Endocrine (1995) 3, 751–754

8-OH-DPAT regulates the amplitude and the phase of LH surge in ovariectomized steroid-primed rats

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Precise interactions between ovarian steroids and neurotransmitters are required for the secretion of phasic LH surge. Previous data suggested the existence of an interactive stimulatory effect of progesterone (P) and serotonin (5-HT) on LH release. In the present work the effects of 8-OH-DPAT, a selective 5-HT_{1A} agonist, on phasic LH secretion were tested in ovariectomized rats implanted for 6 days with a pellet of 17 β estradiol (OVX-E_2) and in OVX-E_2 treated with progesterone (OVX-E_2-P). Intraperitoneal injection of 8-OH-DPAT at 11.00 h in the morning of the expected LH surge had no effect on circadian plasma levels of LH in OVX-E_2 rats, whereas it induced a phase advance and an increase in LH surge in OVX-E_2-P rats. Administration of the antiprogestin RU 38486 in OVX-E_2-P rat, totally abolished the combined effects of P and 8-OH-DPAT on phasic LH release. SDZ 216-525, a specific 5-HT_{1A} antagonist administered 60 min before 8-OH-DPAT, inhibited the stimulatory effect of the 5-HT_{1A} agonist on the amplitude of LH surge. The present data suggest that progesterone is required for the regulation of phasic LH release by 5-HT_{1A} agonists and that under this hormonal condition the activation of 5-HT_{1A} receptors induces a phase advance and an increase in LH surge.

Keywords: phasic LH release; 8-OH-DPAT; progesterone; phase shift; rat

Introduction

The effects of gonadal steroids on gonadotropin release are believed to be exerted at the level of the hypothalamus via altering the activity of a number of neurotransmitters. Considerable evidence suggest that serotonin (5-HT) plays an integral role in the generation of phasic LH release (for a review see Vitale & Chiocchio, 1993). Ovarian steroids have been shown to alter 5-HT synthesis, turnover, release and binding sites (Cone *et al.*, 1981; Héry *et al.*, 1982; Biegon & McEwen, 1982; Johnson & Crowley, 1986) suggesting that the influence of gonadal steroids on LH release might be at least, partially mediated by a modulation of the serotoninergic transmission.

In brain areas involved in the control of LH secretion, serotoninergic neurotransmission is modulated by progesterone (P) (Walker & Wilson, 1983; James *et al.*, 1989). Furthermore, P and 5-HT are believed to stimulate LH release in an interactive way. Joint administration of P and 5-hydroxytryptophan, the precursor of 5-HT, increased LH secretion, whereas anti 5-HT drugs blunted the facilitatory action of P on LH release (Johnson & Crowley, 1986). This may result from an interactive stimulatory effect of P and 5-HT on LHRH neurons, since we recently reported that P potentiated the increase in LHRH release induced by a 5-HT_{1A} receptor agonist in fetal hypothalamic cells in culture (Héry *et al.*, 1995).

The following experiments were performed in order to determine whether P could interact with a 5-HT_{1A} receptor agonist, 8-OH-DPAT (8-Hydroxy-2-(di-n-propylamino) tetralin) (Middlemiss & Fozard, 1983), to stimulate phasic LH release *in vivo*.

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Received 4 May 1995; accepted 17 July 1995

Results

Effects of 8-OH-DPAT on phasic LH release

In OVX-E_2 rats which received no further treatment, plasma LH levels showed high amplitude daily fluctuations, with maximal levels at 19.00 h (Figure 1A). Under these hormonal conditions, 8-OH-DPAT modified neither the kinetic, nor the amplitude of LH surge (Figure 1A).

The amplitude of LH surge was greater in OVX-E_2-P rats, than in OVX-E_2 ($F_{1,95} = 14.05$ Figure 1B). Although the onset of LH surge was early in OVX-E_2-P, the highest levels of LH were found at 19.00 h in OVX-E_2-P and OVX-E_2 rats (Figure 1B). A single injection of 8-OH-DPAT at 11.00 h increased the amplitude of LH surge in OVX-E_2-P ($F_{1,127} = 18.969$), with maximal levels at 17.00 h (Figure 1B). 8-OH-DPAT became unable to increase the magnitude of the LH surge when RU 38486 a progesterone antagonist, was adminsitered together with P at 09.00 h in OVX-E_2-P rats. ($F_{1,74} = 38.731$, Figure 1B).

Effect of SDZ-216525 on phasic LH release in OVX-E_2-P rats

In OVX-E_2-P rats, administration of a 5-HT_{1A} receptor antagonist (SDZ 216-525, 1 mg/kg) at 10.00 h in the morning of the expected LH surge delayed the onset of the phasic LH surge and decreased its magnitude (Figure 2A). A one way analysis of variance showed that the LH surge measured in the group of OVX-E_2-P rats treated with SDZ 216-525 was not significantly different from that measured in OVX-E_2 rats. ($F_{1,61} = 1.354$ Figure 2A compared to Figure 1A). Administration of SDZ 216-525 1 h before 8-OH-DPAT injection in OVX-E_2-P rats dramatically reduced the magnitude of LH surge ($F_{1,75} = 29.38$), without altering the time of peak (Figure 2B).

Effect of 8-OH-DPAT on hypothalamic LH-RH contents in OVX-E_2-P rats

The administration of 8-OH-DPAT at 11.00 h in OVX-E_2-P rats significantly decreased hypothalamic levels of LHRH 30 min later (1.85 ± 0.06 compared to 2.45 ± 0.12 ng/hypothalamus).

Discussion

The present results show that a single injection of 8-OH-DPAT in the morning of the expected LH surge induces a phase advance in the phasic surge of LH and an increase in its amplitude in OVX steroid primed rats. A pretreatment of OVX-E_2 animals with P is required to observe these 5-HT_{1A} receptor agonist effects.

The involvement of 5-HT in LH release has long been investigated with several techniques but contradictory results

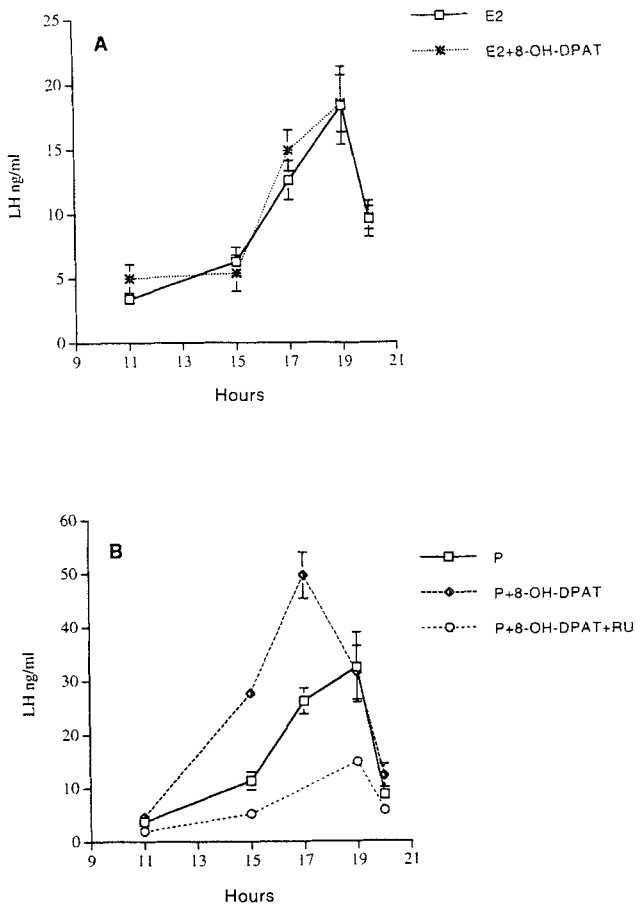

Figure 1 (**A**) Effect of 8-OH-DPAT on phasic LH release in ovariectomized estradiol (E_2) treated rats. 8-OH-DPAT was injected at 11.00 h. (**B**) Effect of 8-OH DPAT or 8-OH-DPAT + RU 38486 (RU) in ovariectomized estradiol, progesterone (P) treated rats. P or P + RU 38486 were injected at 9.00 h, 8-OH-DPAT was injected at 11.00 h. Each point represents the mean ± SEM of 8 to 15 rats (corresponding to 2 or 3 experiments)

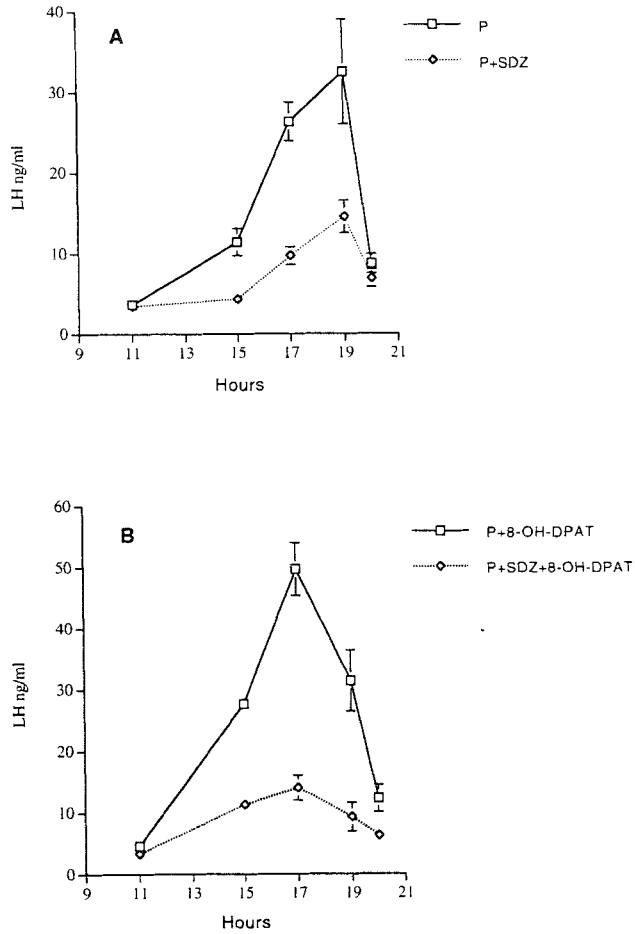

Figure 2 (**A**) Effect of SDZ 216-525 on phasic LH release in ovariectomized estradiol progesterone (P) treated rats. P and SDZ 216-525 were injected at 9.00 h and 10.00 h respectively. (**B**) Effect of SDZ 216-525 on 8-OH-DPAT effect in ovariectomized estradiol progesterone (P) treated rats. SDZ 216-525 and 8-OH-DPAT were injected at 10.00 h and 11.00 h respectively. Each point represents the mean ± SEM of 8 to 15 rats (corresponding to 2 or 3 experiments)

were obtained (Vitale & Chiocchio, 1993). These discrepancies could be explained by the differential effects of 5-HT on specific receptor subtypes involved in LHRH and LH release mechanisms and by differences in the hormonal status of the animals used in each study. Nevertheless there is general consensus about the fact that pharmacological manipulations of the serotonergic transmission indicate a facilitatory role of 5-HT on preovulatory LH surge, although the 5-HT receptor subtypes involved in this regulation have not been established yet.

An inhibition of the preovulatory LH surge by ketanserin or ritanserin was recently reported and showed the involvement of 5-HT_2 receptors in the 5-HT-induced stimulation of phasic LH release (Tanaka *et al.*, 1993; Dow *et al.*, 1994). Nevertheless Dow *et al.* (1994) emphasized the fact that these drugs inhibit both phasic and basal LH release, and that 5-HT_2 receptor antagonists also block α1 adrenoreceptors antagonist properties. To our knowledge, the effects of 5-HT_{1A} receptor agonists on the regulation of LH release have been only reported in studies analysing the short term effect of 8-OH-DPAT (Johnson & Sanders, 1987; Aguilar *et al.*, 1993). These studies reported an increase in LH secretion 15 to 30 min after the systemic injection of 8-OH-DPAT in prepubertal female rats or in OVX-E_2 primed rats, whereas LH release is decreased in OVX rats.

The positive effect of 8-OH-DPAT on phasic triggering of LH release, reported here, in OVX-E_2-P rats, appears to be specifically mediated by 5-HT_{1A} receptors activation since the effect of 8-OH-DPAT was inhibited by the specific 5-HT_{1A} antagonist SDZ 216-525 (Schoeffter *et al.*, 1993). It is well admitted that 5-HT_{1A} receptors may function as auto (Sotelo *et al.*, 1990) as well as heteroreceptors (Verge *et al.*, 1986; Frankfurt *et al.*, 1993) being located in both cases on neuronal cell bodies and dendrites. The present effect of 8-OH-DPAT is unlikely to be induced by activation of 5-HT_{1A} somatodendritic autoreceptors since similar effects were observed by others using non specific 5-HT agonists whose affinities for autoreceptors are very low (Chen *et al.*, 1981; Lenahan *et al.*, 1986).

We recently reported a stimulatory effect of 8-OH-DPAT on LHRH release from fetal hypothalamic cells (Héry *et al.*, 1995). It is therefore reasonable to hypothesize that the decrease in hypothalamic LHRH content observed 30 min following 8-OH-DPAT administration is associated with an increased release of the neurohormone, and that the increase in LH release could result at least partially from an action of 8-OH-DPAT in the hypothalamus.

Under our experimental conditions the presence of P was required in order to observe the stimulatory effect of 8-OH-DPAT treatment on LH release in OVX-E_2 rats. Indeed, 8-OH-DPAT treatment alone had no effect in OVX-E_2 rats and we showed that the 8-OH-DPAT induced stimulation of LH release in OVX-E_2-P animals was inhibited by a pretreatment with the potent antiprogestin RU 38486. This is not the

only physiological response whose modulation requires a combined action of P and 5-HT_{1A} receptors agaonists. P modulates the ability of various 5-HT_{1A} agonists to inhibit lordosis (Mendelson & Gorzalka, 1986) and we reported a potentiation of the stimulatory effect of 8-OH-DPAT on LHRH release by P in hypothalamic cells in culture (Héry *et al.*, 1995).

Many studies suggest that 5-HT mediates the actions of progesterone on LH surge in estrogen primed rats. Walker & Wilson (1983) reported that P stimulates 5-HT synthesis whereas an antiprogestin decreases the levels of 5-hydroxyindolacetic acid, the main 5-HT metabolite, and that such variations of the serotoninergic activity are correlated with phasic LH release. Combined administration of the serotonin precursor, 5-hydroxytryptophan and P in the morning significantly potentiated the increase in LH release induced by P (Franks *et al.*, 1980), whereas destruction of 5-HT nerve terminals in the medial preoptic stria terminalis (Johnson & Crowley, 1986) or administration of non-specific serotonin receptor antagonists (Franks *et al.*, 1980; Iyengar & Rabii, 1983) blocked or at least reduced the increase in LH surge induced by P. The inhibitory effect of SDZ 216-525 on P-induced increase in LH release observed here is consistent with these previous works, moreover our results show that the facilitatory effect of 5-HT on the P-induced increase in LH surge involves the 5-HT_{1A} receptors.

However, the mechanism involved in P interaction with 5-HT_{1A} receptors is unknown. The binding of P to membranes of hypothalamic neurones induces an increase in LHRH release (Ke & Ramirez, 1987; Héry *et al.*, 1995). Considering that LHRH cell bodies are contacted synaptically by 5-HT nerve terminals (Kiss & Halasz, 1985) the interaction between P and 5-HT_{1A} receptors activity could take place at the level of LHRH neurons. However, Wright & Jennes (1993) failed to detect any expression of 5-HT_{1A} receptor mRNA in LHRH neurons of OVX-E_2 rats. In light of the present results showing that the presence of P is required in order to detect the effect of 5-HT_{1A} receptor activation it is possible that a P treatment would unmask the expression of 5-HT_{1A} mRNA in LHRH neurons. Nevertheless, the existence of indirect neuronal pathways involved in the control of LHRH release by the combined action of P and 5-HT_{1A} agonists cannot be ruled out.

Administration of 8-OH-DPAT, induced a phase advance in the surge of LH, in OVX-E_2-P rats. Similar magnitude phase advances in drinking and wheel running patterns were reported following the administration of a 5-HT_{1A} receptor agonist at the same time in the morning (Edgar *et al.*, 1993). Pharmacological manipulations of the circadian rhythm control system made it clear that the effect could be mediated both directly at the level of the suprachiasmatic nucleus (SCN) and indirectly via afferent projections arising from other brain regions (Edgar *et al.*, 1993). The SCN which constitute a central pacemaker of the circadian timing receives an important serotoninergic innervation involved in phasic LH release (Héry *et al.*, 1978, 1982). Considering that serotoninergic agonists, including 8-OH-DPAT can phase shift the SCN pacemaker *in vitro* (Prosser *et al.*, 1990), the SCN might be involved in the 8-OH-DPAT-induced phase advance in LH surge. However, 5-HT_{1A} receptors activation also increase wakefulness (Lerman *et al.*, 1986; Edgar *et al.*, 1990) and a contribution of 5-HT-induced behavioural changes in the observed phase shift cannot be ruled out. The timing of LH surge was not altered in OVX-E_2 rats treated with 8-OH-DPAT, suggesting that 5-HT_{1A} receptors were involved in association with P in the phase shift of LH surge. If we assume that the SCN is involved in the phase shift effect of 8-OH-DPAT, the detection of neurons containing progesterone receptor mRNAs within the suprachiasmatic portion of the preoptic area (Lauber *et al.*, 1991) has to be considered.

It is important to notice that the 5-H_{1A} receptors antagonist SDZ 216-525 failed to inhibit the 8-OH-DPAT-induced phase shift in LH surge, suggesting that other 5-HT receptor subtypes with high affinity for 8-OH-DPAT, could be involved in this effect. The 5-HT_7-receptor type, which was recently cloned, and was shown to induce a phase advance in spontaneous activity of the SCN neurons (Lovenberg *et al.*, 1993) is a good candidate for the mediation of 8-OH-DPAT-induced phase advance in LH surge.

The present data shows that progesterone is required in order to observe the regulation of phasic LH release induced by 5-HT_{1A} receptor activation and that under this steroidal condition, the activation of 5-HT_{1A} receptors induces a phase advance and an increase in LH surge.

Materials and methods

Experimental model

Adult female Sprague Dawley rats (180–200 g) were housed in a temperature and light controlled environment (21 ± 1°C, light on 05.00–19.00 h). Food and water were supplied ad libitum. Two weeks after bilateral ovariectomy, the animals were implanted with 17 β estradiol (E_2) contained in silastic capsule inserted under the skin. Six days later at 09.00 h the animals were injected subcutaneously with either 5 mg of progesterone (P) dissolved in 0.5 ml of sesame oil (OVX-E_2-P group), or P (5 mg) together with RU 38486 (5 mg), or vehicle alone (OVX-E_2 group). Two hours later, at 11.00 h animals from each group were injected intraperitoneally (ip) with either 8-OH-DPAT (0.5 mg/kg) or saline. Some animals of the OVX-E_2-P group were injected ip with SDZ 216-525 (Methyl-4(4-[4-(1,1,3-trioxo-2H-1,2-benziosothiazol-2-yl) buthyl]-1piperazinyl)1H-indole-2-carboxylate) (1 mg/kg) one hour before 8-OH-DPAT or saline. Blood samples were collected from each animal by jugular puncture under light ether anesthesia at different times between 11.00 h and 20.00 h. Blood samples collected in heparinized tubes were centrifugated and plasma samples were frozen until LH assay.

In order to measure hypothalamic LHRH levels, some animals of the OVX-E_2-P group were sacrificed by decapitation 30 min after 8-OH-DPAT ($n = 10$) or saline injection ($n = 10$). Immediately after dissection, hypothalami were homogenized in 0.1 N HCl and stored at 4°C for 2 h. After centrifugation (4000 g–4°C) the supernatants were neutralized, frozen and stored at −20°C until LHRH assay.

Hormone assay and statistical analysis

Serum levels of LH were measured in duplicate by double antibody radioimmunoassay and expressed as ng/ml of NIAMDD rat LH-RP 2. Anti rat LH serum CSU 120 was provided by Dr G.D. Niswender (Colorado State University). The sensitivity of the assay was 25 pg. Intra and interassay variabilities were below 10%. All samples from one experiment were analysed in the same assay.

LHRH was measured using radioimmunoassay procedure (Hartter & Ramirez, 1980). LHRH antiserum no. B73 was kindly provided by Dr V.D. Ramirez, Intra and interassay variabilities were 6.2 and 8.3%, respectively.

Data were expressed as means ± SEM and the significance of differences among means was analysed by two or one way analysis of variance (ANOVA) followed by Scheffe test. Control groups of different experiments were combined after checking with one way analysis of variance that there was no significant difference between them.

Drugs

8-OH-DPAT was obtained from RBI (Natick, Mass., USA) and SDZ 216-525 generously from Sandoz. RU 38486 was purchased from Roussel Uclaf.

Acknowledgements

The authors wish to thank Dr P. Boulenguez for his helpful advice during the preparation of the manuscript and Mrs Loutrein for the typing.

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