

## ROLE OF 5-HT<sub>7</sub> RECEPTORS ON LORDOSIS BEHAVIOUR AND LH RELEASE IN THE FEMALE RATS

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**Background:** The present study was designed to investigate the role of the 5-HT<sub>7</sub> receptors in lordosis and release of LH and compare the lordotic responses with 5-HT<sub>1A</sub> agent. **Methods:** Ovariectomised but oestradiol benzoate (OB) (10 ug) for 48 h plus by progesterone (0.5 mg)-primed receptive rats were used for the study. Thirty min. prior to progesterone, 5-HT<sub>1A</sub> and 5-HT<sub>7</sub> agonists were administered intra-peritoneally (i.p.). Lordotic quotient and release of LH were measured. Agonistic effect was then antagonized by respective antagonists. Effects on the above parameters were noted and correlated for possible interplay. **Results:** 5-HT<sub>7</sub> agonist mimicked inhibitory effect of 8-OH DPAT on lordosis in receptive rats, however, the response was generally attenuated. Treatment by 5-HT<sub>1A</sub> antagonist, WAY 100135 causing a protective effect was evident transiently. Attenuation of lordotic quotient was again evident in rats treated with 5-HT<sub>7</sub> antagonist. 5-HT and the 5-HT<sub>1A/7</sub> receptor agonist, 8-OH-DPAT, injected i.p. into the female rat inhibit the LH release and the effects of both are blocked by 5-HT<sub>1A</sub> antagonist, WAY 100135 and 5-HT<sub>7</sub> antagonist, SB 269970-A as both 5-HT<sub>1A</sub> agonist, 8-OH-DPAT and 5-HT<sub>7</sub> agonist, 5-CT have moderate activity at the 5-HT<sub>7</sub> receptor subtype, indicating the possibility that this subtype might mediate these effects has been investigated. Ovariectomised but steroids primed rats induces an LH surge. (5-CT), a potent but non-selective agonist at 5-HT<sub>7</sub> receptors, like 5-HT and 8-OH-DPAT inhibited the LH surge at 2 mg injected i.p. The selective 5-HT<sub>7</sub> receptor antagonist, SB-269970-A blocked LH surge when given systemically at both 5-HT<sub>1A</sub> and 5-HT<sub>7</sub> receptor subtypes. **Conclusion:** These data indicate that 5-HT<sub>7</sub> receptors play a role in the regulation of lordosis and release of LH and there exist a direct correlation between the two.

**Key words:** 5-HT, 5-HT<sub>1A</sub> receptors, 5-HT<sub>7</sub> receptors, lordosis, Lordotic quotient, LH

### INTRODUCTION

Highly oestrous female rats show lordosis and soliciting behaviour with male mounting. These characteristics sexual behaviours are modulated by oestrogen-dependant serotonergic system in the hypothalamus. Both facilitatory and inhibitory influences are exerted via ventromedial hypothalamic nuclei (VMN) and preoptic area (POA), respectively.<sup>1,2</sup> These facilitatory and inhibitory mechanisms in the forebrain are modified by the limbic system and the lower brainstem.

At least three 5HT receptor families are known to influence lordosis by modulating the oestrogen sensitive receptive field in some sensory neurones of the VMN in the hypothalamus.<sup>3</sup> Activation of 5-HT<sub>1A</sub> receptors within VMN inhibits lordosis.<sup>4,5</sup> In sexually receptive rats a 5-HT<sub>2A/2C</sub> receptors antagonist also inhibits lordosis<sup>6</sup> whereas 5-HT<sub>2A/2C</sub> receptors agonist facilitates the behavior in non-sexually receptive females.<sup>7</sup>

An LH surge is also known to have been preceded beside enhanced lordotic response at oestrous. However, 5-HT<sub>2A/2C</sub> mediated facilitatory effect on lordosis had been reported to have no effect on LH release, but the 5HT<sub>2</sub> receptor antagonist, ritanserin, antagonized the inhibitory effect of both 5-HT and 5-HT<sub>1A</sub> agonist, 8-OH DPAT on LH release.<sup>8</sup> Conflicting reports thus indicate possibility of more

careful considerations of other possibilities including involvement of 5-HT receptors subtype other than 5-HT<sub>1A</sub> receptors in mediating the inhibitory effect of 5-HT. Nonetheless, the presence of 5-HT<sub>7</sub> receptors in the hypothalamus and their potential involvement in circadian rhythm<sup>9</sup> makes them a likely candidate for regulation of steroid hormone release and lordosis behavior. An appropriate steroidal milieu has been reported as a pre-requisite for a role in 5-HT-dependent modulation of lordosis. Although, lordotic response requires steroids priming, the response may vary depending not only on the dose regimen of steroids but also the serotonergic transmitter system involved.

Involvement of 5-HT<sub>7</sub> receptor subtype with moderate affinity to 8-OH DPAT, a 5HT<sub>1A</sub> agonist which also has 5-HT<sub>1A/7</sub> affinity, requires attention to investigate the role of 5HT<sub>7</sub> receptors in lordosis and control of LH release in female rats. Moreover, any correlation, if existing between 5-HT<sub>7</sub> mediated lordotic response and LH release also needed to be determined.

### MATERIAL AND METHODS

The serotonergic receptor agonists were 5-hydroxytryptamine creatinine sulphate (5-HT); Oestradiol benzoate; progesterone; Sigma Chemicals Ltd., Poole, Dorset, UK, (+)-8-hydroxy-2-(di-n-propylamino) tetralin. HBr (8-OH DPAT; Research

Biochemicals International (RBI) Ltd., Natwick, Mass, USA, and 5-carboxamidotryptamine maleate (5-CT); RBI Ltd., Natwick, Mass, USA. The serotonergic receptor antagonists were (R)-3-(2-(2-(4-methyl-piperidin-1-yl)-pyrrolidine-1-sulfonyl)-phenol(SB-269970-A)); Glaxo Smith Kline Beecham Pharmaceuticals, Harlow, Essex, n-tert-butyl-3-(4-methoxyphenyl) piperazin-1-yl (WAY 100135); Wyeth Research Centre, Taplow, Bucks., UK, and ritanserin (6-[2-[4-[bis-(4-fluorophenyl) methylene]-1-piperidinyl]-ethyl]-7-methyl-5H-thiazole[3,2-1]pyrimidin-5-one; RBI Ltd., Natwick, Mass, USA.

### **Animals, housing conditions and surgical procedures**

Female Wistar rats from litters born and raised in the animal housing facility at Aga Khan University were weaned at 25 days of age. The rats were housed in polycarbonate shoebox cages, four to five like-sex littermates per cage. The colony room was maintained at about 24°C on a 12 h light-dark cycle with lights off at 6:00 p.m. Rat chow and water were available ad lib.

Female rats of 75-80 days of age, (160-200g) were anaesthetized with pentobarbitone, and then bilaterally ovariectomized via bilateral lumbar incision. Immediately post-surgery, these females were housed exactly in a way, described above.

### **Steroids -priming**

Following recovery, groups of rats (n=120-140) were primed with 10µg OB subcutaneously (s.c.) 50 h before the experiment. Administration of OB alone exerts a negative feedback effect on LH release and induces relatively low levels of plasma LH. 48 h after the OB and 2 h before the experiment, this treatment stimulates LH release with a peak concentration about 2-3.5 h after P (0.5 mg, s.c.), associated with enhanced sexual receptivity. Steroids were dissolved in ethanol and raised in 0.1 ml corn oil as solvent vehicle. Two hours after P, lordosis was monitored 15 min. prior to testing against 5-HT agents. Female rats belonging to treatment regimen of OB followed by P, showing an LQ of >55 % were regarded as receptive rats.

### **Experimental Procedures**

Receptive rats (n=110) identified in this fashion were subdivided into 8 groups in the two categories and were then injected with 5-HT<sub>1A</sub> and 5-HT<sub>1A/7</sub> receptor agonist, 8-OH DPAT, (0.5 and 2.0 mg/kg), 5-HT<sub>1A</sub> antagonist, WAY 100135 (0.5 and 2.0 mg/kg), a 5-HT<sub>7</sub> agonist, 5CT (0.5 and 2.0 mg/kg), a 5-HT<sub>7</sub> antagonist, SB 269970-A (0.5 and 2.0 mg/kg), or saline (1 ml/kg) (n=6-12 in each group) to assess lordosis modulating effects of 5-HT agents. All

treatments and observations after that were carried out 3 times with a gap of 8-10 days.

After the OB injection (2h after P administration), rats were injected intraperitoneally (i.p) with 8-OH DPAT, a 5-HT<sub>1A</sub> agonist and tested for lordosis against sexually active male rats. Rats were then anaesthetized with pentobarbitone (2ml / kg) and blood sample (0.1 ml) was collected from the tail vein. During this period, the anaesthetized rats were placed on an electric pad in order to maintain body temperature (at 35.5 ± 0.5°C) and induce a degree of vasodilatation. The blood samples were centrifuged at 2000 rpm for 10 min and the plasma stored at -20°C until assayed for LH.

### **Behavioural testing procedures**

Behavioral testing involved presentation of a test female to a sexually active male rat in a cylindrical pyrex arena measuring 50cm in height and 30cm in diameter. Detailed procedure of lordosis testing is reported elsewhere.<sup>10</sup> Each experimental female remained with a single male for 10 min. and observed for pelvic thrusting as an index of lordosis. If a male would not mount, the female was placed in a different arena containing another male. A female's response to a mount was considered a lordosis response if some degree of concavity of the back was observed.

### **Radioimmunoassay for LH**

Ten microlitre plasma samples were assayed in duplicate employing reagents kindly supplied by the national hormone and pituitary programme (Baltimore, MD, USA). The standard was NIADDK-rLH-RP3 and the antibody, NIADDK-anti-rLHS10. The inter and intra-assay coefficient of variations were 7.7% and 9.0% respectively and the sensitivity was 10 pg/tube (1ug/ml).

### **Statistical analyses**

Results of tests with the different dose regimens were quantified as LQ by calculating the number of lordotic responses (pelvic thrusting by the female) divided by the number of mounts by the male, an LQ of >55% was regarded as an index of good sexual receptivity by the female. Student's 't' test was used for two groups comparisons.

## **RESULTS**

### **I. The effect of serotonergic agents on lordosis behaviour and LH release in sexually receptive rats primed with OB plus P**

5-HT, 8-OH DPAT and 5-CT as serotonergic agonists were injected systemically (i.p.) to ovariectomized rats primed with 10 ug OB followed

at 48 h by 0.5 mg P. The receptor agonists were given 2 h after the P (2.0, 0.5 mg/kg, 5-HT; 2.0 and 0.5 mg/kg, 8-OH DPAT; 2.0 and 0.5 mg/kg, 5-CT). The mean LQ in sexually receptive rats given the prescribed steroids priming regimen, following 5-HT treatment was inhibited significantly ( $p < 0.01$ ) relative to their pre-treatment LQ observed in the first test (LQ 27% vs 46%) (Fig.1A). In the subsequent tests, LQ percentage in the treated and control group remained unchanged (Fig. 1A).

Since the actual peak of the LH surge induced by P is variable, the results in Table 1 have been presented as the difference between the basal plasma LH levels measured just before the injection of the compounds and the highest concentration noted in the 3 h interval after the injection. Thus the rise in LH in the saline controls was  $9.0 \pm 1.0$  ng/ml and the receptor agonist, 5-HT, at 2.0 mg significantly reduced this increase ( $p < 0.01$ ). The lower dose of 0.5 mg, 5-HT no longer had a significant effect.

**II. Effects of 5 HT<sub>1A</sub> agents on lordosis behaviour and LH release in sexually receptive rats primed with OB plus P**

In sexually receptive rats given the standard steroids priming regimen, 8-OH DPAT treatment significantly inhibited ( $p < 0.01$ ) the mean LQ relative to their pre-treatment LQ when observed in the first test (LQ 36% vs 60%) (Fig.1B). Magnitude of inhibition in the second test, however, noted a considerable attenuation following 8-OH DPAT treatment (from 36% to 47%).

5 HT<sub>1A</sub> antagonist, WAY 100135 had a clearly protective effect on the mean LQ in sexually receptive rats primed with OB plus P. When compared to control, an increase of up to 62% was observed in WAY 100135 treated rats during all three tests (Fig. 2A).

LH concentration again noted a decrease in the difference in increase, following treatment with

8-OH DPAT given at the dose of 2 mg (Table 1). At a lower treatment dose of 0.5 mg/kg, decrease in the difference between pre- and post 8-OH DPAT treatment was further reduced but a reduction in release of LH was evident (see Table 1).

Treatment with 5-HT<sub>1A</sub> antagonist, WAY 100135 at 2.0 mg dose following 8-OH DPAT treatment caused a significant attenuating effect on LH release leading to a decrease in the difference of up to 6.4 vs 8.3 ng/ml in the saline treated control group (Table 2). However at 0.5 mg dose, difference with the pre-treated level was insignificant (7.7 vs 8.3 ng/ml).

**III. Effects of 5 HT<sub>7</sub> agents on lordosis behaviour and LH release in sexually receptive rats primed with OB plus P**

The mean LQ in sexually receptive rats with OB plus P-priming regimen, following 5-CT, a 5 HT<sub>7</sub> agonist treatment is given in Fig. 1C. Sexually receptive rats responded to 5-CT treatment by reducing of the mean LQ in the first test, from 61% to 44% (Fig. 1C). Decline in second and third test was not discernable when compared to controls (54% vs 57%) and a mild attenuating effect was evident though animals were treated with a 5HT agonist.

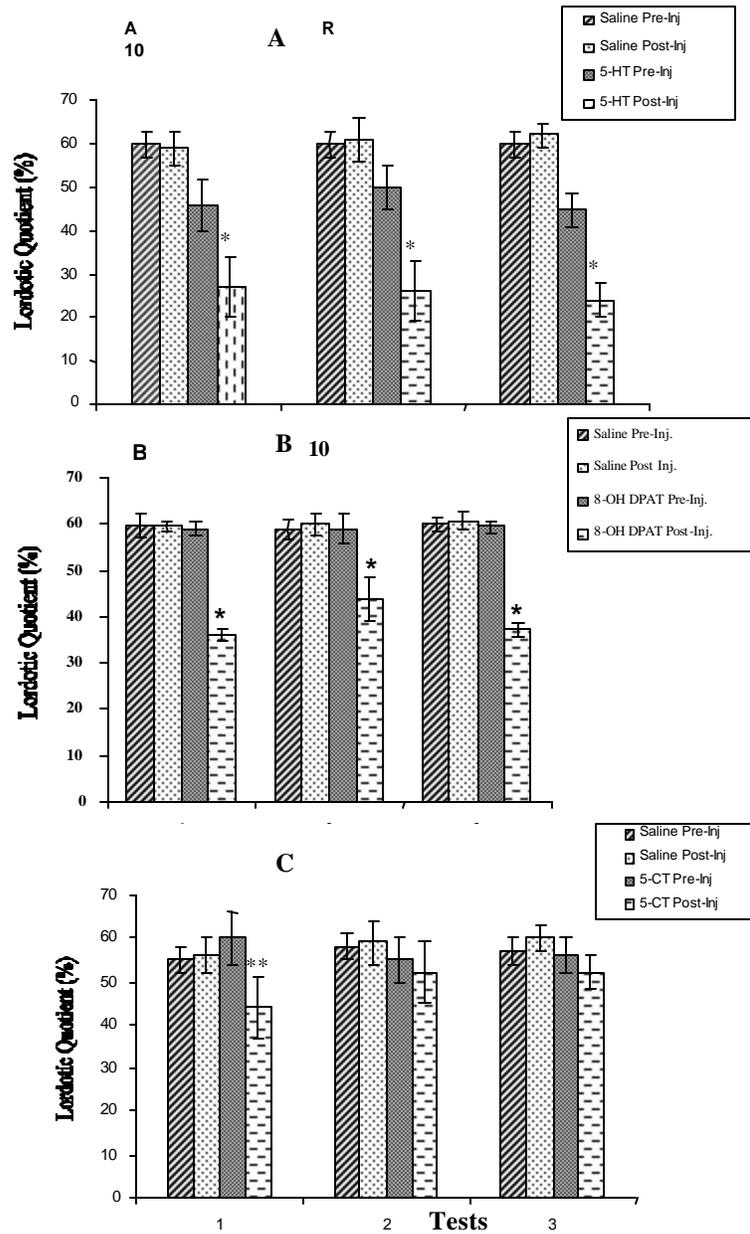
Treatment with 5-HT<sub>7</sub> antagonist, SB 269970-A, sexually receptive rats cause a rise in LQ from pre-treated value of 58% to 66% in second and third test (Fig. 2B).

Table 2 shows that systemic administration of SB-269970-A attenuated the inhibitory effect on LH release exerted by 8-OH DPAT. The treatment was given to groups of rats over a dose range of 0.5 to 2.0 mg/kg, 30 min. prior to the 8-OH DPAT administration. The lowest dose of 0.5 mg/kg significantly antagonised the effect of 8-OH DPAT more significantly ( $p < 0.01$ ), while the dose of 2.0 mg/kg was less effective.

**Table-1: The inhibitory effect of serotonergic receptor agonists on the release of LH**

Treatment (in 0.5 ml saline)	Plasma LH ng/ml $\pm$ S.E.M.				
	Dose	No. rats per group	Basal pre-inj conc	Peak post-inj conc	Difference
Saline	-	11	3.5 $\pm$ 0.4	12.5 $\pm$ 0.5	9.0 $\pm$ 1.0
5-HT	2.0 mg/kg	9	3.0 $\pm$ 0.4	7.1 $\pm$ 0.8	4.1 $\pm$ 0.7 b
„	0.5 mg/kg	6	3.6 $\pm$ 0.6	11.1 $\pm$ 1.6	7.0 $\pm$ 1.0
8-OH-DPAT	2.0 mg/kg	5	3.1 $\pm$ 0.8	7.9 $\pm$ 0.5	4.8 $\pm$ 0.4 b
„	0.5 mg/kg	6	2.0 $\pm$ 0.5	5.1 $\pm$ 0.8	3.1 $\pm$ 0.5 c
5-CT	2.0 mg/kg	8	2.0 $\pm$ 1.1	7.1 $\pm$ 1.0	5.1 $\pm$ 0.6 b
„	0.5 mg/kg	6	2.4 $\pm$ 0.3	7.0 $\pm$ 0.8	4.6 $\pm$ 0.8 b

**Legend for Table 1:** The inhibitory effect on LH release of 5-HT, 8-OH-DPAT and 5-CT at the given doses administered intraperitoneally(i.p.) to ovariectomised rats primed with 10  $\mu$ g/rat oestradiol benzoate followed at 48 h by 0.5 mg progesterone (P). 5-HT agents were administered i.p. after the P and blood samples were collected at 30 min. intervals for 3 h after the P injection. ANOVA:  $F(6,62) = 4.45, P = 0.0008. b = p < 0.05, c = p < 0.01$  compared to saline controls



**Fig. 1:** Effect of 5-HT (A), 8-OH DPAT a 5HT<sub>1A</sub> agonist (B) and 5-CT, a 5HT agonist (D) (n=6-10) on lordosis behaviour in receptive rats primed with 10 ug OB plus P for three consecutive tests. Data is shown as LQ with \* as p <0.01 and \*\* as p<0.05 as level of significance.

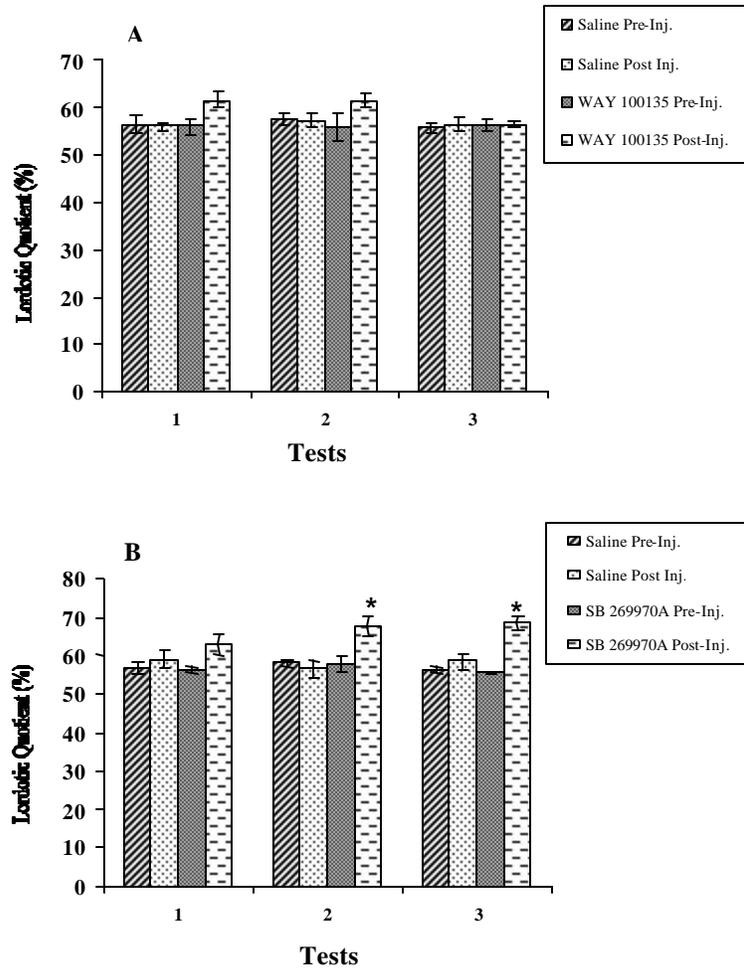


Fig. 2: Effect of WAY 100135 (A), a 5HT<sub>1A</sub> antagonist, and SB 269970A a 5HT<sub>7</sub> antagonist (B) (n=7-10) on lordosis behaviour in receptive rats primed with 10 ug OB plus P for three consecutive tests. Data is shown as LQ with \* as p < 0.01 as level of significance.

Table-2: The antagonistic effect of WAY 100135 and SB 269970-A on the inhibitory actions of serotonergic receptor agonists 5-HT, 8-OH DPAT and 5-CT on LH release

Treatment (in 0.5 ml saline)	Plasma LH ng/ml ± S.E.M.				
	Dose of antagonist (mg)	No. rats per group	Basal pre-inj. Conc	Peak post-inj. conc	Difference
Saline	-	10	4.5 ± 0.6	12.8 ± 1.4	8.3 ± 1.1
Saline + WAY 100135	2.0	11	4.9 ± 0.6	10.1 ± 1.1	5.2 ± 0.6
Saline + WAY 100135	0.5	8	3.7 ± 0.8	7.4 ± 0.5	3.7 ± 0.8
8-OH DPAT + WAY 100135	2.0	11	4.3 ± 0.9	11.7 ± 1.7	6.4 ± 1.6 b
8-OH DPAT + WAY 100135	0.5	9	4.8 ± 0.5	12.5 ± 1.8	7.0 ± 1.0
8-OH DPAT + SB 269970-A	2.0	9	3.7 ± 0.6	7.6 ± 0.9	3.9 ± 0.6 a
8-OH DPAT + SB 269970-A	0.5	12	4.1 ± 0.6	10.2 ± 1.2	6.1 ± 0.8 b
Saline + SB 269970-A	0.5	7	3.5 ± 0.5	11.1 ± 1.1	7.6 ± 0.4 b
Saline + SB 269970-A	2.0	5	2.5 ± 0.6	9.2 ± 1.1	6.7 ± 1.0 a
5-CT + WAY 100135	0.5	6	3.8 ± 0.6	6.9 ± 1.0	3.1 ± 0.7
5-CT + WAY 100135	2.0	8	4.9 ± 0.6	9.1 ± 0.8	4.2 ± 0.9
5-CT + SB 269970-A	2.0	7	3.8 ± 0.9	6.9 ± 2.2	3.1 ± 1.4
5-CT + SB 269970-A	0.5	6	6.9 ± 2.1	13.2 ± 2.9	6.2 ± 0.9 a

Legend for Table 2: The antagonistic effect of WAY 100135 and SB-269970-A on the inhibitory actions of 2.0 mg 5-HT, 8-OH DPAT and 5-CT given i.p. on LH release in ovariectomized rats primed with 10 µg oestradiol benzoate and 0.5 mg progesterone. ANOVA: 5-HT, F (5, 42) = 3.5, P=0.0099; 8-OH-DPAT, F (4, 30) = 9.75; P<0.0001; 5-CT, F (4, 33) = 3.31, P = 0.022. a = p < 0.01, b = p < 0.05 compared to saline controls

Treatment with SB 269970-A to rats pre-treated with 5-HT<sub>7</sub> agonist, 5-CT showed a protective effect on LH release at dose regimen of 0.5 mg whereas at the higher dose of 2.0 mg difference from the pre-treated values was insignificant.

The overall effect of 5-HT antagonists used show that SB-269970-A had an almost identical antagonizing effect on the action of 8-OH DPAT and 5-CT in that it significantly prevented their inhibition of LH release at higher dose used, as noted following SB 269970-A, 5-HT<sub>7</sub> antagonist and at lower dose of WAY 100135, a 5-HT<sub>1A</sub> antagonist on LH release in the OB plus P primed model (Table 2).

## **DISCUSSION**

Several studies have suggested that activation of some selective 5-HT receptor subtypes may inhibit or activate lordosis behaviour.<sup>4,7,10,11</sup> A latest candidate amongst the various 5-HT receptor subtypes is the 5-HT<sub>7</sub> receptor subtype whose involvement in regulation of LH is attributed to its partial affinity to 5HT<sub>1A</sub> receptor subtypes. Present study therefore evaluates corresponding changes in lordosis along with LH release. Major objective of the present study therefore was first to evaluate the effects on lordotic response elicited via 5-HT<sub>1A</sub> receptor agonist 8-OH DPAT and antagonist WAY 100135 in sexually receptive rats and then to compare these responses to 5-HT<sub>7</sub> agonist, 5-CT and SB 269970-A as 5-HT<sub>7</sub> antagonist. The animal models chosen for this study were female rats just at the margin of receptive behaviour as these might be more vulnerable to events that disrupt the behaviour.

In agreement with earlier studies,<sup>4,12</sup> the 5HT<sub>1A</sub> agonist, 8-OH DPAT, was a potent inhibitor of lordosis in receptive rats; the effect was described as more potent than that of buspirone.<sup>5,13</sup> Buspirone has been reported to act on 5HT<sub>1A</sub>, dopamine (DA) and noradrenaline (NA) receptors.<sup>14-16</sup> Amongst all these, DA receptors contribute to the stimulatory mechanism of lordosis, because direct activation of dopaminergic pathways in the CNS was found to enhance lordosis.<sup>17</sup> The noradrenergic system is also known to be involved in a lordosis facilitating mechanism<sup>18,19</sup> Therefore, stimulation of monoaminergic receptors other than those for 5-HT, may weaken the lordosis inhibiting effect of buspirone. 8OH DPAT acts on 5HT<sub>1A</sub> receptors specifically in the VMN.<sup>5</sup> Thus, 8OH DPAT and buspirone are thought to inhibit lordosis by binding to the hypothalamic 5-HT<sub>1A</sub> receptors in the female rat brain.

Activation of 5-HT<sub>1A</sub> receptors by 8-OH DPAT produces two major effects on 5-HT<sub>1A</sub> receptive neurons. Cells in the VMN, an important target area increases K<sup>+</sup> conductance as a result of G-

protein coupling between 5-HT<sub>1A</sub> receptors and K<sup>+</sup> channels.<sup>20,21</sup> Another mechanism involves a decrease in cAMP, probably resulting from 8-OH DPAT's activation of the Gi-coupled 5-HT<sub>1A</sub> receptor.<sup>22</sup> It is not firmly established yet whether 8-OH DPAT decreases lordosis by increasing K<sup>+</sup> conductance or by decreasing cAMP, but either event cause the effect.

Treatment with WAY 100135, a known selective 5-HT<sub>1A</sub> antagonist, quite convincingly demonstrates prevention of lordosis inhibitory effect. Attenuation in the response was evident in second test in 10 ug OB-primed rats and not seen in test 1 and 3. In agreement with these findings, Uphouse et al<sup>6</sup> have already indicated that a change in functional activation of 5-HT<sub>1A</sub> receptors may accompany neural responses to estrogen that are essential for priming of the lordosis reflex. It may be that, endogenous activation of 5-HT<sub>1A</sub> receptors should be relatively high in the sub-optimally hormone-primed ovariectomized rat with relatively low sexual receptivity.

This finding shows that WAY 100 135, a 5-HT<sub>1A</sub> antagonist has potency to attenuate inhibitory influence of 8-OH DPAT by enhancing lordosis behavior acutely in female rats with a low estrous state. Treatment with 5-CT and SB 269970-A as 5-HT<sub>7</sub> agonist and antagonist respectively have mimicked 5-HT-mediated lordotic response as moderate affinity towards 5-HT<sub>1A</sub> receptors has been reported. This offers a comparable effect on lordosis as a result of two 5-HT agents used.

Previous results obtained in this laboratory, indicate that serotonergic neurones in the dorsal hypothalamus exert an inhibitory effect on LH release and this is mediated via 5HT<sub>1A</sub> receptors, since the 5-HT<sub>1A</sub> receptor agonist, 8-OH-DPAT mimics the inhibitory effect of 5-HT and the effect of both receptor agonists is prevented by a selective 5-HT<sub>1A</sub> receptor antagonist, WAY 100135.<sup>8</sup> However, the same studies indicated that another receptor subtype may be involved in 5-HT-mediated inhibitory effect. The 5-HT<sub>7</sub> receptor was a candidate for this role because 5-HT<sub>7</sub> receptors are present in the dorsal hypothalamus and the inhibitory effect of 8OH-DPAT (as well as 5-HT) could be reversed by ritanserin.<sup>23</sup> Although 8-OH-DPAT has a high affinity and potency at the 5-HT<sub>1A</sub> receptor and ritanserin has a high affinity for the 5-HT<sub>2</sub> receptor,<sup>24</sup> they also share the common property of moderate affinity for the 5-HT<sub>7</sub> receptor<sup>9,25</sup> and the experiments described in this report investigated the possibility that 5-HT<sub>7</sub> receptors might mediate serotonergic regulation of LH release.

Another approach to whether lordosis is modulated by a particular OB milieu when the effect

is mediated by 5-HT<sub>7</sub> receptor subtype an experiment was designed to demonstrate that 5-CT, a non-selective 5-HT<sub>7</sub> receptor agonist, inhibits lordosis in a manner similar to that of 8-OH DPAT. Moreover, if the effects are mimicked, is it because lordotic response is modulated when the 5HT agents are allowed to function in a certain specific OB milieu? Like 8-OH DPAT, 5-CT clearly mimicked behavioural inhibition in receptive female rats that were primed with 10 ug of OB in the first test of observation. The attenuating effect of the OB milieu was evident in tests 2 and 3, indicating that like 8-OH DPAT, few days of OB-priming at the dose used is a pre-requisite to develop the attenuation in non-receptive female rats where the effect is mediated via 5-HT<sub>7</sub> receptor subtype..

Earlier studies indicate that serotonergic neurons in the hypothalamus exert an inhibitory effect on LH release via 5-HT<sub>1A</sub> receptors, since 8-OH DPAT mimics the inhibitory effect of 5HT.<sup>8</sup> Similarly such influences on lordosis behavior were also mimicked by certain 5-HT agents.<sup>26</sup> Both of these effects are prevented by a selective 5HT<sub>1A</sub> receptor antagonist WAY 100135.<sup>6,8</sup> Distribution of 5-HT<sub>7</sub> receptors in the hypothalamus and amygdala<sup>27</sup> indicate the possibility that, 5-CT may have mimicked 5-HT's inhibitory effect possibly on neuronal firing in the brain areas involved in regulation of lordotic response.<sup>28</sup> This would be consistent with effects of 8-OH DPAT on 5-HT<sub>7</sub> receptors.

If exogenous 8-OH DPAT exerts an inhibitory effect on lordosis via agonist effect on 5HT<sub>1A</sub> receptors, it is possible that 5CT, a 5HT<sub>7</sub> receptor agonist, exerts a similar response on lordosis. This would be revealed by administering 5-HT antagonists to see if they remove the putative inhibition facilitate lordosis.

The approach was to show that the serotonergic receptor agonist, 5-CT which has highest affinity and potency for 5-HT<sub>7</sub> receptors, although it is still non-selective, had a similar inhibitory effect on LH release to that of 5-HT and 8-OH DPAT. In vitro functional and binding studies indicate that the rank order of 5-HT<sub>7</sub> potency of the three compounds is 5-CT > 5-HT > 8-OH-DPAT. However, this was not demonstrated in vivo, with 5-HT the natural ligand, being the least potent; perhaps because it is more rapidly degraded than the synthetic compounds. In addition none of the agents are selective and their additional effect on the 5-HT<sub>1A</sub> receptors may have affected the final outcome.

SB-269970-A is a highly potent 5-HT<sub>7</sub> receptor antagonist with selectivity of 100 fold or greater across a wide range of receptors and enzymes, except for the 5-HT<sub>5A</sub> receptor for which selectivity

is 50-fold. On rat cortical membranes it has a K<sub>D</sub> of 0.9 ± 0.1nM and B<sub>max</sub> of 30 ± 20.1 fmol/mg protein. When SB-269970-A was given systemically, it was more effective at 2 mg/kg i.p. It is difficult to explain the lack of effect of other dose used. It is possible that the effect on the 5-HT<sub>5</sub> receptor comes into play and masks the action on the 5HT<sub>7</sub> subtype when tissue concentration is high. Alternatively perhaps the 5-HT<sub>7</sub> receptor mediates the regulation of other neuroendocrine functions and at the higher doses SB-269970-A affects endogenous 5-HT activity on these other systems which then interact and/or mask its effect on LH release.

Previous findings showed that the selective 5-HT<sub>1A</sub> receptor antagonist WAY 100135 was also ineffective in this model indicating that neither 5HT<sub>1A</sub> nor 5-HT<sub>7</sub> receptors are involved in mediating any endogenous inhibitory control of LH release at this site and in these conditions. This is in contrast to the effect of naloxone (a. μ-opioid receptor antagonist) which induces a rise in plasma LH levels indicating it has disinhibited the LH system from a tonic inhibitory effect of endogenous β-endorphin.

In conclusion, it is suggested that in addition to the well-known 5-HT<sub>1A</sub> inhibitory effect on lordosis 5-HT<sub>7</sub> receptors mediate part of the response. These findings support the previous suggestion that as is the case for 5-HT<sub>1A</sub>,<sup>12</sup> 5-HT<sub>7</sub> receptor function plays an important role in the modulation of female sexual behavior. It can therefore be suggested that the inhibitory effect of 5-HT in the dorsal hypothalamus may be mediated, in part, by 5-HT<sub>7</sub> receptors. This is based on the pharmacological evidence that the inhibitory effects of 5-HT and two other serotonergic agents with affinity for the 5-HT<sub>7</sub> receptor (5-CT and 8-OH DPAT) are blocked by a highly selective 5-HT<sub>7</sub> receptor antagonist SB-269970-A. These data may be of significant value in indicating modulatory effect of 5HT receptors on fertility regulation with real possibilities of a potential therapeutic application.

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