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DOSE DEPENDENT D2 EFFECTS ON GENITAL REFLEXES AFTER MPOA INJECTIONS OF QUINELORANE AND APOMORPHINE

T.J. Bazzett, R.C. Eaton, J.T. Thompson, V.P. Markowski, L.A. Lumley, E.M. Hull*

Department of Psychology, SUNY at Buffalo
Buffalo, NY 14260

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Summary

This study investigated the effects on genital reflexes of unilateral MPOA injections of 0.1, 1, 3, and 10 μg of the D2 agonist quinelorane (LY-163502), and of 3 μg quinelorane administered together with 3 μg of the D1 antagonist SCH-23390. In addition, the effects of an MPOA injection of 10 μg apomorphine were tested. All but the lowest dose of quinelorane significantly decreased the latency to the first reflex. The 3 and 10 μg doses of quinelorane, and the combination of quinelorane and SCH-23390, decreased the total number of reflexes. In addition, 10 μg quinelorane increased the number of seminal emissions. 10 μg apomorphine, like 10 μg quinelorane, decreased the latency to the first reflex and increased the number of seminal emissions, but did not decrease the numbers of erections or penile movements. The ratio of D1/D2 activity may influence the number of erections displayed during ex copula testing.

Dopamine (DA) agonists have been shown to enhance libido and/or decrease ejaculatory threshold in a variety of species including humans (1,2), nonhuman primates (3), and rats (4,5,6,7,8). Microinjections of the mixed D1 and D2 DA agonist apomorphine into the medial preoptic area (MPOA) facilitated sexual behavior in male rats (9). Increases in both the rate of copulation and the number of mounts that resulted in intromission contributed to an increased number of ejaculations. Administration of apomorphine at a number of other sites was without significant effects. High doses of the mixed D1 and D2 DA antagonist cis-flupenthixol inhibited sexual behavior when injected into the MPOA (10). This inhibition was reflected in a slowing of copulatory rate, a decreased number of ejaculations, and a decrease in number of animals copulating.

One component of copulatory behavior is properly timed and integrated genital reflexes. Although it is difficult to observe genital reflexes in copula, penile reflexes (erections and penile movements, sometimes called flips) occur spontaneously after retraction of the penile sheath in supine restrained male rats. These reflexes appear to be functionally related to penile action in copula (11). Infrequently a seminal emission may also occur under these conditions. The functional relationship between penile reflexes during copulation and those observed ex copula has been discussed by Sachs and Garinello (12). MPOA injections of 1 μg and 2 μg apomorphine increased the numbers of erections and penile movements in tests of restrained supine male rats; the number of seminal emissions was not affected (13). On the other hand, cis-flupenthixol in the MPOA decreased the numbers of erections and penile movements (14). Thus, apomorphine facilitated both copulation and ex copula reflexes and cis-flupenthixol inhibited both.

The present experiments tested the effects of a range of doses of the selective D2 agonist quinelorane (LY-163502), microinjected into the MPOA, on genital reflexes; the same doses had previously been used in copulatory behavior tests (15). In an additional test, the D1 antagonist SCH-23390 was injected together with quinelorane. Since low and high doses of quinelorane produced different patterns of genital responses in Experiment 1, and since we had previously used only low doses of apomorphine, we tested the effects of a 10 μg dose of apomorphine, microinjected into the MPOA, on genital reflexes.

*Correspondence Author

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Methods

Animals and Housing

For Experiment 1, twenty male Long-Evans rats (Harlan Sprague Dawley/Blue Spruce Farm) weighing approximately 400 g were chosen based on their display of penile reflexes during a 30 min screening. They were tested for reflexes following counterbalanced administration of 0, 1, 3, and 10 μg quinelorane. At the completion of quinelorane testing, all animals were tested for reflexes following a single injection of 3 μg quinelorane + 3 μg SCH-23390. Separate groups of 20 animals that had previously been used in other similar experiments were tested for the effects of 0.1 μg quinelorane in Experiment 1, and for the effects of 10 μg apomorphine in Experiment 2. All rats were housed individually in large plastic cages with free access to food and water, in a temperature controlled colony room with a 14 h light, 10 h dark cycle.

Surgical Procedure

Each rat was anesthetized with ketamine hydrochloride (50 mg/kg) and xylazine hydrochloride (4 mg/kg) i.m. prior to implantation of a stainless steel guide cannula ending 1 mm above the left MPOA (AP:2.4, ML:0.2, DV:-6.6, incisor bar: +5 mm; see ref 16). An obturator, cut even with the guide cannula, prevented entry of foreign material into the cannula. Details of surgery and cannula construction are described elsewhere (9). In addition, each rat's suspensory ligament was excised in order to allow full exposure of the glans penis during testing.

Drugs

Quinelorane was dissolved in 0.5 μl sterile water. Apomorphine was dissolved in 0.5 μl sterile water with 0.2% ascorbate. The combination of SCH-23390 and quinelorane was dissolved in 0.5 μl sterile water with 10% dimethyl sulfoxide (DMSO). Drugs were mixed immediately before testing. Quinelorane was generously donated by Dr. Mark Foreman of the Eli Lilly Company; SCH-23390 was a generous gift of Dr. Allen Barnett of the Schering Corporation.

Testing Procedure

Ten days following surgery, all rats were given a single postoperative baseline test for penile reflexes. One week following postoperative testing a schedule of weekly tests was begun in which all animals received 0, 1, 3, and 10 μg quinelorane in counterbalanced order; the same animals received an additional test following 3 μg quinelorane + 3 μg SCH-23390. A Harvard infusion pump was used to administer drug injections at a rate of 1.0 μl/min. The injection cannula, which extended 1 mm beyond the guide cannula, was left in place for 30 sec following injection to allow for drug diffusion. Testing took place immediately after drug injection.

Rats were restrained in a supine position in a rectangular metal tube (8.5 x 5.5 x 20 cm). The lower half of the rat's body protruded from the device, and was secured with Velcro straps. Following retraction of the penile sheath, the latency to the first erection was recorded, as well as the numbers of each of three gradations of erections: E1 - reddening and distention of the glans, E2 - tumescence of the base and tip of the glans, E3 (cup) intense erection resulting in a flaring of the tip of the glans. Numbers of penile movements and seminal emissions were also scored. Seminal plugs present upon sheath retraction were removed and included in the total number of seminal emissions for that test. For additional details of scoring procedures see Pehek et al. (13). Tests lasted 15 min from the first reflex or 20 min if no reflexes occurred.

Histology

Upon completion of all behavioral testing, animals were anesthetized and sacrificed by decapitation. Coronal sections of 40 μm were stained with cresyl violet, and examined for accuracy of cannula placement under a projection magnifier. Animals with cannula placements more than .5 mm from the MPOA were excluded from data analysis.

Statistics

Data from counterbalanced quinelorane doses in Experiment 1 were analyzed using a one way, repeated measures, ANOVA. A Newman-Keuls test for multiple comparisons was used to compare individual doses. Data from the 0.1 μg dose of quinelorane and the 3 μg quinelorane + 3 μg SCH-23390 were analyzed using a Student's t test comparison with the appropriate vehicle scores. Data from Experiment 2, were also analyzed using Student's t test.

Results

Experiment 1. Effects of quinelorane and of quinelorane + SCH-23390 on genital reflexes.

Analysis of the 0, 1, 3, and 10 μg doses of quinelorane showed that the total numbers of erections and penile movements were significantly decreased by quinelorane (Erections: $F(3,51)=7.52$, $p<.001$; Penile movements: $F(3,51)=4.64$, $p<.01$; Total Reflexes: $F(3,51)=6.20$, $p<.001$; Fig. 1). The decrease in total erections reflected decreases in mild erections (E1s, $F(3,51)=3.09$, $p<.05$) by the 10 μg dose ($p<.05$), and in moderate (E2s, $F(3,51)=4.06$, $p<.02$) and intense erections (E3s, $F(3,51)=3.85$, $p<.02$) by both the 10 μg ($p<.05$) and 3 μg ($p<.05$) doses. In addition, these three doses significantly decreased the latency to the first reflex ($F(3,51)=6.17$, $p<.005$; Fig. 2). Seminal emissions were increased by 10 μg quinelorane ($F(3,51)=3.02$, $p<.05$; Fig. 3).

The 3 μg SCH-23390 + 3 μg quinelorane dose also decreased the total number of reflexes ($t(13)=5.53$, $p<.005$; Fig. 1) and decreased reflex latency ($t(13)=3.04$; $p<.01$; Fig. 2). Student's t test revealed no significant differences in genital reflexes of animals receiving 0.1 μg quinelorane (Figs. 1 and 2). Vehicle scores for these animals were not significantly different from vehicle scores for the former group and are included in a single graph for clarity of comparison.

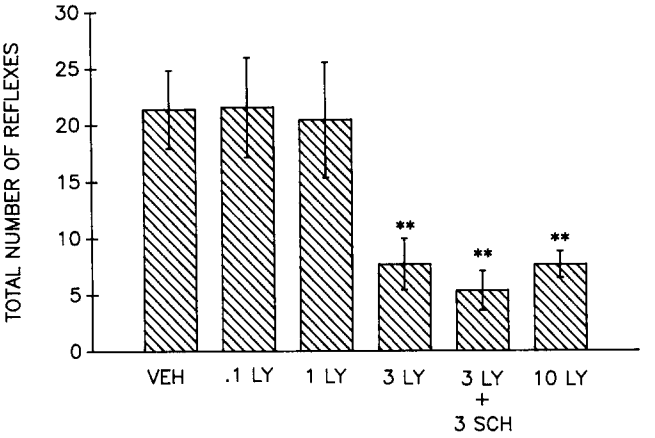

Fig. 1.
Effects on total number of penile reflexes. Both the 3 and 10 μg doses of quinelorane significantly decreased number of reflexes (**$p<.01$), as did 3 μg quinelorane + 3 μg SCH-23390 (**$p<.005$).

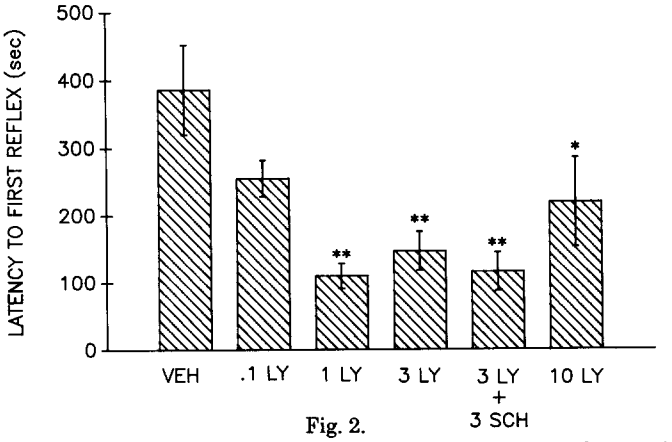

Fig. 2.
Effects on the latency to begin penile reflexes. 1, 3, and 10 μg quinelorane significantly decreased latency, as did 3 μg quinelorane + 3 μg SCH-23350. (*$p<.05$; **$p<.01$)

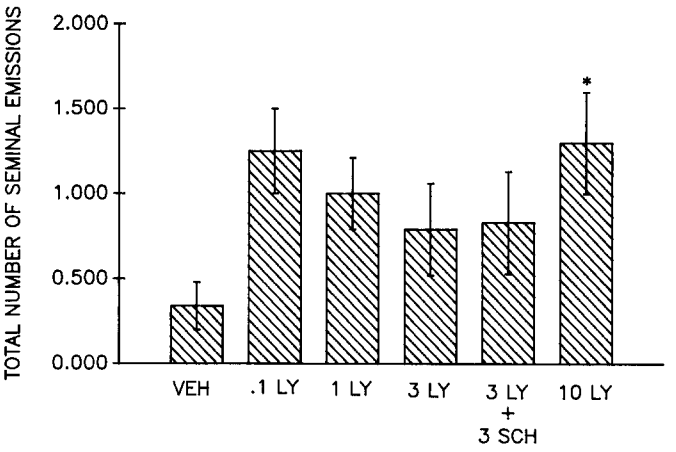

Fig. 3.
Effects on number of seminal emissions. Only 10 μg quinelorane significantly increased the number of seminal emissions. (*$p < .05$)

In order to determine whether the occurrence of seminal emission affected the onset of erections, latency to the first erection for animals having at least one seminal emission was compared to latency to the first erection in animals having no seminal emissions. Similarly, to determine if occurrence of seminal emission affected the number of erections, total number of erections for animals having at least one seminal emission was compared to total number of erections for animals having no seminal emissions. Student's t test revealed no significant differences in latency or number of erections for animals with or without seminal emission, within test groups ($p > .3$ in all cases). In addition, the temporal distribution of reflexes was not affected by quinelorane, or by a preceding seminal emission. 47% of all reflexes occurred in the first 5 min period, 30% in the second period, and 23% in the final 5 min period.

Experiment 2. Effects of 10 μg apomorphine on genital reflexes.

10 μg apomorphine significantly decreased the latency to the first reflex ($t(15) = 3.65$, Fig. 4a) and increased the number of seminal emissions ($t(19) = 3.09$, Fig. 4b). No other measures were affected.

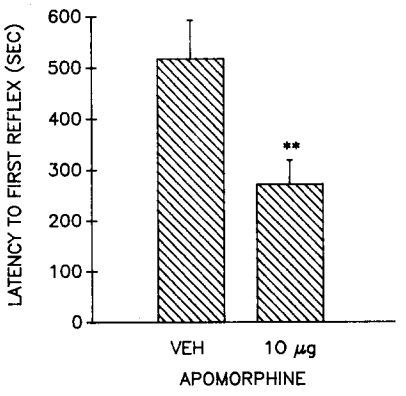

Fig. 4a.

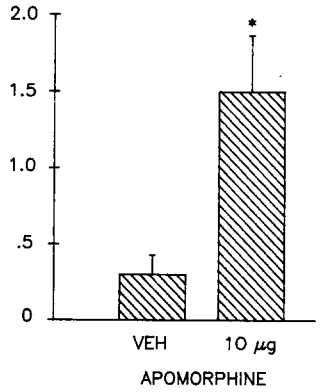

Fig. 4b.

10 μg apomorphine significantly decreased the latency to the first reflex (**$p < .005$, Fig. 4a) and increased the number of seminal emissions (*$p < .01$, Fig. 4b).

Discussion

The selective D2 agonist quinelorane affected genital reflexes in a dose dependent fashion. The 3 and 10 μg doses significantly decreased the total numbers of erections and penile movements. Only the 10 μg dose increased the number of seminal emissions. In addition, the latency to the first reflex was significantly shortened by 1, 3, and 10 μg quinelorane, with the 1 and 3 μg doses producing the shortest latencies. The effects of 3 μg quinelorane were not blocked by coadministration of 3 μg of the D1 antagonist SCH-23390. A dose of 0.1 μg quinelorane was without effect when tested in a separate group of animals. 10 μg apomorphine increased the number of seminal emissions and decreased the latency to the first reflex, without affecting the numbers of reflexes.

Our lab has previously shown that quinelorane injections into the MPOA preceding copulation increased the latency to the first intromission and decreased the number of intromissions preceding ejaculation (15). We hypothesized that the increase in intromission latency may have reflected an impairment of erectile mechanisms and/or penile movements, both of which are associated with intromission (17). On the other hand the decrease in number of intromissions preceding ejaculation may have been correlated with an enhancement of seminal emission. The data are, for the most part, consistent with that hypothesis.

However, there appears to be considerable complexity in the regulation of sexual behavior. Although the decrease in numbers of erections and penile movements is consistent with the hypothesized inhibition of those mechanisms, the shorter latency to the first reflex suggests a facilitation of reflexes. We would have expected an increase in reflex latency, rather than a decrease. However, the apparent paradox is not unique to this study. We observed this pattern with MPOA injections of a high dose (10 μg) of quinelorane in an earlier experiment (18). Furthermore, Sachs and Bitran (19) reported a similar pattern after acute spinal block. Several investigators have also elicited this pattern when testing genital reflexes following two or more ejaculations in copula (12,20,21). The latter results suggested the possibility that the reduction in reflexes following intracranial quinelorane may have been a behavioral artifact resulting from increased seminal emission. However, the decreased latency and decreased numbers of erections and movements following quinelorane treatment occured even in animals not displaying seminal emission. Thus, the pattern appears to be caused directly by the drug treatment, and not indirectly as a result of previous seminal emission.

Sachs and Bitran (19) offered a theoretical model to explain decreases in both reflex latency and number of erections following spinal block. Such a block was said to interrupt both the descending inhibition of a "starter" mechanism and the descending excitation of a "generator" mechanism. Releasing the "starter" mechanism from inhibition would shorten the latency to the first erection, whereas blocking excitation of the "generator" would decrease the number of erections. One possible interpretation of Sachs and Bitran's "starter" and "generator" mechanisms is that they may represent separate autonomic and striated muscle influences on the reflexes.

Neurons controlling genital reflexes are located in the spinal cord and are subject to descending influences from the brain. Erections are mediated primarily by parasympathetic innervation, although they are augmented by actions of the bulbocavernosus muscle (22). Penile movements result from the action of the ischiocavernosus muscle (17). It appears that the parasympathetic action may be synchronized with striatal muscle activity to promote erections and penile movements, both of which are required for intromission (17). It is of interest that erections and movements were affected in parallel fashion by quinelorane in this experiment, and by apomorphine and cis-flupenthixol in previous experiments (12,14). On the other hand, the sympathetic system elicits seminal emission, but also some erections (23). It was further noted by Benson (23) that sympathetic innervation affecting genital response originates in the lower thoracic area, whereas parasympathetic innervation originates in the sacral (S2-4) region. Thoracic spinal block would interrupt descending influences to both areas.

While quinelorane's effects on copulatory parameters appear to be related to the number of ex copula reflexes and seminal emissions, the temporal patterning of reflexes and of copulatory behavior are quite different. Quinelorane lengthened the latency to the first intromission in tests of copulatory behavior (15) but shortened the latency to the first reflex in the ex copula tests. Furthermore, penile reflexes recorded in supine restrained rats occur in clusters of 1-7 reflexes in several seconds. During copulation, however, intromissions are paced at intervals of 30 seconds or more. It is possible that the discrete

reflexive responses to tonic stimulation (i.e. sheath retraction) ex copula are internally paced, whereas reflexes during copulation are influenced by external cues such as motor activity, the presence of a female, and pheromones. Sachs and Garinello (12) have noted that although penile actions occurring during copulation and in supine reflex tests seem to share a functional relationship, temporal patterning of such reflexes are considerably different in the two conditions.

The finding that D1 antagonism did not diminish the effect of D2 stimulation contrasts with the synergistic relationship between D1 and D2 receptors in the nigrostriatal pathway (reviewed in 24,25). D1 antagonism also failed to decrease the effects of D2 stimulation of the MPOA in copulatory behavior tests; indeed two copulatory measures (reduction of intromissions preceding ejaculation and slowing of copulatory rate) showed additivity of effects of the D1 antagonist and D2 agonist (15). Opposite effects of D1 and D2 stimulation have also been observed on vacuous chewing (26) and grooming behavior (27).

A 10 μg dose of the mixed D1 and D2 agonist apomorphine in the MPOA increased the number of seminal emissions and decreased the latency to the first reflex. The similarity between these effects and those seen after MPOA injection of 10 μg quinelorane seem to indicate shared aspects of a D2 pattern of responding. In support of this idea, seminal emission elicited by 10 μg apomorphine was blocked by coadministration of a selective D2, but not a D1, antagonist in another study (Hull et al., in preparation). The fact that 10 μg apomorphine did not decrease the numbers of erections and penile movements, as did quinelorane, may be the result of concurrent D1 and D2 stimulation; we have observed that a D1 agonist injected into the MPOA increased the number of erections (Hull et al., in preparation). We have previously observed that low doses (1 or 2 μg) of apomorphine increased the numbers of erections and penile movements, but not of seminal emissions (13). Thus, seminal emission elicited by D2 mechanisms may have a higher threshold than D1 mediated erectile mechanisms.

The effects of D2 stimulation in these experiments does not appear to be due to selective stimulation of autoreceptors, which are thought to be of the D2 type (reviewed in 25). Doses of apomorphine between .5 and 10 μg have consistently increased the number of ejaculations per test (9,10,15,28). However, a very low dose of apomorphine (.1 μg) injected into the MPOA decreased the number of ejaculations, presumably by selectively stimulating presynaptic autoreceptors; this effect was blocked by 6-OHDA lesions of dopamine terminals in the MPOA (28). The effects of the very low (autoreceptor selective) dose were similar to those of the dopamine antagonist cis-flupenthixol (10), suggesting that .1 μg apomorphine had decreased the release of dopamine.

In summary, data presented here are consistent with previous findings that erections and seminal emissions are mediated by two separate mechanisms. Microinjections of quinelorane into the MPOA evoked a response similar to acute spinal block, suggesting that these results may have been due to a decrease in descending influences. The 10 μg dose of quinelorane evoked not only the seminal emission response normally associated with sympathetic activity, but also decreased the numbers of erections and penile movements, which occurred with short latencies. The 10 μg dose of apomorphine similarly increased seminal emissions and decreased the latency to first the first reflex, but did not affect numbers of erections and penile movements. It is possible that responses seen after 10 μg apomorphine or quinelorane may simulate D2 mechanisms associated with ejaculation during copulation. Finally, we have shown that the MPOA is a likely site of integration for central influences on spinal reflexive mechanisms.

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