The Journal of Neuroscience, June 1991, 11(6): 1531–1539

Septal Arginine Vasopressin (AVP) Receptor Regulation in Rats Depleted of Septal AVP following Long-term Castration

Paule Poulin and Quentin J. Pittman

Neuroscience Research Group, Department of Medical Physiology, University of Calgary, Calgary, Alberta, Canada T2N 4N1

Arginine vasopressin (AVP) causes severe motor disturbances, including barrel rotations and myotonic/myoclonic convulsions, following repeated injections into either a lateral cerebral ventricle or the ventral septal area (VSA) of the rat brain. Because the AVP content of the rat septal area has been shown to be virtually eliminated following long-term castration, and because removal of a receptor ligand typically results in receptor upregulation and behavioral supersensitivity to the ligand, we tested the hypothesis that long-term castrated rats may be supersensitive to the motor actions caused by centrally injected AVP and may have upregulated septal AVP receptors. In these experiments, adult male Wistar rats were used 5 months after castration or, as controls, after sham castration. The effectiveness of long-term castration in eliminating AVP content of the VSA was indicated by the observation that a priming hypertonic saline stimulus (known to induce the central release of AVP and sensitize the rat brain) sensitized the brains of sham control rats but not of the castrated rats to the motor actions of a subsequent intracerebroventricular injection of AVP. The motor actions of centrally injected AVP, as well as septal AVP receptor characteristics (number and affinity), and AVP-stimulated phosphoinositide (PI) hydrolysis were then investigated in long-term castrated and sham control rats. Motor disturbances induced by either a first or a second injection of AVP were not greater in long-term castrated rats than in sham controls. VSA AVP receptor affinity and density (as measured by ^{3}H-AVP binding) and AVP-stimulated PI hydrolysis in septal slices were not significantly changed following long-term castration. These results suggest that the loss of AVP content of the septal area of the rat brain does not result in a compensatory supersensitivity to AVP.

Arginine vasopressin (AVP) has potent antipyretic (Kovacs and DeWied, 1983; Naylor et al., 1987a) and motor actions (Kasting et al., 1980; Burnard et al., 1983, 1985, 1986; Wurpel et al., 1986) when injected into a lateral cerebral ventricle of the rat brain. The ventral septal area (VSA; an area extending from the area of the diagonal bands of Broca to the anterior hypothalamus) of the rat basal forebrain appears to be a major site at which AVP is capable of causing both antipyresis and motor disturbances (Naylor et al., 1985; for review, see Pittman et al., 1988b). The motor actions of centrally injected AVP, however, involve a sensitization process in that centrally injected AVP has greatly enhanced motor effects if given after the rat brain has been preexposed (sensitized), 24–48 hr earlier, with a central injection of AVP (Kasting et al., 1980; Burnard et al., 1983, 1985, 1986; Naylor et al., 1985; Wurpel et al., 1986). In addition, potent stimuli causing the release of central AVP, for example, hemorrhage or hypertonic saline (Koob et al., 1985; Demotes-Mainard et al., 1986; Landgraf et al., 1988), have also been shown to mimic the effect of a central injection of AVP in sensitizing the rat brain to the motor action of centrally injected AVP (Burnard et al., 1983).

Structure-activity studies suggest that the central receptor mediating the actions of AVP in the VSA resembles the V1 (vasopressor) rather than the V2 (antidiuretic) peripheral AVP receptor (Michell et al., 1979), or the oxytocin-vasopressin receptor (Audigier and Barberis, 1985), in that (1) an antagonist of the V1 receptor, [1-(β-mercapto-β,β-cyclopentamethylenepropionic acid), 2-(*O*-methyl)tyrosine] AVP, [$d(CH_2)_5$ Tyr(Me)AVP] (Kruszynski et al., 1980), prevents the antipyretic (Naylor et al., 1987b), motor (Burnard et al., 1986), and electrophysiological (Disturnal et al., 1987) of AVP in the VSA; (2) the V2 agonist 1-desamino-8-D-arginine vasopressin (DDAVP) and oxytocin were shown to be inactive, either as antipyretic (Kovacs et al., 1983; Naylor et al., 1987b) or motor agents (Poulin and Pittman, 1988). Furthermore, AVP receptors have been localized in the VSA of the rat brain (for review, see Jard et al., 1987) and further characterized as V1 type (Raggenbass et al., 1987; Poulin et al., 1988).

In agreement with the hypothesis that AVP acts in the VSA as a neurotransmitter, immunocytochemical and transport studies have demonstrated that the septal area, which includes the VSA, contains fibers immunoreactive for AVP (DeVries et al., 1985). The major source of AVP to the septal area appears to be a vasopressinergic pathway originating in the bed nucleus of the stria terminalis (BST; DeVries and Buijs, 1983; DeVries et al., 1985; Disturnal et al., 1985). A special aspect of this AVP fiber system is its sensitivity to circulating steroids. Following long-term castration, both immunoreactive AVP cell bodies in the BST and immunoreactive AVP fibers projecting to the septal area were virtually eliminated (DeVries et al., 1985). In keeping with these findings, both immunoassayed AVP content of the septal area (Pittman et al., 1988a) and perikaryal AVP mRNA content in the BST (Miller et al., 1989a,b) were shown to be

Received Aug. 31, 1990; revised Dec. 18, 1990; accepted Dec. 27, 1990.

This research was supported by the Medical Research Council of Canada (MRCC). P.P. is an AHFMR student, and Q.J.P. is an AHFMR Scientist. Thanks to Dr. D. Dorsa and Dr. S. Weiss for their help in setting up the inositol assay and to Dr. D. McMaster, Dr. D. Burnard, and Dr. R. Auer for their valuable suggestions in writing the manuscript.

Correspondence should be addressed to Paule Poulin, Neuroscience Research Group, Department of Medical Physiology, University of Calgary, 3330 Hospital Drive Northwest, Calgary, Alberta, Canada T2N 4N1.

reduced in long-term castrated rats. The reduction of the AVP content in the VSA appears to be functionally significant in that, in the absence of AVP in the VSA, the typical antipyretic action of endogenous AVP appears reduced in long-term castrated rats, because they display enhanced fever when challenged with a pyrogen (Pittman et al., 1988a).

Many neurotransmitter receptors have been shown to be regulated by the concentration of ligand to which they are exposed. For instance, target cells typically respond to a decrease in ligand levels by "upregulating" their receptors (either by increasing the number and/or altering the affinity of the ligand surface receptors or by increasing the ligand responsiveness at the postreceptor level; for reviews, see Catt et al., 1979; Klein et al., 1989), and animals typically respond to a decrease in ligand levels by developing "supersensitive" responses to the ligand (e.g., see Jenner and Marsden, 1987). In agreement with this, homozygous Brattleboro rats (HO-BB), which are genetically deficient in AVP, have been reported to have upregulated septal AVP receptors (Shewey and Dorsa, 1986; Shewey et al., 1989) and to be supersensitive to the motor effects of centrally injected AVP (Burnard et al., 1985).

Thus, because long-term castration drastically reduces the AVP content of the VSA (DeVries et al., 1985), in the present study we tested the possibility that the brain of long-term castrated rats would not be sensitized to AVP by stimulus (hypertonic saline)-induced release of endogenous AVP, but that the brain of long-term castrated rats would, however, have upregulated VSA AVP receptors that (1) following a central injection of AVP would result in a supersensitive response to the motor actions of AVP, (2) would show an increase in receptor numbers and/or affinity as measured by ^{3}H-AVP binding studies, and/or (3) would show an increase in AVP-stimulated phosphoinositide (PI) hydrolysis measured in septal slices. We also measured AVP receptor properties in the lateral septal area (LS) and the hippocampus (HPC) because immunocytochemical studies of long-term castrated rats have demonstrated that immunoreactive AVP fibers are also depleted in these regions (DeVries et al., 1985). Furthermore, because plasma testosterone level has been shown to alter AVP gene expression (Miller et al., 1990) in the cells of the BST within 24 hr of treatment, and because steroid hormones have been shown to alter significantly central neurohypophysial receptor expression within 24 hr of steroid treatment (Johnson et al., 1989), we also measured the characteristics of AVP receptors in short-term (24-hr castrated) testosterone-depleted rats.

Materials and Methods

Adult male Wistar rats (200–250 gm) were anesthetized with sodium pentobarbital (50 mg/kg), and under aseptic conditions, half of the rats were castrated. The remaining rats underwent sham surgery, in which the scrotum was opened and then sutured closed. The rats were housed in groups of four per cage on a 12-hr light cycle and allowed food and water ad libitum.

AVP-induced motor disturbances. Twenty weeks following castration, the animals were reanesthetized with sodium pentobarbital, and a stainless-steel 23-gauge guide cannula was implanted stereotaxically to allow access to a lateral cerebral ventricle. Rats were then allowed 5–7 d to recover. Prior to experimentation, synthetic AVP (Bachem, Torrance, CA) was made up into the appropriate concentrations using sterile, pyrogen-free physiological saline. Behavioral experiments were conducted 2 d apart in order not only to examine the effect long-term castration may have on the initial motor responses to a central AVP injection (day 1), but also to reveal possible effects of castration on the "sensitization" phenomenon of AVP-induced motor disturbances (day 3) previously described (Kasting et al., 1980; Burnard et al., 1983; Naylor et al., 1985; Wurpel et al., 1986). On each experimental day following each injection, the animals were placed in a large Plexiglas chamber and observed for the development of motor disturbances for a 10-min period. Motor disturbances were defined and scored at 1-min intervals on a scale modified from that developed by Kasting et al. (1980) as follows: 0, no effect; 1, pauses as defined by periods (10 sec or longer) of absence of activity; 2, prostration; 3, large head swaying and locomotor difficulties; 4, barrel rotation as indicated by the animal's rotation along the long axis of the body; 5, myotonus (extreme limb extension) or myoclonus; 6, death. This scoring procedure was previously validated in our laboratory using a double-blind experiment (Burnard et al., 1983). Experiments were all performed between 1000 and 1800 hours. Results are presented as the highest score each animal received throughout a 10-min observation period. Results were statistically analyzed by the nonparametric Mann–Whitney U test and the Wilcoxon matched-pairs signed-ranks test.

Using the method described above, two series of behavioral experiments were conducted. In the first set of experiments, we tested the possibility that the brain of long-term castrated rats would not become sensitized by a stimulus known to release endogenous AVP. On the first experimental day (day 1), castrated rats ($n = 6$) or sham controls ($n = 7$) were given an intraperitoneal injection of 1.0 ml 1.5 M hypertonic saline to stimulate endogenous release of AVP into the brain (Burnard et al., 1983; Koob et al., 1985; Demotes-Mainard et al., 1986; Landgraf et al., 1988) or, as control, 1.0 ml of physiological saline ($n = 4$ for each group). Two days later (day 3), all animals received an intracerebroventricular injection of 100 pmol of AVP in 5 μl saline or physiological saline alone, and their motor responses were recorded as described above.

In the second set of experiments, we tested the possibility that the brain of long-term castrated rats would have upregulated VSA AVP receptors, which, following a central injection of AVP, would result in a supersensitive motor response to AVP. On the first experimental day (day 1), castrated rats ($n = 18$) or sham controls ($n = 14$) were given an intracerebroventricular injection of AVP (10 pmol in 5 μl saline). Two days later (day 3), all animals received an intracerebroventricular injection of AVP [10 pmol ($n = 8$, controls; $n = 9$, castrated) or 1 pmol ($n = 6$, controls; $n = 8$, castrated)] in 5 μl saline, and the behavior of the animals was scored as described above. As additional controls, on each experimental day, in addition to receiving AVP intracerebroventricularly, each animal was given a control injection of the vehicle alone (physiological saline, 5 μl, i.c.v.), and the behavior of the animals was scored as described above.

Radioligand binding studies. ^{3}H-AVP binding studies were conducted as previously described (Poulin et al., 1988). Briefly, 20 weeks or 24 hr following castration or sham surgery ($n = 30$ per group), rats were decapitated, and the brains were rapidly removed. Three brain areas were dissected as follows: (1) the LS, delineated by the coordinates 1.2 mm anterior to and −0.3 mm posterior to bregma, laterally at this level by the lateral ventricles and ventrally by a cut just above the anterior commissure; (2) the VSA, defined as the area ventral to the LS sample, between the anterior commissure and the ventral surface of the brain and within 2.0 mm of the midline; and (3) complete HPC. Partially purified synaptic plasma membranes (SPM) from the VSA, LS, and HPC of rat brains were prepared. In addition, because receptor upregulation may also be observed as an increase in the rate of receptor synthesis or as a decrease in receptor degradation, and because the presence of receptors in the "microsomal" membrane fraction has been suggested to reflect receptors that may be in the process of synthesis or degradation (Burt, 1985), we also prepared microsomal membrane fractions of the VSA and LS. Membranes [100–200 μg protein/tube; protein concentration determined by the method of Bradford (1976)] were incubated in a final volume of 300 μl of a medium composed of 50 mM Tris-HCl (pH, 7.4), containing $MgCl_2$ (3 mM), bovine serum albumin (BSA; 1 mg/ml), bacitracin (1 mg/ml), and various amounts (0.1–10 nM of ^{3}H-AVP (specific activity, 70 mCi/mmol; New England Nuclear Corp.) in the presence or absence of 1.0 μM AVP. The assays, in triplicate, were initiated by addition of membranes. Incubation was at 22°C for 60 min and was terminated by the addition of 5 ml of buffer (50 mM Tris-HCl; pH, 7.4) at 4°C. The contents of the assay tubes were then quickly filtered through Whatman GF/B glass filters that had been presoaked in BSA-containing buffer. Filters were washed rapidly three times with 5 ml rinsing buffer, dried by high vacuum, and placed in scintillation vials. Radioactivity retained on the filters was measured by liquid scintillation

spectrometry using an LKB β-counter with a counting efficiency of 37%. Nonspecific binding was defined as the binding occurring in the presence of 1.0 μM AVP. Data from saturation experiments were analyzed using a computerized, interactive curve-fitting procedure to generate representative Scatchard plots. Statistical analysis comparing Scatchard plot data from castrated rats and sham controls was performed using regression analysis (Kleinbaum et al., 1988, Chap. 14) to consider two basic questions: (1) Are the two slopes ($1/K_d$) the same or different? (2) Are the two x intercepts (B_{max}) the same or different?

Assay of ^{3}H-inositol-1-phosphate (IP_1). The method used was essentially that of Shewey and Dorsa (1988) with slight modifications as described below. Briefly, brain septal slices (250 × 250 μm) from long-term castrated rats or sham controls (n = 5 animals per group) were prepared using a Sorval tissue chopper and dispersed in a modified Krebs–Ringer bicarbonate (KRB) buffer containing (in 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 (pH, 7.4; 37°C under 95% O_2, 5% CO_2) for 30 min. Dispersed slices were labeled with 0.3 μM ^{3}H-inositol (Myo-[2-^{3}H(N)]-inositol; 20.0 Ci/mmol; New England Nuclear Corp.) in KRB buffer under 95% O_2, 5% Co_2 for 1 hr at 37°C and then washed three times with fresh KRB buffer to remove extracellular label. Gravity-packed labeled slices (25 μl; approximately 400–600 μg of protein/well) were then added to tissue-culture wells (to maximize gas exchange) containing 250 μl KRB buffer containing 10 mM LiCl under 95% O_2, 5% CO_2 at 37°C. After 10 min, agonists were added in the appropriate concentrations, and the incubation continued for an additional 60 min. The reaction was stopped by the addition of ice-cold chloroform : methanol (1:2). The mixture was then transferred to polypropylene tubes, vortexed, and separated into two phases by centrifugation. Labeled inositol phosphates, in the water-soluble phase, were analyzed using modifications of the method described by Berridge et al. (1983) employing anion-exchange resin columns. Briefly, a 1-ml aliquot of the water-rich phase of each tube was diluted to 3.2 ml with distilled water and loaded onto a 1-ml Dowex formate column (Dowex AG1-X8, Bio-Rad Laboratories). The columns were washed with an additional 1 ml of distilled water. Three milliliters of 5 mM sodium tetraborate in 60 mM sodium formate, followed by 1 ml distilled water, were used to elute the glycero-phosphoinositol (GPI) fraction. Inositol monophosphate (IP_1) was then eluted from the columns by the addition of 4 ml of 0.2 M ammonium formate in 0.1 M formic acid. The remaining inositol phosphates were eluted with 4 ml of 1 M ammonium formate in 0.1 M formic acid. The radioactivity contained in aliquots of the GPI, IP_1, and inositol phosphates fractions, and in the chloroform-rich phase containing the phosphoinositides (PI), was measured by scintillation counting after the addition of 6 ml of Ready Gel (Beckman, Missisauga, Ontario). In order to compensate for the variability associated with pipetting slice suspensions, the radioactivity accumulating in the IP_1 fraction was expressed as a percentage of the label incorporated in the PI fraction. The remaining inositol phosphates and GPI accounted for less than 1% of the total radioactivity. Results were statistically analyzed using two-way analysis of variance (two-way ANOVA) for all dose–response curves, and where further post hoc testing was required, the Scheffé multiple-range test was used. Values of $p < 0.05$ were considered significant.

Results

AVP-induced motor disturbances

A first experiment was designed to establish whether long-term castrated rats were functionally deficient (in terms of AVP-induced motor disturbances) in AVP content of the VSA. In this first experiment, the method used was essentially that of Burnard et al. (1983), in which a hypertonic stimulus is used to stimulate the central release of AVP from endogenous stores to sensitize the rat brain to the motor effects of subsequent injections of AVP. Figure 1 illustrates the behavioral scores of long-term (20 weeks) castrated rats and of sham controls in response to an intracerebroventricular injection of 100 pmol AVP 2 d after intraperitoneal injection of either 1.0 ml of hypertonic (1.5 M) saline or 1.0 ml of physiological saline as control. As seen in Figure 1, AVP-induced motor disturbances in sham control rats that received the hypertonic saline pretreatment are significantly higher ($p < 0.008$, Mann–Whitney U test) than the AVP-induced motor disturbances observed in sham controls that received the control physiological saline pretreatment. The behavioral scores show that sham control rats that received the hypertonic saline pretreatment displayed locomotor difficulties and head swaying (n = 4), barrel rotation (n = 1), myotonus and myoclonus (n = 1), and even death (n = 1). Motor disturbances of this magnitude are not normally seen following a first intracerebroventricular injection of 100 pmol AVP (P. Poulin and Q. J. Pittman, unpublished observations). This suggests that, in sham controls, hypertonic saline pretreatment on day 1 mimics the previously described effect (Burnard et al., 1983) of an intracerebroventricular injection of AVP on day 1 in causing sensitization and hence increased behavioral responses on day 3 to an intracerebroventricular injection of AVP.

In contrast, the motor responses of long-term castrated rats to the intracerebroventricular injection of 100 pmol of AVP, following either the physiological saline or the hypertonic saline pretreatments, are not significantly different. All rats exhibited minor motor disturbances rating scores of 1 (pauses) and 2 (prostration), and one rat exhibited some locomotor difficulties. These behaviors are also seen following a first intracerebroventricular injection of 100 pmol AVP (Poulin and Pittman, unpublished observations), indicating that in castrated rats, no sensitization occurred following the hypertonic saline pretreatment. It can therefore be seen in Figure 1 that sham control rats displayed increased sensitivity to 100 pmol of intracerebroventricular AVP when compared to castrated rats ($p < 0.004$, Mann–Whitney U test) following intraperitoneal hypertonic saline pretreatment.

A second series of behavioral experiments was conducted in order to examine whether the prolonged lack of AVP in the VSA has rendered the AVP receptor supersensitive to exogenous AVP administration. Figure 2 illustrates the behavioral scores of long-term (20 weeks) castrated rats and sham controls following a first (day 1) and a subsequent (day 3) intracerebroventricular injection of AVP. As indicated in Figure 2, on the first day of exposure to intracerebroventricular AVP (10 pmol), both sham controls and castrated rats displayed minor behavioral responses consisting of pauses, prostration, and some locomotor difficulties. No significant differences were observed between sham controls and castrated rats ($p > 0.5$, Mann–Whitney U test). When the animals were retested 2 d later (day 3), however, it can be seen that a second intracerebroventricular injection of AVP induced enhanced motor responses in a dose-dependent manner, in that (1) at the highest doses of AVP (10 pmol AVP, i.c.v., on days 1 and 3; Fig. 2, top), the motor responses of the animals on day 3 were significantly increased ($p < 0.001$, Wilcoxon matched-pairs signed-ranks test) from those of day 1, with both groups of animals displaying severe motor disturbances including barrel rotation, convulsions, and death. In Figure 2, top, it can be seen that no significant differences in the AVP-induced motor disturbances either on day 1 or on day 3 were observed between castrated and sham control rats ($p >$ 1.0, Mann–Whitney U test). Thus, though it appears that there are no significant differences in the motor actions of centrally injected AVP either on day 1 or on day 3, it could be that on day 3 too high a dose was used, because near-maximal motor disturbances were observed even in the control group. Therefore, a submaximal dose of AVP was used on day 3 in another group of rats. As can be seen in Figure 2, bottom, when lower doses of AVP (10 pmol AVP, i.c.v., on day 1 and 1 pmol AVP, i.c.v., on day 3) were used, on day 3 castrated rats were shown

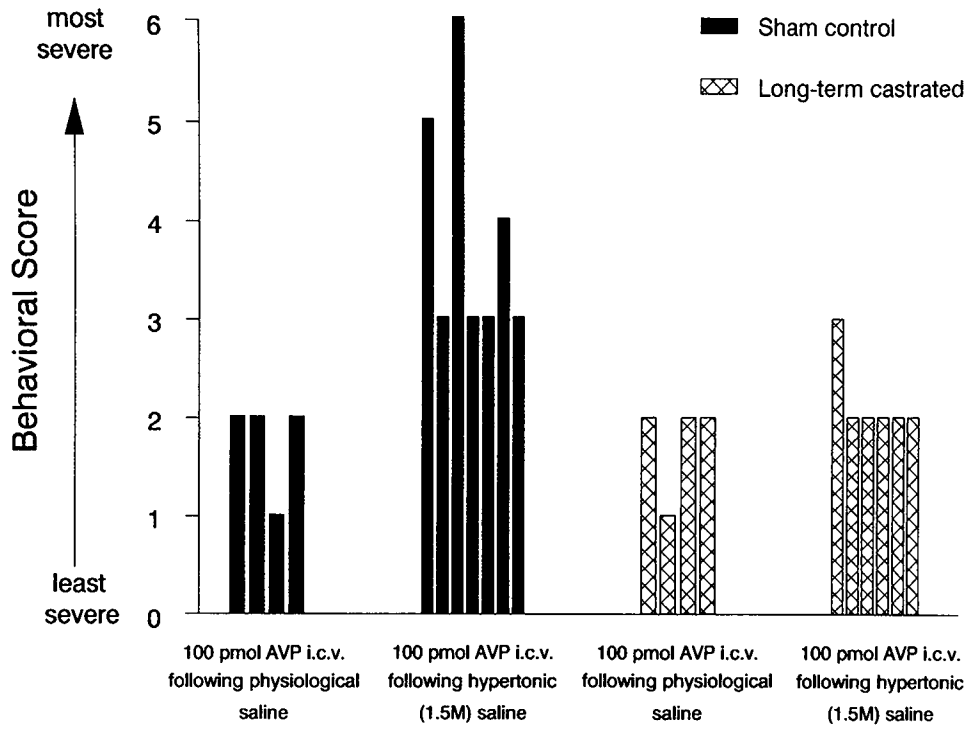

Figure 1. Endogenous release of AVP. Each *bar* represents the most severe behavioral score of a sham control (*solid bars*) or a long-term castrated male Wistar rat (*cross-hatched bars*) in response to 100 pmol intracerebroventricular AVP 2 d after receiving, intraperitoneally, either 1 ml of physiological saline ($n = 4$, each group) or 1 ml of 1.5 M hypertonic saline ($n = 6$, controls; $n = 7$, castrated). Each *bar* represents the behavioral score of an individual animal. The scoring was as follows: *0*, no effect; *1*, pauses; *2*, prostration; *3*, head swaying and locomotor difficulties; *4*, barrel rotation; *5*, myotonus/myoclonus; *6*, death. The sham control rats received significantly higher behavioral scores than did the castrated rats following the hypertonic saline pretreatment ($p < 0.008$, Mann–Whitney *U* test). No significant difference was seen in the response of the two groups to physiological saline.

to be somewhat less sensitive to the motor effects of 1 pmol AVP than were sham controls ($p < 0.02$, Mann–Whitney *U* test). In all cases, intracerebroventricular saline injections did not elicit any motor disturbances rating greater than a score of 1 (data not shown). An additional control experiment was conducted in which one group of animals was given an intracerebroventricular saline injection on day 1 followed by 10 pmol of intracerebroventricular AVP on day 3. In this experiment, the behavioral scores of animals on day 3 to 10 pmol intracerebroventricular AVP did not significantly differ from behavioral scores of animals given 10 pmol intracerebroventricular AVP on day 1, indicating that an injection of intracerebroventricular saline on day 1 is insufficient to sensitize the rat brain to AVP-induced motor disturbances (data not shown). It can therefore be concluded that, when compared to sham controls, long-term castrated rats did not develop a supersensitive response to the motor effects of centrally injected AVP. Long-term castrated rats may even be somewhat less sensitive to the motor actions of centrally injected AVP.

Binding studies

The effect long-term castration may have on AVP receptor number and/or affinity was measured. The binding of ^{3}H-AVP to SPMs prepared from the VSA, LS, and HPC of long-term castrated rats or sham controls was measured using saturation experiments. In all cases, binding of ^{3}H-AVP to SPMs was saturable over ligand concentrations from 0.1 to 10 nM, and specific binding of up to 70% was observed. Scatchard analysis of ^{3}H-AVP binding to VSA, LS, and HPC SPMs yielded linear plots (Figs. 3, 4), which is consistent with a single-binding-site model. Each linear plot could be used for estimating apparent dissociation constants (K_d; 1/slope) and binding-site concentrations (B_{max}; x intercept). Figure 3 shows that the K_d values of the binding site for ^{3}H-AVP in brain tissues of sham controls were not significantly different from the K_d values obtained from brain tissues of long-term castrated rats. The K_d values of the AVP binding sites from sham controls and long-term castrated rats were calculated to be 3.2 versus 3.0 nM, $p > 0.94$, VSA; 0.76 versus 0.82 nM, $p > 0.89$, LS; and 0.47 versus 0.57 nM, $p > 0.49$, HPC, respectively. Figure 3 also shows that the binding-site concentration (B_{max}) for ^{3}H-AVP from sham controls was not significantly different from the B_{max} for ^{3}H-AVP from long-term castrated rats. The B_{max} values were calculated to be 30 versus 33 fmol/mg protein, $p > 0.48$, VSA; 44 versus 45 fmol/mg protein, $p > 0.84$, LS; and 22 versus 20 fmol/mg protein, $p > 0.89$, HPC, respectively. These results suggest that long-term castration does not appear to alter the ^{3}H-AVP binding-site apparent dissociation constant (K_d) or ^{3}H-AVP binding-site concentration (B_{max}) in SPMs of any regions of the rat brain studied.

Because the presence of receptors in the "microsomal" membrane fraction has been suggested to reflect receptors that may be in the process of synthesis or degradation (Burt, 1985), we also investigated AVP binding-site concentrations in the microsomal membrane fraction in the VSA and in the LS of sham controls and long-term castrated rats. ^{3}H-AVP binding-site concentrations (B_{max}) of the microsomal membrane fraction from sham controls and from long-term castrated rats were calculated to be 40 and 38 fmol/mg protein for VSA and 42 and 50 fmol/mg protein for LS, respectively (data not shown).

AVP receptor regulation was also measured in short-term testosterone-depleted (24 hr castration) rats. As can be seen in Figure 4, the K_d for ^{3}H-AVP binding sites measured in membranes prepared from sham control VSA SPMs is not significantly different from the K_d of ^{3}H-AVP binding sites from short-term castrated rat VSA SPMs (1.5 vs. 1.2 nM; $p > 0.64$). Similar results were obtained when binding studies were performed in membranes prepared from the LS (0.95 vs. 1.4 nM; $p > 0.44$) or the HPC (0.49 vs. 0.48 nM; $p > 0.41$). Taken together, the K_d values of the binding site for ^{3}H-AVP in brain tissues of sham

controls were not significantly different from those obtained from brain tissues of short-term castrated rats. Figure 4 also shows that the B_{max} for ^{3}H-AVP in VSA membranes prepared from sham controls was not significantly different from the B_{max} for ^{3}H-AVP in VSA membranes prepared from short-term castrated rats (38 vs. 30 fmol/mg protein; $p > 0.2$). Similar results were obtained when binding studies were performed in membranes prepared from LS (38 vs. 41 fmol/mg protein; $p > 0.17$) or HPC (23 vs. 23 fmol/mg protein; $p > 0.85$). These results indicate that short-term hormone depletion (castration) does not appear to alter the AVP binding-site dissociation constant or concentration in any of the regions of the rat brain studied.

Assay of IP_1

To determine the effect that septal AVP depletion, caused by long-term castration of rats, has on septal AVP postreceptor mechanisms, we measured AVP-induced ^{3}H-IP_1 accumulation in septal slice preparations. We have previously shown (Poulin et al., 1990) that, though PI hydrolysis induced by AVP is not detectable in control rats, our assay procedure is sensitive to detect the enhanced PI hydrolysis in sensitized rats. In keeping with these observations, Figure 5 shows that AVP induced no significant ($p > 0.9$, two-way ANOVA) ^{3}H-IP_1 accumulation in septal slices either from long-term castrated rats or from sham controls over AVP concentrations of 10^{-9} to 10^{-6} M. Although carbachol (1 mM) resulted in an increase in the stimulation of ^{3}H-IP_1 accumulation over basal (85 ± 10% in sham controls, 105 ± 28% in castrated rats), no significant differences were observed between the two groups ($p > 0.89$, Scheffé multiple-range test).

Discussion

The VSA has been shown to be a site in which AVP acts to cause motor disturbances (Naylor et al., 1985). We and others (Kasting et al., 1980; Burnard et al., 1985, 1986; Wurpel et al., 1986) have previously demonstrated that the motor disturbances produced by central injections of AVP involve a "sensitization" phenomenon in that a first injection of AVP increases the severity of the motor disturbances caused by subsequent central injections of AVP. This phenomenon was also shown following central release of endogenous AVP. For example, Burnard et al. (1983) demonstrated that, in rats, an intraperitoneal hypertonic saline pretreatment can mimic the effect of a central injection of AVP in sensitizing the brain to the motor effects of subsequent injections of AVP. These results were interpreted to suggest that hypertonic saline pretreatment caused release of central AVP such that the rat brain became "sensitized." In agreement with this, additional evidence from several laboratories has also shown that intraperitoneal hypertonic saline treatment causes release of central AVP (Koob et al., 1985; Demotes-Mainard et al., 1986; Landgraf et al., 1988). We report here that more than 5 months after castration, in the absence of AVP in the VSA, no sensitization of the rat brain was obtained using stimulus-induced release of endogenous AVP. We interpret our results to suggest that intraperitoneal hypertonic saline pretreatment in long-term castrated animals does not sensitize the rat brain to AVP-induced motor disturbances, possibly because no AVP was released in the VSA. The lack of stimulus-induced sensitization of the rat brain to AVP further supports previous findings suggesting that the reduction of the AVP content in the VSA following long-term castration appears to be functionally significant (Pittman et al., 1988a).

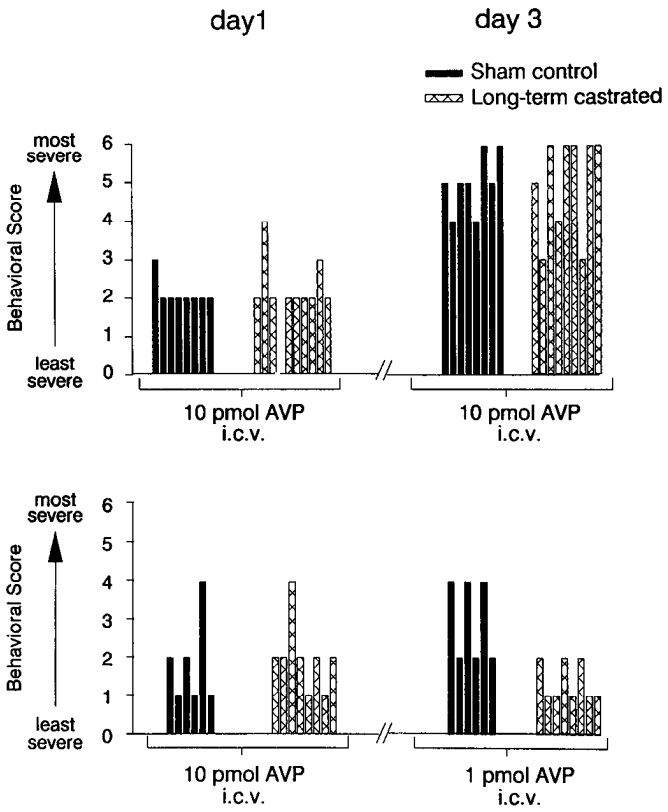

Figure 2. Lack of behavioral supersensitivity following castration. Each *bar* represents the most severe behavioral score of a sham control (*solid bars*) or a long-term castrated (*cross-hatched bars*) male Wistar rat in response to intracerebroventricular AVP. The scoring was as follows: *0*, no effect; *1*, pauses; *2*, prostration; *3*, head swaying and locomotor difficulties; *4*, barrel rotation; *5*, myotonus/myoclonus; *6*, death. In the *top graph*, animals received 10 pmol intracerebroventricular AVP on days 1 and 3. While the behavioral scores obtained from both sham controls and castrated animals on day 3 were significantly higher ($p < 0.001$; Wilcoxon matched-pairs signed-ranks test) than on day 1, on day 3 the sham and castrated groups did not differ from each other ($p > 0.05$, Mann–Whitney U test). In the *bottom graph*, animals received 10 pmol intracerebroventricular AVP on day 1 and 1 pmol intracerebroventricular AVP on day 3. Sham control rats given 1 pmol intracerebroventricular AVP on day 3 received higher behavioral score ($p < 0.02$, Mann–Whitney U test) than did long-term castrated rats, indicating an increased severity of the motor disturbances.

The present study was also designed to investigate the properties of brain AVP receptors following removal of its ligand, because removal or absence of a receptor ligand has been shown to result typically in an upregulation of receptors and in the development of behavioral supersensitivity (Catt et al., 1979; Klein et al., 1989). Thus, in view of the fact that long-term castration of adult male rats causes a dramatic reduction in the AVP content of the VSA where sensitive sites for the motor effects of centrally injected AVP have been identified (Naylor et al., 1985), we hypothesized that long-term castration of adult male rats could result in the animals becoming "supersensitive" to the motor effects of centrally injected AVP and in the "upregulation" of VSA AVP receptors. We report here that no "supersensitivity" in VSA AVP receptors was found in that (1) the motor effects of centrally injected AVP were no greater in long-term castrated rats than in sham controls; (2) neither the number of AVP receptors nor the apparent affinity of the receptor sites, as determined by ^{3}H-AVP binding, was altered by long-term

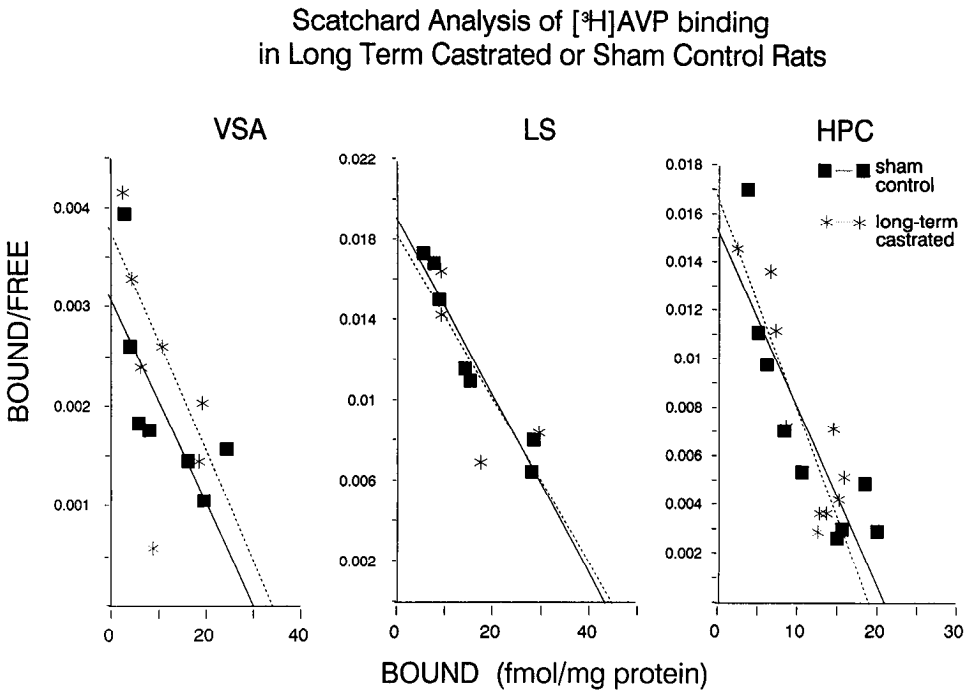

Figure 3. Scatchard plot analysis of ^{3}H-AVP specific binding in SPM prepared from the *VSA, LS,* and *HPC* of sham controls or long-term (20 weeks) castrated male Wistar rats. SPMs were incubated with increasing concentrations of ^{3}H-AVP (0.1–10 nM) in the presence or absence of 1.0 μM AVP. Each *point* is the mean of triplicate determinations. Each linear plot could be used for estimating apparent dissociation constants (K_d; 1/slope) and maximal binding-site concentration (B_{max}; x intercept). Data were analyzed using regression analysis. No differences in either K_d or B_{max} for ^{3}H-AVP binding were observed between long-term castrated animals and sham controls.

castration; and (3) AVP stimulation of PI hydrolysis in septal slices was not increased in long-term castrated rats. In addition, because receptor upregulation may also be observed as an increase in the rate of receptor synthesis or as a decrease in receptor degradation, and because the presence of receptors in the "microsomal" membrane fraction has been suggested to reflect receptors that may be in the process of synthesis or degradation (receptor in vesicular compartments derived from receptor-mediated endocytosis and recycling; Burt, 1985), we also investigated AVP binding-site concentration in the microsomal membrane fraction in the VSA and in the LS of sham controls and long-term castrated rats. When ^{3}H-AVP specific binding concentration is compared between the SPM and the microsomal membrane fraction, equivalent concentrations of ^{3}H-AVP binding sites are observed in these membrane fractions. These results are similar to what has previously been shown (Poulin et al., 1988) and further suggest that the rate of synthesis and degradation of ^{3}H-AVP binding sites does not appear to be altered in sham controls and in long-term castrated rats.

The VSA of long-term castrated rats contains some residual AVP (Pittman et al., 1988a), though markedly reduced from that of the control animals; thus, it is possible that it may be

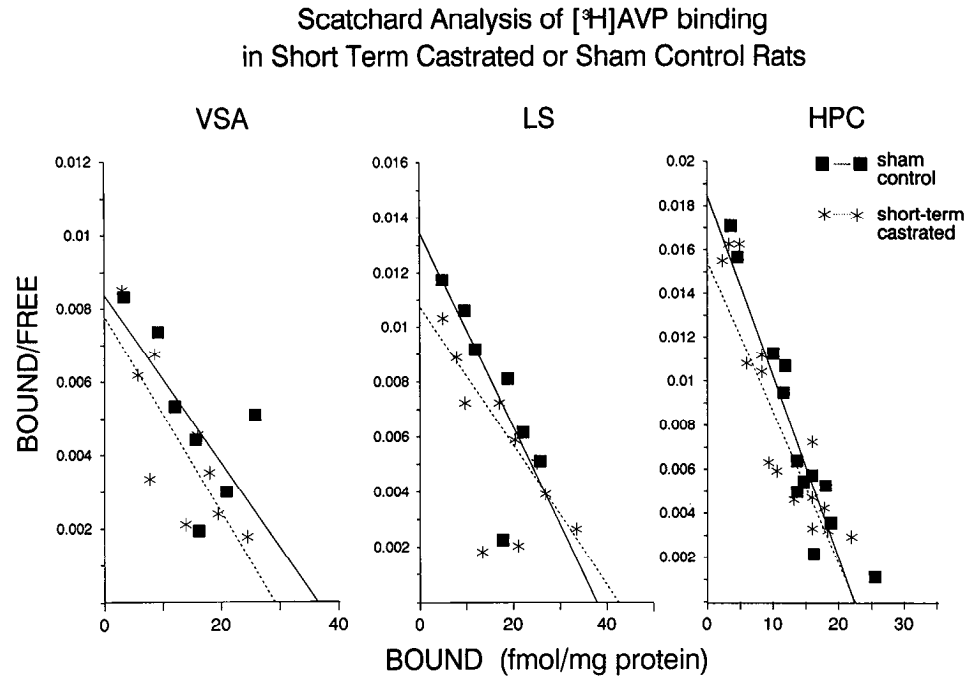

Figure 4. Scatchard plot analysis of ^{3}H-AVP specific binding in SPM prepared from the *VSA, LS,* and *HPC* of sham controls or short-term (24 hr) castrated male Wistar rats. SPMs were incubated with increasing concentrations of ^{3}H-AVP (0.1–10 nM) in the presence or absence of 1.0 μM AVP. Each *point* is the mean of triplicate determinations. Each linear plot could be used for estimating apparent dissociation constants (K_d; 1/slope) and binding-site concentration (B_{max}; x intercept). Data were analyzed using regression analysis. No differences in either K_d or B_{max} for ^{3}H-AVP binding were observed between short-term castrated animals and sham controls.

sufficient to maintain AVP receptors under normal regulation. It does not seem, however, that we can explain our failure to find an "upregulation" of VSA AVP receptors by a failure of the long-term castration to reduce functionally the AVP content of the VSA (see above discussion).

Our study therefore suggests that the septal AVP receptor system displays potentially unique properties in that the reduction in the concentration of its ligand, during adulthood, fails to "upregulate" the receptor and fails to result in a behavioral "supersensitivity" to AVP. In this respect, it resembles the V1 receptor in the periphery, which also appears to be relatively independent of the level of circulating AVP (Butlen et al., 1984). To explain the lack of dynamic regulation of the V1 receptor, one might consider that receptor supersensitivity has been proposed to be a compensatory homeostatic response to the loss of stimulation by ligand. Thus, supersensitivity to a ligand would be expected to occur in tonically active systems. In contrast, in systems not tonically active, the removal of the ligand may not be expected to produce a supersensitivity response because no great loss in basal ligand stimulation would occur. Because the loss of vasopressinergic innervation of the VSA did not result in a compensatory supersensitivity response in VSA AVP receptors, our results would therefore suggest that the central VSA AVP system may not be tonically active. This may well be true, because several months after castration are required before immunoreactive vasopressin is eliminated from the BST cells (DeVries et al., 1985), despite an almost immediate arrest in AVP synthesis (Miller et al., 1990).

Receptor "supersensitivity" has been described mainly under conditions (including denervation and chronic treatment with antidepressants and neuroleptics; e.g., see Wonnacott, 1990) that decrease the synaptic concentration of transmitter in a very short period of time (days). It is therefore possible that, because the depletion of central AVP level is occurring very slowly after castration (DeVries et al., 1985), the receptor system may have the time to "adjust" to this slowly changing agonist concentration, which may explain why no alteration in receptor is observed 5 months following castration despite an almost complete depletion of VSA AVP stores.

It is also possible that castration may cause a transient alteration in central AVP receptors. Our results, however, demonstrated that AVP receptor regulation is not affected 24 hr after castration. This is an important observation because neurohypophysial hormone receptor expression has been shown to be directly influenced by steroid hormones within 24 hr of steroid treatment (Johnson et al., 1989). Direct steroid influences on neurohypophysial hormone receptors have also been observed in other systems. For example, Voorhuis et al. (1988) have shown that testosterone increases the density of ^{3}H-AVP binding sites in certain brain nuclei of birds. In the rat, deKloet et al. (1986) have shown that estradiol treatment increases the density of the central oxytocin-vasopressin receptor in discrete brain regions. It is interesting to note that these investigators have suggested that the putative V1 type of AVP receptor, located in the septal area of the rat brain, does not appear to be responsive to this steroid treatment (deKloet et al., 1986). A recent report by Tribollet et al. (1990) also indicates that, whereas the oxytocin receptor in the brain is regulated by steroids, the V1 receptor concentration is unaltered by manipulating gonadal hormone levels.

While in our study the elimination of androgen hormones following castration did not alter the affinity and/or number of central AVP receptors, nor did it affect the postreceptor response of VSA AVP receptors, this does not argue against the possibility that AVP receptor *regulation* may, in itself, be dependent upon steroid hormones derived from the testes. It may be that the absence of such circulating steroids (e.g., androgens) would prevent the upregulation of the AVP receptors that might normally result from reduced ligand exposure. In fact, Antoni et al. (1985) demonstrated that rat anterior pituitary AVP receptors appear to be regulated through a mechanism that requires the presence of the adrenal glucocorticoid hormone corticosterone. Although some central actions of AVP have been shown to be altered by gonadal steroids (Albers et al., 1988), the present results do indicate that the induction of the "sensitization" phenomenon following central AVP administration does not appear to be dependent upon circulating steroids, because a second exposure to AVP causes enhanced behavioral responses in both sham controls and long-term castrated rats.

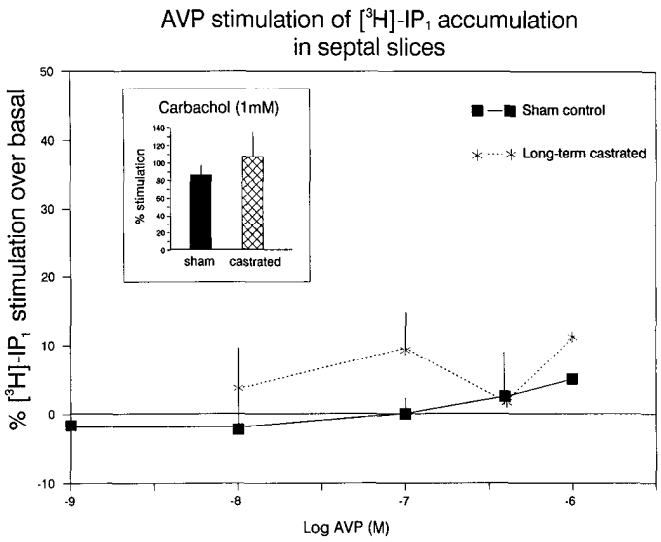

Figure 5. Dose–response curves for AVP-stimulated ^{3}H-IP$_1$ accumulation in septal slices from long-term castrated (*) and sham control (■) male Wistar rats. Slices were prelabeled with 0.3 μM ^{3}H-inositol for 60 min followed by incubation with AVP (0.001–1.0 μM) or carbachol (1 mM) for an additional 60 min in the presence of Li^+. The accumulation of ^{3}H-IP$_1$ was measured as described in the text. Values are means ± SEM of three separate experiments performed in triplicate (no SEM is shown at 0.001 and 1 μM AVP because the experiment was done only once). Data were analyzed using two-way ANOVA in conjunction with Scheffé multiple-range test. AVP did not induce any significant ^{3}H-IP$_1$ stimulation in septal slices of either group. *Inset,* Although carbachol significantly stimulated ^{3}H-IP$_1$ accumulation over basal values, no significant difference ($p > 0.89$, Scheffé multiple-range test) was observed between groups.

Burnard et al. (1985) have shown that HO-BB rats, which are also deficient (genetically) in AVP, are "supersensitive" to the motor effects elicited from central AVP injections when compared to Long–Evans rats. In keeping with these behavioral observations, Shewey and Dorsa (1986) have shown that HO-BB rats have an apparent increase in the number of septal AVP receptors (as well as a lower affinity) when compared to their heterozygous littermates. In addition, HO-BB rats were also shown to display increased AVP-stimulated phosphoinositol hydrolysis (Shewey et al., 1989) in septal slices. While these results suggest that the absence of AVP may result in septal AVP-receptor upregulation, it is possible that the absence of AVP during a "critical" neonatal period in HO-BB rats, but not

on long-term castrated rats, results in differences in receptor regulation. In fact, neonatal AVP exposure has been shown to decrease the number of vasopressin binding sites in adult animals (Handelman and Sayson, 1984).

Besides self-regulation, the regulation of central AVP receptors may depend on other factors. For example, Antoni et al. (1985) showed that oxytocin was equipotent with AVP in regulating anterior pituitary AVP receptors. Interestingly, long-term castration has been shown to be without effect on the oxytocin content of the VSA, LS, or HPC (DeVries et al., 1986; Pittman et al., 1988a). It is therefore possible that this ligand is capable of maintaining normal regulation of central AVP receptors in long-term castrated animals. In this respect, we have obtained preliminary evidence that oxytocin may indeed be important in the regulation of central AVP receptor function in the rat brain (Poulin and Pittman, 1988).

It is also possible that age may alter AVP receptor regulatory mechanisms, most particularly because it has been shown that age affects AVP levels in a number of areas of the rat brain (Fliers et al., 1985). Thus, an additional consideration is that both sham control and castrated rats used in our study were over 8 months of age. Our results indicate that VSA AVP receptors in older animals (both long-term castrated or sham controls) appear to have a somewhat lower affinity for AVP (3.2 and 3.0 nM, respectively) as compared to VSA AVP receptor affinity of younger (both short-term castrated and sham controls; 1.5 and 1.2 nM, respectively) animals. Although it is possible that as animals age receptor regulation becomes less responsive, such animals were, in our study, able to display the expected "sensitization" to AVP seen in younger animals.

In conclusion, our study shows that the septal AVP receptor displays potentially unique properties. First, as has been previously shown, repeated treatments with AVP result in the "sensitization" of the rat brain to AVP. Second, our study further suggests that the septal AVP receptor may not behave in a typical fashion because it is not subject to the development of the upregulation typically observed after removal of a ligand (even though the removal of the ligand appears functionally significant).

References

Albers HE, Liou SY, Ferris CF (1988) Testosterone alters the behavioral response of the medial preoptic-anterior hypothalamus to microinjection of arginine vasopressin in the hamster. Brain Res 456: 382–386.

Antoni FA, Holmes MC, Kiss JZ (1985) Pituitary binding of vasopressin is altered by experimental manipulations of the hypothalamo-pituitary-adrenocortical axis in normal as well as homozygous (di/di) Brattleboro rats. Endocrinology 117:1293–1299.

Audigier S, Barberis C (1985) Pharmacological characterization of two specific binding sites for neurohypophyseal hormones in hippocampal synaptic plasma membranes of the rat. EMBO J 4:1407–1412.

Berridge MJ, Dawson MC, Downes CP, Hesslop JP, Irvine RF (1983) Changes in the levels of inositol phosphates after agonist-dependent hydrolysis of membrane phosphoinositides. Biochem J 212:472–482.

Bradford MM (1976) A rapid sensitive method for the quantisation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal Biochem J 72:248–254.

Burnard DM, Pittman QJ, Veale WL (1983) Increased motor disturbances in response to arginine vasopressin following haemorrhage or hypertonic saline: evidence for central AVP release in rats. Brain Res 273:59–65.

Burnard DM, Pittman QJ, Veale WL (1985) Brattleboro rats display increased sensitivity to arginine vasopressin-induced motor disturbances. Brain Res 342:316–322.

Burnard DM, Veale WL, Pittman QJ (1986) Prevention of arginine-vasopressin-induced motor disturbances by a potent vasopressor antagonist. Brain Res 362:40–46.

Burt DR (1985) Criteria for receptor identification. In: Neurotransmitter receptor binding, 2d ed (Yamamura HI, Enna SJ, Kuhar M, eds), pp 41–60. New York: Raven.

Butlen D, Baddouri K, Rajerison RM, Guillon G, Cantau B, Jard S (1984) Plasma antidiuretic hormone levels and liver vasopressin receptors in the Jerboa, *Jaculus orientalis*, and rats. Gen Comp Endocrinol 54:216–229.

Catt KJ, Harwood JP, Aguilera G, Dufau ML (1979) Hormonal regulation of peptide receptors and target cell responses. Nature 280: 109–116.

deKloet ER, Voorhuis DAM, Boschma Y, Elands J (1986) Estradiol modulates density of putative oxytocin receptors in discrete rat brain regions. Neuroendocrinology 44:415–421.

Demotes-Mainard J, Chauveau J, Rodriguez F, Vincent JD, Poulain DA (1986) Septal release of vasopressin in response to osmotic, hypovolemic and electrical stimulation in rats. Brain Res 381:314–321.

DeVries GJ, Buijs RM (1983) The origin of the vasopressinergic and oxytinergic innervation of the rat brain with special reference to the lateral septum. Brain Res 273:307–317.

DeVries GJ, Buijs RM, Van Leeuwen FW, Caffe AR, Swaab DF (1985) The vasopressinergic innervation of the brain in normal and castrated rats. J Comp Neurol 233:236–254.

DeVries GJ, Duetz W, Buijs RM, Van Heerikhuize J, Vreeburg JTM (1986) Effects of androgens and estrogens on the vasopressin and oxytocin innervation of the adult rat brain. Brain Res 399:296–302.

Disturnal JE, Veale WL, Pittman QJ (1985) Electrophysiological analysis of potential arginine vasopressin projections to the ventral septal area of the rat. Brain Res 342:162–167.

Disturnal JE, Veale WL, Pittman QJ (1987) Modulation by arginine vasopressin of glutamate excitation in the ventral septal area of the rat brain. Can J Physiol Pharmacol 65:30–35.

Fliers E, DeVries GJ, Swaab DF (1985) Changes with aging in the vasopressin and oxytocin innervation of the rat brain. Brain Res 348: 1–8.

Handelman GE, Sayson SC (1984) Neonatal exposure to vasopressin decreases vasopressin binding sites in the adult kidney. Peptides 5: 1217–1219.

Jard S, Barberis C, Audigier S, Tribollet E (1987) Neurohypophyseal hormone receptor systems in brain and periphery. Prog Brain Res 72:173–187.

Jenner P, Marsden CD (1987) Chronic pharmacological manipulation of dopamine receptors in brain. Neuropharmacology 26:931–940.

Johnson AE, Ball GF, Coirini H, Harbaugh CR, McEwen BS, Insel TR (1989) Time course of the estradiol-dependent induction of oxytocin receptor binding in the ventromedial hypothalamic nucleus of the rat. Endocrinology 125:1414–1419.

Kasting NW, Veale WL, Cooper KE (1980) Convulsive and hypothermic effects of vasopressin in the brain of the rat. Can J Physiol Pharmacol 58:316–319.

Klein WL, Sullivan J, Skorupa A, Aguilar JS (1989) Plasticity of neuronal receptors. FASEB J 3:2132–2140.

Kleinbaum DG, Kupper LL, Muller KE (1988) Applied regression analysis and other multivariable methods, 2d ed, pp 260–296. Boston: PWS-KENT.

Koob GF, Dantzer R, Rodriguez F, Bloom FE, LeMoal M (1985) Osmotic stress mimics effects of vasopressin on learned behavior. Nature 316:750–752.

Kovacs GL, De Wied D (1983) Hormonally active arginine-vasopressin supresses endotoxin-induced fever in rats: lack of effect of oxytocin and a behaviorally active vasopressin fragment. Neuroendocrinology 37:258–261.

Kruszynski M, Lammek B, Manning M, Seto J, Haldar J, Sawyer WH (1980) [1-(β-mercapto-β,β-cyclopentamethylenepropionic acid), 2-(*O*-methyl)tyrosine] arginine-vasopressin and [1-(β-mercapto-β,β-cyclopentamethylenepropionic acid)] arginine-vasopressin, two highly potent antagonists of the vasopressor response to arginine-vasopressin. J Med Chem 23:364–368.

Landgraf R, Neumann I, Schwarzberg H (1988) Central and peripheral release of vasopressin and oxytocin in the conscious rat after osmotic stimulation. Brain Res 457:219–225.

Michell RH, Kirk CJ, Billah MM (1979) Hormonal stimulation of

phosphatidylinositol breakdown, with particular reference to the hepatic effects of vasopressin. Biochem Soc Trans 7:861–865.

Miller MA, Urban JH, Dorsa DM (1989a) Steroid dependency of vasopressin neurons in the bed nucleus of the stria terminalis by *in situ* hybridization. Endocrinology 125:335–340.

Miller MA, Urban JH, Dorsa DM (1989b) Testosterone regulation of vasopressin mRNA in the bed nucleus of the stria terminalis is dose dependent. Soc Neurosci Abstr 15:1271.

Miller MA, Dorsa DM, DeVries GJ (1990) Decline of vasopressin mRNA and immunoreactivity in the BNST following castration: a time-course study. Soc Neurosci Abstr 16:661

Naylor AM, Ruwe WD, Burnard DM, McNeely PD, Turner SL, Pittman QJ, Veale WL (1985) Vasopressin-induced motor disturbances: localization of a sensitive forebrain site in the rat. Brain Res 361:242–246.

Naylor AM, Cooper KE, Veale WL (1987a) Vasopressin and fever: evidence supporting the existence of an endogenous antipyretic system in the brain. Can J Physiol Pharmacol 65:1333–1338.

Naylor AM, Gubitz GJ, Dinarello CA, Veale WL (1987b) Central effects of vasopressin and 1-desamino-8-D-arginine vasopressin (DDAVP) on interleukin-1 fever in the rat. Brain Res 401:173–177.

Pittman QJ, Malkinson TJ, Kasting NW, Veale WL (1988a) Enhanced fever following castration: possible involvement of brain arginine vasopressin. Am J Physiol 254:R513–R517.

Pittman QJ, Naylor A, Poulin P, Disturnal J, Veale WL, Martin SM, Malkinson TJ, Mathieson B (1988b) The role of vasopressin as an antipyretic in the ventral septal area and its possible involvement in convulsive disorders. Brain Res Bull 20:887–892.

Poulin P, Pittman QJ (1988) Oxytocin receptors sensitize the brain to vasopressin's convulsive actions. Soc Neurosci Abstr 14:1263.

Poulin P, Lederis K, Pittman QJ (1988) Subcellular localization and characterization of vasopressin binding sites in the ventral septal area, lateral septum and hippocampus of the rat brain. J Neurochem 50: 889–898.

Poulin P, Weiss S, Pittman QJ (1990) Enhanced vasopressin (AVP)-induced phosphoinositide (PI) hydrolysis in rats pretreated with i.c.v. oxytocin (OT). Soc Neurosci Abstr 16:372.

Raggenbass M, Tribollet E, Dreifuss JJ (1987) Electrophysiological and autoradiographical evidence of V1 vasopressin receptors in the lateral septum of the rat brain. Proc Natl Acad Sci USA 84:7778–7782.

Shewey LM, Dorsa DM (1986) Enhanced binding of ^{3}H-arginine-8-vasopressin in the Brattleboro rat. Peptides 7:701–704.

Shewey LM, Dorsa DM (1988) V1-type vasopressin receptors in rat brain septum: binding characteristics and effects on inositol phospholipid metabolism. J Neurosci 8:1671–1677.

Shewey LM, Brot MD, Szot P, Dorsa DM (1989) Enhanced phosphoinositol hydrolysis in response to vasopressin in the septum of the homozygous Brattleboro rat. Brain Res 478:95–102.

Tribollet E, Audigier S, Dubois-Dauphin M, Dreifuss JJ (1990) Gonadal steroids regulate oxytocin receptors but not vasopressin receptors in the brain of male and female rats. An autoradiographical study. Brain Res 511:129–140.

Voorhuis TAM, deKloet ER, DeWied D (1988) The distribution and plasticity of [^{3}H]vasopressin-labelled specific binding sites in the canary brain. Brain Res 457:148–153.

Wonnacott S (1990) The paradox of nicotinic acetylcholine receptor upregulation by nicotine. Trends Pharmacol Sci 11:216–219.

Wurpel JND, Dundore RL, Barbella YR, Balaban CD, Keil LC, Severs WB (1986) Barrel rotation evoked by intracerebroventricular vasopressin injections in conscious rats. I. Description and general pharmacology. Brain Res 365:21–29.