Pergamon

Pharmacology Biochemistry and Behavior, Vol. 47, No. 3, pp. 467–470, 1994

0091-3057/94 $6.00 + .00

Hypothalamic Infusion of the 5-$HT_{2/1C}$ Agonist, DOI, Prevents the Inhibitory Actions of the 5-HT_{1A} Agonist, 8-OH-DPAT, on Lordosis Behavior

L. UPHOUSE,[1] M. ANDRADE, M. CALDAROLA-PASTUSZKA AND S. MASWOOD

Department of Biology, Texas Woman's University, Denton, TX 76204

Received 5 April 1993

UPHOUSE, L., M. ANDRADE, M. CALDAROLA-PASTUSZKA AND S. MASWOOD. *Hypothalamic infusion of the 5-$HT_{2/1C}$ agonist, DOI, prevents the inhibitory actions of the 5-HT_{1A} agonist, 8-OH-DPAT, on lordosis behavior.* PHARMACOL BIOCHEM BEHAV 47(3) 467–470, 1994.—Sexually receptive, intact, proestrous rats were infused bilaterally into the ventromedial nucleus of the hypothalamus (VMN) with 200 ng of the 5-HT_{1A} agonist, 8-hydroxy-2-(di-*n*-propylamino)tetralin (8-OH-DPAT), with 2000 ng of the 5-$HT_{2/1C}$ agonist, (±)-(2,5-dimethoxy-4-iodophenyl)-2-aminopropane HCl (DOI), or with both 8-OH-DPAT and DOI. Alone, VMN infusions of 8-OH-DPAT, but not DOI, inhibited lordosis behavior. When 2000 ng DOI was infused simultaneously with 8-OH-DPAT, the inhibitory effects of 8-OH-DPAT were completely abolished. These results suggest that neural sites responsible for the reported facilitatory effects of 5-$HT_{2/1C}$ agonists on lordosis behavior coexist in the VMN with those sites in which 5-HT_{1A} agonists are effective in reducing lordosis behavior. In contrast to the protective action of the 5-$HT_{2/1C}$ receptor agonist following VMN infusion, no protection was seen when both DOI and 8-OH-DPAT were administered intraperitoneally. Thus, the interaction of these two receptor subtypes in the control of lordosis behavior may be different in regions outside the VMN.

Serotonin Serotonin$_{1A}$ receptors Serotonin$_2$ receptors Receptivity Proceptivity Intact proestrous females Sexual behavior

A DUAL control by serotonin (5-HT) of female reproductive function has been recognized for several years. A robust inhibitory function of 5-HT_{1A} receptors in the control of female sexual behavior (1,8) has been attributed in part to postsynaptic 5-HT_{1A} receptors located in the ventromedial nucleus of the hypothalamus (VMN) (2,13–15). Serotonin's facilitation of female lordosis behavior appears to involve 5-$HT_{2/1C}$ receptors (9,10), but the neural sites responsible for this facilitation have not been identified. However, Mendelson and Gorzalka (10) hypothesized that 5-HT's effect on lordosis behavior was dependent on its relative degree of activation of 5-HT_{1A} inhibitory and 5-$HT_{2/1C}$ facilitatory sites. This hypothesis, indirectly, leads to the suggestion of regional colocalization of inhibitory and facilitatory 5-HT receptor sites. This suggestion was supported by recent findings from our laboratory (14) and was strengthened considerably by Kow et al.'s (7) report that hypothalamic slices, containing the VMN, respond in vitro to both 5-HT_{1A} and 5-$HT_{2/1C}$ compounds.

In a previous report (15), we suggested that 8-OH-DPAT infusions into the VMN inhibited lordosis behavior by reducing the firing of VMN neurons thought to be responsible for this brain area's facilitation of female sexual behavior. Therefore, Kow et al.'s (7) finding that the decreased firing of VMN neurons in response to 5-HT was mimicked by in vitro application of 8-OH-DPAT is particularly interesting. Moreover, in neurons showing both facilitatory and inhibitory responses to 5-HT, the inhibitory effect was mimicked by 8-OH-DPAT and the facilitatory effect was mimicked by the 5-$HT_{2/1C}$ agonist, (2,5-dimethoxy-4-iodophenyl)-2-aminopropane HCl (DOI). Furthermore, in some cases, DOI was able to partially attenuate the inhibitory effects of 5-HT. Thus, it might be anticipated that DOI could also provide a protective action

[1] To whom requests for reprints should be addressed.

against the inhibitory effects of 5-HT_{1A} agonists on lordosis behavior. In the following experiment, we provide evidence that, within the VMN, such protection does, in fact, occur.

METHOD

Materials

(±)-8-Hydroxy-2-(di-*n*-propylamino)tetralin (8-OH-DPAT) and (±)-(2,5-dimethoxy-4-iodophenyl)-2-aminopropane HCl (DOI) were purchased from Research Biochemicals Inc. (Natick, MA). Serotonin creatinine sulfate (5-HT) was purchased from Sigma Chemical Co. (St. Louis, MO). Intracranial cannulae were purchased from Plastic Products Inc. (Roanoke, VA) and dental acrylic was purchased from Reliance Dental Mfg. Co. (Worth, IL). Methoxyflurane (Metofane) was purchased from Pitman Moore (Mundelein, IL). All other supplies came from Fisher Scientific (Houston, TX).

Animals and Housing Conditions

Adult, female rats (CDF-344), bred in our laboratory from stock obtained from Sasco Laboratories (Omaha, NE), were housed three or four per cage with like-sex littermates in a colony room maintained at 22.2°C and 55% humidity on a 12 L : 12 D cycle with lights off at 12 noon. Food and water were available ad lib. Twenty-nine rats were implanted bilaterally with 22-ga stainless steel guide cannulae advanced stereotactically into the ventromedial nucleus of the hypothalamus [atlas coordinates from König and Klippel (6) AP 4.38; DV 7.8; ML 0.4] as previously described (13,15). Twenty-seven rats, used for IP treatments, remained in their home cage until the day of the experiment. The vaginal smear of each rat was monitored daily for at least 7 days prior to use in the experiment. Only females with proestrous smears (evidencing nucleated cells or primarily nucleated with a few cornified cells but an absence of leucocytes) and with a high sexual receptivity prior to the drug treatment were used in the experiments.

Intracranial Treatment

Five females were infused with 200 ng 8-OH-DPAT, alone, and five were infused with 2000 ng DOI, alone. Fifteen females received a simultaneous infusion of 200 ng 8-OH-DPAT and 2000 ng DOI; 12 of these same females received an infusion of 200 ng 8-OH-DPAT 1.5 to 2 h after the first infusion. Four additional females received a simultaneous infusion of 200 ng 8-OH-DPAT and 200 ng DOI. All drugs were dissolved in 0.9% saline and all infusions were delivered at a rate of 0.24 to 0.26 μl/min to a final infusion volume of 0.5 μl per bilateral site.

Sexual behavior was tested within a CMA/120 containment system (Bioanalytical Systems) as previously described (13, 15). The female's dummy cannulae were replaced with 28-ga stainless steel internal cannulae (terminating 0.5 mm below the guide cannulae), attached by tubing (i.d. = 0.58 mm; o.d. = 0.96 mm) to a CMA/100 (Bioanalytical Systems, Lafayette, IN) microinjector. The female was allowed to adjust to the chamber for 5–10 min before the male was placed with the female. The female's behavior was recorded continuously for five to 10 mounts prior to infusion, during the infusion and for 30 min after the infusion.

Intraperitoneal Treatment

Seven proestrous females were injected with 1.5 mg/kg DOI; 10 females were injected with 0.15 mg/kg 8-OH-DPAT; and 10 females were injected with 1.5 mg/kg DOI plus 0.15 mg/kg 8-OH-DPAT. Drugs were dissolved in 0.9% saline and were given IP in a volume of 1 ml/kg. The sexual behavior of the proestrous rats was tested in the home cage (polycarbonate shoe-box) of a sexually active male. After a minimum of 10 mounts by the male, the female was removed and was injected with 8-OH-DPAT, DOI, or a combination of DOI and 8-OH-DPAT. The effect of the drug treatments on sexual behavior was tested 30 min after injection. Testing sessions continued for 10 min or for a minimum of 10 mounts by the male.

Sexual Behavior

Sexual receptivity was quantified as the lordosis to mount (L/M) ratio (e.g., number of lordosis responses by the female divided by the number of mounts by the male) as previously described (13,15).

Histological Procedures

Females were anesthetized with Metofane and were perfused intracardially with 0.9% saline followed by 10% buffered formalin. The brain was excised and placed in 10% buffered formalin for a minimum of 24 h before sectioning (100 μm). Tissue sections were stained with cresyl violet and cannulae locations were verified according to König and Klippel (6). The location of each cannula was determined by an individual without knowledge of the experimental treatment or behavioral results. Only rats with both cannulae located in ventromedial nucleus sites where 8-OH-DPAT was previously found to inhibit lordosis behavior (13) were included in the studies.

Statistical Procedures

Following VMN infusions, L/M data were organized into the preinfusion period, infusion period, and consecutive 5-min intervals after infusion. The data were ranked and the ranks were subjected to repeated measures ANOVA (as recommended by Zar, 1987) with time as the repeated measure and treatment as the independent factor. The time-dependent decline in the L/M ratio (within treatment groups) was then analyzed with Friedman's chi-square followed when appropriate by Mann–Whitney *U* comparisons to the pretreatment interval. For IP treatments, data were grouped into preinjection period and 30 min after injection. Since all pretest data in the IP study showed L/M ratios of 1.0, only the data for the 30-min time point were compared. Data were analyzed by Kruskal–Wallis ANOVA followed by Mann–Whitney *U* comparisons of individual differences. The statistical reference was Zar (16) and an alpha level of 0.05 was required for rejection of the null hypothesis.

RESULTS

By 10 min after infusion, females treated with 200 ng 8-OH-DPAT, alone, or with 200 ng 8-OH-DPAT plus 200 ng DOI, had reduced L/M ratios, which continued throughout the testing interval (Fig. 1). VMN infusions of 2000 ng DOI, alone, had no effect on lordosis behavior but, when combined with 8-OH-DPAT, completely prevented the 8-OH-DPAT-induced decline in lordosis behavior [nonparametric ANOVA of ranks, respectively, for treatment, time, and treatment × time interaction: $\chi^2(4) = 49.29$, $p \leq 0.001$; $\chi^2(7) = 40.28$, $p \leq 0.001$; $\chi^2(28) = 20.12$, $p > 0.05$]. Relative to the pretest interval, 8-OH-DPAT reduced the L/M ratio by 5 min after infusion, and the L/M ratio remained suppressed throughout

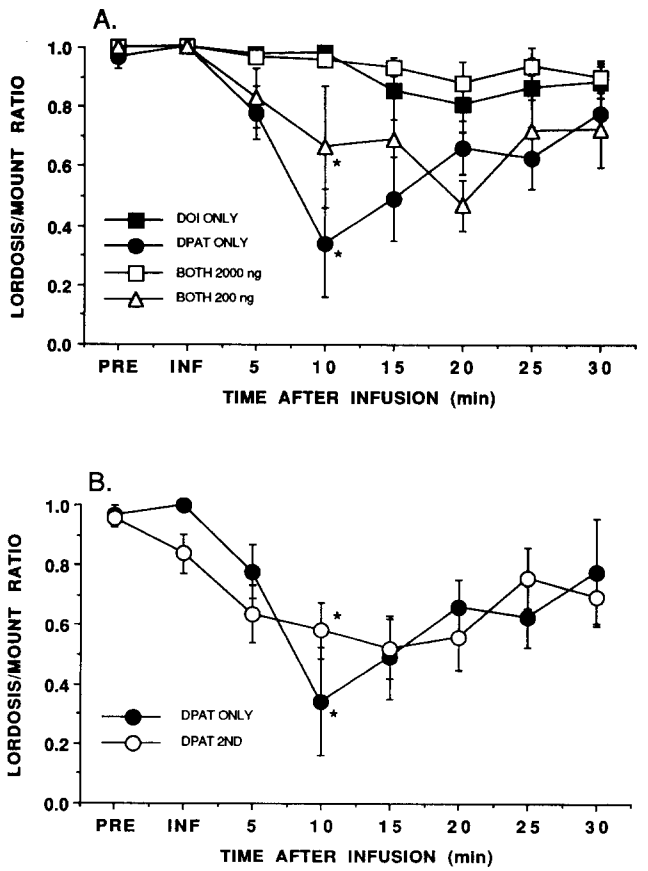

FIG. 1. Effects of VMN infusions of DOI, 8-OH-DPAT, or their combination on lordosis behavior. (A) The mean ± SE L/M ratios for proestrous female rats infused bilaterally into the VMN with 200 ng 8-OH-DPAT (n = 5), 200 ng 8-OH-DPAT plus 200 ng DOI (n = 4), 200 ng 8-OH-DPAT plus 2000 ng DOI (n = 15), or 2000 ng DOI (n = 5). The data indicate the L/M ratios prior to infusion (PRE), during the infusion (INF), and for six consecutive 5-min intervals following infusion. Asterisks indicate the first testing interval in which the L/M ratio was significantly different from the preinfusion interval. (B) The data for rats infused for the first time with 200 ng 8-OH-DPAT (A) vs. 12 rats given 200 ng 8-OH-DPAT 2 h after the previous infusion with the combination of 200 ng 8-OH-DPAT and 2000 ng DOI.

the testing interval (Mann–Whitney U comparisons within treatment, all $p \leq 0.05$). When 2000 ng DOI was infused simultaneously with 8-OH-DPAT, the L/M ratio was never significantly different from the pretest interval (Mann–Whitney U, all $p > 0.05$). The lower dose (200 ng) of DOI was substantially less effective in preventing the effects of 8-OH-DPAT.

That cannulae were located in sites effective for 8-OH-DPAT's inhibition of lordosis behavior and that 2000 ng DOI had, in fact, prevented a decline in lordosis behavior was confirmed by infusion of 200 ng 8-OH-DPAT 1.5 to 2 h later into the same rats that had previously received both 8-OH-DPAT and 2000 ng DOI. As shown in Fig. 1B, in the absence of 2000 ng DOI, 8-OH-DPAT was highly effective in reducing the L/M ratio.

The dose of 2000 ng DOI also partially attenuated the heightened resistance seen following infusions of 8-OH-DPAT

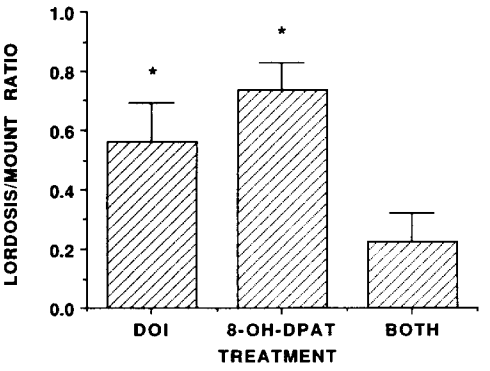

FIG. 2. Effects of IP treatments with DOI, 8-OH-DPAT, or their combination on lordosis behavior. Proestrous female rats were pretested for sexual receptivity and were then injected IP with 1.5 mg/kg DOI (n = 7), 0.15 mg/kg 8-OH-DPAT (n = 10), or 1.5 mg/kg DOI plus 0.15 mg/kg 8-OH-DPAT (n = 10). The figure shows the mean ± SE L/M ratio 30 min after the respective treatments. Asterisks indicate significant differences from the animals treated with both DOI and 8-OH-DPAT.

into the VMN. Most females infused with both 8-OH-DPAT and 2000 ng DOI did show some kicking or vocalizing, but extreme resistance such as boxing, rolling over, and wrestling was not seen. In contrast, when females were infused only with 8-OH-DPAT or with 8-OH-DPAT 1.5 to 2 h after the combination of 8-OH-DPAT and 2000 ng DOI, such severe resistance was present. As in previous studies (13), 8-OH-DPAT had minimal effects on lordosis quality (data not shown).

Unlike the effects of the VMN treatments, DOI failed to attenuate the lordosis-inhibiting effects of 8-OH-DPAT 30 min following IP treatment (Fig. 2). Instead, DOI, alone, suppressed lordosis behavior and appeared to accentuate the inhibitory effects of 8-OH-DPAT. Lordosis behavior following the combined treatment with 1.5 mg/kg DOI and 0.15 mg/kg 8-OH-DPAT was less than that following either 5-HT agonist, alone [Kruskal–Wallis ANOVA, $\chi^2(3) = 11.56$, $p \leq 0.05$; Mann–Whitney U, $p \leq 0.05$]. However, nine out of 10 of the females given the combined treatment of DOI and 8-OH-DPAT showed flattened posture 30 min after treatment; thus, these more global motor disturbances may have interfered with elicitation of the lordosis posture. Flattened posture was present in only one female treated IP with DOI alone and in only a single female treated with 8-OH-DPAT, alone. Thus, flattened posture was also accentuated by the combined IP treatment with 8-OH-DPAT and DOI.

DISCUSSION

Although functional interactions between the 5-HT_{1A} and 5-$HT_{2/1C}$ receptor have been previously reported (3–5), most such observations have followed systemic treatment with the 5-HT compounds. The present findings that the 5-$HT_{2/1C}$ agonist, DOI, can attenuate the inhibitory effects of direct intracranial infusions of the 5-HT_{1A} agonist, 8-OH-DPAT, on female sexual behavior demonstrate a behaviorally significant interaction, within a select brain region, between the 5-$HT_{2/1C}$ and 5-HT_{1A} sites in the control of lordosis behavior. That the attenuation was evident following VMN infusions of the drugs, and not after systemic treatment, may indicate a regional specificity in the nature of the 5-$HT_{2/1C}$ and 5-HT_{1A}

interaction. Although, in the VMN, inhibition of lordosis behavior occurs at relatively lower doses of 8-OH-DPAT, a reduction in lordosis behavior is also seen following infusion of the drug into the medial preoptic area (11) or into the midbrain central gray (12). Agonists acting at 5-$HT_{2/1C}$ receptors may be unable to attenuate these lordosis-inhibiting effects of 8-OH-DPAT outside the VMN. The uniqueness of the current findings to the VMN cannot be determined until additional brain areas are examined.

In contrast to the VMN effects, systemic treatment with both DOI and 8-OH-DPAT accentuated the inhibition of lordosis behavior, but this may have been secondary to the production of flattened posture. Components of the serotonin behavioral syndrome, such as forepaw treading, have been reported to be enhanced by the combined treatment of DOI and 8-OH-DPAT (3,5). In the present experiment, forepaw treading was only occasionally seen in females treated IP with DOI and 8-OH-DPAT. However, the male's continuous attempts to mount the female may have precluded our detection of this behavior.

While multiple neural locations may be responsible for effects following IP treatment with both DOI and 8-OH-DPAT, the results following IC infusions clearly demonstrate a functional interaction between the 5-$HT_{2/1C}$ and 5-HT_{1A} receptors within the VMN. Kow et al. (7) suggested that a relatively large proportion of the VMN neurons that contained one receptor subtype also contained the other. Our findings are consistent with this suggestion. However, we cannot rule out the possibility of intrinsic interactions among neuronal populations, each of which contained a single receptor subtype, but each of which resided within the diffusion area of the cannula tip. What is clear, however, is that a larger dose of the 5-$HT_{2/1C}$ agonist, relative to the 5-HT_{1A} agonist, was required to attenuate the effects of 8-OH-DPAT. Although doses of DOI between 200 and 2000 ng were not examined in combination with 200 ng 8-OH-DPAT, the failure of 200 ng DOI to reduce the effectiveness of the VMN infusions with 8-OH-DPAT suggests that a relatively large excess of DOI relative to 8-OH-DPAT may be required for the attenuation to occur. This observation is consistent with Kow et al.'s (7) conclusion that the 5-HT_{1A} subtype in the VMN may be more sensitive than its 5-$HT_{2/1C}$ counterpart.

In summary, the VMN is a neural site in which both facilitatory and inhibitory components of 5-HT's dual control over female lordosis behavior converge. Within this brain area, it is the relative balance between inhibitory and facilitatory mechanisms (rather than the absolute degree of activation of either) that appears to determine the nature of the female's response to a sexually active male. While the 5-HT receptor subtypes mediating the facilitatory and inhibitory actions of 5-HT are functionally linked and, thereby, able to reduce the effectiveness of the opposing receptor subtype(s), an inhibitory bias appears to predominate. Such a bias may be functionally significant in restricting the female's mating behavior to appropriate stages of the reproductive cycle.

ACKNOWLEDGEMENTS

Special appreciation is given to Ms. Nekayla Moore and Ms. Sharon Wilson for technical assistance and to Mr. Tim Lair for his care of the animals. The research was supported by NIH GM08256 and by NIH RO1 HD28419 to L.U.

REFERENCES

1. Ahlenius, S. N.; Fernandez-Guasti, A.; Hjorth, S.; Larsson, K. Suppression of lordosis behavior by the putative 5-HT receptor agonist 8-OH-DPAT in the rat. Eur. J. Pharmacol. 124:361–363; 1986.
2. Aiello-Zaldivar, M.; Luine, V.; Frankfurt, M. 5,7-DHT facilitated lordosis: Effects of 5-HT agonists. Neuroreport 3:542–544; 1992.
3. Arnt, J.; Hyttel, J. Facilitation of 8-OH-DPAT-induced forepaw treading of rats by the 5-HT_2 agonist DOI. Eur. J. Pharmacol. 161:45–51; 1989.
4. Backus, L. I.; Sharp, T.; Grahame-Smith, D. G. Behavioural evidence for a functional interaction between central 5-HT_2 and 5-HT_{1A} receptor. Br. J. Pharmacol. 100:793–799; 1990.
5. Berendsen, H. H. G.; Broekkamp, C. L. E. Behavioural evidence for functional interactions between 5-HT-receptor subtypes in rats and mice. Br. J. Pharmacol. 101:667–673; 1990.
6. König, J.; Klippel, R. The rat brain. A stereotaxic atlas of the forebrain and lower parts of the brain stem. Baltimore: Williams and Wilkins; 1963.
7. Kow, L.-M.; Tsai, Y.-F.; Wang, L.; Pfaff, D. W. Electrophysiological analyses of serotonergic actions on neurons in hypothalamic ventromedial nucleus in vitro: Receptor subtypes involved and implications for regulation of feeding and lordosis behaviors. Chin. J. Physiol. 35:105–121; 1992.
8. Mendelson, S. D.; Gorzalka, B. B. 5-HT_{1A} receptors: Differential involvement in female and male sexual behavior in the rat. Physiol. Behav. 37:345–351; 1986.
9. Mendelson, S. D.; Gorzalka, B. B. Serotonin type 2 antagonists inhibit lordosis behavior in the female rat: Reversal with quipazine. Life Sci. 38:33–39; 1986.
10. Mendelson, S. D.; Gorzalka, B. B. Sex differences in the effects of 1-(m-trifluoromethylphenyl)piperazine and 1-(m-chlorophenyl)-piperazine on copulatory behavior in the rat. Neuropharmacology 29:783–786; 1990.
11. Uphouse, L.; Caldarola-Pastuszka, M. Female sexual behavior following intracerebral infusion of the 5-HT_{1A} agonist, 8-OH-DPAT, into the medial preoptic area. Brain Res. 601:203–208; 1993.
12. Uphouse, L.; Caldarola-Pastuszka, M.; Droge, M. 8-OH-DPAT in the midbrain central gray inhibits lordosis behavior. Pharmacol. Biochem. Behav. 43:833–838; 1992.
13. Uphouse, L.; Caldarola-Pastuszka, M.; Montanez, S. Effects of the 5-HT_{1A} agonist and of the partial agonist/antagonist, NAN-190, on female sexual behavior. Neuropharmacology 31:969–981; 1992.
14. Uphouse, L.; Caldarola-Pastuszka, M.; Moore, N. Inhibitory effects of the 5-HT_{1A} agonists, 5-hydroxy- and 5-methoxy-(3-(di-n-propylamino)chroman), on female lordosis behavior. Neuropharmacology 32:641–651; 1993.
15. Uphouse, L.; Montanez, S.; Richards-Hill, R.; Caldarola-Pastuszka, M.; Droge, M. Effects of 8-OH-DPAT on sexual behaviors of the proestrous rat. Pharmacol. Biochem. Behav. 39: 635–640; 1991.
16. Zar, J. H. Biostatistical analysis, second edition. Englewood Cliffs, NJ: Prentice Hall, Inc.; 1984.