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Short Communication

The nitric oxide synthase inhibitor L-NAME suppresses androgen-induced male-like pseudocopulatory behavior in whiptail lizards

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Abstract

The synthesis of nitric oxide by the enzyme nitric oxide synthase (NOS) is involved in the androgen-dependent gating of male-typical copulatory behavior, both centrally, particularly in the preoptic area, and peripherally, notably through its role in penile erection. In the all-female whiptail lizard species *Cnemidophorus uniparens*, individuals display copulatory behaviors indistinguishable from males of similar species if gonadectomized and treated with testosterone. In this experiment, androgenized individuals were treated with a NOS inhibitor, which eliminated male-like behavior in half the individuals, suggesting that the central role of nitric oxide synthesis is conserved in this species. The deficit was principally in mounting, suggesting that sexual motivational systems were affected, rather than consummatory mechanisms.

Theme: Neural basis of behavior
Topic: Hormonal control of reproductive behavior

Keywords: Testosterone; NOS; Nitric oxide; L-NAME; Lizard; Pseudocopulation

Male mammals in breeding condition will, in response to a receptive female, display a series of stereotyped behaviors culminating in intromission and ejaculation, while animals deprived of androgens by castration will not. It is thought that the preoptic area (POA) plays a central role in this testosterone-dependent gating [7] by a mechanism involving the facilitation of dopaminergic transmission by nitric oxide (NO) [8]. NO is produced in neurons from arginine by the enzyme nitric oxide synthase (NOS) and is thought to play critical roles in both peripheral and central control of reproductive behavior [1]. While in male rats, NO is involved in the control of androgen-dependent copulatory behavior [8], in female rats, it has been shown to influence progesterone-mediated lordosis behavior [11]. The involvement of NO in copulatory behavior and its role in mediating the behavioral effects of both androgen and progesterone suggested that it might be involved in the neural control of the unusual male-like copulatory behavior observed in *Cnemidophorus uniparens*, an all-female lizard species. *C. uniparens* individuals display male-like pseudosexual behavior under the influence of progesterone normally [6], but can be made to display the same behavior with testosterone treatment [14]. This display of male-like copulatory behavior is known as pseudocopulation [5]. The experiment described below was designed to determine whether NO is involved in the male-like pseudocopulatory behavior of whiptail lizards under the influence of circulating androgens.

Adult *C. uniparens* were captured in the environs of Portal, Arizona, transported to the University of Texas at Austin and housed in environmentally controlled chambers as described by Wade and Crews (1991) [13]. Animals were ovariectomized under hypothermic anesthesia and each implanted with a Silastic tube (Helix Medical, 10 mm long, internal diameter 1.47 mm, external diameter 1.96 mm) packed with crystalline dihydrotestosterone (DHT, Sigma).

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This treatment has been shown to produce male-typical levels of hormone in plasma and results in the robust expression of male-like copulatory behavior [10]. Animals to be used as receptive stimulus animals were ovariectomized and after 3 days to recover from surgery were fed three times each week one waxworm injected with 0.5 μg of estradiol (Sigma) dissolved in peanut oil. This treatment robustly induces receptivity in female and parthenogenic whiptails.

After 3 weeks to recover from surgery, behavioral tests with receptive animals were commenced. The sexual behavior of whiptails lizards has been described by Lindzey and Crews (1986) [10]. Tests were conducted by introducing stimulus animals to the subjects' home vivaria (25 cm × 30 cm) with the water bowls and planks removed to leave only the sand substrate. Before conducting tests, each stimulus animal's receptivity was verified by testing with a long-term androgenized stud animal. Behaviors scored for analysis (described by Crews and Fitzgerald, 1980 [5]) were APPROACH (subject moves towards stimulus animal and makes contact with her); MOUNT (subject mounts on top of the stimulus animal with forelimbs on either side of her trunk); and PSEUDOCOPULATION (the circular position wrapped around the abdomen of the female that is assumed by males of sexual whiptail species during intromission and ejaculation). Each test lasted 10 min, or until the subject displayed pseudocopulation, and the time at which each behavior occurred was recorded. All animals were given three screening tests and those that failed to mount on at least two of these three tests were eliminated from the study (n = 7), leaving (n = 10) vigorous courters only.

N-nitro-L-arginine methyl ester (L-NAME), or its inactive isomer D-NAME (both obtained from Sigma), was dissolved in 0.9% saline and injected intraperitoneally 1 h before the beginning of the test. The target of this experiment was the nitrergic neurons inervating the preoptic area, but L-NAME exhibits little specificity for any one isoform of nitric oxide synthase, so in addition to its effects on brain mechanisms other than those mediating sexual behavior, it affects a number of other physiological processes such as the control of blood pressure. Pilot experiments were therefore conducted to establish a dose that affected sexual behavior without being broadly debilitating. L-NAME administered at 600 μg per individual was effective in suppressing copulatory behavior, while doses greater than this appeared to have effects on the animals' behavior other than on copulatory endpoints, as assessed by observing each animal's ability to seize a live, moving cricket presented after the termination of the test. Generally, unmanipulated lizards will seize this prey avidly, and successful capture within a few seconds was taken as evidence of intact motor and motivational systems.

All animals were first given a baseline test without drug treatment to establish typical latencies to the three behavioral endpoints. Each subject was then tested on two more occasions, once 5 days later and once 2 days after that (i.e., 5 and 7 days after the first test). Each subject was tested after treatment with L-NAME on one of these later tests, and after treatment with D-NAME on the other test. Order of drug versus control treatment was balanced across subjects.

All experimental animals regardless of treatment seized crickets following their tests within 5 s (data not shown). Fig. 1 shows mean approach, mount, and pseudocopulatory posture latencies for the three testing conditions, and shows that animals responded in a similar manner when treated with D-NAME as they did in the baseline test. For this figure, animals that did not show a behavior were assigned the maximum latency of 600 s. All animals approached, mounted, and achieved the copulatory posture when treated with D-NAME, as was the case in the baseline test. When treated with L-NAME, half the animals failed to mount. All animals that mounted despite L-NAME treatment also went on to achieve the pseudocopulatory posture. The expression of this latter behavior being dependent on mounting, all animals that failed to mount also failed to show pseudocopulation. The effect of L-NAME treatment on approach behavior was somewhat weaker; in L-NAME trials, 8 out of 10 subjects approached the stimulus animal, compared to 10 out of 10 during the D-NAME and baseline trials. Latencies for the three behaviors appeared not to differ between the three different trials; although half the animals failed to pseudocopulate when given L-NAME, the individuals that did express the behavior did so with similar latencies whether given L-NAME, D-NAME, or untreated. For each behavior, latencies were subjected to repeated measures ANOVA for all individuals that did express the behavior in all three trials (individuals that did not express a behavior were not included in this analysis because the assignment of the full 600 s latency for

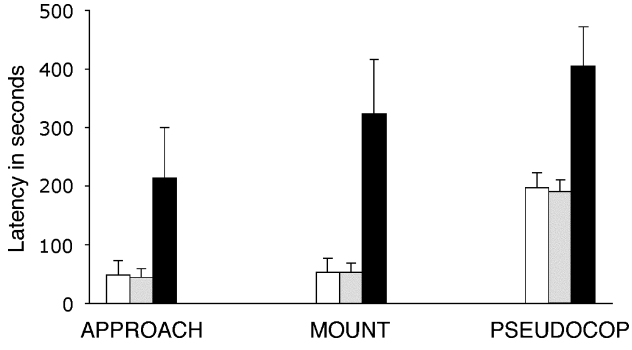

Fig. 1. Mean latencies to exhibit copulatory behaviors under baseline, drug, and control conditions. Small bars show standard errors. Results of baseline tests are represented by clear bars, results from animals treated with D-NAME (control) are by grey bars, and from animals treated with L-NAME (nitric oxide synthase inhibitor) by black bars. Animals that failed to show a behavior were assigned the maximum latency of 600 s.

animals not expressing a behavior was considered too arbitrary). For APPROACH, $n = 8$, $F = 0.18$, $P > 0.841$; MOUNT, $n = 5$, $F = 0.33$, $P > 0.73$; for PSEUDOCOPULATION, $n = 5$, $F = 0.61$, $P > 0.565$. Since the principal difference between L-NAME and other groups was the proportion of individuals that exhibited the various behaviors, the categorical responses under the three conditions (pre-test, D-NAME, or L-NAME) were subjected to Cochran's Q test for each behavior; APPROACH was not significantly different between the treatments ($P < 0.135$); mounting and pseudocopulation were both observed less frequently in animals that had been treated with L-NAME ($n = 10$, $P < 0.007$).

The suppression of pseudocopulation by L-NAME in this species is consistent with the idea that NO synthesis may be involved in the control of expression of this behavior. Results of reported experiments examining the effects of NO synthesis inhibition on male copulatory behavior in rats are somewhat heterogeneous (see [7]). One source of variation in reported results is probably the effect of previous sexual experience. Benelli et al. (1995) [2] found that systemic L-NAME treatment reduced the percentage of individuals mounting and ejaculating in both experienced and naive rats, while intracerebroventricular administration had this effect only in naive animals. Bialy et al. (1996) [3] report that the impact of L-NAME treatment was largely on the later consummatory aspects of the mating sequence, suggesting that sexual motivation was intact, while Ratnasooriya et al. (2000) [12] found that L-NAME caused a marked reduction in precopulatory behaviors such as pursuit and anogenital investigation, suggesting the opposite. The results of a recent thorough investigation of the influence of experience on the effect of central NOS inhibitor administration in male rats [9] suggest that while sexual experience does attenuate the effect of NOS inhibition on the display of mounting, even sexually experienced animals show greatly decreased levels of consummatory copulatory behaviors after infusion of L-NAME to the medial preoptic area. The animals in the present study had been given the opportunity to express pseudosexual behavior on at least the three screening tests, in addition to an unknown amount of pseudosexual experience in the wild before their capture, and are perhaps best considered "experienced" for the purposes of this discussion. This interpretation is consistent with the observations of Lagoda et al. (2004) [9], who found that while in naïve male rats mounting was almost eliminated by L-NAME treatment, in experienced animals, slightly less than half exhibited mounting despite L-NAME treatment, a proportion similar to that seen in the present study. What, mechanistically, is different between experienced and naïve rats, or between those experienced animals sensitive to NOS inhibition and those that appear refractory to such treatment, remains an interesting question.

Another important confound that is latent in experiments involving systemic injection of NOS inhibitors is the effect of the drug on peripheral physiology, particularly penile erection. Since NO synthesis is critically involved in the local control of erection [4], the effect of its systemic suppression on male copulatory behavior is only partly attributable to mechanisms in the central nervous system. Two observations make it likely that the suppression of male-like copulatory behavior in *C. uniparens* is independent of penile erection. Firstly, the animals that failed to pseudocopulate did so because they failed to mount, suggesting that the deficit was more in motivation than in consummatory ability. Secondly, these animals have entirely female-typical morphology and no erectile or intromittent structures with which the drug might interfere. The results observed are therefore compatible with an effect of NO synthesis suppression on the central mechanisms of appetitive motivation. A significant problem in thus attributing the observed effect, particularly given the fact that suppression of NO synthesis was not verified other than by the observation of the behavioral effect, is that the drug L-NAME, which is not specific to the neuronal isoform of NOS, also has the potential to interfere with other physiological systems that depend on NO synthesis. When administered systemically, the contribution of such unintended effects to a behavioral observation cannot be ruled out, although the fact that the animals' normal feeding behavior was unimpaired argues against such an interpretation in this case.

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