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Modulation of the Behavioral Effects of 8-OH-DPAT by Estrogen and DOI

NAVIN MASWOOD AND LYNDA UPHOUSE[1]

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

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MASWOOD N., AND L. UPHOUSE. *Modulation of the behavioral effects of 8-OH-DPAT by estrogen and DOI.* PHARMACOL BIOCHEM BEHAV **58**(4) 859–866, 1997.—The effects of female gonadal hormones on 8-hydroxy-2-(di-n-propylamino)tetralin (8-OH-DPAT)-induced flat body posture, hypothermia, and eating behavior were examined. Ovariectomized rats were injected with estradiol benzoate, estradiol benzoate, and progesterone, progesterone or vehicle on each of 2 consecutive weeks. On each week, the behavioral effects of the 5-HT_{1A} receptor agonist, 8-OH-DPAT, or the combination of both 8-OH-DPAT and 1-(2,5-dimethoxy-4-iodophenyl)-2-aminopropane hydrochloride (DOI), a 5-HT_2 receptor agonist, were examined. 8-OH-DPAT produced flat body posture, hypothermia, and eating behavior on each week of the experiment. Female gonadal hormones modulated eating behavior (e.g., rats treated with estradiol benzoate showed less eating after 8-OH-DPAT), but had no effect on either flat body posture or hypothermia. The 5-HT_2 receptor agonist, DOI, attenuated 8-OH-DPAT's effect on flat body posture and on hypothermia, but not on eating behavior. 8-OH-DPAT's effect on all behaviors declined during the second week of the experiment. © 1997 Elsevier Science Inc.

Female rats Serotonin receptor agonists Presynaptic Postsynaptic Receptor interaction

FEMALE gonadal hormones exert a variety of effects on serotonergic function, including an apparent modulation of the density and/or functioning of 5-HT receptors (4,14,27,28). Lakoski (21) reported that the ability of the 5-HT_{1A} receptor agonist, 8-hydroxy-2-(di-n-propylamino) tetralin (8-OH-DPAT), to reduce firing of dorsal raphe neurons was reduced by estrogen. Consistent with this observation are the findings of Uphouse et al. (32) that the hyperphagia induced by 8-OH-DPAT (thought to be mediated by the drug's action at somatodendritic autoreceptors) (11,12) was lower in proestrous relative to diestrous rats and the observation by Salamanca and Uphouse (24) that estrogen reduced 8-OH-DPAT–induced hyperphagia in ovariectomized, hormone-primed rats. Whether or not this hormonal modulation of 5-HT_{1A} receptor function is unique to those events that result from 8-OH-DPAT's activation of somatodendritic autoreceptors or includes behaviors modulated by postsynaptic 5-HT_{1A} receptors is unknown. However, 2 consecutive weeks of estrogen plus progesterone priming reduced the ability of 8-OH-DPAT to inhibit lordosis behavior in ovariectomized rats (32). Because 8-OH-DPAT–mediated inhibition of lordosis behavior reflects an action at postsynaptic 5-HT_{1A} receptors (1,29), functioning of postsynaptic 5-HT_{1A} receptors may also be modulated by female gonadal hormones. The present study was designed to compare the effects of female gonadal hormones on 8-OH-DPAT–mediated behaviors that reflect primarily activation of either presynaptic or postsynaptic 5-HT_{1A} receptors. 8-OH-DPAT–induced flattened body posture and hypothermia were measured as examples of postsynaptic receptor-mediated responses (33). To obtain a simultaneous estimate of eating behavior, 8-OH-DPAT–induced eating of food pellets was also recorded as an indication of the drug's action at somatodendritic autoreceptors (11,12). Finally, because several investigators have reported an interaction between 5-HT_{1A} and 5-HT_2 receptor subtypes (2,3), the effect of a low dose of the 5-HT_2 receptor agonist, 1-(2, 5-dimethoxy-4-iodophenyl)-2-aminopropane hydrochloride (DOI), on 8-OH-DPAT–induced behaviors was also examined.

METHOD

Materials

8-hydroxy-2-(di-n-propylamino-tetralin (8-OH-DPAT) and 1-(2,5-di-methoxy-4-iodophenyl)-2-aminopropane hydrochloride (DOI) were purchased from Research Biochemicals (Natick, MA). Estradiol benzoate, progesterone, and sesame seed oil were purchased from Fisher Scientific (Houston, TX).

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

Methods

Animals, housing, and hormone treatments. Adult, female rats (CDF-344) were bred in our laboratory from stock obtained from Sasco Laboratories (Omaha, NE). At 25 days of age, rats were weaned and housed with like-sex littermates in polycarbonate shoebox cages. Animals were housed two or three per cage in a colony room with a 12-L–12-D cycle (lights on at 2400 h) with ad lib access to food (rat chow) and water. Female rats, 60–90 days old, were anesthetized with methoxyflurane and were bilaterally ovariectomized. Two weeks after ovariectomy, the rats received a subcutaneous (SC) injection of either sesame seed oil or estradiol benzoate (25 μg/animal) in sesame seed oil at about 1000 h. Forty-eight hours later, the rats were injected with either sesame seed oil or progesterone (500 μg/animal) in sesame seed oil. All hormone injections were administered in a volume of 0.1 ml per rat. Hormonal treatments resulted in four different groups: oil–oil, oil–progesterone, estradiol benzoate–progesterone, and estradiol benzoate–oil. The same hormone treatments were repeated 1 week later.

Drug treatments. Each week, 4–6 h after the second hormone treatment (i.e., oil or progesterone), the rats were treated as follows: (a) 40 rats were injected SC with 8-OH-DPAT (0.25 mg/kg) on each week of the experiment; (b) 40 rats were injected each week with both 8-OH-DPAT (0.25 mg/kg) and DOI (0.08 mg/kg); (c) 22 rats received 0.08 mg/kg kg DOI on each week of the experiment; (d) 20 rats received saline on each week of the experiment; and (e) 20 rats received saline on the first week and 0.25 mg/kg 8-OH-DPAT on the second week of the experiment. Injections were given SC at 0.1 ml/100 g rat.

Behavioral methods. Behavioral observations and measurement of rectal temperature occurred immediately after drug treatment as detailed below. In all cases, rats from all four hormone-treated groups were tested within the same hour. Two rats were observed for each 30-min period and the hormone treatments were counterbalanced during the 60-min observation period. A rectal probe (Physitemp, Ret-2) and a digital temperature recorder (Sensortek, BAT-2) were used for measurement of rectal temperature as previously described (30). Rectal temperature was recorded immediately prior to injection and again at 15 and 30 min after injection. After injection, the rat was placed back into its cage and the incidences of flat body posture and eating behavior were recorded for the next 30 min. For each 5-min observation interval, eating and flat body posture were recorded as either present or absent. When a rat had a food pellet or part of a food pellet inside her mouth, eating behavior was recorded. Gnawing on a nonfood items (feces or bedding) were also recorded and scored separately as gnawing. Flat body posture (17,26) was recorded when the rat showed an outstretched posture with the abdomen resting close to the cage floor.

Statistical analyses. Treatment effects on flat body posture and eating were quantified as the number of 5-min intervals in which the behavior was present after injection. Group differences due to hormone treatment were evaluated by repeated-measures ANOVAs with hormone as the main effect and week of treatment as the repeated factor. Data for rectal temperature were analyzed by two-way repeated measures ANOVA with time after 8-OH-DPAT as the repeated factor and week and hormone treatment as independent factors. The effects of DOI plus 8-OH-DPAT relative to 8-OH-DPAT were compared by transforming the data to a ratio to that of the oil–oil (O O) controls given 8-OH-DPAT alone in week 1. When the contribution of agonist pretreatment to the drug-induced behavioral decline in the second week of the study was evaluated, data for the second week were compared by ANOVA with pretreatment and hormone as main effects. For assessment of drug effects relative to saline, data were subjected to ANOVA with treatment as the independent factor. The statistical reference was Zar (34) and an alpha level of 0.05 was required for rejection of the null hypothesis.

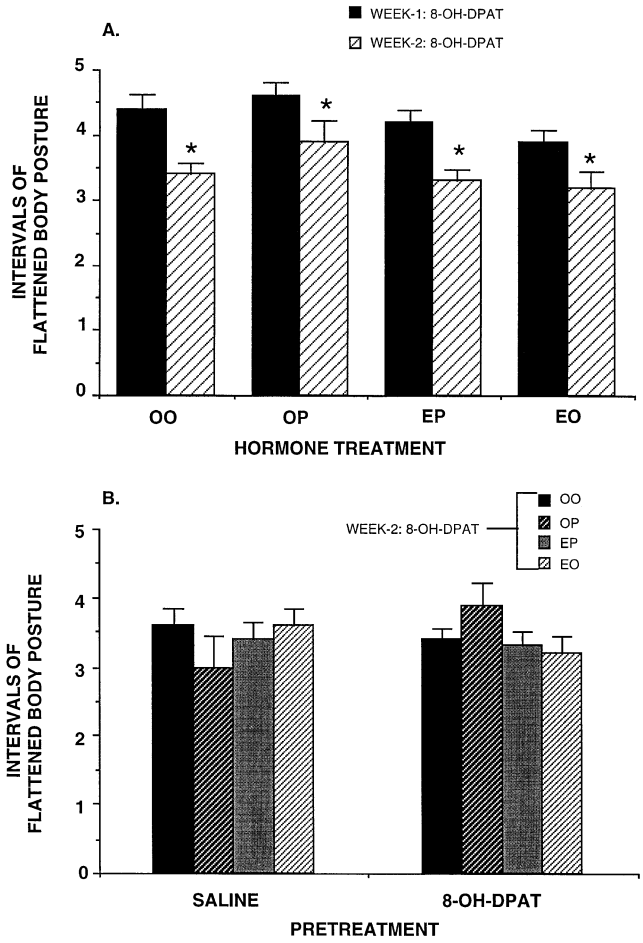

FIG. 1. Effects of female gonadal hormones on 8-OH-DPAT–induced flat body posture. Shown in A are the mean ± SE intervals of flat body posture in hormone-primed ovariectomized rats (n = 10/group) injected with 0.25 mg/kg of 8-OH-DPAT. Two weeks after ovariectomy, rats were injected SC with either sesame seed oil or estradiol benzoate (25 μg). Forty-eight hours later, rats received either sesame seed oil or progesterone (500 μg) SC. Hormone treatments resulted in four different groups: oil–oil (O O), oil–progesterone (O P), estradiol benzoate–progesterone (E P), and estradiol benzoate–oil (E O). Four to six hours after the second hormone treatment, all rats were injected with 8-OH-DPAT. The presence or absence of flat body posture was recorded for each 5-min interval for 30 min after injection with 8-OH-DPAT. The entire procedure was repeated 1 week later. Asterisks indicate significant differences between the same hormone treatment in week 1 and week 2. In B, the mean ± SE intervals of flat body posture for rats treated the prior week with 8-OH-DPAT (from A) are plotted together with rats given saline on the first week of the study and 0.25 mg/kg 8-OH-DPAT on the second week of the study. Data are for the second week of the experiment when both groups of rats received 0.25 mg/kg 8-OH-DPAT. Data for saline-pretreated rats are for five rats in each of the four hormone conditions.

RESULTS

Effects of 8-OH-DPAT

There were no effects of hormone treatment on 8-OH-DPAT–induced flat body posture or hypothermia. Female rats in all hormone groups showed flat body posture after 8-OH-DPAT treatment (Fig. 1A). Although estradiol benzoate tended to reduce the number of 5-min intervals in which flat body posture was present after 8-OH-DPAT, the main effect of hormone treatment was not significant, $F(3, 36) = 2.76$, $p > 0.056$. Relative to the first week of treatment, 8-OH-DPAT–induced flat body posture significantly declined in week 2 $F(1, 36) = 36.16$, $p \leq 0.05$, and this was true for all hormone treatments, Tukey's, all $q(36, 2) > 2.85$, $p \leq 0.05$. The hormone by week interaction was not significant, $F(3, 36) = 0.299$, $p > 0.05$. The decline in 8-OH-DPAT–induced flat body posture on the second week may have been in part the result of adaptation to the testing condition because there was no significant difference between rats pretreated with saline and those pretreated with 8-OH-DPAT, $F(1, 52) = 0.61$, $p > 0.05$ (Fig. 1B).

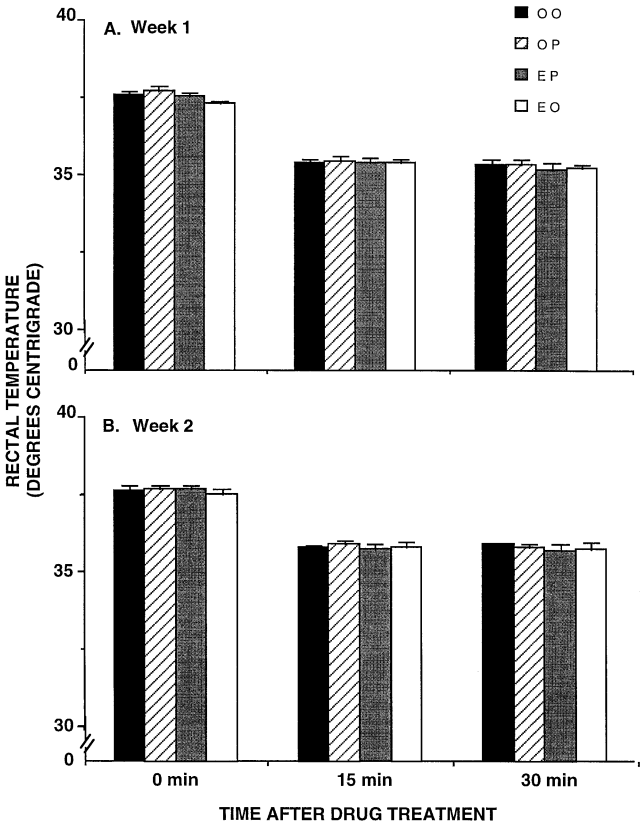

FIG. 2. The effects of 8-OH-DPAT on the rectal temperature of hormone-primed ovariectomized rats. Shown are the mean ± SE rectal temperature before and 15 min and 30 min after treatment with 0.25 mg/kg of 8-OH-DPAT in the same rats shown in Fig. 1A. Ovariectomized rats were treated with oil–oil (O O), oil–progesterone (O P), estradiol benzoate–progesterone (E P), or estradiol benzoate–oil (E O), as described for Fig. 1. (A) The effects of 8-OH-DPAT on rectal temperature (°C) in the first week of the experiment; (B) the data for the second week of the experiment.

The effect of 8-OH-DPAT on rectal temperature is shown in Fig. 2. There was significant decline in rectal temperature after 8-OH-DPAT treatment, $F(2, 144) = 1347.57$, $p \leq 0.001$, but neither the overall effect of hormone $F(3, 72) = 0.56$, $p > 0.05$, nor the hormone by week interaction were significant $F(3, 72) = .042$, $p > 0.05$. There was, however, a significant effect of the week of treatment, $F(1, 72) = 10.35$, $p \leq 0.002$, and a significant time after 8-OH-DPAT by week of treatment interaction, $F(2, 144) = 11.28$, $p \leq 0.001$. Both appeared to result from the slightly smaller effect of 8-OH-DPAT during the second week of treatment.

The reduced hypothermic effect of 8-OH-DPAT during the second week of the experiment appeared to result from the prior agonist treatment (Fig. 3). When the change in rectal temperature 30 min after injection was compared in rats given saline on both weeks of the experiment (SS rats), saline in the first week and 8-OH-DPAT in the second week (SD rats), or 8-OH-DPAT (DD rats) in both weeks of the experiment, there were significant group effects, $F(2, 136) = 377.6$, $p \leq 0.0001$, significant effects of the week of the experiment, $F(1, 136) = 99.65$, $p \leq 0.0001$, and a significant group by week interaction, $F(2, 136) = 164.7$, $p \leq 0.0001$). Because no effects of hormone treatment were present ($p > 0.05$), data in Fig. 3 are collapsed across the hormone conditions. Rats treated with 8-OH-DPAT for the second time showed a smaller decline in rectal temperature than they had shown in the first week of the experiment and were also significantly different from rats treated with saline in week 1 and 8-OH-DPAT in week 2 [both $q(136, 6) \geq 4.8$, $p \leq 0.05$]. However, even in rats pretreated with 8-OH-DPAT, a significant decline in rectal temperature was present following the second drug treatment ($p \leq 0.05$).

In contrast to the findings for flat body posture and hypothermia over both weeks, there was a significant effect of hormone on eating behavior after treatment with 8-OH-DPAT (Figs. 4 and 5). When animals treated with saline each week, saline in week 1 followed by 8-OH-DPAT in week 2, or 8-OH-DPAT in

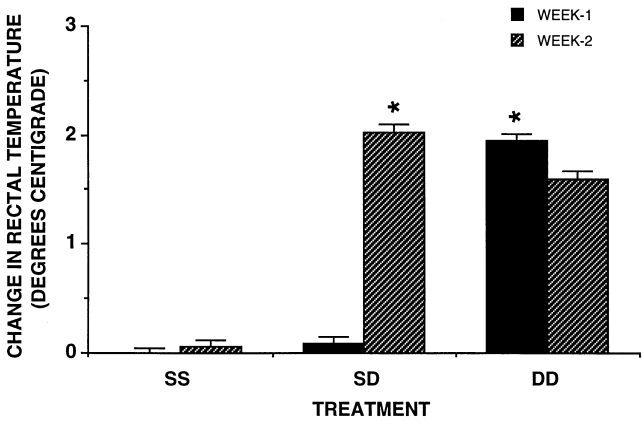

FIG. 3. Treatment effects on 8-OH-DPAT–induced hypothermia. Data for rectal temperature are plotted as a difference between the rectal temperature before injection and the temperature 30 min after injection. Data are the mean ± SE for 20 rats (collapsed across hormone treatments) that were injected each week with saline (SS) and 20 rats (collapsed across hormone treatments) injected with saline in week 1 and 0.25 mg/kg 8-OH-DPAT in week 2 (SD). Data for the rats given 8-OH-DPAT each week (DD) are shown for comparison. Asterisks indicate a significant difference in the response to 8-OH-DPAT relative to rats given 8-OH-DPAT on each week (DD-week 2).

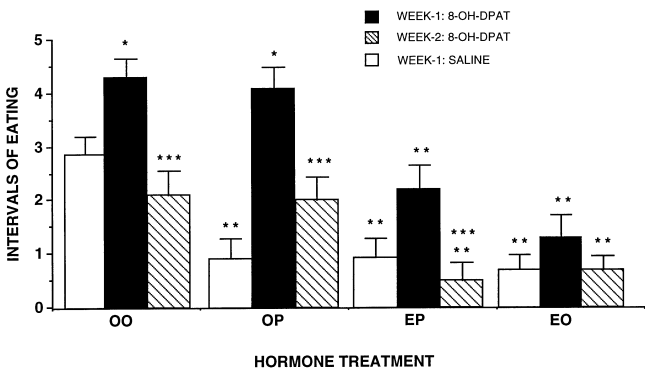

FIG. 4. Effects of female gonadal hormones on 8-OH-DPAT-induced eating. Shown are the mean ± SE 8-OH-DPAT–induced intervals of eating in ovariectomized rats, primed with oil–oil (O O), oil–progesterone (O P), estradiol benzoate–progesterone (E P), or estradiol benzoate–oil (E O). Data are for the rats treated with 8-OH-DPAT on the first and second week of the experiment and for 10 saline-treated rats in each of the four hormone conditions during the first week of the experiment. Single asterisks indicate significant differences in week 1 relative to saline (within the same hormonal condition). Double asterisks indicate a significant difference relative to the oil–oil (O O) group of the same week. Triple asterisks indicate significant differences, within hormone treatments, between week 1 and week 2.

both weeks were compared, there were significant interactions between week of treatment and hormone, $F(3, 68) = 2.92$, $p \leq 0.04$, between week of treatment and type of treatment, $F(2, 68) = 27$, $p \leq 0.0001$, as well as a significant week of treatment by type of treatment by hormone interaction, $F(6, 68) = 2.39$, $p \leq 0.04$. Following the first treatment with 8-OH-DPAT, a significant increase in eating behavior was seen in OO and OP-treated rats but not in EP or EO-treated animals, $q(72, 8) \geq 4.363$, $p \leq 0.05$. The overall main effect of hormone treatment, $F(3, 68) = 14.37$, $p \leq 0.0001$, reflected the generally lower eating behavior of rats that had been injected with estradiol benzoate, progesterone, or both hormones. In the second week of the experiment, there was a decline in eating behavior elicited by 8-OH-DPAT in 8-OH-DPAT–pretreated rats (Fig. 5). This decline was significant for all hormone groups except for the EO group. The effect of prior agonist treatment on 8-OH-DPAT–induced eating is best seen in Fig. 5 where eating behavior is plotted as a difference between eating behavior in week 1 and in week 2.

Because 8-OH-DPAT has been reported to increase gnawing, as well as eating behavior (7), gnawing on nonfood items was also recorded in the experiment. However, no animal showed more than one interval of gnawing on any item other than the food pellet (data not shown).

Effects of DOI on 8-OH-DPAT-Elicited Behaviors

Similar to the findings with 8-OH-DPAT alone, animals given 8-OH-DPAT plus DOI showed flat body posture, hypothermia, and an increase in eating behavior. However, the effects were not identical to those following 8-OH-DPAT alone. Comparisons between 8-OH-DPAT and 8-OH-DPAT plus DOI are shown in Figs. 6, 8, and 9.

When rats were treated with DOI alone, no animal showed any flat body posture alone (data not shown). However, DOI significantly reduced 8-OH-DPAT–induced flat body posture,

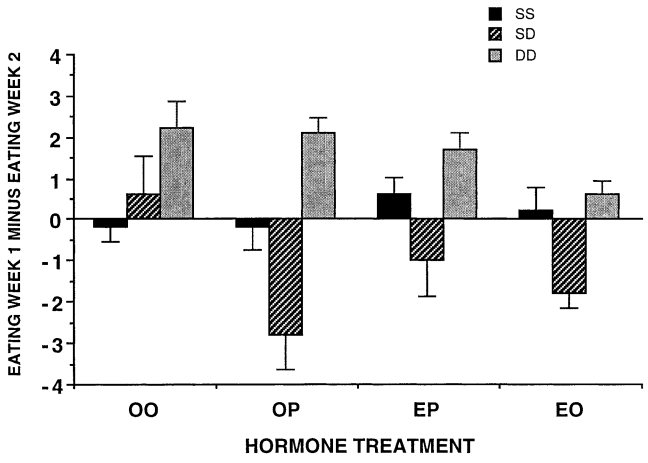

FIG. 5. Change in eating behavior between the first and second week of treatment. Shown are the differences in eating behavior between week 1 and week 2 for all hormone conditions. Five rats in each hormone condition were injected with saline each week (SS) and five rats (SD) per hormone condition were injected with saline in week 1 and with 0.25 mg/kg 8-OH-DPAT in week 2. Also shown are the data for the 10 rats per hormone condition that received 0.25 mg/kg 8-OH-DPAT each week. Data are the mean ± SE difference in eating behavior (e.g., for each rat, intervals of eating in week 1 minus the intervals of eating in week 2). When the bar lies below zero, there was more eating behavior in week 2 than in week 1. When the bar lies above zero, there was more eating behavior in week 1 than in week 2.

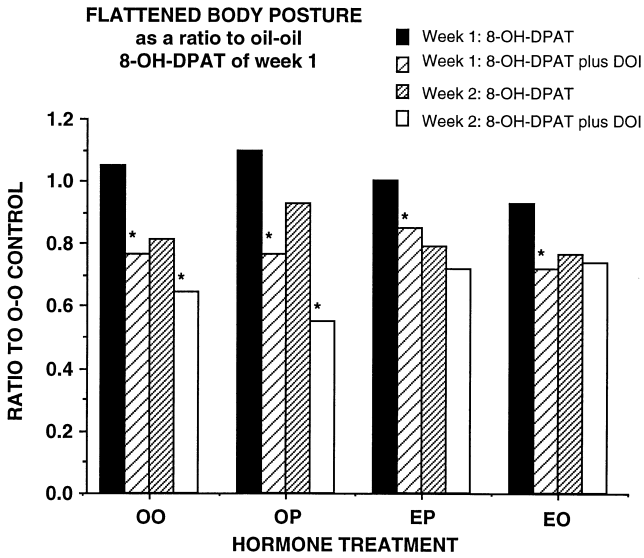

FIG. 6. Comparison of the effects of 8-OH-DPAT vs. 8-OH-DPAT plus DOI on flat body posture. Ovariectomized rats were hormonally primed as described in Fig. 1 and were injected with 8-OH-DPAT (0.25 mg/kg) or with 8-OH-DPAT (0.25 mg/kg) plus DOI (0.08 mg/kg) SC as described in the Methods section. Mean ± SE intervals of flat body posture for rats given oil–oil, oil–progesterone, estradiol benzoate–progesterone, or estradiol benzoate–oil are shown for both weeks of the study. Data for all groups are plotted as a ratio to that of the oil–oil (O O) animals given 8-OH-DPAT only, in week 1. Asterisks indicate a significant difference between 8-OH-DPAT, alone, and 8-OH-DPAT plus DOI within the same hormone condition.

$F(1, 71) = 43.61$, $p \leq 0.001$ (Fig. 6). There was also a significant effect of the week of treatment, $F(1, 71) = 41.83$, $p \leq 0.001$, and a significant drug by hormone interaction, $F(3, 71) = 3.47$, $p \leq 0.02$. During the first week of treatment, all 8-OH-DPAT–treated rats showed significantly more episodes of flat body posture than did the corresponding 8-OH-DPAT plus DOI-treated animals [Tukey's, all $q(71,2) \geq 3.73$, $p \leq 0.05$]. However, in week 2, a significant difference was present only in the oil–oil (O O) and the oil–progesterone (O P) groups [Tukey's, $q(71,2) =$ respectively 3.5, 8.0, $p \leq 0.05$].

Relative to saline-treated rats, DOI produced a slight increase in rectal temperature, $F(1, 54) = 15.15$, $p \leq 0.05$, but there were no interactions between the 5-HT_2 receptor agonist and hormone treatment ($p > 0.05$), and the effects of DOI alone did not vary between week 1 and week 2. Rectal temperature of rats treated in week 1 with either saline or DOI are shown in Fig. 7.

Shown in Fig. 8 are the effects of 8-OH-DPAT and 8-OH-DPAT plus DOI on rectal temperature. There were significant effects of drug, $F(1, 43) = 60.86$, $p \leq 0.001$, time after drug treatment, $F(2, 286) = 2017$, $p \leq 0.001$, week of treatment, $F(1, 143) = 23.08$, $p \leq 0.001$, and the week of treatment by time after drug interaction $F(2, 286) = 8.21$, $p \leq 0.001$). A significant time by drug treatment interaction, $F(2, 286) = 42.75$, $p \leq 0.001$, as well as the time by drug by week of treatment interaction, $F(2, 286) = 3.71$, $p \leq 0.03$, resulted from the lesser effect of DOI plus 8-OH-DPAT relative to 8-OH-DPAT alone.

Because rats injected with DOI alone averaged less than a single interval of eating behavior (data not shown), quantitative comparisons between groups treated with DOI alone were not performed. The effects of 8-OH-DPAT plus DOI relative to 8-OH-DPAT alone on eating behavior are shown in Fig. 9. There was no significant difference between the two drug treatments, $F(1, 71) = 0.07$, $p > 0.05$. A significant effect of hormone was present, $F(3, 71) = 20.24$, $p \leq 0.001$, but none of the interaction terms between hormone and drug treatment were significant.

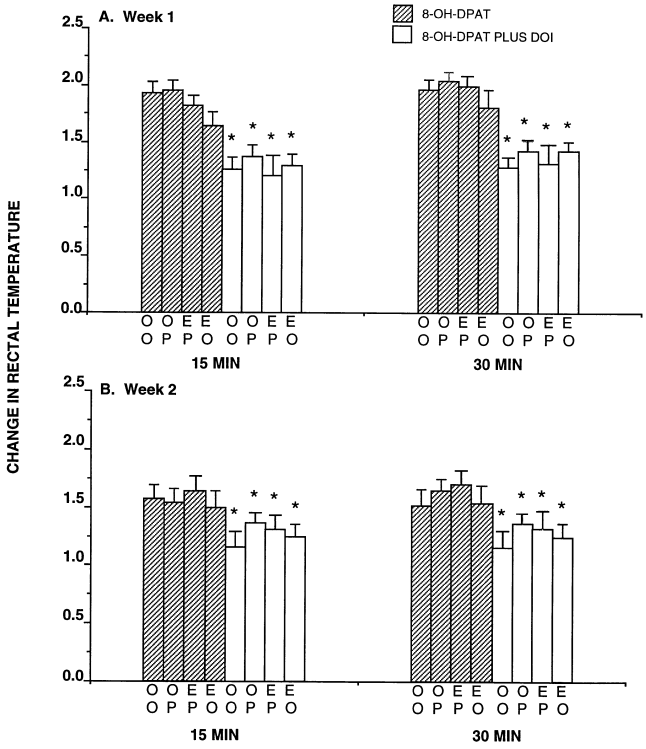

FIG. 8. Comparison of the effects of 8-OH-DPAT vs. 8-OH-DPAT plus DOI on 8-OH-DPAT-induced decline in rectal temperature. Ovariectomized rats were hormonally primed as described in Fig. 1 and were injected with 8-OH-DPAT (0.25 mg/kg) or with 8-OH-DPAT (0.25 mg/kg) plus DOI (0.08 mg/kg) SC as described in the Methods section. Mean ± SE decline in rectal temperature at 15 and 30 min after injection for rats given oil–oil, oil–progesterone, estradiol benzoate–progesterone, or estradiol benzoate–oil are shown for both weeks of the study. Data for all groups are plotted as a ratio to that of the oil–oil (O O) animals given 8-OH-DPAT only, in week 1 (see Fig. 2). Asterisks indicate a significant difference between 8-OH-DPAT and 8-OH-DPAT plus DOI within the same treatment conditions.

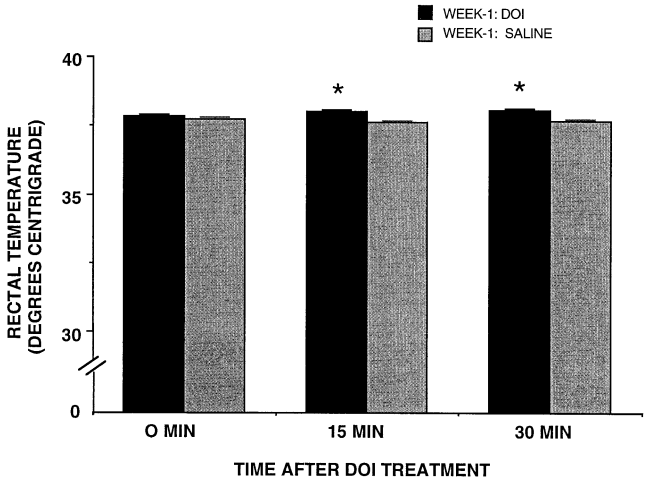

FIG. 7. Effects of saline or DOI injection on rectal temperature. The mean ± SE rectal temperature (degrees Centrigrade) for rats given saline ($n = 40$) or DOI ($n = 22$) in the first week of the experiment are shown. Data are collapsed across the four hormone treatments. Asterisks indicate significant differences (within time after injection) between saline-treated and DOI-treated rats.

DISCUSSION

A major objective of the present studies was to evaluate the effects of gonadal hormones on several behaviors elicited by 5-HT_{1A} receptor agonists. The current findings are consistent with previous suggestions that behaviors mediated by activation of somatodendritic 5-HT_{1A} autoreceptors may be sensitive to modulation by estradiol benzoate (21,24,30). In the present study, treatment with estradiol benzoate reduced the effects of 0.25 mg/kg 8-OH-DPAT on eating behavior while having little effect on 8-OH-DPAT–induced flat body posture or hypothermia. Although the mechanisms whereby estradiol benzoate reduces 8-OH-DPAT–induced food intake are not known, increased food intake after 8-OH-DPAT is thought to result from the drug's activation of somatodendritic 5-HT_{1A} receptors (12,15,22). Drugs that increase synaptic availability of 5-HT decrease eating (6), and 5-HT synthesis inhibitors generally increase eating (9,11). By reducing the firing of 5-HT neurons, 5-HT_{1A} autoreceptors are thought to decrease release of 5-HT and thereby reduce the inhibitory effect of 5-HT on food intake. Lakoski (21) reported that estradiol benzoate reduced the ability of 8-OH-DPAT to inhibit firing of dorsal raphe neurons. It is, therefore, possible that estrogen's attenuation of

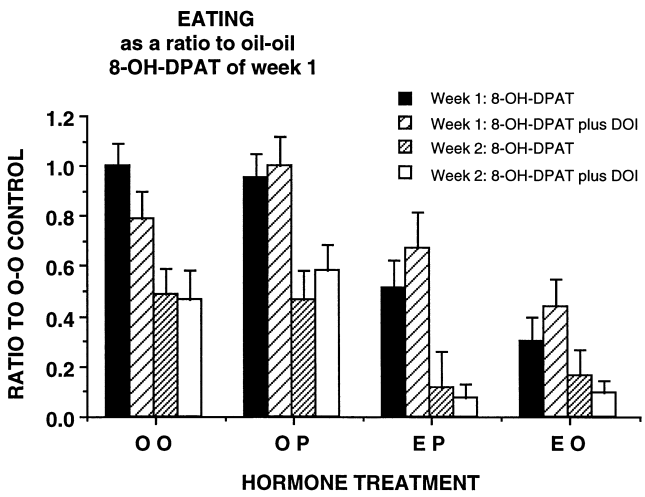

FIG. 9. Comparison of the effects of 8-OH-DPAT vs. 8-OH-DPAT plus DOI on 8-OH-DPAT-induced eating. Ovariectomized rats were hormonally primed as described in Fig. 1 and were injected with 8-OH-DPAT (0.25 mg/kg) or with 8-OH-DPAT (0.25 mg/kg) plus DOI (0.08 mg/kg) SC as described in Methods section. Data are plotted as a ratio to the mean of the oil–oil (O O) animals given 8-OH-DPAT only in week 1 (Fig. 4). Data are the mean ± SE of these ratios for rats given oil–oil, oil–progesterone, estradiol benzoate–progesterone, or estradiol benzoate–oil in both weeks of the study.

8-OH-DPAT–induced hyperphagia results from the hormone's modulation of somatodendritic 5-HT_{1A} autoreceptors.

It is important to note that in the current study 8-OH-DPAT's effect on eating was measured by the appearance of eating behavior and not by the amount of food eaten, which has been more commonly reported in studies of 8-OH-DPAT–induced hyperphagia (11,12,24). Because this measure of eating behavior could be quite different from the hyperphagia attributed to 8-OH-DPAT's action at somatodendritic autoreceptors, we cannot be sure that the behavior reflected the drug's action at that site. However, the hormonal effects on the present measure of eating behavior (after the first treatment with 8-OH-DPAT) mirror precisely the pattern shown by Salamanca and Uphouse (24) for the amount of food eaten in response to 8-OH-DPAT. A second distinction between the present studies and many others is that eating behavior was measured during the dark portion of the light/dark cycle when rats eat a majority of their food. There has been one other study in which hyperphagia was seen even when 8-OH-DPAT was administered during the dark portion of the light/dark cycle (9), and there is a general agreement between the findings of the present studies and those of prior reports. Therefore, the present method of measuring 8-OH-DPAT's effect on eating behavior appears to be consistent with other measures and may provide a complementary approach to the study of 8-OH-DPAT–induced hyperphagia. However, in spite of the rare presence of gnawing behavior on items other than food pellets, we cannot rule out the possibility that gnawing behavior contributed to the 8-OH-DPAT–induced eating observed in the current study.

In contrast to estradiol benzoate's modulation of 8-OH-DPAT–induced eating, there was no evidence that estradiol benzoate modulated the 5-HT_{1A} agonist's effect on flat body posture or hypothermia. Consistent with the findings of the present study, Fischette et al. (13) reported that although female rats were more likely than male rats to show elements of the serotonin syndrome after treatment with L-tryptophan, estradiol benzoate had no effect on this behavior. These findings also agree with the report of Uphouse et al. (30) that 8-OH-DPAT–induced hypothermia was gender, but not estrous cycle, dependent. However, the possible inference that behaviors mediated by postsynaptic 5-HT_{1A} sites are not modulated by estradiol benzoate is at variance with the reports by Uphouse et al. (32) and Jackson and Uphouse (16) that 2 consecutive weeks of estradiol benzoate plus progesterone treatment reduced 8-OH-DPAT's ability to inhibit lordosis behavior. Moreover, Clarke and Maayani (8) reported that chronic treatment of ovariectomized rats with estradiol benzoate enhanced 5-HT_{1A} receptor-mediated inhibition of pyramidal cells in the dorsal hippocampal area and accentuated the 8-OH-DPAT–induced increase in corticosterone secretion. These divergent reports of estradiol benzoate's effect on postsynaptic 5-HT_{1A} receptor-mediated responses may evidence a regional specificity in the degree and manner with which postsynaptic 5-HT_{1A} receptor-mediated behaviors are altered by the hormone. In fact, such a regional variation would be consistent with the findings of Biegon et al. (4) that 5-HT_1 receptors were increased, decreased, or unchanged by estradiol benzoate, depending on the brain region examined. However, because a single drug dose was examined in the present experiment, we cannot rule out the possibility that a hormone-dependent shift in the dose response effect of 8-OH-DPAT occurred for all behaviors.

The mechanisms whereby estradiol benzoate alters some indices of 5-HT_{1A} receptor function are currently unknown. Estradiol benzoate may affect the synthesis and/or degradation of 5-HT_{1A} receptors. It is also possible that by increasing the release of 5-HT, estradiol benzoate increases agonist activation of the 5-HT_{1A} receptor and thus initiates an agonist-induced desensitization of 5-HT_{1A} receptors. Because pre- and postsynaptic 5-HT_{1A} receptors have been reported to differ in the degree to which agonist-induced desensitization occurs (10), estradiol benzoate's differential effects on behaviors mediated by pre- and postsynaptic 5-HT_{1A} receptor activation may reflect this difference. Although it is generally agreed that long-term treatment with a 5-HT_{1A} receptor agonist will downregulate/desensitize both pre- and postsynaptic 5-HT_{1A} receptors (10,25), a single treatment with 8-OH-DPAT was reported to reduce behaviors mediated by presynaptic, but not postsynaptic, 5-HT_{1A} receptors (18). In the present studies, all 8-OH-DPAT–elicited behaviors were reduced on the second week of the experiment, and for both hypothermia and eating behavior, this decline clearly resulted from the prior agonist treatment.

Because in the previously mentioned studies male rats have been used, gender differences may be present in the degree to which postsynaptic 5-HT_{1A} receptors respond to agonist activation. Females may be more likely than males to show evidence of agonist-induced postsynaptic 5-HT_{1A} receptor modulation. Females are more sensitive to treatments that elicit the "serotonin syndrome" (13) and Haleem (14) reported that the quantitative scores for forepaw treading, flat body posture, and head weaving induced by 8-OH-DPAT were higher in females than in males and that the effects of 8-OH-DPAT on hippocampal 5-HT synthesis were also greater in females. Because females generally have higher levels of 5-HT than males and may have a more active 5-HT system (23), addition of an exogenous agonist may have a greater effect in females than in males.

The 5-HT_2 receptor agonist, DOI, significantly attenuated 8-OH-DPAT–induced flat body posture and hypothermia. This finding agrees with that of Uphouse et al. (31), who

found that direct infusion of DOI into the VMN of the hypothalamus reduced the inhibitory effect of 8-OH-DPAT on female sexual behavior. Kidd et al. (19) also reported that repeated DOI treatments attenuated the inhibitory effects of the 5-HT_{1A} receptor agonist, 8-OH-DPAT, on both cortical 5-HT release and dorsal raphe 5-HT neuronal firing. DOI's ability to decrease the effect of 8-OH-DPAT also agrees with the findings of Kow et al. (20), who showed that DOI reversed 8-OH-DPAT–induced inhibition of firing of VMN neurons. Moreover, in the present experiment, there were some interesting interactions between the effects of DOI and the effects of gonadal hormones. For example, in week 1, 8-OH-DPAT–treated females showed significantly more episodes of flat body posture than the corresponding 8-OH-DPAT plus DOI-treated animals. However, in week 2, a significant difference between the effects of 8-OH-DPAT and 8-OH-DPAT plus DOI was present only for the oil–oil and oil–progesterone groups. Thus, although female gonadal hormones did not seem to significantly modulate 8-OH-DPAT–induced flat body posture and hypothermia, in the presence of both DOI and estradiol benzoate, the effect of prior treatment with 8-OH-DPAT appears to have been attenuated. This is especially interesting in view of the report by Biegon et al. (5) that chronic exposure to estradiol benzoate or progesterone reduced 5-HT_1 receptors, but increased 5-HT_2 receptors, in the cortex.

Unlike DOI's attenuation of 8-OH-DPAT's effect on flat body posture and hypothermia, DOI did not reduce 8-OH-DPAT's effect on eating. This was surprising because activation of 5-HT_2 receptors has been found to produce hypophagia (33,35). The dose of DOI (0.08 mg/kg) used in the present study may have been too low (3) to produce robust effects on the eating parameter measured. It is also possible that the drug's failure to attenuate 8-OH-DPAT–induced eating is indicative of a greater impact of 5-HT_2/5-HT_{1A} receptor interaction for those behaviors that are mediated by postsynaptic 5-HT_{1A} receptor activation.

In summary, three major findings emerged from the current studies: 1) female gonadal hormones modulated 8-OH-DPAT's effect on eating behavior without altering the drug's action on flat body posture or hypothermia; 2) the 5-HT_2 receptor agonist, DOI, attenuated the effects of 8-OH-DPAT on hypothermia and flat body posture, but not eating behavior; and 3) a single treatment with the 5-HT_{1A} receptor agonist, 8-OH-DPAT, reduced the drug's effect 7 days later.

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