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The Roles of Nitric Oxide in Sexual Function of Male Rats

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Summary—Nitric oxide (NO) may mediate penile erection by inhibiting smooth muscle of the corpora cavernosa, thereby allowing vasodilation of the corpora. In order to test the role of NO in the sexual function of intact male rats, either the precursor of NO (L-arginine, L-Arg) or an inhibitor of its synthesis (N^G-nitro-L-arginine methyl ester, NAME) was administered systemically before tests of copulation, ex copula genital reflexes, or sexual motivation/motor activity. NAME impaired copulation in a dose dependent manner. It also decreased the number of ex copula erections, but it increased the number of ex copula seminal emissions and decreased the latency to the first seminal emission. L-Arg marginally increased the number of penile reflexes, but had no other effects. NAME had no effect on sexual motivation or motor activity. The results indicate that nitric oxide promotes erection in intact male rats, probably by mediating filling of the corpora cavernosa. The data also suggest that NO inhibits seminal emission, probably by decreasing sympathetic nervous system activity; this may help prevent premature ejaculation.

Keywords—Nitric oxide, sexual function, L-arginine, NAME.

Nitric oxide (NO) has been suggested to be the primary physiological mediator of penile erection (Azadzoi *et al.*, 1992; Ignarro, 1992; Ignarro *et al.*, 1990; Kim *et al.*, 1991; Rajfer *et al.*, 1992). Electrical stimulation of the non-adrenergic, non-cholinergic (NANC) pathway, which is responsible for about 90% of the erectile response (reviewed in Benson, 1988; Ignarro, 1992), leads to smooth muscle relaxation in isolated strips of human, dog, rabbit or rat corpus cavernosum. This response is abolished by the inclusion of NO synthesis inhibitors in the bath solution (Burnett *et al.*, 1992; Ignarro *et al.*, 1990; Kim *et al.*, 1991; Rajfer *et al.*, 1992). Therefore, it has been proposed that NO, either liberated from the NANC neurons or formed in the postsynaptic endothelium or smooth muscle, stimulates cGMP formation in the smooth muscle, which in turn relaxes and allows blood to fill the corpus cavernosum.

A better understanding of the role of NO in sexual function may lead to more effective treatment of impotence or ejaculatory problems. The present experiments tested the role of nitric oxide in the sexual behavior of intact rats. N^G-nitro-L-arginine methyl ester (NAME), an inhibitor of NO synthase, or L-arginine (L-Arg), the precursor of NO, was administered systemically before copulation tests, ex copula genital reflex tests, or X-maze tests of sexual motivation and motor activity. Some of these data have been presented in abstract form (Hull *et al.*, 1992).

METHODS

Adult male Long–Evans rats (300–400 g, Harlan Sprague–Dawley, Blue Spruce Farms) were housed individually in large plastic cages. A 14:10 light:dark cycle was in effect, with lights out at 11.00 hr. Food and water were available *ad libitum*. N^G-nitro-L-arginine methyl ester (NAME), and L-arginine (L-Arg) (both from Sigma Chemical) were dissolved in sterile saline and injected intraperitoneally (i.p.) 30 min before behavioral tests. All animals received all drug treatments. L-Arg and vehicle were administered in counterbalanced order; since N^G-nitro-L-arginine binds irreversibly (Dwyer *et al.*, 1991), NAME was administered at the end of each experiment in which it was used. Females of the same strain were ovariectomized under ketamine hydrochloride (50 mg/kg i.p.) and xylazine hydrochloride (4 mg/kg i.p.) anesthesia, using bilateral flank incisions. The ovary and surrounding fatty tissue were tied off and cut distal to the ligature. Overlying muscle and skin were sutured. Bacitracin ointment was spread over the site of incision. The animals were injected with Combiotic antibiotic and allowed at least two weeks for recovery. They were injected with 20 μg estradiol benzoate 48 hr before copu-

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lation or X-maze tests. All males were weighed and handled daily, and females were checked daily for signs of infection.

In Experiment 1a, a pilot group of 6 sexually experienced animals that had been used in a previous experiment received injections of 50 mg/kg NAME or vehicle 30 min before copulation tests with an estrous female. In Experiment 1b, 18 sexually experienced males were tested for copulatory behavior following injections of 25 mg/kg NAME, 500 or 1000 mg/kg L-Arg, or vehicle. Tests for Experiments 1a and 1b were conducted in each male's home cage, and lasted for 30 min after the first vaginal intromission, or for 30 min after introduction of the estrous female if no intromission occurred. The frequency and latency of mounts, intromissions, and ejaculations were recorded using a program for the IBM-XT by Stephen Yeoh. A mount was scored when a male approached the female from the rear, clasped her flanks, and performed a series of rapid, shallow pelvic thrusts. Vaginal intromissions were distinguished from mounts by the presence of a final deep thrust followed by a rapid, springing dismount. Ejaculations were distinguished from mounts and intromissions by a deeper thrust followed by a prolonged grasp, slow dismount and a 5–10 min period of inactivity.

In Experiment 2a, 18 males were tested for ex copula genital reflexes following i.p. injections of 25 or 50 mg/kg NAME, or vehicle. In Experiment 2b, 26 males were tested following i.p. injections of 10, 50, or 100 mg/kg L-Arg. For all genital reflex tests, animals were restrained in a supine position, with their torso inside a cylinder that was mounted on a larger plexiglass platform. Their hindlimbs and tail were taped to the platform with masking tape. The preputial sheath was retracted and held in the retracted position. Typically, a series of penile erections and anteroflexions occur spontaneously within a few minutes after sheath retraction; occasionally, a seminal emission occurs. Animals were accustomed to this procedure at least three times, for 30 min per day, before testing began. The occurrence and time of the following responses were recorded using a program for the IBM-XT by Stephen Yeoh: (1) seminal emission, a discharge of seminal fluid usually accompanied by several rapid, brief penile erections and rostral movement of the testes within the scrotal sac; (2) anteroflexions of the penis; and (3) glans erections of three intensities—E1, reddening and distention of the base of the glans; E2, tumescence of the base and tip of the glans; and E3 (sometimes called cups), intense erection of the glans resulting in flaring of the tip of the glans. Latencies to the first penile reflex (erection or anteroflexion) and the first seminal emission were measured from the time of preputial sheath retraction. Animals were tested for 15 min after the first erection or anteroflexion, or for 20 min if no reflex occurred.

In Experiment 3, 10 sexually experienced male rats were tested in an X-maze, which contained a receptive female in one goal box, a stud male in the opposite goal box, and two empty goal boxes. Animals were trained and tested as previously described (Hull *et al.*, 1991). Briefly, a male was allowed 60 sec after placement in the center of the maze to run to one of the four goal boxes. If he chose the female's goal box, he was allowed one intromission, or a total of 5 min if no intromission occurred, before being replaced in the central start area of the maze. If he chose any of the other 3 goal boxes, he was given 30 sec in that box before being replaced into the start area. The test was ended after the male either ejaculated, failed to ejaculate after 25 trials, failed to intromit within 5 min of choosing the female's goal box on 3 trials, or failed to move from the start area on 10 trials. The percentage of trials on which the male chose the female's goal box was considered to be a measure of sexual motivation. The latency to cross a line in front of the goal box with at least two paws (constituting choice of the goal box), and the number of trials on which the male failed to leave the start area, were used as measures of motor activity. Copulatory parameters were scored as in Experiment 1. Drug doses were 5, 10, or 20 mg/kg L-Arg, 25 or 50 mg/kg NAME, or vehicle.

Statistical analyses consisted of repeated measures analyses of variance, followed by Neuman–Keuls comparisons. Analyses of scores for measures on which numerous animals failed to show the behavior utilized only animals that did exhibit the behavior. Uncorrelated analyses of variance were used for these measures; the numbers of animals in each group are given in the table or figure caption. For example, mounts preceding ejaculation in Experiment 1 were scored only for those animals that did ejaculate, and seminal emission latency in Experiment 2 was scored only for those that had a seminal emission. Cochrans Q was used to compare the numbers of animals that ejaculated in Experiment 3. In Experiment 1a, t-tests were used to compare NAME with vehicle; a t-test was also used to compare the lowest dose of L-Arg with vehicle in Experiment 2.

RESULTS

Experiment 1. Effects of NAME and high doses of L-Arg on copulation

In Experiment 1a, no animal receiving 50 mg/kg NAME ejaculated, whereas all animals ejaculated following vehicle injections [$t(5) = 12.85$, $P < 0.0001$]. (See Table 1. Data for Experiments 1a and 1b were

Table 1. Effects of NAME and high doses of L-Arg on copulation

Treatment	Ejaculations	Intromissions	Mounts
Vehicle	2.3 ± 0.2	24.4 ± 1.4	21.2 ± 2.1
25 mg/kg NAME	1.2 ± 0.3*	14.3 ± 2.0**	53.8 ± 6.0*
50 mg/kg NAME	0.0 ± 0.0**	2.0 ± 1.8**	37.8 ± 14.2
500 mg/kg L-Arg	2.7 ± 0.1	27.1 ± 1.5	19.4 ± 2.3
1000 mg/kg L-Arg	2.6 ± 0.2	25.2 ± 2.1	20.2 ± 2.4

*$P < 0.05$, **$P < 0.01$, compared to vehicle. Values are means ± SE. Data are from 6 animals that received 50 mg/kg NAME or vehicle, and 18 animals that received 25 mg/kg NAME, 500 or 1000 mg/kg L-arginine or vehicle.

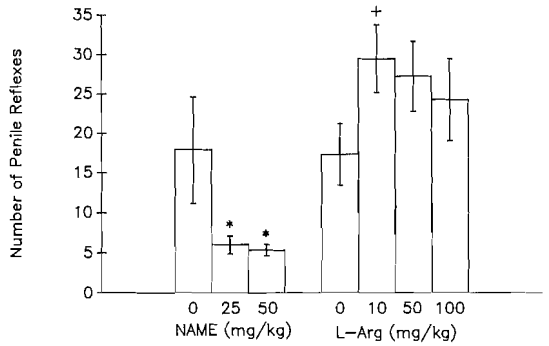

Fig. 1. Effects of N^G-nitro-L-arginine methyl ester (NAME) or L-arginine (L-Arg) on ex copula penile reflexes (erections and anteroflexions). Separate groups of animals received NAME [0 (vehicle), 25 or 50 mg/kg i.p.; $n = 18$], or L-Arg [0 (vehicle), 10, 50 or 100 mg/kg i.p.; $n = 26$] 30 min before tests of ex copula reflexes. Both doses of NAME decreased the number of reflexes, whereas the lowest dose of L-Arg marginally increased reflexes. Values are means ± SE. $^*P < 0.05$, compared to vehicle $^+P < 0.05$, based on t-test comparison of 10 mg/kg L-Arg and vehicle.

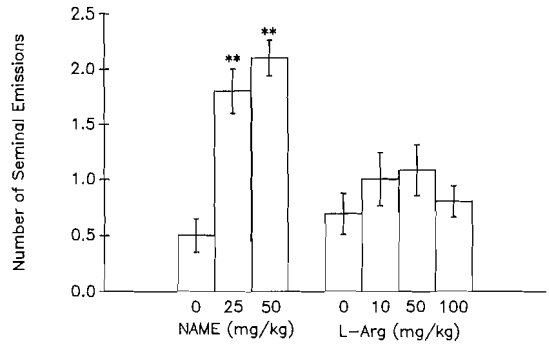

Fig. 2. Effects of N^G-nitro-L-arginine methyl ester (NAME) or L-arginine (L-Arg) on ex copula seminal emissions. Separate groups of animals received NAME [0 (vehicle), 25 or 50 mg/kg i.p.; $n = 18$], or L-Arg [0 (vehicle), 10, 50 or 100 mg/kg i.p.; $n = 26$] 30 min before tests of ex copula reflexes. Both doses of NAME increased the number of seminal emissions; L-Arg was ineffective. Values are means ± SE. $^{**}P < 0.01$, compared to vehicle.

analyzed separately, but are combined in one table for easier comparison.) Only 2 animals intromitted (10 and 2 times, respectively) after injections of 50 mg/kg NAME; however, most mounted repeatedly.

In Experiment 1b, 25 mg/kg NAME also decreased the number of ejaculations [$F(3,51) = 9.93$, $P < 0.001$] and the number of intromissions per test [$F(3,51) = 15.3$, $P < 0.0001$]. NAME increased the total number of mounts per test [$F(3,51) = 25.21$, $P < 0.0001$] and the number of mounts preceding ejaculation for those animals that ejaculated on all tests [$F(3,27) = 3.18$, $n = 10$, $P < 0.05$]. There were no effects of high doses of L-Arg on copulation.

Experiment 2. Effects of NAME and L-Arg on ex copula genital reflexes

In Experiment 2a, NAME decreased the number of penile reflexes (erections and anteroflexions) [$F(2,34) = 3.18$, $P < 0.01$; Fig. 1]. This reflected primarily decreases in moderate erections (E2) [$F(2,34) = 4.31$, $P < 0.025$] and intense erections (E3) [$F(2,34) = 3.46$, $P < 0.01$] (see Table 2). On the other hand, NAME increased the number of seminal emissions [$F(2,34) = 26.25$, $P < 0.00001$; Fig. 2] and decreased the latency to the first seminal emission [$F(2,40) = 8.69$, $P < 0.001$; Fig. 3]. NAME decreased the latency to the first penile reflex (erection or anteroflexion) only when the erections accompanying seminal emission were included [$F(2,44) = 13.6$, $P < 0.001$; Table 2]. Thus, the decreased reflex latency resulted largely from the decreased latency of the erections that accompanied seminal emission.

In Experiment 2b, there was a dose related trend towards an increase in the number of total penile reflexes with the lowest doses being most effective. Although the overall F value was not statistically significant ($P < 0.18$), a comparison of the lowest dose (10 mg/kg) with vehicle achieved a marginal level of significance [$t(25) = 2.18$, $P < 0.05$; Fig. 1].

Experiment 3. Effects of NAME and L-Arg on X-maze performance

Neither NAME nor L-Arg affected percent choice of the female's goal box, latency to reach the female's goal box, or number of trials on which the male failed to leave the start area (see Table 3). However, as in Experiment 1, NAME dramatically increased the number of non-intromissive mounts [$F(2,18) = 10.6$; $P < 0.001$], increased the latency to intromit [$F(2,18) = 3.71$; $P < 0.05$], decreased the number of intromissions [$F(2,18) = 5.24$, $P < 0.025$], and decreased the number of males that ejaculated within the allotted 25 trials [$Q(2) = 12.25$; $P < 0.01$] (see Table 3). NAME did not

Table 2. Effects of NAME on ex copula genital reflexes

Treatment	Moderate (E2) erections	Intense (E3) erections	Reflex latency incl. erections w/sem. em.	Reflex latency excl. erections w/sem. em.
Vehicle	5.61 ± 2.3	3.2 ± 1.5	647.8 ± 69.9	678.3 ± 38.9
25 mg/kg NAME	0.94 ± 0.3*	0.5 ± 0.4*	371.3 ± 57.4**	745.3 ± 52.5
50 mg/kg NAME	0.61 ± 0.3*	0.0 ± 0.0*	248.6 ± 33.5**	611.0 ± 17.9

$^*P < 0.05$, $^{**}P < 0.01$. Values are means ± SE. Reflex latency is calculated either including or excluding the erections that accompanied seminal emissions. Data are from 18 animals.

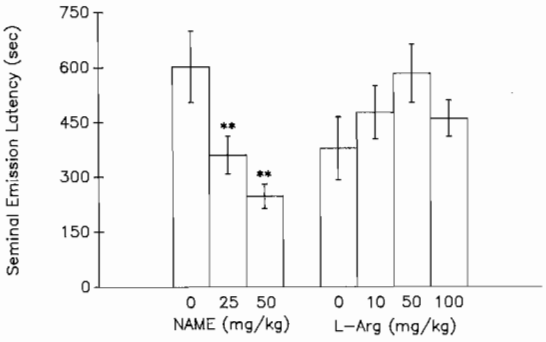

Fig. 3. Effects of N^G-nitro-L-arginine methyl ester (NAME) or L-arginine (L-Arg) on the latency to the first ex copula seminal emission. Separate groups of animals received NAME [0 (vehicle), 25 or 50 mg/kg i.p.], or L-Arg [0 (vehicle), 10, 50 or 100 mg/kg i.p.] 30 min before tests of ex copula reflexes. Both doses of NAME decreased the latency to the first seminal emission. NAME data are from 8 animals that had seminal emissions after vehicle injections; 17, after 25 mg/kg NAME; and 18, after 50 mg/kg NAME. L-Arg was ineffective. L-Arg data are from 12 animals that had seminal emissions after vehicle injections; 14, after 10 mg/kg L-Arg; 17, after 50 mg/kg L-Arg; and 16, after 100 mg/kg L-Arg. Values are means ± SE. $^{**}P < 0.01$ compared to vehicle.

affect mount latency, and L-Arg did not affect any measure.

DISCUSSION

Inhibition of NO synthesis dramatically and dose dependently impaired copulation by male rats. NAME increased the latency to intromit, decreased the total number of intromissions and ejaculations, and increased the number of nonintromissive mounts in both Experiments 1 and 3. This impairment appears to reflect primarily inhibition of erectile function, since NAME also decreased the number of ex copula erections in Experiment 2a. No measure of sexual motivation or motor activity was affected by either NAME or the NO precursor L-Arg in Experiment 3. Animals receiving NAME mounted frequently and with short latencies; they chose the female's goal box as consistently and ran as rapidly as when they were treated with vehicle. These results are consistent with an inhibition by NAME of relaxation of smooth muscle in the corpora cavernosa, leading to erectile dysfunction (Azadzoi *et al.*, 1992; Ignarro, 1992; Ignarro *et al.*, 1990; Kim *et al.*, 1991; Rajfer *et al.*, 1992).

On the other hand, NAME injections actually increased the number of ex copula seminal emissions. Furthermore, the increase in seminal emissions probably resulted from stimulation of sympathetic nerves (Benson, 1988). This suggests that NO maintains a tonic inhibitory influence on at least some measures of sympathetic function. There have been several suggestions that endogenous NO may inhibit sympathetic function. Microinjection of the NO donor *S*-nitrosocysteine into the nucleus tractus solitarius decreased mean arterial pressure and heart rate (Lewis *et al.*, 1991). On the other hand, injections of *N*-monomethyl-L-arginine (NMMA, an inhibitor of NO synthase), into the nucleus tractus solitarius, but not into the area postrema, increased renal sympathetic nerve activity, arterial pressure and heart rate (Harada *et al.*, 1993). Similarly, intracisternal injections of NMMA increased sympathetic renal nerve activity, an effect blocked by cervical spinal cord transection or L-Arg (Togashi *et al.*, 1992). In addition, the hypertensive effects of systemic NMMA were diminished by ganglionic blockade (Lacolley *et al.*, 1991). Finally, NADPH diaphorase, a marker of NO synthase, was localized in neurons of the rostral ventral medulla, a site important for central autonomic regulation (Iadecola *et al.*, 1993).

The sympathetic nervous system's control of seminal emissions and reflex latency may also be affected by NO in the medial preoptic area (MPOA). NMMA injection into the MPOA increased the number of ex copula seminal emissions and decreased reflex latency; however, the number of erections was not affected (Moses and Hull, unpublished observations).

NO may act centrally to affect penile erection, as well as seminal emission. In a study of freely moving animals, erections elicited by systemically injected apomorphine or oxytocin were blocked by injections of NAME into the paraventricular nucleus of the hypothalamus, though not into the caudate nucleus, septum, preoptic area, or hippocampus (Melis *et al.*, 1994). However, the relation between these drug induced erections in freely moving animals (which occur at the rate of 2–4 per hour), to the spontaneous erections observed in supine animals with preputial sheath retraction (which occur at rates of 10–30 within 20 min) is not clear.

The relative ineffectiveness of L-Arg in these exper-

Table 3. Effects of NAME and low doses of L-Arg on X-maze performance

Treatment	Latency to first intromission (sec)	Total mounts	Total intromissions	Animals that ejaculated	Percent choice of female
Vehicle	40.1 ± 7.5	6.4 ± 1.4	7.6 ± 1.1	10	87.0 ± 1.6
25 mg/kg NAME	160.1 ± 84.9	12.4 ± 4.4	4.2 ± 0.9*	7	91.7 ± 4.7
50 mg/kg NAME	394.9 ± 137.6*	29.6 ± 4.5**	2.5 ± 1.0*	2**	82.7 ± 4.8
5 mg/kg L-Arg	58.8 ± 20.4	3.5 ± 1.0	6.2 ± 1.0	10	86.7 ± 2.8
10 mg/kg L-Arg	22.9 ± 5.0	6.3 ± 1.6	6.8 ± 1.0	10	91.5 ± 3.2
20 mg/kg L-Arg	61.8 ± 22.5	6.2 ± 2.1	6.1 ± 0.7	10	90.5 ± 2.8

$^{*}P < 0.05$, $^{**}P < 0.01$ compared to vehicle. All values are means ± SE. Data are from 10 animals.

iments suggests that availability of the precursor usually is not a rate limiting step in the production of NO, as has been suggested in the context of the regulation of vascular tone (e.g. Katusic, 1992; Panza *et al.*, 1993). On the other hand, the low dose of L-Arg in Experiment 2 did slightly increase the number of ex copula erections. Thus, increased precursor availability may marginally facilitate erectile function. The ineffectiveness of higher doses may have been due to competing responses. The very high doses administered in Experiment 1 were based on a report that similar doses were necessary to affect food intake in mice (Morley and Flood, 1991). However, observers in Experiment 1 reported that some animals appeared to be slightly impaired motorically with those doses. Therefore, doses of L-Arg were lowered in Experiments 2 and 3. The doses in Experiment 3 were chosen to bracket the dose that had marginally facilitated penile reflexes in Experiment 2. L-Arg has been reported to affect neurotransmitter release (Lauth *et al.*, 1993; Lorrain and Hull, 1993; Zhu and Luo, 1992), convulsions (Mollace *et al.*, 1991), drinking (Calapai *et al.*, 1992), and antinociception (Xu and Tseng, 1993). Therefore, precursor availability does appear to affect some functions.

In summary, inhibition of NO synthesis impaired copulation in male rats primarily by inhibiting erectile function. Neither sexual motivation nor motor activity were affected. On the other hand, inhibition of NO synthesis increased the number of seminal emissions and decreased their latency, effects attributed to a probable increase in sympathetic nerve activity. Therefore, NO probably acts peripherally, and perhaps centrally, as well, to promote erectile function. It may act centrally to inhibit sympathetic elicitation of seminal emission, thereby decreasing the likelihood of premature ejaculation.

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