V₁-Type Vasopressin Receptors in Rat Brain Septum: Binding Characteristics and Effects on Inositol Phospholipid Metabolism

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Specific binding sites for ³H-arginine⁸-vasopressin (AVP) have been characterized in rat septal membranes. Scatchard analyses revealed a single class of high-affinity binding sites having an equilibrium dissociation constant of 1.7 ± 0.3 nM and total binding capacity of 22.6 ± 4.2 fmol/mg protein. Binding displacement studies with peptide analogs of AVP indicate that this binding site is similar to the V₁ (pressor)-type receptor for AVP. When added to rat brain septal slices that had been prelabeled with ³H-myo-inositol, vasopressin stimulated the accumulation of ³H-inositol-1-phosphate (IP₁) in the presence of 7 mM lithium. This effect was dose dependent with maximal stimulation (65% over basal) occurring at a concentration of 0.5 μ M AVP. Higher concentrations, however, tended to inhibit phosphoinositide hydrolysis. The vasopressin-stimulated accumulation of ³H-IP₁ was completely inhibited by the vasopressin V₁ antagonist, d(CH₂)₅[Tyr(Me)²]AVP, in a concentration-dependent manner. Oxytocin, at concentrations of 10⁻⁸ and 10⁻⁵ M, only slightly increased ³H-IP₁ accumulation (17–20% over basal). In contrast, the V₂ agonist deamino-D-arginine vasopressin (dDAVP), failed to produce significant stimulation of ³H-IP₁ accumulation, even at high concentrations. The effects of these analogs on phosphoinositide hydrolysis is consistent with their potencies in displacing ³H-AVP from septal binding sites. These results indicate that vasopressin stimulates hydrolysis of inositol phospholipids in rat brain septum through an interaction with V₁-type vasopressin receptors.

The neurohypophyseal hormone vasopressin (AVP), with well-known peripheral endocrine functions, has also been postulated to serve as a neuromodulator or neurotransmitter in the CNS. AVP has been implicated as a neurotransmitter that can enhance certain forms of learning and memory consolidation (de Wied, 1965). One of the brain areas thought to be involved in learning and memory processes which is also a suggested site of action of AVP, is the septum. The lateral septum has been shown by immunohistochemical techniques to be innervated by AVP-containing neurons (Sofroniew and Weindl, 1981). Microinjection of AVP into the dorsal septal nucleus facilitates passive

avoidance behavior (de Wied, 1983), and iontophoretically applied AVP produces excitation of lateral septal neurons (Joëls and Urban, 1982). AVP also has several neuromodulatory effects in the septum, such as increasing catecholamine turnover (Kovacs et al., 1979) and enhancing the efficacy of excitatory amino acid transmission on lateral septal neurons (Urban and Joëls, 1983; Joëls and Urban, 1984). AVP has other central actions, including an antipyretic effect when perfused into the septal area (Kasting et al., 1979; Cooper et al., 1979) and induction of severe motor disturbances, such as barrel rotations and myoclonic/myotonic movements when microinjected into the ventral septal area (Burnard, 1985).

Receptors for AVP have been classified into 2 major subtypes (V₁ and V₂) based on their transduction mechanisms (Michell et al., 1979). The intracellular effects of V₁ receptors (glycogenolysis and vasoconstriction) appear to be mediated by hydrolysis of phosphatidylinositol, which leads to cytosolic Ca²⁺ mobilization (de Wulf et al., 1980), whereas V₂ receptors promote antidiuretic effects by activating adenylate cyclase and increasing cAMP levels (Jard, 1983). Recent development of a large series of AVP structural analogs that can distinguish between the 2 subtypes has supported this hypothesis (Kirk et al., 1981; Manning and Sawyer, 1983). Subsequently, differences in ligand selectivity have led to the further subdivision of the V₁ receptor into "V_{1a}" (liver, vascular smooth muscle, testicular interstitial cells, superior cervical ganglia, and adrenal cortex: Kirk et al., 1981; Balla et al., 1985; Kiraly et al., 1986; Meidan and Hsueh, 1985; Villet et al., 1986) and "V_{1b}" (adenohypophysis: Jard et al., 1986).

Specific binding sites for AVP have been demonstrated autoradiographically in the lateral septum, amygdala, nucleus of the solitary tract, olfactory nucleus, ventral subiculum, hippocampus, cerebellum, adenohypophysis, and spinal cord, as well as in the paraventricular and supraoptic nuclei of the hypothalamus (Baskin et al., 1983; Dorsa et al., 1983, 1984; van Leeuwen and Wolters, 1983; Yamamura et al., 1983; Biegon et al., 1984; Brinton et al., 1984; de Kloet et al., 1985). AVP binding sites have been further characterized in several brain areas (hippocampus, amygdala, and dorsal hindbrain) using membrane binding assays (Barberis, 1983; Dorsa et al., 1983, 1984; Pearlmutter et al., 1983; Constantini and Pearlmutter, 1984; Cornett and Dorsa, 1985). Potency profiles of AVP analogs suggest that the brain AVP receptor appears to be best described as a V₁ receptor (Cornett and Dorsa, 1985). In support of this hypothesis is the observation that AVP does not alter adenylate cyclase activity in various brain regions (Courtney and Raskind, 1983). Furthermore, AVP has been shown to stim-

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Figure 1. Saturation isotherm of the specific binding of ^3H -AVP to septal membrane preparations from Long-Evans rats. Figures shown are representative of typical values obtained in an experiment. *Insert*, Computerized Scatchard analysis of the data. Values for K_d and B_{max} are 1.7 ± 0.3 nM and 22.6 ± 4.2 fmol/mg protein, respectively, and represent means $\pm$ SEM from 5 separate experiments.

ulate phosphatidylinositol turnover through an interaction with a V_1 -type receptor in the superior and cervical ganglia (Bone et al., 1984), anterior pituitary (Raymond et al., 1985), and hippocampus (Stephens and Logan, 1986).

Since phosphoinositide hydrolysis has been shown to be important in mediating the action of AVP at V_1 receptors, we asked whether this pathway was also involved in mediating the CNS effects of AVP. This paper presents the characterization of AVP binding sites and stimulation of ^3H -IP $_1$ accumulation in the septum, an area known to contain a high density of receptors for AVP (Baskin et al., 1983; van Leeuwen and Wolters, 1983; Biegon et al., 1984; de Kloet et al., 1985).

Materials and Methods

Male Long-Evans rats (160–180 gm) were decapitated and their brains placed immediately in ice-cold, freshly gassed (95% O_2 –5% CO_2) buffer containing (mM): 118 NaCl, 4.7 KCl, 1.3 CaCl_2 , 1.2 KH_2PO_4 , 1.2 MgSO_4 , 5.0 MgCl_2 , 25.0 NaHCO_3 , 11.7 glucose (final pH = 7.4). The MgCl_2 was included in this buffer in light of the Mg^{2+} dependency of AVP binding (Gopalakrishnan et al., 1986). The brains were rapidly dissected over ice and tissue containing the septum was cross-chopped at 0.25 mm intervals with a McIlwain tissue chopper (Brinkman/Mickle Co., Westburg, NY). The slices were transferred to a flask containing 30 ml of warm buffer and preincubated for 30 min at 37°C in a gently shaking water bath with 2 intermediate changes of buffer.

The slices were allowed to settle and the medium removed. Fresh buffer was added to the slices to equal 3–4 times the initial incubation volume of packed slices in order to achieve a protein concentration of approximately 400–600 $\mu\text{g}/\text{tube}$. While gently agitating, 40 μl of the slices were transferred to 12 $\times$ 75 mm polypropylene tubes containing 230 μl buffer containing 0.3 μM ^3H -inositol. After 60 min of incubation at 37°C, LiCl (7 mM) was added. Agonists were added after 20 min in a total volume of 300 μl , and the incubation continued for an additional 60 min. When present, antagonists were added 10 min prior to agonists.

The reaction was terminated after washing the slices 3 times with 3 ml of fresh buffer and by the addition of 0.94 ml chloroform : methanol

(1:2 vol/vol) according to the method of Berridge et al. (1982). An additional 0.31 ml water and 0.31 ml chloroform were added and the tubes capped tightly and vortexed for 2 min. The tubes were then centrifuged ($500 \times g$ for 5 min at 4°C) to facilitate phase separation, and an aliquot (1 ml) of the aqueous phase (diluted 1:3 with water) was added to polypropylene columns containing 1 ml of Dowex 1-X8 (100–200 mesh) in the formate form. The columns were washed with 10 ml of 5 mM myo-inositol. ^3H -IP $_1$ was eluted directly into 20 ml scintillation vials with 5 ml of 0.2 M ammonium formate/0.1 M formic acid, and the radioactivity was measured after addition of 10 ml of hydrofluor (National Diagnostics, Mannville, NJ). Proteins were measured by the method of Bradford (1976).

Competitive binding experiments and saturation analyses were performed using membrane preparations from rat liver and brain tissues as previously described (Dorsa et al., 1983).

Myo-[2- ^3H]-inositol (15 Ci/mmol) and ^3H -AVP (17.1 Ci/mmol) were obtained from New England Nuclear (Boston). Aliquots of the trace were dried down the day of the experiment to remove the ethanol and resuspended in buffer to the desired concentration. Arginine 8 -vasopressin, oxytocin, and the V_1 antagonist $\text{d}(\text{CH}_2)_5[\text{Tyr}(\text{Me})^2]\text{AVP}$ were obtained from Peninsula Laboratories (Palo Alto, CA). Deamino-D-arginine 8 -vasopressin (dDAVP), a V_2 agonist, was obtained from USV Laboratories (Tarrytown, NY). Anion exchange resin and columns were obtained from BioRad Labs (Berkeley, CA). Long-Evans rats were purchased from Simonson Labs (Gilroy, CA). All other chemicals were obtained from Sigma Chemical Company (St. Louis).

Statistics. Data were statistically analyzed using one-way analysis of variance for all dose-response curves and where further *post hoc* testing was required, the Neuman-Keuls multiple range test was used. In some instances, Student's *t* test was performed. A value of $p < 0.05$ was required to consider differences significant.

Results

Receptors for ^3H -AVP were characterized in rat septal membrane preparations (Fig. 1). Binding of ^3H -AVP to septal membranes was saturable over ligand concentrations from 0.25 to 10.0 nM. Specific binding of up to 60% was observed. A com-

Figure 2. Inhibition of ^3H -AVP binding by vasopressin analogs and related peptides. Membrane preparations from septum (A) and liver (B) tissue were incubated with 2 nM ^3H -AVP and increasing concentrations of either AVP ($\circ$), $\text{d}(\text{CH}_2)_5\text{Tyr}(\text{Me})\text{AVP}$ ($\blacksquare$), oxytocin ($\diamond$), or dDAVP ($\bullet$). Points are the means from duplicate determinants from representative experiments.

puterized iterative curve-fitting procedure (Lundon Software, Cleveland, OH) was used to analyze Scatchard plots of AVP bound versus the ratio of bound to free AVP. A single class of high-affinity binding sites was revealed with a K_D of 1.7 ± 0.3 nM and B_{max} of 22.6 ± 4.2 fmol/mg protein ($n = 5$).

The interaction of AVP, oxytocin, dDAVP, and the V_1 antagonist $\text{d}(\text{CH}_2)_5[\text{Tyr}(\text{Me})^2]\text{AVP}$ with the septal AVP binding sites was investigated by comparing their ability to compete with ^3H -AVP for binding to septal membrane preparations (Fig. 2A). The potency profiles obtained in the septum were virtually identical to those from liver membrane preparations when measured in the same assay (Fig. 2B). The rank order of potency of these peptides was $\text{d}(\text{CH}_2)_5[\text{Tyr}(\text{Me})^2]\text{AVP} > \text{AVP} \gg \text{oxytocin} \geq \text{dDAVP}$.

In rat septal slices, AVP was found to induce a dose-dependent increase in ^3H -IP₁ formation over basal levels (Fig. 3) (1-way ANOVA, $f = 12.56$; 5,37, $p < 0.005$). Maximum stimulation of AVP-induced ^3H -IP₁ accumulation ($64.9 \pm 6.6\%$ over basal; $n = 7$) was observed at a concentration of $0.5 \mu\text{M}$ AVP (Neuman-Keuls multiple range test, $p < 0.01$). Higher doses of AVP, however, resulted in a decrease in ^3H -IP₁ accumulation. In a typical experiment, basal values were 700 and 1200 dpm in the presence of $0.5 \mu\text{M}$ AVP.

Preliminary experiments have been performed to optimize the assay time and tissue concentration. After 60 min of incubation with AVP, the AVP-stimulated ^3H -IP₁ levels were maximal and continued at this level for an additional 60 min (data not shown). A tissue concentration of approximately $600 \mu\text{g}/40 \mu\text{l}$ aliquot was required for the maximal response to AVP (data not shown). Concentrations both higher and lower resulted in submaximal responses. The extent of the response to AVP (65% stimulation) is much less than that observed for 2.0 mM car-

bachol ($268 \pm 22.6\%$, $n = 7$) in the septum (Table 1 and Fig. 5).

In the cerebral cortex, however, an area containing a high density of cholinergic muscarinic receptors but few, if any, AVP receptors, a large carbachol stimulation of ^3H -IP₁ accumulation was observed ($345 \pm 37.5\%$ over basal, $n = 3$) comparable to that observed in the septum ($t = 1.82$, $df = 8$, NS), while AVP failed to elicit a significant response ($5.6 \pm 3.2\%$, $n = 3$; Table 1, $t = 0.287$, $df = 4$, NS).

In order to examine the specificity of the AVP-induced response, oxytocin and dDAVP₁ were tested to determine their ability to stimulate ^3H -IP₁ accumulation. Oxytocin induced only slight increases in ^3H -IP₁ accumulation (17–20% stimulation above basal) (Fig. 4a), and the effects were not significant (1-way ANOVA, $f = 0.76$; 6, 35, NS). The renal V_2 agonist, dDAVP

Figure 3. Concentration-effect curves for AVP-stimulated ^3H -IP₁ accumulation in rat septal slices. Slices were preincubated for 30 min followed by incubation with $0.3 \mu\text{M}$ ^3H -inositol for 60 min. Lithium (7 mM) was then added, followed 20 min later by AVP, and incubation continued for an additional 60 min. Extraction of ^3H -IP₁ was performed as described in the text. Values are means $\pm$ SEM of 4–10 separate experiments performed in triplicate. Asterisks indicate significant differences in relation to lowest dose tested ($0.001 \mu\text{M}$) using 1-way ANOVA in conjunction with the Neuman-Keuls multiple range test: $*p < 0.05$, $**p < 0.01$.

Table 1. Stimulation of ^3H -IP₁ formation from rat septal and cerebral cortical slices in the presence of AVP and carbachol

Ligand	^3H -IP ₁ , % stimulation over basal level	
	Septum	Cerebral cortex
AVP ($0.5 \mu\text{M}$)	64.9 ± 6.6 (7)	5.6 ± 3.2 (3)
Carbachol (2 mM)	268 ± 22.6 (7)	345 ± 37.5 (3)

Values are means $\pm$ SEM of (n) determinations.

Figure 4. Concentration-effect curves for oxytocin (A), dDAVP (B), and $[\text{d}(\text{CH}_2)_5\text{Tyr}(\text{Me})^2\text{AVP}]$ (C) on $^3\text{H}\text{-IP}_1$ accumulation in rat septal slices. Conditions of incubation and extraction were as in Figure 2. Values are means $\pm$ SEM of at least 3 separate experiments.

failed to produce any significant dose-related stimulation of $^3\text{H}\text{-IP}_1$ levels (Fig. 4b) (1-way ANOVA, $f = 0.61$; 2, 7, NS).

To confirm that the AVP-induced $^3\text{H}\text{-IP}_1$ accumulation was mediated by the V_1 receptor, we tested the ability of the V_1 antagonist $[\text{d}(\text{CH}_2)_5\text{Tyr}(\text{Me})^2\text{AVP}]$ to inhibit the maximal response to AVP. The V_1 antagonist completely inhibited AVP-stimulated accumulation of $^3\text{H}\text{-IP}_1$ in a concentration-dependent manner with 90% inhibition of the response occurring at a concentration of 0.1 μM (Fig. 4). This compound alone did not significantly stimulate $^3\text{H}\text{-IP}_1$ accumulation at any of these concentrations (Fig. 4c) (1-way ANOVA, $f = 1.09$, 3, 8, NS). The V_1 antagonist, however, also produced a partial, but significant (Neuman-Keuls multiple range test, $p < 0.05$), inhibition of the carbachol-stimulated response (40%) at a concentration of 1.0 μM , a dose at which the muscarinic antagonist atropine completely inhibited the response (99.9% at 1.0 μM ; Fig. 5).

Discussion

The results obtained in this study provide strong evidence that AVP induces phosphoinositide hydrolysis in rat brain septum through activation of a V_1 -like receptor. Using a slightly modified revision of the procedure of Berridge et al. (1982), we have shown that in rat septal slices, AVP induces a dose-dependent increase in $^3\text{H}\text{-IP}_1$ accumulation in the presence of lithium. Other areas of the brain known to contain AVP receptors (hindbrain, hippocampus, and amygdala) also produced an AVP-stimulated accumulation of $^3\text{H}\text{-IP}_1$ (data not shown), but these responses have not yet been fully characterized. In the septum, maximal stimulation was obtained at a concentration of 0.5 μM AVP,

promoting a 60–70% increase over basal values. This response appears to be complex in that higher concentrations of AVP inhibit the response.

At present, we have no explanation for the shape of this dose curve response but suggest that it may result from overlapping stimulatory and inhibitory effects of AVP on phosphoinositide turnover. This could be due to feedback inhibition of some compound that builds up during AVP stimulation. One candidate might be the diacylglycerol formed by phosphatidylinositol-4,5-bisphosphate (PIP_2) hydrolysis, which has been postulated to act through protein kinase C as an endogenous inhibitor of hormone-stimulated phosphoinositide metabolism (Orellana et al., 1985). Alternatively, a build-up of intracellular Ca^{2+} concentrations at higher doses of AVP ($>10^{-7}$ M) could increase the activity of a Ca^{2+} -activated inhibitory protein, which has been reported to inhibit AVP binding (Fishman et al., 1986) and presumably phosphoinositide hydrolysis as well (Creba et al., 1983; Kirk et al., 1984). Dose-response curves for AVP-induced Ca_i^{2+} elevation in smooth muscle cells show that maximal Ca^{2+} concentrations are obtained at AVP concentrations between 10^{-7} and 10^{-6} M (Aiyar et al., 1986). Similar results are seen with platelets (Pollock and MacIntyre, 1986) and hepatocytes (Thomas et al., 1984).

Another possibility is that AVP might interact with another binding site that inhibits phosphoinositide hydrolysis. Audiger and Barberis (1985) have recently described 2 distinct populations of binding sites for neurohypophyseal hormones in the hippocampus: one population with a high affinity for AVP, oxytocin and arginine vasotocin (AVT) and the other with a high affinity for AVP and AVT and a low affinity for oxytocin. Since AVP binds to both sites with similar affinity, it would be possible

Figure 5. Antagonism by the V_1 antagonist $d(\text{CH}_2)_5[\text{Tyr}(\text{Me})^2]\text{AVP}$ of AVP-stimulated $^3\text{H}\text{-IP}_1$ accumulation in rat septal slices. Results are expressed as the percentage of maximal effect produced by $0.5 \mu\text{M}$ AVP. Conditions of incubation and extraction were as in Figure 2, with the addition of the antagonist 10 min prior to AVP. Values are the means $\pm$ SEM from 3 separate experiments. Antagonism was significant by 1-way ANOVA ($f = 16.5$, 3, 8, $p < 0.05$).

for one site to cause stimulation and the other inhibition of phosphoinositide hydrolysis; this could account for the bimodal response observed with AVP. We intend to explore these possibilities further with the use of more selective AVP and oxytocin agonists and antagonists.

The unusual response of agonist-induced $^3\text{H}\text{-IP}_1$ accumulation seen for AVP is not unique to the literature. Similar responses have been reported for glucagon in hepatocytes (Wakelam et al., 1986) and 5-HT in the hippocampus (Janowsky et al., 1984). Comparable responses to AVP-stimulated IP_1 accumulation, however, have not been observed for the other V_1 receptors mediating phosphoinositide hydrolysis: hepatocytes (Kirk et al., 1981), smooth muscle (Villet et al., 1986), platelets (Pollock and MacIntyre, 1986), sympathetic ganglia (Bone et al., 1984), anterior pituitary cells (Raymond et al., 1985), adrenal cortex (Balla et al., 1985), or WRK₁ cells (derived from a mammary tumor) (Kirk et al., 1986); however, the concentration of AVP eliciting the maximal response is typically in the range of 10^{-8} – 10^{-6} M, in agreement with the present results.

The response in the septum seen with AVP (65%) is much smaller than that seen for AVP stimulation of phosphoinositide hydrolysis in other tissues. For example, concentrations of 10^{-7} and 10^{-6} M AVP elicit 600 and 700% stimulation of IP_1 accumulation in hepatocytes and smooth muscle, respectively (Thomas et al., 1984; Aiyar et al., 1986). This is not surprising since there seems to be a good correlation between receptor occupancy and phosphoinositide hydrolysis (Creba et al., 1983; Kirk et al., 1984; Lynch et al., 1985). In liver tissue from male Long-Evans rats the concentration of AVP binding sites is approximately 300 fmol/mg protein (Shewey, unpublished observation), and in the septum, 23 fmol/mg protein (Fig. 1). The AVP response in the septum is also much smaller than that seen with (2 mM) carbachol in our assay (270%) or to the reported responses for other agonists in the brain such as excitatory amino acids (750%), norepinephrine (250%), histamine (125%), or 5-HT (133%) (Brown et al., 1984; Janowsky et al., 1984; Claro et al., 1986; Nicoletti et al., 1986) but is similar to the response elicited by another neuropeptide, substance P (Mantyh et al., 1984). It is possible that these differences can be explained by the large discrepancies between the receptor densities for compounds eliciting the largest responses (i.e., carbachol: 1.1 pmol/mg pro-

Figure 6. Carbachol-stimulation (2 mM) of $^3\text{H}\text{-IP}_1$ accumulation in rat septal slices in the absence (A) or presence of atropine $0.1 \mu\text{M}$ (B), $1 \mu\text{M}$ (C), $10 \mu\text{M}$ (D), and $1.0 \mu\text{M}$ $d(\text{CH}_2)_5[\text{Tyr}(\text{Me})^2]\text{AVP}$ (E). Antagonists were added 10 min prior to the addition of carbachol. Values are means $\pm$ SEM of 3 separate experiments.

tein in cortex; Costa et al., 1986) and the smallest (i.e., AVP: 23 fmol/mg protein), as has been shown for substance P (Mantyh et al., 1984). This is also supported by the observation that in the cerebral cortex, an area that does not contain measurable AVP receptors, very little stimulation of $^3\text{H}\text{-IP}_1$ accumulation with AVP is observed; however, a large response to carbachol is elicited. Nonetheless, various other possibilities can account for the variability in agonist-induced responses, such as stimulation of different pools of phospholipids, as has been shown for AVP and muscarine in the superior cervical ganglion (Horwitz et al., 1986), down-regulation of one of the receptors, or differences in the metabolism of the agonists.

Selective agonists and antagonists were employed to characterize further the AVP-stimulated phosphoinositide hydrolysis in the septum. Since the AVP V_1 receptor subtype by classical definition (Michell et al., 1979) is the subtype that mediates AVP stimulation of phosphoinositide hydrolysis and Ca^{2+} mobilization, we wanted to affirm that the AVP-stimulated $^3\text{H}\text{-IP}_1$ accumulation in septal slices was mediated by a V_1 -like receptor. The failure of the selective V_2 agonist dDAVP to stimulate phosphoinositide hydrolysis even at high concentrations, the small stimulatory effect of oxytocin, and the inhibition of the AVP-stimulated response by the specific V_1 antagonist $d(\text{CH}_2)_5[\text{Tyr}(\text{Me})^2]\text{AVP}$ strongly support the theory that AVP stimulates phosphoinositide hydrolysis in the septum by interaction with a V_1 -type receptor. The potencies of the agonists and antagonist tested for their abilities to displace $^3\text{H}\text{-AVP}$ from binding sites in the septum were very similar to the profiles for displacement in the liver and were in excellent agreement with their potencies in stimulating or inhibiting phosphoinositide hydrolysis mediated by the septal AVP receptor, again indicating that this response is mediated via AVP receptors that are similar to hepatic V_1 receptors.

One interesting and unexplained phenomenon we observed was that the V_1 antagonist also partially inhibited the carbachol stimulation of $^3\text{H}\text{-IP}_1$ accumulation, though not to the same extent as atropine. Since it seems unlikely that the V_1 antagonist would interact with muscarinic receptors, it may be that some portion of the carbachol response is due to endogenous AVP

release. Cholinergic agonists are known to be potent stimulators of neuronal AVP release (Nordmann et al., 1971; Sladek, 1983), and preliminary evidence (Shewey et al., unpublished observations) indicates that carbachol may cause release of vasopressin from these septal slices during the incubation.

In summary, we have demonstrated that AVP is able to stimulate phosphoinositide hydrolysis in rat septal slices. The specificity of this effect—as shown by the partial stimulation by oxytocin, the inability of dDAVP (a V_2 agonist) to elicit this response, and the complete inhibition of the AVP stimulation of ^3H -IP₁ accumulation by the V_1 antagonist—is in agreement with the potency profiles for these peptides in displacing ^3H -AVP from septal binding sites and is consistent with an action on V_1 -type receptors.

For some time now, AVP has been postulated to play a role as a neurotransmitter or neuromodulator in the CNS. In the septum, AVP receptors are thought to be involved in mediating AVP-induced antipyresis, neuronal excitation, potentiation of excitatory amino acid effects, and modulation of memory processes. Although much data has been accumulated on localization, behavioral and physiological effects, and demonstration of specific binding sites for AVP in the brain, the mechanism of action of AVP in the brain was unknown. This paper provides the first demonstration that AVP receptors in the rat brain are coupled to inositol phospholipid hydrolysis, which may be the second messenger system involved in mediation of the CNS actions of AVP.

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