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European Journal of Pharmacology 330 (1997) 1–9

ejp

Flesinoxan: a prosexual drug for male rats

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Received 3 March 1997; revised 25 April 1997; accepted 2 May 1997

Abstract

Two tests were carried out to compare the stimulatory (i.e., prosexual) effects of the 5-HT_{1A} receptor agonists flesinoxan and 8-hydroxy-2-(di-*n*-propylamino)tetralin (8-OH-DPAT) on sexual behavior in male Wistar rats. Two groups of rats were used: normal males and males with impaired masculine sexual behavior due to neonatal treatment with the aromatase inhibitor 1,4,6-androstatriene-3,17-dione (ATD). In Experiment 1, flesinoxan (0.3 and 1.0 mg/kg) stimulated ejaculation frequency and number of animals displaying this behavior, both in controls and ATD males. With 0.3 mg/kg flesinoxan ATD males did not differ from controls in ejaculation frequencies. There was a concomitant decrease in latency to first ejaculation. No 'premature' ejaculations (i.e., at first or second intromission) were observed. In Experiment 2, the effects of 0.4 mg/kg 8-OH-DPAT, 0.3, 1.0 and 3.0 mg/kg flesinoxan and saline were tested in two ejaculation series. 'Premature' ejaculations only occurred during first ejaculation series with 8-OH-DPAT in 8 of 10 controls and in 6 of 9 ATD males; it did not occur during flesinoxan treatment nor in the second ejaculation series with 8-OH-DPAT treatment. Thus, flesinoxan stimulates sexual behavior in control rats and in rats with impaired sexual behavior. Unlike 8-OH-DPAT flesinoxan does not render them into 'premature' ejaculators. Therefore, flesinoxan could be considered a prosexual drug for male rats. © 1997 Elsevier Science B.V.

Keywords: Sexual behavior; Ejaculation; Flesinoxan; 8-OH-DPAT (8-hydroxy-2-(di-*n*-propylamino)tetralin); ATD (1,4,6-androstatriene-3,17-dione); (Wistar rat)

1. Introduction

The neurotransmitter serotonin (5-hydroxytryptamine, 5-HT) has been known for its involvement in male rat sexual behavior since the late sixties (e.g., Tagliamonte et al., 1969; Gessa and Tagliamonte, 1974). By now, there is quite an extensive literature on the proclaimed stimulatory effects of serotonergic agents, especially the various 5-HT_{1A} receptor agonists (e.g., 8-hydroxy-2-(di-*n*-propylamino)tetralin (8-OH-DPAT), buspirone, ipsapirone, flesinoxan) on male rat sexual behavior (Ahlenius and Larsson, 1988; Mos et al., 1990). The 5-HT_{1A} receptors are somatodendritic and terminal autoreceptors in the central nervous system, with the highest density in the dorsal raphe nuclei (Marsden and Kendall, 1992). Facilitation of these autoreceptors results in an inhibition of neuronal firing of rat raphe neurons, and subsequently in a fall of extracellular 5-HT in the striatum and ventral hippocampus (Marsden and Kendall, 1992; Kreiss and Lucki, 1994).

In male rats 8-OH-DPAT increases ejaculation frequency, but concomitantly decreases intromission frequency (Ahlenius et al., 1981; Haensel et al., 1991). Quite often such ejaculation behavior resulted in abnormal deposition of the ejaculate, suggesting that no proper penile intromission had occurred (Haensel et al., 1991). Therefore, we earlier suggested that 8-OH-DPAT renders male rats to become 'premature' ejaculators (Haensel et al., 1991) which could plead against naming this drug 'prosexual' (i.e., stimulating sexual behavior without altering the specific pattern of male rat sexual behaviors, for instance more animals to be sexually active, higher ejaculation frequencies, shorter latencies to ejaculation, shorter

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PII S0014-2999(97)00170-2

post-ejaculatory intervals, etc.; Kwong et al., 1986; Mos et al., 1990).

Flesinoxan, also a selective 5-HT_{1A} receptor agonist, is reported to react similarly as 8-OH-DPAT in behavioral and pharmacological male rat studies (Mos et al., 1990; Ybema et al., 1990; Ahlenius et al., 1991). However, critical reading of these studies indicates differences from 8-OH-DPAT: flesinoxan also increases ejaculation frequency but does not significantly affect other sexual behaviors. The present study was designed to investigate this in more detail.

The presumed prosexual properties of flesinoxan were studied in control rats and in male rats with impaired sexual behavior. Impaired sexual behavior can be found in male rats which were neonatally deprived of endogenous estrogen (Vreeburg et al., 1977; Davis et al., 1979). Such deprivation can be achieved by treating newborn male rats with the aromatase inhibitor ATD (1,4,6-androstatriene-3,17-dione), which blocks the aromatization of testicular testosterone to estradiol in specific areas of the central nervous system (Bakker et al., 1996). In adulthood, such ATD males show normal frequencies of mounts and intromissions, but no or very low frequencies of ejaculation behavior when tested early in the dark phase with a female rat in heat (Bakker et al., 1993).

2. Materials and methods

2.1. Animals and treatments

Female albino Wistar rats (HSD, Zeist, Netherlands) were time mated. Within 3 h after birth male pups received subcutaneously in the neck under hypothermic anesthesia a Silastic implant (inner diameter 1.5 mm, outer diameter 2.1 mm, effective length 5 mm) randomly filled with either ATD or left empty (placebo group). At 21 days of age, the implants were removed, the animals weaned and housed 2–3 of same sex and treatment to a cage with food and water available ad libitum. In adulthood, these males were behaviorally tested. Earlier studies revealed that adult ATD and control males have similar serum levels of endogenous testosterone, follicle stimulating hormone, luteinizing hormone and inhibin (Bakker et al., 1995). The day/night cycle was artificially maintained (dark: 5:30 p.m.–7:30 a.m.). Temperature in the air-conditioned room ranged from 20 to 22°C.

Adult stimulus female rats were ovariectomized via bilateral incisions under ether anesthesia. They were brought into behavioral estrus by injecting 20 μg estradiol benzoate in 0.1 ml olive oil 24 h prior to testing, followed by 2.5 mg progesterone in 0.1 ml oil 3–4 h before testing.

A fresh solution of flesinoxan (kindly provided by Dr J. Mos, Solvay-Duphar, Weesp, Netherlands) or 8-OH-DPAT ((±)-8-hydroxy-2-(di-*n*-propylamino)tetralin · HBr; Research Biochemicals International, Natick, MA, USA) was dissolved in saline approximately 1 h before testing to a volume of 1 ml/kg body weight. Flesinoxan was administered intraperitoneally (i.p.) and 8-OH-DPAT was given subcutaneously (s.c.).

2.2. Behavioral testing

Pair tests with an estrous female were carried out in a semicircular arena, measuring $62 \times 40 \times 36$ cm, with a wire mesh floor and a transparent front. Testing started about 1 h after the onset of the dark cycle and the males were allowed to adapt to the test cage for at least 15 min. The test room was dimly illuminated with indirect white light. At least 3 pair tests preceded the onset of experimental testing.

The following sexual behaviors were recorded: mount latency (time from start of test till first mount with pelvic thrusts); intromission latency (time from start of test till first intromission); ejaculation latency (time from first mount or intromission till first ejaculation); mounts (number of mounts with pelvic thrusts); number of intromissions; number of ejaculations; post-ejaculatory interval (time from ejaculation to first following sexual behavior).

2.3. Statistical analysis

The data were subjected to two- or three-way analyses of variance (ANOVA) for repeated measures (Perlman, 1986). If there was an overall statistically significant effect, the least significant difference (LSD) test was used to make pairwise comparisons amongst means (Kirk, 1968). The 0.05 level of probability (2-tailed) was adopted as the level of statistical significance.

3. Results

3.1. Experiment 1: Flesinoxan and sexual behavior in ATD male rats and controls

It was earlier reported that 0.2 and 0.4 mg/kg 8-OH-DPAT increased ejaculation frequencies in ATD males (Brand et al., 1991). Ahlenius et al. (1991) reported facilitation of male rat ejaculatory behavior by flesinoxan similar to the effect of 8-OH-DPAT in normal male rats. Experiment 1 was carried out to investigate the behavioral effects of flesinoxan (two doses) in ATD males and controls.

3.1.1. Methods

Twenty-three heterosexually experienced male rats were used, 12 controls and 11 ATD males, aged 47 weeks, with a mean body weight of 490 g (range: 375–575 g). In six weekly pair tests the animals received consecutively: noth-

ing, saline, 0.3 mg/kg flesinoxan, saline, 1.0 mg/kg flesinoxan and saline. The drug or vehicle was administered intraperitoneally, in a volume of 1 ml/kg. Behavioral testing lasted 15 min.

3.1.2. Results

As can be learned from Table 1, all sexual behaviors except mount latency showed a statistically significant difference over the tests. Most striking effects were found with flesinoxan treatment in ejaculation behavior: almost all animals displayed the behavior. With flesinoxan the mean frequencies of ejaculations per test increased and the mean latencies to first ejaculation decreased in both groups. Although not statistically different (LSD(5%) = 1.1), highest mean ejaculation frequencies were seen with flesinoxan treatment (0.3 and 1.0 mg/kg) both in ATD males and controls. The number of animals ejaculating increased during flesinoxan treatment: with the low dose 9 of 11 (82%) ATD males, and 11 of 12 (92%) controls, and with the high dose all animals ejaculated. In the blank and saline tests clearly less ATD males displayed ejaculatory behavior. Post-hoc analysis with McNemar's test revealed that with 1.0 mg/kg flesinoxan significantly more ATD males ejaculated (11 of 11) than in the blank test (3 of 11; $P < 0.01$) and than in the first saline test (5 of 11; $P < 0.05$).

The shortest latencies to first ejaculation were found in both groups during flesinoxan treatment. This effect was most outspoken in the ATD males. Post-hoc analysis revealed that during flesinoxan treatment (0.3 and 1.0 mg/kg) the latencies to first ejaculation no longer differed between controls and ATD males (LSD(5%) = 137). Combining data of ATD males and controls, shortest latencies (mean = 278 s) were found with 1.0 mg/kg versus 360 s with 0.3 mg/kg treatment, but this difference was not statistically significant (LSD(5%) = 314).

To investigate a possible period effect of testing, a subsequent 2-way analysis of variance was performed on the three saline tests only. No statistically significant differences were found in latencies. There was an effect of groups ($P < 0.04$) but not of tests in the number of ejaculations. In the ATD group, the number of animals ejaculating did not differ between the first and third saline test (5/11 vs. 8/11, Fisher exact probability test, n.s.). The number of mounts was significantly higher in the first saline test in both control and ATD males than the two subsequent saline tests ($P = 0.002$). With respect to the number of intromissions during the three saline tests the only significant difference appeared between the first and second saline test in both groups of males ($P < 0.02$); the first and second saline test did not differ from the third test.

Surprisingly, during flesinoxan treatment, none of the males ejaculated 'prematurely', i.e., during the first or second intromission. To investigate this phenomenon in more detail, a second experiment was performed.

3.2. Experiment 2: A comparison between the effects of 8-OH-DPAT and flesinoxan on sexual behavior in ATD males and control rats

From Experiment 1 it became clear that flesinoxan had prosexual (i.e., sexually stimulating) effects in adult male rats. The stimulatory effects seemed different from what has been described for 8-OH-DPAT: with flesinoxan the rats showed higher frequencies and shorter latencies of sexual behaviors, and 8-OH-DPAT rendered them into 'premature' ejaculators (Haensel et al., 1991). Experiment 2 was carried out to compare the behavioral effects of 0.4 mg/kg 8-OH-DPAT and flesinoxan (in three different doses) in control and ATD males. The dose of 0.4 mg/kg was chosen on the basis of various earlier experiments (Brand et al., 1991; Haensel et al., 1991, 1993; Mos et al., 1990). Testing lasted normally till the second ejaculation. This enabled us to study the presumed stimulatory effects of both 5-HT_{1A} receptor agonists in two consecutive ejaculation series.

3.2.1. Methods

Nine ATD male rats and ten controls (aged 5 months, mean body weight 355 g, range: 295–395 g) were pair-tested once weekly with an estrous female and received various consecutive drug treatments 30 min prior to testing: blank, 0.4 mg/kg 8-OH-DPAT s.c., 1 ml/kg saline s.c., 0.3 mg/kg flesinoxan i.p., 1.0 mg/kg i.p., 3.0 mg/kg flesinoxan i.p., 1 ml/kg saline i.p. Animals were tested till second ejaculation or for 25 min. The first ejaculation series is defined as the time from start of testing till first ejaculation. The second ejaculation series is defined as the time from first sexual behavior after first ejaculation till second ejaculation. Between first ejaculation and the onset of the second ejaculation series is the (first) refractory period.

3.2.2. Results

Various sexual behaviors are presented in Table 2. All control male rats ejaculated twice in each test. Only during 5-HT_{1A} receptor agonist treatment all ATD males ejaculated twice. From inspecting the data it is clear that 5-HT_{1A} receptor agonist treatment affected sexual behaviors of ATD and control males, both during the first and the second ejaculation series. In general, mount and intromission frequencies were lower, and mount, intromission and ejaculation latencies were shorter during 5-HT_{1A} receptor agonist treatment than during blank or saline treatment. Because the objective of this experiment was to compare the effects of two different 5-HT_{1A} receptor agonists, these data were subjected to 3-way analysis of variance, with factors groups, test and ejaculation series (see Fig. 1 and Table 3).

When comparing 8-OH-DPAT with flesinoxan treatment, some interesting differences were found. During the first ejaculation series (Fig. 1, hatched bars) the differences

Table 1
Experiment 1. Various sexual behaviors (mean ± S.E.M. (*n*)) of male rats: 12 control Wistar rats (Contr) and 11 rats neonatally treated with ATD

Behavior	Male groups	Consecutive treatments												Statistics	
		Blank		Saline		Flesinoxan		Saline		Flesinoxan		Saline		2-way	
			(*n*) [a]	i.p.	(*n*)	0.3 mg/kg i.p.	(*n*)	i.p.	(*n*)	1.0 mg/kg i.p.	(*n*)	i.p.	(*n*)	ANOVA:	*P*
Mounts	Contr	25.9 ± 4.2	(12)	24.2 ± 5.1	(12)	22.9 ± 3.6	(12)	13.4 ± 2.2	(12)	14.1 ± 2.2	(12)	14.9 ± 2.6	(12)	Test	< 0.001
	ATD	19.9 ± 2.0	(11)	17.8 ± 2.1	(11)	25.5 ± 3.1	(11)	12.2 ± 3.7	(11)	16.8 ± 1.9	(11)	10.1 ± 1.7	(11)	group	n.s.
														t × g	n.s.
Intromissions	Contr	10.8 ± 1.5	(12)	15.8 ± 1.2	(12)	13.2 ± 1.5	(12)	11.2 ± 1.6	(11)	14.2 ± 1.5	(12)	14.5 ± 1.8	(12)	Test	0.007
	ATD	11.3 ± 2.0	(11)	14.5 ± 1.8	(11)	12.7 ± 1.0	(11)	12.3 ± 1.5	(11)	12.6 ± 1.3	(11)	14.4 ± 1.1	(11)	group	n.s.
														t × g	n.s.
Ejaculations	Contr	1.0 ± 0.2	(9)	1.8 ± 0.2	(11)	2.2 ± 0.3	(11)	1.8 ± 0.3	(10)	2.3 ± 0.2	(12)	2.2 ± 0.3	(10)	Test	< 0.001
	ATD	0.5 ± 0.2	(3)	0.9 ± 0.3	(5)	1.7 ± 0.4	(9)	1.1 ± 0.3	(6)	1.7 ± 0.2	(11)	1.3 ± 0.4	(8)	group	0.034
														t × g	n.s.
Mount latency (s)	Contr	9.5 ± 2.1		6.4 ± 1.1		6.2 ± 0.8		21.8 ± 13.2		5.2 ± 1.0		4.8 ± 0.8		Test	(0.075)
	ATD	5.6 ± 0.7		6.0 ± 0.7		7.0 ± 1.6		13.5 ± 6.9		5.3 ± 1.5		8.8 ± 3.5		group	n.s.
														t × g	n.s.
Intromission latency (s) [b]	Contr	47.6 ± 17.7		13.8 ± 3.6		20.1 ± 5.9		119.8 ± 73.4		13.0 ± 4.7		10.5 ± 2.9		Test	0.021
	ATD	77.1 ± 32.2		23.8 ± 14.7		19.3 ± 5.2		93.3 ± 75.4		7.6 ± 1.2		15.2 ± 5.5		group	n.s.
														t × g	n.s.
Ejaculation latency (s) [b]	Contr	600 ± 74		326 ± 62		339 ± 87		365 ± 81		222 ± 32		339 ± 79		Test	< 0.001
	ATD	748 ± 79		641 ± 106		381 ± 95		520 ± 113		333 ± 69		474 ± 92		group	(0.071)
														t × g	n.s.

Animals were pair-tested for 15 min with an estrous female in a semicircular arena, 30 min after drug administration.
[a] Number of animals displaying the behavior.
[b] Non-responders: 900 s.

Table 2
Experiment 2. Various sexual behaviors (mean ± S.E.M. (*n*)) of male rats: 10 control Wistar rats (Contr) and 9 rats neonatally treated with ATD

Behavior	Male groups	Consecutive treatments													
		Blank		8-OH-DPAT		Saline		Flesinoxan		Flesinoxan		Flesinoxan		Saline	
			(*n*) [a]	0.4 mg/kg s.c.	(*n*)	s.c.	(*n*)	0.3 mg/kg i.p.	(*n*)	1.0 mg/kg i.p.	(*n*)	3.0 mg/kg i.p.	(*n*)	i.p.	(*n*)
First ejaculation series															
Mounts	Contr	13.9 ± 1.8	(10)	0.5 ± 0.2	(10)	11.8 ± 1.8	(10)	7.0 ± 1.2	(10)	7.0 ± 1.2	(10)	3.5 ± 0.7	(10)	10.1 ± 2.9	(10)
	ATD (ejac. only) [b]	25.0 ± 0.7	(2)	1.1 ± 0.6	(9)	20.5 ± 3.0	(6)	8.9 ± 1.3	(9)	8.3 ± 2.4	(9)	2.3 ± 0.8	(9)	13.1 ± 3.2	(8)
	ATD (all)	23.2 ± 3.9	(9)			24.0 ± 2.0	(9)							16.6 ± 2.8	(9)
Intromissions	Contr	10.9 ± 1.0	(10)	0.3 ± 0.2		13.5 ± 1.1	(10)	10.9 ± 1.3		9.6 ± 1.2		6.5 ± 1.1		12.3 ± 1.6	(10)
	ATD (ejac. only)	14.0 ± 1.4	(2)	0.8 ± 0.3		19.0 ± 1.9	(6)	13.8 ± 1.4		7.7 ± 1.4		5.4 ± 0.8		14.1 ± 1.9	(8)
	ATD (all)	13.7 ± 2.5	(9)			21.3 ± 1.7	(9)							18.4 ± 1.7	(9)
Mount	Contr	24.2 ± 9.5	(10)	17.8 ± 2.7		18.9 ± 8.0	(10)	7.4 ± 1.9		8.4 ± 3.5		6.1 ± 1.5		9.7 ± 3.8	(10)
latency (s)	ATD (all)	25.1 ± 15.4	(9)	11.6 ± 1.6		15.0 ± 6.0	(9)	7.3 ± 2.8		13.0 ± 4.3		23.4 ± 7.4		15.4 ± 5.5	(9)
Intromission	Contr	39.5 ± 9.4	(10)	21.1 ± 2.9		27.5 ± 9.2	(10)	15.6 ± 4.4		16.6 ± 3.9		15.0 ± 4.2		19.3 ± 3.5	(10)
latency (s)	ATD (all)	90.7 ± 46.2	(9)	34.4 ± 18.1		22.4 ± 5.8	(9)	10.9 ± 3.0		19.0 ± 6.6		31.8 ± 11.2		39.9 ± 19.0	(9)
Ejaculation	Contr	412 ± 51	(10)	13 ± 7		413 ± 37	(10)	201 ± 22		161 ± 24		129 ± 23		382 ± 63	(10)
latency (s)	ATD (ejac. only)	536 ± 99	(2)	47 ± 20		825 ± 128	(6)	337 ± 54		210 ± 41		103 ± 10		664 ± 125	(8)
	ATD (all) [c]	> 1264	(9)			> 1043	(9)							> 756	(9)
Post-ejaculatory	Contr	416 ± 10	(10)	462 ± 18	(10)	307 ± 15	(10)	263 ± 9	(10)	230 ± 11	(10)	314 ± 23	(10)	286 ± 19	(10)
interval (s)	ATD (ejac. only)	390 ± 51	(2)	222 ± 31	(9)	265 ± 20	(6)	214 ± 7	(9)	152 ± 22	(9)	160 ± 20	(9)	276 ± 23	(8)
Second ejaculation series															
Mounts	Contr	11.2 ± 1.6	(10)	2.6 ± 0.4	(10)	13.0 ± 3.2	(10)	4.7 ± 0.9	(10)	3.8 ± 0.8	(10)	2.9 ± 0.4	(10)	6.8 ± 1.5	(10)
	ATD (ejac. only)	6.0 ± 2.1	(2)	3.4 ± 0.5	(9)	7.3 ± 2.2	(3)	6.3 ± 1.2	(9)	7.7 ± 2.4	(9)	4.0 ± 0.5	(9)	5.0 ± 1.3	(5)
Intromissions	Contr	6.4 ± 0.9	(10)	2.8 ± 0.3		6.4 ± 0.9	(10)	5.2 ± 0.7		3.7 ± 0.4		3.4 ± 0.3		5.6 ± 0.8	(10)
	ATD (ejac. only)	6.0 ± 0.7	(2)	2.8 ± 0.3		4.0 ± 0.9	(3)	6.1 ± 0.5		5.7 ± 0.8		4.3 ± 0.8		5.4 ± 0.4	(5)
Ejaculation	Contr	269 ± 26	(10)	134 ± 20		276 ± 60	(10)	113 ± 18		84 ± 8		117 ± 22		153 ± 23	(10)
latency (s)	ATD (ejac. only)	165 ± 0	(2)	178 ± 36		203 ± 11	(3)	215 ± 23		243 ± 55		151 ± 22		190 ± 24	(5)

Animals were tested with an estrous female during 25 min, or till second ejaculation. Drug administration occurred intraperitoneally (i.p.) or subcutaneously (s.c.) 30 min before start of test. First ejaculation series was defined as time from start of test till first ejaculation, and second ejaculation series as time from first sexual behavior after first ejaculation to second ejaculation. The time between first and onset of second ejaculation series was defined as post-ejaculatory interval.

[a] Number of animals displaying the behavior.

[b] Ejac. only: ejaculators only.

[c] Non-ejaculators: 1500 s.

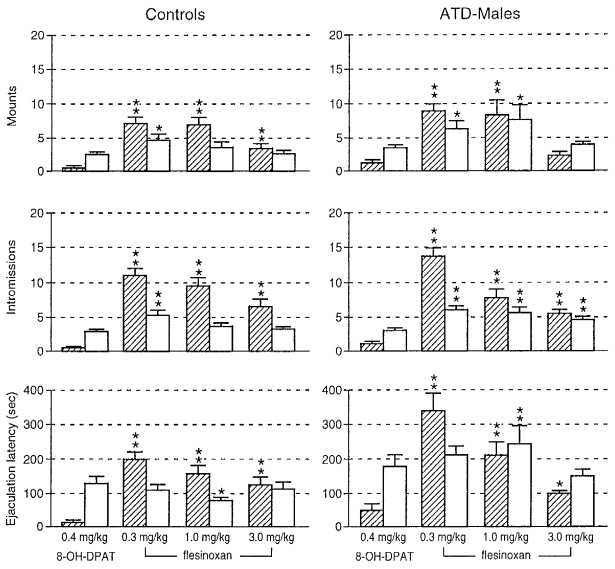

Fig. 1. Various sexual behaviors (mean ± S.E.M.) of two groups of male rats: controls ($n = 10$) and neonatally ATD-treated males ($n = 9$) during the first (hatched bars) and second (open bars) ejaculation series. Asterisks indicate a statistically significant difference ($^{*}P < 0.05$, $^{**}P < 0.01$) between flesinoxan and 8-OH-DPAT treatment, per group and per ejaculation series. Animals were pair-tested with an estrous female until second ejaculation. Drugs were administered 30 min before testing.

between 8-OH-DPAT and flesinoxan are most obvious, both in control and ATD males. Compared to flesinoxan, lower mount and intromission frequencies and a shorter ejaculation latency to first ejaculation were found with 8-OH-DPAT (Fig. 1).

During the first ejaculation series there appeared to be a dose-dependent effect of flesinoxan: with higher doses of flesinoxan there was a decrease in mean number of mounts (LSD(5%) = 1.9), of intromissions (LSD(5%) = 1.1) and in mean ejaculation latency (LSD(5%) = 41) in all cases. Post-hoc analyses revealed a significant effect for all behaviors with 0.3 vs. 3.0 mg/kg flesinoxan, and for all three doses of flesinoxan with intromissions prior to first ejaculation.

Ejaculation with the first or second intromission (i.e., 'premature' ejaculations) occurred only with 8-OH-DPAT treatment and only during the first ejaculation series in 8 of 10 control males, and in 6 of 9 ATD males. Premature ejaculations were not observed with 0.3, 1.0 or 3.0 mg/kg flesinoxan treatment.

With regard to the post-ejaculatory interval after first ejaculation it was found that there was a difference of groups ($P < 0.001$), of tests ($P < 0.001$) and a significant group × test interaction ($P < 0.001$). With 0.4 mg/kg 8-OH-DPAT controls had a post-ejaculatory interval that was more than twice as long as ATD males ($P < 0.001$), whereas there was no significant difference between the groups with 0.3 mg/kg flesinoxan (LSD(5%) = 45: n.s.).

During the second ejaculation series (Fig. 1, open bars) the mean differences between 8-OH-DPAT treatment and flesinoxan were smaller. In mean number of mounts prior to second ejaculation, 0.3 mg/kg flesinoxan differed from all other treatment tests in control rats; in ATD males, 0.3 and 1.0 mg/kg flesinoxan differed from 0.4 mg/kg 8-OH-DPAT and 3.0 mg/kg flesinoxan (LSD(5%) = 1.9). When comparing the mean number of intromissions prior to second ejaculation, 0.4 mg/kg 8-OH-DPAT differed from 0.3 mg/kg flesinoxan in control rats, and from all doses of flesinoxan treatment in ATD males (LSD(5%) = 1.1). Latency to second ejaculation was significantly longer with 1.0 mg/kg flesinoxan than with 0.4 mg/kg 8-OH-DPAT in both groups (LSD(5%) = 41). In ATD males, 0.3 mg/kg flesinoxan also differed from 3.0 mg/kg flesinoxan.

4. Discussion

The main findings of the two experiments can be summarized as follows. Flesinoxan treatment stimulated ejaculation frequency and decreased ejaculation latency in normal male rats and in males with impaired sexual behavior (neonatal ATD treatment). This was in line with results of earlier experiments with another 5-HT_{1A} receptor ago-

Table 3
Statistical results of data collected in Experiment 2

Three-way ANOVA	Prior to ejaculation		Ejaculation latency: *P*
Factor	Mounts: *P*	Intromissions: *P*	
Test (t)	< 0.001	< 0.001	< 0.001
Group (g)	n.s.	n.s.	0.019
1st vs. 2nd ejaculation series (e)	n.s.	< 0.001	n.s.
Interactions:			
t × g	n.s.	n.s.	0.026
t × e	< 0.001	< 0.001	< 0.001
g × e	n.s.	n.s.	(0.069)
t × g × e	n.s.	(0.054)	n.s.

See footnote to Table 2. Three-way analyses of variance were performed on tests with flesinoxan and 8-OH-DPAT only. Two-tailed *P*-values of ≤ 0.10 are indicated. *P*-values of ≤ 0.05 are considered to be statistically significant.

nist, 8-OH-DPAT (Ahlenius et al., 1991; Brand et al., 1991; Haensel et al., 1991). With flesinoxan treatment, sexual behavior of ATD males did not differ from control rats. Surprisingly, with flesinoxan no 'premature' ejaculations (i.e., ejaculation with the first or second intromission) were observed in either group in both experiments. The most striking differences between 8-OH-DPAT and flesinoxan appeared during the first ejaculation series: nine of ten controls and six of nine ATD males ejaculated 'prematurely' with 8-OH-DPAT but none of the animals did so with flesinoxan. When looking at the second ejaculation series, the differences between 8-OH-DPAT and flesinoxan were remarkably smaller. The most striking differences between 8-OH-DPAT and flesinoxan on the stimulatory properties on male rat sexual behavior were seen during the first ejaculation series. For example, in control animals, mounts, intromissions and ejaculation latency were significantly higher with the highest dose of flesinoxan than with 8-OH-DPAT during the first ejaculation series, but were not significantly different during the second ejaculation series.

When testing moderately experienced rats, Mos et al. (1990) found similar results for the effect of 8-OH-DPAT and flesinoxan on sexual behavior in male rats. Administration of various serotonergic drugs, including 8-OH-DPAT and flesinoxan, resulted in an increase of ejaculation frequencies, and a decrease of ejaculation latency and mount and intromission frequencies prior to ejaculation. Compared to flesinoxan, the effect of 8-OH-DPAT appeared to be 'stronger'. Unfortunately, Mos et al. (1990) gave no details on how many rats ejaculated at the first or second intromission with the different drugs, and only the first ejaculation series was described.

Ahlenius et al. (1991) concluded that flesinoxan and 8-OH-DPAT facilitated sexual behavior in a similar way, with flesinoxan being about an order of magnitude less potent than 8-OH-DPAT. This differs from the present study on some major points (e.g., intromissions prior to first ejaculation, post-ejaculatory interval). There is no easy explanation for these discrepancies: animal strains, housing and testing conditions and drug doses were all comparable. The only difference between the two studies is the administration route of flesinoxan: intraperitoneally in the present study versus subcutaneously by Ahlenius et al. (1991).

The difference in facilitation of male rat sexual behavior between the two selective 5-HT_{1A} receptor agonists flesinoxan and 8-OH-DPAT can possibly be explained by an administration route effect or a different pharmacological affinity pattern. The latter possibility would imply a role for dopamine, since flesinoxan has a limited co-affinity for the dopamine D_2 receptor (Olivier et al., 1995). Administration of various dopamine receptor agonists facilitates several aspects of copulatory behavior and ex copula genital responses (e.g., Napoli-Farris et al., 1984; Bitran and Hull, 1987). When testing male rat sexual behavior with a selective dopamine D_2 receptor agonist, like SND 919 (pramipexol) there was a significant decrease of mounts and intromissions prior to ejaculation, and of ejaculation latency in normal male rats (Ferrari and Giuliani, 1994). However, none of the studies report ejaculations at the first or second intromission with dopaminergic agents. The stimulatory properties of flesinoxan on both the 5-HT_{1A} autoreceptor and the dopamine D_2 receptor could work synergistically to decrease ejaculation latency, and the (limited) affinity for the dopamine D_2 receptor of flesinoxan could prevent male rats from ejaculating prematurely. Recently, 8-OH-DPAT is described to have a limited co-affinity for the 5-ht_7 receptor, which is mainly located in the rat hypothalamus (Sleight et al., 1995). To our knowledge, no selective 5-ht_7 receptor agonists have been described.

Administration of 8-OH-DPAT renders male rats to become premature ejaculators: very short latency to ejaculation, and often ejaculation during the first or second intromission (Haensel et al., 1991; present study). From the present study, flesinoxan could be considered a prosexual drug for male rats: normal sexual behavioral pattern, shorter latencies, higher ejaculation frequency and a shorter post-ejaculatory interval.

Recently, several studies have been published on the treatment of premature ejaculation in humans with (selective) serotonin reuptake inhibitors like clomipramine (Althof et al., 1995; Haensel et al., 1996), paroxetine (Waldinger et al., 1994; Ludovico et al., 1996), and fluoxetine (Graziottin et al., 1996; Lee et al., 1996). Behavioral experiments with selective 5-HT reuptake inhibitors on rats have shown inhibition of male rat sexual behavior (Yells et al., 1994), a long-lasting decrease of the ejaculatory response after a single dose of fluoxetine (Rényi, 1986) and increase of intermount-bout intervals, time-outs, grooming time, ejaculation latency, number of mounts per mount bout, and number of mount bouts prior to ejaculation (Yells et al., 1995). Hence, the administration of serotonin reuptake inhibitors results in a higher concentration of 5-HT in the synapse with a decrease of various ejaculatory behaviors, and administration of 5-HT_{1A} receptor agonists results in a lower concentration of 5-HT in the synapse (Kreiss and Lucki, 1994) with a subsequent increase in ejaculatory behaviors. Apparently, the 5-HT levels in the synapse determine the ejaculatory latency. It remains to be demonstrated whether or not the effects on sexual behavior of 8-OH-DPAT and flesinoxan in male rats could be antagonized by simultaneous administration of a selective serotonin reuptake inhibitor.

Flesinoxan is currently being tested in humans on neuroendocrinological effects and body temperature (e.g., De Koning and De Vries, 1995), on depression (e.g., Grof et al., 1993) and suicidal behavior (Pitchot et al., 1995). From the present study it is clear that these human studies should also investigate possible effects on the sexual behavior of the male subjects.

Acknowledgements

The authors would like to thank J. van Ophemert for the animal care and assistance throughout the experiment, Dr. J. Bakker for performing the neonatal operations, Dr. W.C.J. Hop for statistical advice and help, and Dr. J. Mos for critical reading of an earlier version of the manuscript.

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