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Nitric oxide promotes medial preoptic dopamine release during male rat copulation

Daniel S. Lorrain, Leslie Matuszewich, Ryan V. Howard,[1] Jianfang Du and Elaine M. Hull[CA]

Department of Psychology, Park Hall, State University of New York at Buffalo, Buffalo, NY 14260; [1]Current address: State University of New York Health Science Center, 750 East Adams Street, Syracuse, NY 13210, USA

[CA]Corresponding Author

DOPAMINE (DA) is released in the medial preoptic area (MPOA) of male rats during copulation. DA agonists infused into the MPOA facilitate, and antagonists impair, copulatory behavior. Local administration of the nitric oxide (NO) precursor L-arginine also increases DA release in the MPOA. The present experiment used microdialysis to test whether NO promotes DA release during copulation. Males received either an NO synthesis inhibitor, nitro-L-arginine methyl ester (L-NAME, 400 μM), or its inactive isomer D-NAME (400 μM) into the MPOA via a microdialysis probe for 3 h prior to the introduction of a female. Following D-NAME administration, DA increased during copulation, while L-NAME prevented this increase. NO may therefore promote DA release in the MPOA of male rats, thereby facilitating copulation.

Key words: Dopamine; HPLC-EC; Male rats; Medial preoptic area; Nitric Oxide; Sexual behavior

Introduction

The medial preoptic area (MPOA) is a major integrative site for male sexual behavior in virtually all vertebrate species, including the rat.[1] Dopamine (DA) may contribute to sexual behavior by enhancing sensorimotor processing in the MPOA. Using *in vivo* microdialysis, we observed increases in extracellular DA and its two main metabolites, 3,4-dihydroxyphenylacetic acid (DOPAC) and homovanillic acid (HVA), in the MPOA of male rats during an anticipatory precopulatory period and during copulation.[2] Furthermore, DA agonists microinjected into the MPOA facilitated copulation[3] and *ex copula* genital reflexes,[4] whereas DA antagonists in the MPOA impaired copulation, *ex copula* genital reflexes and sexual motivation.[5] The mechanisms regulating MPOA DA activity during copulation have not been clarified.

One factor that may affect DA release is nitric oxide (NO). NO was originally recognized as the endothelial-derived relaxing factor,[6,7] and has since been implicated in many physiological functions, including brain mechanisms of male and female reproduction.[8–11] NO synthase, the enzyme that synthesizes NO from L-arginine, is distributed throughout the brain, including in scattered fibers in the MPOA.[12] NO has been suggested to modulate the release of several neurotransmitters in specific brain areas, including DA in striatal slices,[13,14] norepinephrine and acetylcholine in hippocampal slices,[15] glutamate and aspartate in the medulla[16] and glutamate and norepinephrine in cerebral cortex synaptosomes.[17]

We previously reported that NO may enhance DA release in the MPOA.[18] Specifically, administration of the precursor of NO, L-arginine, by microdialysis probe increased extracellular DA. This increase was blocked by co-administration of the NO synthesis inhibitor *N*-monomethyl-L-arginine (L-NMMA); L-NMMA administered alone decreased basal levels of DA and its metabolites.

The present experiment examined whether inhibition of NO synthesis by nitro-L-arginine methyl ester (L-NAME) blocks the dopamine release in the MPOA of male rats that normally occurs during, and facilitates, copulatory activity. L-NAME or its inactive isomer D-NAME was perfused into the MPOA through a microdialysis membrane before and during copulation, while DA diffused into the dialysate and was assayed using high performance liquid chromatography with electrochemical detection (HPLC-EC).

Materials and Methods

Seventeen adult male Long-Evans/Blue Spruce rats (300–350 g), purchased from Harlan Sprague–Dawley (Indianapolis, IN), were housed individually in a controlled environment with food and water available *ad lib*. Their light cycle was reversed, with lights

off at 11.00 h and on at 21.00 h. One week before microdialysis testing, subjects were anesthetized with ketamine hydrochloride (50 mg kg^{-1}, i.m.) and xylazine hydrochloride (4 mg kg^{-1}, i.m.) and placed in a Kopf stereotaxic frame with incisor bar at +5 mm. All rats were implanted with a 15 mm, 23 G thin-wall stainless steel guide cannula aimed to end 2 mm above the left MPOA (mm from bregma: AP, +2.2; ML, +0.2; DV, –6.2). Details of the surgery and cannula construction have been described previously.[3] An obturator fashioned from 27 G tubing, ending flush with the guide cannula, was inserted after surgery. All procedures were in accordance with the NIH Guide for the Care and Use of Laboratory Animals and were approved by the local Institutional Animal Care and Use Committee.

Microdialysis probes using a concentric flow design were used. A 3 mm length of dialysis membrane (mol. wt cut-off 12 000, 210 μm o.d., Spectra-Por) was glued to one end of a 27 G 15 mm stainless steel shaft with waterproof epoxy. The end was plugged and 2 mm were inactivated with epoxy to maintain an active dialyzing length of 1 mm. Samples were collected into 250 μl centrifuge tubes and analyzed immediately. *In vitro* recovery averaged 30% at the flow rate used in these experiments.

The dialysis perfusion medium was a modified Dulbecco's phosphate buffered saline solution (Sigma) (138 mM NaCl, 2.7 mM KCl, 0.5 mM $MgCl_2$, 1.5 mM KH_2PO_4, 8.1 mM Na_2HPO_4, 1.2 mM $CaCl_2$; pH 6.5). Dissolved in the perfusion medium was either D-NAME (400 μM) or L-NAME (400 μM). Perfusion flow was controlled by a Harvard syringe infusion pump (model 22) at the rate of 0.5 μl min^{-1}.

On the day of testing, animals were briefly anesthetized with ketamine hydrochloride (12.5 mg kg^{-1}) and xylazine hydrochloride (1 mg kg^{-1}) to facilitate insertion of the microdialysis probe. The flow of the perfusion medium was started immediately after probe insertion, and a 3 h stabilization period was allowed prior to dialysate collection. Twelve animals received D-NAME and eight received L-NAME throughout the entire microdialysis procedure: three of the eight L-NAME animals were previously tested with D-NAME, resulting in a total of 20 tests on 17 animals. All animals were sexually naive, except three of the eight in the L-NAME group tested once previously with D-NAME.

All animals were tested in their home cage with a Plexiglas addition that increased the height of the walls; all copulation experiments were conducted during the rat's dark phase of the light cycle. After the stabilization period, dialysate was collected every 6 min and analyzed until three consecutive samples showed $\leqslant$ 10% variation in DA levels. At this time, an estrous female was placed behind a perforated Plexiglas barrier in the male's testing cage for an 18 min precopulatory period. The barrier was then removed and the animals allowed to copulate for 20 min. One precopulatory sample was analyzed (12–18 min interval) and three consecutive copulatory samples were analyzed during the 20 min copulation test, starting 2 min after removal of the barrier (to allow for dead volume in the probe). At the end of the experiment, rats were given a lethal injection of Somlethol, decapitated and the brains removed and frozen. Probe placements were histologically verified.

DA and its main metabolites DOPAC and HVA were assayed using HPLC-EC. Samples were loaded into a Rheodyne injector valve delivering a 500 nl volume to an LC Packings C18 reverse phase capillary column, using a mobile phase consisting of 30 mM citric acid, 50 mM sodium acetate, 0.027 mM Na_2EDTA, 0.25 mM octyl sodium sulfate and 2.5% acetonitrile v/v (pH 3.8). A Gilson model 307 pump, operating at 0.5 ml min^{-1}, was equipped with an Acurate flow splitter which delivered 6 μl min^{-1} to the column. Compounds were detected with an Antec microcell detector, using a glassy carbon working electrode maintained at a potential of +0.7 V relative to a Ag/AgCl reference electrode.

Data are expressed as a percentage of the average of the final two baseline samples preceding exposure to the female. Data were analyzed only from animals that copulated (10 treated with D-NAME and seven with L-NAME), using a two-way repeated measures analysis of variance (ANOVA) for drug × sample period. Further analysis used a one-way repeated measures ANOVA for each drug condition, followed by Neuman–Keuls' pairwise comparisons.

Results

During copulation with a female, rats receiving the inactive isomer D-NAME showed a significant increase in extracellular DA ($F(4,36) = 3.97$, $p < 0.05$, Fig. 1), while those receiving the nitric oxide inhibitor L-NAME failed to show such an increase. The results of the two drugs differed significantly from each other in the two-way repeated measures ANOVA ($F(1,60) = 5.02$, $p < 0.05$). All animals analyzed copulated to at least one ejaculation during the 20 min test. There were no differences between the groups in copulatory measures. Two animals in the D-NAME and one in the L-NAME condition failed to copulate; this difference was not statistically significant, and their microdialysate data were not analyzed.

Discussion

DA released in the MPOA of male rats in response to an estrous female contributes to the initiation of

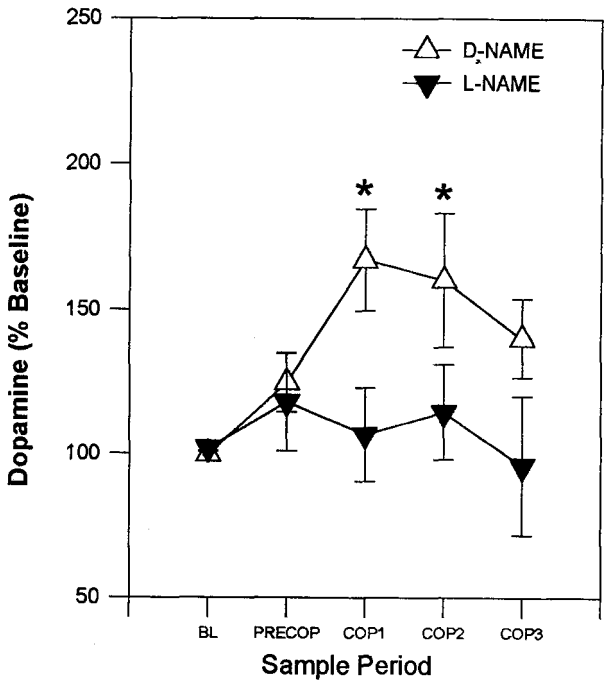

FIG. 1. Extracellular levels of MPOA dopamine during baseline, a precopulatory period (when an estrous female was separated from the male by a perforated barrier), and three copulatory intervals (when the female was freely accessible). Dialysate contained either 400 μM L-NAME (NO synthesis inhibitor) or 400 μM D-NAME (inactive isomer). Each value represents the mean dialysate concentration, presented as a percentage of baseline. *$p < 0.05$ compared with final baseline for D-NAME animals.

copulation and efficient copulatory performance.[2,3] This release may be regulated by NO, since administration of L-arginine into the MPOA increases the levels of DA and its metabolites.[18] In the present experiment, inhibition of NO synthesis by L-NAME blocked copulation-induced DA release in the MPOA. The effect was stereospecific, with DA increasing in subjects receiving the inactive isomer D-NAME. Therefore, NO activation within the MPOA may be necessary for the DA release associated with male rat copulatory behavior.

During copulation, NO may increase extracellular DA by promoting its release and/or inhibiting its uptake. NO has been shown to activate cyclic nucleotide-gated (CNG) channels via the second messenger guanylate cyclase.[19] Once activated, CNG channels can depolarize the membrane to allow calcium influx, thereby enhancing transmitter release. It has also been suggested that NO may evoke neurotransmitter release by facilitating fusion of synaptic vesicles to the presynaptic membrane directly, not merely by regulating calcium concentratations.[20] On the other hand, increases in extracellular DA may be due to decreased uptake. In striatal slice preparations, NO blocked [^{3}H]DA uptake into synaptosomes[21] and caused an efflux of DA via a reversal of the transporter.[22] Using *in vivo* microdialysis, we have shown that inhibiting DA uptake with cocaine decreased levels of DA metabolites in the MPOA while increasing DA levels.[23] In our experiments, L-arginine[18] and copulation[2] increased both DA and metabolite levels, suggesting that release was enhanced, rather than uptake inhibited.

While our previous studies have indicated that blocking NO activity in the MPOA, using microinjections of large doses of L-NMMA, impaired sexual behavior in male rats,[24] all animals included in this experiment copulated to ejaculation. There were no differences in behavioral measures or in the number of animals that copulated. The lack of behavioral impairment in this experiment may be due to the small, local area of the MPOA that is influenced by infusing drug through the microdialysis probe. Blocking NO activity with L-NAME may be able to prevent the proximal neurons from releasing DA, but other neurons in the MPOA, not in contact with the probe, should still release DA and permit normal copulation to occur. In behavioral tests, only large lesions of the MPOA abolish male sexual behavior; smaller lesions are not as effective.[1] Furthermore, the dose of L-NMMA that inhibited copulation in our previous study (400 μg in 1 μl)[24] was much larger than that administered here. Thus, a larger portion of the DA-releasing neurons in the MPOA may have to be inhibited by an NO blocker to observe behavioral effects.

There appears to be a consistent relationship between a precopulatory DA release in the MPOA of sexually experienced male rats and the animals' subsequent ability to copulate.[2] All males that showed a precopulatory DA increase copulated after removal of the barrier separating the animals, while no animal that failed to show the precopulatory increase was able to copulate. In the present experiment, the subjects that received D-NAME showed a significant increase in extracellular DA during copulation, but not during the precopulatory period. The lack of the precopulatory DA rise in the those receiving D-NAME may be due to their lack of sexual experience. Preliminary data from our laboratory have indicated that DA release in the MPOA of sexually naive males does not rise significantly during a precopulatory period, although it does during copulation. Therefore, the precopulatory DA rise may occur only after the male has learned to associate the estrous female with copulation. Males in the present experiment were not given previous sexual experience because L-NMMA microinjections in a previous experiment disrupted copulation only in sexually naive animals.[24] It is of interest that the three males that copulated first under the D-NAME condition, and then under the L-NAME condition, showed the typical DA increase during their first copulation

experiment, but not during their second experiment. Therefore, even in males with some sexual experience, NO appears to be necessary for the DA release during copulation. The lack of DA increase in the L-NAME condition can not be attributed to tissue damage from the previous probe insertion, since we have frequently observed DA increases during a second microdialysis test.

Conclusion

Administration of L-NAME, an NO synthase inhibitor, into the MPOA of male rats blocked the increase in DA release normally seen during copulation. Animals perfused with D-NAME, the inactive isomer, showed normal dopamine increases. Thus, endogenous NO release appears to be necessary to enhance DA release in the MPOA during male sexual behavior.

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