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Facilitation and inhibition of male rat ejaculatory behaviour by the respective 5-HT_{1A} and 5-HT_{1B} receptor agonists 8-OH-DPAT and anpirtoline, as evidenced by use of the corresponding new and selective receptor antagonists NAD-299 and NAS-181

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1 Ejaculatory problems and anorgasmia are well-known side-effects of the SSRI antidepressants, and a pharmacologically induced increase in serotonergic neurotransmission inhibits ejaculatory behaviour in the rat. In the present study the role of 5-HT_{1A} and 5-HT_{1B} receptors in the mediation of male rat ejaculatory behaviour was examined by use of selective agonists and antagonists acting at these 5-HT receptor subtypes.

2 The 5-HT_{1A} receptor agonist 8-OH-DPAT (0.25–4.00 μmol kg^{-1} s.c.) produced an expected facilitation of the male rat ejaculatory behaviour, and this effect was fully antagonized by pretreatment with the new selective 5-HT_{1A} receptor antagonist (*R*)-3-*N*,*N*-dicyclobutylamino-8-fluoro-3,4-dihydro-2*H*-1-benzopyran-5-carboxamide hydrogen (2*R*,3*R*) tartrate monohydrate (NAD-299) (1.0 μmol kg^{-1} s.c.). NAD-299 by itself (0.75–3.00 μmol kg^{-1} s.c.) did not affect the male rat ejaculatory behaviour.

3 The 5-HT_{1B} receptor agonist anpirtoline (0.25–4.00 μmol kg^{-1} s.c.) produced a dose-dependent inhibition of the male rat ejaculatory behaviour, and this effect was fully antagonized by pretreatment with the 5-HT_{1B} receptor antagonist isamoltane (16 μmol kg^{-1} s.c.) as well as by the new and selective antagonist (*R*)-(+)-2-(3-morpholinomethyl-2*H*-chromene-8-yl)oxymethylmorpholino methansulphonate (NAS-181) (16 μmol kg^{-1} s.c.). Isamoltane (1.0–16.0 μmol kg^{-1} s.c.) and NAD-181 (1.0–16.0 μmol kg^{-1} s.c.) had no, or weakly facilitatory effects on the male rat ejaculatory behaviour. The non-selective 5-HT_1 receptor antagonist (−)-pindolol (8 μmol kg^{-1} s.c.), did not antagonize the inhibition produced by anpirtoline.

4 The present results demonstrate opposite effects, facilitation and inhibition, of male rat ejaculatory behaviour by stimulation of 5-HT_{1A} and 5-HT_{1B} receptors, respectively, suggesting that the SSRI-induced inhibition of male ejaculatory dysfunction is due to 5-HT_{1B} receptor stimulation.

Keywords: Serotonin; ejaculation; 5-HT_{1A} receptors; 5-HT_{1B} receptors; male rat

Introduction

Disturbances in mechanisms of ejaculation in men, as well as anorgasmia in both sexes, are well-known side effects of the selective serotonin reuptake inhibitor (SSRI) antidepressants (Modell *et al*., 1997). An enhancement of synaptic availability of 5-hydroxytryptamine (5-HT), as produced by 5-hydroxytryptophan (5-HTP), in combination with a monoamine oxidase inhibitor and/or inhibition of neuronal 5-HT reuptake by tricyclic antidepressants or SSRIs, results in an inhibition of male rat ejaculatory behaviour (e.g. Malmnäs, 1973; Ahlenius *et al*., 1980). This is in all probability due to an increased stimulation of postsynaptic 5-HT receptors in the CNS since: (1) The local application of 5-HT in the basal forebrain terminal areas of serotonergic projections produce a similar delay of ejaculation, as seen after systemic 5-HTP administration (Hillegaart *et al*., 1991); (2) The local application of 5-HT onto inhibitory autoreceptors on serotonergic cell bodies of origin for ascending projections, is followed by a facilitation of male rat ejaculatory behaviour (Hillegaart *et al*., 1989). Available evidence suggests that the postsynaptic receptor is a 5-HT_{1B} receptor (Fernandez-Guasti *et al*., 1992; Ahlenius & Larsson, 1998), and the presynaptic receptor a 5-HT_{1A} receptor (Hillegaart *et al*., 1991).

Direct confirmation of the 5-HT receptor subtypes involved, however, has been hampered by lack of selective pharmacological tools. This situation has changed recently by the advent of new selective agonists and antagonists. Thus, NAD-299 (Johansson *et al*., 1997) represents a new antagonist at the 5-HT_{1A} receptor site. Anpirtoline (Engel *et al*., 1989; Swedberg *et al*., 1992) has been characterized as a 5-HT_{1B} receptor agonist, whereas isamoltane (Renyi *et al*., 1991) and NAS-181 (Berg *et al*., 1998) in particular, represents new 5-HT_{1B} receptor antagonists. As 5-HT_{1A} receptor agonist we used the well defined prototype agent 8-hydroxy-2-di-*n*-propylamino)tetralin (8-OH-DPAT) (Arvidsson *et al*., 1981; Hjorth *et al*., 1982). The availability of these selective tools made the present study possible, where we examined the specific roles of brain 5-HT_{1A} and 5-HT_{1B} receptor subtypes in the mediation of male rat ejaculatory behaviour.

Methods

Animals

Adult male and female Wistar rats (B&K Universal AB, Sollentuna, Sweden) were used. The animals arrived in the laboratory at least 10 days prior to start of the experiments, in

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order to adapt to the laboratory environmental conditions of controlled light–dark cycle (12:12 h, lights off 10.00 h), relative humidity (55–65%) and temperature (21.0±0.4°C). The animals were housed five per cage (Makrolon® IV). Food (R36, Ewos, Södertälje, Sweden), and tap water were available *ad libitum* in the home cage.

The studies were approved by the Stockholm South Local Ethical Committee on Animal Experiments.

Drugs

(±)-8-Hydroxy-2-(di-*n*-propylamino)tetralin HBr (8-OH-DPAT), mol wt 328.29 (RBI, Natick, MA, U.S.A.); anpirtoline HCl, mol wt 265.21 (Tocris Cookson, Bristol, U.K.); isamoltane HCl, mol wt 250.7 (Novartis, Basel, Switzerland); (*R*)-(+)-2-(3-morpholinomethyl-2*H*-chromene-8-yl) oxymethylmorpholino methansulphonate (NAS-181), mol wt 442.52 (Astra Arcus AB, Sweden); (*R*)-3-*N*,*N*-dicyclobutylamino-8-fluoro-3,4-dihydro-2*H*-1-benzopyran-5-carboxamide hydrogen (2*R*,3*R*) tartrate monohydrate (NAD-299), mol wt 486.50 (Astra Arcus AB). The above drugs were all dissolved in 0.9% NaCl. (−) Pindolol, mol wt 248.33 (RBI), was dissolved in a minimum of glacial acetic acid, and the volume was made up with 5.5% glucose. Controls were given the appropriate vehicle. All drugs were injected s.c. in a volume of 2 ml kg^{-1}. Females were brought into estrus by sequential treatment with estradiol benzoate (Fluka, Buchs, Switzerland) 12.5 μg $animal^{-1}$ 48 h, and progesterone (Fluka) 0.5 mg $animal^{-1}$ 6 h, before the observations. The hormones were dissolved in fractionated coconut oil (Miglyol®-812, Nobel Chemicals, Karlskoga, Sweden), and injected s.c. in a volume of 0.1 ml $animal^{-1}$.

Behavioural observations

Male rats, sexually experienced in at least four pretests over a 2-week period, were presented with a receptive female brought into estrus by sequential treatment with estradiol benzoate and progesterone in a circular perspex arena (∅ = 500 mm), the floor covered with wood shavings, and in a dimly lit room. The male was presented with the female in the observation arena at time 0 (Figure 1), and the following items of the male rat sexual behaviour were recorded: Mounts (M), number of mounts without vaginal penetration; Intromissions (I), number of mounts with vaginal penetration; Intromission latency (IL), time from presentation of the female to the first intromission; Ejaculation latency (EL), time from the first intromission to ejaculation; Postejaculatory interval (PEI), time from ejaculation to the following intromission. The observations were ended when one of the following conditions was fulfilled: (1) If, after the first ejaculation, the animals had initiated a new copulatory series by an intromission; (2) If the male made an intromission, but no ejaculation occurred within 30 min; (3) If the male did not initiate copulation by an intromission within 15 min upon presentation of the female. The animals served as their own controls in a change-over design (Li, 1964), and were tested twice a week.

Statistical procedures

Statistical analysis was performed by means of a Friedman two-way analysis of variance, followed by the Wilcoxon matched-pairs signed-ranks test for comparisons between groups (Siegel, 1956).

Results

Effects of 5-HT$_{1A}$ receptor agonists and antagonists, alone and in combination, on male rat sexual behaviour

Effects of 8-OH-DPAT As expected, the 5-HT$_{1A}$ receptor agonist 8-OH-DPAT produced a facilitation of the ejaculatory behaviour, as evidenced by a marked and statistically significant reduction in number of mounts and intromissions, as well as in time to ejaculation (Figure 2). There were no statistically significant effects on intromission latency (not shown in figure) or in the postejaculatory interval.

Effects of NAD-299 There were no statistically significant effects by NAD-299 (0.75–3.0 μmol kg^{-1} s.c.), on any aspect of the male rat sexual behaviour, as observed here (Table 1).

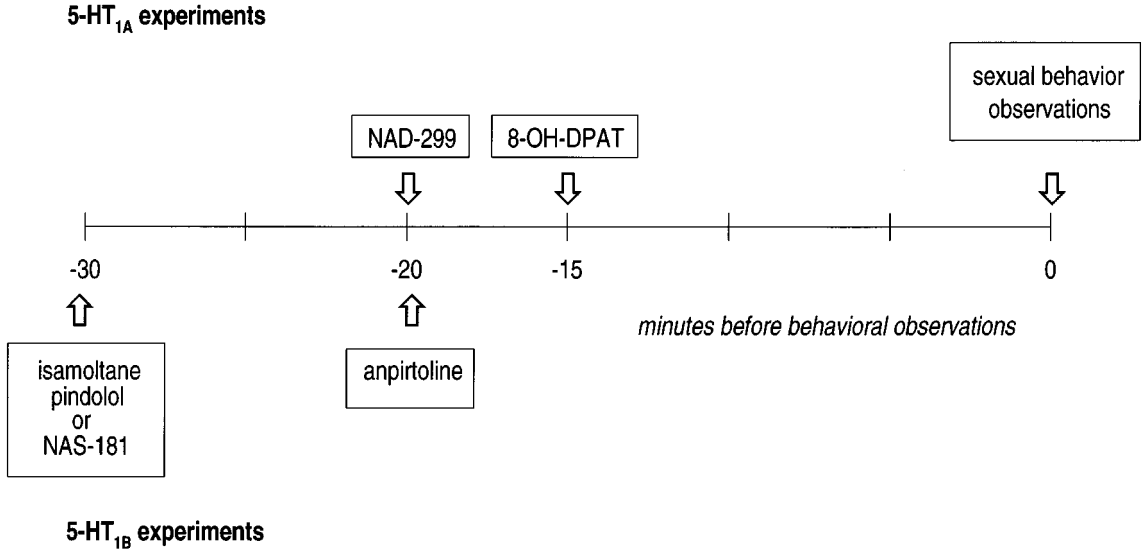

Figure 1 Schedule of drug injections in relation to behavioural observations.

Antagonism by NAD-299 of the 8-OH-DPAT-induced facilitation of male rat ejaculatory behaviour As shown in Figure 3, NAD-299 completely antagonized the 8-OH-DPAT-induced reduction in time to ejaculation ($P<0.01$), as well as partially antagonized the effects on intromissions ($P<0.01$). There were no statistically significant effects between groups as regards number of mounts, intromission latency or the postejaculatory interval.

Effects of 5-HT$_{1B}$ receptor agonists and antagonists, alone and in combination, on male rat sexual behaviour

Effects of the 5-HT$_{1B}$ receptor agonist anpirtoline Anpirtoline (0.25–4.0 μmol kg^{-1} s.c.) produced a dose-dependent inhibition of male rat ejaculatory behaviour, as evidenced by an increase in number of mounts preceding ejaculation, and in time to ejaculation. In addition, there was a statistically

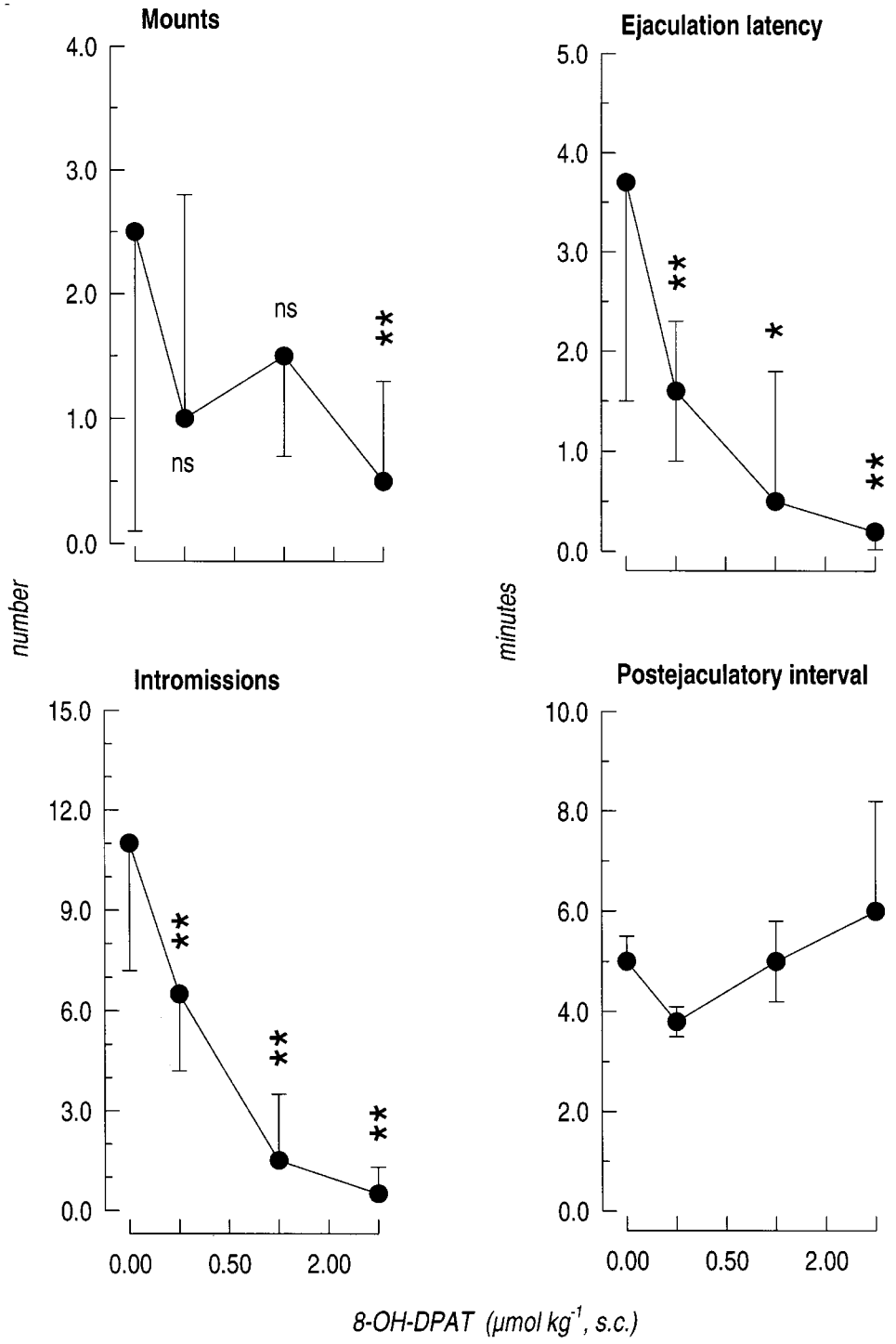

Figure 2 Effects of 8-OH-DPAT on male rat copulatory behaviour. 8-OH-DPAT was administered s.c., 15 min before observations started. The results are presented as medians ± semi-interquartile range based on repeated observations of 12 rats. The animals served as their own controls in a change-over design (Li, 1964). Statistical analysis was performed by means of the Friedman two-way ANOVA followed by the Wilcoxon matched-pairs signed-ranks *T*-test (Siegel, 1956) for comparisons with the respective control group, as shown in the figure. Mounts: $\chi^2(3)=8.13$, $P<0.05$; Intromissions: $\chi^2(3)=28.03$, $P<0.01$; Intromission latency: $\chi^2(3)=10.68$, $P<0.05$; Ejaculation latency: $\chi^2(3)=17.50$, $P<0.01$; Postejaculatory interval: $\chi^2(3)=6.70$, n.s. ns $P>0.05$; *$P<0.05$; **$P<0.01$.

Table 1 Effects of NAD-299, isamoltane and NAS-181 on male rat ejaculatory behaviour

	NAD-299 (μmol kg^{-1} s.c.)				
	0.00	*0.75*	*1.50*	*3.00*	χ^2
M	3.0±1.5	4.0±5.5	5.0±5.0	1.0±3.0	7.16ns
I	9.0±3.0	11.0±4.5	10.0±2.0	9.0±3.5	2.88ns
IL	0.17±0.13	0.17±0.13	0.42±0.25	0.33±0.34	4.98ns
EL	2.8±0.8	4.3±2.1	3.9±1.3	4.7±1.7	5.46ns
PEI	4.1±0.7	4.3±0.9	4.5±0.8	4.1±0.8	2.94ns
	Isamoltane (μmol kg^{-1} s.c.)				
	0.0	*1.0*	*4.0*	*16.0*	χ^2
M	3.0±2.5	6.0±4.5	5.0±2.5	5.0±3.5	3.94ns
I	12.0±3.5	11.0±3.5	12.0±3.5	12.0±3.5	2.06ns
IL	0.15±0.05	0.08±0.17	0.13±0.09	0.17±0.07	1.78ns
EL	4.3±1.3	4.6±0.9	4.5±1.6	4.6±0.9	0.94ns
PEI	5.2±0.7	4.8±0.5	4.9±0.5	4.9±0.8	1.70ns

	NAS-181 (μmol kg^{-1} s.c.)					
	0.0	*1.0*	*4.0*	*16.0*	*64.0*	χ^2
M	3.0±2.8	3.0±3.0ns	1.0±3.0ns	2.0±2.0ns	1.0±2.0*	10.77*
I	8.5±3.5	8.0±3.5	8.0±1.5	8.0±2.0	6.0±3.0	9.20ns
IL	0.15±0.06	0.12±0.06	0.15±0.16	0.12±0.09	0.13±0.08	1.05ns
EL	4.0±1.9	4.0±1.8ns	2.8±1.4ns	2.3±2.2ns	2.5±0.8*	13.81**
PEI	4.5±0.5	4.9±0.6	4.2±1.0	4.9±0.8	4.2±0.5	3.97ns

The table shows medians±semi-interquartile range, based on repeated observations of 15 animals in each of the three experiments presented in the table. Statistical evaluation was performed by means of the Friedman two-way ANOVA, followed by the Wilcoxon matched-pairs signed-ranks *T*-test, as shown in the table. ns $P>0.05$; *$P<0.05$; **$P<0.01$

significant prolongation of the intromission latency at the highest dose of anpirtoline (4.0 μmol kg^{-1}) (Figure 4). No statistically significant effects were found on number of intromissions, or on the postejaculatory interval.

Effects of isamoltane and NAS-181 There were no statistically significant effects by the 5-HT$_{1B}$ receptor antagonist isamoltane (1.0–16.0 μmol kg^{-1} s.c.) on any of the behavioural items observed here (Table 1). The more selective 5-HT$_{1B}$ receptor antagonist NAS-181 (1.0–64.0 μmol kg^{-1} s.c.), however, produced a slight reduction in number of mounts preceding ejaculation, and of the ejaculation latency, effects that were statistically significant at the highest dose used (64 μmol kg^{-1} s.c.). No other statistically significant effects were found after administration of NAS-181 (Table 1).

Antagonism by isamoltane, or NAS-181, of the anpirtoline-induced inhibition of male rat ejaculatory behaviour Isamoltane, in the dose of 16 μmol kg^{-1} s.c., completely antagonized the anpirtoline induced increase in number of mounts, the increased ejaculation latency, and in the post-ejaculatory interval (Figure 5). A similar, and dose-dependent, antagonism of the anpirtoline-induced inhibition of the male rat ejaculatory behaviour was found after NAS-181 administration (Figure 6).

Failure to antagonize the anpirtoline-induced inhibition of male rat ejaculatory behaviour by (−)pindolol As shown in Figure 7, (−)pindolol (8 μmol kg^{-1} s.c.) did not antagonize any of the effects produced by anpirtoline (1 μmol kg^{-1} s.c.).

Discussion

As expected, the 5-HT$_{1A}$ receptor agonist 8-OH-DPAT produced a marked, and statistically significant, facilitation of the male rat ejaculatory behaviour, as evidenced by a reduction in number of intromissions preceding ejaculation, and in the ejaculation latency (for example Ahlenius & Larsson, 1991b). That the effects of 8-OH-DPAT indeed are due to its properties as a 5-HT$_{1A}$ receptor agonist, receives support from the observation that the effects were fully antagonized by the selective 5-HT$_{1A}$ receptor antagonist NAD-299, and confirm results from previous studies, using the less selective 5-HT$_{1A}$ receptor antagonists (−)-pindolol (Ahlenius & Larsson, 1988) *S*-(−)-5-fluoro-8-hydroxy-2-(di-*n*-propylamino)tetralin HCl (UH-301) (Johansson *et al*., 1991) and *N*-(2-(4-(2-methoxyphenyl)-1-piperazinyl ethyl)-*N*-(2-pyridyl)cyclohexa-necarboxamide 3HCl (WAY-100635) (Ahlenius & Larsson, 1998; *c.f*. Ahlenius *et al*., 1999). Finally, it should be noted that NAD-299, by itself, had no statistically significant effects on the male rat ejaculatory behaviour. This observation suggests that the 5-HT$_{1A}$ receptors involved in the mediation of this behaviour are not normally under tonic activation.

In stark contrast to the above effects of 8-OH-DPAT, activation of the 5-HT$_{1B}$ receptor by anpirtoline administration, resulted in a prolongation of the ejaculation latency. In addition, there was an increase in the number of mounts preceding ejaculation, and a tendency for a similar effect on the number of intromissions. This effect could be linked to the 5-HT$_{1B}$ receptor by its complete sensitivity to either of the 5-HT$_{1B}$ receptor antagonists, isamoltane or NAS-181. Generally, there were few, or no, effects by the antagonists themselves. It should be noted, however, that at the highest dose of NAS-181 (64 μmol kg^{-1}), there was a statistically significant reduction in the ejaculation latency, and in the number of mounts preceding ejaculation. This could be a sign of a certain tonus at this receptor subtype, in contrast to the 5-HT$_{1A}$ receptor, as noted above.

An interesting aspect of the results obtained in the present study are the very clear and opposite effects obtained by use of the 5-HT$_{1A}$ and the 5-HT$_{1B}$ receptor agonists. Thus, the male rat ejaculatory behaviour offers a sensitive and reliable model to pharmacologically differentiate 5-HT$_{1A}$ from 5-HT$_{1B}$ receptor agonists. The selectivity of the model receives support from the fact that 5-HT$_2$ receptor agonists, or antagonists, do

not have specific effects on male rat ejaculatory behaviour (Watson & Gorzalka, 1991; Klint *et al*., 1992; Fernandez-Guasti & Rodriguez-Manzo, 1992; Fernandez-Guasti *et al*., 1992; Ahlenius & Larsson, 1998). However, the possible role of a number of recently cloned 5-HT receptor subtypes, such as 5-HT_4, 5-HT_5, 5-HT_6 and 5-HT_7 receptors (for example Lucas & Hen, 1995), in male rat ejaculatory behaviour must await the development of selective and specific pharmacological tools to study the functional importance of these newly identified receptors.

The β-blocking agent (−)-pindolol has often been used for its 5-HT_{1A} receptor blocking properties. Thus, for example, (−)-pindolol can antagonize the 8-OH-DPAT-induced facilitation of male rat ejaculatory behaviour (Ahlenius & Larsson, 1988). In addition, (−)-pindolol treatment will enhance the 5-HTP-induced inhibition of male rat ejaculatory behaviour (Ahlenius & Larsson, 1991a;1998), presumably due to disinhibition of autoreceptor-mediated inhibition of firing in serotonergic neurons (see Aghajanian, 1995), as well as in synthesis (Hjorth *et al*., 1982; Hillegaart *et al*., 1990) and release (Hutson *et al*., 1989; Sharp *et al*., 1989) of forebrain serotonin. In support for this view, the inhibition of male rat ejaculatory behaviour produced by 5-HTP was also enhanced by pretreatment with the 5-HT_{1A} receptor antagonist WAY-100635 (Ahlenius & Larsson, 1998). (−)-Pindolol, however,

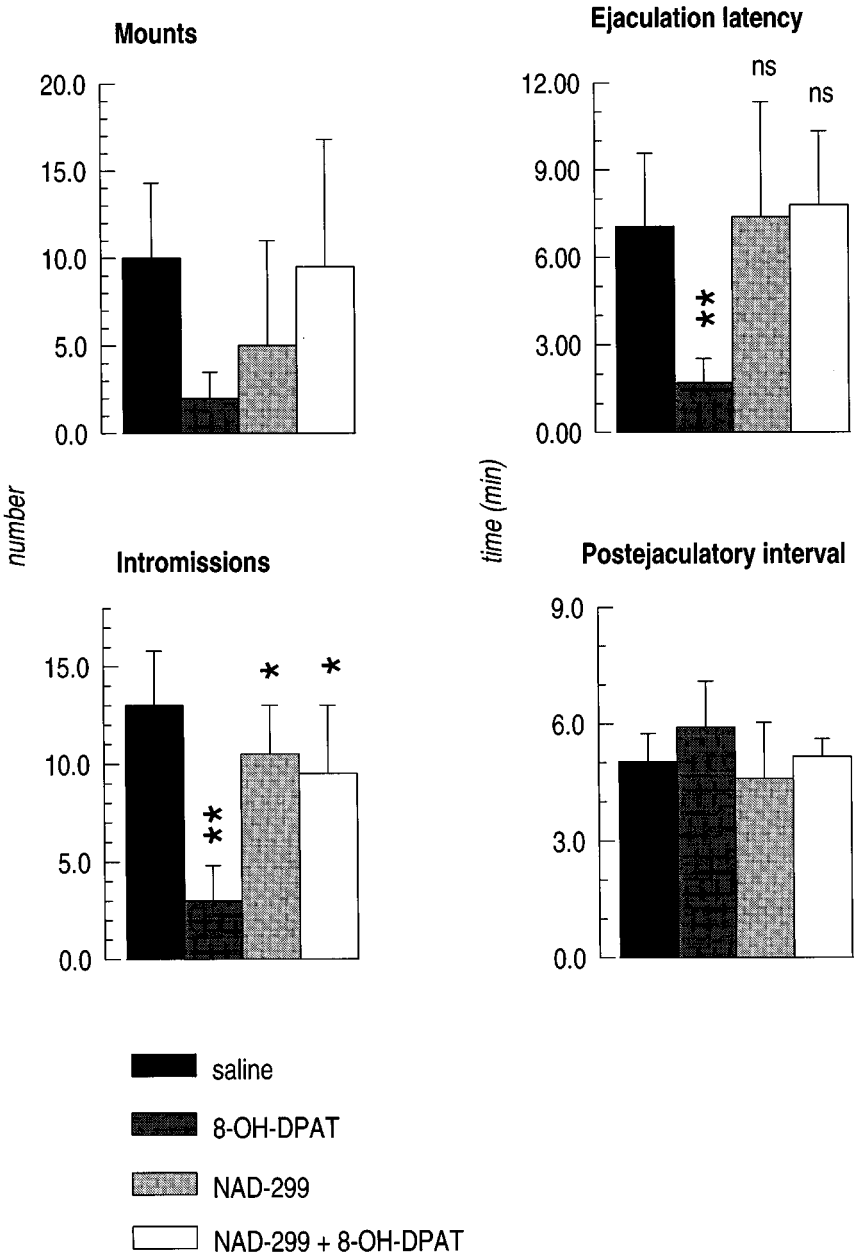

Figure 3 Antagonism of the effects induced by 8-OH-DPAT on male rat sexual behaviour by the 5-HT_{1A} receptor antagonist NAD-299. NAD-299 (1.0 μmol kg^{-1} s.c.) was administered 20 min, and 8-OH-DPAT (0.8 μmol kg^{-1} s.c.) 15 min before observations started. Shown are the medians ± semi-interquartile range, based on repeated observations of 12 rats in a change-over design (see Li, 1964). Statistical evaluation by means of the Friedman two-way ANOVA, followed by the Wilcoxon matched-pairs signed-ranks *T*-test for comparisons with saline treated controls. Mounts: $\chi^2(3) = 5.88$, n.s.; Intromissions: $\chi^2(3) = 24.03$, $P < 0.01$; Intromission latency: $\chi^2(3) = 0.90$, n.s. (not shown in the figure); Ejaculation latency: $\chi^2(3) = 11.70$, $P < 0.01$; Postejaculatory interval: $\chi^2(3) = 1.90$, n.s. ns $P > 0.05$; *$P < 0.05$; **$P < 0.01$.

is not selective for the 5-HT_{1A} receptor, and displays high affinity also for the 5-HT_{1B} receptor (see Hoyer *et al.*, 1994). The present results clearly demonstrate that 5-HT_{1B} receptor antagonism is not a prominent feature of (−)-pindolol. On the contrary, some of the observations with (−)-pindolol, alone and in combination with 5-HTP, is compatible with partial agonist properties of (−)-pindolol at this 5-HT receptor subtype. Thus, for example, (−)-pindolol by itself produces an anpirtoline-like effect on male rat ejaculatory behaviour (Ahlenius & Larsson, 1991a).

In contrast to the opposite effects by 8-OH-DPAT and anpirtoline on male rat ejaculatory behaviour, both the 5-HT_{1A} and the 5-HT_{1B} receptor agonists stimulate spontaneous locomotor activity in rats (Hillegaart *et al.*, 1996; O'Neill & Parameswaran, 1997). Furthermore, these two 5-HT_1 receptor agonists combined interact synergistically in the locomotor

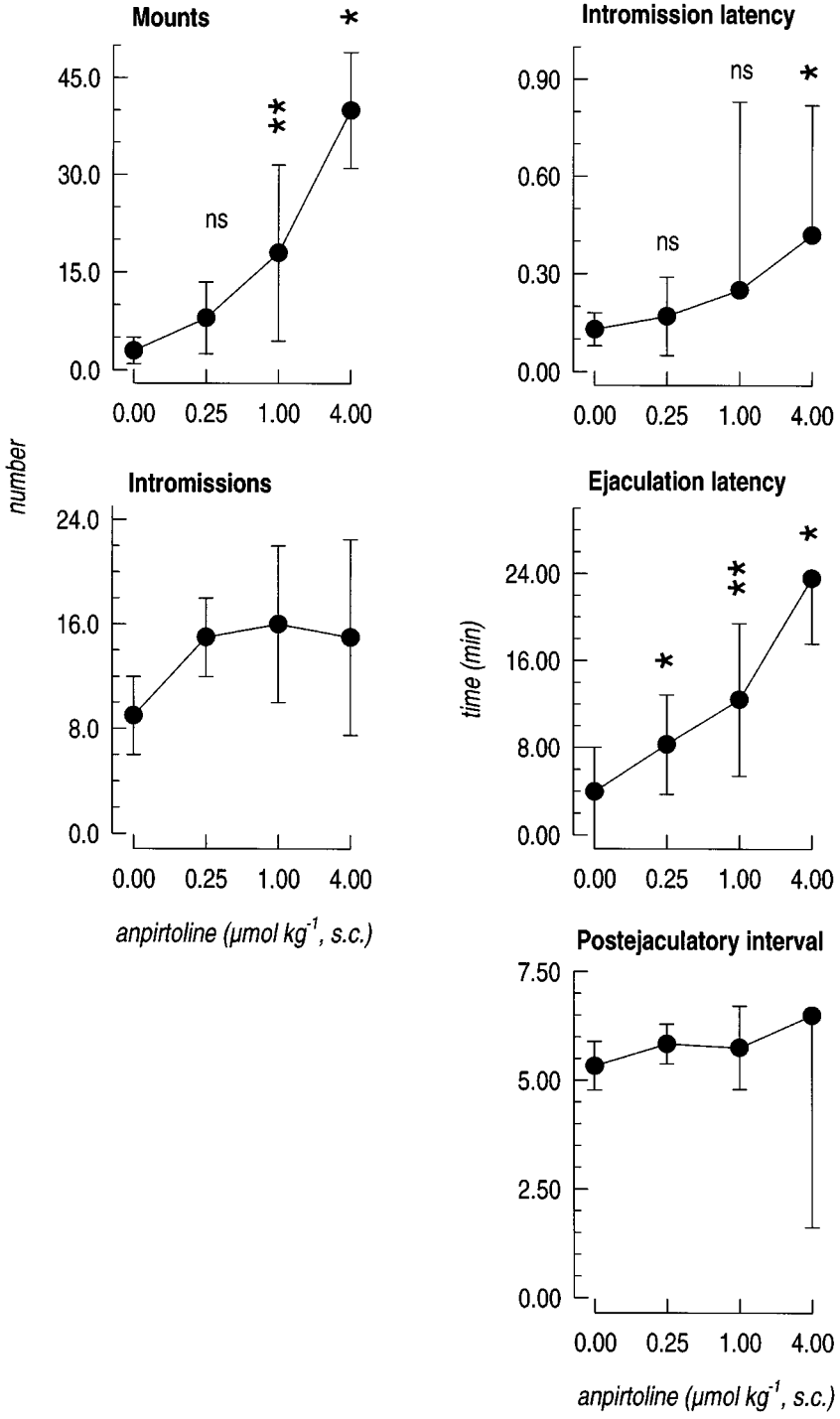

Figure 4 Effects of anpirtoline on male rat sexual behaviour. The animals were injected with anpirtoline (0.00−4.00 μmol kg^{-1} s.c.) 20 min prior to placement together with the female in the observation arena. The results are presented as medians ± semi-interquartile range, based on observations of 15 animals, in a change-over design. Statistical analysis was performed by means of a Friedman two-way ANOVA followed by the Wilcoxon matched-pairs signed-ranks *T*-test for comparisons with the saline-treated controls (Siegel, 1956). Mounts: $\chi^2(3) = 15.30$, $P < 0.01$; Intromissions: $\chi^2(3) = 7.33$, n.s.; Intromission latency: $\chi^2(3) = 8.06$, $P < 0.05$; Ejaculation latency: $\chi^2(3) = 19.29$, $P < 0.001$; Post-ejaculatory interval: $\chi^2(3) = 3.51$, n.s. ns $P > 0.05$; $^*P < 0.05$; $^{**}P < 0.01$.

activity experiments (O'Neill & Parameswaran, 1997; Jackson *et al.*, in preparation). These observations strongly suggest that different populations of 5-HT_{1A} and/or 5-HT_{1B} receptors are recruited in mechanisms of male rat sexual and locomotor behaviours.

The 5-HT_{1A} and 5-HT_{1B} receptor agonists, 8-OH-DPAT and anpirtoline, already display a high degree of selectivity for their respective targets within the 5-HT_1 receptor family (Schlicker *et al.*, 1992; see Hoyer *et al.*, 1994). The corresponding antagonists, NAD-299 and NAS-181, appear to provide an even higher selectivity, also in comparison with non-5-HT_1 receptor subtypes (Johansson *et al.*, 1997; Berg *et al.*, 1998). Together, this provides strong support for the specific roles here ascribed to the 5-HT_{1A} and 5-

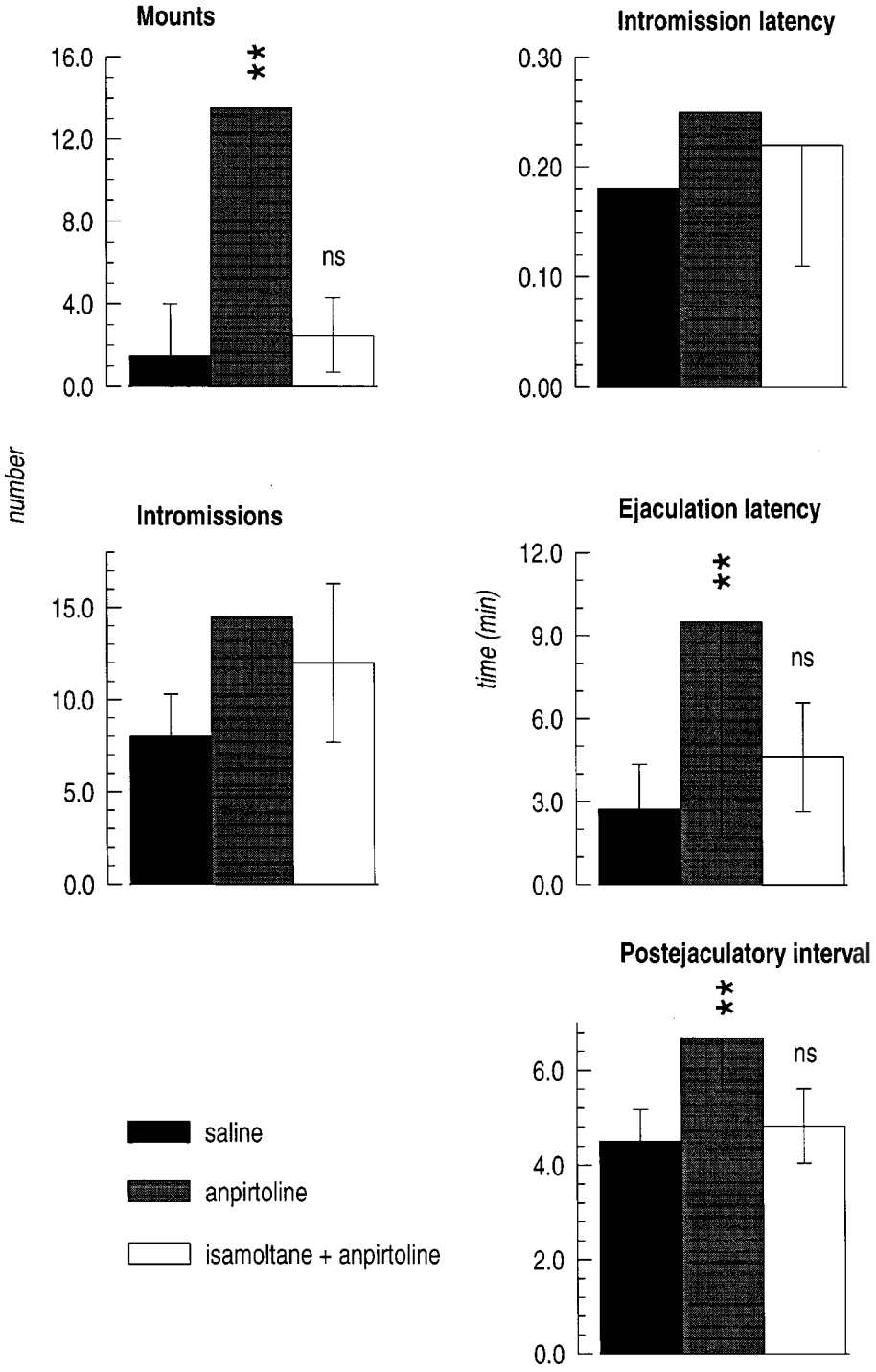

Figure 5 Antagonism by isamoltane of the anpirtoline-induced effects on male rat sexual behaviour. Isamoltane (16 μmol kg^{-1}, s.c.) was administered 30 min, and anpirtoline (1 μmol kg^{-1}, s.c.) 20 min, before observations started. Shown are the medians $\pm$ semi-interquartile range, based on repeated observations of 15 rats in a change-over design (see Li, 1964). Statistical evaluation by means of the Friedman two-way ANOVA, followed by the Wilcoxon matched-pairs signed-ranks *T*-test (see Siegel, 1956) for comparisons with saline treated controls. Mounts: $\chi^2(2)=14.23$, $P<0.01$; Intromissions: $\chi^2(2)=4.57$, n.s.; Intromission latency: $\chi^2(2)=9.18$, $P<0.01$; Ejaculation latency: $\chi^2(2)=21.40$, $P<0.01$; Postejaculatory interval: $\chi^2(2)=19.45$, $P<0.01$. ns $P>0.05$; $*P<0.05$; $**P<0.01$.

HT_{1B} receptors in the mediation of male rat ejaculatory behaviour.

It is worth noting that the drug effects on male rat ejaculatory behaviour, reported here, are very different from their effects on penile erections. Thus, 8-OH-DPAT, which facilitates the ejaculatory behaviour, inhibits penile erections (Mathes *et al*., 1990; Finberg & Vardi, 1990). Furthermore, the non-selective 5-HT_{1B} receptor agonist 1-(3′-chlorophenyl)-piperazine (mCPP) induces penile erections (Berendsen & Broekkamp, 1987, *c.f.* Berendsen *et al*., 1990), whereas the 5-HT_{1B} receptor agonist anpirtoline inhibited ejaculatory behaviour in the present study. Finally, stimulation of 5-HT_{2C} receptors (formerly the 5-HT_{1C} receptor), induces penile erections (Berendsen *et al*., 1990; Stancampiano *et al*., 1994; Millan & Perrin-Monneyron, 1997; Bös *et al*., 1997). The corresponding receptor agonists lack specific effects on the male rat ejaculatory behaviour. Together with other results, as those presented here, male rat sexual functions presents

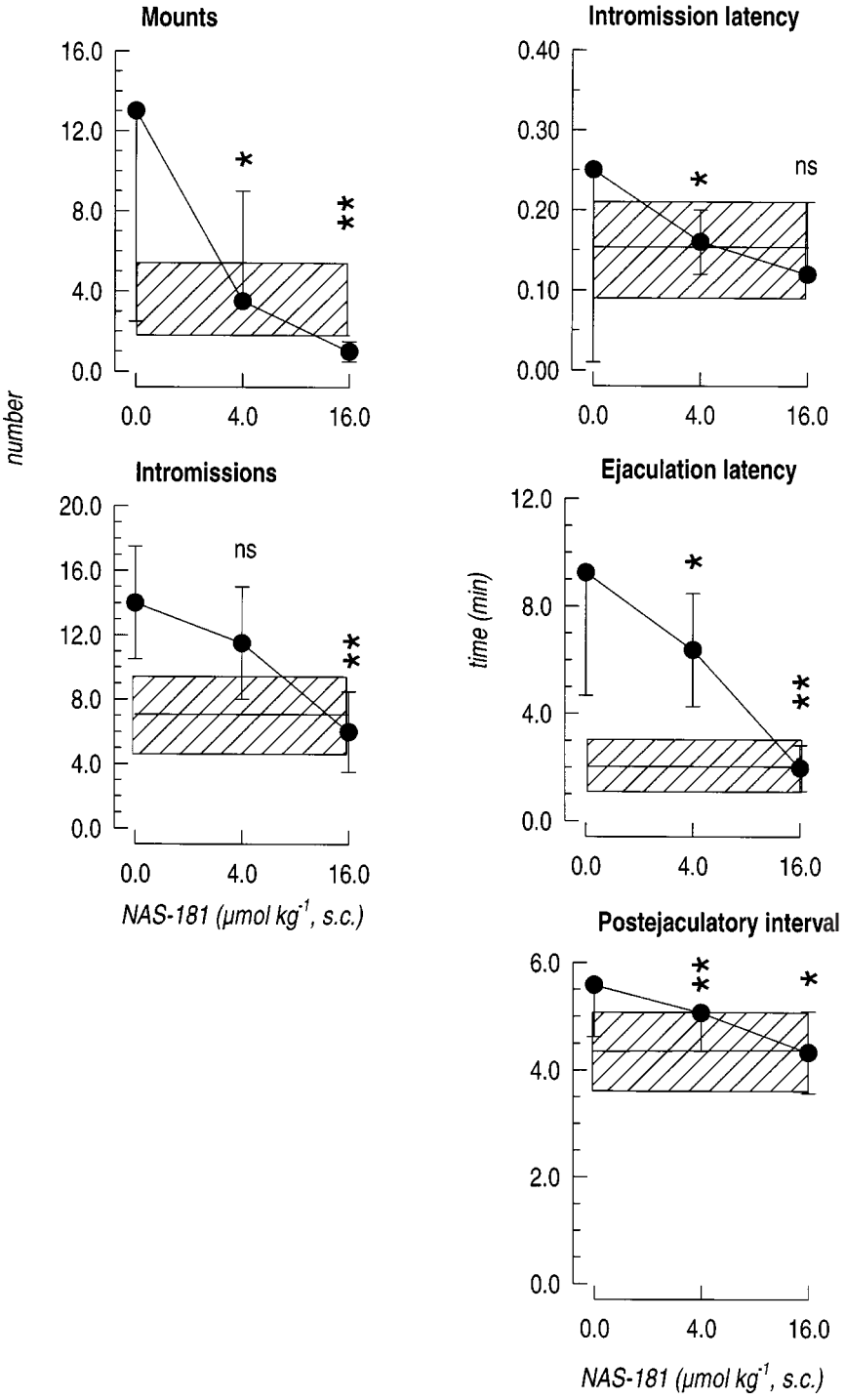

Figure 6 Antagonism by NAS-181 of the anpirtoline-induced effects on male rat sexual behaviour. NAS-181 was administered s.c. 30 min, and anpirtoline (1 μmol kg^{-1} s.c.) 20 min, before observations started. Shown are the medians ± semi-interquartile range based on repeated observations of 12–15 rats in a change-over design. Saline controls are represented by the hatched area. Statistical comparisons with anpirtoline-treated controls by means of the Wilcoxon matched-pairs signed-ranks *T*-test, as shown in the figure. ns $P>0.05$; *$P<0.05$; **$P<0.01$.

interesting possibilities when looking for pharmacological models able to distinguish *in vivo* effects of new serotonin receptor ligands within the 5-HT_1 and 5-HT_2 receptor families.

The effects of tricyclic antidepressants on male ejaculatory mechanisms and libido are well known side-effects in the clinic, and have been used in the treatment of ejaculatio preacox (see Barnes & Harvey, 1993). These effects have been retained in the more recently developed SSRIs, and are in fact more pregnant due to the generally more benign side-effect profile of this new generation antidepressants (Modell *et al*., 1997). These side-effects include anorgasmia and loss of libido in both sexes, as well as ejaculatory disturbances in males, possibly affecting as much as half of the patient population (Zejacka *et al*., 1997). Laboratory studies suggest that at least some of

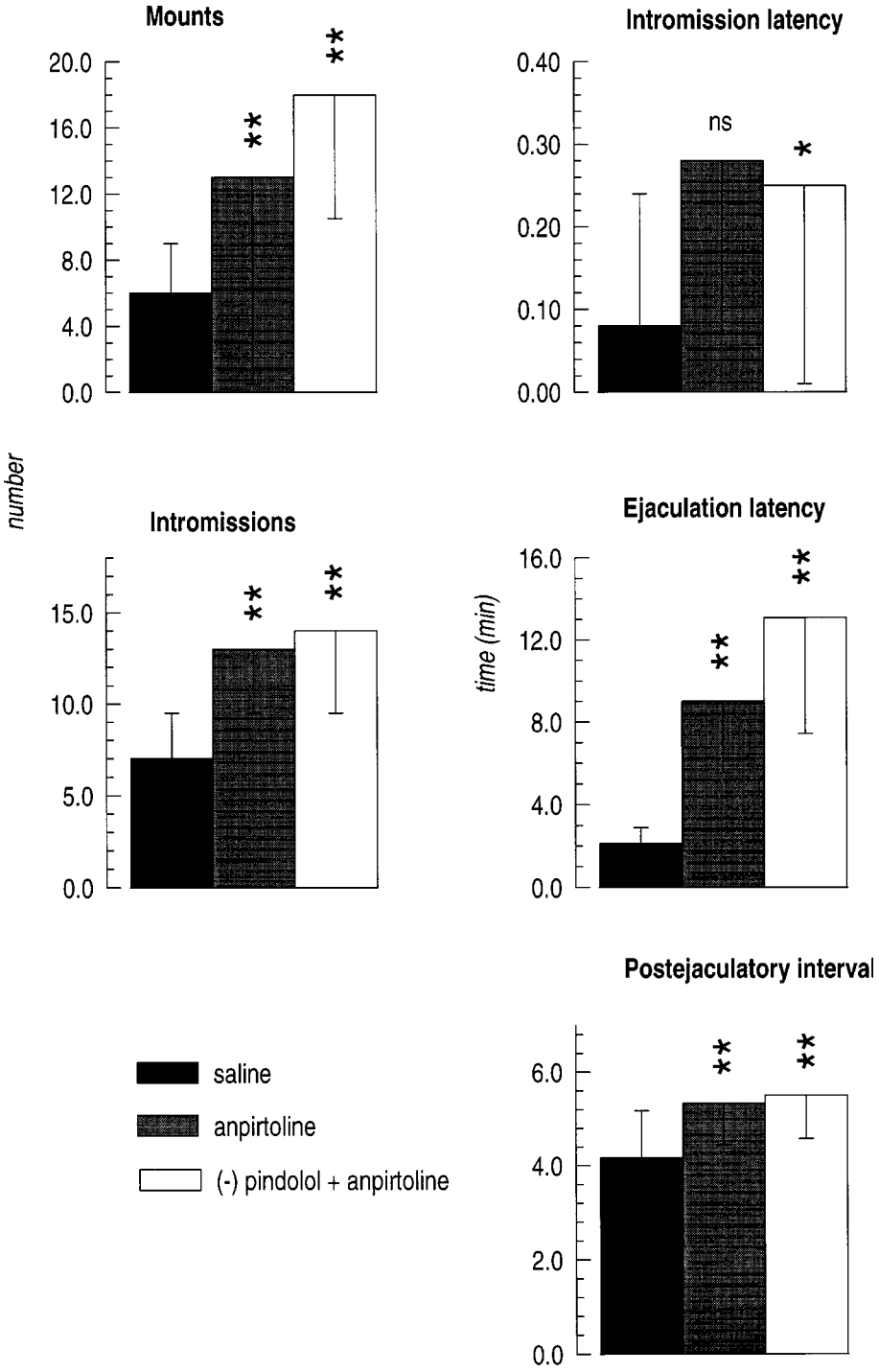

Figure 7 Failure to antagonize the anpirtoline-induced inhibition of male rat ejaculatory behaviour by (−)-pindolol. Anpirtoline (1 μmol kg^{-1} s.c.) and (−)-pindolol (8 μmol kg^{-1} s.c.) were administered as shown in Figure 1. Shown are the medians ± semi-interquartile range, based on repeated observations of 15 rats. Statistical evaluation was performed by means of the Friedman two-way ANOVA, followed by the Wilcoxon matched-pairs signed-ranks *T*-test, for comparisons with saline treated controls. Mounts: $\chi^2(2) = 13.17$, $P < 0.01$; Intromissions: $\chi^2(2) = 2.38$, n.s.; Intromission latency: $\chi^2(2) = 3.79$, n.s.; Ejaculation latency: $\chi^2(2) = 17.17$, $P < 0.01$; Postejaculatory interval: $\chi^2(2) = 15.13$, $P < 0.01$. ns $P > 0.05$; *$P < 0.05$; **$P < 0.01$.

these effects are due to activation of central 5-HT_{1B} receptors since the inhibitory effects of 5-HTP on male rat ejaculatory behaviour are antagonized by isamoltane (Ahlenius & Larsson, 1998).

In conclusion 5-HT_{1A} and 5-HT_{1B} receptors have opposite roles in the mediation of male rat ejaculatory behaviour, 5-HT_{1A} being facilitatory and 5-HT_{1B} being inhibitory.

The generic name for NAD-299 is robalzotan.

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