Enhancement of Sexual Motivation in Male Rats by Yohimbine

Abstract. *Yohimbine hydrochloride, an α_2-adrenoceptor antagonist, increased sexual motivation in male rats as evidenced by increased mounting performance in mating tests conducted after genital anesthetization, increased percentage of male rats ejaculating in their first heterosexual encounter, and induction of copulatory behavior in sexually inactive male rats. These observations lead to the suggestion that α-adrenoceptors are important modulators of sexual arousal in intact male rats. These results indicate that pharmacological treatment of sexual (libido) dysfunction may be useful.*

That monoamines play an important role in regulation of male and female sexual behavior has long been suspected (*1*). Monoaminergic mechanisms are probably related to the sexual dysfunction commonly associated with antihypertensive and psychotropic medication (*2*). In recent years, pharmacologic studies on male rats have generated various relevant hypotheses, the most common of which are that serotonergic transmission is inhibitory (*3*) and dopaminergic transmission is facilitatory (*4*) to masculine sexual behavior. However, the neurochemical substrates of the individual components of the male's behavior, primarily arousal or motivation (termed libido in men) and erectile and ejaculatory responses [potency in men (*5*)], have remained elusive. Yet a single pharmacologic agent can have opposing effects on different behavioral components (*6–8*).

Yohimbine, an alkaloid found in the bark of *Corynanthe johimbe* and other plants, has a history of popular use for its supposed aphrodisiac (sexual desire–stimulating) properties (*9*). Pharmacologists have generally disparaged this use and have ascribed any sexual actions of the drug to placebo effects or to increased peripheral genital vasocongestion rather than to a true stimulation of libido (*10*). However, no accounts of controlled studies of human response are available, and we know of only one account (giving negative results) of studies on male rats (*11*). Recently, yohimbine has been widely used in pharmacologic research because of its preferential α_2-adrenergic blocking properties (*12*). In a previous experiment we observed that yohimbine (2 mg per kilogram of body weight) prevented the inhibitory effect of the α-adrenergic agonist clonidine on copulatory behavior of male rats and, when used alone, increased the rate of copulatory events in intact, sexually experienced male rats (*13*). The alterations in the mating pattern suggested that yohimbine may facilitate sexual arousal in the male rat and, consequently, that noradrenergic transmission may play an important role in male sexual behavior.

We therefore examined whether yohimbine had aphrodisiac properties in male rats by using a specific test for sexual arousal or motivation: the rate of mounting behavior after application of a local anesthetic [tetracaine hydrochloride (*14*)] to the glans penis. During the males' exposure to females, this procedure eliminates erections and intromissive or ejaculatory behavior, thereby allowing measurement of sexual arousal as reflected by mount frequency uninfluenced by other behavioral components. Mount frequency is linearly related to amounts of plasma testosterone in castrated rats (*15*). Such mounting tests and copulatory behavior tests were conducted in standard semicircular testing arenas during the first half of the dark period (*16*). Stimulus females were rendered sexually receptive by treatment with estradiol benzoate and progesterone before exposure to males.

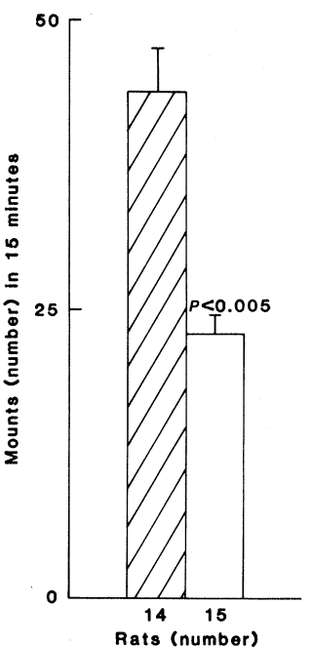

Fig. 1. Effect of yohimbine (2 mg per kilogram of body weight) (▨) or vehicle (0.1 ml per 100 g of body weight) (□) on the number of mounts (mean ± standard error of the mean) exhibited in tests of mounting behavior after genital anesthetization. Tests were initiated 20 minutes after administration of yohimbine or vehicle. Statistical analysis was by the Mann-Whitney *U* test.

Thirty sexually vigorous male Long-Evans rats were injected intraperitoneally with yohimbine or vehicle (2 mg per kilogram of body weight). Twenty minutes later they were anesthetized in the genital area and subjected to mounting tests (15 minutes). The rats treated with yohimbine exhibited about twice as much mounting behavior in the 15-minute test period as did the controls (Fig. 1). No evidence of seminal emission was seen. Yohimbine thus appears to be a potent stimulator of sexual arousal in the intact, sexually vigorous male rat in the absence of feedback from the genitalia.

A significant though variable proportion of adult rats do not mate upon first exposure to a sexually receptive female, and those that do mate show prolonged behavioral latencies relative to experienced males (*17*). Fifty-nine male rats, naïve to heterosexual behavior and drugs, were randomly divided into two groups and subjected to mating tests 20 minutes after they were injected with yohimbine (2 mg per kilogram of body weight) or vehicle. Surprisingly, most of the animals in each treatment group mated on this initial copulatory test. However, more of the animals treated with yohimbine mounted and intromitted and significantly more ejaculated (Fig. 2A). As for the parameters of copulatory behavior, the yohimbine-treated animals initiated copulatory activity significantly sooner (*18*) and showed nonsignificant trends toward decreased ejaculatory latency and intercopulatory interval (time between intromissions). In tests conducted 7 days after treatment, the two groups did not differ on any behavioral parameter. These data indicate that yohimbine has a stimulatory effect on sexual behavior in the sexually inexperienced male rat.

In any population of adult male rats, a certain percentage does not copulate even after repeated testing. These noncopulators show normal penile reflex activity (including erections in tests conducted in nonmating situations with no overt sexual stimulation), deposit spontaneous seminal emissions in the normal manner, and have amounts of circulating testosterone within the normal range (*8, 19*). Male rats of various ages that did not mate when tested at 3 months of age during at least six exposures to receptive females over a period of several weeks

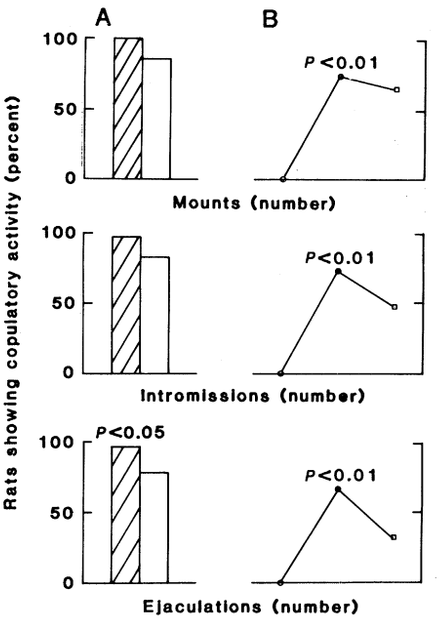

Fig. 2. Effects of yohimbine (2 mg per kilogram of body weight administered 20 minutes before testing) (▨) and vehicle (□) on the percentage of rats showing copulatory activity. (A) Effect in heterosexually inexperienced (naïve) male rats (n = 30 and 29 for yohimbine- and vehicle-treated males, respectively). Statistical treatment was by Fisher's Exact Probability test. (B) Effect in male rats (n = 15) that had been sexually inactive in at least seven exposures to receptive females 7 days before administration of yohimbine (○), at the time of yohimbine administration (●), and 7 days after yohimbine administration (□). Statistical analysis was by Fisher's Exact Probability test comparing (○) and (●).

were tested 20 minutes after an injection of vehicle. No animals mated in this test. One week later they were tested again, this time 20 minutes after treatment with yohimbine (2 mg per kilogram of body weight). The drug induced copulatory activity in most of these previously sexually inactive animals (Fig. 2B). Further, the behavior of animals that copulated was within the normal range (*20*). Finally, in tests with vehicle performed 1 week after the tests with yohimbine, some of the animals continued to copulate.

These data suggest that yohimbine may be a true aphrodisiac since it inceases arousal in sexually experienced male rats, facilitates copulatory behavior (including ejaculation) in sexually naïve males, and induces sexual activity in males that were previously sexually inactive.

The discrepancy in results from our study and that of Johnson and Diamond (*11*), in which no effects of yohimbine on sexual behavior of male rats were shown, is presumably due to the different treatment they used (35 days of treatment; 20 mg per kilogram of body weight for 10 days and 10 mg per kilogram for 25 days). We have examined only acute effects of a lower dose, which did not produce gross changes in ongoing behavior. Also, the period between drug administration and behavioral tests was not specified by Johnson and Diamond.

In light of considerable evidence indicating the specificity of yohimbine as an α_2-adrenoceptor antagonist (*12*) and its reversal of copulatory suppression by clonidine (*13*), it is likely that yohimbine exerts its effects on sexual behavior via blockade of α-adrenergic autoreceptors. The result of blockading these receptors, most of which are located presynaptically, should be potentiation of the postsynaptic actions of endogenously released norepinephrine by attenuation of negative feedback mechanisms (*21*). Our results further suggest that conditions of sexual inactivity may be related to decreased activity of noradrenergic neurons. Because yohimbine has a complex pharmacologic profile, which includes reports of stimulation (*22*) and blockade (*23*) of serotonergic receptors, increased dopamine catabolism in the striatum (*24*), blockade of dopamine receptors (*25*), and increased amounts of serum prolactin [apparently not through dopaminergic mechanisms (*26*)], it will be of interest to ascertain whether this enhancement of sexual arousal is peculiar to yohimbine or is shared by other α-adrenergic agents. Although preliminary clinical data are suggestive of a libido-promoting effect in human males (*27*), caution should be exercised—especially concerning possible interactions between yohimbine and other medications [for example, yohimbine competitively antagonizes the antihypertensive action of clonidine (*28*)]—in extrapolating these data for application to humans. Previous reports of apparent libido-promoting agents have generally been limited to effects of long-term treatment [for example, para-chlorophenylalanine (*3*)], effects in castrated male rats [for example, lisuride, pergolide, apomorphine, and RDS-127 (*7, 29*)], or the interpretation of facilitation of ejaculation during mating (*7, 29*) or erection in nonmating situations (*30*) as a stimulation of libido. Further research could lead to developments in the pharmacologic treatment of sexual dysfunction.

JOHN T. CLARK*
ERLA R. SMITH
JULIAN M. DAVIDSON
Department of Physiology,
Stanford University,
Stanford, California 94305

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6. RDS-127 (2-*N*,*N*-di-*n*-propylamine-4,7-dimethoxyindane) facilitates seminal emission *ex copula* (nonmating situations with no overt sexual stimulation) and ejaculation in mating tests (*7*), but inhibits penile reflexes, including erection, *ex copula* [M. Stefanick, E. Smith, J. Clark, J. Davidson, *Physiol. Behav.* **29**, 973 (1982)]. Also, quanethidine abolishes seminal emission *in* and *ex copula*, does not alter penile reflex activity, and reduces the number of intromissions preceding ejaculation (*8*).
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16. Copulatory behavior tests consisted of exposure to receptive females and monitoring all mounts, intromissions, and ejaculations. Measurements derived from the records are mount and intromission latencies, ejaculation latency, postejaculatory interval, and mount and intromission frequencies. Additionally, intercopulatory interval (time between intromissions) and copulatory efficiency are calculated [see (*7*)]. Tests were terminated if an intromission did not occur within 15 minutes or if ejaculation latency exceeded 30 minutes.
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18. Mount latency, 1.3 ± 0.2 minutes for yohim-

bine-treated and 2.6 ± 0.5 minutes for vehicle-treated ($P < 0.02$); intromission latency, 1.6 ± 0.3 minutes for vehicle-treated ($P < 0.02$); ejaculation latency, 6.9 ± 0.9 minutes for yohimbine-treated and 8.5 ± 0.8 minutes for vehicle-treated. The intercopulatory interval was 0.65 ± 0.04 minute for yohimbine-treated and 0.87 ± 0.08 minute for vehicle-treated. Most males that intromitted exhibited ejaculation (29 of 29 yohimbine-treated and 23 of 24 vehicle-treated). Values are the mean ± standard error of the mean.
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20. Mount latency, 1.5 ± 0.8 minutes; intromission latency, 2.4 ± 1.0 minutes; ejaculation latency, 8.2 ± 1.6 minutes; postejaculatory interval, 6.6 ± 0.4 minutes; mount frequency, 5.3 ± 1.2; intromission frequency, 6.5 ± 1.1; intercopulatory interval, 1.27 ± 0.17 minutes; copulatory efficiency, 0.58 ± 0.06 (n = 10). Values are means ± standard error of the mean.
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* Present address: Department of Obstetrics and Gynecology, University of Florida College of Medicine, P.O. Box J-294 JHMHC, Gainesville 32610.

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Enhanced Neural Response to Familiar Olfactory Cues

Abstract. *Norway rat pups have an enhanced olfactory bulb response to a familiar odor. A specific complex of glomeruli showed increased carbon-14–labeled 2-deoxy-D-glucose uptake in response to peppermint odor in 19-day-old pups exposed to peppermint on days 1 to 18 after birth, relative to control pups that had been exposed to clean air. The increased activity was not due to increased respiration of the familiar odor.*

Norway rat mothers emit an odor that attracts their young from the second through the fourth postpartum weeks (*1*), a period that corresponds to the time when the pups return to the mother to nurse (*2*). Mothers initially emit the attractant in low quantities, inducing the pups to orient toward it during the first 2 weeks (*3*). The principal source of the maternal odor is the cecotrophe portion of the maternal anal excreta (*4*). Synthesis of the cecal odor depends on cecal bacteria populations (*5, 6*), the composition and metabolic products of which differ with different diets (*7*). Since there is no single maternal odor, the pups must become attracted to the odor that they will approach through postnatal experience. Leon (*6*) found that pups raised with mothers on a particular diet are attracted specifically to the odor of mothers eating that diet. In fact, brief daily experience of young rats with either a maternal or an arbitrarily selected odor induces a preferential response by the pups (*8, 9*).

It seemed possible that this early olfactory experience could induce the developing olfactory system to have a special, perhaps enhanced, response to that odor. If one accepts the proposition that elevated use of glucose by neurons reflects neural activity and that 2-deoxy-D-glucose (2DG) uptake reflects glucose use, then 2DG autoradiography is a powerful technique for determining the locations of differentially active cells (*10*). We used this technique to determine whether there is a differential neural response to familiar and unfamiliar odors by young rats.

An artificial odor, peppermint, was chosen as the olfactory stimulus because its presentation could be controlled more precisely than maternal odors. We gave young rats experience with peppermint odor in a manner similar to that which had previously induced a strong behavioral preference (*9*). For 10 minutes each day for the first 18 days after birth, eight rat pups were exposed to peppermint-scented air delivered through a flow-dilution olfactometer (*11*). Exposure was accompanied by perineal stimulation, a procedure that mimics the licking that rat mothers do as part of their maternal care and facilitates the acquisition of an olfactory preference in neonates (*12*). Six control pups were exposed daily to clean air in the olfactometer while receiving perineal stimulation.

We then used 2DG autoradiography to determine whether these different olfactory experiences had influenced the responsiveness of the olfactory bulbs to peppermint. On day 19, both the pups experienced with peppermint and the naïve pups received, for the only time, a single subcutaneous injection of ^{14}C-labeled 2DG (200 μCi/kg). Both groups were then exposed to peppermint odor for 45 minutes, without perineal stimulation. We used a 45-minute exposure period to avoid the artifact associated with measuring unphosphorylated 2DG (*13*). At the end of the exposure period, pups were decapitated and their brains were quickly removed and frozen in Freon-12 at −40°C. Autoradiographs of the olfactory bulbs were prepared and developed according to standard techniques, which included exposing a set of calibrated ^{14}C-labeled standards with each film (*14*). The sections then were counterstained with thionin.

Autoradiographs, coded to prevent experimental bias, were analyzed with a computer-based image processor that allowed pseudocolor imaging and two-dimensional quantitative optical densitometry. Because the autoradiographs could be aligned by the image processor with the matching thionin-stained sections, 2DG uptake could be associated with specific lamina in the olfactory bulb (*15*). As a first step in quantifying 2DG uptake, the computer constructed a calibration curve that related the gray value of each ^{14}C-labeled standard exposure to its previously determined ^{14}C-labeled tissue equivalent. It then linearized this function so that the gray values of the autoradiograph could be translated into ^{14}C concentrations, and hence 2DG taken up by the tissue.

The entire bulb was scanned for areas of relatively high 2DG uptake. When an active glomerular complex was noted, sections throughout it were analyzed and the average uptake of 2DG by the complex was calculated. In every section, five readings (each of 40 pixels) were taken in each area of high activity within the glomerular layer, five readings were taken from the rest of the glomerular layer, and five more readings were taken within the periventricular core of the bulb. After the uptake of ^{14}C by an area of interest was determined, it was expressed relative to ^{14}C uptake in the periventricular core of the same section. Expressing uptake in terms of this ratio minimizes errors due to variations in section thickness or to nonuniformity of background illumination. Previous reports have shown that 2DG uptake in the core of the bulb is consistently low regardless of the odor stimulus used (*16*). Even short-term unilateral closure of a naris during odor presentation does not alter 2DG uptake in this region (*17*). We observed no significant difference in the concentration of ^{14}C in the periventricular core between experienced ($\overline{X}$ ±