Pharmacology Biochemistry and Behavior, Vol. 63, No. 3, pp. 345–348, 1999

0091-3057/99/$–see front matter

ELSEVIER

PII S0091-3057(98)00252-4

A Nitric Oxide Synthesis Inhibitor Administered Into the Medial Preoptic Area Increases Seminal Emissions in an Ex Copula Reflex Test

JASON MOSES[1] AND ELAINE M. HULL[2]

Department of Psychology, State University of New York at Buffalo, Buffalo, NY 14260

Received 5 December 1997; Accepted 28 September 1998

MOSES, J. AND E. M. HULL. *A nitric oxide synthesis inhibitor administered into the medial preoptic area increases seminal emissions in an ex copula reflex test.* PHARMACOL BIOCHEM BEHAV **63**(3) 345–348, 1999.—Nitric oxide synthesis inhibitors, when administered systemically or into the ventricles of the brain, affect several indices of male sexual behavior. Some of the systemic effects are assumed to be due to local vasoconstriction at the penis. Others are suggested to be mediated within the brain. In these experiments, the nitric oxide synthesis inhibitor L-NMMA, and its less active enantiomer, D-NMMA, were microinjected into the medial preoptic area of male rats. In an ex copula test of genital reflexes, L-NMMA increased the number of seminal emissions, while D-NMMA had no effect. These results are consistent with the hypothesis that nitric oxide is a tonic inhibitor of sympathetic nervous system tone, possibly in part through an influence on dopamine synthesis or release. © 1999 Elsevier Science Inc.

Autonomic nervous system Copulation Dopamine Erection Genital reflexes Male Medial preoptic area Nitric oxide Rats Seminal emission Sexual behavior

NITRIC OXIDE (NO) is formed from the amino acid L-arginine through the actions of the enzyme nitric oxide synthase [NOS; reviewed in (16)]. Initially recognized for a primary role in the relaxation of peripheral vasculature, NO has since been implicated in the control of several activities that are at least partially mediated by the preoptic area or hypothalamus (1,3,14). Nitric oxide may influence these behaviors through release of a variety of neurotransmitters and hormones—among them, dopamine (13).

Nitric oxide has been implicated in the control of male sexual behavior. Systemic administration of the NOS inhibitor N^G-nitro-L-arginine methyl ester (L-NAME) impaired copulation in a dose-dependent manner (10). This probably was due to an inhibition of erectile ability; drug-treated males were unable to achieve vaginal intromissions during most mounts, and had fewer erections in an ex copula reflex test. These results suggest that NO promotes erection in intact males, probably by mediating filling of the corpora cavernosa of the penis (19). In addition to these peripheral effects are probable central influences of NO. Systemic administration of L-NAME also increased the number of seminal emissions in the ex copula reflex test mentioned above (10). This may have been due to increased activation of the sympathetic system, centrally and/or peripherally, and is consistent with the suggestion that NO maintains tonic inhibition of the sympathetic nervous system (20).

NO may affect neural activity in brain areas that promote male sexual behavior. One site that may mediate this effect is the medial preoptic area (MPOA), which is critical for male sexual behavior [reviewed in (15)]. Administration of L-NAME into this site reduced mount rate in male rats (21). There are scattered NOS-immunoreactive cells in the MPOA (25), which decrease in number following castration (6). The following experiment was undertaken to elucidate the role of NO within the MPOA in male sexual behavior.

[1]Current address: Department of Psychology, University of Texas at El Paso, El Paso, TX 79968-0553.
[2]To whom requests for reprints should be addressed at Psychology Department, Park Hall, SUNY at Buffalo, Buffalo, NY 14260-4110; e-mail: emhull@acsu.buffalo.edu

METHODS

Subjects

Twenty-one male Long–Evans/Blue Spruce rats (300–350 g, Harlan–Sprague–Dawley) were housed in individual cages in a temperature- and humidity-controlled environment on a 14-h light–dark cycle (lights off at 1100 h). Food and water were available ad lib.

Drugs

The NOS inhibitor N^G-monomethyl-L-arginine (L-NMMA) and its less active enantiomer D-NMMA were purchased from Sigma Chemical Company. In Experiment 1a, the drug conditions were saline vehicle, L-NMMA (25, 50, 100, and 200 μg), and D-NMMA (200 μg), administered in counterbalanced order. Following a delay of 10 days, Experiment 1b tested the effects of vehicle vs. L-NMMA (400 μg). Because the 100-μg dose produced a greater effect than the 200-μg dose, this additional test was conducted to extend the dose–response curve. Drugs were microinjected in 0.5 μL sterile saline at the rate of 1 μL/min via a stainless steel injection cannula protruding 1 mm below the level of the guide cannula. The injection cannula remained in place for 30 s following the injection.

Surgery

Subjects were anesthetized with ketamine hydrochloride (50 mg/kg) and xylazine hydrochloride (4 mg/kg), and a stainless steel guide cannula was stereotaxically implanted to end 1 mm above the left MPOA at coordinates AP 2.4, ML 0.2, and DV −0.7 mm from bregma (18), following the procedure of Hull et al. (8). A stainless steel obturator was inserted into the guide cannula. After surgery subjects were injected with Gentamicin antibiotic (0.02 mg/kg twice daily, for 3 days).

Procedure

A 10-inch tube affixed to a Plexiglas base served as the restrainer. The rat's head was placed in the tube and its legs were strapped in a supine position. The penis was retracted to allow measurement of penile reflexes. Subjects were habituated to restraining devices on three occasions prior to surgery. Only those rats that exhibited penile reflexes and/or seminal emission during at least two of the three habituation sessions were used as subjects. Surgery was conducted and, 4 days later, the subjects were again placed into the restrainers for a final habituation session. Four days after this the experiment began, and each subject was tested on every fourth day, between 1200 and 1500 h. After drug administration, the subject was immediately placed into a restrainer. The following measures were quantified: erections, consisting of E1s, in which only the base of the glans engorges; E2s, in which both the base and the tip of the glans engorge; and E3s, in which the base of the glans engorges while the tip flares, so that the diameter of the tip is greater than that of the base of the glans (also termed a cup); anteroflexions, consisting of short flips, in which the penis suddenly arches <90 degrees from the resting position; and long flips, in which the penis arches >90 degrees from the resting position; and seminal emissions, in which a white, viscous fluid or coagulated plug is ejected from the penis. Additionally, the latencies to the first erection and seminal emission were recorded. A test session lasted 15 min following the first erection or anteroflexion, or 30 min if no erections or anteroflexions occurred.

Following the experiment, subjects were administered an overdose of sodium pentobarbital and were sacrificed by decapitation. Their brains were sectioned, stained, and examined with a projection magnifier. Only those animals with histologically verifiable cannula placements in the MPOA were included in data analysis; one subject was excluded because the cannula placement was inaccurate.

Data Analysis

Only those subjects that displayed erections and/or seminal emissions during at least $\frac{1}{4}$ of the testing sessions were used in data analysis. Thus, two additional subjects were excluded from data analysis from Experiment 1a ($n = 18$), and four additional subjects were excluded from Experiment 1b ($n = 16$). Analyses of variance for unequal sample sizes were used to analyze latency for each behavior, using only animals that demonstrated those behaviors. Newman–Keuls comparisons among groups were used if the ANOVA was statistically significant. These results are presented as means plus standard errors. Cochran's Q-tests, followed by McNemar comparisons among groups, were utilized to compare the numbers of animals that displayed erections and/or seminal emissions.

RESULTS

In Experiment 1a, L-NMMA (100 μg) increased seminal emission frequency, $F(5, 85) = 2.68$, $p < 0.05$ (see Fig. 1). This was due to an increase in the number of subjects displaying one or more seminal emissions in the L-NMMA (100 μg) condition, $Q(5) = 11.25$; $p < 0.05$.

Furthermore, in Experiment 1b, L-NMMA (400 μg), compared to vehicle, significantly increased seminal emission frequency, $F(1, 15) = 49$, $p < 0.001$ (see Fig. 1), and the number of subjects displaying one or more seminal emissions, $\chi^2(1) = 9.09$, $p < 0.01$.

During Experiment 1a, L-NMMA (200 μg) significantly decreased erection latency among those subjects that displayed erections, $F(5, 53) = 2.38$, $p < 0.05$ (see Fig. 2), and the latency to the first behavior, whether erection or seminal emission, among those subjects that displayed such behaviors, $F(5, 69) = 3.95$, 100 μg, $p < 0.05$; 200 μg, $p < 0.01$. On the other hand, L-NMMA (400 μg) administered in Experiment 1b did not decrease either of these measures. There were no differences in erection frequency in either Experiment 1a or 1b. Similarly, there were no significant differences in seminal

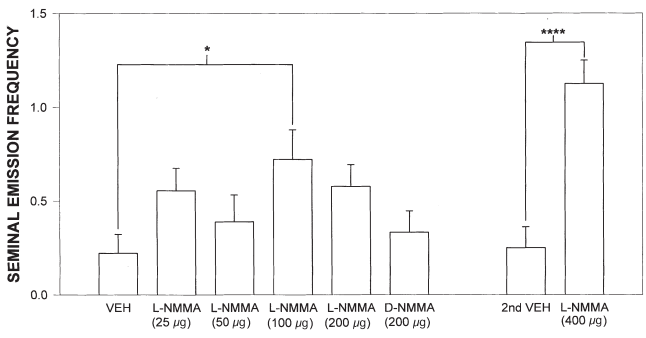

FIG. 1. The effects of various doses of L-NMMA (25, 50, 100, 200, and 400 μg) and D-NMMA (200 μg), injected into the MPOA in two separate tests, on seminal emission frequency. In Experiment 1a, L-NMMA (100 μg) increased seminal emission frequency. D-NMMA was ineffective. In Experiment 1b, L-NMMA (400 μg) increased seminal emission frequency. VEH, vehicle. *$p < 0.05$, ****$p < 0.001$.

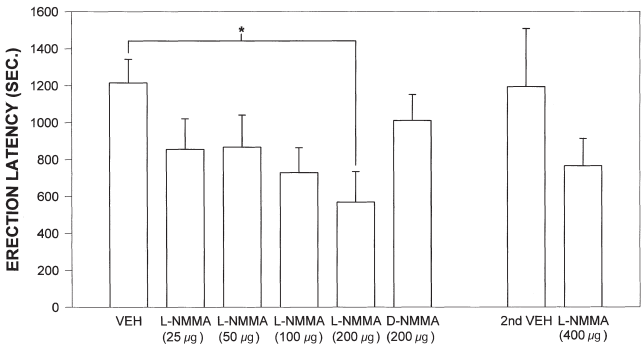

FIG. 2. The effects of various doses of L-NMMA (25, 50, 100, 200, and 400 μg) and D-NMMA (200 μg), injected into the MPOA in two separate tests, on erection latency. In Experiment 1a, L-NMMA (200 μg) decreased the latency to the first erection. D-NMMA was ineffective. There were no differences in Experiment 1b. VEH, vehicle. $*p < 0.05$.

emission latency or the number of subjects that displayed erections in either Experiment 1a or 1b.

DISCUSSION

The administration of the NOS inhibitor L-NMMA into the MPOA increased instances of seminal emission and decreased the latency to the first reflex (erection or seminal emission) in this ex copula reflex test. In a previous experiment, systemic administration of another NOS inhibitor, L-NAME, decreased erections, decreased the latency to the first reflex, and increased seminal emissions in an ex copula reflex test (10) [L-NAME was not used in this experiment due to the suggestion that L-NAME promoted neuronal breakdown, resulting in a mechanical release of neurotransmitter (11)]. The reduction in erections following systemic administration of a NOS inhibitor may be explained by local inhibition of NO-mediated filling of the corpora cavernosa of the penis (19,24), but the increase in seminal emissions and decrease in reflex latency were suggested to have a central source. The current experiment would suggest that the MPOA may at least partially mediate the increase in seminal emissions and the decrease in reflex latency. The MPOA is critical for male sexual behavior [reviewed in (15)], and there are scattered NOS-immunoreactive cells in the MPOA (25), which decrease in number following castration (6). We have previously shown that administration of the dopamine D_2 agonist quinelorane into the MPOA decreased reflex latency and increased the number of seminal emissions (2), similar to the effects of L-NMMA in the present experiment. Quinelorane also decreased the number of erections, an effect not observed here, possibly because of the relatively small number of erections in vehicle conditions.

A potential explanation for the increase in seminal emissions and the decrease in reflex latency is enhanced sympathetic nervous system activity. Intracisternal injection of the NO synthesis inhibitor, L-NMMA, increased arterial blood pressure and sympathetic renal nerve activity (23). It was suggested (17) that one central site at which NO may produce its sympathoinhibition is the nucleus tractus solitarius (NTS), where L-glutamate caused an L-NMMA and methylene blue reversible decrease in arterial blood pressure and heart rate in the rat (12), presumably mediated by NO (5). Moreover, L-NMMA injected directly into the NTS increased renal sympathetic nerve activity in rabbit (7). The NTS has reciprocal connections with a number of central structures, and is in a unique position to react to sensory information with the necessary alterations in autonomic tone. One such connection is with the paraventricular nucleus of the hypothalamus (PVN), which in turn, is reciprocally connected with the MPOA (22). It is possible that NO may have a similar function in each of these interconnected areas, namely, the inhibition of sympathetic function. Although the same doses of L-NMMA used in the present experiment had no effect on genital reflexes when injected into the PVN, these doses in the PVN did increase body temperature and resulted in considerable struggling during restraint (Moses, unpublished observations), suggestive of sympathetic activation. Thus, by inhibiting the inhibitor (NO), L-NMMA in the MPOA enhances seminal emission, which is mediated by the sympathetic nervous system [reviewed in (4)]. In addition, L-NMMA (400 μg) injected into the MPOA blocked intromissions in a copulation test, although only among sexually naïve males (Moses, unpublished observations), confirming a recent report (21). Erection, required for successful intromission, is controlled in part by the parasympathetic nervous system [reviewed in (4)]. Thus, with little or no NO to inhibit sympathetic activity, sexually naïve animals may have had difficulty obtaining an erection. Although there was no inhibition of erection in the ex copula reflex experiment, there were relatively few erections following vehicle injection; thus, there may have been a floor effect.

The decrease in latency to the first erection in Experiment 1a may seem to indicate a facilitation of erection. However, we have previously reported that low doses of a dopamine D_2/D_3 agonist (quinelorane) decreased the latency to the first erection without affecting the number of erections; higher doses also decreased the latency to the first erection, but actually decreased the number of erections (9). Thus, a decrease in latency may not indicate a facilitation of erection.

In summary, L-NMMA injected into the MPOA increased seminal emissions in an ex copula reflex test. This may reflect disinhibition of sympathetically mediated seminal emission, suggesting that NO in the MPOA may normally inhibit some functions of the sympathetic nervous system.

ACKNOWLEDGEMENTS

We thank Juan Dominguez for assistance with behavioral testing and figure preparation. This work was supported by NIMH Grant MH-40826 to E.M.H.

REFERENCES

1. Amir, S.; De Blasio, E.; English, A. M.: N^G-Monomethyl-L-arginine co-injection attenuates the thermogenic and hyperthermic effects of E_2 prostaglandin microinjection into the anterior hypothalamic preoptic area in rats. Brain Res. 556:157–160; 1991.
2. Bazzett, T. J.; Eaton, R. C.; Thompson, J. T.; Markowski, V. P.; Lumley, L. A.; Hull, E. M.: Dose dependent D2 effects on genital reflexes after MPOA injections of quinelorane and apomorphine. Life Sci. 48:2309–2315; 1991.
3. Calapai, G.; Squadrito, F.; Altavilla, D.; Zingarelli, B.; Campo, G. M.; Cilia, M.; Caputi, A. P.: Evidence that nitric oxide modulates drinking behavior. Neuropharmacology 31:761–764; 1992.
4. Dail, W. G.: Autonomic innervation of male reproductive genita-

lia. In: Burnstock, G.; Maggi, C. A., ed. The autonomic nervous system, vol. 3. Nervous control of the urogenital system. Switzerland: Harwood Academic Publishers; 1993:69–101.
5. Di Paola, E. D.; Vidal, M. J.; Nistico, G. L.: L-Glutamate evokes the release of an endothelium-derived relaxing factor-like substance from the rat nucleus tractus solitarius. J. Cardiovasc. Pharmacol. 17(Suppl. 3): S269–S272; 1991.
6. Du, J.; Lumley, L. A.; Hull, E. M.: Effects of castration and hormone replacement on nitric oxide synthase in the medial preoptic area of male rats. Soc. Neurosci. Abstr. 22:1414; 1996.
7. Harada, S.; Tokunaga, S.; Momohara, M.; Masaki, H.; Tagawa, T.; Imaizumi, T.; Takeshita, A.: Inhibition of nitric oxide formation in the nucleus tractus solitarius increases renal sympathetic nerve activity in rabbits. Circ. Res. 72:511–516; 1993.
8. Hull, E. M.; Bitran, D.; Pehek, E. A.; Warner, R. K.; Band, L. C.; Holmes, G. M.: Dopaminergic control of male sex behavior in rats: Effects of an intracerebrally-infused agonist. Brain Res. 370:73–81; 1986.
9. Hull, E. M.; Eaton, R.; Markowski, V. P.; Moses, J.; Lumley, L.; Loucks, J. A.: Opposite influence of medial preoptic D1 and D2 receptors on genital reflexes: Implications for copulation. Life Sci. 51:1705–1713; 1992.
10. Hull, E. M.; Lumley, L. A.; Matuszewich, L.; Dominguez, J.; Moses, J.; Lorrain, D. S.: The roles of nitric oxide in sexual function of male rats. Neuropharmacology 33:1499–1504; 1994.
11. Izumi, Y.; Clifford, D. B.; Zorumski, C. F.: Inhibition of long-term potentiation by NMDA-mediated nitric oxide release. Science 257:1273–1276; 1992.
12. Lewis, S. J.; Ohta, H.; Machado, B.; Bates, J. N.; Talman, W. T.: Microinjection of S-nitrosocysteine into the nucleus tractus solitarii decreases arterial pressure and heart rate via activation of soluble guanylate cyclase. Eur. J. Pharmacol. 202:135–136; 1991.
13. Lorrain, D. S.; Hull, E. M.: Nitric oxide increases dopamine and serotonin release in the medial preoptic area. Neuroreport 5:87–89; 1993.
14. Mani, S. K.; Allen, J. M. C.; Rettori, V.; McCann, S. M.; O'Malley, B. W.; Clark, J. H.: Nitric oxide mediates sexual behavior in female rats. Proc. Natl. Acad. Sci. USA 91:6468–6472; 1994.
15. Meisel, R. L.; Sachs, B. D.: The physiology of male sexual behavior. In: Knobil, E.; Neill, J. D., eds. Physiology of reproduction, 2nd ed. New York: Raven Press; 1994:3–106.
16. Moncada, S.; Palmer, R. M. J.; Higgs, E. A.: Nitric oxide: Physiology, pathophysiology, and pharmacology. Pharmacological Rev. 43:109–142; 1991.
17. Okamoto, H.; Hoka, S.; Kawasaki, T.; Okuyama, T.; Takahashi, S.: L-arginine attenuates ketamine-induced increase in renal sympathetic nerve activity. Anesthesiology 81:137–146; 1994.
18. Pellegrino, L. J., Pellegrino, A. S.; Cushman A. J.: A stereotaxic atlas of the brain, 2nd ed. New York: Raven Press; 1979.
19. Rajfer, J.; Aronson, W. J.; Bush, P. A.; Dorey, F. J.; Ignarro, C. J.: Nitric oxide as a mediator of relaxation of the corpus cavernosum in response to nonadrenergic, noncholinergic neurotransmission. New Engl. J. Med. 326:90–94; 1992.
20. Sakuma, I.; Togashi, H.; Yoshioka, M.; Saito, H.; Yanagida, M.; Tamura, M.; Kobayashi, T.; Yasuda, H.; Gross, S. S.; Levi, R.: N^G-methyl-L-arginine, an inhibitor of L-arginine-derived nitric oxide synthesis, stimulates renal sympathetic nerve activity in vivo: a role for nitric oxide in the central regulation of sympathetic tone? Circ. Res. 70:607–611; 1992.
21. Sato, Y.; Horita, H.; Kurohata, T.; Adachi, H.; Tsukamoto, T.: Effect of the nitric oxide level in the medial preoptic area on male copulatory behavior in rats. Am. J. Physiol. 274:R243–7; 1998.
22. Simerly, R. B.; Swanson, L. W.: Projections of the medial preoptic nucleus: A phaseolus vulgaris leucoagglutinin anterograde tract-tracing study in the rat. J. Comp. Neurol. 270:209–242; 1988.
23. Togashi, H.; Sakuma, I.; Yoshioka, M.; Kobayashi, T.; Yasuda, H.; Kitabatake, A.; Saito, H.; Gross, S. S.; Levi, R.: A central nervous system action of nitric oxide in blood pressure regulation. J. Pharmacol. Exp. Ther. 262:343–347; 1992.
24. Trigo–Rocha, F.; Hsu, G. L.; Donatucci, C. F.; Lue, T. F.: The role of cyclic adenosine monophosphate, cyclic guanosine monophosphate, endothelium and nonadrenergic, noncholinergic neurotransmission in canine penile erection. J. Urol. 149:872–877; 1993.
25. Vincent, S. R.; Kimura, H.: Histochemical mapping of nitric oxide synthase in the rat brain. Neuroscience 46:755–784; 1992.