptides. Binding reactions were terminated by rapid filtration through Whatman GF/B filters (Clifton, NJ) that had been presoaked in ice-cold 50 mM Tris, pH 7.4, in 0.1% BSA, using the M-48R Cell-Harvester (Brandel, Gaithersburg, MD). Filters were washed twice with 3 ml of ice-cold 50 mM Tris, pH 7.4. Bound radioactivity was measured in a liquid scintillation counter (Tri-Carb Packard Instrument Co., Downers Grove, IL) in 10 ml scintillation fluid (Filtercount, Packard Instrument Co., Downers Grove, IL).

Adenylate Cyclase Assay

Plasma membrane fractions were prepared according to Barnes et al (1974). Seminal vesicles or renal medulla were homogenized in 3 vol of a medium consisting of 0.25 M sucrose — 1 mM EDTA, pH 7.4, using a Ultra-Turrax homogenizer. The homogenate was filtered through cheesecloth and centrifuged at 1500 × g for 10 minutes. The resulting pellet was resuspended in 2 M sucrose and centrifuged at 13,000 × g for 15 minutes. The supernatant was then collected, diluted to a final concentration of 0.25 M sucrose and spun at 35,000 × g for 15 minutes. The resulting pellet was suspended in 0.25 M sucrose—1 mM EDTA, pH 7.4 and washed twice with the same buffer. The final pellet was suspended as before, at an approximate protein concentration of 1 mg/ml, divided in small aliquots, quickly frozen, and stored in liquid nitrogen.

Adenylate cyclase activity was measured as previously described (Baldi et al., 1988). Aliquots of membranes (30–60 μg of protein) were incubated in a final volume of 100 μl containing 80 mM Tris-HCl, pH 7.4, 10 mM theophylline, 5 mM MgSO₄, 0.1 mM ATP, 1 mM cAMP, 0.58 μCi [14C]ATP, 3.6 mg/ml creatine phosphate, 200 U/ml creatine kinase, 0.1% BSA. Incubations were carried out at 37°C for 20 minutes, in the absence or presence of increasing concentrations of arginine vasopressin (10⁻¹⁰ to 10⁻⁵ M). The incubation was stopped by adding 100 μl of a stopping solution (40 mM ATP, 1.4 mM cAMP, 2% sodium dodecyl sulfate, 10 mM Tris-HCl, pH 7.4) and [3H]cAMP. Labeled cAMP was separated from labeled ATP by sequential chromatography (Salomon et al., 1974) and counted in a beta counter (Tri-Carb, Packard Instrument Co., Downers Grove, IL). Each value was corrected for cAMP recovery (70–80%) as estimated from the recovered [3H]cAMP added to the tubes, and for the blank value determined in the absence of membranes.

Protein Concentration

Protein concentration was estimated using the Pierce protein assay reagent (Pierce Chemical Co., Rockford, IL) based on the method of Bradford (1976) with BSA as standard.

Analysis of Experimental Results

The dose-response curves from adenylate cyclase assays were analyzed using the computer program ALLFIT (De Lean et al., 1978). This program uses the constrained four-parameter logistic model to obtain estimates of ED₅₀ values, the logit-log slope ("pseudo-Hill coefficient") and relative potencies. Simultaneous analysis of families of self- and cross-displacement binding curves was performed using the computer program LIGAND (Munson and Rodbard, 1980). Models involving one, two, or three independent classes of sites were evaluated using weighted nonlinear least-squares curve fitting. Selections of the best model and significance of differences for parameter values were based on an F test using the "extra sum of squares" principle (Munson and Rodbard, 1980).

Results

Binding Studies

Seminal Vesicles. In human seminal vesicles [³H]arginine vasopressin binds to a single class of very high affinity, low capacity vasopressin sites, with Kᵣ = 0.2 ± 0.06 nM, and binding capacity R = 14.2 ± 2 fmol/mg protein (mean ± SE; seven experiments). Figure 1 (upper panel) shows the results obtained in one representative experiment. Specific binding sites for [³H]oxytocin were not detectable.

To characterize further the vasopressin binding sites in human seminal vesicles, we generated several families of competition curves using [³H]arginine vasopressin and [³H]d(CH₂)₅TyrMeAVP displaced by the corresponding unlabeled peptides and by dVDAVP, the vasopressin agonist that showed the highest affinity for the V₂ vasopressin receptor present in human renal membrane (Fig. 2, upper panel). The simultaneous computer modeling of 17 curves of self- and cross-displacement strongly indicates the presence of a homogeneous class of vasopressin receptor that differs from the vasopressin receptor identified in the medullary portion of human kidney (Table 1). Indeed, in human seminal vesicles d(CH₂)₅TyrMeAVP competes with virtually equal affinity as arginine vasopressin for [³H]arginine vasopressin and [³H]d(CH₂)₅-TyrMeAVP binding (Kᵣ = 0.3 ± 0.06 nM), while the V₂ agonist dVDAVP is 26-fold less potent (Kᵣ = 6.5 ± 2.14 nM) (Table 1). The V₁ vasopressin
receptor concentration in human seminal vesicles is inversely related to the age of the patients. This relationship can be described as linear (r = 0.9, P < 0.001) (Fig. 3).

Renal Medulla. In the medullopapillary portion of human kidney, Scatchard analysis of homologous competition curves of arginine vasopressin suggests the presence of a single class of high affinity, high capacity arginine vasopressin binding sites with $K_d = 1.6 \pm 0.2$ nM and binding capacity $R = 347$ fmoles/mg protein ± 50 (mean ± SE for four experiments). A representative Scatchard plot is shown in Fig. 1 (lower panel). No specific binding sites for $[^3H]$oxytocin have been observed.
Computer modeling of families of competition curves between arginine vasopressin, the V2 agonists dVDAVP or dDAVP and the V1 antagonist d(CH2)5TyrMeAVP using membranes from renal medulla from four patients confirmed that a model involving a single class of site was satisfactory in all cases. Figure 2, lower panel, shows a typical family of competition curves obtained using human renal medulla. To characterize further the vasopressin receptor subtype, several additional competition studies were performed in one membrane preparation using arginine vasopressin, dVDAVP, d(CH2)5TyrMeAVP and eight additional analogs with different selectivities for the V1 and V2 vasopressin receptors and for the oxytocin receptor (Table 1). This pharmacologic characterization of vasopressin receptors in human kidney confirms that the V2 subtype of vasopressin receptors is present: the V2 agonist dVDAVP and the V2 antagonist [d(CH2)5D-Ile2,Ile4]AVP compete with high affinity for [3H]arginine vasopressin binding, while the V1 agonist [Phe2,Orn9]VT and the V1 antagonist d(CH2)5TyrMeAVP show 2000- and 50-fold lower potencies, respectively. The antidiuretic hormones LVP and AVT have Ks in the nanomolar range for human renal membranes, while oxytocin and the selective oxytocin agonist [Thr4,Gly7] oxytocin are effective only when used at micromolar concentrations. No significant correlation was found between the concentration of arginine vasopressin binding sites in renal medulla and patient age (data not shown).

Adenylyl Cyclase Activity. In human renal membranes, arginine vasopressin stimulates adenylyl cyclase to 164 ± 12% of control values, with an ED50 of 0.5 ± 0.07 nM (mean ± SE, for three

![Graph](image)

Fig. 3. Correlation between the concentration of V1 vasopressin receptors in human seminal vesicles and the age of the patients. Regression analysis indicates a statistically significant negative relationship with a correlation coefficient of 0.9 (p < 0.001). Dashed lines indicate the 95% confidence limits for the line.
kidneys) (Fig. 4). The V2 agonist dDAVP stimulated adenylate cyclase activity in human renal medulla to a similar extent as arginine vasopressin, although with lower potency (ED$_{50}$ = 45 nM, one membrane preparation, data not shown). In contrast, increasing concentrations of arginine vasopressin up to 10$^{-6}$ M were not able to stimulate adenylate cyclase activity in plasma membranes derived from the seminal vesicles of three patients (Fig. 4).

Discussion

This is the first demonstration and characterization of vasopressin receptors in human seminal vesicles. These binding sites are present in the seminal vesicles from all seven patients investigated and their concentrations are inversely correlated with the age of the patients. In contrast, we did not identify any specific [H]oxytocin binding sites in these glands. The density of vasopressin receptors in human seminal vesicles is 10-100-fold lower than in porcine seminal vesicles and porcine or human kidney (Maggi et al, 1986, 1988a). The vasopressin receptors we have identified in human seminal vesicles bind both arginine vasopressin and the V1 antagonist d(CH$_2$)$_5$TyrMeAVP with high affinity, while showing lower affinity for the selective V2 agonist dVDAVP. These results, together with the lack of any significant effect of arginine vasopressin on adenylate cyclase activity, indicate that a low concentration of very high affinity vasopressin binding sites similar to the V1 subtype of vasopressin receptors are present in human seminal vesicles.

Several reports suggest that physiologic concentrations of vasopressin are able to increase both the volume and sperm count of the ejaculate (Kihlstrom and Agmo, 1974; Agmo, 1975). The V1 vasopressin receptors present in human seminal vesicles are similar to those previously identified in other tissues, such as blood vessels (Penit et al, 1983; Schiffrin and Genest, 1983), uterus and vagina (Maggi et al, 1988b) and that mediate the contractility of these tissues. A similar action might be considered for human seminal vesicles. Release of vasopressin at the time of coitus in men was first postulated by Friborg (1953) and subsequently confirmed by Fitzpatrick (1966). Recently, Murphy et al (1987) measured plasma arginine vasopressin in men, during sexual arousal and ejaculation using a very sensitive RIA. They observed a significant increase of arginine vasopressin at the time of sexual arousal, which declined at the time of ejaculation. Further studies are needed to clarify whether the V1 vasopressin receptors, described by us, in human seminal vesicles are involved in the modulation of the contractility of these accessory glands. In contrast, different findings have been reported in other mammalian species. Stoneham et al (1985) failed to detect any change in the plasma concentration of arginine vasopressin in male rabbits during sexual intercourse. Further, arginine vasopressin was unable to stimulate the motility of seminal vesicles either in vitro or in vivo in the rabbit (Cross and Glover, 1958; Melin, 1970; Stjernquist et al, 1983). In the pig, vasopressin receptors present in seminal vesicles belong to the V2 subtype and are localized in the epithelial cells of the glands (Maggi et al, 1988a). Our observation of the presence of V2 vasopressin receptors in human kidney, functionally coupled to adenylate cyclase activity, is consistent with those of Dousa (1974) and Guillon et al (1982). Moreover, we extended the pharmacologic characterization of this site by using selective ligands for the different neurohypophyseal hormone receptors. Our results also confirm the lack of any specific binding sites for the neurohypophyseal hormone oxytocin in renal medulla, and suggest that the modest antidiuretic activity of this peptide is likely to be due entirely to the crossreactivity of oxytocin with the V2 vasopressin receptors. Further, we find that the V2 agonist dVDAVP is 10-fold more potent in displacing [H]arginine vasopressin binding from renal membranes than dDAVP, which
is the vasopressin analog widely used clinically for therapy of patients with central diabetes insipidus. We confirmed that the latter compound is less potent than the natural antidiuretic hormone arginine vasopressin in human kidney, both in terms of ligand potency and adenylate cyclase stimulation (Guillon et al, 1982). Thus, the pronounced antidiuretic activity of dDAVP is likely related to its enhanced metabolic stability.

In conclusion, we have identified vasopressin receptors in human seminal vesicles that are distinct from the V2 vasopressin isoreceptor present in human kidney in terms of ligand specificity and adenylate cyclase activation. The density of the receptor in seminal vesicles is inversely correlated with patient age. These findings, together with the reported rise of plasma arginine vasopressin during sexual arousal in man, suggest the possibility of a role for vasopressin in the regulation of human reproductive function at the time of ejaculation.

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