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The adrenergic α_2 receptor and sexual incentive motivation in male rats

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Abstract

The purpose of the present series of experiments was to determine whether drugs acting at the α_2-adrenoceptor modify unconditioned sexual incentive motivation in the male rat. To that end a highly specific agonist, dexmedetomidine, a corresponding antagonist, atipamezole, and a less specific antagonist, yohimbine, were administered to groups of sexually inexperienced male rats. The subjects were tested in a large rectangular arena, where a sexually receptive female and an intact male were employed as incentives. The incentive animals were confined behind a wire mesh in opposite corners of the arena. The animals could see, hear and smell each other, but no sexual interaction was possible. Approach to the incentives constituted the measure of incentive motivation. In addition, the test provided data on ambulatory activity and general arousal. Dexmedetomidine, at a dose of 8 μg/kg, produced a slight reduction of sexual incentive motivation. Ambulatory activity and general arousal were also inhibited. Atipamezole, in doses of 0.1 and 0.3 mg/kg enhanced the positive incentive properties of the receptive female. A high dose of 1 mg/kg did not have any significant effect. Ambulatory activity was slightly reduced by the two larger doses of atipamezole. Yohimbine had a slight stimulatory effect on sexual incentive motivation at a dose (4 mg/kg) that also reduced ambulatory activity and general arousal. It is concluded that blockade of the adrenergic α_2 receptor stimulates sexual incentive motivation in the male rat whereas stimulation of it has the opposite effect. At present it is not clear if these drug effects are caused by pre- or postsynaptic actions of the drugs, and the importance of secondary changes in other neurotransmitter systems remains unknown.

Keywords: Atipamezole; Dexmedetomidine; Yohimbine; α_2-Adrenoreceptor; Sexual motivation; Ambulatory activity

1. Introduction

There is a substantial amount of data suggesting that blockade of the adrenergic α_2 receptor stimulates male rat sexual behavior. The original report (Clark et al., 1984) suggested that the stimulatory effect was mainly on motivation. This proposal was based on the observation that male rats with anesthetized penis showed increased mounting after treatment with the α_2-adrenergic antagonist yohimbine. Such animals are unable to achieve intromission. Mounting in this context is sometimes considered an indicator of sexual motivation. Several other studies have confirmed that yohimbine enhances the intensity of male rat copulatory behavior (Peters et al., 1988; Tallentire et al., 1996). Intracerebroventricular administration is as efficient as systemic administration (Saito et al., 1991; Sala et al., 1990), suggesting that yohimbine acts within the central nervous system and not through the sympathetic nervous system.

Yohimbine is not only an α_2-adrenergic antagonist but also a serotonin$_{1A}$ agonist in vitro (Millan et al., 2000) and in vivo (Shannon and Lutz, 2000; Winter and Rabin, 1993). A prominent effect of 5-HT$_{1A}$ agonists like 8-OH-DPAT is to reduce the number of pre-ejaculatory intromissions and thereby the ejaculation latency. This is also the most consistent effect of yohimbine (Clark et al., 1985a; Koskinen et al., 1991). It is, therefore, likely that some or all of the effects of yohimbine on male sexual behavior can be attributed to actions at the 5-HT$_{1A}$ receptor (Tallentire et al., 1996). However, other α_2-adrenergic antagonists, such as efaroxan and delequamine, have also been found to stimulate some aspects of male rat copulatory behavior (Benelli et al., 1993; Tallentire et al., 1996) while α_2 agonists, like clonidine or guanabenz, have an inhibitory effect (Benelli et al., 1993; Clark, 1991; Clark and Smith, 1990). It is unlikely

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that the actions of all these compounds can be attributed entirely to the 5-HT_{1A} receptor, suggesting that the adrenergic α_2 receptor indeed is of importance in the control of copulatory behavior.

None of the above-mentioned studies has specifically addressed the question of whether the stimulatory effects of yohimbine and other α_2-adrenergic antagonists are on sexual incentive motivation or on the execution of sexual reflexes, i.e. copulation. With sexual incentive motivation is understood the animal's urge to approach a potential sexual partner (Ågmo, 1999; Hetta and Meyerson, 1978; Meyerson and Lindström, 1973; Pfaff and Ågmo, 2002) rather than the ease by which copulatory reflexes are activated. Enhanced mounting would represent easier activation of such reflexes. In fact, an incentive motivational effect of yohimbine has not been established in a context where the execution of sexual reflexes (including mounts) was not the indicator of sexual motivation. Furthermore, the purported motivational effects were obtained in studies where the males' sexual behavior was subnormal, either because of penile anesthesia (Clark et al., 1984) or because of low concentrations of circulating androgens brought about by castration, and/or lack of sexual experience (Clark et al., 1985b; Tallentire et al., 1996).

We decided to clarify the role of the adrenergic α_2 receptor in the control of sexual incentive motivation by observing the effects of a highly specific α_2-adrenergic antagonist, atipamezole (Haapalinna et al., 1997; Virtanen et al., 1989), in a procedure specially designed for the evaluation of sexual incentive motivation (Ågmo, 2003b; Ågmo et al., 2004). Atipamezole has no functionally relevant effect on the 5-HT_{1A} receptor or on any other receptor with known importance for sexual behavior (Newman-Tancredi et al., 1998; Winter and Rabin, 1992). The effects of a highly specific α_2-adrenergic agonist, dexmedetomidine (Newman-Tancredi et al., 1998; Virtanen et al., 1988), were also evaluated. A group of males treated with yohimbine was also included. The purpose of this was to determine whether yohimbine affects sexual incentive motivation in the same way as it has been found to affect copulatory behavior, and to evaluate the potential incentive motivational effects in animals that had not been selected or manipulated for obtaining subnormal levels of sexual activity.

2. Methods

2.1. Subjects

Wistar rats were purchased from Harlan, Oxon, UK. The animals were housed in pairs in Macrolon® cages under a reversed light/dark cycle (12/12 h, lights off 11:00) with constant access to drinking water and commercial rodent pellets (B&K Universal, Sollentuna, Sweden). Females were ovariectomized under midazolam/fentanyl (3 and 0.2 mg/rat, respectively) anesthesia at least 2 weeks before use. They were brought into estrous by sequential treatment with estradiol benzoate, 25 μg rat, and progesterone, 1 mg/rat, about 48 and 4 h before tests, respectively. Both steroids were from Sigma, St. Louis, MO, USA and they were injected s.c. in 0.2 ml of peanut oil. The experiments reported herein were authorized by the local ethics committee and performed in agreement with the EEC council directive 86/609.

2.2. Apparatus

Sexual motivation was evaluated in a rectangular arena (100 × 50 × 45 cm high) with rounded corners. The walls consisted of metal sheet covered with a black plastic surface and the floor was made of dark-gray polyvinyl chloride. At the long sides, 15 cm from opposite corners, were openings measuring 25 × 25 cm. On the outside of the observation arena, incentive animal cages could be fitted to the openings. The incentive animal was separated from the experimental subject by a 1 × 1 cm wire mesh. Thus, the animals could see, smell, and hear each other. Some limited physical contact with the snout was possible. Outside each incentive animal cage, a virtual zone of 35 × 25 cm was defined. The subject was considered to be within the zone whenever its point of gravity was inside. A video camera located in the ceiling above the observation arena was connected to a computer and a video track system (Ethovision Pro, Noldus, Wageningen, The Netherlands) determined the experimental subject's position with a frequency of 5 Hz. The program determined the time the experimental subjects spent in each incentive zone, the distance moved during the test, the mean velocity of movement, and the time moving.

For a detailed description and discussions of the incentive motivation test, see Ågmo (2003b) and Ågmo et al. (2004).

2.3. Drugs

Atipamezole HCl and dexmedetomidine HCl (both from Orion Pharma, Turku, Finland) and yohimbine HCl (Sigma, St. Louis, MO, USA) were dissolved in physiological saline. Yohimbine was injected i.p., because this was the route employed in the earlier studies (see Introduction). Atipamezole and dexmedetomidine were given s.c. as was the case in most other studies with these drugs (Kauppila et al., 1991; Viitamaa et al., 1995). Injection volume was always 1 ml/kg body weight. Preinjection time was 30 min for atipamezole and dexmedetomidine and 20 min for yohimbine. Again, these times were chosen so as to coincide with earlier studies. Atipamezole was administered at the doses of 0.1, 0.3 and 1 mg/kg, dexmedetomidine at the doses of 2, 4 and 8 μg/kg, and yohimbine was given in doses of 1, 2 and 4 mg/kg. All these doses were within the range normally used in behavioral studies, and in the case of yohimbine the two larger doses employed have been found to stimulate copulatory behavior (see references in the Introduction).

2.4. Procedure

The males were familiarized to the observation arena during 3 sessions of 5 min each separated by 24 h. Incentive animal cages were empty. The experiment was then initiated. Now, one incentive animal cage contained a proceptive and receptive

female and the other contained an intact male. The position of the incentive animal cages was semi-randomly changed between subjects. Within each treatment, half of the animals had the incentives in one position and the other half had them in the opposite position.

Before every experimental session, the arena and the incentive animal cages were cleaned with a 0.1% (v/v) glacial acetic acid solution in water. At the start of a test the experimental subject was put in the middle of the observation arena and the video track system was activated. Data were collected for a period of 10 min. Thereafter the subject was removed and the next subject was immediately introduced.

After the end of the experiments all males were subjected to a standard mating test with a receptive female. The purpose of this was to assure that the males showed sexual activity within the range normally observed in animals of similar age. The 30 min test was performed in rectangular cages (40 × 60 × 40 cm high), and the following parameters of copulatory behavior were recorded with an in-house software: Mount latency, time from introduction of the female until the first mount; intromission latency, time from introduction of the female until the first intromission; ejaculation latency, time from the first intromission until the ejaculation; postejaculatory interval, time from the ejaculation until the following intromission. The number of mounts with pelvic thrusting and the number of intromissions were also registered. The ovariectomized females used as copulation partners had received the same hormone treatment as the female incentives.

2.5. Design

Groups of 10–16 animals each were used. One group received yohimbine, two groups were treated with atipamezole, and another with dexmedetomidine. All subjects received all doses of a given drug + saline. The order of the doses was counterbalanced. There was at least 48 h between experimental sessions (72 h at weekends).

The reason for including a second group treated with atipamezole was to confirm the data obtained with the first group, especially with regard to the ambiguous effect on ambulatory activity. Due to a corrupted video file, indices of ambulatory activity as well as the number of visits to the incentives could not be determined for 2 animals in the first group. Thus, only 8 subjects were included in the analyses of these parameters. Since atipamezole enhanced sexual incentive motivation, it was considered to be most important to determine whether this effect occurred in the absence of motor effects or not. It is not evident that eight subjects would have been enough for a firm conclusion.

2.6. Data preparation and statistics

Sexual incentive motivation was quantified in 2 ways: The *preference score* (time spent in the female incentive zone/(time spent in the female incentive zone + time spent in the male incentive zone)) and *time spent in the female incentive zone*. In addition, the number of entries into each incentive zone, and the mean duration of visits to the incentives were calculated. As

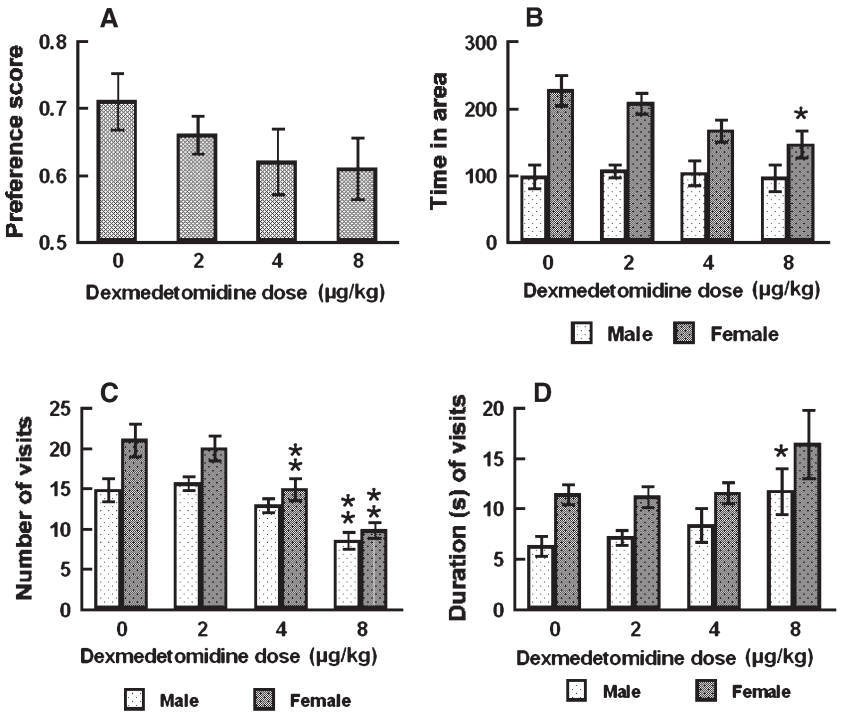

Fig. 1. (A) Mean ± S.E.M. preference score in male rats treated with varying doses of dexmedetomidine and tested in the incentive motivation test. Doses are expressed in µg/kg. $n = 16$. (B) Mean ± S.E.M. time spent in the male incentive area and in the female incentive area. (C) Mean ± S.E.M. number of visits to the male and receptive female incentives. (D) Mean ± S.E.M. duration of visits to the male and to the receptive female incentives. *, different from control treatment (dose 0), $P < 0.05$; **, $P < 0.01$, Tukey's HSD test.

indicator of motor function we employed ambulatory activity, viz. the total distance moved during the test, while the mean velocity of movement and the time spent moving constituted measures of general arousal. The calculation of the total distance moved was based on all displacements of the subject's point of gravity that were larger than 5 cm. This means that small movements, like turning on the spot or adjusting body posture, were not included. Therefore, the distance moved represents essentially forward locomotion. The mean velocity of movement included all displacements of the point of gravity without any minimum distance specified. Small movements, including turning on the spot or adjustments of body posture associated or not with grooming or sniffing were thereby included. A consequence of this difference is that distance moved and velocity of movement are mathematically independent. Furthermore, there are data showing that forward locomotion and general motor activity can be differentially affected by experimental manipulations (e.g. Salmi and Ahlenius, 2000) and that general activity (here represented by the velocity of movement and the time in movement) may be more appropriate indicators of arousal than forward locomotion (see e.g. Garcia-Rill et al., 1996; van der Harst et al., 2003).

The preference score and the indices of ambulatory activity and arousal were evaluated with one-factor ANOVA for repeated measures. A posteriori comparisons were made with Tukey's HSD procedure. The time spent in the incentive zones, the number of visits to these zones and the mean duration of visits were analyzed with two-factor ANOVAs for repeated measures on both factors. The factors were dose (4 levels) and incentive (male vs. female). In case of significance, the means were compared with Tukey's HSD procedure.

Finally, the preference score was compared to the score indicating no preference for any incentive, 0.5, using the *t*-test. There were 4 treatment conditions (saline + 3 doses of the respective drug) in each experiment. With the purpose of avoiding false significances because of the use of multiple *t*-tests the nominal *P* was adjusted with the Bonferroni correction. Thus, a $P<0.0125$ was required for considering a difference significant at the 0.05 level. All probabilities mentioned are two-tailed.

3. Results

3.1. Dexmedetomidine

Fig. 1A–D shows the sexual motivation data obtained with dexmedetomidine. The drug failed to significantly affect the preference score ($F(3,45)=1.14$, NS). Nevertheless, when the

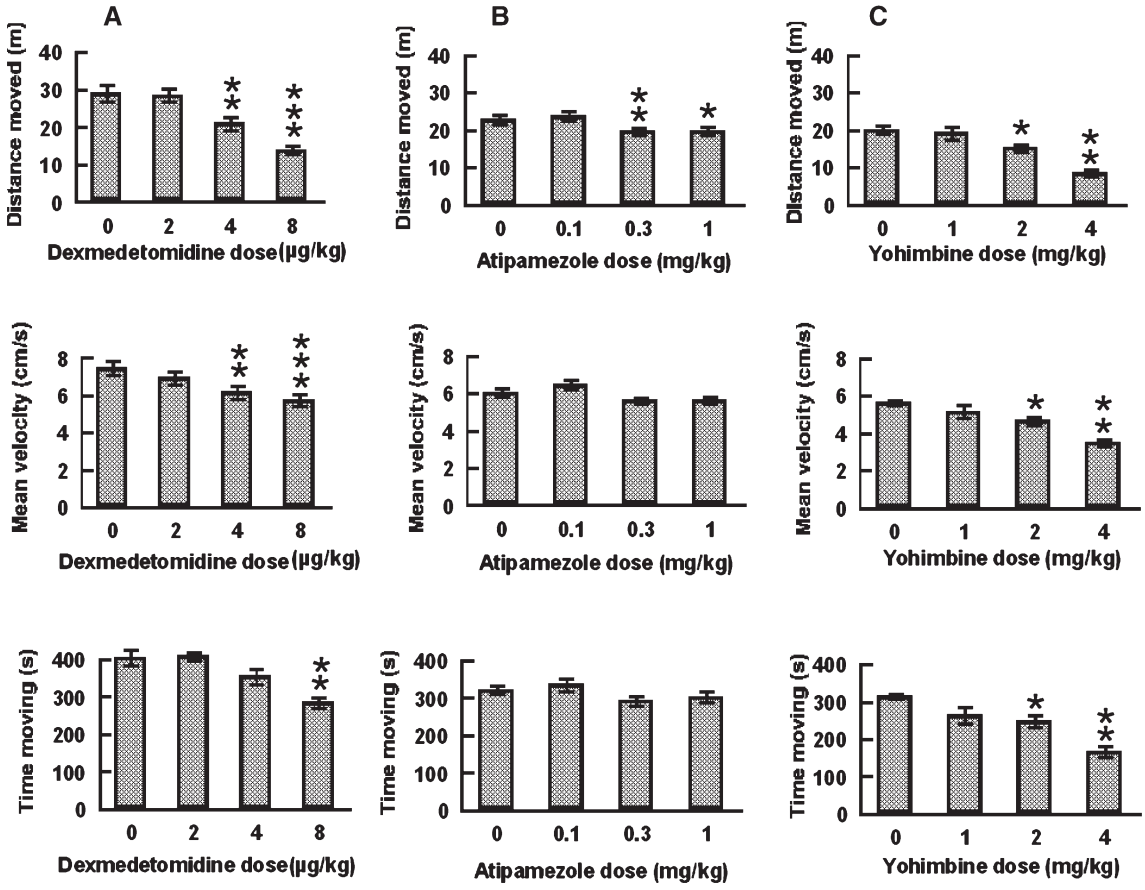

Fig. 2. Distance moved, mean velocity of movement while moving, and the time spent in movement during the motivation test in male rats treated with varying doses of dexmedetomidine (A, $n=16$), atipamezole (B, $n=20$), or yohimbine (C, $n=10$) . Data are mean±S.E.M.∗, different from control treatment (dose 0), $P<0.05$;∗∗, $P<0.01$;∗∗∗, $P<0.001$, Tukey's HSD test.

preference score was compared to 0.5 (no preference) after each dose it was found that the score obtained after dexmedetomidine 4 and 8 μg/kg did not differ from chance level. Thus, after these treatments the sexual incentive motivation was not significantly different from zero.

When analyzing the time spent in the incentive zones, a difference between the male and the female incentive zones was found ($F(1,15)=23.72$, $P<0.001$). The drug modified the time spent in the incentive zones ($F(3,45)=3.24$, $P<0.05$), but the interaction between incentive and dose of drug was not significant ($F(3,45)=2.13$, NS). The Tukey HSD test revealed that the time spent in the receptive female incentive zone was reduced by the largest dose, 8 μg/kg. No change in the time spent in the male incentive zone was found. Furthermore, the animals spent more time in the female incentive zone than in the male zone only after saline and dexmedetomidine, 2 μg/kg.

Concerning the number of visits, there was a difference between incentives ($F(1,15)=15.27$, $P=0.001$) and an effect of dose ($F(3,45)=31.26$, $P<0.001$). The interaction incentive × dose was not significant ($F(3,45)=2.03$, NS). A posteriori comparisons determined that only the largest dose reduced the number of visits to the male, while both this dose and the 4 μg/kg dose reduced the number of visits to the female. Moreover, only saline treated animals made more visits to the female than to the male incentive.

There was also a difference between incentives with regard to mean duration of visits ($F(1,15)=9.49$, $P<0.01$). The effect of dose was also significant ($F(3,45)=4.47$, $P<0.01$), but there was no interaction incentive × dose ($F(3,45)=0.16$, NS). A posteriori comparisons revealed that the effect of dose was limited to the 8 μg/kg dose. Furthermore, the mean duration of visits to the female was unchanged while those to the male were much reduced. After saline and dexmedetomidine, 2 μg/kg, the subjects stayed longer at visits in the female incentive zone than they did at visits to the male zone. This difference was not present after the two largest doses of the drug.

Ambulatory activity ($F(3,459=33.50$, $P<0.001$) as well as velocity of movement ($F(3,459=18.39$, $P<0.001$) and time moving ($F(3,45)=12.09$, $P<0.001$) were reduced by dexmedetomidine. The Tukey HSD test revealed that the doses of 4 and 8 μg/kg reduced ambulatory activity and velocity of movement while the 8 μg/kg dose reduced the time spent moving. Data are shown in Fig. 2A.

3.2. Atipamezole

The two groups treated with atipamezole were similar in all respects. In fact, comparison of the groups with *t*-tests failed to detect any significant difference (all *P*'s > 0.28). Furthermore, when the drug effects were evaluated they turned out to be identical (in terms of statistical significance) in both groups. In order to avoid a double presentation of the atipamezole results, the groups have been pooled both for presentation and analyses.

ANOVA showed a significant effect on the preference score ($F(3,63)=3.52$; $P<0.05$). A posteriori tests showed that the dose of 0.1 mg/kg increased the preference score (Fig. 3A). At

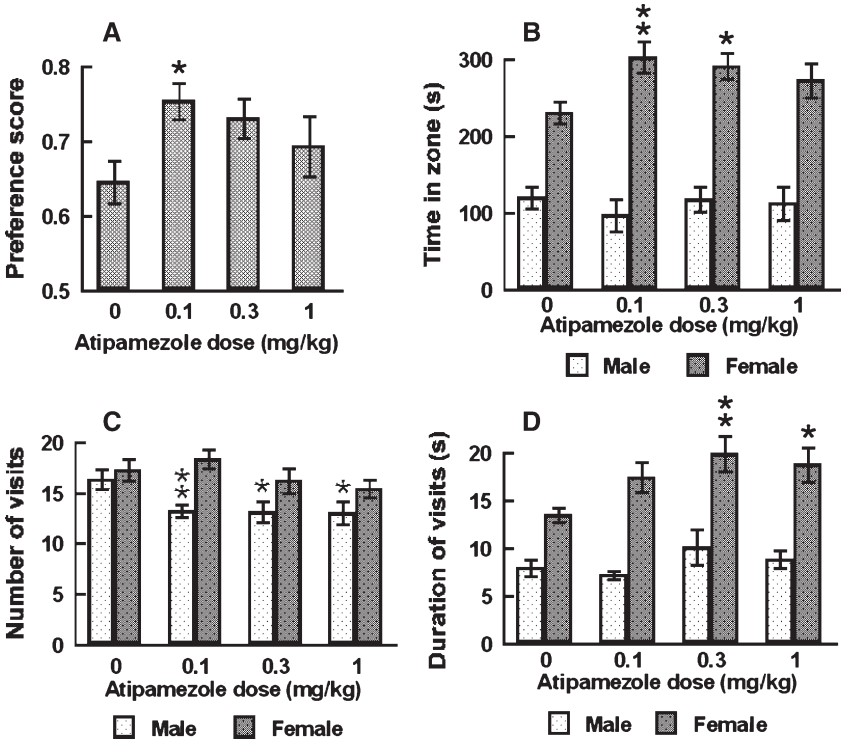

Fig. 3. (A) Mean ± S.E.M. preference score in male rats treated with varying doses of atipamezole and tested in the incentive motivation test. $n=22$. (B) Mean ± S.E.M. time spent in the male incentive area and in the female incentive area. $n=22$. (C) Mean ± S.E.M. number of visits to the male and receptive female incentives. $n=20$. (D) Mean ± S.E.M. duration of visits to the male and to the receptive female incentives. $n=20$. ∗, different from control treatment (dose 0), $P<0.05$;∗∗, $P<0.01$, Tukey's HSD test.

all treatments the subjects showed a significant preference for the female, i.e. the preference score was significantly above 0.5.

There was a difference between incentives with regard to the time spent in incentive zones ($F(1,21)=66.48$; $P<0.001$). There was also a difference between atipamezole doses ($F(3,63)=5.45$; $P<0.001$) and the interaction incentive × dose was likewise significant ($F(3,63)=3.07$; $P<0.05$). A posteriori analyses showed that the doses of 0.1 and 0.3 mg/kg increased the time spent in the female incentive zone. To the contrary, the drug did not modify the time spent in the male incentive zone. Intra-treatment comparisons showed that the subjects spent longer time in the female incentive zone than in the male zone after all treatments. Data can be seen in Fig. 3B.

ANOVA of the number of visits to the incentives revealed a significant effect of incentive ($F(1,19)=11.02$; $P<0.01$) as well as of dose ($F(3,57)=9.85$; $P<0.001$). The interaction incentive × dose was also significant ($F(3,57)=3.28$; $P<0.05$). A posteriori comparisons revealed that all doses of atipamezole reduced the number of visits to the male incentive. However, none of the doses differed from saline with regard to the number of visits to the female incentive. Intra-treatment comparisons revealed that there was no difference in the number of visits to the male and female incentives after treatment with saline or atipamezole 0.3 and 1 mg/kg whereas the subjects made more visits to the female than to the male after 0.1 mg/kg of atipamezole. These results are shown in Fig. 3C.

There was an effect of incentive on the mean duration of visits to the incentives ($F(1,19)=54.54$; $P<0.001$). The main effect of atipamezole dose was also significant ($F(3,57)=9.73$; $P<0.001$) while there was no interaction incentive × dose ($F(3,57)=1.60$; NS). A posteriori analyses showed that the 0.3 and 1 mg/kg doses increased the mean duration of visits to the female. Intra-treatment comparisons established that visits to the female were longer than visits to the male after all treatments. Data are summarized in Fig. 3D.

Atipamezole also affected the total distance moved during the test ($F(3,57)=8.38$; $P<0.001$). A posteriori comparisons showed that the doses of 0.3 and 1 mg/kg differed from saline (Fig. 2 B). When the indicators of general arousal were analyzed, it was found that atipamezole affected both the mean velocity of movement ($F(3,57)=7.19$, $P<0.001$) and the time spent moving ($F(2,57)=4.43$, $P<0.01$). However, there was no difference between saline and atipamezole. With regard to velocity of movement, the animals moved faster after atipamezole 0.1 mg/kg than they did after 0.3 and 1 mg/kg. Concerning the time moving, the subjects moved a longer time after 0.1 mg/kg than after 0.3 mg/kg. These data show that effects on ambulatory activity were quite modest while indices of general arousal did not differ between saline and drug treatment.

3.3. Yohimbine

Yohimbine had mixed effects on sexual incentive motivation. There was no significant change in the preference score ($F(3,27)=2.86$; NS), but as can bee seen in Fig. 4 A, there was a tendency for a dose-dependent increase. In fact the ANOVA gave a P of 0.056. Within each treatment condition, the

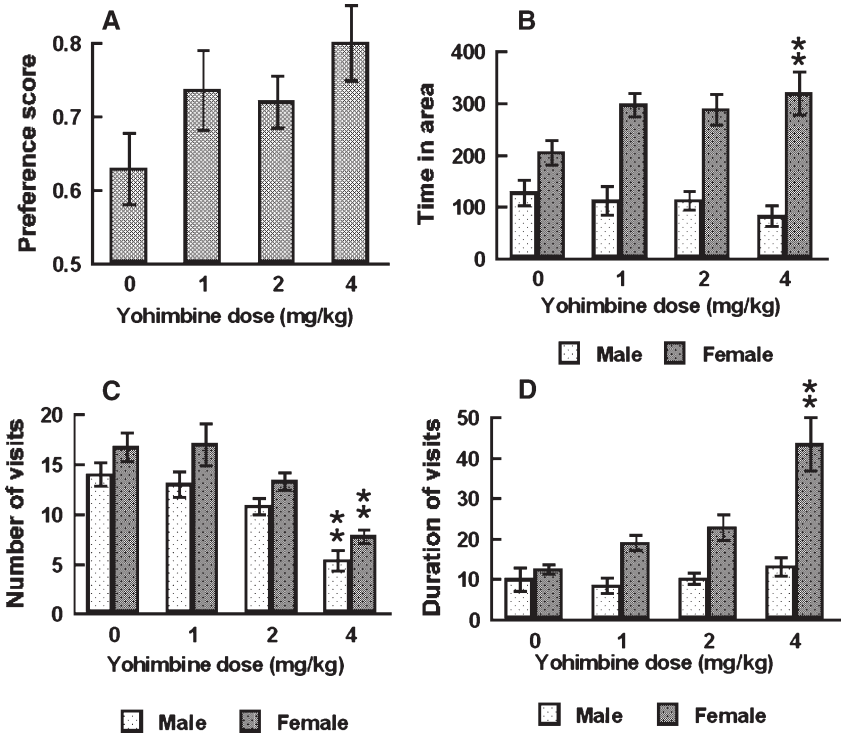

Fig. 4. (A) Mean ± S.E.M. preference score in male rats treated with varying doses of yohimbine and tested in the incentive motivation test. $n=10$. (B) Mean ± S.E.M. time spent in the male incentive area and in the female incentive area. (C) Mean ± S.E.M. number of visits to the male and receptive female incentives. (D) Mean ± S.E.M. duration of visits to the male and to the receptive female incentives.**, different from control treatment (dose 0); $P<0.01$,***, $P<0.001$, Tukey's HSD test.

preference score was significantly different from chance level in the way that the female was always preferred.

When the time spent in the incentive zones was analyzed, it was found that the incentives differed ($F(1,9)=52.22$; $P<0.001$). Furthermore, there was a main effect of dose ($F(3,27)=3.23$, $P<0.05$). The interaction incentive × dose approached significance ($F(3,27)=2.71$; $P=0.06$). Tukey's HSD test showed that the time spent in the female incentive zone was increased after the dose of 4 mg/kg but not after lower doses, while the time spent in the male zone was not modified. When the time spent in the female incentive zone was compared to that spent in the male incentive zone within each treatment it was found that the subjects spent more time in the female zone after all doses of yohimbine but not after saline. Data are illustrated in Fig. 4B.

There was a difference between the incentives with regard to the number of visits ($F(1,9)=12.85$; $P<0.01$). Likewise, there was a difference between doses ($F(3,27)=18.49$; $P<0.001$). The interaction incentive × dose was not significant ($F(3,27)=0.44$; NS). A posteriori tests showed that the largest dose, 4 mg/kg, reduced the number of visits to both the male and female incentive. Furthermore, there was no difference in the number of visits to the female compared to the male after treatment with saline. After all doses of yohimbine, though, the experimental subjects made more visits to the female than to the male incentive. Data are depicted in Fig. 4C.

The mean duration of visits differed between the incentives ($F(1,9)=53.32$; $P<0.001$), and the main effect of dose was also significant ($F(3,27=14.65$; $P<0.001$). This was also the case with the interaction between incentive and dose ($F(3,27=6.60$; $P<0.01$). The Tukey test showed that there was no effect of yohimbine on the duration of the visits to the male incentive while the duration of visits to the female incentive increased after the dose of 4 mg/kg (Fig. 4 D). Within-treatment comparisons showed that the animals made longer visits to the female than to the male after all doses of yohimbine but not after saline.

There was a reduction in ambulatory activity after yohimbine ($F(3,27)=21.91$; $P<0.001$). Tukey's HSD test showed that this effect was obtained after treatment with the doses of 2 and 4 mg/kg. The mean velocity of movement ($F(3,27)=16.56$, $P<0.001$) as well as the time moving ($F(3,27)=13.87$, $P<0.001$) were also reduced by these doses of yohimbine. These data show that yohimbine dose-dependently reduced all indicators of activity or general arousal. Data are displayed in Fig. 2C.

The results of the yohimbine experiment show that the drug has a tendency to stimulate sexual incentive motivation. The preference score was not significantly modified, but the time spent in the receptive female incentive zone as well as the mean duration of visits to it was increased.

3.4. Post-experimental test for copulatory behavior

Results from the post-experimental mating test showed that the animals in all groups copulated with about the same intensity as would be expected for inexperienced animals of similar age. More than 90% of the males mounted, about 75% displayed intromissions and 68% achieved ejaculation. There was no difference between groups in any parameter of sexual behavior (all P's > 0.18). These data show that the males employed in the experiments reported here were not sexually sluggish or performing at a "subnormal" level.

4. Discussion

The α_2 agonist dexmedetomidine had a slight inhibitory effect on sexual incentive motivation at the largest dose employed, 8 μg/kg, albeit this dose reduced ambulatory activity and general arousal. It is not likely, though, that the sedative effect was the cause of the drug's actions on sexual motivation. Other drugs, at doses having larger effects on ambulatory activity, leave sexual incentive motivation unaffected (Ågmo, 2003a). The data obtained in the yohimbine experiment reinforce the idea that reduced activity is not necessarily associated with reduced sexual incentive motivation. In view of this we propose that stimulation of the α_2-adrenergic receptor may reduce sexual incentive motivation. Earlier data show that α_2-adrenergic agonists also reduce copulatory behavior (Clark, 1991).

Atipamezole had a substantial effect on parameters reflecting the female's sexual incentive value for the male at a dose that did not affect ambulatory activity. The preference score as well as the time spent in the receptive female incentive zone were increased but there was no change in the time spent in the male incentive zone after a dose of 0.1 mg/kg. None of the indices of ambulatory activity or general arousal was modified by this dose. A larger dose, 0.3 mg/kg, failed to significantly enhance the preference score but increased the time spent in the receptive female incentive zone. This dose, as well as the 1 mg/kg dose, slightly reduced the distance moved. However, the other indicators of general activity or arousal were not modified, suggesting that effects on motor systems were limited to forward locomotion and that reactivity to the test environment was not seriously affected. This coincides with an earlier report where similar doses of atipamezole, while reducing activity in an open field, left activity on an elevated plus-maze and in a two-compartment exploratory test unaffected (Kauppila et al., 1991). The effects of atipamezole on sexual incentive motivation observed in the present study cannot, therefore, be attributed to some non-specific action on arousal or general activity.

Interestingly, atipamezole has been shown to induce sexual behavior in very old rats with almost no sexual activity in the drug free state (Viitamaa and Haapalinna, 1996). Similar effects were observed in middle-aged rats selected for low sexual activity (Viitamaa et al., 1995). Moreover, the number of ejaculations during a 30 min test was enhanced by atipamezole in rather old male *Macaca arctoides* (Linnankoski et al., 1992). Such effects are consistent with the hypothesis that atipamezole stimulates sexual motivation. The lack of data concerning the effects of atipamezole on copulatory behavior in young adult animals, like those employed in the present study, makes it impossible to determine whether the stimulatory effect on sexual incentive motivation in such animals is associated with enhanced intensity of copulatory behavior or not.

Yohimbine did not significantly enhance the preference score, but the time spent with the receptive female as well as the mean duration of visits to her were increased. Thus, yohimbine seems to have produced some stimulation of sexual incentive motivation. It should be noted, though, that this effect was obtained with a dose which substantially reduced indices of ambulatory activity and general arousal. Such effects are commonly reported after administration of yohimbine in similar doses (Bowes et al., 1992). Nevertheless, a stimulatory effect on sexual motivation coincides with numerous studies showing enhanced intensity of copulatory behavior after treatment with yohimbine (see Introduction). It seems unlikely, then, that the effects on motivation observed here are solely consequences of sedation. It is also interesting to note that drugs having similar effects on indicators of motor activity and arousal, viz. dexmedetomidine and yohimbine, have opposite effects on indicators of the intensity of sexual incentive motivation. This fact strengthens the notion that the motivational effects of these drugs are independent of their motor effects.

While atipamezole is highly specific for the adrenergic α_2 receptor, yohimbine has many other pharmacological actions as already mentioned (see also Millan et al., 2000). Despite different pharmacological profiles these drugs have a common action on sexual incentive motivation, and this action is more evident for the highly specific α_2-adrenergic antagonist, atipamezole, than for the less specific one, yohimbine. These data warrant the suggestion that blockade of the α_2-adrenergic receptor stimulates sexual incentive motivation, and that other actions of yohimbine are not contributing to this effect. The inverted U-shaped dose response curve is very typical in behavioral studies. Because of the unspecific properties of yohimbine it has a very sharp dose–effect curve, as reported also in earlier studies (Haapalinna et al., 1997).

Like other α_2-adrenergic receptor antagonists, atipamezole and yohimbine enhance noradrenaline release throughout the brain, including hypothalamic areas of importance for sexual incentive motivation (Hurtazo et al., 2003; Laitinen et al., 1995). This effect is presumably mediated by the α_{2A}-adrenergic receptor subtype. Indeed, much data show that the α_{2A}-adrenergic receptor is responsible for the effects on noradrenaline release of drugs acting on the α_2-adrenergic receptor, both in locus coeruleus (Callado and Stamford, 1999; Chiu et al., 1995) and elsewhere in the central nervous system (Palij and Stamford, 1993; Talley et al., 1996; Umeda et al., 1997). Dexmedetomidine has the opposite effect, i.e. like other α_2-adrenergic agonists the drug reduces noradrenaline release throughout the brain (Gobert et al., 1998; Jorm and Stamford, 1993). It could be assumed, then, that enhanced noradrenaline release stimulates sexual incentive motivation while reduced release impairs it. This hypothesis would be strengthened if enhanced noradrenaline release also facilitated copulatory behavior and reduced release inhibited it. Unfortunately, available data are inconsistent.

Reduced noradrenergic transmission, brought about by inhibition of dopamine-β-hydroxylase within the medial preoptic area/anterior hypothalamus, dramatically reduces male copulatory behavior (Clark, 1995), while intracerebral infusions of noradrenaline facilitate it (Gulia et al., 2002). However, other data show that enhanced availability of noradrenaline, caused by systemic administration of the specific noradrenaline precursor dihydroxyphenylserine, inhibits copulatory behavior (Ågmo and Picker, 1990; Ågmo and Villalpando, 1995; Malmnäs, 1973), while a noradrenergic neurotoxin, DSP4, facilitates it (Ågmo and Picker, 1990). These effects were presumably of central nervous origin, since peripheral decarboxylase was blocked in the precursor studies, and since the test was performed at a time when peripheral noradrenergic neurons had regenerated in the neurotoxin studies. The contradictory results obtained after manipulations of the availability of noradrenaline preclude any generalization from effects on copulatory behavior to effects on sexual incentive motivation.

If we assume that noradrenaline indeed is stimulatory to sexual incentive motivation, then we must examine at which receptor the transmitter might act to have this effect. The postsynaptic α_2-adrenergic receptors may be excluded because of the results obtained at low doses of dexmedetomidine. Stimulation of the α_1-adrenergic receptors may be inhibitory to sexual behavior (Clark, 1995), so this site of action can also be excluded. The role of the β-adrenergic receptors is not entirely clear, but much data suggest that nonselective antagonists are inhibitory both after peripheral and central administration while agonists may have a stimulatory action on copulatory behavior (Gulia et al., 2002; Mallick et al., 1996; Smith et al., 1995). The selective β_1-adrenergic antagonists atenolol, labetalol and metoprolol are ineffective (Smith et al., 1990, 1996), suggesting that the β_2-adrenergic receptor is the one involved in the control of male sexual behavior. Thus, it is reasonable to propose that enhanced noradrenergic neurotransmission facilitates copulatory behavior, at least in part, through stimulation of central β_2-adrenergic receptors. If this conclusion applies also to sexual incentive motivation remains to be established.

At present, it is not possible to determine which of the explanations presented above is correct. Moreover, presynaptic α_2-adrenergic heteroreceptors may modulate the release of acetylcholine, dopamine, serotonin or GABA (Gobert et al., 1998, 2003), also at brain sites crucial for the control of male sexual behavior like the preoptic area (Manns et al., 2003), making indirect actions on any of these transmitter systems a putative target. Only a far more sophisticated pharmacological approach could answer these questions. However, according to the results obtained in the experiments described here, it is evident that manipulations of the adrenergic α_2 receptor may modify sexual incentive motivation. The importance of that observation is not necessarily reduced by lack of knowledge of the exact brain areas and mechanisms downstream from the α_2-receptors involved in the net effect.

The main finding in the present experiments is that atipamezole and also yohimbine enhance sexual incentive motivation in the male rat. This suggests that the α_2-adrenergic antagonist might be a useful treatment for hypoactive sexual desire. This dysfunction is increasingly recognized as a clinical problem severely reducing the quality of life of those affected (O'Donohue et al., 1999), and although the disorder has its

highest prevalence among women (Rosen and Leiblum, 1995; Spector and Carey, 1990) it also affects a substantial proportion of adult men (see Ågmo et al., 2004 for a discussion).

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