

Electrophysiological and autoradiographical evidence of V₁ vasopressin receptors in the lateral septum of the rat brain

(binding sites/brain slices/oxytocin/receptor subtypes/synthetic structural analogues)

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ABSTRACT Extracellular recordings were obtained from single neurons located in the lateral septum, an area known to receive a vasopressinergic innervation in the rat brain. Approximately half of the neurons tested responded to 8-L-arginine vasopressin (AVP) by a marked increase in firing rate at concentrations >1 nM. The effect of vasopressin was blocked by synthetic structural analogues possessing antagonistic properties on peripheral vasopressin and oxytocin receptors. Oxytocin was much less potent than vasopressin in firing septal neurons, and a selective oxytocic agonist was totally ineffective. The action of vasopressin on neuronal firing was mimicked by the vasopressor agonist [2-phenylalanine,8-ornithine]vasotocin but not by the selective antidiuretic agonist 1-deamino[8-D-arginine]vasopressin. In a parallel study, sites that bind [³H]AVP at low concentration (1.5 nM) were found by *in vitro* autoradiography in the lateral septum. Adjacent sections were also incubated with 1.5 mM [³H]AVP and, in addition, with 100 nM [2-phenylalanine,8-ornithine]vasotocin or 1-deamino[8-D-arginine]vasopressin—i.e., the same compounds as those used for the electrophysiological study. Results showed that the vasopressor agonist, but not the antidiuretic agonist, displaced [³H]AVP, thus indicating that the vasopressin binding sites detected by autoradiography in the septum were V₁ (vasopressor type) rather than V₂ (antidiuretic type) receptors. Based on the electrophysiological evidence, we conclude that these receptors, when occupied, lead to increased firing of lateral septal neurons.

The neurohormone vasopressin is synthesized in the hypothalamus and carried by axoplasmic transport to the neurohypophysis, where the arrival of action potentials triggers its release from nerve terminals into the bloodstream (1). Vasopressin then acts upon various peripheral tissues after its binding to membrane receptors. The hypertensive effect of vasopressin is mediated by V₁ receptors located on vascular smooth muscle; in addition, V₁ receptors have been detected in several other tissues, such as liver, where vasopressin causes glycogenolysis (2). Binding of vasopressin to V₁ receptors stimulates Ca²⁺ mobilization through a process that involves phosphatidylinositol breakdown (3). The antidiuretic effect of vasopressin is mediated by V₂ receptors located in the kidney that activate a membrane adenylate cyclase (2).

Furthermore, it has become clear that vasopressin is synthesized by neurons in hypothalamic and extrahypothalamic nuclei the axons of which project to numerous areas within the mammalian central nervous system (4, 5). In rats, the lateral division of the septum is densely innervated by vasopressin-immunoreactive axons and axon terminals (6, 7). Some of these axons originate in the bed nucleus of the stria terminalis (8), whereas others may derive from the paraven-

tricular nucleus or from the amygdala (9, 10). Vasopressin can be released in a calcium-dependent manner from rat septum *in situ* (11) and *in vitro* (12). In addition, binding sites for vasopressin have been detected by light microscopic autoradiography in the lateral septum (13–16). Taken together, these data suggest that vasopressin may function as a peptide transmitter in the lateral septum.

To corroborate this conjecture we have carried out extracellular single-unit recordings from slices of the rat brain and have observed that vasopressin increases the excitability of neurons located in the lateral septum. Next, to characterize the receptor type(s) mediating this stimulatory effect of vasopressin, we have tested various synthetic structural analogues of vasopressin that possess receptor-specific, agonistic properties in peripheral tissues (17). In a parallel study, we determined the distribution of [³H]vasopressin and [³H]oxytocin binding sites in the septum by *in vitro* light microscopic autoradiography and assessed the ligand specificity of these binding sites by performing displacement tests using the same structural analogues.

MATERIALS AND METHODS

Electrophysiological Recordings. Experiments were done using male adult rats (200–300 g) from a Sprague–Dawley-derived strain. The animals were decapitated, their brains were excised, and a block of brain tissue containing the septum was prepared. Coronal slices, 300–400 μm thick, were cut using a vibrating microtome. Slices were transferred to a thermoregulated (34–35°C) recording chamber and placed at the interface between a humidified oxygenated atmosphere and a perfusion solution of 130 mM NaCl/5 mM KCl/20 mM NaHCO₃/1.2 mM KH₂PO₄/10 mM glucose/1 mM MgSO₄/0.7 mM CaCl₂. The solution was gassed with 95% O₂/5% CO₂, pH 7.35–7.45. Peptides were tested by dissolving them in the perfusion solution, which flowed at ≈2 ml/min and could be exchanged in 2.5–3.0 min. Under microscopic guidance, extracellular single-unit recordings were obtained from the lateral septum using glass micropipettes filled with 4 M NaCl and having tip dc resistances of 5–25 MΩ. Voltage signals were amplified, filtered, and displayed on an oscilloscope. Records of single-unit activity were gathered with a rate meter and monitored on chart.

Light Microscopic Autoradiography. The brains of animals from the same stock were rapidly removed and frozen in

Abbreviations: AVP, 8-L-arginine vasopressin; OXT, oxytocin; [Phe²,Orn⁸]VT, [2-phenylalanine,8-ornithine]vasotocin; HO[Thr⁴,Gly⁷]OXT, [1-(L-2-hydroxy-3-mercaptopropionic acid), 4-threonine,7-glycine]OXT; deamino-D-*AVP*, 1-deamino[8-D-arginine]vasopressin; dEt₂Tyr(Me)-*AVP*, [1-(β-mercaptopropionic acid),2-O-methyltyrosine,8-D-arginine]vasopressin; d(CH₂)₅Tyr(Me)-*AVP*, [1-(β-mercaptopropionic acid),β-cyclopentamethylene propionic acid],2-O-methyltyrosine]arginine vasopressin.

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Table 1. Endocrine activities (international units per mg) of AVP, OXT, and agonists

Peptide	Vasopressor (V ₁)	Antidiuretic (V ₂)	Oxytocic*
AVP [†]	369	323	25.5
[Phe ² ,Orn ⁸]VT [‡]	121	1.6	5.3
Deamino-DAVP [†]	0.4	1200	2.9
OXT [§]	4.3	4	486
HO[Thr ⁴ ,Gly ⁷]OXT [§]	<0.01	0.004	1002

*Tested on rat uterus *in vitro* in the presence of 0.5 mM Mg²⁺.

[†]Manning *et al.* (20).

[‡]Berde *et al.* (21).

[§]Lowbridge *et al.* (22).

isopentane at -25°C. Series of 10- to 15- μ m-thick sections were cut, mounted on gelatin-coated slides, dried for 2-12 hr at 4°C, and stored at -80°C until use. Control sections of kidney were also prepared. Sections were preincubated for 20 min in 50 mM Tris-HCl (pH 7.4) containing 100 mM NaCl and 50 nM guanosine 5-triphosphate, then rinsed in 50 mM Tris-HCl. Incubation was then carried out for 1 hr in a humid chamber by covering each section with 100-200 μ l of medium of 50 mM Tris-HCl/0.1 mM bacitracin/5 mM MgCl₂/0.1% bovine serum albumin containing various labeled and unlabeled peptides.

For differentiation of oxytocin (OXT) and 8-L-arginine vasopressin (AVP) binding sites, one series of sections was incubated with 1.5 nM [³H]AVP, and another series was incubated with 3.5 nM [³H]OXT. Adjacent sections were incubated with the same concentration of one of these labeled peptides and, in addition, with 150 nM of the specific oxytocic agonist [1-(L-2-hydroxy-3-mercaptopropionic acid),4-threonine,7-glycine]oxytocin (HO[Thr⁴,Gly⁷]OXT).

For V₁/V₂ receptor discrimination, one series of sections was incubated with 1.5 nM [³H]AVP; adjacent sections were incubated with the same concentration of vasopressin and, in addition, with 100 nM of either the V₁ agonist [2-phenylalanine,8-ornithine]vasotocin ([Phe²,Orn⁸]VT) or the V₂ agonist 1-deamino[8-D-arginine]vasopressin (deamino-DAVP).

Incubation was followed by two washes in ice-cold incubation medium and a quick rinse in distilled water. The slides were then dried with cold air, put for 2 hr in a vacuum desiccator containing paraformaldehyde powder preheated at 80°C, and then placed in an x-ray cassette in contact with tritium-sensitive LKB Ultrafilm for 3-4 mo at 4°C.

Chemicals. AVP and OXT were purchased from Bachem Fine Chemicals (Bubendorf, Switzerland). The synthetic structural analogues [Phe²,Orn⁸]VT; deamino-DAVP; HO-[Thr⁴,Gly⁷]OXT; [1-(β -mercapto- β , β -diethylpropionic acid),2-O-methyltyrosine,8-D-arginine]vasopressin [dEt₂Tyr(Me)-DAVP]; and [1-(β -mercapto- β , β -cyclopentamethylene propionic acid),2-O-methyltyrosine]arginine vasopressin [d(CH₂)₅-Tyr(Me)DAVP] were provided by M. M. Manning (Department of Biochemistry, Medical College of Ohio, Toledo, OH). Three of these compounds are agonists possessing enhanced receptor specificity, as is shown in Table 1. The last two compounds are powerful V₁ and oxytocic antagonists and possess extremely weak V₂ agonistic activity (18, 19).

For autoradiography, AVP tritiated on the phenylalanyl residue (specific activity, 40 Ci/mmol; 1 Ci = 37 GBq) and tritiated OXT labeled on the tyrosyl residue (specific activity, 35 Ci/mmol) were purchased from New England Nuclear. Their radiochemical purity was checked by HPLC, the fractions corresponding to the tritiated peptide being separated on an affinity-chromatography column containing neurophysin bound to Sepharose.

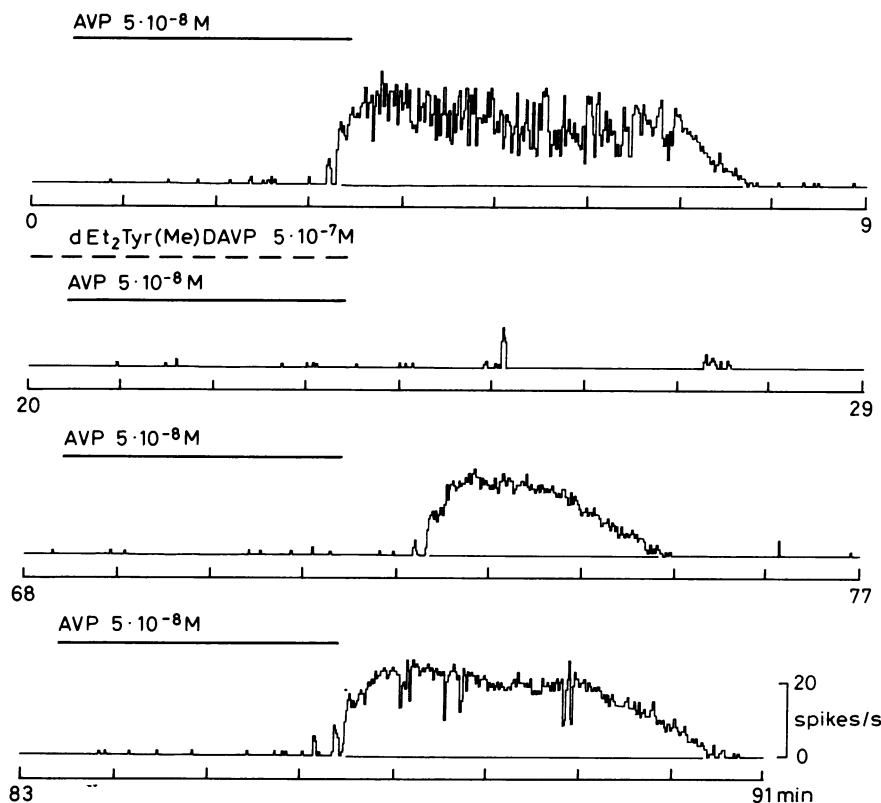


FIG. 1. Effects of AVP and of the synthetic structural analogue dEt₂Tyr(Me)DAVP, on a septal neuron. In this and the two following illustrations, the records are consecutive, but not continuous. AVP was added to the perfusion solution at the concentration indicated and for the period of time shown by a solid horizontal line above each record. The synthetic analogue, present at 500 nM for 6.5 min, starting from min 17 completely and reversibly suppressed the AVP-induced increase in firing.

RESULTS

Electrophysiology. Stable unitary extracellular recordings were obtained from over 100 neurons located in the dorsal and intermediate areas of the lateral septum. They were either silent or fired irregularly. Vasopressin, at concentrations >1 nM, excited 52 neurons; 45 neurons did not respond, and 8 neurons were inhibited. The vasopressin-induced excitation was concentration-dependent and fully reversible. This increase in firing rate was probably caused by membrane depolarization because at concentrations >100 nM, the excitation brought about by vasopressin could lead to partial action potential inactivation. Evidence that the action of vasopressin was receptor-mediated was provided by testing 15 neurons without and with the synthetic structural analogues, $dEt_2Tyr(Me)DVP$ and $d(CH_2)_5Tyr(Me)AVP$. These compounds did not by themselves affect the spontaneous firing of the cells but fully and reversibly antagonized the excitation induced by vasopressin (Fig. 1).

In order to assess which receptors were involved, the effects of vasopressin, of OXT, and of the selective oxytocic agonist were tested on eight neurons, which were all excited by vasopressin. OXT also had an excitatory action, but it was less potent by a factor of 10–200 than vasopressin (Fig. 2, third trace). The selective oxytocic agonist had no effect, even when applied at a high concentration (Fig. 2, fourth trace).

To further characterize the vasopressin-induced response, the action of vasopressin was compared with that of a V_1 agonist, $[Phe^2, Orn^8]VT$, and with that of the selective

antidiuretic agonist, deamino-DVP. These tests were done on nine neurons. The V_2 agonist was without effect even when applied at a high concentration; in contrast, all nine neurons tested were readily excited by the V_1 agonist: at two to five times higher concentrations $[Phe^2, Orn^8]VT$ produced an effect comparable to that of AVP (Fig. 3).

Autoradiography. In coronal sections that included the septum, the distribution of vasopressin and OXT binding sites differed markedly. Thus, whereas the septum contained no binding sites for OXT (Fig. 4A), the lateral septum was one of areas of the brain in which $[^3H]$ vasopressin labeling was most intense (Fig. 4C). OXT binding was detected in the dorsal part of the bed nucleus of the stria terminalis, and this binding could be displaced by $HO[Thr^4, Gly^7]OXT$ (Fig. 4B). In contrast, $HO[Thr^4, Gly^7]OXT$ could not successfully compete with vasopressin binding (Fig. 4D).

In two more animals, the V_1 character of $[^3H]AVP$ binding in septum could be ascertained by using V_1 and V_2 agonists in competition studies. Results are shown in Fig. 5, with kidney slices included for comparison. In contrast to renal labeling, vasopressin binding in septum was displaced by the V_1 agonist (Fig. 5D) but not by the V_2 agonist (Fig. 5H).

DISCUSSION

We have shown that the predominant effect of vasopressin on neurons located in the lateral septum of rats is excitatory; moreover, the effects of receptor specific agonists indicated that this action of vasopressin was mediated by V_1 receptors.

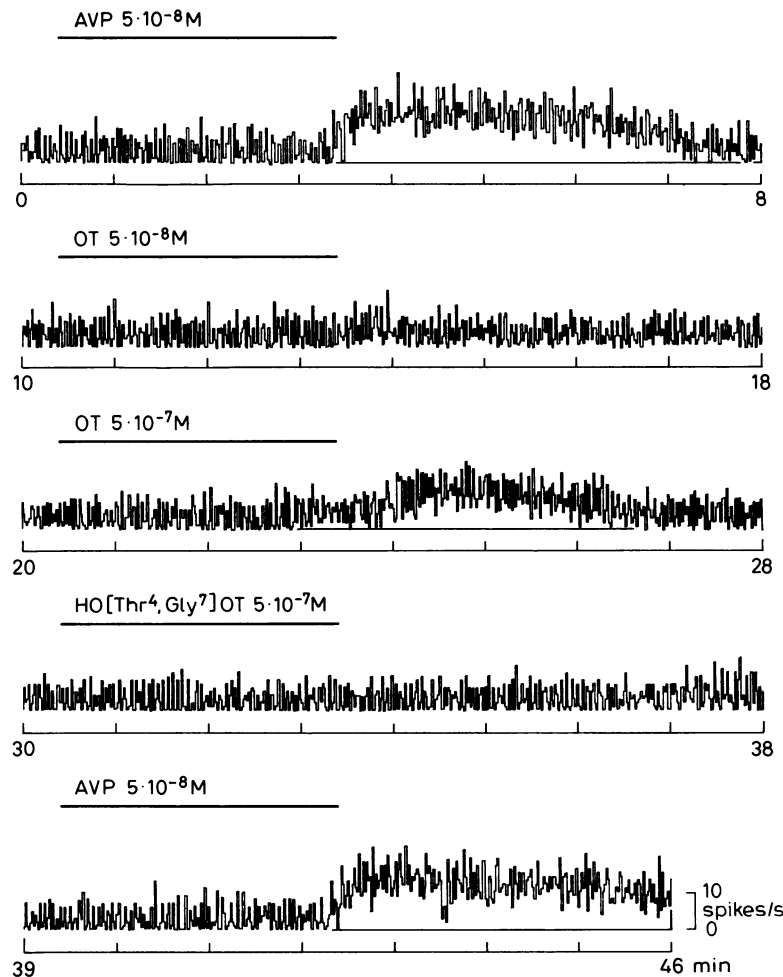


FIG. 2. Effects of AVP, of OXT (OT), and of the selective oxytocic agonist $HO[Thr^4, Gly^7]OXT$ (OT) on the firing activity of a septal neuron. Note that the selective oxytocic agonist had no effect and that OXT was weaker by a factor of >10 than AVP in exciting the cell.

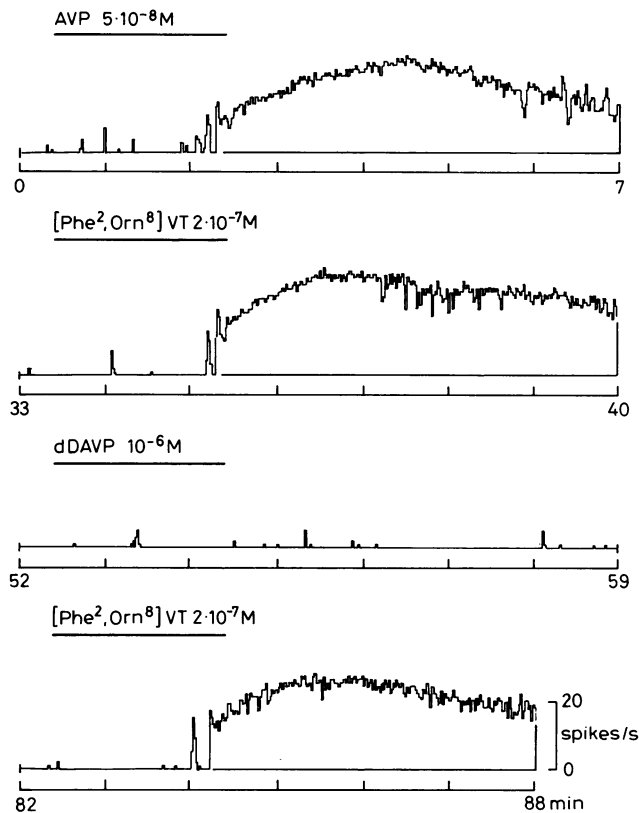


FIG. 3. Effects of AVP and of synthetic structural analogues on a septal neuron. $[Phe^2, Orn^8]VT$, a V_1 agonist, added to the perfusion solution at 200 nM, caused an increase in firing comparable to that brought about by AVP at 50 nM. In contrast, deamino-D AVP (dDAVP), 1000 nM, was without effect.

The autoradiographic data were in accordance with the results of the electrophysiological studies. A high density of $[^3H]AVP$ binding sites was detected in the lateral septum, and competitive studies using unlabeled structural analogues suggested that these binding sites were of the V_1 type because

a V_1 agonist, but neither a V_2 nor an oxytocic agonist, displaced the labeled compound. Taken together, our results corroborate the notion that endogenous vasopressin might act as a peptide transmitter in the lateral septum.

Earlier reports had suggested that vasopressin and OXT exert an excitatory effect on a small proportion of neurons located in the lateral septum (23, 24). In addition, both peptides were said to enhance the glutamate-induced excitation of septal neurons and to increase the number of action potentials evoked by orthodromic activation of these neurons (24, 25). In contrast, however, other workers reported that, in the same brain area, vasopressin suppressed synaptically evoked action potentials (26). In these studies, the neuropeptides were administered locally by microiontophoresis, and their effective concentration was therefore unknown; moreover, neither agonists nor antagonists were tested to ascertain the selectivity of the effects. More recently, Urban and De Wied (27) showed that vasopressin, superfused on the dorsal surface of the septum, could increase the negative wave of a field potential evoked by stimulation of the fimbria. The results were somewhat surprising, however, because OXT was as powerful as vasopressin in eliciting this effect.

Previous work, using light microscopic autoradiography, has shown that the lateral septum of rats contains $[^3H]$ vasopressin binding sites (13–16). Biegon *et al.* (15) used displacement studies to report that $d(CH_2)_5Tyr(Me)AVP$, but not OXT, competed efficiently with vasopressin for binding. These findings lead the authors to conclude, in accordance with the present study, that septal vasopressin binding sites are of the V_1 type. In contrast to our results, De Kloet *et al.* (16) reported that $[^3H]OXT$ labeled the lateral septum, though less effectively than $[^3H]$ vasopressin. This discrepancy may be due, at least in part, to the fact that these authors used 10 nM of tritiated peptide, a value much higher than those used in the present study.

High-affinity binding sites for vasopressin, with a K_d in the nanomolar range, have been characterized in at least two laboratories on isolated membrane preparation. In the amygdala and in the dorsal hindbrain of rats, $d(CH_2)_5Tyr(Me)AVP$, but not OXT, was an effective competitor of $[^3H]$ vasopressin binding, a finding that indicated that the sites present in these areas of the brain resemble V_1 rather than V_2 ,

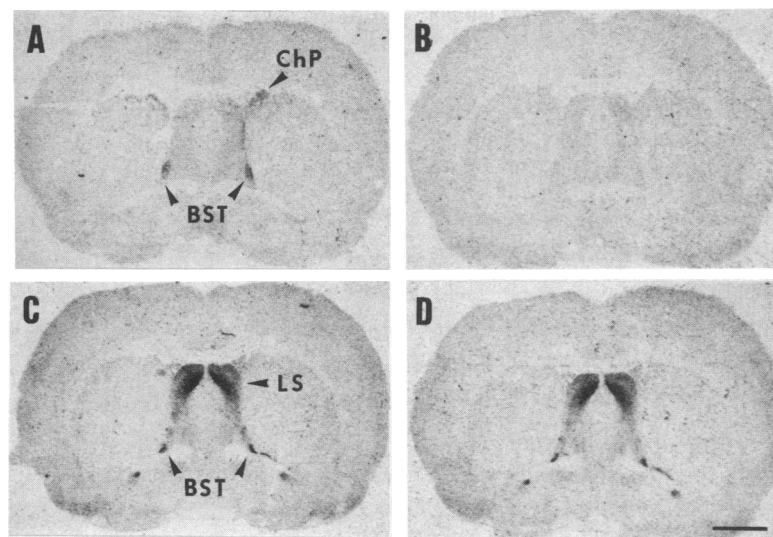


FIG. 4. Autoradiographs revealing the presence of binding sites for OXT and AVP in sections of the rat brain. Two adjacent sections were exposed to 3.5 nM $[^3H]OXT$ alone (A) or to 3.5 nM $[^3H]OXT$ and to 150 nM $HO[Thr^4, Gly^7]OXT$ (B). In A, OXT binding sites are present in the bed nucleus of the stria terminalis (BST) and in the choroid plexuses that span the wall of the lateral ventricle (ChP); (B) binding in these sites was displaced by the selective oxytocic agonist. Note the absence of $[^3H]OXT$ binding in the lateral septum (A), and contrast it with an adjacent section, incubated with 1.5 nM $[^3H]AVP$ (C). $[^3H]AVP$ binding in septum and BST was not displaced by coapplication of 150 nM $HO[Thr^4, Gly^7]OXT$ (D). Calibration line: 2 mm.

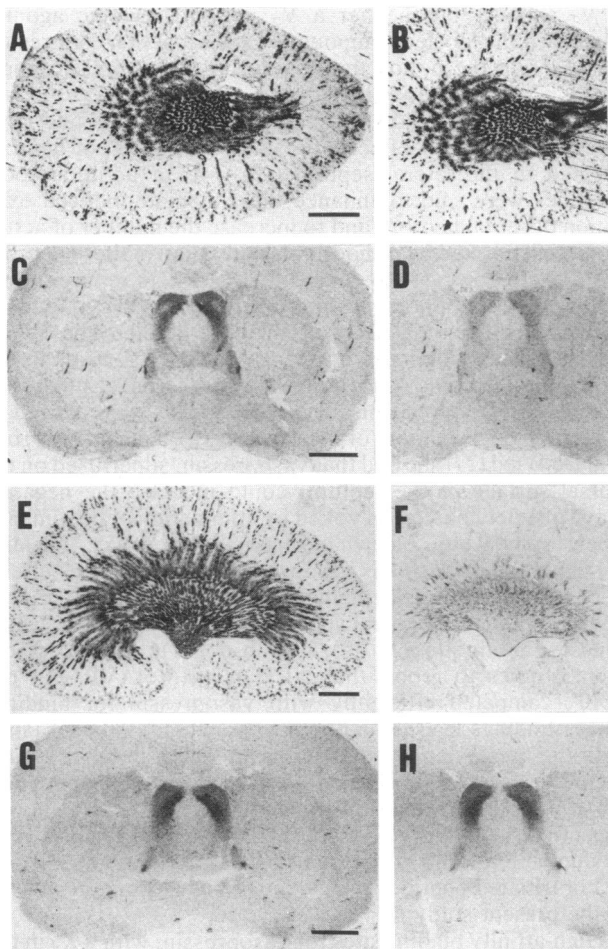


FIG. 5. AVP binding sites in lateral septum and their pharmacological identification as V_1 receptors. Sections from kidney (A, B, E, and F) and brain (C, D, G, and H) were incubated with 1.5 nM [3 H]AVP. B and D were incubated with the same amount of tritiated AVP and in addition with 100 nM of [$\text{Phe}^2, \text{Orn}^8$]VT. This V_1 agonist displaced label in septum (D) but not in kidney (B). Sections F and H were exposed to 1.5 nM [3 H]AVP and to 100 nM deamino-DAVP. Note that the V_2 agonist competed with AVP in kidney (F) but not in septum (H). Calibration lines: 2 mm.

or OXT receptors (28, 29). In a very thorough study in which synaptic membranes from rat hippocampus were used and the binding of nanomolar concentrations of labeled vasopressin, labeled OXT, and various synthetic structural analogues was studied, Audigier and Barberis (30) reported the existence of two distinct populations of binding sites, which probably correspond to V_1 and OXT receptors, respectively. To our knowledge, no comparable studies have yet been done using membranes isolated from the septum.

The autoradiographic and electrophysiological data reported in the present article show convincingly the existence in the rat septum of V_1 receptors similar to those present on smooth muscular, hepatic, and other nerve cells but markedly different from V_2 receptors found in the medulla of the kidney. We conclude that this part of the brain, which

receives a sexually dimorphic dense vasopressinergic innervation appears to be particularly suited for studies aimed at better understanding the role(s) of vasopressin in the central nervous system.

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