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Role of presynaptic serotonergic receptors on the mechanism of action of 5-HT1A and 5-HT1B agonists on masculine sexual behaviour: physiological and pharmacological implications

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Summary. In order to establish whether the 5-HT1A or the 5HT1B agonists, 8-OH-DPAT or TFMPP, produce their facilitatory or inhibitory actions on masculine sexual behaviour via a mechanism involving: (a) the serotonin synthesis or release; (b) the stimulation of presynaptic receptors, or (c) the stimulation of somatodendritic receptors, three series of experiments were performed. The administration of the serotonin synthesis inhibitor, p-chlorophenylalanine (p-CPA, 300 mg/kg × 3 days), facilitated sexual behaviour but does not interfere neither with the inhibitory nor with the facilitatory effects of TFMPP (0.5 mg/kg) or 8-OH-DPAT (0.5 mg/kg), respectively. The icv or the intraraphé administration of the serotonergic neurotoxin, 5,7-dihydroxytryptamine (5,7-DHT), slightly stimulated masculine sexual behaviour and produced a decrease in serotonin and its metabolite levels. In lesioned animals TFMPP (0.5 mg/kg) resulted in an inhibitory effect reflected as a prolongation of the ejaculation latency. The inhibitory effect of this drug on mounting behaviour was not observed in 5,7-DHT treated rats. In lesioned animals 8-OH-DPAT (0.5 mg/kg) produced the same facilitatory effect. Present data indicate that serotonergic postsynaptic receptors mediate both the inhibitory and the facilitatory actions of TFMPP or 8-OH-DPAT in copulation. All data further support the idea that endogenous serotonin acts via the stimulation of 5-HT1B receptors to induce its inhibitory effects on masculine sexual behaviour.

Keywords: 5HT1A and 5HT1B agonists, male sexual behaviour, presynaptic serotonin receptors, p-CPA, 5,7-DHT.

Introduction

It is well known that the serotonergic system plays an inhibitory role in the neural control of masculine sexual behaviour (cf. Bitran and Hull, 1987; Larsson

and Ahlenius, 1986). Thus, an increase in the serotonergic transmission produced by the administration of the serotonin (5-HT) precursor, 5-hydroxytryptophan (5-HTP) or by the intrabrain administration of 5-HT, produces an inhibition of masculine sexual behaviour mainly reflected as a prolongation of the intromission and ejaculation latencies, and the postejaculatory interval (Malmnäs, 1973; Ahlenius and Larsson, 1987). Conversely, a decrease of the serotonergic activity provoked by the systemic injection of the serotonin synthesis inhibitor p-chlorophenylalanine (p-CPA) (Ahlenius et al., 1971; Salis and Dewsbury, 1971) or by electrolytic or neurotoxic lesions of the dorsal raphé nucleus produces a facilitation of masculine sexual behaviour (Larsson et al., 1978; McIntosh and Barfield, 1984).

Although the large body of evidence indicating an inhibitory role of this system on copulation, the receptor subtype on which serotonin acts to produce its inhibitory actions remains unclear. At present various 5-HT receptor subtypes have been established: 5-HT1 A, B, and C; 5-HT2 and 5-HT3 (cf. Bradley et al., 1986; Hoyer et al., 1985 a; Sills et al., 1984). As to the 5-HT1A subtype, it has been reported that the administration of various agonists to this site facilitates copulatory behaviour by drastically reducing the number of preejaculatory intromissions and by shortening the ejaculation latency (cf. Fernández-Guasti et al., 1990), suggesting that the inhibitory action of endogenous serotonin is not mediated via the stimulation of this receptor. Additionally, Mendelson and Gorzalka (1985) reported that the 5-HT2 receptor antagonists inhibit the copulatory behaviour leading to the conclusion that the stimulation of this receptor may also result in a facilitation of this behaviour and thus excluding the possibility that endogenous 5-HT acts through this subtype. On these bases, together with recent data showing that serotonin possesses high affinity for the 5-HT1B receptor subtype (Hoyer et al., 1985 a; Sills et al., 1984), we proposed that the 5-HT1B subtype could mediate the inhibitory actions of endogenous 5-HT on copulation. In support of this idea, we have shown that various 5-HT1B agonists produce clear inhibitory responses similar to those observed after increasing the endogenous serotonergic transmission (Fernández-Guasti et al., 1989).

Since the 5-HT1B agonists possess a high intrinsic activity, it could be argued that their actions are not the result of a direct postsynaptic stimulation but rather an effect mediated through the release of endogenous 5-HT. Therefore, the first experiment was performed to study this possibility. To that purpose the effect of the 5-HT1B agonist, TFMPP, was assessed in animals treated with the serotonin synthesis inhibitor p-CPA (Koe and Weissman, 1966).

It has been proposed that the presynaptic serotonin receptors primarily belong to the 5-HT1B subtype (Engel et al., 1983; Hoyer et al., 1985 b). Thus, another possible interpretation for the inhibitory action of 5-HT1B agonists on copulation could be based on the stimulatory effect of these drugs at a presynaptic site. To analyze this possibility the serotonergic terminals were lesioned by the intracerebroventricular administration of the selective serotonin neurotoxin, 5,7-dihydroxytryptamine (5,7-DHT).

Electrophysiological (Sprouse and Aghajanian, 1986), biochemical (Nishikawa and Scatton, 1986) and behavioural (Dourish et al., 1985; Hutson et al., 1987) data support the idea that the stimulation of the somatodendritic 5-HT 1 receptors produce a suppression of the firing of the raphé cells leading to a reduction in the 5-HT function in terminal regions. Although most of the somatodendritic serotonergic receptors belong to the 5-HT1A receptor subtype, some 5-HT1B receptors have been identified in this brain region (Verge et al., 1985; Weissman-Nanopoulos et al., 1985). Thus, in the third part of this study we analyzed whether these receptors could mediate the inhibitory actions of the 5-HT1B agonists on male sexual behaviour. The neuronal somas (including the somatodendritic receptors) were lesioned by injecting the neurotoxin, 5,7-DHT directly into the dorsal raphé nucleus. In both experiments involving 5,7-DHT lesions the proper neurochemical and histological controls were made.

In all experiments an extra group using the 5-HT1A agonist 8-OH-DPAT was included with the double purpose of comparing the effects of two different serotonergic agonists and to study the possible mechanisms underlying the paradoxical actions of 8-OH-DPAT on copulatory behaviour.

Materials and methods

General

Animals

Male Wistar rats (300–450 gr) were used in this experiment. The male rats were sexually trained with three sexual behaviour tests and only those males copulating in all tests and with an ejaculation latency (for definition *vide infra*) shorter than 15 min in the last test, were selected. All animals were maintained under controlled light-dark cycle (12 hr light/12 hr dark) conditions, and individually housed in a humidity and temperature regulated room. Animals had free access to water and food (Purina rat chow) all over the experiment. Female Wistar rats (injected with oestradiol valerianate, 5 μg/rat, −48 h and with progesterone, 1 mg/rat −4 h) were used as stimulus.

Drugs and steroid hormones

Steroids. Oestradiol valerianate (Schering Pharmaceuticals, Berlin) and progesterone (Sigma Chemical, St. Louis, USA) were sc injected in a volume of 0.1 ml of corn oil.
Drugs. TFMPP [(1-m-trifluoromethylphenyl)piperazine], 8-OH-DPAT [8-hydroxy-2-(di-n-propylamino)tetralin], p-CPA (p-chlorophenylalamine), 5,7-DHT (5,7-dihydroxytryptamine) and desimipramine. TFMPP and 8-OH-DPAT were purchased from Research Biochemicals, Natick, USA. p-CPA, 5,7-DHT and desimipramine were purchased from Sigma Chemicals, St. Louis, USA.

Sexual behaviour tests

The behavioural observations were done 3–5 hrs after the onset of darkness. Males were introduced in a cylindrical cage 5 min before the beginning of the experiment. A receptive female was settled with the male and the following parameters registered: intromission latency (IL), number of preejaculatory mounts (NM) and intromissions (NI), ejaculation latency (EL), and postejaculatory interval (PEI) (Larsson and Ahlenius, 1986). The IL,

EL, and PEI data are expressed in minutes. Tests were finished at the end of the PEI or if any of the following conditions occurred: IL longer than 15 min or EL longer than 30 min.

Statistical analysis

Sexual behaviour parameter comparisons were made by means of the Mann-Whitney U-test. The proportion of copulating animals was compared using the Fisher F test (Siegel, 1956). The biochemical analysis data were compared by help of the Student's T test (Steel and Torrie, 1985).

Experiment 1: Effect of TFMPP and 8-OH-DPAT on the sexual behaviour of p-CPA pretreated rats

Three independent groups of animals were injected for two consecutive days with p-CPA (300 mg/kg) in a volume of 4 ml/kg of methylcellulose (0.2%) (Koe and Weissman, 1966). Copulatory tests were performed 72 hrs after the second injection. 8-0H-DPAT (0.5 mg/kg), TFMPP (0.5 mg/kg) or saline (2.0 ml/kg) were administered ip 15 min before the beginning of the observations. The doses of the serotonergic agonists were chosen on the basis of previous results (Ahlenius et al., 1981; Fernández-Guasti et al., 1989).

Experiment 2: Effect of TFMPP or 8-OH-DPAT on the copulatory behaviour of animals with presynaptic receptor lesions produced by the intracerebroventricular injection of 5,7-DHT

Sexually active males (250–275 g body weight) were anesthetized with pentobarbital (35 mg/kg) and mounted in a stereotaxic apparatus and injected into the right lateral ventricle (coordinates DV 3.9; AP 0.8; L 1.4 mm; Paxinos and Watson, 1982) with the neurotoxin, 5,7-DHT (10 µg/10 µl ascorbic acid 0.2% according to McIntosh and Barfield, 1984). In order to prevent noradrenergic reuptake of the neurotoxin all animals were treated with desimipramine (25 mg/kg) 45 min before the surgery. Five days after the injection the animals were randomly divided into three groups. Each group received either of the following ip treatments: saline (2.0 ml/kg), 8-OH-DPAT (0.5 mg/kg) or TFMPP (0.5 mg/kg). An extra control-control group was included in which ascorbic acid (0.2%) was intraventricularly injected. In this group, five days after the icv injection, saline was ip administered. In all groups the sexual behaviour tests were performed 15 min after the ip treatments. Immediately after the tests the animals were sacrificed. To assure the localization of the injection an extra histology-control group was injected with sky pontamine blue and the tissue analyzed using the rapid procedure described by Sánchez-Alvarez et al. (1988).

Noradrenaline (NA), 5-HT and 5-hydroxyindolacetic acid (5-HIAA) were analyzed by HPLC with electrochemical detection following the technique described by Saligaut et al. (1986) with minor modifications. The animals belonging to the control-control and to the 5,7-DHT-control groups were sacrificed by decapitation. The brain was removed, placed on a cold plate and hippocampus (Hc), hypothalamus (Ht), brain stem (BS) and frontal cortex (Cx) dissected according to the method of Iversen and Glowinski (1966). After thawing, cerebral structures were placed in an antioxidant solution containing 0.1% w/w Na2S2O5 in 0.05 M perchloric acid, 0.3 ml for Ht, 0.7 for Hc and BS and 1.0 for Cx. The tissue was then homogenized by sonication and centrifuged at 8000 g for 10 min, the supernatant was stored frozen until analysis. All samples were filtered on millipore filters (0.22 µM) prior to injection into the HPLC apparatus that consisted of a Perkin-Elmer series 3B liquid chromatograph with a 20 µl sample loop. Monoamines signal was monitored with a Metrohom amperometric detector using an oxidation potential of 0.8 V vs Ag/AgCl reference electrode at 5 nA of sensitivity scale. Peaks were integrated with a Sigma 10 chromatography data station. Mobile phase consisted of 0.03 M phosphate buffer

Table 1. Effect of p-chlorophenylalanine (p-CPA) on the inhibitory and facilitatory actions of the 5-HT 1 B and 5-HT 1 A receptor agonists TFMPP and 8-OH-DPAT

Treatment	% E	IL	NM	NI	EL	PEI
Vehicle	100	0.85	5	12	8.00	5.12
p-CPA	100	0.40	2	8*	3.36*	6.85
p-CPA + TFMPP	50#	1.21*	11***	10	10.10*	8.08**
p-CPA + 8-OH-DPAT	100	0.24	4	6*	3.18	6.22

Table shows percentage of animals ejaculating (% E) and median values for each copulatory behaviour parameter. *IL* intromission latency; *NM* number of mounts; *NI* number of intromissions; *EL* ejaculation latency; *PEI* postejaculatory interval. p-CPA treated group was compared with the vehichle treated group, all other groups were statistically compared with the p-CPA pretreated group. For all groups $n = 8$. Proportions were compared using the Fisher-F-test, # $p < 0.05$. The various copulatory behavioural parameters were compared by help of the Mann Whitney U test. * $p < 0.05$; ** $p < 0.02$; *** $p < 0.01$

(pH = 3.5) containing 0.08% w/v sodium octyl sulphate, 0.03% EDTA and 15% methanol. The column was an Alltech C-18 reversed phase (100 × 4.6 mm, 3 µm of average particle size), and the flow rate was 1.4 ml/min. The results were expressed as ng of substance/ g fresh tissue.

Experiment 3: Effect of TFMPP and 8-OH-DPAT on the copulatory behaviour of animals with serotonergic somatodendritic receptor lesions produced by the injection of 5,7-DHT in the raphé nucleus

The surgical procedure followed in this experiment was similar to that described in experiment 2. The main difference was that the neurotoxin 5,7 DHT (4 µg/0.5 µl according to McIntosh and Barfield, 1984) was administered directly into the raphé nucleus following these coordinates: DV: 5.9; AP: −7.8; L: 0.0 (Paxinos and Watson, 1982). As in the previous experiment three main groups were studied: saline (2.0 ml/kg); TFMPP (0.5 mg/kg) and 8-OH-DPAT (0.5 mg/kg). An extra control-control group in which ascorbic acid (0.2%) was injected in to the raphé nucleus followed five days later by saline (2.0 ml/kg) was also included. The behavioural, histological and neurochemical analyses were performed as previously described (see experiment 2).

Results

Experiment 1: Effect of TFMPP or 8-OH-DPAT on the sexual behaviour of p-CPA pretreated animals

The results of this experiment are shown in Table 1. Clearly, as previously demonstrated (Ahlenius et al., 1971; Salis and Dewsbury, 1971) administration of p-CPA (300 mg/kg × 2) resulted in a facilitation of sexual behaviour evidenced as a reduction in the number of intromissions and ejaculation latency. The injection of TFMPP (0.5 mg/kg) resulted in a statistical significant reduction of the proportion of males showing ejaculatory behaviour (50%, Fisher F test, $p < 0.05$). Additionally, those males ejaculating showed an inhibition of the behaviour reflected as a statistical significant increase in the following param-

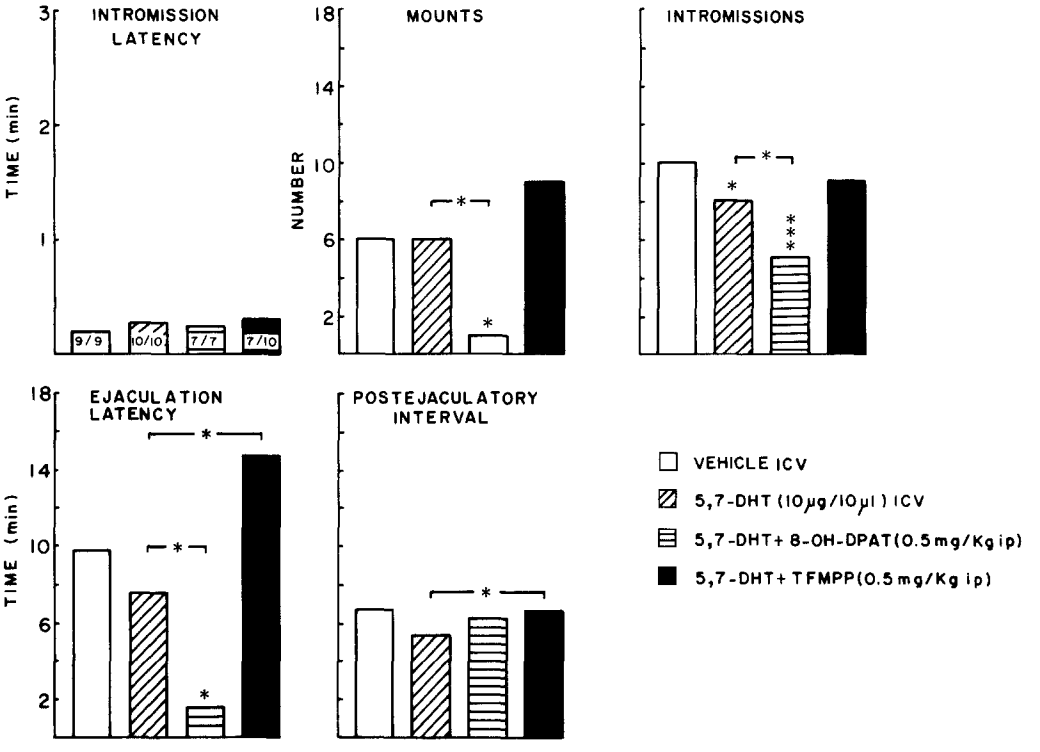

Fig. 1. Effect of the 5-HT1A and 5-HT1B agonists 8-OH-DPAT and TFMPP on the sexual behaviour of male rats treated icv with 5,7 DHT (see Methods section). Proportions within colums represent the number of copulating animals after each treatment. Asterisks over colums show statistical significant differences as compared with the control-control groups. Brakets show comparisons between the experimental (5,7 DHT-drug-treated) and their respective control (5,7 DHT-saline-treated). Mann Whitney U test, * $p<0.05$; ** $p<0.02$; *** $p<0.01$

eters: intromission latency, number of mounts preceding ejaculation, ejaculation latency and length of the postejaculatory interval. Finally, the injection of 8-OH-DPAT (0.5 mg/kg) to p-CPA pretreated animals, produced a facilitation of copulation expressed as a reduction of the number of intromissions preceding ejaculation.

Experiment 2: Effect of TFMPP or 8-OH-DPAT on the copulatory behaviour of males with presynaptic receptor lesions produced by the intracerebroventricular administration of 5,7-DHT

The results of this experiment are shown in Fig. 1. Also, as previously demonstrated (McIntosh and Barfield, 1984), lesions with 5,7-DHT produced a facilitation of copulatory behaviour. Clearly, none of the treatments, but TFMPP, resulted in a decrease in the proportion of males displaying ejaculation in a 30 min test (proportions in parenthesis within each column). This reduction, however, did not reach statistical significance (Fisher F test, non significant).

Table 2. Monoamine assays (ng/g tissue) on the brain stem, hippocampus, hypothalamus and frontal cortex of 5,7-DHT (10 µg/10 µl, intraventricular) treated rats

	Groups	Serotonin	5 HIAA	Noradrenaline
Brain stem	Control	311 ± 22	224 ± 24	540 ± 75
	5,7-DHT	117 ± 36*** (38)	108 ± 32** (48)	650 ± 61^{ns} (120)
Hippocampus	Control	239 ± 63	223 ± 33	476 ± 115
	5,7-DHT	104 ± 33* (44)	125 ± 19* (56)	525 ± 99^{ns} (110)
Hypothalamus	Control	635 ± 125	361 ± 71	1,712 ± 199
	5,7-DHT	203 ± 59*** (32)	162 ± 32** (45)	1,576 ± 195^{ns} (92)
Frontal cortex	Control	457 ± 76	414 ± 53	524 ± 51
	5,7-DHT	108 ± 17*** (24)	108 ± 16*** (26)	758 ± 205^{ns} (144)

The neurotoxin was injected 60 min after desipramine (25 mg/kg, ip). Values are expressed as means ± S.E. Values in parenthesis indicate percentage of control. Student T test, *ns* non significant; * $p \leqslant 0.05$; ** $p \leqslant 0.02$; *** $p \leqslant 0.002$

As to the various parameters of sexual behaviour it is interesting to observe that the administration of TFMPP (0.5 mg/kg) to 5,7-DHT lesioned animals resulted in an inhibition of the behaviour primarily reflected as a prolongation of the ejaculation latency and of the postejaculatory interval. Finally, treatment with the 5-HT1A agonist, 8-OH-DPAT (0.5 mg/kg) produced a facilitation of the behaviour evidenced as a shortening of the ejaculation latency.

Table 2 shows the neurochemical analysis of NA, 5-HT and 5-HIAA in various brain areas after the icv injection of 5,7-DHT. Five days after the neurotoxin injection a reduction in 5-HT and 5-HIAA, without altering the NA levels, was produced.

Experiment 3: Effect of TFMPP or 8-OH-DPAT on the copulatory behaviour of animals with lesions of the serotonergic somatodendritic receptors produced by the injection of 5,7-DHT into the raphé nucleus

Figure 2 shows the effect of TFMPP or 8-OH-DPAT in animals with lesions of the somatodendritic serotonergic receptors by the intraraphé injection of 5,7-DHT. As in the previous experiment, none of the treatments, but TFMPP, resulted in a decrease in the proportion of copulating animals (numbers in parenthesis within each column), such decrease, however, did not reach statistical significance (Fisher F test, non significant). The intraraphé injection of the neurotoxin did not result in any statistical significant effect on copulation. However, treatment with TFMPP (0.5 mg/kg) produced a clear inhibitory effect by drastically prolonging the intromission and ejaculation latencies. The systemic administration of 8-OH-DPAT (0.5 mg/kg) to 5,7-DHT lesioned animals facilitates the copulatory behaviour by shortening the intromission and ejaculation latencies and the postejaculatory interval.

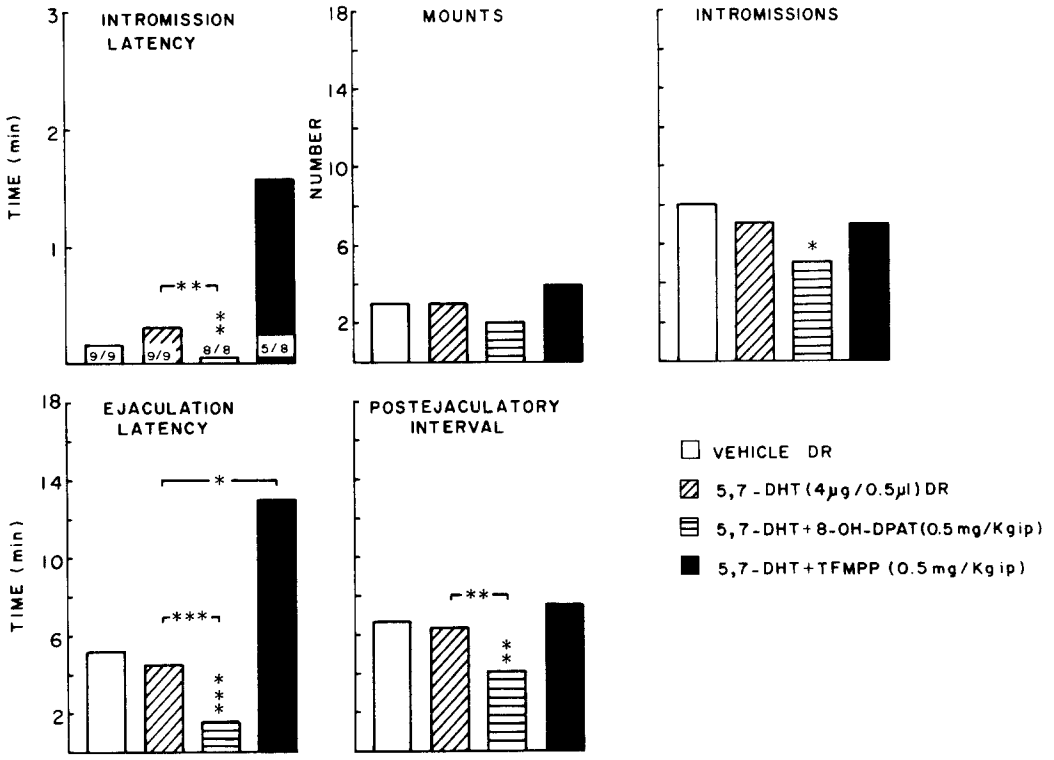

Fig. 2. Effect of the 5-HT1A and 5HT1B agonists 8-OH-DPAT and TFMPP on the sexual behaviour of male rats with dorsal raphé lesion after administration of 5,7 DHT (see Methods section). Proportions within colums represent the number of copulating animals after each treatment. Asterisks over colums show statistical significant differences as compared with the control-control groups. Brakets show comparisons between the experimental (5,7 DHT-drug-treated) and their respective control (5,7 DHT-saline-treated). Mann Whitney U test, * $p<0.05$; ** $p<0.02$; *** $p<0.01$

Table 3 shows the neurochemical analysis of 5-HT, 5-HIAA and NA after the intraraphé injection of 5,7-DHT. A statistical singificant reduction in 5-HT and 5-HIAA in frontal cortex and hypothalamus was observed. The levels of 5-HT and 5-HIAA were not changed in hippocampus neither in brain stem. In no case the NA titers were different between the control and the 5,7 DHT-treated groups.

Discussion

From present data the following conclusions could be drawn: a) neither the facilitatory nor the inhibitory effects of the 5-HT1A and 5-HT1B agonists, respectively, are mediated via serotonin release; b) the inhibitory effect of the 5-HT1B agonist, TFMPP, results after the stimulation of both pre- (auto and somatodendritic receptors) and postsynaptic receptors, and c) in the facilitation of copulatory behaviour induced by 8-OH-DPAT the presynaptic (auto and somatodendritic) receptors are not involved.

Table 3. Monoamine assays (ng/g tissue) on the brain stem, hippocampus, hypothalamus and frontal cortex of 5,7-DHT (4 µg/0.5 µl, intra raphé) treated rats

	Groups	Serotonin	5 HIAA	Noradrenaline
Brain stem	Control	322 ± 41	321 ± 47	580 ± 43
	5,7-DHT	222 ± 35^{ns} (68)	222 ± 18^{ns} (69)	496 ± 29^{ns} (85)
Hippocampus	Control	329 ± 41	144 ± 7	265 ± 41
	5,7-DHT	277 ± 30^{ns} (84)	182 ± 37^{ns} (126)	337 ± 50^{ns} (127)
Hypothalamus	Control	328 ± 43	334 ± 80	1,775 ± 242
	5,7-DHT	160 ± 53* (48)	81 ± 34* (24)	1,492 ± 109^{ns} (84)
Frontal cortex	Control	430 ± 39	292 ± 43	445 ± 59
	5,7-DHT	271 ± 37* (63)	109 ± 13*** (36)	307 ± 55^{ns} (69)

The neurotoxin was injected 60 min after desipramine (25 mg/kg, ip). Values are expressed as means ± S.E. Values in parenthesis indicate percentage of control. Student T test, *ns* non significant; * $p \leqslant 0.05$; *** $p \leqslant 0.005$

Interestingly, the administration of TFMPP to p-CPA pretreated rats does not affect the inhibitory action of this drug on mating, indicating that serotonin synthesis or release is not involved in its mechanism of action and suggesting a direct stimulation of postsynaptic receptors. In spite that the effect of 8-OH-DPAT was not suspected to be mediated via serotonin release, the results revealing that 8-OH-DPAT facilitatory action is present in p-CPA pretreated animals, suggests that the action of this drug is not presynaptically mediated. Other experiments in this direction (*vide infra*) further support this conclusion.

The 5-HT1B receptor subtype has been identified pre- and postsynaptically (Engel et al., 1983; Hoyer et al., 1985 b; Kennett et al., 1987; Middlemiss, 1985). The inhibitory actions of TFMPP on masculine sexual behaviour are mainly reflected as a reduction in the proportion of copulating animals, and a drastic increase in mounting behaviour accompanied by a prolongation of the ejaculation latency (Fernández-Guasti et al., 1989). Present data revealing that after lesioning the presynaptic or somatodendritic receptors, by the intracerebroventricular or intraraphé 5,7-DHT injection, prevent the action of TFMPP on mounting behaviour, suggest that the increase in this parameter depends upon the integrity of the presynaptic terminal. By contrast, in lesioned animals a decrease in the proportion of males copulating and an increase in the ejaculation latency was observed making possible to propose that these inhibitory effects are mediated via the stimulation of 5-HT1B postsynaptic receptors. The analogy between the 5-HTP and TFMPP effects on masculine sexual behaviour would further strengthens the proposition that endogenous 5-HT acts directly on postsynaptic 5-HT1B receptors to produce its inhibitory actions. Further studies, however, should be undertaken to fully confirm this idea.

Although the stimulation of the 5-HT1A receptor subtype seems to produce non-physiological responses (Carlsson, 1987) it is interesting to discuss the

possible mechanisms of action underlying the effect of these agonists. Ahlenius and coworkers in their original report (Ahlenius et al., 1981) proposed that the mechanism through which 8-OH-DPAT acts to facilitate masculine sexual behaviour could involve three non exclusive possibilities: (A) the stimulation of a subgroup of postsynaptic receptors; (B) the activation of 5-HT presynaptic receptors and (C) an antagonistic effect on 5-HT postsynaptic receptors. Surprisingly, few experimental work has been made in regard to these possibilities. Thus, Ahlenius and Larsson (1987) have found that 8-OH-DPAT is able to antagonize the inhibitory action of 5-HTP on sexual behaviour, therefore suggesting that 8-OH-DPAT possesses antagonistic properties. Furthermore, these same authors have recently reported that the facilitatory action of 8-OH-DPAT can be effectively blocked by some beta-5-HT1A antagonists (Ahlenius and Larsson, 1989).

8-OH-DPAT has been presented as a ligand for 5-HT autoreceptors (Gozlan et al., 1983). In spite that this is still a matter of controversy (Middlemiss, 1984), it has been suggested (Ahlenius et al., 1981) that the stimulation of this receptor could underly its facilitatory effect on copulation. Present results showing that the stimulatory action of this drug is not altered in animals with serotonergic fibers lesions suggests that a presynaptic autoreceptor mediation is not involved.

Another plausible interpretation could be based on the actions of 8-OH-DPAT on somatodendritic serotonergic receptors. In this regard it has been shown that stimulation of the 5-HT1A somatodendritic receptors by 8-OH-DPAT results in a decrease of the serotonergic transmission from those fibers arising from the nucleus raphé (Higgins et al., 1988). Thus, the facilitation of sexual behaviour observed after this compound could be interpreted as a reduction in the serotonergic transmission caused by the stimulation of these receptors. Present results showing a drastic clear facilitation of the sexual behaviour by 8-OH-DPAT in raphé nucleus lesioned-animals suggests that such a mechanism does not underly the actions of this drug on copulation.

Recently it has been demonstrated that the stimulation of the 5-HT1A and 5-HT1B receptor subtypes produces exactly opposite actions on various behaviours. Generally, the 5-HT1B agonists produce very similar behavioural effects to those elicited by increasing the serotonergic transmission. The inconsistency between the action of the 5-HT1A agonists and that produced by serotonin would importantly question the putative physiological role of the 5-HT1A receptor subtype (Carlsson, 1987). Accordingly, the facilitatory action of various 5-HT1A agonists on masculine sexual behaviour (Ahlenius et al., 1981; Fernández-Guasti et al., 1990) could be of pharmacological interest but difficult to interpret on physiological basis.

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References

Ahlenius S, Eriksson H, Larsson K, Modigh K, Södersten P (1971) Mating behaviour in the male rat treated with p-chlorophenylalanine methyl esther alone and in combination with pargyline. Psychopharmacologia 20: 383–388

Ahlenius S, Larsson K, Svensson L, Hjorth S, Carlsson A, Lindberg P, Wikström H, Sánchez D, Arvidsson LE, Hacksell U, Nilsson JLG (1981) Effects of a new type of 5-HT receptor agonist on male rat sexual behaviour. Pharmacol Biochem Behav 15: 785–792

Ahlenius S, Larsson K (1987) Evidence for a unique pharmacological profile of 8-OH-DPAT by evaluation of its effects on male rat sexual behaviour. In: Dourish CT, Ahlenius S, Hutson P (eds) Brain 5-HT1A receptors: behavioural and neurochemical pharmacology. Ellis Horwood, Chichester, pp 186–198

Ahlenius S, Larsson K (1989) Antagonism by pindolol, but not by betaxolol, of 8-OH-DPAT-induced facilitation of male rat sexual behaviour. J Neural Transm 77: 163–170

Bitran D, Hull E (1987) Pharmacological analysis of male rats sexual behaviour. Neurosci Biobehav Rev 11: 365–389

Bradley PB, Engel G, Fenuik W, Fozard JR, Humphrey PPA, Middlemiss DN, Mylecharane EJ, Richardson PB, Saxena PR (1986) Proposals for the classification and nomenclature of functional receptors for 5-hydroxytryptamine. Neuropharmacology 25: 563–576

Carlsson A (1987) Introduction. In: Dourish CT, Ahlenius S, Hutson PH (eds) Brain 5-HT1A receptors: behavioural and neurochemical pharmacology. Ellis Horwood, Chichester, pp 15–18

Dourish CT, Hutson PH, Curzon G (1985) Characteristics of feeding induced by the serotonin agonist 8-hydroxy-2-(di-n-propylamino) tetralin (8-OH-DPAT). Brain Res Bull 15: 377–384

Engel G, Göthert M, Müller-Schweinitzer E, Schlicker E, Sistonen L, Stadler PA (1983) Evidence for common pharmacological properties of [3H]5-hydroxytryptamine binding sites, presynaptic 5-hydroxytryptamine autoreceptors in CNS and inhibitory presynaptic 5-hydroxytryptamine receptors on sympathetic nerves. Naunyn Schmiedebergs Arch Pharmacol 324: 116–124

Fernández-Guasti A, Escalante A, Agmo A (1989) Inhibitory action of various 5-HT1B receptor agonists on rat masculine sexual behaviour. Pharmacol Biochem Behav 34: 811–816

Fernández-Guasti A, Escalante A, Agmo A, Hong E (1990) Behavioural actions of indorenate, a new putative 5-HT receptor agonist. Pharmacol Biochem Behav 37: 83–88

Gozlan H, El Mestikawi S, Pichat L, Glowinski J, Hamon M (1983) Identification of presynaptic serotonin autoreceptors using a new ligand: [3H]-PAT. Nature 305: 140–142

Higgins GA, Bradbury AJ, Jones BJ, Oakley NR (1988) Behavioural and biochemical consequences following activation of 5-HT1-like and GABA receptors in the dorsal raphe nucleus of the rat. Neuropharmacology 27: 993–1001

Hoyer D, Engel G, Kalkman H (1985a) Molecular pharmacology of 5-HT1 and 5-HT2 recognition sites in rat and pig brain membranes: radioligand binding studies with [3H] 5-HT, [3H] 8-OH-DPAT, (−) [125I] iodocyanopindolol, [3H] mesulergine and [3H] ketanserin. Eur J Pharmacol 118: 13–23

Hoyer D, Engel G, Kalkman H (1985b) Characterization of the 5-HT1B recognition site in the rat brain: binding studies with (−) [125I] iodocyanopindolol. Eur J Pharmacol 118: 1–12

Hutson PH, Dourish CT, Curzon G (1987) 8-Hydroxy-2-(di-n-propylamino) tetralin (8-OH-DPAT)-induced hyperphagia: neurochemical and pharmacological evidence for an involvement of 5-hydroxytryptamine somatodendritic autoreceptors. In: Dourish CT, Ahlenius S, Hutson PH (eds) Brain 5-HT1A receptors: behavioural and neurochemical pharmacology. Ellis-Horwood, Chichester, pp 211–232

Iversen LL, Glowinski J (1966) Regional studies of catecholamines in the rat brain. J Neurochem 13: 655–669

Kennett GA, Dourish CT, Curzon G (1987) 5-HT1B agonists induce anorexia at a post synaptic site. Eur J Pharmacol 141: 429–435

Koe B, Weissman A (1966) p-Chlorophenylalanine: a specific depletor of brain serotonin. J Pharmacol Exp Ther 154: 499–525

Larsson K, Fuxe K, Everitt BJ, Holmgren M, Södersten P (1978) Sexual behaviour in male rats after intracerebral injection of 5,7-dihydroxytryptamine. Brain Res 141: 293–303

Larsson K, Ahlenius S (1986) Masculine sexual behaviour and brain monoamines. In: Segal M (ed) Psychopharmacology of sexual disorders. John Libbey, London, pp 15–32

Malmnäs CO (1973) Monoaminergic influence of testosterone activated copulatory behaviour in castrated male rats. Acta Physiol Scand [Suppl] 395: 1–128

McIntosh TK, Barfield RJ (1984) Brain monoaminergic control of male reproductive behaviour. I. Serotonin and the postejaculatory refractory period. Behav Brain Res 12: 255–265

Mendelson SD, Gorzalka BB (1985) Serotonin antagonist pirenperone inhibits sexual behaviour in the male rat: attenuation by quipazine. Pharmacol Biochem Behav 22: 565–571

Middlemiss DN (1984) Stereoselective blockade at [3H] 5-HT binding sites and at the autoreceptors by propranolol. Eur J Pharmacol 101: 289–293

Middlemiss DN (1985) The putative 5-HT1 receptor agonist RU 24969, inhibits the efflux of 5-hydroxytryptamine from rat frontal cortex slices by stimulation of the 5-HT autoreceptor. J Pharm Pharmacol 37: 434–445

Nishikawa T, Scatton B (1985) Inhibitory influence of GABA on central serotonergic transmission. Raphé nuclei as the anatomical site of the GABAergic inhibition of cerebral serotonergic neurons. Brain Res 331: 91–103

Paxinos G, Watson C (1982) The rat brain in stereotaxic coordinates. Academic Press, New York

Saligaut C, Chretien P, Daoust M, Moore N, Boismare F (1986) Dynamic characteristics of dopamine, norepinephrine and serotonin metabolism in axonal endings of rats hypothalamus and striatum during hypoxia: a study using HPCL with electrochemical detection. Meth Find Exp Clin Pharmacol 8: 343–349

Salis PJ, Dewsbury DA (1971) p-Chlorophenylalanine facilitates copulatory behaviour in male rats. Nature 232: 400–401

Sánchez-Alvarez M, León-Olea M, Condés-Lara M, Briones M, Fernández-Guardiola A (1988) Localization of the microelectrode tip combining a rapid procedure method and marking with pontamine sky blue. Bol Est Med Biol (Méx) 36: 55–59

Siegel S (1956) Non parametric statistics for the behavioural sciences. McGraw Hill, New York

Sills MA, Wolfe BB, Frazer A (1984) Determination of selective and nonselective compounds for the 5-HT1A and 5-HT1B receptor subtypes in rat frontal cortex. J Pharmacol Exp Ther 231: 480–487

Sprouse JS, Aghajanian GK (1986) (−) Propranolol blocks the inhibition of serotonergic dorsal raphé cell firing by 5-HT1A selective agonists. Eur J Pharmacol 128: 295–298

Steel RGD, Torrie JH (1985) Principles and procedures of statistics. A biometrial approach. McGraw-Hill, New York

Verge D, Daval G, Patey A, Gozlan H, El Mestikawi S, Hamon M (1985) Presynaptic

5-HT autoreceptors on serotonergic cells bodies and/or dendrites but not terminals are of the 5HT1A subtype. Eur J Pharmacol 113: 463–464

Weissmann-Nanopoulos D, Mach E, Magre J, Demassey Y, Pujol JF (1985) Evidence for the localization of 5-HT1A binding sites on serotonin containing neurons in the raphé dorsalis and raphé centralis nuclei of the rat brain. Neurochem Int 7: 1061–1072

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