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Pharmacology, Biochemistry and Behavior 82 (2005) 427 – 433
PHARMACOLOGY BIOCHEMISTRY AND BEHAVIOR
www.elsevier.com/locate/pharmbiochembeh

Differential effects of simultaneous or sequential administration of paroxetine and WAY-100,635 on ejaculatory behavior

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Received 3 May 2005; received in revised form 8 September 2005; accepted 27 September 2005
Available online 25 October 2005

Abstract

Clinical treatment of depression or anxiety with selective serotonin reuptake inhibitors (SSRIs) often results in delayed ejaculation or anorgasmia. Co-treatment with subtype-selective serotonin receptor antagonists may alter the timing of onset of action and potentiate or reduce sexual side effects. Sexual behavior in male Sprague–Dawley rats was examined after acute administration of the SSRI, paroxetine and the serotonin$_{1A}$ antagonist, WAY-100,635. Acute administration of paroxetine alone did not alter male ejaculatory behavior. However, administration of paroxetine plus WAY-100,635 resulted in a significant delay in mounting behavior and increased the time to ejaculation. Simultaneous administration of paroxetine and WAY-100,635 produced a greater delay in initiation of mounting behavior and ejaculation compared to sequential administration of paroxetine followed by WAY-100,635. The differential effect on sexual behavior or addition of specific serotonin receptor antagonists may be relevant for clinical treatment therapies of premature ejaculation.

Keywords: Serotonin; Ejaculation; Selective serotonin reuptake inhibitors; Rat; 5-HT$_{1A}$ antagonist

1. Introduction

Selective serotonin reuptake inhibitors (SSRIs) given to treat depression, anxiety or obsessive/compulsive disorder may cause sexual dysfunction in particular a delayed ejaculation or anorgasmia is frequently reported (e.g. Waldinger et al., 1998a). This effect is thought to be due to increased availability of 5-hydroxytryptamine (5-HT) in the central nervous system since serotonin inhibits sexual behavior (Ahlenius et al., 1980; Hillegaart and Ahlenius, 1998; Marson and McKenna, 1992; Waldinger et al., 1998a). This side effect has been explored as therapy for premature ejaculation (Waldinger et al., 2004a; Waldinger and Olivier, 2004). This side effect has been explored as therapy for premature ejaculation (Waldinger et al., 2004a; Waldinger and Olivier, 2004). Daily, chronic treatment with the SSRI, paroxetine ((−)-*trans*-4-(*p*-fluorophenyl)-3-[[3,4-methylenedioxy) phenoxy]-methyl]piperadine) appears to be more effective than other SSRIs tested to date in treating premature ejaculation (Montague et al., 2004; Waldinger et al., 2004a).

Clinical improvement of depression with SSRIs takes several weeks. One explanation may be related to the time taken to desensitize 5-HT$_{1A}$ autoreceptors (Celada et al., 2004; Waldinger et al., 1998a). Some research suggests that SSRI antidepressants have a more rapid onset of action when the β-adrenoreceptor antagonist, pindolol, which also antagonizes 5-HT$_{1A}$ receptors, is co-prescribed (Artigas et al., 1996; Ballesteros and Callado, 2004; Perez et al., 1997). Pindolol is generally co-administered with the SSRI, but it is given more frequently than the SSRI (Perez et al., 1997).

In the acute administration of multiple pharmacotherapies the sequence of administration of drug treatment therapies may impact the clinical outcome. Microdialysis studies of the rat forebrain demonstrated that systemic administration of 5-HT$_{1A}$ receptor antagonists augment the increase in 5-HT produced by SSRI treatment (Hjorth, 1993; Hjorth et al. 1996, 1997; Romero and Artigas, 1997). The extent of augmentation reported is highly variable, from 100% to 1900% (Hjorth, 1993; Hjorth et al., 1996; Gundlah et al., 1997; Romero and Artigas, 1997). In one microdialysis study, when the 5-HT$_{1A}$

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doi:10.1016/j.pbb.2005.09.014

receptor antagonist, WAY-100,635 (*N*-[2-[4-(2-methoxyphenyl)-L-piperazinyl]ethyl]-*N*-(2-pyridinyl) cyclohexanecarboxamide 3HCl), was administered after fluoxetine, the increase in local serotonin levels was more than twice that observed when the compounds were administered simultaneously (Taber et al., 2000). This suggests that sequential drug treatment may have a greater effect on SSRI treatment therapy compared to simultaneous administration, but the impact on sexual behavior and depression should also be investigated.

The timing of the administration of SSRIs and 5-HT_{1A} antagonists on ejaculatory behavior has not been investigated. A potential use of a 5-HT_{1A} antagonist could allow the reduction of the dosage of the SSRI for premature ejaculation and thus reduce side effects. Previously Ahlenius and Larsson (1999) demonstrated that sequential administration of the SSRI citalopram, followed by WAY-100,635, resulted in a significant delay in ejaculation in rats. Acute single administration of SSRIs or the 5-HT_{1A} antagonist, WAY-100,635, does not significantly affect male rat sexual behavior (Ahlenius and Larsson, 1998, 1999; Sura et al., 2001). In the present study, we examined the effects of sequential or simultaneous administration of the SSRI, paroxetine, and WAY-100,635 on male rat ejaculatory behavior.

2. Materials and methods

2.1. Animals

Studies were performed on male and female Sprague–Dawley rats (250 g to 350 g, Charles River Laboratories, Inc.). Female rats were ovariectomized by Charles River Laboratories prior to arrival. Animals were given ~2 weeks to adapt to the light–dark cycle, with lights off at 08:00; on at 20:00 h. Male rats were sexually experienced on 3 prior occasions, and only males that consistently ejaculated during preliminary trials were selected for the studies. The animals served as their own controls in a cross over design. The experiments and procedures were approved by the University of North Carolina Institutional Animal Care and Use Committee in accordance with the NIH policies guide for the care and use of laboratory animals.

2.2. Drugs

Paroxetine (1 mg/ml, Toronto Research Chemicals) was dissolved in 12.5% Polyethylene glycol-15-hydroxystearate solution (Solutol, BASF, Ludwigshafen, Germany). WAY-100,635 (0.1 mg/ml, Sigma, St. Louis) was dissolved in normal saline. Solutol vehicle was used to prepare the paroxetine and WAY-100,635 mixture (Paroxetine/WAY) for simultaneous drug administration. Paroxetine (10 mg/kg) and WAY-100,635 (0.1 mg/kg) were given subcutaneously (1.0 ml/kg) 30 min prior to the onset of sexual behavior except in the case of sequentially administered drugs where paroxetine and WAY-100,635 were given 60 and 30 min, respectively, before the onset of behavior. Two control groups were tested in order to parallel the injection regimes. One group received Solutol 30 min before the onset of behavior and the other group received Solutol and saline, 60 and 30 min, respectively, before the onset of behavior. These doses and times were similar to previous studies (Mos et al., 1999; Ahlenius and Larsson, 1998, 1999; de Jong et al., 2005a,b; etc).

Females were given estradiol 3-benzoate (20 μg/kg s.c. in sesame oil; Sigma, St. Louis) 48 h prior to testing, followed by progesterone (2 mg/kg s.c. in sesame oil; Sigma, St. Louis) at least 3 h prior to testing.

2.3. Behavioral observations

The sexual behavior was recorded during the early phase of the dark cycle by video under red light for 30 min. A trained, validated observer that was blinded to the treatment group scored the male rats' sexual behavior. The number of attempted mounts (number of mounts without male thrusting behavior); mounts (number of mounts with thrusting behavior but without vaginal penetration); intromissions (number of mounts with vaginal penetration); and ejaculations were recorded. In addition, the intromission latency (time from presentation of the female to the first intromission); ejaculation latency (time from the first intromission to an ejaculation); post ejaculatory interval (time from an ejaculation to the next intromission) and copulatory efficiency (the number of intromissions divided by the number of mounts plus intromissions) were calculated.

2.4. Data analysis

Data is presented for each ejaculatory series and the total observation time (30 min). Animals that did not ejaculate within the 30 min observation period were given a value of 30 min for the ejaculation latency. Similarly, if a rat did not intromit during the testing period a 30 min intromission latency was assigned. Statistical analyses were performed using one-way ANOVA (SPSS statistical program) followed by Scheffe's post hoc test and unpaired *t*-tests as appropriate. The *Z*-test was used to compare 2 proportional values (i.e. number of animals ejaculating per group) (Bruning and Kintz, 1997). A value of $p<0.05$ was considered statistically significant and differences of $p<0.05$, $p<0.01$ and $p<0.001$ are shown.

3. Results

3.1. Effects of paroxetine on male rat sexual behavior

Administration of paroxetine did not significantly alter either ejaculation latency or intromission latency when compared to the control group (vehicle (V); Fig. 1, Table 1). No significant differences were found in the number of intromissions or post ejaculatory interval during each ejaculatory series and the number of mounts only tended to increase in the 2nd ejaculatory series (Fig. 2). When we examined sexual behavior over the 30 min total recording period, no significant differences were found (Tables 1 and 2). All rats in the paroxetine and vehicle group ejaculated an average of 3 times within the 30 min observation period (Table 2).

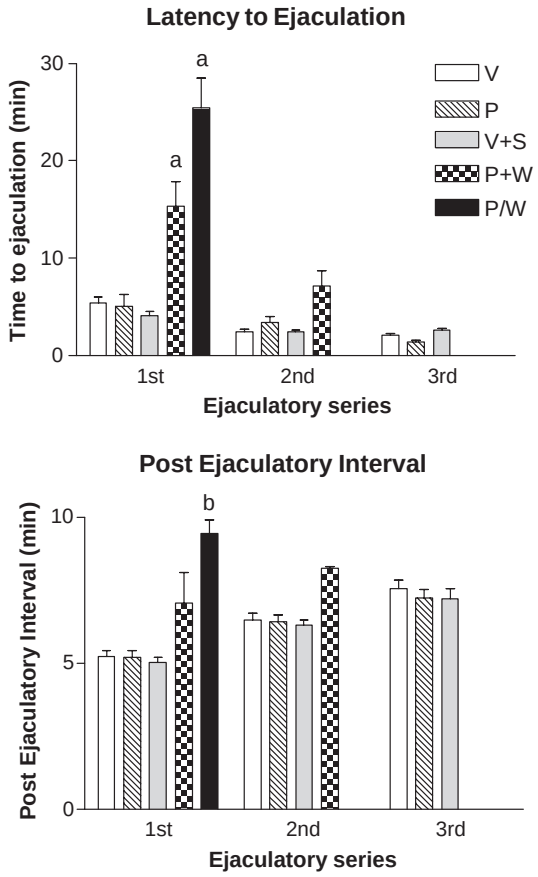

Fig. 1. Effects of paroxetine and sequential (P+W) or simultaneous (P/W) administration of paroxetine and WAY100,635 on latency to ejaculation and post ejaculatory interval. Data represents the first 3 ejaculatory series. Data were analyzed using one-way ANOVA with Scheffe's post hoc test. Values are mean±S.E. $N=8-14$ (except post ejaculatory interval for 1st series P/W and 2nd series P+W where $N=2$). The following significant differences were found: (a) significant difference from all other groups ($p<0.001$); (b) significant difference from V, V+S and P ($p<0.001$). Abbreviations: S—saline, P—paroxetine, W—WAY-100,635, V—Solutol vehicle. Note: missing bars from 2nd and 3rd ejaculatory series in P/W and P+W groups represent no 2nd or 3rd ejaculation in that group.

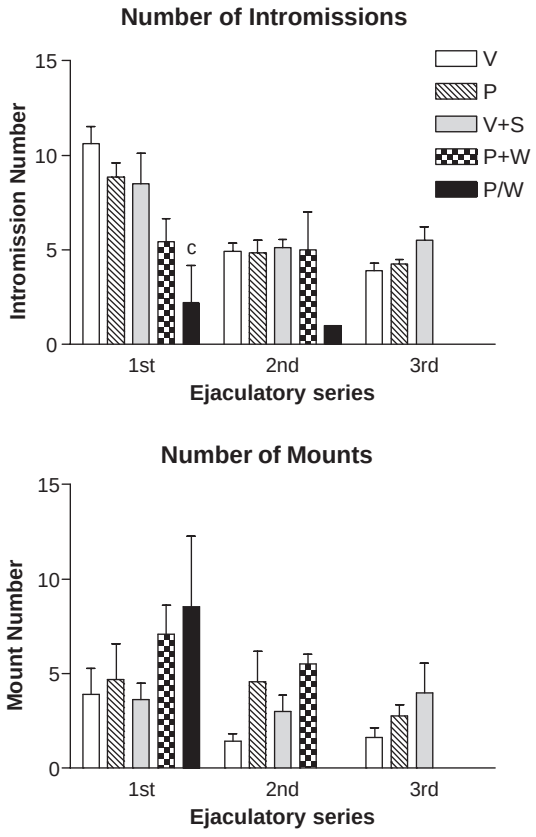

Fig. 2. Effects of paroxetine and sequential (P+W) or simultaneous (P/W) administration of paroxetine and WAY100,635 on mounting behavior. Data were analyzed using one-way ANOVA with Scheffe's post hoc test. Values are mean±S.E. $N=8-14$ (except for P/W 2nd ejaculatory series where $N=2$). The following significant differences were found: c—significant difference from V ($p<0.001$), V+S and P ($p<0.05$). Abbreviations: S—saline, P—paroxetine, W—WAY-100,635, V—Solutol vehicle. Note: missing bars indicate an absence of mounting behavior.

3.2. Effects of sequential administration of paroxetine followed by WAY-100,635 on male rat sexual behavior

The administration of paroxetine followed 30 min later by WAY-100,635 (Paroxetine+WAY) inhibited ejaculatory behavior (Fig. 1, Table 2). Seventy-eight percent of animals given Paroxetine+WAY ejaculated, however this group only averaged one ejaculation during the observation period, which was significantly less than the control and paroxetine alone groups (Table 2). In addition, the latency to ejaculation was significantly longer in this group compared to controls (Fig. 1). Even when the two animals that failed to ejaculate were removed from the data analysis, there was still a statistically significant difference in the ejaculation latency (1st ejaculatory series: 11:24±1:20 (min:s), mean±S.E.; unpaired t-test, $p<0.001$) compared to vehicle+saline controls (4.08±0.45). In addition, in those animals that did ejaculate, the post ejaculatory interval was significantly longer in the Paroxetine+WAY group compared to controls (Fig. 1; unpaired t-test, $p<0.05$). Only two rats from the Paroxetine+WAY group had

Table 1
Summary of the latency to first intromission, copulatory efficiency and time spent in sexual behavior

Treatment (n)	Latency to first intromission (min:s)	Copulatory efficiency	Duration of sexual behavior (min:s)
Vehicle (14)	0:22±0:48	0.734±0.156	10:18±3:32
Paroxetine (7)	0:23±0:24	0.602±0.179	11:32±4:37
Vehicle+saline (9)	0:17±0:07	0.648±0.211	12:21±4:03
Paroxetine+Way (9)	7:59±10:25	0.407±0.170*, b	16:43±7:35
Paroxetine/Way (9)	15:56±13:47**, a	0.157±0.127***, a	11:53±11:41

Data were calculated for the 30 min observation period. Values are mean±S.D. Significant differences were found using one-way ANOVA with Scheffe's post hoc: ***$p<0.0001$; **$p<0.001$; *$p<0.05$. [a]Different from vehicle, paroxetine and vehicle+saline; [b]different from vehicle group.

Table 2
Summary of the total mounting behavior and number of ejaculations

Treatment (*n*)	Number of intromissions	Number of mounts	Number of attempted mounts	Number of ejaculations
Vehicle (14)	20±4	8±6	13±10	3.1±0.8
Paroxetine (7)	19±4	14±8	10±5	3.3±0.8
Vehicle+saline (9)	20±6	12±9	22±14	3.3±0.7
Paroxetine+Way (9)	7±5**,[a]	11±7	13±9	1.0±0.7**,[a]
Paroxetine/Way (9)	2±3***,[a]	10±11	5±5	0.2±0.4***,[a]

Data were calculated for the 30 min observation period. Values are mean±S.D. Significant differences were found using one-way ANOVA with Scheffe's post hoc: ***$p<0.0001$; **$p<0.001$. [a]Different from vehicle, paroxetine and vehicle+saline.

a second ejaculation during the 30 min observation period. Both the latency to ejaculation and post ejaculatory interval in these 2 rats was longer than the average responses seen in the control groups (Fig. 1, 2nd ejaculatory series). The intromission latency tended to be longer after Paroxetine+WAY compared to controls but this did not reach statistical significant (Table 1 (ANOVA) and $p=0.0550$, unpaired *t*-test compared to vehicle+saline control group). Rats treated with Paroxetine+WAY tended to display an increased number of mounts and a decreased number of intromissions during the first ejaculation series (Fig. 2) which led to a reduction in overall copulatory efficiency (Table 1). Analysis of the mounting behavior over the total 30 min observation period confirmed that the Paroxetine+WAY group had significantly fewer intromissions compared to the control groups (Table 1).

3.3. Effects of simultaneous administration of paroxetine and WAY-100,635 on male rat sexual behavior

Simultaneous administration of Paroxetine and WAY (Paroxetine/WAY) inhibited ejaculatory behavior (Fig. 1 and Table 2). Only 22% of animals given Paroxetine/WAY ejaculated within the 30 min observational period. This was significantly different to the control groups in which every animal had at least 2 ejaculations. Animals without an ejaculation were given 30 min ejaculation latency; thus the group treated with Paroxetine/WAY had a significant longer latency to ejaculation compared to the control groups (Fig. 1). In addition, the mean ejaculation latency of the 2 animals that did ejaculate was 10:38±7.32 min, which was still longer than the controls (~5 min). The post ejaculatory interval in these 2 animals was also extremely prolonged (Fig. 1). The lack of ejaculatory behavior was related to the increased latency to initiate intromissions and, significantly fewer intromissions were observed with Paroxetine/WAY compared to control groups (Fig. 2; Tables 1 and 2). Interestingly, the mean number of mounts was not statistically different which resulted in a decreased copulatory efficiency in the Paroxetine/WAY group (Table 1).

3.4. Comparison of sequential administration and simultaneous administration of paroxetine and WAY-100,635

Significantly fewer animals (22%) ejaculated after simultaneous administration of Paroxetine/WAY compared to sequential drug administration (78%) (*Z*-test , $p<0.05$). The latency to the 1st intromission tended to be longer in the Paroxetine/WAY group and the simultaneous administration of Paroxetine/WAY resulted a decreased copulatory efficiency compared to the Paroxetine+WAY group (unpaired *t*-test, $p<0.01$) (Table 1). In addition, the simultaneous administration of Paroxetine/WAY resulted in an overall reduction in the number of intromissions (unpaired *t*-test, $p<0.05$) and attempted mounts compared to the sequential administration of Paroxetine+WAY (Table 2). Therefore, simultaneous administration of Paroxetine/WAY had a greater effect on sexual behavior compared to sequential drug administration.

Locomotion and general behavior was not part of the structured analysis during the present study. However, no statistically difference in the time spent in sexual behavior was observed between the groups (Table 2). More than half of the rats in each of the paroxetine plus WAY-100,635 treatment groups showed normal behavior; i.e. they were moving around the cage, sniffing the bedding and the female, grooming and occasionally lied down. All of these behaviors were observed in the control and paroxetine groups. The remaining rats treated with paroxetine and WAY-100,635 showed normal mounting behavior, but spent more time lying down compared to the animals in the control groups. However, it is important to note that rats treated with the 5-HT_{1A} agonist (8-hydroxy-2-(di-*n*-propylamino)tetralin (8-OH-DPAT)) spend a considerable amount of time lying down between bouts of sexual behavior, but this does not inhibit their ability to ejaculate (unpublished observations).

4. Discussion

The present study demonstrates that acute administration of the SSRI paroxetine, and the 5-HT_{1A} antagonist WAY-100,635 act in a synergistic manner to delay ejaculation and reduce the number of intromissions seen in male rats. Moreover, we have shown that simultaneous administration of the drugs produced a greater inhibition of mounting behavior and ejaculation compared to sequential administration of paroxetine followed by WAY-100,635.

Chronic administration of SSRIs, in rodents, primarily delays ejaculation, increases the post ejaculatory interval and decreases copulatory efficiency (Cantor et al., 1999; de Jong et al., 2005a,b; Waldinger et al., 2002). This is due to an increase in serotonin levels at both pre- and post-synaptic receptor sites. However, acute administration of the traditional SSRIs, at doses shown to increase extracellular 5-HT, either does not

alter, or has minor effects on, male sexual behavior (Ahlenius and Larsson, 1998; de Jong et al., 2005a,b; Mos et al., 1999; Yells et al., 1994). The effect of chronic SSRI treatment in rodents agrees with clinical studies in men by demonstrating a continuous decrease in sexual behavior over time (Rosen et al., 1999; Waldinger and Olivier, 1998; Waldinger et al., 1998a,b, 2004a,b). The present study agrees with previous studies, in that acute treatment with paroxetine alone did not significantly alter rodent sexual behavior. Also, acute treatment with paroxetine had no clinically relevant delay in ejaculation in men with lifelong premature ejaculation (Waldinger and Olivier, 2004; Waldinger et al., 2004b). Time-dependent desensitization of 5-HT_{1A} post-synaptic receptors that occurs during chronic SSRI administration may be necessary for mediation of the delay in ejaculation seen with chronic SSRI treatment.

Multiple serotonin receptors are present in various brain and spinal cord regions that have been shown to regulate sexual behavior. The most prominent effect on male sexual behavior is through 5-HT_{1A} receptor activation which intensely facilitates ejaculation (e.g. Hillegaart and Ahlenius, 1998, Sura et al., 2001). Other serotonin receptors, 5-HT_{1B} and possibly 5-HT_{2C}, may regulate the inhibition of ejaculatory behavior (Ahlenius et al., 1980; Ahlenius and Larsson, 1998; Foreman et al., 1989; Hillegaart and Ahlenius, 1998). Serotonin receptors are also involved in other aspects of sexual behavior such as penile erection (5-HT_{2C}) and initiation of copulation (5-HT_{2A}) (Bancila et al., 1999; Berendsen and Broeklamp, 1987; Gonzalez et al., 1994; Millan et al., 1997). In addition, serotonin may regulate sexual behavior, either alone or in coordination with other neurotransmitters. For example, dopamine is released into the hypothalamus in response to 5-HT_{1A} receptor stimulation which may contribute to the enhanced sexual behavior (Hull et al., 2004). Serotonin reuptake inhibitors have been shown to facilitate erections in rodents. Acute administration of paroxetine or fluoxetine enhances neurally evoked increases in intracaverous pressure and elicits penile erections (Angulo et al., 2003; Ahn et al., 2005; Millan et al., 1997). Therefore, the SSRI induced effects on erectile and ejaculatory behavior may be mediated by different CNS mechanisms.

The net effect of a delayed ejaculation seen with administration of SSRIs is probably mediated via inhibitory 5-$HT_{1B/2C}$ receptors (Ahlenius et al., 1980; Ahlenius and Larsson, 1998; Foreman et al., 1989; Hillegaart and Ahlenius, 1998). It is reasonable to speculate that the combination of a 5-HT_{1A} antagonist and SSRI augments the ejaculatory delay seen with SSRIs alone through two mechanisms. First, blockade of the negative feedback system through 5-HT_{1A} autoreceptors (Cremers et al., 2000; Hjorth et al., 1997) would increase synaptic levels of 5-HT available to stimulate the inhibitory 5-$HT_{1B/2C}$ receptors. Secondly, blockade of the post-synaptic 5-HT_{1A} receptors in the spinal cord that facilitate ejaculation would allow the delaying effects of the 5-$HT_{1B/2C}$ receptor activation to be more pronounced (Marson and McKenna, 1992). Previous studies consistently found that administration of WAY-100,635 at doses selective for 5-HT autoreceptors does not alter rodent sexual behavior when administered alone (Ahlenius and Larsson, 1999; de Jong et al., 2005a; Sura et al., 2001). However, WAY-100,635 blocks the actions of 5-HT_{1A} agonists that facilitate ejaculation (Ahlenius and Larsson, 1998; Rehman et al., 1999). In addition, co-administration of WAY-100,635 with SSRIs expose an inhibitory effect on ejaculation that is not observed when the SSRIs are given alone (Ahlenius and Larsson, 1999; de Jong et al., 2005a; present study). A potential conclusion is that under basal conditions 5-HT_{1A} receptors do not play a critical role in ejaculation; another is that the enhanced specific 5-HT receptor subtype interactions concomitant with the blockade of pre- versus post-synaptic receptors lead to the synergistic inhibition of ejaculation.

When both paroxetine and WAY-100,635 were acutely administered to male rats in the present study, a profound inhibition of male ejaculatory behavior was observed. Intromission latency was significantly increased and copulatory efficacy was reduced. Sequential administration of paroxetine followed by WAY-100,635 produced a significant delay in ejaculatory behavior but this effect was not as pronounced as after simultaneous drug administration. We speculate that in the scenario where paroxetine is administered prior to the WAY-100,635, the pre-synaptic 1A autoreceptors are available to immediately decrease the synaptic concentration of 5-HT through binding some of the free 5-HT. The subsequent administration of the 1A antagonist provides for a slow increase in the synaptic concentration of 5-HT through competitive displacement of the 1A-bound 5-HT. With simultaneous administration, the high affinity 1A ligand WAY-100,635 is able to successfully compete with the synaptic 5-HT for the 1A autoreceptor and thus leave a higher concentration of 5-HT for modulation of other 5-HT receptors like the sexually inhibitory 1B/2C receptors.

This synergistic effect has been previously reported with the SSRI, citalopram and WAY-100,635 (Ahlenius and Larsson, 1999; de Jong et al., 2005a). Around 40% of males that received sequential administration of citalopram (10 mg/kg) and WAY-100,635 (0.04–0.08 mg/kg) failed to ejaculate, while 75% of the male rats that received citalopram and WAY-100,635 (0.1 mg/kg) within 1 min of each did not ejaculate (Ahlenius and Larsson, 1999; de Jong et al., 2005a). These results are comparable to the present study where simultaneous administration of paroxetine and WAY-100,635 resulted in a greater proportion of males failing to ejaculate compared to sequential administration.

Numerous studies have examined synaptic serotonin levels after administration of a variety of SSRIs and serotonin antagonists (e.g. Hjorth, 1993; Hjorth et al., 1996, 1997; Romero and Artigas, 1997; Taber et al., 2000). One microdialysis study that examined serotonin levels after administration of fluoxetine and WAY-100,635 stressed that while WAY-100,635 potentiated the effect of fluoxetine on serotonergic neurotransmission, sequential drug treatment resulted in far greater levels of serotonin in the cortex compared to simultaneous drug treatment (Taber et al., 2000). In addition, systemic administration of fluoxetine reduced the firing rate of

dorsal raphe neurons, which was reversed by subsequent administration of WAY-100,635. However, WAY-100,635 failed to antagonize the effects of the SSRI when given at the same time (Taber et al., 2000). Therefore, the sequence of drug administration differentially affects the serotonergic system.

The various SSRIs differ in their ability to treat depression and delay ejaculation (e.g. Leonard, 1997; Maurel et al., 1999; Thor, 2001; Waldinger et al., 1998b, 2001, 2002, 2004a). The time course of sexual side effects with antidepressant drugs is very short implying that distinct and different behavioral outcomes may follow from interactions with the serotonin receptor system. Sequential drug treatments with serotonin antagonists and SSRIs have either shown a potentiation or no change in the anxiolytic effects of the SSRIs (Hashimoto et al., 1997; Tatarczynska et al., 2002). This may be related to their affinity for the various 5-HT receptor subtypes in the central nervous system. It is evident from published studies that SSRIs differ in their ability to treat depression and inhibit ejaculation and that different serotonin receptor subtypes probably contribute to anxiety and sexual behavior.

In conclusion, the present study demonstrated that paroxetine plus WAY-100,635 inhibits ejaculatory behavior and that the degree of inhibition varies after simultaneous or sequential drug administration. This may be relevant for assessment of clinical treatment and side effects of multiple drug treatment therapies.

Acknowledgments

This work was supported in part by a short-term research training grant from NIDDK to C. Looney and NIH Grant NS39166. The authors would like to thank Karla Gravitt for her excellent technical assistance.

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