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Participation of 5-HT_{1B} receptors in the inhibitory actions of serotonin on masculine sexual behaviour of mice: pharmacological analysis in 5-HT_{1B} receptor knockout mice

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1 The role of the 5-Hydroxytryptamine$_{1B}$ (5-HT_{1B}) receptor subtype in masculine sexual behaviour in mice was analysed in both 5-HT_{1B} receptor knockout (KO_{1B}) and wild-type (WT) animals.

2 Comparison of male copulatory behaviour of WT and KO_{1B} strains revealed that KO_{1B} mice become interested earlier in sexual behaviour, but require more stimulation to achieve ejaculation than its corresponding WT strain.

3 The pharmacological manipulation of male sexual activity in the WT strain showed that the serotonin precursor 5-Hydroxytryptophan (5-HTP), the 5-HT_{1B} agonist (1-(m-trifluoromethylphenyl) piperazine (TFMPP) and the 5-Hydroxytryptamine$_{1A}$ (5-HT_{1A}) receptor agonist 8-hydroxy-2-di-n-propylamino-tetralin (8-OH-DPAT) all inhibited male copulatory behaviour in mice.

4 In KO_{1B} mice, TFMPP lacked an effect, 5-HTP exerted a mild inhibitory effect while 8-OH-DPAT provoked only a tendency towards a reduction in the percentage of animals that achieved ejaculation. In general, KO_{1B} mice were less sensitive to the inhibitory actions of 5-HTP and 8-OH-DPAT than the WT strain.

5 Based on these results, we can suggest that serotonin plays a general inhibitory role in the sexual behaviour of male mice and that both 5-HT_{1B} and 5-HT_{1A} receptor subtypes participate in the inhibitory actions of this neurotransmitter.

6 The absence of the 5-HT_{1B} receptor subtype affected both components of mouse masculine sexual behaviour, motivation and execution, further confirming the involvement of this receptor subtype in the control of this behaviour. In addition, the diminished sensitivity to serotonergic stimulation exhibited by KO_{1B} mice suggests the occurrence of compensatory changes as a consequence of the absence of the 5-HT_{1B} receptor subtype.
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Keywords: Masculine sexual behaviour in mice; 5-HT_{1B} knockout; 5-HT_{1B} receptors; 5-HT_{1A} receptors; 5-HTP; 8-OH-DPAT; TFMPP; serotonin

Abbreviations: CD, carbidopa; EL, ejaculation latency; 5-HT_{1A}, 5-Hydroxytryptamine$_{1A}$ receptor subtype; 5-HT_{1B}, 5-Hydroxytraptamine$_{1B}$ receptor subtype; |5-HTP, 5-Hydroxytryptophan; I, intromissions; IL, intromission latency; KO_{1B}, 5-HT_{1B} receptor knockout; mCPP, 1-(m-chlorophenyl) piperazine; 5MeODMT, 5-methoxy-*N*, *N*-dimethyltryptamine; M, mounts; ML, mount latency; 8-OH-DPAT, 8-hydroxy-2-di-n-propylamino-tetralin; RU 24969, 5-methoxy-3-(1,2,3,6-tetrahydro-4-pyridinyl) indole; SSRIs, selective serotonin reuptake inhibitors; TFMPP, 1-(m-trifluoromethylphenyl) piperazine; TME, total mounting events; WAY 100635, N-[2-[4-(2-methoxyphenyl)-1-piperazinyl]ethyl]-N-2-pyridinyl-cyclohexane-carboxamide maleate; WT, wild-type

Introduction

A series of studies have shown that experimental manipulations directed to increase serotonergic transmission inhibit male sexual behaviour, while those directed to impair it result in a facilitation of copulatory activity. Thus, the administration of serotonin (Fernández-Guasti *et al*., 1992; Hillegaart *et al*., 1989; 1991), its precursor, 5-hydroxytryptophan (5-HTP) (Ahlenius & Larsson, 1998; Fernández-Guasti & Rodríguez-Manzo, 1992) or the chronic treatment with selective serotonin reuptake inhibitors (SSRIs) all impair masculine sexual behaviour of rats and humans (Ahlenius *et al*., 1979; Modell *et al*., 1997; Rosen *et al*., 1999). On the other side, the neurotoxic lesion of the serotonergic system (Fernández-Guasti & Escalante, 1991; Larsson *et al*., 1978), the selective electrolytic lesion of the midbrain raphe nuclei (McIntosh & Barfield, 1984) or the inhibition of serotonin synthesis (Fernández-Guasti & Escalante, 1991; Salis & Dewsbury, 1971) facilitate copulation. These data indicate that increased serotonergic transmission inhibits rat masculine sexual behaviour.

5-HT_{1B} receptors have been proposed to mediate the inhibitory actions of endogenous serotonin on male rat sexual activity. This proposal is experimentally sustained by the following observations: (a) The systemic or intrabrain administration of various 5-HT_{1B} agonists [1-(m-trifluoromethylphenyl) piperazine (TFMPP), 1-(m-chlorophenyl) piperazine (mCPP), 5-methoxy-3-(1,2,3,6-tetrahydro-4-pyridinyl) indole (RU 24969) and anpirtoline] produces an

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inhibitory effect on male sexual behaviour similar to the one observed after the injection of serotonin or its precursor 5-hydroxytryptophan (5-HTP) (Fernández-Guasti *et al*., 1989; 1992; Hillegaart & Ahlenius, 1998; Mendelson & Gorzalka, 1990); (b) co-administration of 5-HTP plus a 5-HT_{1B} agonist, at doses that are sub-threshold to inhibit male rat sexual activity, synergistically interact to inhibit this behaviour (Fernández-Guasti & Rodríguez-Manzo, 1992) and (c) the selective 5-HT_{1B} receptor antagonist isamoltane completely blocks the inhibitory effect of 5-HTP on copulation, while antagonists to other 5-HT receptor subtypes do not (Ahlenius & Larsson, 1998).

Although 5-HT_2 receptors have also been associated with an inhibition of male rat sexual behaviour, stimulation of this receptor subtype seems to produce a disruption of the copulatory behaviour, rather than an inhibition of its components (Watson & Gorzalka, 1991). Besides, the inhibitory effects of 5-HTP are not antagonised by 5-HT_2 antagonists (Ahlenius & Larsson, 1998).

Central serotonin is also coupled to facilitatory mechanisms in the regulation of male rat sexual activity. Thus, stimulation of 5-HT_{1A} receptors by 8-hydroxy-2-di-n-propylamino-tetralin (8-OH-DPAT) and other 5-HT_{1A} agonists such as buspirone, 5MeODMT, indorenate, ipsapirone and lisuride produces a dramatic facilitation of male rat copulatory behaviour (Ahlenius & Larsson, 1991; Ahlenius *et al*., 1981; Fernández-Guasti *et al*., 1986; 1990). The facilitatory effect of 8-OH-DPAT can be effectively prevented by pre-treatment with selective 5-HT_{1A} antagonists, such as WAY 100635 and NAD-299, confirming the participation of this receptor subtype in that action (Ahlenius *et al*., 1999; Hillegaart & Ahlenius, 1998). It is important to mention, however, that species differences might exist in the role played by serotonin receptor subtypes in the mediation of male sexual activity. For example, a work by Svensson *et al*. (1987) reports that 5-HTP treatment facilitated some aspects of male sexual behaviour in mice and that the stimulation of the 5-HT_{1A} receptor subtype, by 8-OH-DPAT, had inhibitory effects. No data regarding the role of 5-HT_{1B} receptors in male mouse copulatory activity have been published.

The use of knockout mice, with a targeted deletion of specifc genes encoding for receptor proteins provides a new approach to analyse the possible role of those receptors in the regulation of a selected behaviour (Nelson & Young, 1998). The recent availability of 5-HT_{1B} receptor gene knockout mice allows the study of this specific receptor subtype (Saudou *et al*., 1994). In previous studies, a variety of behaviours that are thought to be modulated by the 5-HT_{1B} receptor, such as locomotion, aggression, anxiety and depression (Brunner *et al*., 1999; Mayorga *et al*., 2001; Ramboz *et al*., 1996; Sceance-Levie *et al*., 1999), have been analysed using these mice. The data obtained reveal that 5-HT_{1B} knockout mice (KO_{1B}) show increased aggressive behaviour, lower levels of anxiety and hyperactivity when compared to its wild-type (WT) control strain (Brunner *et al*., 1999; López-Rubalcava *et al*., 2000; Ramboz *et al*., 1996; Sceance-Levie *et al*., 1999). An analysis of the role played by 5-HT_{1B} receptors in the control of male sexual behaviour in mice has not been conducted.

On these bases, the first purpose of the present series of experiments was to compare the masculine sexual behaviour of KO_{1B} mice with that of its corresponding WT strain. The second purpose of this study was to establish the effect of the serotonin precursor 5-HTP and agonists to the 5-HT_{1A} and 5-HT_{1B} receptor subtypes, previously tested in our laboratory on rat masculine sexual behaviour (TFMPP and 8-OH-DPAT, respectively), on the copulatory behaviour of both KO_{1B} and WT mice. The possibility of drug-induced motor actions that could interfere with display of sexual behaviour was excluded by running motor co-ordination tests.

Methods

General

Male and female wild-type (WT 129/Sv-ter strain) and KO_{1B} mice (20–30 g b wt) were used in these series of experiments. Breeding pairs of mice were shipped from the colonies of Dr R. Hen at Columbia University (Center for Neurobiology and Behavior, New York, NY, U.S.A.) and bred in our animal facilities at the 'Centro de Investigación y Estudios Avanzados' (Mexico City, Mexico). Animals in the colony were routinely tested by means of a Southern blot analysis to guarantee that the 5-HT_{1B} gene remained knocked out in the mutant mice. Male mice were individually housed and kept in a room under inverted and controlled light dark cycle conditions (12 h light: 12 h dark. Lights off at 1000 h). Females were kept 4–6 per cage. All animals received Purina mouse chow and water *ad libitum* all over the experiment. The Local Committee of Ethics on Animal Experimentation approved all experimental procedures, which followed the regulations established in the Mexican official norm for the use and care of laboratory animals 'NOM-062-ZOO-1999'.

Sexual behaviour tests

Observations of sexual behaviour were made in a room under dim red light and in the males' home cages. Male mice were individually tested for sexual behaviour with females brought into sexual receptivity by the sequential s.c. injection of oestradiol valerianate (20 μg 0.05 ml^{-1}, at −48 h) followed by progesterone (0.5 mg 0.05 ml^{-1}, at −4 h). Stimulus females and experimental males were of the same strain. Previous to experimental sessions males of both strains were trained twice for sexual behaviour, separated by one week, and only those males that ejaculated in both training sessions were considered sexually experienced and selected for the study.

In each experimental group, the percentage of males that displayed mounts, intromissions and ejaculation was established. The term 'intromission' is used to refer to the behavioural pattern consisting of repeated deep pelvic thrusting, associated with penile insertion. Similarly, ejaculation refers to the behaviour that occurs at the end of an intromission characterized by the reflexive grasping of the female often followed by the male falling off the female to the side (Clemens *et al*., 1988; McGill, 1962). For the animals that ejaculated, the following specific sexual behaviour parameters were determined: (a) mount latency (ML, the time from the introduction of the female to the first mount), (b) intromission latency (IL, the time from the introduction of the female to the first intromission), (c) ejaculation latency (EL, the time from the first intromission to ejaculation), (d) number of mounts before ejaculation (M), (e) number of

intromissions before ejaculation (I) and (f) total mount events (TME, sum of mounts and intromissions).

Tests were ended when an animal ejaculated or after the fulfilment of one of the following criteria: mount, intromission or ejaculation latencies longer than 30 min. Behavioural recording was conducted by an experienced observer who was unaware of the strain and the pharmacological treatment of the experimental subjects.

Drugs

The following drugs were used for the pharmacological treatments: Carbidopa (Sigma Chemicals Co., St. Louis, MO, U.S.A.), 5-hydroxytryptophan (5-HTP, Sigma Chemicals Co., St. Louis, MO, U.S.A.) [1-(m-trifluoromethylphenyl) piperazine (TFMPP, Biochemical Research, Natick, MA, U.S.A.) and 8-hydroxy-2-di-n-propylamino-tetralin (8-OH-DPAT, Biochemical Research, Natick, MA, U.S.A.). All drugs were dissolved in physiological saline and injected in a volume of 4.0 ml kg^{-1}.

Experiment 1. Comparison of male sexual behaviour between KO_{1B} and WT mice

For this study, a total of 78 WT and 92 KO_{1B} male mice, both sexually experienced animals, were subjected to two consecutive sexual behaviour tests separated by one week. The sexual behaviour parameters were recorded as previously described and comparisons were made between strains in a same session. Statistical comparisons for each parameter of sexual behaviour were made by means of the Mann Whitney *U* test. Proportions were statistically compared using the Chi square test.

Experiment 2. Effect of 5-HTP, TFMPP and 8-OH-DPAT on male sexual behaviour and motor co-ordination of KO_{1B} and WT mice

A total of 38 WT and 28 KO_{1B} mice were randomly divided into three experimental groups. On the basis of the great variability in execution of sexual behaviour exhibited by mice (McGill, 1962; Mosig & Dewsbury, 1976) Latin square designs were used for each experiment. Sexually experienced male mice of each strain were subjected to one treatment per week and pharmacological treatments were common to both mice strains.

In the first experiment, the effect of saline + saline, carbidopa (a peripheral decarboxylase inhibitor) + saline and of carbidopa + three different doses of 5-HTP (the serotonin precursor) was assessed in a group of 10 WT (Group 1) and eight KO_{1B} (Group 2) mice. Carbidopa (25 mg kg^{-1}, i.p.) was injected 15 min before 5-HTP (12.5, 25 or 50 mg kg^{-1}, s.c.) that was administered 45 min before the behavioural test.

In the second experiment the effect of the 5-HT_{1B} receptor agonist TFMPP on male mouse sexual behaviour was analysed. Thus, a group of 18 WT (Group 3) and 10 KO_{1B} mice (Group 4) was i.p. injected with either saline, 0.5 or 1.0 mg kg^{-1} of TFMPP, 30 min previous to behavioural observations.

Finally, in the last experiment, the effect of different doses of the 5-HT_{1A} receptor agonist 8-OH-DPAT (0, 0.0625 or 0.125 mg kg^{-1}) was evaluated in 10 WT (Group 5) and 10 KO_{1B} (Group 6) mice. In this case, animals were s.c. injected 20 min before observations of sexual behaviour.

The percentages of male mice showing mounts, intromissions and ejaculation within each Latin square were statistically compared by means of the Cochran Q ANOVA followed by the binomial test. The specific parameters of sexual behaviour in these groups were analysed by means of a repeated measure ANOVA followed by the Wilcoxon signed rank test. Comparison of the effects of a given treatment between WT and KO_{1B} mice was established with the Mann Whitney *U* test.

Motor co-ordination tests

Independent groups of both strains of mice ($n=10$, each) were examined for motor co-ordination in a treadmill apparatus (rotarod) after receiving the highest dose of either 5-HTP, TFMPP or 8-OH-DPAT. The procedure was the same as previously described (López-Rubalcava & Fernández-Guasti, 1994). Briefly, in this test animals are placed upon a cylinder (7 cm diameter) rotating at a speed of 11 r.p.m. Mice are trained to walk on the cylinder for three consecutive sessions and on the fourth, they receive the drug treatment. The number of falls during a 5 min period is counted. Statistical comparisons were performed by means of the Wilcoxon signed rank test between the third training and the treatment sessions.

Results

Experiment 1. Comparison of male sexual behaviour of KO_{1B} and WT mice

Figure 1 compares the spontaneous sexual behaviour of sexually experienced WT and KO_{1B} mice on two consecutive sessions. In general a higher proportion of KO_{1B} animals exhibited mounts, intromissions and ejaculation as compared with the WT strain (panel A). These differences were statistically significant in the first session for the percentage of animals that mounted ($X^2=7.05$, d.f. $=1$, $P=0.008$) and, in the second session, for the percentage of mice showing intromissions ($X^2=5.69$, d.f. $=1$, $P=0.017$).

As it can be seen in panel B, KO_{1B} mice initiated mounting and intromission behaviour earlier than the WT strain, the difference being statistically significant in the second test (Mann Whitney *U* test, $P<0.001$ for ML and $P<0.01$ for IL). On the contrary, the ejaculation latency was consistently longer in KO_{1B} animals than in the WT strain (Mann Whitney U test, $P<0.001$ in the 1st session and $P<0.01$ in the 2nd). In addition, KO_{1B} mice required a higher number of mounts and intromissions, considered independently or as total mount events, to achieve ejaculation in both sessions as compared to WT mice (Mann Whitney *U* test, $P<0.05$ for M; $P<0.001$ for I and TME in the 1st session; $P<0.01$ for I and TME in the 2nd session) (Figure 1C).

Experiment 2. Effect of 5-HTP, TFMPP and 8-OH-DPAT on male sexual behaviour and motor co-ordination of KO_{1B} and WT mice

Table 1 shows the analyses of variance (Cochran Q) for each sexual behaviour response within the Latin squares after the different pharmacological treatments. Figure 2 shows the percentage of males showing mounts, intromissions and

ejaculation after treatment with different doses of 5-HTP (panel A), TFMPP (panel B) or 8-OH-DPAT (panel C) in KO_{1B} (right columns) and WT mice (left columns). Clearly, the serotonin precursor 5-HTP (panel A) induced a dose-dependent decrease in the proportion of WT mice showing mounts, intromissions and ejaculation, the diminution being statistically significant only for the percentages of WT mice showing intromissions and ejaculation. Thus, at the dose of 25 mg kg^{-1}, 60% of the WT males (6/10) showed mounts, but only 20% (2/10) were able to intromit and ejaculate, while at the highest dose tested (50 mg kg^{-1}) only mounting behaviour could be observed. By contrast, in KO_{1B} mice, only the highest dose of 5-HTP (50 mg kg^{-1}) significantly

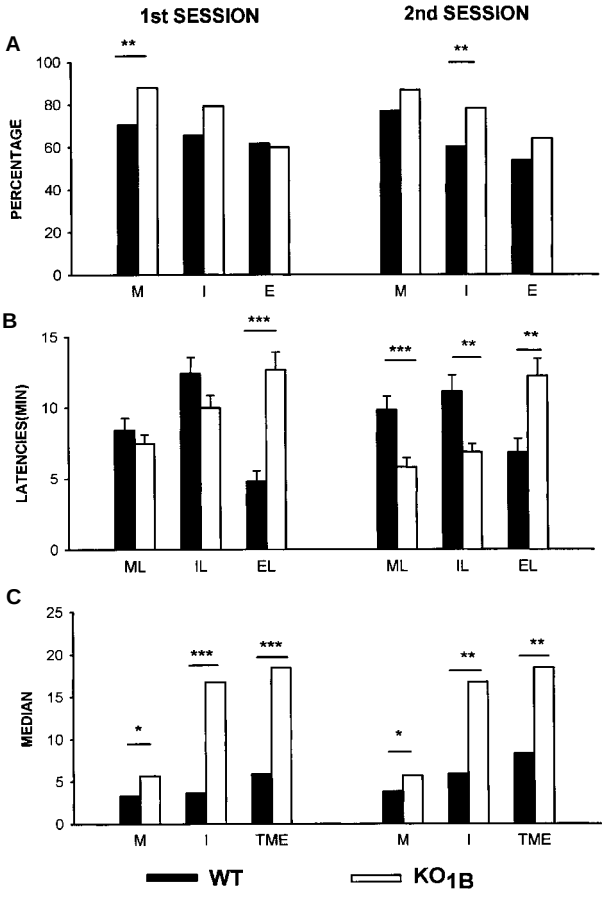

Figure 1 Spontaneous sexual behaviour of sexually experienced WT ($n=78$, filled bars) and KO_{1B} ($n=92$, empty bars) mice on two consecutive testing sessions. (A) Percentage of animals that showed mounts (M), intromissions (I) and ejaculation (E) in each session. (B) Latencies to the occurrence of the first mount (ML) or intromission (IL) and to achievement of ejaculation (EL) in an ejaculatory series expressed as mean ± s.e.mean. (C) Median number of mounts (M), intromissions (I) and total mount events (TME, M + I) that preceded ejaculation. (A) Chi square test, $^{**}P<0.02$, (B and C) Mann Whitney U test $^{*}P<0.05$; $^{**}P<0.02$, $^{***}P<0.001$.

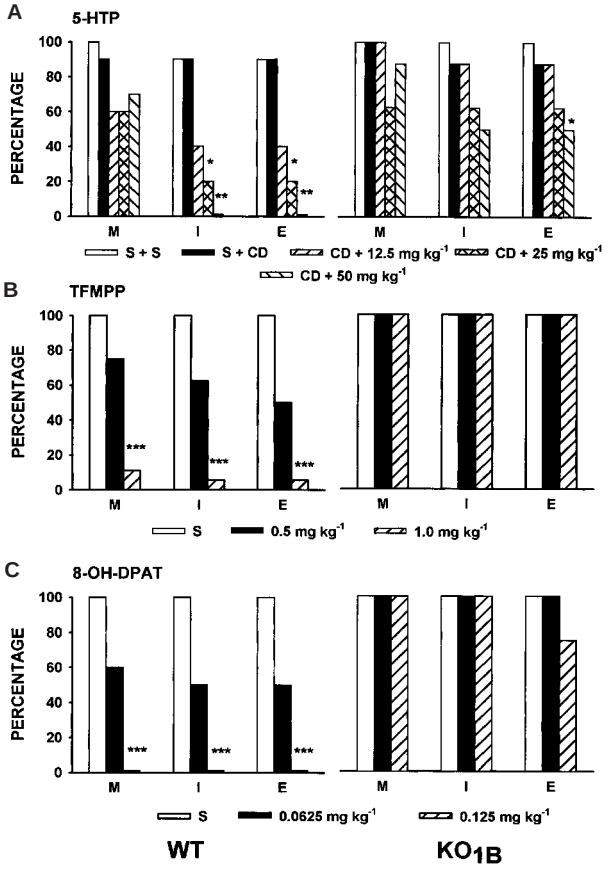

Figure 2 Percentage of KO_{1B} (right columns) and WT (left columns) male mice showing mounts (M), intromissions (I) and ejaculation (E) after treatment with different doses of either 5-HTP (A; $n=10$ for WT, $n=8$ for KO_{1B}); TFMPP (B; $n=18$ for WT; $n=10$ for KO_{1B}) or 8-OH-DPAT (C; $n=10$ for both WT and KO_{1B}). Cochran Q ANOVA followed by the binomial test. $^{*}P<0.05$; $^{**}P<0.002$; $^{***}P<0.001$. S, saline; CD, carbidopa (25 mg kg^{-1}).

Table 1 Analyses of variance (Cochran Q) of the percentage of male mice that showed each sexual behaviour response (M, I, E) within the Latin squares after the different pharmacological treatments.

		Male sexual behaviour responses								
		M			*I*			*E*		
Treatment	*Strain*	*Q value*	*d.f.*	P *value*	*Q value*	*d.f.*	P *value*	*Q value*	*d.f.*	P *value*
5-HTP (0 + 0, CD + 0, CD + 12.5, CD + 25 & CD + 50 mg kg^{-1})	WT	7.33	4	n.s.	41.75	4	<0.001	41.75	4	<0.001
	KO_{1B}	9.71	4	<0.05	10.8	4	<0.05	14.46	4	<0.01
TFMPP (0, 0.5 & 1.0 mg kg^{-1})	WT	26	2	<0.001	26.24	2	<0.001	25.53	2	<0.001
	KO_{1B}	0	2	n.s.	0	2	n.s.	0	2	n.s
8-OH-DPAT (0, 0.0625 & 0.125 mg kg^{-1})	WT	15.2	2	<0.001	15	2	<0.001	15	2	<0.001
	KO_{1B}	0	2	n.s.	0	2	n.s.	4	2	n.s.

M, mount; I, intromission; E, ejaculation; WT, wild type; KO_{1B}, $5\text{-}HT_{1B}$ receptor knockout; CD, Carbidopa (25 mg kg^{-1}); 5-HTP, 5-Hydroxytryptophan; TFMPP, 1-(m-trifluoromethylphenyl) piperazine; 8-OH-DPAT, 8-hydroxy-2-di-n-propylamino-tetralin; d.f., degrees of freedom; n.s., non-significant.

Table 2 Effect of the pharmacological treatments on parameters of male sexual behaviour of WT and KO_{1B} mice

			Parameters of sexual behaviour					
Treatment	*n*		*ML*	*IL*	*M*	*I*	*EL*	*TME*
Saline + Saline	7	WT	3.33 ± 1.64	5.32 ± 1.71	0	3	3.61 ± 1.27	7
(5 ml kg^{-1})	6	KO_{1B}	4.04 ± 1.49	7.84 ± 2.0	0	6.5	4.10 ± 1.83	10
CD + Saline	7	WT	2.43 ± 0.38	7.97 ± 2.57	0	2	2.16 ± 1.32	5
(+5 ml kg^{-1})	6	KO_{1B}	2.11 ± 0.61	6.22 ± 3.01	0	4	3.81 ± 1.44	10
CD + 5-HTP	7	WT	2.24 ± 0.68	9.11 ± 2.97	1	4	3.44 ± 0.68	7
(+12.5 mg kg^{-1})	6	KO_{1B}	5.32 ± 1.97	9.78 ± 2.64	0	3.5	3.22 ± 1.68	7
Saline	8	WT	4.44 ± 1.43	10.51 ± 2.86	0	1.5	1.96 ± 0.73	3
(5 ml kg^{-1})	10	KO_{1B}	2.31 ± 1.48	3.32 ± 1.51	2	3	3.12 ± 0.75	9
TFMPP	8	WT	11.86 ± 2.98*)+++	12.38 ± 3.2)+++	2	5.5**)+	7.72 ± 3.1**	14.5**)+
(0.5 mg kg^{-1})	10	KO_{1B}	1.03 ± 0.24	2.58 ± 0.66	0	2	2.99 ± 1.09	5
Saline	5	WT	1.94 ± 0.74	10.36 ± 3.86	1	2	3.01 ± 0.32	8
(5 ml/kg)	8	KO_{1B}	2.56 ± 0.82	3.22 ± 1.27	7	17	17.36 ± 3.11	28
8-OH-DPAT	5	WT	6.84 ± 2.72	14.17 ± 3.07)++	4)+	4	6.25 ± 0.63	20
(0.0625 mg kg^{-1})	8	KO_{1B}	1.21 ± 0.39	2.95 ± 1.27	1.5	13.5	12.82 ± 4.2	18

ML, mount latency; IL, intromission latency and EL, ejaculation latency are expressed as mean ± s.e.mean; M, mounts; I, intromission and TME, total mount events (M + I), are expressed as median number. CD, carbidopa (25 mg kg^{-1}). Comparisons within a group: Wilcoxon signed rank test $*P<0.05$, $**P<0.01$. Comparisons between strains: Mann Whitney U tests + $P<0.05$, + + $P<0.01$, + + + $P<0.001$.

diminished the percentage of males displaying ejaculation. Notwithstanding, a tendency towards a reduction in the percentage of mice exhibiting intromission and ejaculation appears already at the dose of 25 mg kg^{-1}.

The effect of the 5-HT_{1B} agonist TFMPP is presented in Figure 2B. In WT subjects 0.5 mg kg^{-1} had no significant effect on copulatory behaviour, but a dose of 1.0 mg kg^{-1} produced a drastic reduction in the proportion of animals displaying all aspects of male sexual behaviour. Conversely, at the same doses, TFMPP had no effect on the sexual behaviour of KO_{1B} mice. Indeed, all animals treated with these doses mounted, intromitted and ejaculated. Therefore, a higher dose of this compound (2.0 mg kg^{-1}) was tested in an independent group of KO_{1B} mice ($n=10$). In this case, a reduction in the proportion of copulating males was observed (data not shown). However, this effect was accompanied by a significant impairment of motor co-ordination (see Table 3).

Figure 2, panel C depicts the effect of 8-OH-DPAT treatment in both strains of mice. This 5-HT_{1A} agonist inhibited the sexual behaviour of WT mice, but not that of KO_{1B} animals. Thus, already at the dose of 0.125 mg kg^{-1} a complete inhibition of the copulatory behaviour of WT mice was obtained, while in KO_{1B} males only a tendency towards a reduction in the percentage of mice ejaculating is seen. Therefore two higher doses of this compound (0.25 and 0.50 mg kg^{-1}, $n=10$ each) were tested in independent groups of KO_{1B} mice. After the highest dose of 8-OH-DPAT, 70% of the KO_{1B} mice (7/10) were still able to mount and intromit and 60% (6/10) achieved ejaculation with no signs of impairment of motor co-ordination (see Table 2).

Table 2 shows the specific parameters of sexual behaviour of WT and KO_{1B} mice after the lowest dose of each pharmacological treatment: 5-HTP (12.5 mg kg^{-1}), TFMPP (0.5 mg kg^{-1}) and 8-OH-DPAT (0.0625 mg kg^{-1}). Statistical analyses were performed only at these dose levels since at higher doses almost no WT mouse copulated, precluding any comparison (see Figure 2A,B,C). Statistical comparisons within each Latin square showed that neither 5-HTP nor 8-OH-DPAT treatment, at these dose levels, modified any specific sexual behaviour parameter in either strain. On the other hand, the inhibitory effects of TFMPP in WT mice were manifested in an augmentation of mount and ejaculation latencies as well as in an increase in the number of intromissions and total mount events that preceded ejaculation. In KO_{1B} mice no specific parameter of sexual behaviour was affected by the 5-HT_{1B} agonist.

Table 3 Effect of serotonergic compounds on motor coordination of mice (rota-rod test)

Treatment	*WT*	*Treatment*	*KO_{1B}*
Control	0	Control	0
5-HTP 50.0 mg kg^{-1}	0	5-HTP 50.0 mg kg^{-1}	0
Control	0	Control	0
TFMPP 1.0 mg kg^{-1}	0	TFMPP 2.0 mg kg^{-1}	4*
Control	0	Control	0
8-OH-DPAT 0.125 mg kg^{-1}	0	8-OH-DPAT 0.50 mg kg^{-1}	0

Wilcoxon signed rank test $*P<0.02$.

In addition, when comparing these parameters between strains it became evident that the most pronounced difference was found after TFMPP treatment. Thus, except for the number of mounts and ejaculation latency, all sexual behaviour parameters appeared inhibited in WT animals when compared with KO_{1B} mice. Also, after 8-OH-DPAT treatment WT mice exhibited increased intromission latency and a larger number of mounts in comparison with the KO_{1B} strain. No significant differences between strains were found after 5-HTP treatment (Table 2).

Table 3 shows the results of the motor co-ordination tests, expressed as median number of falls, after treatment with 5-HTP, TFMPP and 8-OH-DPAT in WT and KO_{1B} mice. Clearly, only the highest dose of TFMPP tested in KO_{1B} mice (2.0 mg kg^{-1}) impaired motor co-ordination. No other treatment had unspecific motor effects in either strain.

Discussion

There is a large body of evidence sustaining that serotonin is coupled to both inhibitory and faciltatory mechanisms in the regulation of male rat sexual activity (Ahlenius & Larsson,

1998). The inhibitory effects of an increased serotonergic transmission appear to be exerted through 5-HT_{1B} receptors and the facilitatory mechanism to be coupled to the 5-HT_{1A} receptor subtype. In the present study, both augmentation of serotonergic transmission by 5-HTP and stimulation of either 5-HT_{1B} or 5-HT_{1A} receptor subtypes in male mice of the 129/Sv-ter strain (WT) resulted in an inhibition of sexual behaviour. These data show the existence of species differences in the role played by the different receptor subtypes in the mediation of the serotonergic influence on male sexual behaviour. On the other hand, apparently, strain differences can also be observed in the effect of serotonergic compounds. Thus, while in the present investigation 5-HTP treatment dose-dependently inhibited the percentage of WT mice showing intromission and ejaculation, a facilitatory effect of the serotonin precursor on specific parameters of the NMRI albino mice strain was reported (Svensson *et al*., 1987). However, in the same work a lack of effect of p-chlorophenilalanine (pCPA) treatment on male sexual behaviour, which inhibits serotonin synthesis, was found. Although the distinct results obtained are not easy to explain, a comparison of both works reveals, at a first glance, differences in the sensitivity to drugs between strains (129/Sv-ter *versus* NMRI). Thus, the 5-HTP doses tested in NMRI mice were 50 and 100 mg kg^{-1}, while present data show that 129/Sv-ter males neither intromitted not ejaculated after 25 mg kg^{-1} of 5-HTP and that the dose of 50 mg kg^{-1} caused a complete inhibition of sexual behaviour. There are no other published data analysing the effect of the enhancement of serotonergic transmission on the male sexual behaviour of mice.

Administration of the 5-HT_{1B} agonist, TFMPP, to WT mice exerted a clear inhibitory action on male sexual activity, suggesting an inhibitory role of this receptor subtype in the mediation of sexual behaviour in male mice. To our knowledge this is the first work testing the effect of stimulating the $5HT_{1B}$ receptor subtype on expression of mouse male sexual behaviour. In rats, stimulation of this receptor subtype has been found to inhibit ejaculatory behaviour, evidenced primarily by an increased number of intromissions preceding ejaculation and an increase in the ejaculation latency (Fernández-Guasti & Escalante, 1991; Gorzalka *et al*., 1990). Present data show that these same parameters are inhibited after TFMPP treatment in male mice of the WT strain, in addition to mount latency, which was significantly augmented. Thus, it seems that the role of the 5-HT_{1B} receptor is very similar in rats and mice.

On the other hand, the stimulation of the 5-HT_{1A} receptor subtype by 8-OH-DPAT produced a dose-dependent inhibition in the percentage of WT mice showing each of the male sexual behaviour responses (mount, intromission and ejaculation). When analysing the specific sexual behaviour parameters exhibited after the lowest dose of 8-OH-DPAT (0.0625 mg kg^{-1}), no statistically significant difference was found as compared to vehicle injection. However, a clear tendency towards an inhibition, very close to significance ($P=0.06$), was obtained in the ejaculation latency and the total mount events. This is an interesting finding, since these are the same parameters reported to be affected after 8-OH-DPAT treatment in rats, but in the opposite direction (Ahlenius *et al*., 1981; Fernández-Guasti & Escalante, 1991). Accordingly, in rats, 8-OH-DPAT produces a dramatic decrease in the ejaculation latency and the number of mounts and intromissions preceding ejaculation (Ahlenius *et al*., 1981; Hillegaart & Ahlenius, 1998; Hillegaart *et al*., 1991). In line with the data obtained in the present study, Svensson *et al*. (1987) found an inhibitory effect of 8-OH-DPAT treatment in male sexual behaviour of albino mice (NMRI strain). An inhibitory role for 5-HT_{1A} receptors in the control of masculine sexual behaviour has also been described for other species such as the rabbit (Paredes *et al*., 2000) and the ferret (Paredes *et al*., 1994). Thus, it appears that in mice both 5-HT_{1A} and 5-HT_{1B} receptors are involved in the inhibitory actions of serotonin on male copulatory behaviour.

Comparison of the spontaneous male copulatory behaviour of sexually experienced WT and KO_{1B} mice revealed that the knockout strain exhibits a certain degree of facilitation of specific copulatory behaviour parameters, but also a clear inhibition of others. The facilitation can be traced in the reduced mount and intromission latencies as well as in the increased percentage of KO_{1B} mice that exhibit mounts or intromissions as compared to its corresponding WT strain. The inhibition, by contrast, is evidenced in the increased ejaculation latency and the higher number of mounts and intromissions required by KO_{1B} mice to achieve ejaculation.

In the experimental analysis of male sexual behaviour a distinction has been made between the processes underlying the activation of this behaviour, the motivational component, and those underlying its execution, the copulatory-ejaculatory component. Sexual behaviour parameters have been considered to reflect either sexual motivation (latencies to the initiation of sexual activity and percentage of copulating animals) or the consummatory component of copulation (number of mount events and the time needed to achieve ejaculation) (Beach, 1956). Analysis of spontaneous male sexual behaviour in these terms shows that in KO_{1B} mice sexual motivation appears facilitated (shortening of ML and IL and increased percentage of mice showing mounts and intromission) and execution inhibited (increased number M and I and lengthened EL) when compared to the WT strain. The changes in the motivational component suggest that the 5-HT_{1B} receptor subtype could be involved in the inhibitory actions exerted by serotonin on sexual motivation, given the fact that in the WT strain, the pharmacological stimulation of this receptor subtype inhibits sexual motivation. By contrast, the consummatory component appears more profoundly inhibited in KO_{1B} mice than in the WT strain, contrary to what could be expected in the absence of the receptor subtype which stimulation inhibits this component in the WT strain. One possible explanation relies on the fact that the inhibitory actions of serotonin on this component could be modulated by more than one receptor. It should be kept in mind that although knockout mice were initially considered to represent some form of biological antagonism (Ramboz *et al*., 1996), when the observed behaviour is a composite result of different actions regulated by several receptor subtypes, then the gene deletion can result in a complex alteration of all the components participating in the regulation of that behaviour (Brunner *et al*., 1999; Nelson & Young, 1998). This could be the case of male sexual behaviour in KO_{1B} mice.

On the other hand, it has been reported that KO_{1B} mice exhibit an increased impulsiveness noticed in an increased aggression and drug abuse potential. Thus, KO_{1B} mice seem to attack faster and more often than do WT controls (Ramboz *et al*., 1996; Saudou *et al*., 1994), show faster acquisition of cocaine self-administration and higher motiva-

tion to self-administer this drug (Rocha *et al*., 1997; 1998). In fact, Brunner & Hen (1997) have proposed that the KO_{1B} strain could be a model of impulsive behaviour due to behavioural disinhibition. Consistent with this proposal, the present study reveals that KO_{1B} male mice mount females faster and more often than the WT strain, a finding that could be interpreted as another manifestation of increased impulsive behaviour.

The pharmacological manipulation of male sexual activity in KO_{1B} mice shows that the 5-HT_{1B} receptor agonist TFMPP lacked an effect, further confirming the absence of this receptor subtype. As described in the results section, when testing a higher dose of this compound (2.0 mg kg^{-1}) in KO_{1B} mice a reduction in the proportion of copulating males accompanied by motor co-ordination impairment was obtained. This last datum precludes any interpretation since copulation is an activity that depends on motor co-ordination. It is important to mention that TFMPP has been reported to have affinity for 5-HT_{1A} and 5-HT_{2C} receptors in addition to the 5-HT_{1B} subtype (Schoeffter & Hoyer, 1989), hence the effects on motor co-ordination could be exerted at these 5-HT receptor subtypes. No other pharmacological treatment altered motor co-ordination in any strain.

The enhancement of serotonergic transmission by 5-HTP had an inhibitory influence on male sexual activity of KO_{1B} mice but only at high doses (50 mg kg^{-1}) clearly manifested in the percentage of animals that achieved ejaculation. Stimulation of the 5-HT_{1A} receptor subtype with 8-OH-DPAT did not alter the copulatory behaviour in KO_{1B} mice. It has been reported that in this strain the 5-HT_{1A} receptor subtype may be desensitized (Dulawa *et al*., 1997; 2000; Knobelman *et al*., 2001). Therefore, two higher doses of 8-OH-DPAT were additionally tested, but even after the highest dose (0.5 mg kg^{-1}) 60% of the KO_{1B} mice were still able to ejaculate. These data show that KO_{1B} mice are less sensitive than the WT strain to the inhibitory actions of 5-HTP and 8-OH-DPAT on male sexual behaviour.

As it has been proposed, the deletion of a gene encoding for a specific receptor could induce plastic changes as a result of the adaptations that the organism may develop to compensate for the lack of the gene product (Brunner *et al*., 1999; Nelson & Young, 1998). Several authors have searched for putative plastic changes in KO_{1B} mice, finding that in these animals the brain serotonin transporter is down-regulated (Ase *et al*., 2001) and the levels of the dopaminergic D_1 receptor subtype increased (Zhuang *et al*., 1999). In relation to serotonergic receptor subtypes, autoradiographic studies have failed to reveal the existence of compensatory changes in brain 5-$HT_{1Dα}$, 5-HT_{1A}, 5HT_{2A}, 5-HT_{2C} receptor densities (Ase *et al*., 2001; Lucas *et al*., 1997). However, a difference between KO_{1B} and WT mice in the regulation of extracellular serotonin levels in the ventral hippocampus, but not in the striatum, has been reported. Thus, the systemic injection of 8-OH-DPAT evokes a significantly diminished response in KO_{1B} mice suggesting the potential desensitisation of 5-HT_{1A} receptors in the median raphe nucleus of this strain (Knobelman *et al*., 2001). In the present study, a diminished sensitivity of KO_{1B} mice to the actions of 5-HTP and 8-OH-DPAT on male sexual activity were also found. These data suggest that changes in the sensitivity of the 5-HT_{1A} receptor subtype can be found depending on the response analysed.

In conclusion, the present study provides evidence for a generalized inhibitory effect of serotonin on sexual behaviour in male mice. This conclusion arises from the fact that enhancement of serotonergic transmission and stimulation of either 5-HT_{1A} or 5-HT_{1B} receptor subtypes inhibited this behaviour in the WT strain. Furthermore, the male sexual behaviour of KO_{1B} mice is altered as compared with its corresponding WT strain, KO_{1B} mice become interested earlier in sexual behaviour, but require more stimulation to achieve ejaculation than the WT strain. Hence, we can assume that the 5-HT_{1B} receptor subtype is involved in the control of sexual activity in male mice. Finally, a diminished sensitivity to serotonergic stimulation was found in KO_{1B} mice that could rely on compensatory changes resulting from the absence of the 5-HT_{1B} receptor. Specific experiments to test this possibility should be conducted.

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