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Mediation of Rat Postejaculatory 22 kHz Ultrasonic Vocalization by Dopamine D2 Receptors

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CAGIANO, R , R J BARFIELD, N R WHITE, E T PLEIM AND V CUOMO *Mediation of rat postejaculatory 22 kHz ultrasonic vocalization by dopamine D2 receptors* PHARMACOL BIOCHEM BEHAV **34**(1) 53–58, 1989 —We investigated the role of dopamine receptor subtypes in the regulation of ultrasonic vocalization and masculine copulatory behavior Intact sexually experienced male Long-Evans rats were treated with saline, selective dopamine D1 (SKF 38393) and D2 (LY 171555) receptor agonists and with selective dopamine D1 (SCH 23390) and D2 (raclopride) receptor antagonists 15 and 30 min before the 30-min test session, respectively Mating stimuli were ovariectomized female rats injected SC with estradiol benzoate (8 μg/0 1 ml/rat) and progesterone (200 μg/0 1 ml/rat), 48 and 4 hr before the test session, respectively We found a decrease in the number of intromissions required to reach ejaculation in animals treated with SKF 38393 (10 mg/kg/IP), LY 171555 (doses ranging from 0 01 to 0 5 mg/kg/SC) and with raclopride (0 1 mg/kg/SC) LY 171555 reduced the postejaculatory vocalization (PEV) in a dose-dependent fashion with complete suppression at the highest dose No other parameters of sexual behavior were affected by this treatment Raclopride, a dopamine D2 receptor antagonist, antagonized the suppressive effects of the D2 agonist LY 171555 on the PEV (and also decreased the number of intromissions to reach ejaculation), whereas SCH 23390, a dopamine D1 receptor antagonist, did not Raclopride, given alone at the dose of 0 5 mg/kg/SC, almost completely suppressed all behavioral activity, whereas the lower dose (0 1 mg/kg) decreased intromission frequency and increased the length of the 22 kHz PEV Therefore, we suggest that 22 kHz PEV is under the control of dopamine D2 receptors

Male sexual behavior Selective dopamine receptor agonists and antagonists Rat Postejaculatory vocalization

IN studies on rat sexual behavior, neuropharmacological approaches have revealed the involvement of several neurotransmitter mechanisms in the control of rat sexual behavior (7,12) Neuropharmacological agents have also been used to determine whether specific types of neurons were important in various aspects of sexual behavior and further to investigate the intimate relationship between hormones and putative neurotransmitters in activating and maintaining hormone-dependent behavior (9,26).

That monoamines play an important role in the regulation of rat sexual behavior was long suspected (24) because monoaminergic mechanisms were thought to underlie sexual dysfunctions commonly associated with antihypertensive and psychotropic medications (6, 30, 31). In recent years, pharmacological studies on male rats have generated various hypotheses, the most common of which is that serotonergic (14, 20, 36) and GABAergic transmission (13) are inhibitory, while dopaminergic (14,21) and noradrenergic transmission (22) are facilitatory for masculine sexual behavior Since rodents communicate with each other by ultrasonic vocalizations that play an important role in intraspecific interactions such as parental (2,28), aggressive (19,33), exploratory (16), sexual (5,23) and stress-elicited behavior (35), pharmacologists have recently come to appreciate that ultrasonic calls have a great potential as a tool in behavioral pharmacology (1, 8, 10)

We therefore undertook this project to investigate whether selective dopamine receptor agonists and antagonists could influence the two following types of ultrasonic vocalizations emitted by male rats during sexual activity

1) *The 50 kHz call (mating call).* This vocalization has a very short duration (3–15 msec) and occurs prior to ejaculation. It is associated with female darting behavior or with male trailing, mounting and intromissive behavior (5,23)

2) *The 22 kHz call (postejaculatory call)* This call can reach a peak-intensity of 80 dB SPL and has a very long duration (1 to 3 sec). This call reliably, but not invariably, occurs after ejaculation during the postejaculatory refractory period (3) and shows a frequency range of 20–30 kHz Sleep-like EEG patterns and little movement are displayed by male rats during the postejaculatory

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vocalization (PEV) (4) This is commonly called the "22 kHz call" because the greatest part of its energy is emitted at 22 kHz It occurs concurrently with the absolute refractory period during which the male is incapable of renewed sexual activity Its communicative function is not yet established, but it is of great interest to note that these calls are detected only in species which display multiple ejaculations It is presumed either that the 22 kHz call serves to discourage other males from mating with the same female or to keep the female at a distance during the total postejaculatory refractory period (3,15) although this latter function has been recently disputed (32)

These ultrasonic calls appear to be very sensitive indices of changes in rat social and sexual motivation which may be difficult to detect with other more traditional behavioral procedures Thus, measurements of changes in the emission patterns of these ultrasonic calls may reveal subtle effects of pharmacological treatments

METHOD

Experimental male and stimulus female Long-Evans rats (Charles River, Wilmington, MA) were singly housed upon arrival under a reversed 12/12 hr light-dark cycle (dark period 09 30–21 30 hr) in suspended stainless steel cages (24 × 19 × 17 cm) Food and water were available ad lib

Thirty stimulus 225–250 g Long-Evans female rats were bilaterally ovariectomized, under Metofane (Pitman-Moore, Washington Crossing, NJ) anesthesia, about one week after their arrival and were allowed to recover in their home-cages for 2 weeks Each female rat was used in several testing periods at least one week apart Behavioral estrus was induced by injecting estradiol benzoate (8 μg/rat/SC) and progesterone (200 μg/rat/SC), dissolved in 0 1 cc sesame oil, 48 and 4 hours, respectively, before experimental tests

Subject Screening

Two weeks after their arrival in the laboratory, 130 experimental 275–300 g Long-Evans male rats were allowed to achieve one ejaculation per pretest session during 4–8 sessions of sexual experience with stimulus females These sessions (maximum duration 15 minutes) took place in glass aquaria (48 × 25 × 30 cm) under dim red illumination and were terminated as soon as ejaculation was achieved

A male rat was considered experienced after he achieved four ejaculations in no more than 8 pretest sessions Ten animals did not meet this criterion and were eliminated from the study Experimental male rats were tested only once under experimental conditions Each experimental group consisted of 6 animals

Experimental Drug Test

Drug tests began 3 hr after lights out and were performed under dim red illumination The treated male rat was placed in the mating cage for a habituation period of five minutes before the introduction of a randomly assigned estrous female The 30-min test session, including the screen of a 3561 A Hewlett Packard Dynamic Signal Analyzer (DSA) which displayed the frequency spectra of the rat ultrasonic calls, was recorded with a Sony Betamax Video Tape Recorder (VTR), along with the audible signals emitted by a QMC S-100 Heterodyne Receiver (Bat Detector) The Bat Detector, connected to an ultrasonic microphone placed over the center of the mating cage 40 cm above its floor, converted rat ultrasonic signals from 20–100 kHz (broadband setting) to humanly audible sounds A slow motion replay was employed when necessary The following parameters were derived and analyzed

Sexual Parameters

IL—Intromission latency in each ejaculatory series (the time between the introduction of the female into the mating cage and the first intromission in the first ejaculatory series),

MF—Mount frequency in each ejaculatory series,

IF—Intromission frequency in each ejaculatory series,

EL—Ejaculation latency in each ejaculatory series (time between the first intromission and ejaculation),

EF—Ejaculation frequency in a 30 minute session,

PEI—Post Ejaculatory Interval (interval between each ejaculation and the next intromission),

TMF—Total mount frequency during a 30 min session,

TIF—Total intromission frequency during a 30 min session,

ICI—Intercopulatory Interval = (EL/IF) in each ejaculatory series

Ultrasonic Parameters

L22—Latency to 22 kHz PEV in each ejaculatory series (time between ejaculation and the beginning of the 22 kHz call),

D22—Duration of the 22 kHz PEV in each ejaculatory series,

TD22—Total 22 kHz PEV duration during a 30 min session,

L50—Latency to first 50 kHz call (time between the introduction of the female into the mating cage and the appearance of the first "mating call")

Drugs

The following drugs, in crystalline form, were dissolved in 0 9% w/v NaCl and administered in a volume of 1 ml/kg 30 min (selective dopamine antagonists SCH and raclopride) or 15 min (selective dopamine agonists SKF and LY) before the test SCH 23390 emimaleate (SCH) 0 01 mg/kg/IP (Schering Corp , Bloomfield, NJ), a dopamine D1 receptor antagonist (17), raclopride tartrate (raclopride) 0 5 and 0 1 mg/kg/SC (Astra Lakemedel, Sodertalje, Sweden), a dopamine D2 receptor antagonist (27), SKF 38393 HCl (SKF) 5 and 10 mg/kg/IP (Smith Kline & French, Philadelphia, PA) a dopamine D1 receptor agonist (25), LY 171555 or quinpirole HCl (LY) 0 01, 0 05, 0 1 or 0 5 mg/kg/SC (Eli-Lilly, Indianapolis, IN) a dopamine D2 receptor agonist (34)

Statistical Analysis

Results of Experiment 1 and Experiment 2 were evaluated using a one-way analysis of variance (ANOVA) When necessary, individual post hoc Newman-Keuls tests were made between groups The results of Experiment 3 were statistically evaluated by two-way analysis of variance (ANOVA) The sexual and ultrasonic parameters scored in each of the three experiments were the same

RESULTS

Experiment 1

Initially, we investigated the influence of the selective dopamine D1 receptor agonist SKF 38393 and the D2 receptor agonist LY 171555 on the sexual and ultrasonic parameters of mating behavior

Since the "mating calls" are also emitted by female rats, it was impossible to determine whether a male or female subject was responsible for the sound In fact, our initial observations suggested that these calls might correlate with the general arousal of

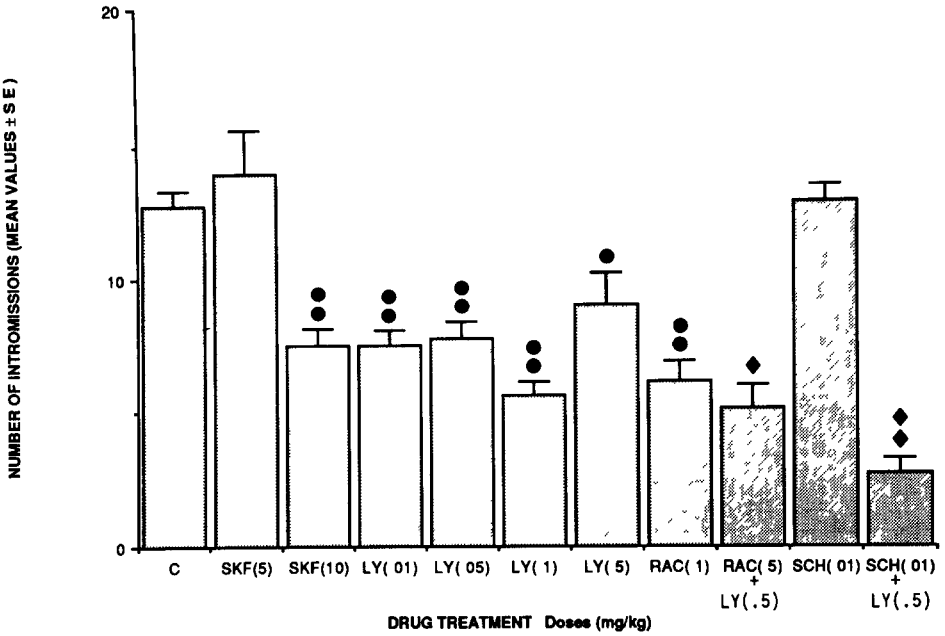

FIG 1 Effect of selective dopamine agonists and antagonists on intromission frequency (IF) of male rats during the first ejaculatory series ● = $p<0.01$, ●● = $p<0.001$ vs C, ◆ = $p<0.05$, ◆◆ = $p<0.001$ vs LY (0 5)

the male rat and that the selective dopamine agonists could affect their occurrence We therefore intend to conduct a future study using devocalized females In this study we took into account only the first ejaculatory series As shown in Fig 1, all doses of LY 171555 significantly decreased IF with respect to the control group. In addition, all doses of LY 171555, except the lowest, significantly decreased the 22 kHz PEV duration with respect to the control group (see Fig 2) Figure 2 also shows that LY 171555

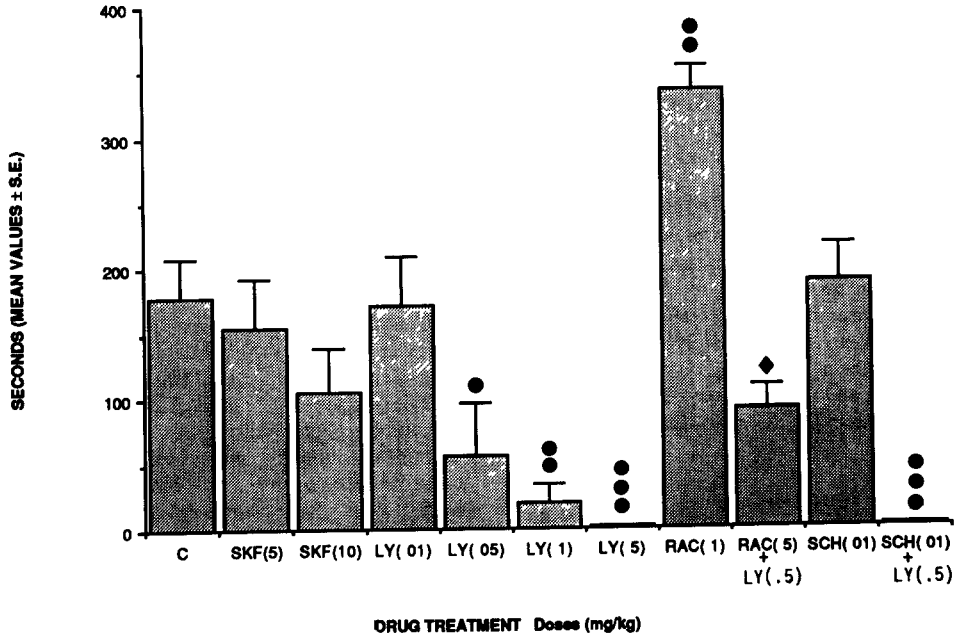

FIG 2 Effect of selective dopamine agonists and antagonists on the duration of the 22 kHz postejaculatory vocalization of male rats during the first ejaculatory series ● = $p<0.05$, ●● = $p<0.01$, ●●● = $p<0.001$ vs C, ◆ = $p<0.001$ vs LY (0 5)

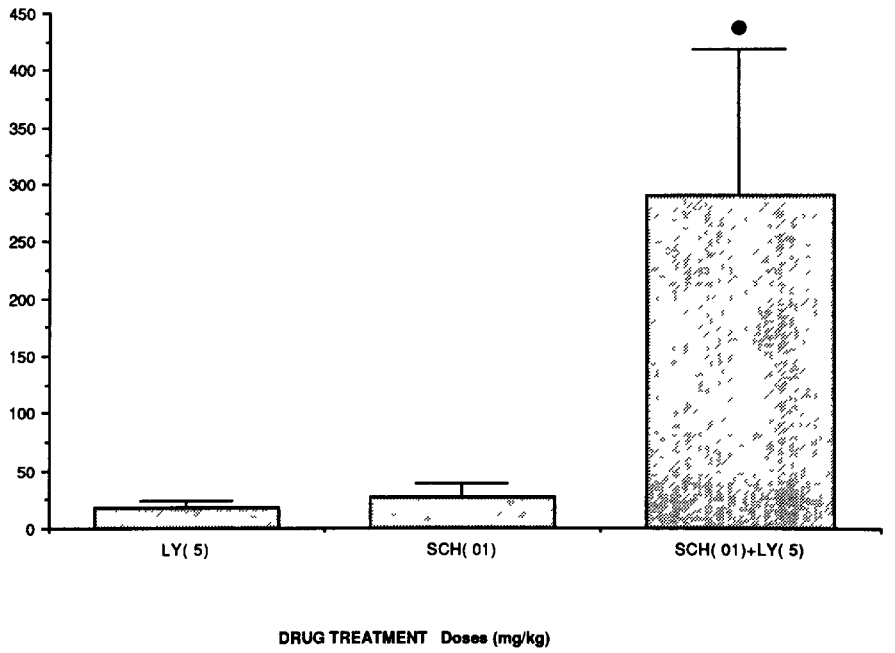

FIG 3 Effects of the combination of a D2 receptor agonist (LY) and a D1 receptor antagonist (SCH) on the intromission latency (IL) of male rats during the first ejaculatory series ● = $p<0.05$ vs LY (0 5)

totally suppressed the 22 kHz PEV at the highest dose

The D1 agonist SKF 38393 had no effect on 22 kHz PEV (Fig 2), but at the dose of 10 mg/kg, significantly decreased IF (Fig 1)

Experiment 2

Next, we wanted to investigate whether the effect of LY 171555 on the 22 kHz PEV was restricted to the dopamine D2 receptors alone or if dopamine D1 receptors were involved as well For this purpose we tried to antagonize the suppressant effect of LY on 22 kHz PEV with a dopamine D2 antagonist (raclopride) and with a dopamine D1 antagonist (SCH 23390) Raclopride 0 5 mg/kg, given in combination with LY 0 5 mg/kg, antagonized the suppressive effect of this drug on the 22 kHz PEV (see Fig 2) and, as shown in Fig 1, further decreased IF with respect to LY 0 5 mg/kg given alone. SCH 0.01 mg/kg, given in combination with LY 0 5 mg/kg, failed to antagonize its suppressive effect on 22 kHz PEV (Fig 2), but more markedly decreased IF (Fig 1) while significantly increasing IL without affecting EL (Fig 3) Raclopride (0 5 mg/kg) given alone almost completely suppressed male rat sexual behavior, however, a lower dose of raclopride (0 1 mg/kg) decreased IF (Fig 1) and increased the duration of the 22 kHz PEV (Fig 2) No changes in EF, MF, EL, PEI, TMF and ICI were found

Experiment 3

Finally, we investigated whether SCH 0 01, which had no antagonistic effect on the 22 kHz PEV suppressed by LY 0 5 mg/kg (a possible basement effect), could have some antagonistic effect on lower doses of LY (0 1 and 0 05 mg/kg) when the PEV was significantly shortened As in Experiment 1, the dopamine D2 agonist LY 171555 (0 05 and 0 1 mg/kg) decreased the duration of the 22 kHz PEV with respect to the control group Figure 4 shows that the dopamine D1 receptor antagonist SCH 23390 had no effect on the duration of 22 kHz PEV shortened by LY

DISCUSSION

The present study shows that the 22 kHz postejaculatory vocalization is under the control of dopamine D2 receptors since LY 171555, a dopamine D2 receptor agonist, was able to suppress the 22 kHz PEV without affecting most other major parameters of sexual behavior Furthermore, raclopride, a dopamine D2 receptor antagonist, but not SCH 23390, a dopamine D1 receptor antagonist, was able to antagonize these effects Consistent with this interpretation, raclopride 0 1 mg/kg given alone increased the duration of the 22 kHz PEV

All doses of LY used in this study caused a reduction of IF This result suggests that LY might have an effect on the ejaculation threshold The higher dose of SKF had the same effect as LY but the lower dose did not Perhaps this dopamine D1 agonist at the higher dose also affects dopamine D2 receptors Raclopride, a dopamine D2 antagonist, reduced IF both at the lower dose, if administered alone, and at the higher dose if administered with LY 0 5 mg/kg

The higher dose of raclopride (0 5 mg/kg) given alone almost completely suppressed sexual behavior This suggests that the dose of raclopride employed (the highest dose that did not decrease sexual behavior 0 1 mg/kg) may have affected one or both dopamine receptor subtypes

SCH 0 01 mg/kg, given in combination with LY 0 5 mg/kg, caused a decrease in IF This did not occur, however, if SCH 0 01 mg/kg was given alone This may reflect the hypothesized reduction of the threshold for ejaculation by LY Since IF is a result of several processes, each of which may be differentially affected by D1 or D2 active agents, such complex findings are not unexpected Moreover, the lack of dose-response relationship between LY and IF obtained in the present study is in agreement with other findings showing that dopamine related drugs produce nonlinear, sometimes biphasic effects (11,18) On the other hand, the present data do not allow us to draw specific conclusions on the rather paradoxical finding that a D2 receptor agonist (LY) and antagonist (raclopride) had the same effect on IF

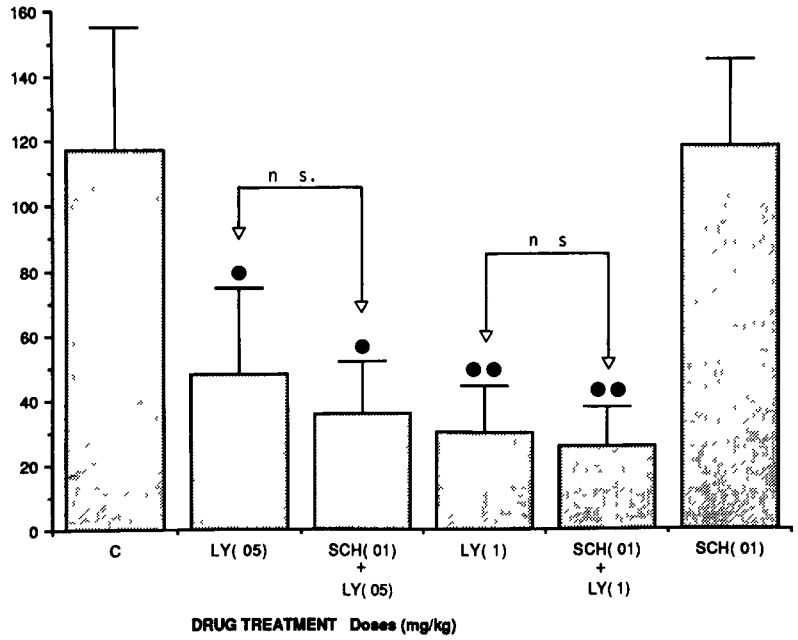

FIG 4 Effect of combined treatment of a D2 receptor agonist (LY) at dose levels able to shorten the 22 kHz PEV of male rats with a D1 receptor antagonist (SCH) ●=$p<0$ 05, ●●=$p<0$ 01 vs controls (C)

However, our data as well as those recently reported by Ogren *et al.* (27) have shown that there exists a separation between doses of raclopride producing distinct behavioral effects In this regard, it has been suggested that the behavioral findings with raclopride could reflect actions on D2 receptor subtypes, yet to be identified, in different projection areas involving functionally different dopamine receptors in the striatum and mesolimbic dopaminergic systems

Furthermore, deactivation of dopamine either by stimulation of D2 receptors (LY) or by blockade of D1 receptors (SCH) did not affect IL, but if the two drugs were administered together, they had a synergistic effect resulting in an increase in IL

Up to now, specific components of male sexual behavior have not been directly linked to the activation or inhibition of a single dopamine receptor type in the central nervous system Finally, these results suggest that it would be worthwhile to explore whether the 22 kHz ultrasonic vocalization emitted in other behavioral situations, such as aggressive and stress-elicited behaviors, could be affected by selective dopamine D2 receptor agonists and antagonists

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