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A Comparison of the Oxytocin and Vasopressin Responses to the 5-HT_{1A} Agonist and Potential Anxiolytic Drug Alnespirone (S-20499)

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VAN DE KAR, L. D., A. D. LEVY, Q. LI AND M. S. BROWNFIELD. *A comparison of the oxytocin and vasopressin responses to the 5-HT_{1A} agonist and potential anxiolytic drug alnespirone (S-20499).* PHARMACOL BIOCHEM BEHAV **60**(3) 677–683, 1998.—The effect of the serotonin1A (5-HT_{1A}) agonist alnespirone (S-20499) on the secretion of both oxytocin and vasopressin was examined in the same conscious, unrestrained male rats. The dose–response and time–course effects on the secretion of oxytocin and vasopressin revealed that alnespirone stimulated oxytocin in a dose-dependent manner, but did not increase vasopressin secretion. Time of maximal effect following injection of alnespirone (5 mg/kg, IP) was as early as 15 min postinjection, with significant stimulation persisting for 30 min. Pretreatment with a low dose of the 5-HT_{1A}/β-adrenoceptor antagonist (−)-pindolol (0.3 mg/kg, SC), 30 min prior to injection of alnespirone (0, 2, 5, and 10 mg/kg, IP) shifted the dose–response curve to the right and inhibited the effect of alnespirone on plasma oxytocin concentration. Furthermore, pretreatment with a low or a high dose of the 5-$HT_{1A/2A}$/dopamine D_2 antagonist spiperone (0.01 or 3 mg/kg, SC) dose dependently shifted the alnespirone dose–response curve effect of alnespirone to the right. None of these drugs, alone or in combination, altered plasma vasopressin levels. These studies suggest that 5-HT_{1A} receptor mechanisms mediate the effect of alnespirone on the secretion of oxytocin. Furthermore, these studies suggest that 5-HT_{1A} receptor mechanisms do not participate in the serotonergic regulation of vasopressin secretion. © 1998 Elsevier Science Inc.

Serotonin Hypothalamus Hormone Secretion Paraventricular nucleus Receptor Neuroendocrine Dose–response Time course 5-HT_{1A} antagonist

THE neurohormones vasopressin and oxytocin are synthesized in magnocellular neurons located in the same hypothalamic regions, the paraventricular and supraoptic nuclei and accessory hypothalamic magnocellular nuclei (16,46). Both send their axons into the posterior lobe of the pituitary gland, where they secrete their respective neurohormone into the circulation. Several stimuli are known to simultaneously increase the secretion of both vasopressin and oxytocin. Such stimuli include increased plasma osmolality and decreased plasma volume (8,26). On the other hand, outside of the role of oxytocin in reproduction, little is known regarding the differentiation between stimuli that increase oxytocin vs. vasopressin secretion (13).

There is agreement from several laboratories that serotonergic mechanisms can stimulate the secretion of both of vasopressin and oxytocin. Injection of 5-HT into the cerebroventricular system of conscious rats stimulates the secretion of both oxytocin (43) and vasopressin (37,39,43). Systemic administration of 5-HT releasers and 5-HT agonists also stimulates the secretion of oxytocin (3,44) and vasopressin (5,9,25). Serotonergic mechanisms also are involved in the osmotic stimulation of both vasopressin and oxytocin secretion (14,42).

Many recent studies have centered on the identification of specific serotonin receptor mechanisms that are involved in stimulating the secretion of either vasopressin or oxytocin.

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Vasopressin secretion can only be increased by activation of 5-HT_{2A} and/or 5-HT_{2C} receptors (5,9). Oxytocin secretion also can be stimulated by 5-$HT_{2A/2C}$ receptor mechanisms (1,2,4,44), as well as by other 5-HT receptor subtypes (27,44).

Although several studies have examined the regulation of either oxytocin or vasopressin separately, only one study has examined the role of 5-$HT_{2A/2C}$ receptors in the regulation of both hormones (43), and one study (3) examined the role of 5-HT_{1A} receptors in the secretion of both vasopressin and oxytocin simultaneously in the same rat. Activation of 5-HT_{1A} receptors also stimulates the secretion of oxytocin (2), but vasopressin secretion may not respond to 5-HT_{1A} receptor activation (2). A complete comparative characterization of the secretion of oxytocin and vasopressin, preferably in the same rats, in response to 5-HT_{1A} agonists is needed to clarify this issue.

The present study was undertaken to examine whether both oxytocin and vasopressin secretion is stimulated by 5-HT_{1A} receptor mechanisms in the same rats. For this study we have selected the 5-HT_{1A} agonist alnespirone (28,29). This drug has been shown to possess anxiolytic properties similar to other 5-HT_{1A} partial agonists such as buspirone, ipsapirone, and tandospirone (10,11,15,20,21,40). We have previously shown that alnespirone increases the secretion of ACTH through activation of 5-HT_{1A} receptor mechanisms (31). We examined the oxytocin and vasopressin responses, in the same conscious rats, to the following: 1) a dose–response experiment, 2) a time-course analysis, and 3) inhibition of the effect of alnespirone with two different 5-HT_{1A} antagonists that have different side effects but share their ability to block 5-HT_{1A} receptors. These 5-HT_{1A} antagonists were (−)pindolol, which also is a β-adrenoceptor antagonist (6,22,24, 30,41), and spiperone, which also is a 5-HT_{2A} and dopamine D_2 antagonist (7,12,18,24). Although it became clear that alnespirone alone does not increase plasma levels of vasopressin, the tests with the two antagonists were undertaken to examine whether these drugs could unmask other effects of alnespirone on the secretion of vasopressin.

METHOD

Animals

Adult male rats (Harlan Sprague–Dawley, Indianapolis, IN, 225–250 g bw) were used in this study. Rats were housed in a lighting (12 L:12 D; lights on at 0700 h), humidity- and temperature-controlled, AALAC approved rat room. Food and water were available ad lib. Animals were housed two per cage. Rats were allowed to recover from shipping for at least 10 days prior to the performance of an experiment. Rats were killed by decapitation using a guillotine and trunk blood was collected into chilled centrifuge tubes containing 0.5 ml of a 0.3 M EDTA (pH 7.4) solution. The blood was centrifuged at 1000 × *g* for 20 min at 4°C and stored at −70°C until extractions and radioimmunoassays were performed for oxytocin and vasopressin. All procedures were conducted in accordance with the NIH Guide for the Care and use of Laboratory Animals (Publication No 85-23, revised 1985) as approved by the Loyola University Institutional Animal Care and Use Committee. All efforts were made to minimize animal suffering, to minimize the number of animals used, and to utilize alternatives to in vivo techniques.

Drugs

Alnespirone [(+)4[N-(5-methoxy-chroman-3-yl)N-propyl-amino]butyl-8-azaspiro-(4,5)-decane-7,9-dione, (S-20499)], was donated by Institut de Recherches Internationales Servier (Courbevoie, France). It was dissolved in normal saline. The 5-HT_{1A} antagonist (−)pindolol (RBI, Nattick MA), was dissolved in a minimal volume of 0.1 N HCl, and diluted to a final volume in saline. (−)pindolol (0.3 mg/kg) was injected subcutaneously 30 min before the injection of alnespirone. Spiperone (Sigma, St. Louis, MO) was initially dissolved in 95% ethanol, then diluted with saline to a 10% ethanol–saline solution. Spiperone (0, 0.01, or 3 mg/kg, SC) was injected SC 30 min before the injection of alnespirone. All injections were in a volume of 1 ml/kg, and control groups received the appropriate vehicle solution.

Experiment 1, Dose Response

Alnespirone was injected in doses of 0.1, 0.5, 1, 5, 10, and 20 mg/kg, IP. Control rats received saline. Blood was collected 30 min following injection.

Experiment 2, Time Course

Alnespirone was injected at a dose of 5 mg/kg, IP, and blood samples were collected by decapitation at 15 and 30 min, and 1, 2, and 4 h following injection. Control rats received saline injection (1 ml/kg, IP) and blood samples were collected at the same time intervals.

Experiment 3, Inhibition With (−)-Pindolol

The specificity of the effect of alnespirone on oxytocin secretion was determined by pretreating rats with the 5-HT_{1A} antagonist/β antagonist (−)-pindolol (6,22,24,30,41). (−)-pindolol (0.3 mg/kg, SC) or its vehicle were administered 30 min prior to alnespirone (2, 5, or 10 mg/kg, IP) or saline. Blood samples were collected 30 min after alnespirone or saline injections.

Experiment 4, Inhibition With the 5-HT_{1A}/5-HT_{2A}/Dopamine D_2 Antagonist Spiperone (7,12,18,24)

Spiperone (0.01 or 3 mg/kg, SC) or its vehicle (10% ethanol in saline) were administered 30 min prior to alnespirone (2, 5, or 10 mg/kg, IP) or saline. Blood samples were collected 30 mins after alnespirone or saline injections.

Plasma Oxytocin and Vasopressin Measurements

The procedures for the extraction and radioimmunoassay of oxytocin and vasopressin have been previously described (9,44). Briefly, plasma was extracted on solid-phase columns (C-18/OH, Varian), equilibrated in triethylamine formate (TEAF, pH 3.0) and eluted in TEAF-isopropanol (1:1). Recovery of extracted peptides was 86.4 ± 2.4% for oxytocin and 88.9 ± 3.8% for vasopressin. Radioimmunoassays were conducted under disequilibrium conditions using ^{125}I labeled synthetic oxytocin and arginine vasopressin (Peninsula, Belmont, CA), prepared by the chloramine-T method, and rabbit antioxytocin (Calbiochem, San Diego, CA) and antivasopressin (MSB 5-13T) sera. Bound tracer was precipitated by centrifugation after addition of second antibody and polyethylene glycol. Minimum detectable level for oxytocin was 0.5 pg/tube and for vasopressin it was 0.06 pg/tube. Crossreactivity in the oxytocin radioimmunoassay for arginine vasopressin and vasotocin was <0.2% and crossreactivity for oxytocin in the vasopressin radioimmunoassay was <0.1%. Crossreactivity for other peptides tested (angiotensin I and II, ACTH, LHRH) in each radioimmunoassay was less than 0.1%.

Statistical Analyses

Each experimental group consisted of eight rats. Data are expressed as mean ± SEM, and were analyzed by one- or two-way analysis of variance (ANOVA) followed by Duncan's new multiple range test (45), using the Statpak statistics program (Northwest Analytical, Portland, OR).

RESULTS

Dose–response studies show that alnespirone significantly elevated plasma oxytocin concentration, but did not elevate plasma vasopressin concentration (Fig. 1). For oxytocin, the one-way ANOVA result was $F(6, 43) = 9.647$, $p < 0.0001$. There was no significant effect of alnespirone on vasopressin, $F(6, 43) = 0.755$, $p > 0.6$.

Time-course data revealed that alnespirone has a maximal stimulatory effect on plasma oxytocin at 15 min postinjection, and the plasma oxytocin levels remained elevated until 30 min postinjection (Fig. 2). Vasopressin secretion was not affected by alnespirone at any time point studied. Injection of saline did not alter plasma levels of either oxytocin or vasopressin at any time point. The one-way ANOVA indicated that oxytocin was significantly elevated over the corresponding saline group at 15 and 30 min postinjection, $F(10, 73) = 14.9123$, $p < 0.001$. Vasopressin was not significantly elevated, $F(10, 74) = 0.69$, $p > 0.6$.

In the third experiment, (−)-pindolol (0.3 mg/kg, SC) pretreatment did not alter basal plasma levels of either oxytocin or vasopressin, but caused a right shift of the alnespirone dose–response curve effect on plasma levels of oxytocin. Again, there was no effect of (−)-pindolol and/or alnespirone

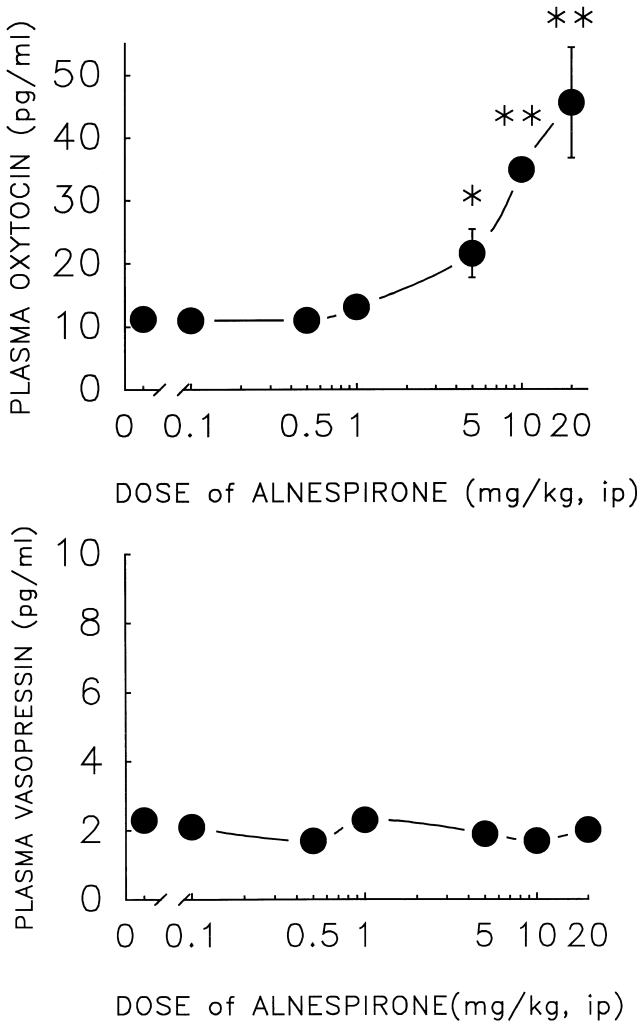

FIG. 1. Dose–response effects of the 5-HT_{1A} agonist alnespirone (0–20 mg/kg, IP) on plasma concentrations of oxytocin and vasopressin. Data represent mean ± SEM, $n = 8$ for each group. Significant difference from saline-injected rats, $*p < 0.05$, $**p < 0.01$ (one-way ANOVA and Duncan's new multiple range test).

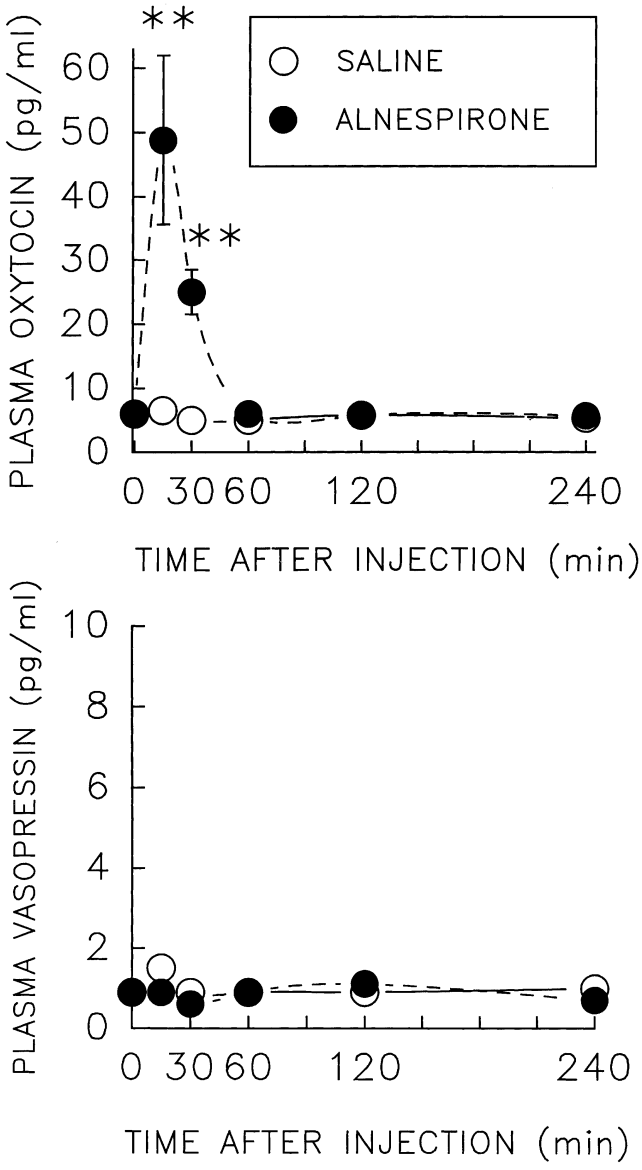

FIG. 2. Time–course effects of alnespirone (5 mg/kg, IP) on plasma oxytocin and vasopressin concentrations. Data represent mean ± SEM, $n = 8$ for each group. **Significant difference from corresponding saline-injected rats, $p < 0.01$ (one-way ANOVA and Duncan's new multiple range test).

on plasma vasopressin concentration. For oxytocin, the two-way ANOVA indicated a significant effect of pindolol, $F(1, 49) = 6.679$, $p < 0.02$, and a significant effect of alnespirone, $F(3, 49) = 16.4059$, $p < 0.001$. A subsequent Duncan's multiple range test indicated that the maximal response to alnespirone (10 mg/kg, IP) was inhibited by (−)-pindolol ($p < 0.05$). The two-way ANOVA for vasopressin revealed no significant main effects for either (−)-pindolol, $F(1, 49) = 0.049$, $p > 0.8$, or alnespirone, $F(3, 49) = 1.97$, $p > 0.1$ (Fig. 3).

Finally, pretreatment with both doses of spiperone (0.01 and 3 mg/kg, SC) did not alter basal levels of oxytocin but shifted the dose–response effect of alnespirone on plasma oxytocin to the right. As seen in all previous studies, there was no elevation in plasma vasopressin levels in any treatment group. For oxytocin, the two-way ANOVA indicated a significant effect of spiperone, $F(2, 76) = 5.966$, $p < 0.01$, and a significant effect of alnespirone, $F(3, 76) = 15.6174$, $p < 0.01$ (Fig. 4). A subsequent Duncan's multiple range test indicated that the response to the alnespirone dose of 5 mg/kg, IP was inhibited by both doses of spiperone ($p < 0.05$). For vaso-

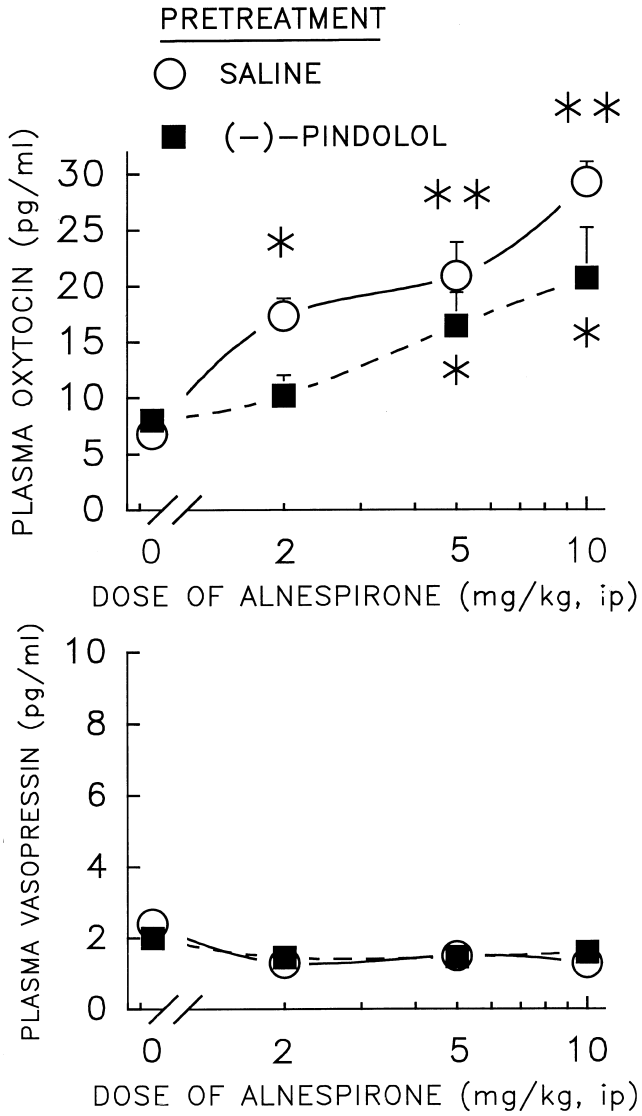

FIG. 3. Effect of (−)-pindolol (5-HT_{1A}/β antagonist) pretreatment (0.3 mg/kg, SC) on plasma oxytocin and vasopressin levels in rats injected with alnespirone. The data represent mean ± SEM of 6–8 rats per group. (−)-pindolol significantly inhibited alnespirone-induced elevation of plasma oxytocin levels. Significant effect of alnespirone *$p < 0.05$; **$p < 0.01$ (two-way ANOVA and Duncan's new multiple range test).

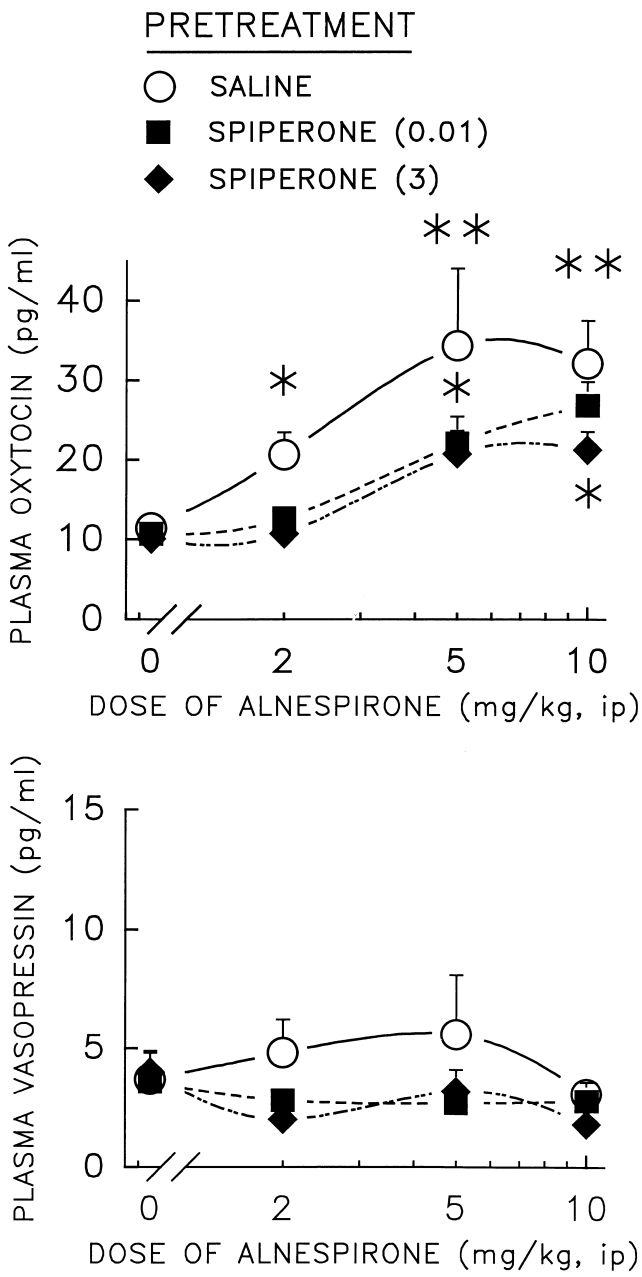

FIG. 4. Effect of spiperone (5-$HT_{1A/2A}$/dopamine D_2 antagonist) pretreatment (0.01 or 3 mg/kg, SC) on oxytocin and vasopressin in rats injected with alnespirone. The data represent mean ± SEM of eight rats per group. Spiperone significantly inhibited alnespirone-induced elevation of plasma oxytocin levels. Significant effect of alnespirone *$p < 0.05$; **$p < 0.01$ (two-way ANOVA and Duncan's new multiple range test).

pressin, the two-way ANOVA revealed no significant main effect for spiperone, $F(2, 79) = 2.905$, $p > 0.6$, and no significant main effect for alnespirone, $F(3, 79) = 1.07$, $p > 0.3$.

DISCUSSION

The present studies show that oxytocin, but not vasopressin secretion is stimulated by a 5-HT_{1A} receptor mechanism in the same conscious male rats. This conclusion was reached because a 5-HT_{1A} agonist stimulated oxytocin, but not vasopressin secretion, in the same rats. Furthermore, pretreatment with (−)-pindolol, a 5-HT_{1A}/β adrenergic antagonist or spiperone, a 5-$HT_{1A/2A}$/dopamine D_2 antagonist (23,50), inhibited the stimulatory effect of alnespirone on oxytocin secretion. The fact that vasopressin and oxytocin were evaluated in the same plasma samples precludes possible interexperimental differences and supports the conclusion that a divergence exist in the neurohypophysial responses to 5-HT_{1A} agonists.

In all the experiments, the blood samples were collected 30 min after the injection of alnespirone instead of 15 min, when a peak effect was observed in the time-course experiment. The reason for this decision was our concern that the stress effects of the injection might elevate plasma levels of oxytocin. Oxytocin is very sensitive to a variety of stressors (35,38,49). Therefore, allowing the rats to recover for a longer duration enabled us to minimize the stress effects, while the pharmacological effects could be clearly evaluated.

The dose–response curve of alnespirone's effects on plasma oxytocin is similar to other physiological effects of alnespirone, such as loss of body weight, increased secretion of ACTH, increased levels of 5-HT and reduced levels of 5-HIAA in the hippocampus, and increased punished drink responses (19,28, 31,40). The studies indicating changes in punished drinking behavior were conducted in mice, and it is unclear whether the doses would be comparable. In contrast, most studies examining the anxiolytic effects of alnespirone suggest that lower doses (0.04–1 mg/kg) are effective (10,15). Somatodendritic 5-HT_{1A} receptors in the raphe are known to be more sensitive to the effects of 5-HT_{1A} agonists, with lower ED_{50} values, than postsynaptic 5-HT_{1A} receptors in the forebrain (36). We deliberately chose a range of doses that will allow the examination of the effect of activating either of these two populations of 5-HT_{1A} receptors (i.e., 0.1–10 mg/kg). The effectiveness of high but not low alnespirone doses suggests that postsynaptic 5-HT_{1A} receptors mediate the oxytocin response to alnespirone. Furthermore, neither population of 5-HT_{1A} receptors seems to be involved in the regulation of vasopressin secretion.

The pharmacological evaluation of antagonist effects relied on two drugs that have a variety of side effects. Spiperone is a potent 5-HT_{1A} antagonist, but it also is a potent 5-HT_{2A} and dopamine D_2 antagonist (7,12,18,24). Similarly, (−)-pindolol is a 5-HT_{1A} antagonist that is a potent β-adrenoceptor antagonist (6,22,24,30,41). Clearly, the only common mechanism that these two antagonists share is a high affinity for 5-HT_{1A} receptors. Low doses of spiperone (0.01 mg/kg, SC) and (−)-pindolol (0.3 mg/kg, SC) were deliberately chosen to avoid high fractional occupancy of any of these receptors and to rely on the ability of both antagonists to compete with alnespirone for 5-HT_{1A} receptors. The reasoning was that if low doses of both antagonists showed the same ability to shift the dose–response curve of alnespirone to the right, this would most likely be due to their common high affinity for 5-HT_{1A} receptors. The results support this approach and suggest that the oxytocin responses to alnespirone are due to activation of 5-HT_{1A} receptors. At the time that these experiments were performed, the more selective 5-HT_{1A} antagonists WAY-100635, (S)-UH-301, and p-MPPF were not available to us.

Previously, a role for 5-HT_{1A} receptors in stimulating oxytocin secretion has been demonstrated by showing that three other 5-HT_{1A} agonists, buspirone, ipsapirone, and 8-OH-DPAT, stimulate oxytocin secretion (1,2,32–34,48). The effect of intravenous 8-OH-DPAT was inhibited by pretreatment with the partial 5-HT_{1A} antagonist NAN-190 (2). These previous observations, combined with the present data, conclusively demonstrate that activation of 5-HT_{1A} receptors increases the secretion of oxytocin. Moreover, the elegant lesion experiments by Bagdy et al. (1) suggest that the hypothalamic paraventricular nucleus contains the cells that are activated by 5-HT_{1A} agonists to secrete oxytocin.

Although several studies indicate that brain serotonergic mechanisms stimulate the secretion of vasopressin, very little information has been available regarding a role of 5-HT_{1A} receptors in the regulation of vasopressin secretion. The present experiments indicate that activation of 5-HT_{1A} receptors does not increase the secretion of vasopressin. In contrast, activation of 5-$HT_{2A/2C}$ receptors can increase the secretion of vasopressin (5,9,43) as well as the secretion of oxytocin (2,44). Lesion experiments have indicated that destruction of the paraventricular hypothalamic nucleus prevents the oxytocin response to the 5-$HT_{2A/2C}$ agonist DOI (1). No data are presently available regarding the neuroanatomical loci responsible for the serotonergic stimulation of vasopressin secretion. Combined, these observations suggest that a clear difference exists between the response of vasopressin and oxytocin neurons to serotonergic stimuli.

The pharmacological differentiation between the oxytocin and vasopressin responses to 5-HT_{1A} agonists might have great usefulness in neuroendocrine challenge tests. Both hormones are secreted by cells that share similar morphological and neuroanatomical characteristics, i.e., magnocellular cells in the hypothalamic paraventricular and supraoptic nuclei. Their nerve terminals in the neural lobe of the pituitary gland are responsible for the release of both vasopressin and oxytocin into the peripheral circulation. Because of concerns with toxicity and/or side effects of most selective 5-HT_{1A} agonists, neuroendocrine challenge tests in humans often depend on less selective agonists than animal studies. For example, buspirone also is a dopamine D_2 antagonist, while ipsapirone is a partial 5-HT_{1A} agonist with low efficacy (6,17,47). Knowing the distinct pharmacological differences between the oxytocin and vasopressin responses to 5-HT_{1A} and 5-$HT_{2A/2C}$ agonists might help interpret the data in humans challenged with less selective 5-HT agonists, or with 5-HT releasing drugs.

In conclusion, the present studies indicate that alnespirone activates postsynaptic 5-HT_{1A} receptor systems to increases the secretion of oxytocin but not of vasopressin. Although vasopressin and oxytocin have very similar neuroanatomical and physiological characteristics, the present study provides evidence for a distinct stimulus differentiation between these two neurohypophysial hormone systems.

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