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Modulation of sexual behaviour in the rat by a potent and selective α_2-adrenoceptor antagonist, delequamine (RS-15385-197)

D. Tallentire, G. McRae, [1]*M. Spedding, R. Clark & B. Vickery

Syntex Research, Palo Alto, California 94304, U.S.A. and *Syntex Research Europe, Research Park Riccarton, Edinburgh, EH14 4AP, Scotland.

1 The contributions of α_2-adrenoceptors and 5-HT_{1A} receptors to sexual behaviour in the rat have been re-evaluated by use of a highly potent and selective α_2-adrenoceptor antagonist, delequamine (RS-15385-197), yohimbine, idazoxan and the partial agonist at 5-HT_{1A} receptors, 8-hydroxy-2(di-n-propylamino)-tetralin (8-OH-DPAT).

2 In a model where naive male rats were introduced to oestrogen-progesterone primed, sexually receptive female rats, delequamine (0.4-6.4 mg kg^{-1}, p.o.) dose-relatedly increased the sexual behaviour score over the entire dose-range whereas yohimbine was effective at only one dose, 2 mg kg^{-1}, p.o.. Idazoxan was active only at 2.5 and 5 mg kg^{-1}, p.o. Yohimbine, but neither delequamine nor idazoxan, decreased ejaculation latency. 8-OH-DPAT (0.1 and 0.25 mg kg^{-1}, s.c.) reduced the time, and the number of intromissions to ejaculation without affecting other parameters. A combination of delequamine (0.4 mg kg^{-1}, p.o.) and 8-OH-DPAT (0.1 mg kg^{-1} s.c.) increased the percentage of rats mounting, intromitting and ejaculating, and reduced ejaculation latency and the number of intromissions.

3 In orchidectomized, sexually experienced rats exposed to sexually receptive females, delequamine, idazoxan and yohimbine increased the number of rats mounting, and there was a tendency to increase the number of animals intromitting, but no effect on ejaculatory behaviour.

4 In ovariectomized female rats brought to low level receptivity by priming with low dose injections of oestradiol benzoate and progesterone, delequamine, at 1.6 and 6.4 mg kg^{-1} p.o., increased lordosis, while yohimbine, at 2, 4 and 8 mg kg^{-1} p.o., reduced lordotic responses to sexually experienced males in a dose-dependent manner. 8-OH-DPAT at 0.1, 0.25 mg kg^{-1}, s.c. reduced lordosis in a dose-dependent manner.

5 These findings may be explained on the basis that yohimbine is an α_2-adrenoceptor antagonist with affinity for 5-HT_{1A} receptors and that the effects of 5-HT_{1A} receptors may modulate the sexual behaviour responses to α_2-receptor antagonism in some models. Thus, in contrast to yohimbine, the highly-selective α_2-adrenoceptor antagonist, delequamine, was very effective in increasing the behavioural score in male and female rats over a wide dose-range.

Keywords: Delequamine; yohimbine; α_2-adrenoceptors; sexual behaviour in rat; impotence; 5-HT_{1A} receptors

Introduction

Male sexual dysfunction is an age-related problem with a large incidence. In the Massachusetts male ageing study of 1709 men aged between 40 and 70 years old, some degree of sexual dysfunction was present in 39% of men aged 40 and in 67% of men aged 70, with complete impotence occurring in 5% of men aged 40 and in 15% of men aged 70 (Feldman *et al.*, 1994). However, there are no proven therapies other than intracavernosal injections or implants. A large but poorly defined proportion of the population suffering sexual dysfunction has been classed as 'psychogenic' and there may also be a large psychogenic population during the early stages of diabetes. There may be up to one million diabetic patients suffering from impotence in the U.S.A. and Europe.

Yohimbine has long been considered to have aphrodisiac functions and has been widely used in the treatment of male dysfunction (Goldberg & Robertson, 1983; The Medical Letter, 1994). However, controlled clinical trials have shown only marginal benefit in patients (Morales *et al.*, 1982; Reid *et al.*, 1987), although lack of toxicity of the compound has encouraged widespread use in some countries. Recently, yohimbine has been shown to reverse fluoxetine-induced sexual dysfunction in man (Jacobsen, 1992). Yohimbine has some effects in animal models, for example the drug has been shown to increase sexual behaviour score in male rats, but only at 1-2 mg kg^{-1}, p.o. (Clark *et al.*, 1985; Smith *et al.*, 1987). The effects of yohimbine have been attributed to α_2-adrenoceptor blockade (pK_i 7.9) but the compound has only 8 fold selectivity for α_2 compared with 5-HT_{1A} receptors (pK_i 7.2; Brown *et al.*, 1992). Furthermore, yohimbine, but not idazoxan, generalizes to a 5-HT_{1A} cue in drug discrimination studies in the rat (Winter & Rabin, 1992) and acts as a full agonist at 5-HT_{1A} receptors in second messenger studies (Kawai *et al.*, 1992). This means that effects of higher doses of yohimbine may be mediated by 5-HT_{1A} receptors.

The question therefore arises as to the mechanism of action of yohimbine in increasing the sexual behaviour score in rats, particularly as the 5-HT_{1A} agonist, 8-hydroxy-(2-di-n-propylamino)tetralin (8-OH-DPAT), has well described effects on sexual behaviour in the male rat, markedly reducing the number of intromissions prior to ejaculation, which has been likened to a state of *ejaculatio preacox* by Ahlenius & Larsson (1991). Are the effects of yohimbine in man due to effects on 5-HT_{1A} receptors, or are they limited by these effects? The recent

[1]Author for correspondence at present address: Institut de Recherches Servier, Croissy Research Centre, 125, Chemin de Ronde, Croissy, 78290, France.

availability of highly selective and potent α_2-adrenoceptor antagonists allows the redefinition of the role of α_2-adrenoceptors in sexual behaviour in the rat. We have therefore examined the effects of delequamine, a highly potent (pK_i 9.5 for α_2-adrenoceptors in rat cortex, Clark *et al.*, 1989; Brown *et al.*, 1992) and selective (>1,000 fold selectivity against 5-HT_{1A} receptors: pK_i 6.5, and 10,000 fold selectivity against α_1-adrenoceptors: pK_i 5.3) α_2-adrenoceptor antagonist. Delequamine is a potent antagonist at both peripheral and central α_2-adrenoceptors in the rat (Redfern *et al.*, 1992) and is devoid of partial agonist effects (Brown *et al.*, 1992). Furthermore the compound, unlike idazoxan, has no affinity for imidazoline sites (MacKinnon *et al.*, 1991), and [^{3}H]-delequamine labels only α_2-adrenoceptors (MacKinnon *et al.*, 1992): thus the effects of delequamine can be specifically attributed to α_2-adrenoceptor antagonism.

In this study we show that, unlike yohimbine, delequamine increases the sexual behaviour score of male and female rats over a wide dose-range.

Methods

Animals

Sprague-Dawley-derived rats were obtained from Bantin and Kingman, Inc., Fremont, CA, U.S.A. (Studies 1–4) or from Charles River Breeding Laboratories, Wilmington, MA, U.S.A. (Studies 5–15). All rats were provided with food and water *ad lib.*

Naive male rat studies: male rats obtained at weaning (22 days of age) were housed 4/cage in a 14 h: 10 h light:dark cycle room (lights on 05 h 00 min) and allowed to mature for 4 weeks before testing. To maintain naiveté no female rats were housed in the same room.

Orchidectomized male rat studies: male rats obtained at 22 days of age were housed 2/cage in a reverse 14 h: 10 h light:-dark cycle room (lights off 10 h 00 min). After a 3 day acclimatization, all rats were tested twice weekly with sexually receptive female rats until they had ejaculated in 4 separate tests. This subpopulation of sexually experienced males was then orchidectomized. Eight and 10 weeks later, they were retested, and only those rats with negative results (no mounts, intromissions, or ejaculatory behaviour) proceeded into experimental use.

Ovariectomized female studies: female rats were ovariectomized at the start of each study and tests were performed at 2 week intervals starting at 2 weeks post-ovariectomy. Females received s.c. injections in 0.05 ml of sesame oil of 1 μg/rat oestradiol benzoate and 50 μg/rat progesterone at 45–48 h and 4 h before testing, respectively, to induce a sub optimal level of sexual receptivity, except in one study evaluating the effect of yohimbine, in which the rats received 1.25 μg/rat oestradiol benzoate and 1 mg/rat progesterone.

Stimulus-female rats: adult female rats, used for the sexual behaviour testing of naive and castrated male rats, were housed 4/cage in a reverse light:dark cycle room. They were brought into sexual receptivity with s.c. injections of 20 μg oestradiol benzoate in 0.1 ml sesame seed oil followed by 1 mg progesterone in 0.1 ml sesame seed oil, at 48 h and 4 h prior to the tests, respectively.

Stimulus-male rats: sexually experienced, adult male rats, used for the sexual receptivity testing of ovariectomized females, were housed 2/cage in the reverse light:dark cycle room. Periodic exposure to receptive females maintained their sexual vigour.

Surgical procedures were performed under ether for anaesthesia from Mallinckrodt (Paris, KY, U.S.A.). These studies were conducted under IACUC reviewed and approved protocols.

Mating behaviour tests

Tests were conducted in hemi-hexagonal observation cages with plexiglass fronts.

Male rat tests: experimental male rats were placed into the observation cages 10 min before the start of the test. Naive males were tested during the light period of the light:dark cycle and castrated males during the dark period under dim lighting. A stimulus female was introduced into each cage and the pattern of male sexual activity recorded using an Esterline-Angus event recorder. Behaviours recorded were mounts, intromissions and ejaculation (Södersten *et al.*, 1977). Intromission-latency (IL, time from the start of the test to the first intromission), ejaculation-latency (EL, time from the first intromission to ejaculation), and the post-ejaculatory interval latency (PEI, time from ejaculation to the first following intromission) were determined. For castrated male rats the mount-latency (ML, time from the start of the test to the first mount) was also recorded. Tests were terminated after the first intromission following ejaculation, or if the IL exceeded 15 min, the EL exceeded 30 min, or the PEI exceeded 15 min. The mean IL for each group was calculated using only those rats that intromitted. The mean EL was calculated using only those rats which ejaculated. A behaviour score (BS) was assigned to each rat as follows: 0 = no sexual activity, 1 = mounting behaviour only, 2 = mounting and intromitting behaviour, and 3 = full behaviour pattern including mounts, intromissions and ejaculation.

Female rat tests: stimulus male rats were placed in the observation cage at 10 min before testing. The experimental female was then introduced and the numbers of mounts (including intromissions and ejaculations) made by the male, and the female response to each mount, were recorded. The female responses were: no lordosis or lordosis (dorsi-flexion of the spine). The lordosis quotient (LQ = ratio of the number of lordoses to the number of mount × 100) was calculated for each rat. Only female rats that were mounted at least once by the male were included in the calculation of LQ. Tests were terminated when the female had received 10 mounts or at the end of 15 min, whichever occurred first.

Dosing regimes of specific studies

Test compounds were administered as a solution in 1 ml kg^{-1} of distilled water or (8 OH-DPAT) 1 ml kg^{-1} of 0.9% saline.

Naive male rat studies: naive male rats were randomly grouped by weight into treatment groups and given a single dose of compound 30 min prior to testing unless otherwise stated. All rats were only tested once, and the observer did not know the drug regimen.

Study 1 Yohimbine was administered at 1, 2 or 4 mg kg^{-1}, p.o. ($n = 33-37$ rats/group).

Study 2 Vehicle or 2 mg kg^{-1} yohimbine was administered to 2 groups of rats p.o. ($n = 36$).

Study 3 Idazoxan was administered at 0.31, 1.25, 2.5, 5.0 or 20 mg kg^{-1}, p.o. ($n = 22-32$).

Study 4 Delequamine was administered at 0.006, 0.025, 0.1, 0.4, or 1.6 mg kg^{-1}, p.o. ($n = 25-26$).

Study 5 Delequamine was administered at 0.025, 0.1, 0.4, or 1.6 mg kg^{-1}, p.o. at 30 min before testing, or at 0.4, 1.6, 6.4 or 25.6 mg kg^{-1}, at 2 h before testing ($n = 20$).

Study 6 Delequamine was administered at 0.1, 0.4, or 1.6 mg kg^{-1} p.o. either daily for 14 days or as a single dose at 30 min before testing ($n=20$).

Study 7 8-OH-DPAT was administered s.c. at 0.1 or 0.25 mg kg^{-1} 10 min, prior to testing ($n=15$).

Study 8 Rats were administered distilled water or delequamine (0.4 mg kg^{-1}) p.o. 30 min before testing and saline or 8-OH-DPAT (0.1 or 0.25 mg kg^{-1}) s.c. 10 min, prior to testing ($n=45$).

Orchidectomized male rat studies: At 11 weeks post castration rats showing no sexual activity were assigned from a common pool to treatment groups for studies 9, 10 and 11, using a random latin square design and tested at three week intervals. The rats used in each treatment group of one study were randomly assigned between all treatment groups of the next study so that no animal was used in the same study more than once. The rats in study 12 comprised a separate group, which were randomly assigned to five treatment groups using a 5×5 latin square design. Thus each rat received each of five treatments over a series of five tests, performed at three week intervals. The compounds were administered p.o. 30 min prior to testing.

Study 9 Yohimbine was administered at 1, 2, 4 or 8 mg kg^{-1}, p.o. ($n=42-45$).

Study 10 Idazoxan was administered at 0.5, 1, 2 or 4 mg kg^{-1}, p.o. ($n=35-50$).

Study 11 Delequamine was administered at 0.025, 0.1, 0.4 mg kg^{-1}, p.o. ($n=57-59$).

Study 12 Delequamine was administered at 0.1, 0.4, 1.6 or 6.4 mg kg^{-1}, p.o. ($n=73$).

Ovariectomized female rat studies Ovariectomized female rats were randomly assigned to treatment schedules using a latin square design. Thus each rat received each treatment over a series of tests. The compounds were administered p.o. at 30 min before testing, except for 8-OH-DPAT which was administered s.c. 10 min before testing.

Study 13 Delequamine was administered at 0.1, 0.4, or 1.6 mg kg^{-1}, p.o. ($n=54$) following priming with 1 μg oestradiol and 50 μg progesterone.

Study 14 Delequamine was administered at 1.6, 6.4, or 25.6 mg kg^{-1}, p.o. ($n=60$) following priming with 1 μg oestradiol and 50 μg progesterone.

Study 15 Yohimbine was administered at 1, 2, or 4 mg kg^{-1}, p.o. ($n=53-61$) following priming with 1 μg oestradiol and 50 μg progesterone.

Study 16 Yohimbine was administered at 1, 2, or 4 mg kg^{-1}, p.o. ($n=67$) following priming with 1.25 μg oestradiol and 1 mg progesterone.

Study 17 8-OH-DPAT was administered at 0.1, 0.25 or 0.625 mg kg^{-1}, s.c. ($n=45$) following priming with 1 μg oestradiol and 50 μg progesterone.

Statistics

Data from the naive male rat studies were analyzed for overall effect of treatment using a nonparametric ANOVA on ranked data (Kruskal-Wallis). Pair comparisons to the vehicle-treated group were made with Fisher's LSD multiple comparison strategy. Data from the orchidectomized male rat studies 9–11 were combined across weeks and those studies involving the use of multiple treatment groups were analyzed by Fisher's exact test for overall effect of treatment and pair comparisons to the vehicle-treated group. Data from the orchidectomized male rat study 12, with a random latin square design, were analyzed with Cochran's Q statistic for overall effect of treatment and McNemar's test for pair comparisons to the vehicle-treated group. The female rat data (lordosis quotients) were analyzed by a one-way non-parametric ANOVA and Fisher's LSD strategy for pair comparisons between groups. All statistical analyses were performed using SAS statistical software (SAS Institute, Inc., Cary, NC, U.S.A.).

Drugs

Yohimbine hydrochloride, oestradiol benzoate and progesterone were obtained from Sigma Chemical Co. (St. Louis, MO, U.S.A.) and idazoxan hydrochloride from Reckitt and Coleman, Ltd (Kingston-upon-Hull, UK). Delequamine hydrochloride (RS 15385-197) (12-ethane sulphonyl-3-methoxy-5,6,7,8,α,9,10,11,12 α-decahydroisoquine[2.1g][1,6] naphthyridine hydrochloride) and 8-OH-DPAT hydrobromide (D. Repke) were synthesized at Syntex, Palo Alto, CA, U.S.A.

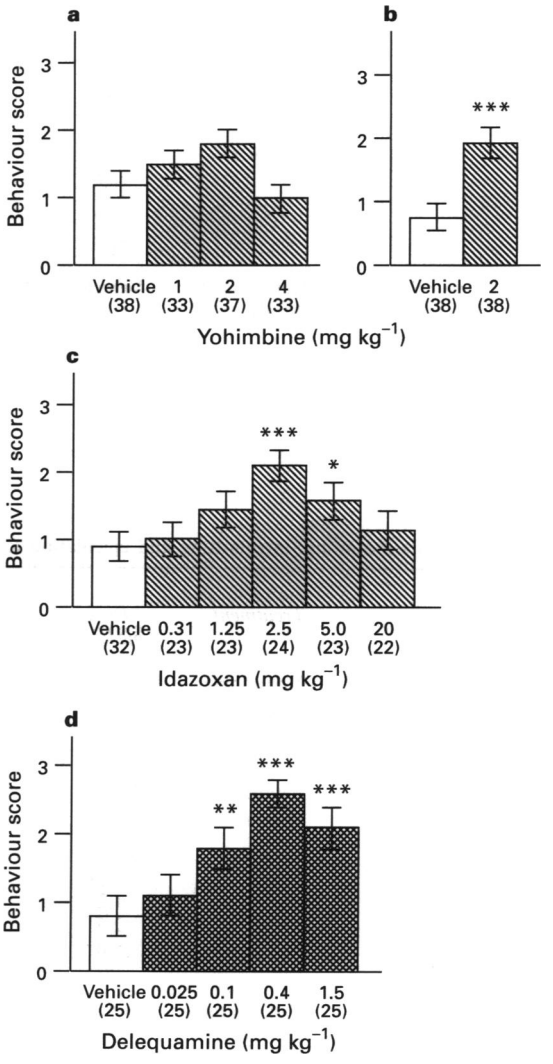

Figure 1 Mean (±s.e.mean) behavioural score of naive male rats after a single oral administration of yohimbine (a,b), idazoxan (c), and delequamine (d). $*P \leq 0.05$, $**P \leq 0.005$, and $***P \leq 0.0005$ compared to the vehicle-treated group. Numbers in parentheses indicate number of animals per group.

Results

Naive male rat studies

Naive male rats showed low sexual behavioural scores (Figure 1), but despite the low scores, yohimbine was only weakly active in enhancing sexual behaviour in the male rat, and only at one dose: 2 mg kg^{-1}, p.o. In two separate studies only weak effects were seen. Thus, although 2 mg kg^{-1} yohimbine tended to increase the mean behaviour score (Figure 1a, study 1), indicating a stimulation of sexual behaviour, this increase was only significant in one of the studies (Figure 1b). However, in the first study, yohimbine did reduce ejaculation latency (Table 1), consonant with some enhancement of sexual behaviour. In study 2, treatment with 2 mg kg^{-1} yohimbine caused a significant increase in the mean behaviour score (Figure 1b; $P<0.0005$), but there was no significant effect on intromission or ejaculation latency. It should be noted that the mean behaviour score (1.2) for the vehicle-treated group in study 1 was higher than that in study 2 (0.8, a value within the normal range for our naive rat model), which may be the reason for the failure to achieve statistical significance. However, the mean behavioural scores for the 2 mg kg^{-1}-treated groups were similar in both studies (1.8 and 1.9, respectively).

Idazoxan, administered at 2.5 and 5.0 mg kg^{-1}, significantly ($P\leqslant 0.0005$ and $P\leqslant 0.05$, respectively) increased the mean behaviour score (Figure 1c, study 3), but there was no significant effect on the mean ejaculation latency or intromission latency (Table 1A). The percentage of rats mounting, intromitting, and ejaculating was increased at 2.5 mg kg^{-1}

Table 1A Mean intromission latency (IL) and mean ejaculation latency (EL) following treatment with yohimbine, idazoxan or delequamine (RS-15385-197)

Treatment	Dose (mg kg^{-1})	IL (min) n	IL (min) Mean±s.e.	EL (min) n	EL (min) Mean±s.e.
Yohimine	Vehicle	16	4.87±0.80	7	24.52±1.19
(Study 1)	1	18	4.80±0.68	9	16.73±2.59*
	2	23	4.68±0.68	16	16.58±1.57*
	4	11	5.71±1.33	8	18.28±2.64
Yohimbine	Vehicle	11	7.72±1.28	5	18.19±1.28
(Study 2)	2	25	5.12±0.66	17	16.88±1.47
Idazoxan	Vehicle	12	4.95±1.07	4	12.97±3.42
(Study 3)	0.31	9	3.19±0.69	3	13.98±2.87
	1.25	13	3.92±0.91	7	21.30±2.08
	2.5	19	4.34±0.82	11	17.11±1.77
	5.0	13	4.12±0.93	8	16.93±2.80
	20	9	5.32±1.12	6	16.32±3.53
Delequamine	Vehicle	7	6.71±1.54	5	22.71±1.39
(Study 4)	0.006	13	3.63±0.74	7	17.05±1.60
	0.025	11	3.87±0.67	5	14.49±4.11
	0.1	17	2.99±0.45*	12	13.49±1.40
	0.4	22	4.14±0.69	20	18.01±1.76
	1.6	19	2.66±0.38*	15	15.14±2.08

*$P<0.05$ compared to the group receiving vehicle.

1B % of rats mounting, intromitting and ejaculating following treatment with yohimbine, idazoxan or delequamine

Treatment	Dose (mg kg^{-1})	n	% of rats Mounting	% of rats Intromitting	% of rats Ejaculating
Yohimbine	Vehicle	36	53	44	19
(Study 1)	1	33	67	55	27
	2	37	73	62	43
	4	33	52	33	24
Yohimbine	Vehicle	36	31	31	14
(Study 2)	2	36	75***	69**	47**
Idazoxan	Vehicle	32	38	38	13
(Study 3)	0.31	22	45	41	14
	1.25	23	57	57	30
	2.5	24	83**	79**	50**
	5.0	23	65	57	35
	20	22	45	41	27
Delequamine	Vehicle	25	28	28	20
(Study 4)	0.006	25	52	52	28
	0.025	25	52	44	20
	0.1	26	69*	65*	46
	0.4	25	88***	88***	80***
	1.6	25	80**	76**	60*

*$P<0.05$, **$P<0.005$, ***$P<0.0005$ using Fisher's exact test.

($P<0.005$; Table 1B). These data indicate that idazoxan effectively stimulates sexual behaviour over a dose-range of less than 4 fold, as doses of 0.31, 1.25 and 20 mg kg^{-1} were inactive. Thus, these α_2-adrenoceptor antagonists were poorly active over a restricted dose range.

In contrast, delequamine administered at 0.1, 0.4, and 1.6 mg kg^{-1}, significantly ($P<0.005$, $P<0.0005$ and $P<0.0005$, respectively) increased the behavioural score and at 0.1 and 1.6 mg kg^{-1} decreased the mean intromission latency ($P<0.05$) (Figure 1d; Table 1B). In contrast to the data with yohimbine, there was no significant effect on the mean ejaculation latency. The stimulatory effect of delequamine was greater than that of either yohimbine or idazoxan and was present over at least a 16 fold dose range.

The question of whether the effects of an α_2-adrenoceptor antagonist are sustained over time was addressed by administration of delequamine at different time intervals prior to testing, or by chronic administration. The relative potency of delequamine over time in naive male rats was compared by testing for sexual behaviour at 30 min and 2 h post administration (study 5; Figure 2). The data indicate that mean behaviour score was significantly increased (Figure 2a) following the administration of 0.4 and 1.6 mg kg^{-1} of delequamine at 30 min before testing ($P\leqslant0.005$, and $P\leqslant0.05$, respectively) (and following 1.6, 6.4, and 25.6 mg kg^{-1} at 2 h before testing; $P\leqslant0.05$, $P\leqslant0.005$, and $P\leqslant0.05$, respectively). In addition, there was a significant decrease in mean intromission latency (Figure 2b) following the 1.6 mg kg^{-1} dose at 30 min and also following the 1.6, 6.4 and 25.6 mg kg^{-1} doses at 2 h ($P\leqslant0.05$ for all comparisons). To obtain the degree of enhancement of sexual behaviour achieved when delequamine was administered at 30 min prior to testing required a 16 times greater dose when given at 2 h before testing. These data are consistent with the metabolism of delequamine in the rat and with the maintenance of a constant degree of α_2-adrenoceptor antagonism (Brown *et al.*, 1992).

The effect on sexual behaviour of 14 daily doses of delequamine was compared to that of a single dose (study 6). Delequamine, administered at 0.1, 0.4, and 1.6 mg kg^{-1} for 14 days and tested 30 min after the last dose, significantly increased the mean behaviour score (Figure 3a) ($P\leqslant0.05$, $P\leqslant0.005$, and $P\leqslant0.0005$, respectively) to the same extent as after a single dose on the day of testing ($P\leqslant0.05$, $P\leqslant0.005$, and $P\leqslant0.0005$, respectively). There was also a significant de-

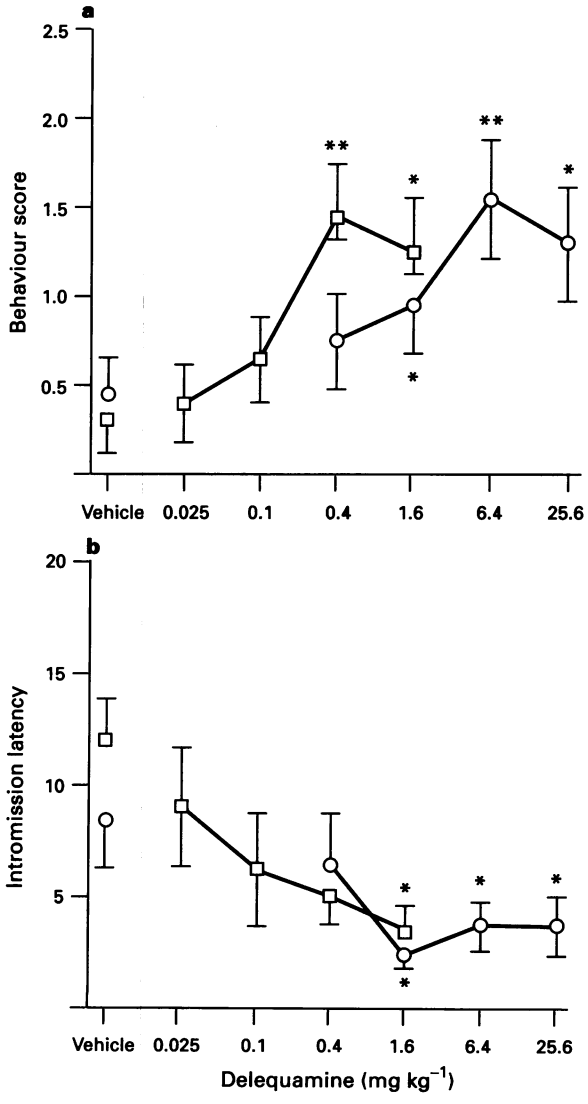

Figure 2 Mean (±s.e.mean) behavioural score (a) and intromission-latency (b) of naive male rats after single oral doses of delequamine at 30 min (□) and 2 h (○) before testing. *$P\leqslant0.05$, **$P\leqslant0.005$, compared to the vehicle-treated group receiving the same dose schedule; 20 rats/group.

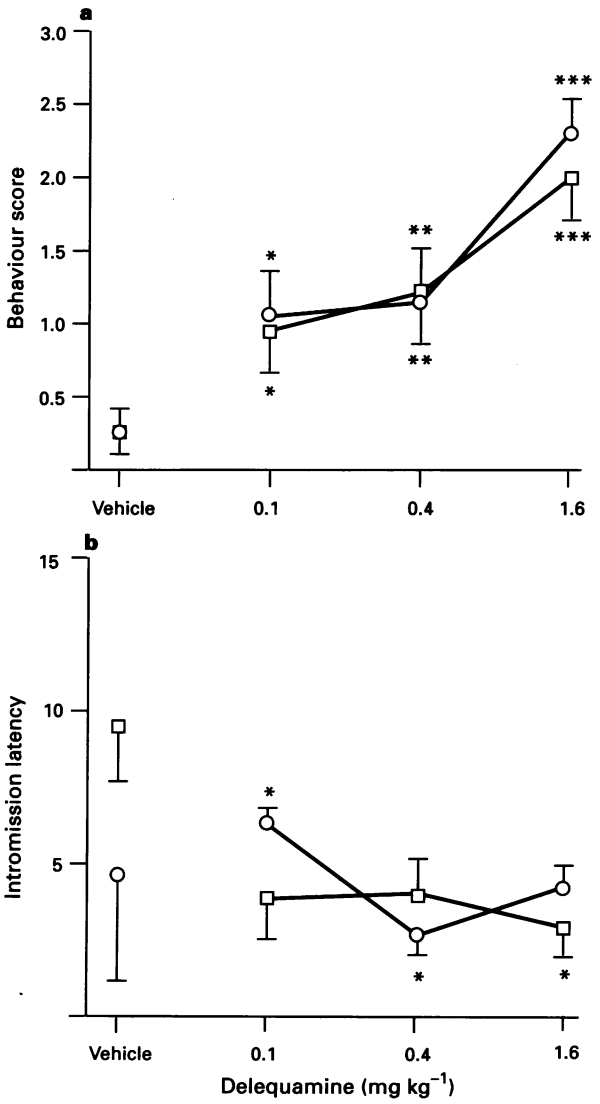

Figure 3 Mean (±s.e.mean) behavioural score (a) and intromission-latency (b) of naive male rats following delequamine administration by a single dose (□) or by 14 daily doses (○). *$P\leqslant0.05$, **$P\leqslant0.005$, compared to the vehicle-treated group receiving the same dose schedule; 20 rats/group.

crease in mean intromission latency (Figure 3b) following 14 days of dosing with 0.4 mg kg^{-1} of delequamine ($P \leqslant 0.05$) or following a single dose of 1.6 mg kg^{-1} ($P \leqslant 0.05$). Thus the data show that the behaviour enhancing effect of delequamine was very similar when dosing was extended from 1 to 14 days.

The agonist at 5-HT_{1A} receptors, 8-OH-DPAT, tended to increase the behavioural score at 0.1 mg kg^{-1}, but not 0.25 mg kg^{-1}, in the naive male rat. However, this effect was associated with a marked reduction in ejaculation-latency at 0.1 mg kg^{-1} and reduced number of intromissions at 0.1 and 0.25 mg kg^{-1} (s.c.) (Figure 4). Higher doses induced the marked behavioural syndrome associated with this compound.

The effects of a combination of delequamine (0.4 mg kg^{-1}, p.o.) and 8-OH-DPAT (0.1 mg kg^{-1}, s.c.) were investigated (Table 2, study 8). As in the previous studies, delequamine alone caused an increase in behavioural score without an effect on the ejaculation latency or the number of intromissions, whereas 8-OH-DPAT caused no change in behavioural score but a reduction in ejaculation latency and decreased the number of intromissions prior to ejaculation. The combination of the two compounds increased behavioural score, reduced intromission latency and ejaculation latency and decreased the number of intromissions (Table 2).

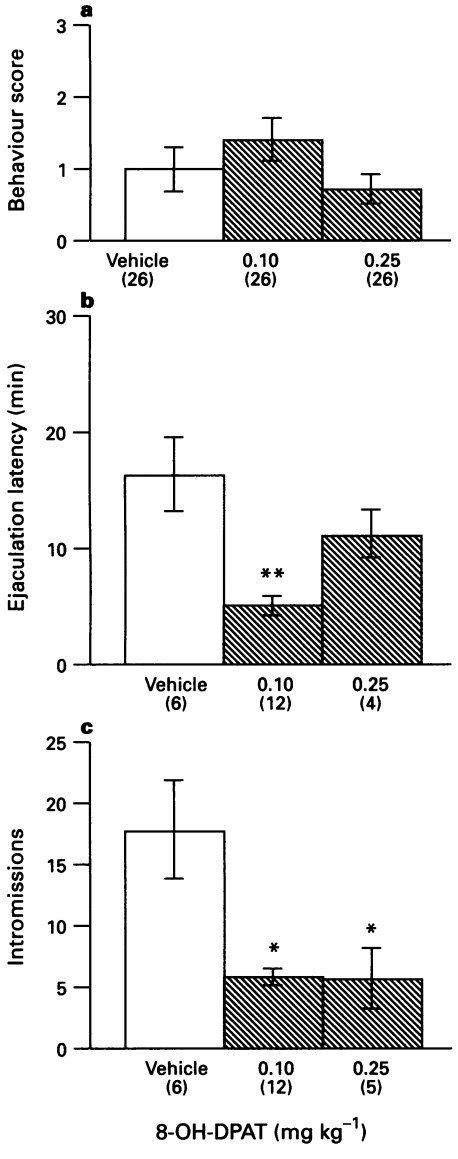

Figure 4 Mean (±s.e.mean) effects of 8-OH-DPAT (0.1 or 0.25 mg kg^{-1}, s.c.) on the overall sexual behaviour score of male rats (a), on the ejaculation latency (b) and on the number of intromissions (c); 15 rats/group.

Orchidectomized male studies

The parameters analyzed statistically for the assessment of enhanced sexual behaviour in the castrated male rat were the percentages of rats mounting and intromitting. The percentage of castrated male rats mounting was significantly ($P \leqslant 0.05$) increased (studies 9 and 10; Table 3) following 2 mg kg^{-1} yohimbine and also with 1 and 2 mg kg^{-1} idazoxan ($P \leqslant 0.05$). However, there was no significant effect of either compound on the percentages of rats intromitting.

Delequamine at 0.4 mg kg^{-1} significantly ($P \leqslant 0.05$) increased the percentage of rats mounting (study 11; Table 3) with no significant effect on the percentage of rats intromitting. However, in a second study (study 12), the percentage of rats intromitting was significantly increased following 1.6 and 6.4 mg kg^{-1} of delequamine ($P \leqslant 0.005$, and $P \leqslant 0.05$, respectively) but there was no significant effect on the percentage mounting (Table 3).

Ovariectomized female studies

The low dose priming with oestradiol benzoate and progesterone used in the ovariectomized rats induced a low level of sexual receptivity (lordosis quotient of 25). Delequamine, administered at 1.6 mg kg^{-1} to such rats significantly ($P \leqslant 0.05$) increased the mean lordosis quotient (Figure 5a). In a second study, significant ($P \leqslant 0.05$) increases in the mean lordosis quotient were observed following the 1.6 and a 6.4 mg kg^{-1} dose of delequamine (Figure 5b). These data show that sexual receptivity was enhanced in the ovariectomized female rat model by 1.6 to 6.4 mg kg^{-1} of delequamine.

Conversely, 4 and 8 mg kg^{-1} yohimbine (study 15) significantly ($P \leqslant 0.005$, and $P \leqslant 0.0005$, respectively) decreased the mean lordosis quotient in a dose-dependent manner (Figure 6a) in ovariectomized female rats primed with low levels of oestradiol benzoate and progesterone. When the priming doses of oestradiol benzoate and progesterone were increased (study 16), the mean lordosis quotient was again significantly ($P \leqslant 0.0005$ for all comparisons) decreased following doses of 2, 4, and 8 mg kg^{-1} of yohimbine (Figure 6b). Thus, the effects of yohimbine in female rats differ markedly from those in male rats, and also from the effects of delequamine. However, the effects of yohimbine in the female rat were similar to those of 8-OH-DPAT, because 0.1, 0.25 and 0.625 mg kg^{-1} of 8-OH-DPAT (study 17; Figure 7) significantly ($P < 0.05$) decreased the mean lordosis quotient on ovariectomized rats primed with low levels of oestradiol and progesterone.

Discussion

The data from the studies in a naive male rat model are entirely consistent with previous data published for the effects of yohimbine and 8-OH-DPAT on sexual behaviour in the rat (Ahlenius *et al.*, 1981; Clark *et al.*, 1984; Bitran & Hull, 1987; Smith *et al.*, 1987; Ahlenius & Larsson, 1991). Yohimbine, a weak α_2-adrenoceptor antagonist, with affinity at the 5-HT_{1A} receptor, enhanced sexual behaviour score and decreased ejaculation latency at the 2 mg kg^{-1} dose with only a narrow window of efficacy. The 5-HT_{1A} agonist, 8-OH-DPAT, decreased the ejaculation threshold (determined by decreases in the ejaculation latency and the number of intromissions prior to ejaculation). Thus both yohimbine and 8-OH-DPAT had very narrow dose-ranges where a trend to an increased behaviour score could be discerned. It is not clear whether the limited dose-range for 8-OH-DPAT is due to the appearance of the 5-HT behavioural syndrome at higher doses, masking sexual behaviour by the imposition of other behaviours, or due to partial agonism or differential effects at pre- and post-

Table 2A % of rats mounting, intromitting and ejaculating, and the mean behavior score, following treatment with 0.4 mg kg^{-1} delequamine p.o., 0.1 mg kg^{-1} 8-OH-DPAT s.c., or a combination of 0.4 mg kg^{-1} delequamine p.o. and 0.1 mg kg^{-1} 8-OH-DPAT s.c.

Treatment	n	*% of rats* *Mounting*	*Intromitting*	*Ejaculating*	*BS* *Mean ± s.e.*
(Study 8)					
Distilled water + saline	45	27	27	9	0.6 ± 0.2
Delequamine + saline	45	51*	40	20	1.1 ± 0.2*
Distilled water + 8-OH-DPAT	45	33	29	27	0.9 ± 0.2
Delequamine + 8-OH-DPAT	45	53*	51*	49**f†	1.5 ± 0.2*†

*$P \leqslant 0.05$, **$P \leqslant 0.0005$ compared to the group receiving distilled water + saline. *f*$P \leqslant 0.05$ compared to the group receiving delequamine + saline. †$P \leqslant 0.05$ compared to the group receiving distilled water + 8-OH-DPAT.

2B Mean intromission latency (IL), mean ejaculation latency (EL) and the number of intromissions prior to ejaculation, following treatment with 0.4 mg kg^{-1} delequamine p.o., 0.1 mg kg^{-1} 8-OH-DPAT s.c. or a combination of 0.4 mg kg^{-1} delequamine p.o. 0.1 mg kg^{-1} 8-OH-DPAT s.c.

Treatment	n	*Number of intromissions* *Mean ± s.e.*	*IL (min)* *Mean* ± s.e.	n	*EL (min)* *Mean* ± s.e.
(Study 8)					
Distilled water + saline	12	13.75 ± 1.87	4.23 ± 1.01	4	7.68 ± 2.88
Delequamine + saline	18	16.50 ± 1.50	3.25 ± 0.65	9	14.54 ± 1.70
Distilled water + 8-OH-DPAT	13	7.92 ± 1.13**ff*	3.95 ± 0.97	12	8.56 ± 2.06*f*
Delequamine + 8-OH-DPAT	23	9.09 ± 0.82**ff*	4.82 ± 0.96	21	7.02 ± 1.22*f*

*$P \leqslant 0.05$, **$P \leqslant 0.005$ compared to the group receiving distilled water + saline. *f* $P \leqslant 0.05$, *ff* $P \leqslant 0.005$ compared to the group receiving delequamine + saline.

Table 3 % of castrate rats mounting, intromitting and ejaculating, and the number of mounts per mounting rat, following treatment with yohimbine, idazoxan or delequamine

Treatment	*Dose (mg kg^{-1})*	n	*% Rats* *Mounting*	*Intromitting*	*Ejaculating*	*Number of mounts per mounting rats* n	*Mean ± s.e.-mean*
Yohimine	Vehicle	44	11	0	0	5	5.6 ± 2.1
(Study 9)	1	44	30	11	2	13	18.3 ± 4.9
	2	44	36*	9	0	16	10.2 ± 2.9
	4	45	16	2	0	7	6.0 ± 1.9
	8	42	10	5	0	4	7.2 ± 4.1
Idazoxan	Vehicle	47	11	2	0	5	5.0 ± 1.7
(Study 10)	0.5	35	11	6	0	4	12.5 ± 7.6
	1	49	29*	14	4	15	14.7 ± 3.7
	2	50	32*	12	6	16	13.9 ± 3.8
	4	47	15	4	4	8	9.2 + 4.0
Delequamine	Vehicle	58	12	3	2	7	13.0 ± 6.2
(Study 11)	0.025	59	22	2	0	13	7.1 ± 2.4
	0.1	57	21	1	2	12	11.8 ± 3.8
	0.4	59	36*	14	5	21	13.9 ± 3.0
Delequamine	Vehicle	73	29	4	1	21	10.4 ± 2.8
(Study 12)	0.1	73	34	10	3	25	16.0 ± 2.8
	0.4	73	27	11	4	20	17.1 ± 3.3
	1.6	73	36	22**	4	26	28.1 ± 3.7
	6.4	73	26	14*	4	19	19.9 ± 4.1

*$P < 0.05$, **$P < 0.005$, using Fisher's exact test (studies 9–11) or McNemar's test (study 12). $P < 0.05$ using Fisher's LSD strategy.

synaptic receptors (Ahlenius & Larsson, 1991).

In contrast, the highly potent and selective α_2-adrenoceptor antagonist, delequamine, effectively enhanced sexual behaviour at 0.1–1.6 mg kg^{-1}, which indicates greater potency over a wider (16 fold) effective dose-range than has been presented for the other α_2-antagonists, but without any effect on ejaculation latency. The dose-ranges are entirely consistent with the α_2-adrenoceptor antagonist potency in the rat (Redfern *et al.*, 1992). We have also shown that delequamine effectively enhances sexual behaviour 2 h after dosing if the dose is increased 16 fold. In addition, a single dose of delequamine is equally as effective as daily dosing for 14 days. The relatively

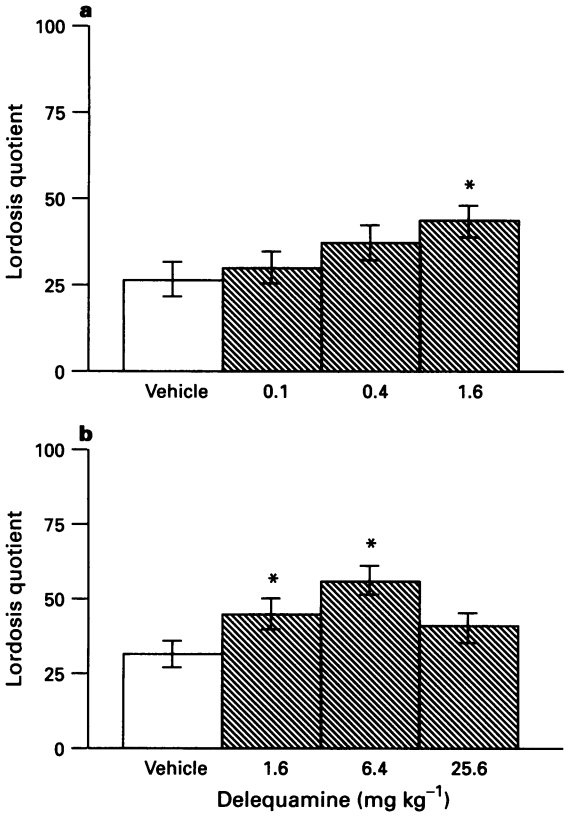

Figure 5 Mean (±s.e.mean) lordosis quotient of ovariectomized female rats primed with 1 μg oestradiol and 50 μg progesterone, after single oral doses of delequamine. *$P \leq 0.05$ compared to the vehicle-treated group; 54 rats/group (a) and 60 rats/group (b).

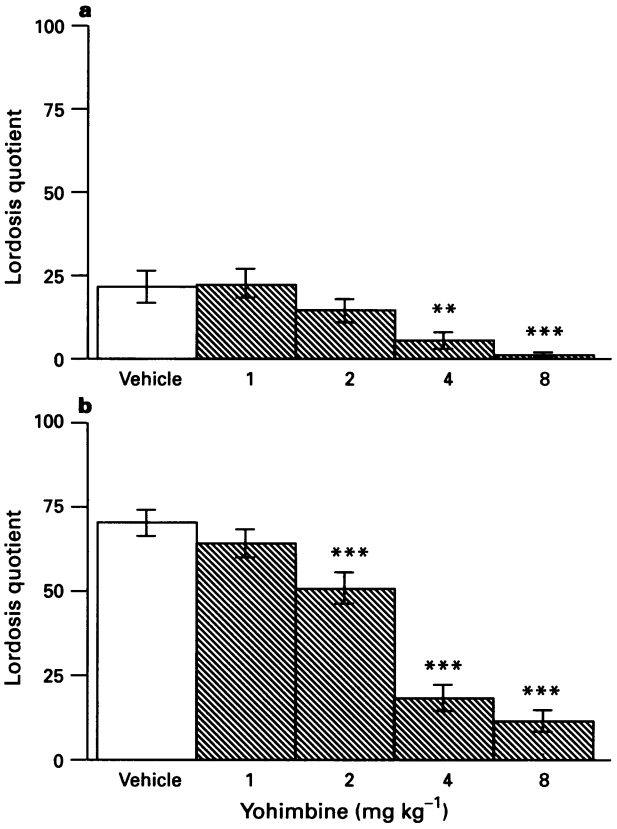

Figure 6 Mean (±s.e.mean) lordosis quotient of ovariectomized female rats, primed with 1 μg oestradiol and 50 μg progesterone (a; $n = 50-61$/group) or with 1.25 μg oestradiol and 1 mg progesterone (b; $n = 67$/group), after single oral doses of yohimbine. **$P \leq 0.005$, ***$P \leq 0.0005$ compared to the vehicle-treated group.

selective α_2-adrenoceptor antagonist, idazoxan, resembled delequamine by increasing the overall behaviour score without changing ejaculation latency; however, idazoxan had a narrow dose window, which may be due to the fact that the compound is less selective for α_2-adrenoceptors than delequamine. These findings are consistent with the hypothesis that selective α_2-adrenoceptor antagonism increases sexual behaviour in the rat without decreasing the ejaculation threshold and that these effects are maintained on chronic administration and are evident over the dose-ranges where the drugs are selective for α_2-adrenoceptor antagonism. These findings also extend to man, because delequamine has been shown to increase sexual arousal and the duration of the erectile response in man at plasma levels which are specific for α_2-adrenoceptor antagonism (Munoz *et al.*, 1994b). Furthermore, delequamine reversed psychogenic erectile failure in young patients, and had a beneficial effect in older patients (Munoz *et al.*, 1994a). Thus α_2-adrenoceptor antagonism would appear to have selective effects on sexual behaviour in rat and man.

In the orchidectomized male rat, yohimbine, idazoxan, and delequamine produced partial restoration of sexual behaviour at similar doses (2 mg kg^{-1}, 1–2 mg kg^{-1}, and 0.4–1.6 mg kg^{-1}, respectively) as in the naive male rat, although delequamine was again effective over a wider dose-range than were the other drugs. The data with yohimbine are similar to data previously obtained by Clark *et al.* (1984) and indicate an exceptional effect whereby in orchidectomized rats the initial mounting components of behaviour are restored by α_2-adrenoceptor antagonism. Thus the behaviour evoked is not testerone-dependent. The present findings extend the findings of Clark *et al.* (1984) to include the more selective α_2-adrenoceptor antagonists and indicate that this component of the effects of yohimbine is probably due to α_2-adrenoceptor antagonism.

Does an effect on the 5-HT$_{1A}$ receptor modify the profile of yohimbine in the male rat? Yohimbine was only weakly effective in the present experimental conditions and the profile of the effects differed from those of delequamine, in that yohimbine decreased ejaculation latency. The 5-HT$_{1A}$ receptor partial agonist, 8-OH-DPAT, also reduces ejaculation latency and has a bell shaped dose-response curve. 8-OH-DPAT also has differential effects in male (reduced ejaculation threshold) and female (reduced receptivity) rats. The effects of 8-OH-DPAT in the male rat are limited by the onset of the characteristic 5-HT behavioural syndrome which becomes apparent as the dose is increased from 0.25 mg kg^{-1}, s.c. At higher doses we were unable to differentiate inhibition of sexual activity from direct locomotor effects but it is clear that the enhancement of sexual activity seen with 8-OH-DPAT has a bell-shaped dose-response curve. In the male rats, the weak effects on mean behaviour score are also predominantly caused by the reduced ejaculation latency, which was seen with yohimbine, but was not a component of the profile of delequamine. As yohimbine has high affinity for 5-HT$_{1A}$ receptors and generalises to 5-HT$_{1A}$ cues in drug discrimination studies (Kawai *et al.*, 1992; Winter & Rabin, 1992), it would appear likely that the effects of yohimbine are due to a combination of α_2-adrenoceptor antagonism and effects at 5-HT$_{1A}$ receptors.

Such a combination of effects is compatible with the findings in female rats, where 8-OH-DPAT markedly reduced the lordosis response in a dose-dependent manner. The effects of yohimbine and delequamine in the primed, ovariectomized, female rat provide very different profiles. Yohimbine suppresses sexual receptivity which corroborates the findings of Davis & Kohl (1977), while delequamine causes a stimulation of receptivity. It would appear that the yohimbine-induced suppression of lordosis may be in part the result of the affinity of the drug for the 5-HT$_{1A}$ receptor. The 5-HT$_{1A}$ receptor

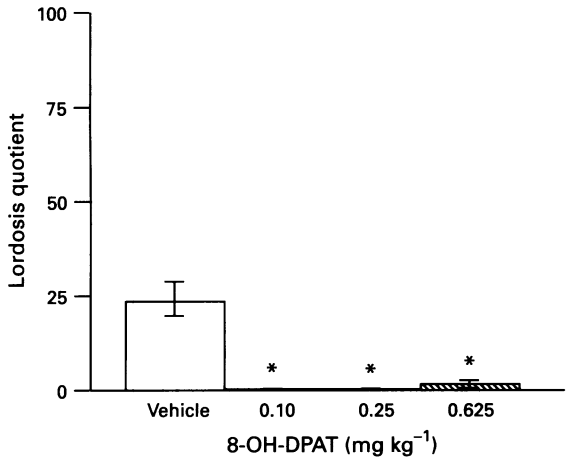

Figure 7 Mean (±s.e.mean) lordosis quotient of ovariectomized female rats primed with 1 μg oestradiol and 50 μg progesterone, after single s.c. doses of 8-OH-DPAT. *$P \leqslant 0.05$ compared to a vehicle-treated group; 49 rats/group.

agonist, 8-OH-DPAT, suppresses the lordosis response in primed, ovariectomized female rats (Ahlenius *et al.*, 1986 and the present studies) and these effects have been localized to the ventromedial nucleus of the hypothalamus and also to the medial preoptic area (Uphouse *et al.*, 1992; Uphouse & Caldorola-Pastuszka, 1993). Thus in the primed, ovariectomized, female rat the effects of yohimbine resemble those of 8-OH-DPAT.

It is not clear whether the limited dose-range for 8-OH-DPAT is due to the appearance of the 5-HT behavioural syndrome at higher doses, masking sexual behaviour by the imposition of other behaviours, or due to partial agonism or differential effects at pre- and postsynaptic receptors. Yohimbine does not show a 5-HT behavioural syndrome at higher doses, and, if the inhibitory effects of higher doses of yohimbine on sexual activity in the rat are related to effects on 5-HT_{1A} receptors; then this would argue against a non-specific reduction of the behaviour score by the imposition of a behavioural syndrome. In the female rat, 5-HT_{1A} receptors may selectively interfere with certain aspects of sexual behaviour. Furthermore, the general behavioural syndrome of forepaw treading and flat body posture induced by 8-OH-DPAT is unaffected by coadministration with delequamine (Redfern *et al.*, 1992), but the effects of a low dose of 8-OH-DPAT on male sexual behaviour may be augmented by delequamine (this study), showing that the enhancing and inhibitory effects of 5-HT_{1A} receptor activation on male sexual behaviour may be mediated by specific mechanisms.

Thus, the effects at 5-HT_{1A} receptors may limit the effectiveness and dose-range of yohimbine. Indeed, the affinity of yohimbine for 5-HT_{1A} receptors may also be responsible for some of the side effects of the drug. Yohimbine is anxiogenic at doses of 3 and 10 mg kg^{-1} in the rat in the elevated X-maze model (Redfern & Williams, 1995) and also causes anxiety in man at high doses (Charney *et al.*, 1982), but delequamine is not anxiogenic in the elevated X-maze model (Redfern & Williams, 1995) and anxiety was not noted in the early clinical trials (Munoz *et al.*, 1994a, b). Redfern & Williams (1995) concluded that the increase in anxiety caused by yohimbine is not associated with α_2-adrenoceptor antagonism. Delequamine is a highly selective agent which has >1000 fold selectivity for α_2-adrenoceptors in binding experiments (Brown *et al.*, 1992) and high selectivity in functional experiments (MacKinnon *et al.*, 1992; Redfern *et al.*, 1992), thus allowing redefinition of the role of specific α_2-adrenoceptor antagonism in man. Delequamine, in contrast to yohimbine and idazoxan, increased sexual behaviour score, under all the conditions tested and over a wide dose-range, in male rats. These effects are compatible with the recent findings reported for delequamine in man (Munoz *et al.*, 1994a, b).

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References

AHLENIUS, S., FERNANDEZ-GUASTI, A., HJORTH, S., & LARSSON, K. (1986). Suppression of lordosis behaviour by the putative 5-HT receptor agonist 8-OH-DPAT in the rat. *Eur. J. Pharmacol.*, **124**, 361–363.

AHLENIUS, S. & LARSSON, K. (1991). Physiological and pharmacological implications of specific effects of 5-HT_{1A} agonists on rat sexual behaviour. In *5-HT_{1A} Agonists, 5-HT_3 Antagonists and Benzodiazepines: Their Comparative Behavioural Pharmacology*. pp. 281–315. ed. Rodgers, RJ & Cooper SJ. Chichester: John Wiley & Sons Ltd.

AHLENIUS, S., LARSSON, K., SVENSSON, L., HJORTH, S., CARLSSON, A., LINDBERG, P., WIKSTROM, H., SANCHEZ, D., ARVIDSSON, L.E., HACKSELL, U. & NILSSON, J.L. (1981). Effects of a new type of 5-HT receptor agonist on male rat sexual behaviour. *Pharmacol. Biochem. Behav.*, **15**, 785–792.

BITRAN, D. & HULL, E.M. (1987). Pharmacological analysis of male sexual behaviour. *Neurosci. Behav. Rev.*, **11**, 365–387.

BROWN, C.M., MACKINNON, A.C., KILPATRICK, A.T., REDFERN, W.S., RAMCHARAN, M., SMALL, C., HICKS, P.E., CLARK, R., MARFARLANE, C.B. & SPEDDING, M. (1992). The pharmacology of Delequamine, a potent and selective α_2-adrenoceptor antagonist. *Br. J. Pharmacol.*, **108**, 516–525.

CHARNEY, D.S., HENINGER, G.R. & REDMOND, JR. DE. (1983). Yohimbine-induced anxiety and increased noradrenergic function in humans: effect of diazepam and clonidine. *Life Sci.*, **33**, 19–29.

CLARK, R.D., REPKE, D.B., KILPATRICK, A.T., BROWN, C.B., DYE, A.D., CLAGUE, R.U. & SPEDDING, M. (1989). 12-Ethane sulfonyl-3-methoxy-5,6,7,8,α,9,10,11,12 α-decahydroisoquine[2.1g][1,6]-naphthyridine. A potent and highly selective α_2-adrenoceptor antagonist. *J. Med. Chem.*, **32**, 2034–2036.

CLARK, T.J., SMITH, E.R. & DAVIDSON, J.M. (1984). Testosterone is not required for the enhancement of sexual motivation by yohimbine. *Physiol. Behav.*, **35**, 517–521.

CLARK, T.J., SMITH, E.R. & DAVIDSON, J.M. (1985). Enhancement of sexual motivation in male rats by yohimbine. *Science*, **225**, 847–849.

DAVIS, G.A. & KOHL, R. (1977). The influence of α-receptors on lordosis in the female rat. *Pharmacol. Biochem. Behav.*, **6**, 47–53.

FELDMAN, H.A., GOLDSTEIN, I., HATZICHRISTOU, D.G., KRANE, R.J. & MCKINLAY, J.B. (1994). Impotence and its medical and psychosocial correlates: results of the Massachusetts male aging study. *J. Urol.*, **151**, 54–61.

GOLDBERG, M.R. & ROBERTSON, D. (1983). Yohimbine: a pharmacological probe for study of the α-adrenoceptor. *Pharmacol. Rev.*, **35**, 143–180.

JACOBSEN, F.M. (1992). Fluoxetine-induced sexual dysfunction and an open trial of yohimbine. *J. Clin. Psychiat.*, **53**, 119–122.

KAWAI, N., YAMAMOTO, T., BABA, A., YAMAMOTO, H., MOROJI, T. & KUMAMOTO, H. (1992). Properties of yohimbine and its stereoisomers as a 5-hydroxytryptamine$_{1A}$ receptor agonist. *Mol. Neuropharmacol.*, **2**, 283–288.

MACKINNON, A.C., BROWN, C.M., KILPATRICK, A.T. & SPEDDING, M. (1991). RS-15385-197, a selective α_2-adrenoceptor antagonist, has low affinity for imidazoline binding sites on hamster adipocytes. *Br. J. Pharmacol.*, **102**, 377P.

MACKINNON, A.C., KILPATRICK, A.T., KENNY, B.A. & BROWN, C.M. (1992). [^{3}H]-RS-15385-197, a selective and high affinity radioligland for alpha2-adrenoceptors: implications for receptor classification. *Br. J. Pharmacol.*, **106**, 1011–1018.

MORALES, A., SURRIDGE, D.H.C., MARSHALL, P.G. & FENEMORE, J. (1982). Nonhormonal pharmacological treatment of organic impotence. *J. Urol.*, **128,** 45–47.

MUNOZ, M., BANCROFT, J. & BEARD, M. (1994a). Evaluating the effects of an alpha2 adrenoceptor antagonist on erectile function in the human male. 2. The erectile response to erotic stimuli in men with erectile dysfunction, in relation to age and in comparison with normal volunteers. *Psychopharmacology*, **115,** 471–477.

MUNOZ, M., BANCROFT, J. & TURNER, M. (1994b). Evaluating the effects of an alpha2 adrenoceptor antagonist on erectile function in the human male. 1. The erectile response to erotic stimuli in volunteers. *Psychopharmacology*, **115,** 463–470.

REDFERN, W.S., MACKINNON, A.C., BROWN, C.M., KILPATRICK, A.T., MARTIN, A.B., WILLIAMS, A., CLAGUE, R.U. & SPEDDING, M. (1992). Modulation of central noradrenergic function by RS-15385-197. *Br. J. Pharmacol.*, **108,** 526–533.

REDFERN, W.S. & WILLIAMS, A. (1995). A re-evaluation of the role of α_2-adrenoceptors in the anxiogenic effects of yohimbine, using the selective antagonist delequamine in the rat. *Br. J. Pharmacol.*, **116,** 2081–2089.

REID, K., SURRIDGE, D.H.C., MORALES, A., CONDRA, M., HARRIS, C., OWEN, J. & FENMORE, J. (1987). Double blind trial of yohimbine in the treatment of psychogenic impotence. *Lancet*, **ii,** 421–423.

SMITH, E.R., LEE, R.L., SCHNUR, S.L. & DAVIDSON, J.M. (1987). α_2-Adrenoceptor antagonists and male sexual behaviour: I. Mating Behaviour. *Physiol. Behav.*, **41,** 7–14.

SÖDERSTEN, P., DAMMASSA, D.A. & SMITH, E.R. (1987). Sexual behaviour in developing male rats. *Horm. Behav.*, **8,** 320–334.

THE MEDICAL LETTER (1994). Yohimbine for male sexual dysfunction. **36,** 115–116.

UPHOUSE, L. & CALDAROLA-PASTUSZKA, M. (1993). Female sexual behaviour following intracerebral infusion of the $5HT_{1A}$ agonist, 8-OH-DPAT, into the medial preoptic area. *Brain Res.*, **601,** 203–208.

UPHOUSE, L., CALDAROLA-PASTUSZKA, M. & MONTANEZ, S. (1992). Intracerebral actions of the $5HT_{1A}$ agonists, 8-OH-DPAT and buspirone and of the $5HT_{1A}$ partial agonist/antagonist, NAN-190, on female sexual behaviour. *Neuropharmacology*, **31,** 10, 969–981.

WINTER, J.C. & RABIN, R.A. (1992). Yohimbine as a serotoninergic agent: evidence from receptor binding and drug discrimination. *J. Pharmacol. Exp. Ther.*, **263,** 682–689.

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