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# D2 Receptors in the Paraventricular Nucleus Regulate Genital Responses and Copulation in Male Rats

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EATON, R. C., V. P. MARKOWSKI, L. A. LUMLEY, J. T. THOMPSON, J. MOSES AND E. M. HULL. *D2 receptors in the paraventricular nucleus regulate genital responses and copulation in male rats.* PHARMACOL BIOCHEM BEHAV **39**(1) 177–181, 1991.—The D2 dopamine receptor agonist quinelorane (LY-163502), microinjected into the paraventricular nucleus (PVN), affected genital responses of restrained supine male rats in a biphasic dose-dependent fashion. A moderate dose (1 μg) facilitated penile responses (intense erections and penile movements), and decreased the latency to the first response. A high dose of quinelorane (10 μg) facilitated seminal emission while inhibiting penile responses. The addition of the D1 antagonist SCH-23390 to the 1 μg dose of quinelorane potentiated quinelorane's increase in seminal emission. We suggest that D1 receptors in the PVN may be antagonistic to D2 receptor-mediated seminal emission, and possibly also penile responses. In copulation tests 1 μg quinelorane decreased mount latency, whereas 10 μg quinelorane increased mount and intromission latencies and slowed copulatory rate. Both 1 and 10 μg quinelorane, and also 1 and 10 μg of the mixed D1 and D2 agonist apomorphine, decreased the number of intromissions preceding ejaculation.

Genital responses Copulation Penile erection Seminal emission Dopamine D2 receptor Quinelorane LY-163502 Paraventricular nucleus

STIMULATION of dopamine receptors in the paraventricular nucleus (PVN) was reported by Melis et al. to increase penile erection and yawning in freely moving male rats (6). In those studies, LY-171555, a specific D2 receptor agonist, was as effective as the mixed D1 and D2 agonist apomorphine in increasing penile erections and yawning when injected into the PVN. Furthermore, facilitation by apomorphine was blocked by both the D2 antagonist haloperidol and the D1 antagonist SCH-23390. Melis et al. suggested that D2 receptors in the PVN facilitate erections, and that D1 receptors "enable" the D2-mediated response.

We have recently examined the effects of medial preoptic area (MPOA) injections of the D2 agonist quinelorane (LY-163502) on genital responses in restrained supine rats (2) and also on copulation (5). A high dose of quinelorane in the MPOA facilitated seminal emission while inhibiting penile erections. In copulation tests the same dose of quinelorane delayed the onset of copulation and slowed its rate, but also decreased the number of intromissions preceding ejaculation. We suggested that the delay in copulatory onset may have been related to an impairment of erectile mechanisms, whereas the decrease in intromissions preceding ejaculation may have reflected an enhancement of seminal emission (5).

The present experiments extended our study of quinelorane's effects on sexual behavior to the PVN. Genital responses of restrained supine rats were observed following a wide range of doses of quinelorane. In addition, the effects of quinelorane on copulation were compared with effects of the mixed agonist apomorphine.

## METHOD

### *Animals*

For each experiment twenty adult male Long-Evans rats weighing 300–375 g were housed individually in large plastic cages. A 14:10 light:dark cycle was in effect, with lights off at 11.00 h. Food and water were available ad lib. Animals were handled daily so that microinjection could be performed without anesthesia. Data from animals that failed to exhibit genital responses on two or more tests in Experiment 1, or that failed to copulate on two or more tests in Experiment 2, were removed from statistical analysis. Data were also removed if an animal's

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cannula was located further than 0.5 mm from the PVN.

*Surgery and Cannulae*

The male rats were anesthetized with ketamine hydrochloride (50 mg/kg) and xylazine hydrochloride (4 mg/kg). Each animal received a 23-ga thin wall unilateral stainless steel guide cannula directed to end 1 mm above the PVN [AP, 0.4; ML, 0.4; DV, −7.0; incisor bar, +5 mm; (8)]. Guide cannulae were implanted using a Kopf stereotaxic frame. Details of surgery and cannula construction are described in Hull et al. (4). An obturator fashioned from 27-ga stainless steel tubing prevented entry of foreign material and kept the guide cannula patent. It was cut to end even with the guide cannula, and held in place by a collar constructed of 23-ga stainless steel tubing and polyethylene. An injection cannula, used at the time of drug administration, was constructed of 27 ga stainless steel tubing and extended 1 mm beyond the guide cannula.

For Experiment 2, female rats of the same strain were ovariectomized under ketamine and xylazine anesthesia. They were housed in a separate room, and were brought into behavioral estrus by an injection of 20 μg estradiol benzoate (Sigma) in oil SC 48 h before behavioral testing.

*Drugs*

Quinelorane (LY-163502) was donated by Dr. Mark Foreman, Eli Lilly and Company (Indianapolis, IN) and dissolved in sterile water immediately before administration. SCH-23390 was donated by Dr. Allen Barnett, Schering Corporation (Bloomfield, NJ) and was dissolved in 10% dimethyl sulfoxide (DMSO) and sterile water prior to administration. Apomorphine (Sigma) was dissolved in sterile water with 0.2% ascorbate.

*Procedures*

*Experiment 1.* Prior to surgery, males were adapted to the restrainer and then given 3 screening tests for penile responses. Males that displayed responses on 2 of the 3 screening tests were used in the experiment. A postoperative baseline was established one week after surgery. Thereafter, behavioral tests following drug (0.1, 1 and 10 μg quinelorane) or vehicle administration were given at one-week intervals. All animals received all doses in counterbalanced order. On the fifth week of testing, all animals received 1 μg quinelorane plus 5 μg SCH-23390.

At the time of testing, the obturator was removed and the injection cannula was inserted. The injection volume was 0.5 μl and was delivered at a rate of 1 μl/min. The injection cannula was left in place for 30 s after the injection to maximize drug diffusion into surrounding tissue. The obturator was replaced, and the male was carried to an adjacent testing chamber where testing began immediately.

During behavioral testing, each rat was placed in a metal restrainer (8.5 × 5.5 × 20 cm) in the supine position and held in place by Velcro straps. The lower portion of the rat's body was left exposed. The penile sheath was retracted and maintained in that position to elicit penile responses. Spontaneous responses usually occured within 10 minutes after exposure of the glans. Responses occurred within clusters, separated by 15 s or longer of inactivity. Three gradations of erections were scored: E1, engorgement of these base of the glans; E2, tumescence of both the base and tip of the glans; E3, tumescence of the base accompanied by extreme flaring of the tip of the glans (sometimes called a cup). Two grades of penile movements (anteroflexions of the penis, sometimes called flips) were scored: short, anteroflexions that did not pass vertical in relation to the body; long, anteroflexions that passed the vertical. Seminal emission was scored by the presence of viscous seminal fluid or a sperm plug.

A test lasted 15 minutes from the first penile response (i.e., erection or penile movement), or 20 minutes if no response occurred. The time and number of each type of erection, penile movement and seminal emission were recorded using a program written by Stephen Yeoh for the IBM microcomputer. Measures derived from the data were the latency to the first response, the number of clusters, the number of seminal emissions, the total number of responses, the total number of erections, the total number of penile movements and the number of each grade of erection (E1, E2, E3) and penile movement (short, long).

*Experiment 2.* Males were given sexual experience in their home cage for two 30-min periods. Copulatory measures were then scored on a preoperative baseline test. Two weeks following surgery, they were given a postoperative test. Thereafter, they were given weekly tests following counterbalanced injections of vehicle, 1 and 10 μg quinelorane, and 1 and 10 μg apomorphine. Injection procedures were the same as for Experiment 1.

Animals were carried to an adjacent room, where a receptive female was placed into each male's home cage. Testing began immediately. Each test lasted for 30 min after the first intromission, or for a total of 30 min if the male failed to intromit. The occurrence and time of each mount, intromission and ejaculation were recorded by a program written by Stephen Yeoh for the IBM microcomputer. Measures derived from the data were: number of ejaculations per test, numbers of mounts and of intromissions preceding each ejaculation, latencies to the first mount and the first intromission, ejaculation latency (latency from the first intromission of an ejaculatory series to the subsequent ejaculation), postejaculatory refractory period (time from the ejaculation to the next intromission), interintromission interval (the average time between intromissions), and intromission ratio (number of intromissions divided by mounts plus intromissions). Intromissions were distinguished behaviorally from mounts by the presence of a deep thrust followed by a rapid, springing dismount. Ejaculation patterns were characterized by longer, deeper thrusts, slow dismounts and a prolonged period of rest after ejaculation.

*Statistical Analysis*

A counterbalanced, repeated measures design was used. Data were analyzed by one-way repeated measures analyses of variance, followed by Dunnett's *t*-statistic comparing treatment means with the control (9). All data are presented as mean ± SEM.

*Histology*

After all behavioral testing, males were anesthetized and sacrificed by decapitation. Cannulae were removed and brains were frozen in an American Optical cryostat. Coronal sections were cut at 40 μm, mounted on glass slides and stained with cresyl violet. Sections were examined with a projection magnifier to verify cannula placement. Data from animals whose cannulae were more than 0.5 mm from the PVN were excluded.

## RESULTS

*Experiment 1*

Intra-PVN injection of quinelorane affected genital responses in a dose-dependent fashion. Administration of 0.1 and 1 μg

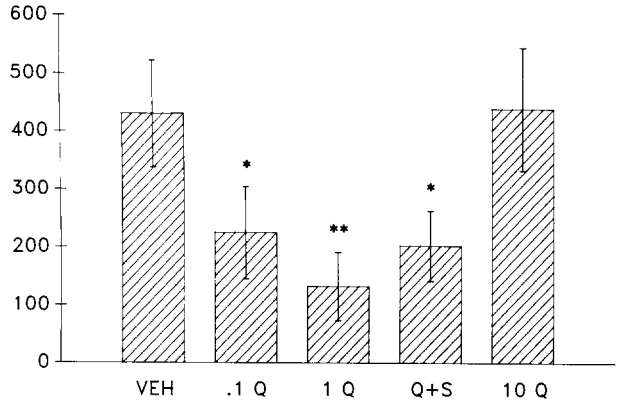


FIG. 1. Effects of intracranial administration of vehicle, quinelorane (0.1 Q, 1 Q and 10 Q) and the combined treatment of quinelorane and SCH-23390 (Q+S) on the latency (seconds) to the first penile response. Data are expressed as mean ± SEM. *$p<0.05$, **$p<0.01$. Q = quinelorane; Q+S = quinelorane (1 μg) + SCH 23390 (5 μg).

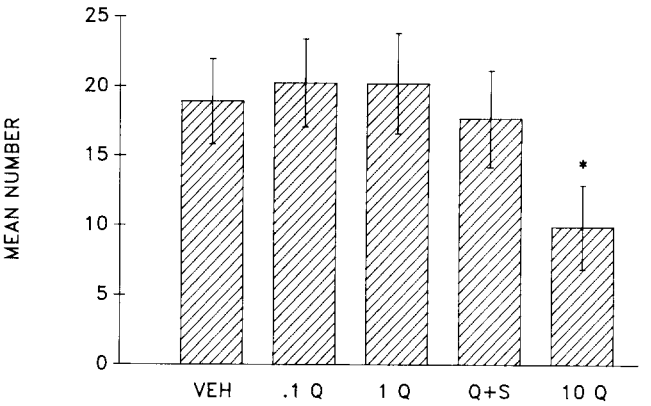


FIG. 3. Effects of intracranial administration of vehicle, quinelorane (0.1 Q, 1 Q and 10 Q) and the combined treatment of quinelorane and SCH-23390 (Q+S) on total erections. Data are expressed as mean ± SEM. *$p<0.05$. Q = quinelorane (μg); Q+S = quinelorane (1 μg) + SCH 23390 (5 μg).

quinelorane, as well as the combined treatment of 1 μg quinelorane plus 5 μg SCH-23390, decreased the time to the first genital response [$F(4,76)=3.85$, $p<0.01$; see Fig. 1]. The 1 μg dose increased the numbers of intense erections (E3) [$F(4,76)=2.96$, $p<0.05$; see Fig. 2a] and long penile movements [$F(4,76)=2.62$, $p<0.05$; see Fig. 2b]. On the other hand, 10 μg quinelorane decreased the total number of erections [$F(4,76)=2.32$, $p<0.06$; $t(5,76)=2.26$, $p<0.05$; see Fig. 3]. The 10 μg dose and the combined treatment increased seminal emissions [$F(4,76)=2.91$, $p<0.05$; see Fig. 4].

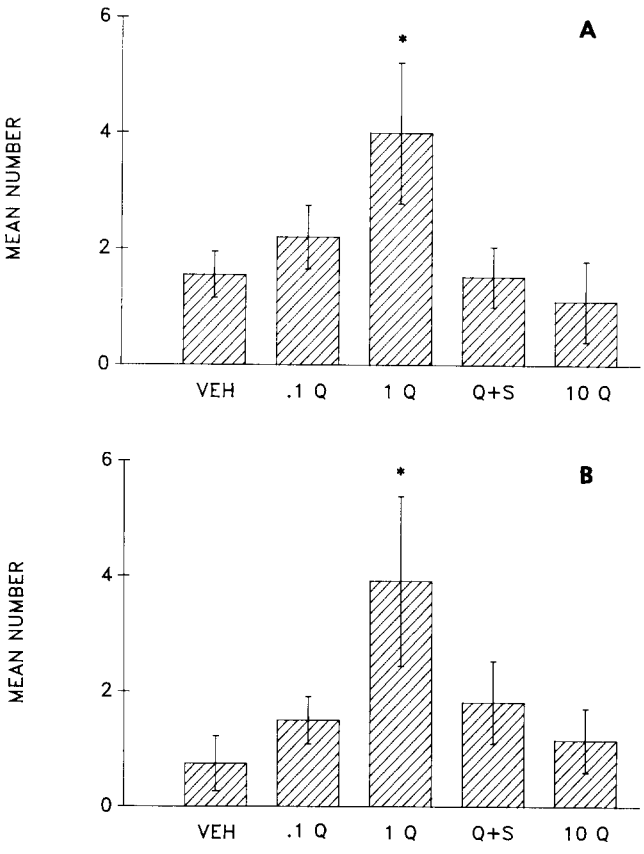


FIG. 2. Mean number of (A) intense erections and (B) penile movements following intracranial administration of vehicle, quinelorane (0.1 Q, 1 Q and 10 Q) and the combined treatment of quinelorane and SCH-23390 (Q+S). Data are expressed as mean ± SEM. *$p<0.05$. Q = quinelorane (μg); Q+S = quinelorane (1 μg) + SCH 23390 (5 μg).

*Experiment 2*

Quinelorane in the PVN produced dose-related effects on mount, $F(4,32)=5.29$, $p<0.005$, and intromission, $F(4,32)=3.17$, $p<0.05$, latencies and on interintromission interval [$F(4,32)=2.93$, $p<0.05$; see Table 1]. The low dose (1 μg) of quinelorane decreased mount latency, whereas the high dose (10 μg) of quinelorane increased mount and intromission latencies and increased interintromission interval. In addition, both doses of quinelorane and of apomorphine decreased the number of intromissions preceding ejaculation [$F(4,32)=4.09$, $p<0.01$; see Table 1].

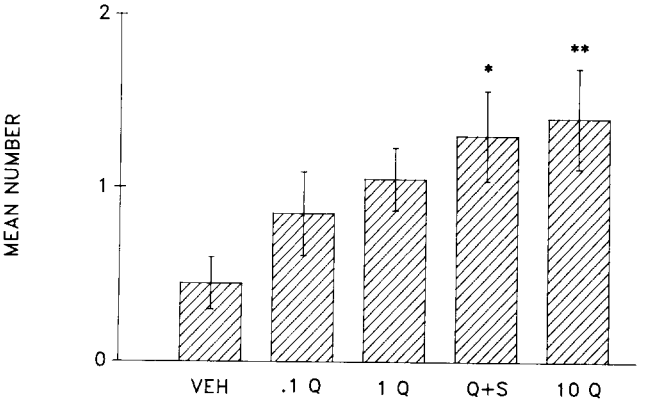


FIG. 4. Effects of intracranial administration of vehicle, quinelorane (0.1 Q, 1 Q and 10 Q) and the combined treatment of quinelorane and SCH-23390 (Q+S) on seminal emission. Data are expressed as mean ± SEM. *$p<0.05$, **$p<0.01$. Q = quinelorane (μg); Q+S = quinelorane (1 μg) + SCH 23390 (5 μg).

TABLE 1

EFFECTS OF QUINELORANE OR APOMORPHINE IN PVN ON COPULATION

| | Veh | 1 μg LY | 10 μg LY | 1 μg APO | 10 μg APO |
|---|---|---|---|---|---|
| Mount Latency (s) | 136.8 ±28.4 | 75.6* ±27.1 | 424.2* ±128.0 | 145.0 ±25.0 | 202.7 ±59.3 |
| Intromission Latency (s) | 267.8 ±78.2 | 176.0 ±71.9 | 653.3* ±125.6 | 379.6 ±129.5 | 389.2 ±128.4 |
| Intromissions per Ejaculation | 9.7 ±1.5 | 6.6* ±0.8 | 5.4† ±0.5 | 7.1* ±0.7 | 6.3* ±0.6 |
| Interintromission Interval (s) | 59.0 ±8.5 | 75.1 ±18.7 | 85.1* ±12.0 | 47.8 ±5.1 | 48.1 ±4.4 |
| Ejaculation Latency (s) | 534.7 ±87.7 | 443.3 ±89.2 | 398.0 ±46.2 | 330.1 ±23.5 | 285.9 ±21.5 |
| Total Ejaculations | 1.9 ±0.2 | 2.0 ±0.2 | 2.4 ±0.2 | 2.2 ±0.3 | 2.0 ±0.4 |

Data expressed as mean ± SEM. *$p<0.05$, †$p<0.01$.

## DISCUSSION

Microinjection of quinelorane into the PVN affected genital responses in a dose-dependent fashion. A facilitation of penile responses by the 1 μg dose was apparent in the increased numbers of intense erections and long penile movements, as well as in the decreased latency to the first genital response. The 0.1 μg dose and the combination of 1 μg quinelorane and 5 μg SCH-23390 also decreased the latency to the first genital response. On the other hand, 10 μg quinelorane decreased total erections but increased the number of seminal emissions. The 0.1 μg and 1 μg doses of quinelorane did not significantly affect seminal emission; however, the addition of 5 μg SCH-23390 to the ineffective 1 μg dose did facilitate seminal emission. Thus blocking D1 receptors enhanced the effectiveness of the D2 agonist, suggesting opposing effects of the two receptor subtypes on seminal emission.

Previous studies have not tested higher doses of dopamine agonists in the PVN. Thus the biphasic nature of the dose-response curve for genital responses (facilitation by 1 μg quinelorane and impairment by 10 μg) has not been observed previously. It is, however, somewhat similar to the dose-response curve that we observed with apomorphine or quinelorane in the MPOA (1, 2, 7). Specifically, low doses increased (apomorphine) or failed to affect (quinelorane) the number of erections and penile movements; low doses of both agonists decreased the response latency. On the other hand, 10 μg of either apomorphine or quinelorane in the MPOA shifted the balance of responses to favor seminal emission, and inhibit erection.

The dose dependency of quinelorane's effects was also observed in copulation tests. The low dose (1 μg) in the PVN facilitated the onset of copulation, whereas the high dose both delayed its onset and slowed copulatory rate (increased interintromission interval). The shorter mount latency following 1 μg quinelorane may have been related to enhanced processing of sensorimotor information, since the same dose increased the numbers of penile responses in Experiment 1. Similarly, the delayed onset and slowed rate observed after 10 μg quinelorane may have reflected a shift from erectile processes (mediated primarily by parasympathetic and striated muscle mechanisms) and towards seminal emission (sympathetically mediated) and ejaculatory processes.

Whereas the latency measures of copulation and numbers of ex copula penile responses appear to be affected similarly, the decrease in intromissions preceding ejaculation observed after both quinelorane and apomorphine may have been related to sympathetically mediated seminal emission. Both quinelorane (Experiment 1) and apomorphine (7) in the PVN increased the number of seminal emissions in supine, restrained male rats. A similar relationship between ex copula reflexes and copulatory measures was observed with manipulations of dopamine receptors in the MPOA, although low doses of quinelorane were ineffective in the MPOA.

Foreman and Hall also observed dose-related effects in copulation tests, using systemic injections of quinelorane (3). Doses ranging from 25 ng/kg to 2.5 mg/kg enhanced ejaculatory mechanisms (decreased ejaculation latency and decreased intromissions preceding ejaculation), whereas a high dose (25 mg/kg)increased ejaculatory latency and decreased the number of animals copulating. None of our doses significantly affected ejaculation latency or number of animals copulating, but all doses decreased intromissions preceding ejaculation. However, it is difficult to compare dose-response curves for systemic and intracranial injections.

Another question addressed in this experiment was the relationship of D1 and D2 receptors in the regulation of genital responses. Melis et al. (6) reported that SCH-23390 blocked the facilitative effects of apomorphine, and inferred that endogenous dopamine stimulation of D1 receptors enable the D2-mediated response. Our data also show that administration of the D1 antagonist blocked the facilitation by 1 μg quinelorane of intense erections and long penile movements.

There are two possible interpretations of our data. In agreement with Melis et al. (6), the decrease in penile erections after administration of the D1 antagonist may have resulted from the loss of the enabling function of the D1 receptors. An alternative interpretation of the data suggests that D1 receptors work in an antagonistic fashion to D2 receptors. Thus the addition of the D1 antagonist may shift the potency of the D2 receptor agonist (quinelorane) toward that of higher doses. In support of this view, we have found that the effectiveness of the combination of 1 μg quinelorane plus 5 μg SCH-23390 was between that of the 1 μg and 10 μg doses of quinelorane for intense erections, long penile movements and seminal emissions. The addition of 5 μg SCH-23390 to the ineffective dose of 1 μg quinelorane resulted in a significant increase in seminal emission, similar to that of the 10 μg dose. It is possible that D1 receptor stimulation enables erections while antagonizing seminal emission; on

the other hand, D1 receptor stimulation may antagonize all D2-mediated effects in the PVN. Additional experiments will be needed to clarify this relationship. However, it is of interest that the mixed D1/D2 agonist apomorphine was generally less effective in Experiment 2 than was the selective D2 agonist quinelorane.

In summary, the present data support a biphasic dose-response curve of quinelorane in the PVN on genital responses and copulation in the male rat. A low dose (1 μg) of quinelorane increased the numbers of intense erections and long penile movements and decreased mount latency. A high dose (10 μg) reduced erections ex copula and delayed and slowed copulation. Both quinelorane (Experiment 1) and apomorphine (7) increased the number of seminal emissions ex copula and enhanced ejaculatory ability in copula (decreased intromissions preceding ejaculation). In addition, we suggest an alternative hypothesis for the interactions of D1 and D2 receptors regulating genital reponses. Melis and co-workers proposed that D1 receptors enable D2-mediated effects on genital responses (6). We, on the other hand, suggest that D1 receptors in the PVN, as well as in the MPOA, may be antagonistic to D2 receptors.

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