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Inhibition of Sexual Reflexes by Lumbosacral Injection of a $GABA_B$ Agonist in the Male Rat

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BITRAN, D., S. A. MILLER, D. B. McQUADE, R. E. LEIPHEIMER AND B. D. SACHS. *Inhibition of sexual reflexes by lumbosacral injection of a $GABA_B$ agonist in the male rat.* PHARMACOL BIOCHEM BEHAV 31(3) 657–666, 1988.—The effects of gamma-aminobutyric acid (GABA) agonists on penile reflexes were investigated. An intrathecal injection of baclofen (0.2, 0.4, or 0.8 μg), a $GABA_B$ receptor agonist, into the subarachnoid space of the lumbosacral spinal cord (L5–S1), resulted in a dose-related decrease in the number of animals responding in a penile reflex test. Doses of 0.2 and 0.4 μg of baclofen decreased the number of erections; 0.4 μg also increased the latency to the first glans erection. The highest dose of baclofen (0.8 μg) completely inhibited penile responses in these tests. None of these doses, however, prevented rats from copulating to ejaculation. Antecedent ejaculation, which facilitated the onset of penile reflexes in saline controls, also blocked the inhibitory effects on penile responses by the lower doses (0.2 and 0.4 μg) of baclofen, but was ineffective in animals treated with 0.8 μg baclofen. In contrast to the inhibitory effects of baclofen in the lumbosacral cord, an intrathecal injection of baclofen (0.8 μg) at thoracic segments (T8–T10) did not affect penile erections elicited following an ejaculation. The role of spinal $GABA_A$ receptors in sexual reflexes was assessed by intrathecal injection of a $GABA_A$ agonist, THIP (0.5, 1, or 2 μg), onto the lumbosacral cord. Only at the largest dose of THIP were slight inhibitory effects on penile reflexes observed. Together, these data indicate that stimulation of $GABA_B$ receptors in the lumbosacral spinal cord inhibits erectile mechanisms ex copula.

Penile reflexes Sexual behavior Intrathecal Spinal cord GABA Baclofen THIP

THE expression of male sexual behavior is regulated by several neurotransmitters [reviewed in (4)]. Recently, an inhibitory role for gamma-aminobutyric acid (GABA) in the neural control of male copulatory behavior and genital reflexes has been proposed. The concentration of GABA in cerebrospinal fluid (CSF) taken from the cisterna magna of male rats increased dramatically during the postejaculatory interval (30), a time when sexual activity is inhibited. GABA, an inhibitory neurotransmitter with a widespread distribution in the central nervous system [see references in (31)], binds to the functionally and pharmacologically distinct $GABA_A$ and $GABA_B$ receptors. Investigations of the effects of GABAergic compounds on male sexual responses have pointed to a differential role of these receptor subtypes. For example, an inhibition of copulatory activity was noted following the administration of muscimol, a $GABA_A$ receptor agonist, into the medial preoptic area (MPOA) of the brain (11). Intra-MPOA injection of (+)bicuculline, a specific $GABA_A$ antagonist, dramatically reduced the postejaculatory refractory period and virtually eliminated the characteristic 22 kHz vocalization (11,12) that occurs during the early phase of the postejaculatory interval (2).

In contrast to the effects of $GABA_A$ agonists on copulatory behavior, the systemic administration of THIP (4, 5, 6, 7-tetrahydroisoxazolo[5,4-c]pyridin-3-ol), a $GABA_A$ receptor agonist, or bicuculline did not reliably affect penile responses elicited from male rats outside the context of copulation (ex copula) (21). However, similar treatment with baclofen, a $GABA_B$ receptor agonist, inhibited ex copula penile erections at doses which did not impair copulatory behavior (21). Thus, preferential activation or inhibition of $GABA_A$ receptors (in the MPOA) affect copulatory behavior, whereas activation of $GABA_B$ receptors inhibits reflexive penile erections. In other studies systemic administration of high doses of baclofen (2.5–5 mg/kg) disrupted copulatory behavior (1,28); however, impaired sexual behavior was also accompanied by deficits in motor execution (28).

The purpose of the following experiments was to determine whether the inhibitory effect on penile erection observed following the systemic administration of baclofen (21)

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is mediated by lumbosacral spinal mechanisms. The presence of GABAergic neurons (23) and receptors (5) in lumbosacral spinal segments previously implicated in the regulation of ex copula penile reflexes forms the basis for the hypothesis that spinal GABAergic mechanisms modulate the expression of penile responses. Thus, we investigated the effects on penile reflexes produced by intrathecal microinjections of a specific $GABA_A$ receptor agonist (THIP) (18) and a $GABA_B$ receptor agonist (baclofen) (8). We now report that intrathecal injections of baclofen onto the lumbósacral spinal cord inhibited penile reflexes in a dose-related fashion. Injections of baclofen onto the thoracic spinal cord were ineffective, and only a slight decrement in erectile potential resulted from lumbosacral injection of THIP.

GENERAL METHOD

Subjects and Experimental Protocol

Adult male Sprague-Dawley rats (350–400 g), purchased from Blue Spruce Farms (Altamont, NY), were housed individually in hanging wire mesh cages (34×18×20 cm) in a temperature-controlled (23°C) colony room on a 12:12 hr light-dark cycle with lights off at 1200 hr. Adult females of the same strain (200–300 g) were housed in groups of three in the same colony room. Water and commercial rat chow were always available.

One week after their arrival, all males were tested for copulatory behavior and ex copula penile reflexes (see below). Three weeks later, males selected for high levels of behavioral responses were prepared for intrathecal cannulation. One week following surgery, all males were retested for copulatory and penile reflex responses and then randomly assigned to drug treatment groups.

Surgery and Materials

Intrathecal cannulae were constructed from polyethylene tubing (PE-10) stretched to approximately 130% of their original length in hot water to decrease the outer diameter (and therefore lessen spinal cord compression). Surgical anesthesia was accomplished by intramuscular injections of ketamine HCl (50 mg/kg) and xylazine HCl (4 mg/kg). Cannulation was performed using the procedure described by Yaksh and Rudy (41). Following intrathecal cannulation, all males had the suspensory ligament of the penis removed. A 1 cm wide incision was made on the ventral surface 5 mm anterior to the preputial sheath. The U-shaped ligament was exposed by tearing apart connective tissue on both sides of the penile body just proximal to the penile flexion. The left and right points of insertion were cut and the ligament was excised. Removal of the suspensory ligament, which keeps the penis retracted within the preputial sheath, facilitates the extrusion of the glans and the distal portion of the penile body, thereby allowing observation of penile body erections.

Behavioral Tests

Tests for penile reflexes were conducted between 0800 and 1200 hr (except when noted). Animals were placed in a supine position with their upper bodies inside a cylinder and restrained with pieces of paper ("masking") tape over the torso, hindlimbs and tail. The preputial sheath was retracted, and the following responses were recorded on a microcomputer with the aid of a data acquisition and analysis program (17): *penile body erections*, distension and elevation of the penile body; *glans erections*, reddening and distention of the glans; *flips*, anteroflexions of the glans penis. The program extracted from the record the latencies to penile body and glans erection, the number of occurrences of each type of event, and the number of penile response clusters, defined as consecutive responses separated by less than 15 sec. Penile reflex tests were terminated 10 min after the first glans response, or 20 min after sheath retraction if no response occurred.

Tests for copulatory behavior were conducted between 1300 and 1700 hr. Males were placed in a glass chamber (50×30×30 cm), littered with pine shavings, 5 min before a sexually receptive female was introduced. Females were rendered sexually receptive with subcutaneous injections of estradiol benzoate (50 μg/rat) and progesterone (300 μg/rat) 48 and 6 hr, respectively, before a sexual behavior test. Tests for copulatory behavior were terminated following the first intromission after the first ejaculation. The following measures were derived from the record: latency to the first mount, latency to the first intromission, latency from the first intromission to ejaculation (ejaculation latency), mean interval between intromissions (interintromission interval), latency from the ejaculation to the next intromission (postejaculatory interval), number of mounts and intromissions preceding ejaculation, and intromissions divided by the sum of mounts and intromissions (intromission ratio).

Drugs and Injection Procedure

Racemic baclofen (CIBA-GEIGY) was dissolved in sterile physiological saline. THIP (H. Lundbeck) was dissolved in sterile physiological saline and the pH was adjusted to 7.2 with 1 N NaOH. Animals were handled daily so that intrathecal injections could be accomplished without stress or anesthesia. The outer portion of the intrathecal cannula was connected to a 15 mm piece of 30 ga hypodermic tubing which was fitted to a length of PE-10 tubing connected to a 1 ml syringe. Drug injections (5 μl) were followed by a sterile saline flush. Flush volumes varied according to cannula length: 6 μl for thoracic injections, 7 μl for lumbosacral injections. Intrathecal injections were delivered at a rate of 1 μl/min.

EXPERIMENT 1

Experimental Design

Thirty-two sexually experienced males that responded positively on 2 preoperative penile reflex tests were chosen randomly for study from a larger pool of animals (43/56 and 52/56 of the total group tested positively for penile reflexes and copulatory behavior, respectively). All males received an intrathecal cannula terminating at L5–L6 spinal segments (ca. 8 mm in length). One week following postoperative (baseline) copulation and reflex tests, males were randomly assigned to one of four groups: saline, 0.2, 0.4, and 0.8 μg of baclofen. Copulation and penile reflex tests were begun 5 and 15 min after intrathecal injection, respectively.

In Test 1, the effects of baclofen on penile reflexes were determined. Test 2, conducted two weeks later, examined the effects of baclofen on penile reflexes evoked following one ejaculation, because this amount of antecedent copulatory activity facilitates the expression of penile responses ex copula (20, 27, 34). At the completion of behavioral testing, animals were sacrificed and cannula placement was verified by exposing the spinal cord and visually locating the tip of the cannula.

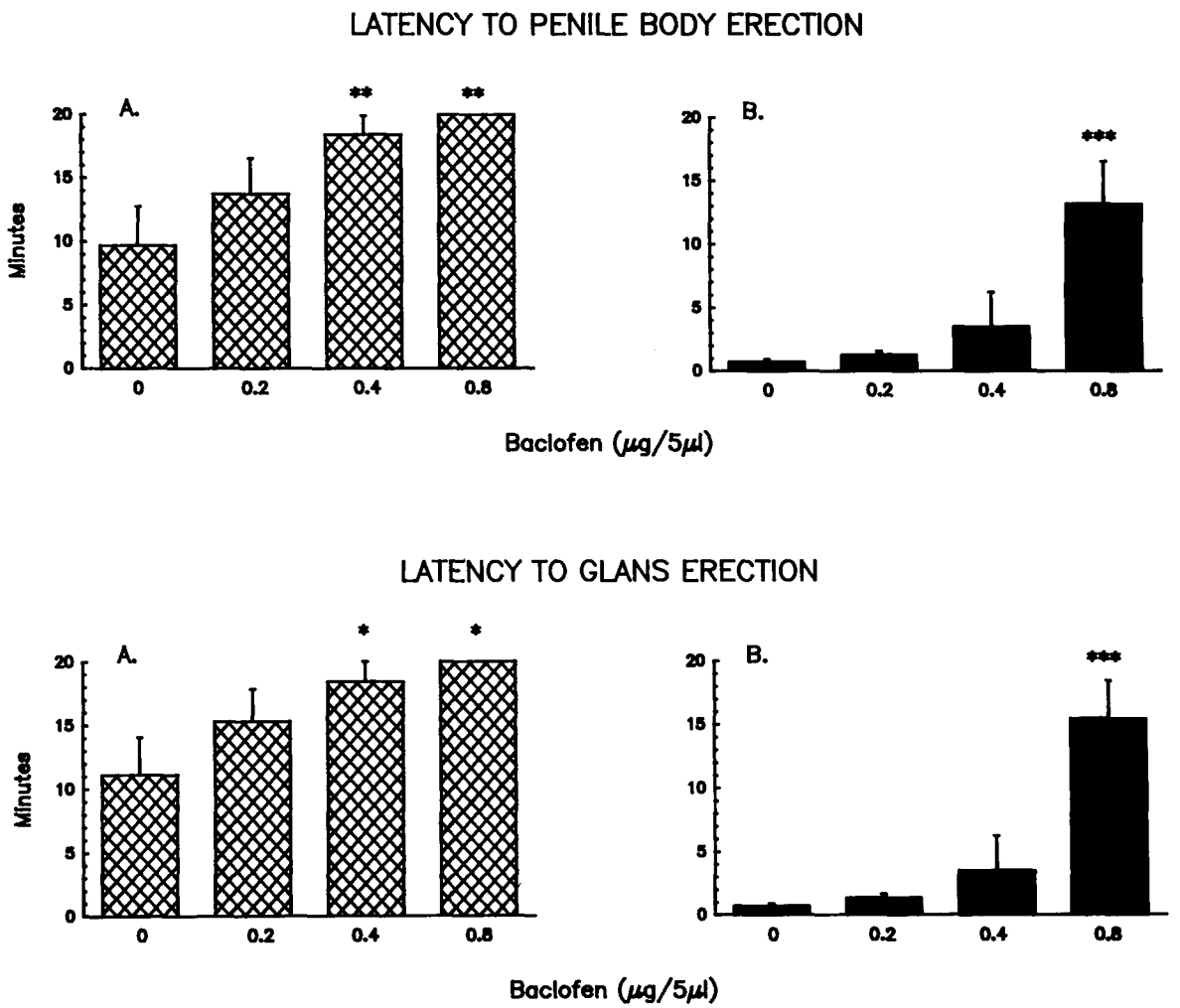

FIG. 1. Latency to penile body erection and glans erection after intrathecal lumbosacral injection of baclofen. (A) Test 1: penile reflex tests conducted on sexually rested animals. (B) Test 2: penile reflex tests conducted on animals immediately after one postejaculatory interval. Values expressed are means±SEM. p Values reflect post hoc comparisons between vehicle and drug dose. *$p<0.05$, **$p<0.02$, ***$p<0.01$.

Statistical Analysis

The proportion of animals responding in reflex tests following intrathecal injection of vehicle or one of three doses of baclofen were compared by Fisher's exact probability test. Parametric data from penile reflex tests and copulatory behavior were analyzed by a one-way analysis of variance and post hoc pairwise comparisons were made using the Newman-Keuls test. In some cases, a Kruskal-Wallis one-way analysis of variance was conducted and significant main effects were further analyzed by the Mann-Whitney U-test. A log transformation was conducted on all latency data in order to normalize the distribution of scores. Statistical significance was attributed when $p<0.05$ (two-tailed).

RESULTS

One animal (0.4 μg group) was excluded from analysis after his cannula was determined to be rostral to the lower lumbar spinal cord. Baclofen produced a dose-related decrease in the number of animals responding in a penile reflex test: vehicle=5/8; 0.2 μg=4/8; 0.4 μg=1/7; 0.8 μg=0/8. The difference in the proportion of responders was reliable only between the vehicle and 0.8 μg group ($p<0.02$). Increased latencies to the onset of penile body erection, $F(3,27)=4.39$, $p<0.02$, and glans erection, $F(3,27)=3.22$, $p<0.05$, were observed following the intrathecal injection of baclofen (Fig. 1A). Post hoc pairwise comparisons revealed that 0.4 and 0.8 μg of baclofen significantly increased the latency to the first penile erection. Although baclofen microinjections resulted in an apparent dose-dependent decrease in the number of penile body erections (Fig. 2A), the differences among groups were unreliable, $F(3,27)=1.94$, $p>0.10$. However, the number of glans erections was significantly reduced by all doses of baclofen, $F(3,27)=4.00$, $p<0.02$ (Fig. 2A). The number of penile reflex clusters was also reduced following the administration of 0.8 μg of baclofen, $F(3,27)=3.76$, $p<0.05$ (Fig. 3A). Similarly, there were fewer glans erections per reflex cluster after 0.4 and 0.8 μg of baclofen, $F(3,27)=4.64$, $p<0.01$, whereas the ratio of glans response clusters to penile body erections was decreased following the

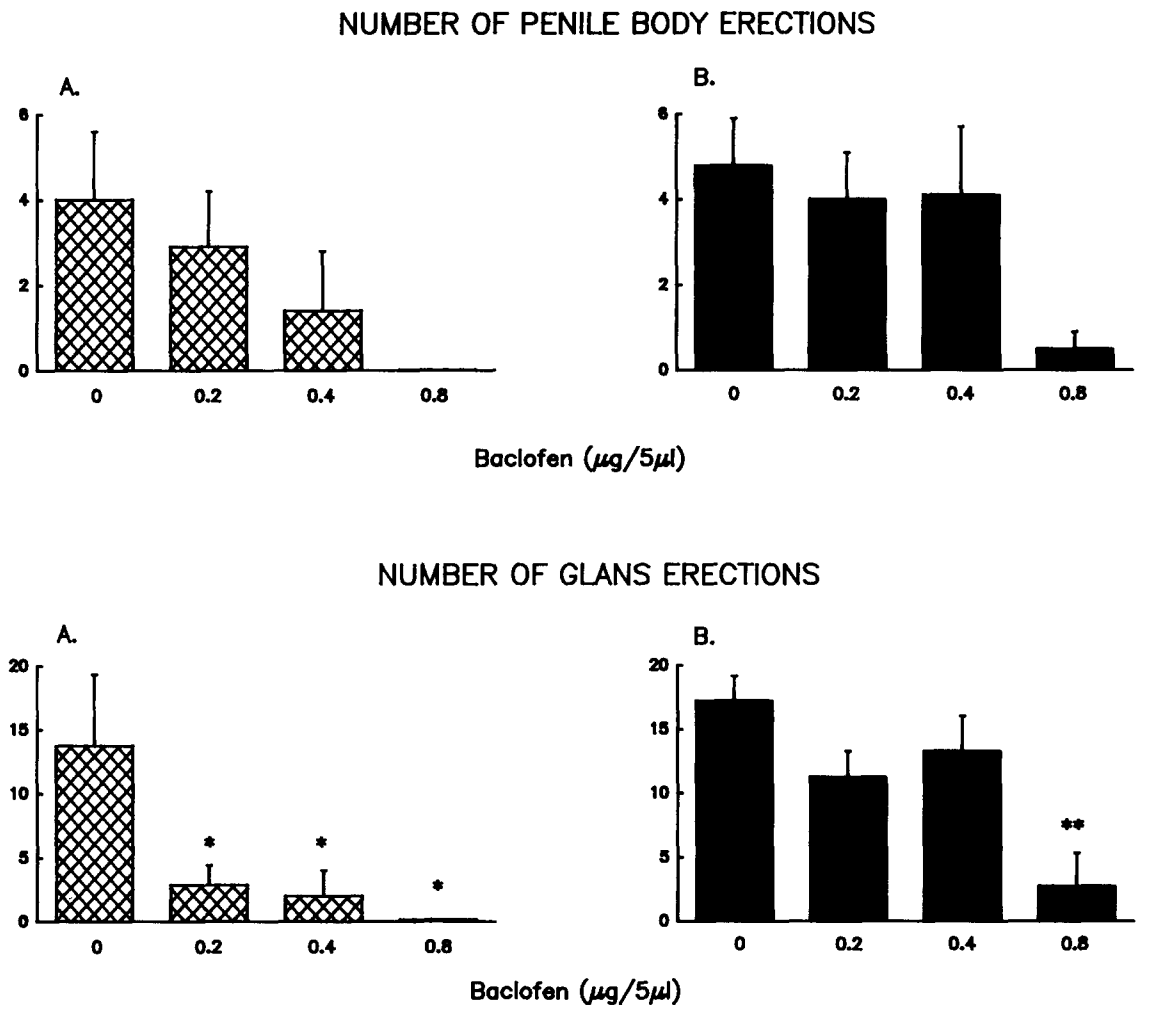

FIG. 2. Number of penile body and glans erections after intrathecal lumbosacral injection of baclofen. Other details of figure are as explained in Fig. 1. $*p<0.05$, $**p<0.005$.

intrathecal injection of all doses of baclofen, $\chi^2(3)=8.04$, $p<0.05$ (Fig. 3A). In intact, untreated males, a glans reflex cluster is usually preceded by a penile body erection, and saline-treated males did not deviate from this pattern.

The effects of an intrathecal injection of baclofen on copulatory behavior are summarized in Table 1. No gross motor impairments were noted following the intrathecal injection, and all animals achieved ejaculation. Baclofen had no effect on mount latency, intromission latency, and postejaculatory interval. However, ejaculation latency, $F(3,27)=6.85$, $p<0.001$, and interintromission interval, $F(3,27)=3.05$, $p<0.05$, were increased by the highest dose of baclofen. Further analysis revealed that these animals displayed an increased number of mounts without intromission, $F(3,27)=6.58$, $p<0.005$; however, neither the number of intromissions preceding ejaculation nor intromission ratio was reliably affected.

Penile reflex tests conducted immediately after copulation revealed that the inhibitory effects of baclofen were attenuated. The proportion of animals responding with a glans erection did not differ following the administration of vehicle (8/8), 0.2 μg (8/8), or 0.4 μg (6/7) of baclofen; however, inhibition of penile reflexes was still evident in animals receiving an intrathecal injection of 0.8 μg baclofen (2/8; $p<0.01$). Increased response latencies to penile body, $F(3,27)=8.59$, $p<0.001$, and glans erection, $F(3,27)=12.05$, $p<0.001$, were associated only with the highest dose of baclofen (Fig. 1B). As in Test 1, intrathecal administration of baclofen did not reliably affect the number of penile body erections elicited following an ejaculation, $F(3,27)=2.70$, $p=0.065$ (Fig. 2B). Only at the highest dose of baclofen was the number of glans erections reduced, $F(3,27)=7.06$, $p<0.002$. Reductions in the number of glans reflex clusters, $F(3,27)=7.97$, $p<0.002$, and glans erections per cluster, $F(3,27)=9.42$, $p<0.001$, were also observed following the intrathecal administration of 0.8 μg of baclofen (Fig. 3B). A similar shift in the dose-response curve was also seen in the proportion of response clusters to penile body erections. Again, significant inhibitory effects were noted only following the intrathecal administration of 0.8 μg of baclofen, $\chi^2(3)=9.3$, $p<0.05$.

EXPERIMENT 2

In Experiment 1 an intrathecal injection of baclofen

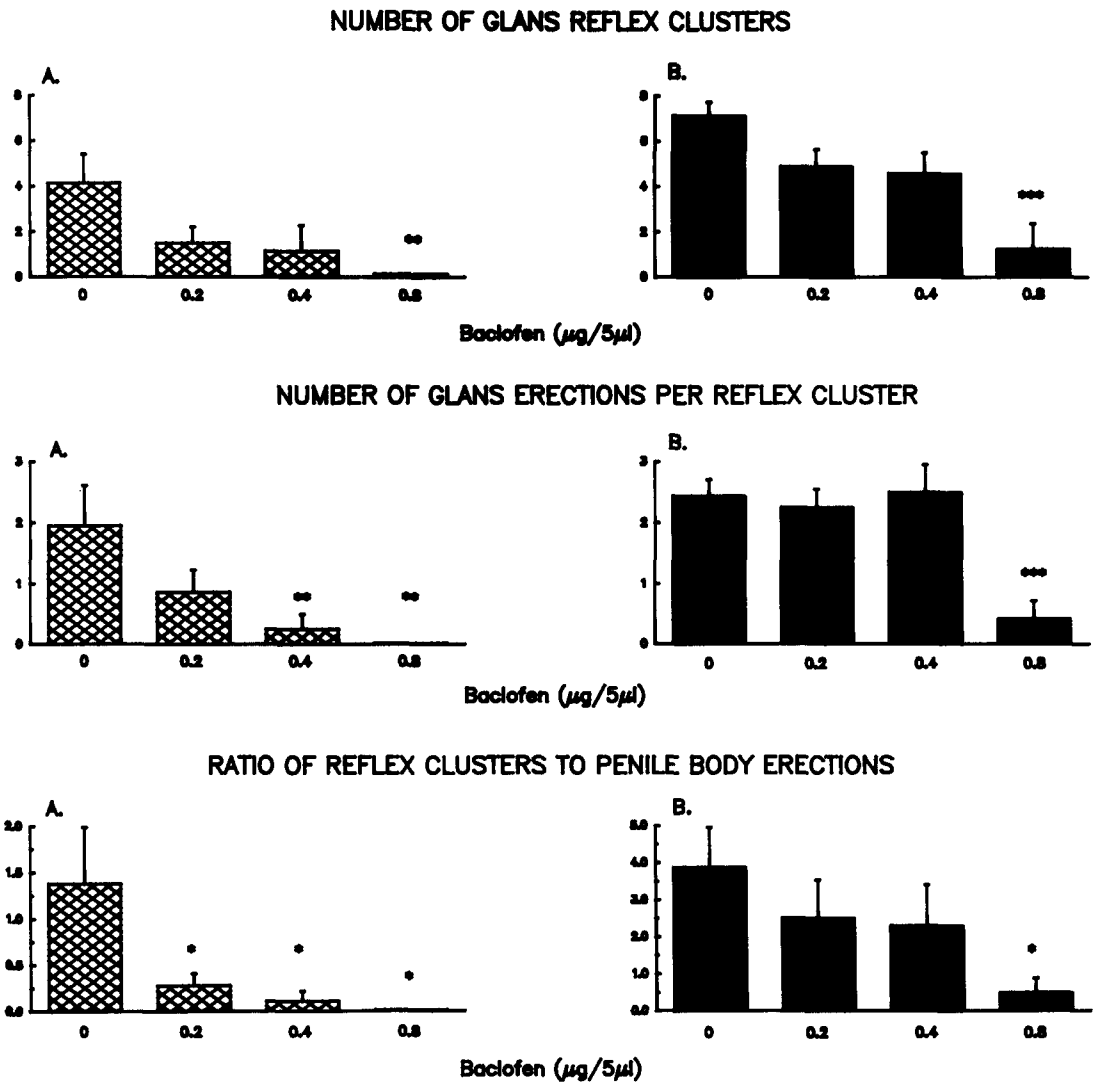

FIG. 3. Number of reflex clusters, glans erections per cluster, and ratio of clusters to penile body erections after intrathecal lumbosacral injection of baclofen. Other details of figure are as explained in Fig. 1. $*p<0.05$, $**p<0.02$, $***p<0.01$.

produced a clear dose-dependent inhibition of penile reflexes that was partially reversed by an antecedent ejaculation. The purpose of the following experiment was to determine whether the inhibitory effect of intrathecal baclofen on postcopulatory penile reflexes was selective to the lumbosacral cord.

Experimental Design and Procedure

Twenty-eight sexually experienced male rats that responded positively on 2 preoperative reflex tests were chosen for the study. (Males used in Experiments 2 and 3 were randomly chosen from a pool of animals in which 62/84 and 78/84 males responded on penile reflex and copulatory behavior tests, respectively.) Eight males were implanted with an intrathecal cannula terminating at the L5–L6 spinal segments, and 20 males received a cannula terminating at the T8–T9 spinal segments (T8–T9 internal cannula length ca. 6 cm). One week later, animals with thoracic cannulae were randomly assigned to receive an injection of 0.8 μg of baclofen or saline vehicle, whereas all animals with lumbosacral cannulae received an injection of 0.8 μg of baclofen. Five minutes following the intrathecal injection, copulatory behavior tests were begun and continued until after the first intromission following the first ejaculation. Animals were then immediately tested for penile reflexes.

Statistical Analysis

The proportion of animals responding in a penile reflex test following intrathecal injection of baclofen or vehicle was compared by Fisher's exact probability test. Parametric data were analyzed by independent *t*-tests. Latency data were subjected to a log transformation in order to normalize their distribution. Statistical significance was attributed when $p<0.05$ (two-tailed).

TABLE 1

COPULATORY BEHAVIOR AFTER INTRATHECAL LUMBOSACRAL INJECTION OF BACLOFEN

	Baclofen (μg/5 μl)			
	0 (n=8)	0.2 (n=8)	0.4 (n=7)	0.8 (n=8)
Mount latency	0.35 ± 0.14	0.65 ± 0.39	1.17 ± 0.59	0.60 ± 0.27
Intromission latency	0.47 ± 0.17	0.66 ± 0.39	1.21 ± 0.58	0.83 ± 0.36
Ejaculation latency	3.35 ± 0.60	2.24 ± 0.41	2.79 ± 0.41	6.74 ± 1.28‡
Interintromission interval	0.39 ± 0.04	0.33 ± 0.05	0.33 ± 0.03	0.62 ± 0.14*
Postejaculatory interval	4.75 ± 0.13	5.24 ± 0.24	4.49 ± 0.19	4.95 ± 0.28
Number of mounts	4.5 ± 1.1	3.4 ± 0.7	3.4 ± 1.0	13.8 ± 3.5†
Number of intromissions	8.9 ± 1.6	7.3 ± 1.1	8.4 ± 1.1	11.0 ± 1.3
Intromission ratio	0.67 ± 0.06	0.69 ± 0.04	0.74 ± 0.05	0.51 ± 0.07

Results are shown in terms of mean ± S.E.M. Latency and interval data are in minutes. Levels of significance: *$p<0.05$; †$p<0.005$; ‡$p<0.001$.

TABLE 2

COPULATORY BEHAVIOR AND PENILE REFLEXES AFTER THORACIC OR LUMBOSACRAL INTRATHECAL INJECTION OF BACLOFEN

	Baclofen (μg/5 μl)		
	0 (T8–T9)	0.8 (T8–T9)	0.8 (L5–L6)
Copulatory Behavior			
Mount latency	2.47 ± 1.64	0.93 ± 0.71	1.00 ± 0.71
Intromission latency	2.80 ± 1.77	1.11 ± 0.71	1.01 ± 0.71
Ejaculation latency	5.85 ± 1.38	2.64 ± 0.33	2.99 ± 0.41
Interintromission interval	0.55 ± 0.09	0.46 ± 0.05	0.49 ± 0.08
Postejaculatory interval	5.47 ± 0.42	5.06 ± 0.25	5.37 ± 0.34
Number of mounts	11.3 ± 3.8	5.0 ± 0.8	4.3 ± 1.2
Number of intromissions	10.4 ± 1.5	6.0 ± 0.7*	6.4 ± 0.5*
Intromission ratio	0.55 ± 0.06	0.56 ± 0.06	0.64 ± 0.06
Penile Reflexes			
Penile reflex responders	10/11	7/9	0/8†
Penile body erection latency	4.22 ± 2.01	6.69 ± 2.72	—
Glans erection latency	4.34 ± 2.01	6.74 ± 2.71	—
Number of penile body erections	3.3 ± 1.3	3.7 ± 1.5	—
Number of glans erections	15.0 ± 3.6	11.3 ± 3.9	—
Number of response clusters	6.0 ± 0.9	4.6 ± 1.4	—

Results are shown in terms of mean ± S.E.M. Latency and interval data are in minutes. Levels of statistical significance: *$p<0.02$; †$p<0.001$.

TABLE 3
PENILE REFLEXES AFTER INTRATHECAL LUMBOSACRAL INJECTION OF THIP

	Experiment 3A						Experiment 3B	
THIP (μg/5 μl)	0	0.5	0	1.0	0	2.0	0	1.0
No. of subjects*	7		8		6		8	8
Penile reflex responders	3/7	4/7	4/8	6/8	5/6	3/6	3/8	5/8
Penile body erection latency	11.8 ± 3.1	10.9 ± 3.3	13.8 ± 2.5	7.9 ± 2.1	8.9 ± 2.6	11.7 ± 3.7	15.0 ± 2.9	9.7 ± 2.4
Glans erection latency	13.9 ± 3.1	11.9 ± 3.2	14.7 ± 2.3	10.2 ± 2.3	9.1 ± 2.5	12.2 ± 3.5	15.0 ± 2.9	11.2 ± 2.6
No. of penile body erections	1.7 ± 0.8	3.0 ± 1.4	3.0 ± 1.2	3.6 ± 1.2	2.8 ± 1.0	2.3 ± 1.5	1.0 ± 0.9	1.8 ± 0.6
No. of glans erections	5.9 ± 3.0	11.1 ± 4.1	6.4 ± 3.1	12.3 ± 4.0[a]	14.3 ± 4.5	5.8 ± 2.9[a]	4.0 ± 2.5	6.9 ± 2.9
No. of response clusters	2.3 ± 1.1	2.7 ± 1.0	1.9 ± 0.9	2.4 ± 0.7	3.9 ± 1.0	2.2 ± 1.1	1.5 ± 0.9	2.4 ± 0.9

Results are shown in terms of mean ± S.E.M. Latency data are in minutes. Significance level: [a]$p<0.05$.
*In Experiment 3A comparisons between vehicle and drug dose were based on repeated measures. However, vehicle and drug groups in Experiment 3B were independent.

RESULTS

The results of intrathecal baclofen administration on copulatory behavior and penile reflex tests are summarized in Table 2. Relative to a thoracic vehicle injection, baclofen administered onto either thoracic or lumbosacral segments decreased the number of intromissions to ejaculation, $F(2,25)=5.28$, $p<0.02$. No other copulatory measures were significantly affected. As in Experiment 1, intrathecal injection of 0.8 μg of baclofen aimed at the lumbosacral spinal cord completely eliminated the display of penile erections (see Table 2). In contrast, the proportion of animals responding with a glans erection was not affected by a thoracic intrathecal injection of baclofen, and none of the measures of penile reflexes was affected significantly.

EXPERIMENT 3

Results from the two previous experiments demonstrated that injections of baclofen into the subarachnoid space of the lumbosacral cord inhibit the display of penile glans erections. In the absence of a well-documented selective $GABA_B$ receptor antagonist (see the Discussion section), our approach to determining the pharmacological specificity of baclofen's inhibition to the $GABA_B$ receptor was to assess the relative role of $GABA_A$ receptor stimulation. The purpose of the following experiment was to test the effects of THIP, a potent and specific $GABA_A$ receptor agonist, on ex copula penile responses. We did not expect THIP to affect penile responses because systemic injection of $GABA_A$ receptor agonists had not reliably affected copulation or penile reflexes (21).

Experimental Design and Procedure

Twenty-one sexually experienced male rats which responded positively on two preoperative reflex tests were implanted with an intrathecal cannula terminating at L5–L6 spinal segments. One week following cannula implantation, males were randomly assigned to one of three THIP dose groups: 0.5, 1, or 2 μg. In a repeated measures design (Experiment 3A), approximately half the animals in each group received the drug and the others received an equal volume of vehicle. Penile reflex tests were conducted 15 min following the intrathecal injection. One week later, treatment schedules were reversed so that each animal served as his own control. Given the unexpected results following the administration of 1 μg THIP (see below), 16 animals were reassigned to one of two groups (vehicle or 1.0 μg THIP) and retested for penile reflexes (Experiment 3B). Males were matched for prior drug treatment.

Statistical Analysis

Results from Experiment 3A were analyzed using the Wilcoxon matched-pairs signed-ranks test (vehicle vs. THIP) for each dose. Data from Experiment 3B were analyzed using the Mann-Whitney U-test. Two-tailed probabilities of 5% or less were considered significant.

RESULTS

Experiment 3A

The proportion of males responding in penile reflex tests following intrathecal injection of THIP at each of the three doses tested did not differ reliably from the proportion of responders following a vehicle injection (Table 3). A dose of 0.5 μg THIP did not affect any measure of penile responses. However, an increased number of glans erections occurred following the administration of 1 μg THIP ($p<0.05$). In contrast, 2 μg THIP decreased the number of glans erections and the number of erections per cluster ($p<0.05$). No other measures were significantly affected.

Experiment 3B

Males treated with 1 μg THIP did not differ from males treated with the saline vehicle on any measure of penile reflexes.

GENERAL DISCUSSION

The results of the present experiments corroborate and extend previous findings from this laboratory which demonstrated inhibitory effects on penile reflexes following the systemic administration of baclofen, but not muscimol or THIP (21). Intrathecal injection of baclofen, a potent and specific $GABA_B$ receptor agonist (8), aimed at the lumbosacral spinal cord produced a dose-related inhibition of penile reflexes, at doses which did not reliably affect copulatory behavior. Copulatory activity immediately preceding a penile reflex test, which facilitated erectile responses of vehicle-treated animals, reduced the inhibitory effects of baclofen. The effects of baclofen on penile reflexes were selective to the lumbosacral spinal cord: Intrathecal injections of baclofen onto the thoracic cord did not affect erectile responses evoked following copulation. Finally, the intrathecal administration of THIP, a potent and specific $GABA_A$ receptor agonist (18), had little effect on penile reflexes. These data support the hypothesis that activity at GABAergic synapses in the lumbosacral spinal cord, and stimulation of $GABA_B$ receptors in particular, inhibit the expression of penile reflexes elicited ex copula.

The occurrence of penile reflexes in supine males depends on the tonic stimulation at the base of the glans caused by the retracted preputial sheath (22). Tactile stimuli from the penis reaches the spinal cord via the dorsal penile nerve (DPN) (29). Erections of the glans penis are augmented by contractions of the ventral bulbospongiosus (=bulbocavernosus) muscle (16,32). Somatic efferents which innervate this muscle originate in the dorsomediolateral nucleus (i.e., spinal nucleus of the bulbocavernous, SNB) of spinal segments L5–L6 (6,38). Thus, the deficits in erectile responses from baclofen administration onto the lumbosacral spinal cord may have resulted from inhibition of: 1) afferent stimulation from the penis; 2) somatic motor efferents mediating contraction of perineal muscles; 3) visceral motor efferents that mediate penile vascular mechanisms (3). These alternatives are not mutually exclusive.

With regard to the first possibility, inhibition of primary afferents in the spinal cord occurs as a result of GABAergic presynaptic inhibition (10). Penile reflexes are eliminated by topical application of a local anesthetic to the glans penis (40) or by transections of the DPN (35). Some DPN afferents terminate in the vicinity of the SNB, and some of these projections may be monosynaptic (26). Thus, GABA may decrease erectile responsiveness by inhibiting primary sensory afference from the the penis. This hypothesis is supported by the following observations: 1) dense GABA-immunoreactive cell bodies and terminals are found in the marginal zone of the dorsal horn (23); 2) there is a high density of $GABA_B$ binding sites, relative to $GABA_A$, in the substantia gelatinosa (5); and 3) baclofen, a $GABA_B$ agonist, is more effective than THIP, a $GABA_A$ agonist, in inhibiting penile reflexes [(21) and present experiments].

The evidence in favor of the second possible mechanism, namely that baclofen inhibited motor efferents whose activity regulates contractions of perineal muscles, lies in the observation that GABA-immunoreactive terminals have been visualized in the ventral horn, in the vicinity of the SNB (23). In addition, $GABA_B$ binding sites were detected in the ventral horn, although their number there was much lower than in the dorsal horn (5). Thus, the neuroanatomical substrate is present for such an inhibitory influence to occur.

The third hypothesis posits that inhibition of autonomic efferents mediated the effects of baclofen on penile reflexes. Indeed, GABA-immunoreactive fibers have been described in the intermediolateral cell column of the lumbosacral cord (23), an area rich in preganglionic parasympathetic nuclei that give rise to the pelvic nerve (14,24). Stimulation of parasympathetic pelvic nerve fibers causes vasodilation of the penile arteries and distention of the corpus cavernosum (7,39). Thus, GABAergic inhibition of visceral efferents may underlie the inhibitory effects observed following the intrathecal administration of baclofen. Our observations provide only partial support for this hypothesis. Following high doses of baclofen (0.4 and 0.8 μg), the proportion of animals displaying penile body erections and glans erections was decreased. However, a smaller dose of baclofen (0.2 μg) reduced the number of glans erections, but not the number of penile body erections. Thus, the somatic components of penile erection that depend on the contraction of the bulbospongiosus muscle are apparently more sensitive to baclofen than are the vascular components.

We must also consider that baclofen may have effects, such as the inhibition of penile reflexes, that are not mediated by $GABA_B$ receptors. Similar questions have been raised with regard to, for example, baclofen-induced spinal analgesia (37), and potent reduction in monosynaptic excitation in the cat spinal cord (9). In order to test whether the inhibitory effects of baclofen on penile reflexes are mediated by activation of the $GABA_B$ receptor, the efficacy of $GABA_B$ receptor blockers in attenuating this response must be assessed. Two compounds, 5-aminovaleric acid and homotaurine, have been described as $GABA_B$ antagonists (13,25). In pilot studies, the systemic administration of 5-aminovaleric acid (20 mg/kg, IP) did not affect penile reflexes, nor did it reverse the inhibitory effects of systemic baclofen administration (S. Miller, Bitran, McQuade and Sachs; unpublished observations). These results must be tempered by the finding that 5-aminovaleric acid is also a weak $GABA_A$ receptor agonist (19). That stimulation of the $GABA_A$ receptor plays little, if any, role in the mediation of penile reflexes is supported by our finding that intrathecal injection of THIP, a specific $GABA_A$ receptor agonist, had weak and unreliable effects on penile responses. Thus, presumptive stimulation of the $GABA_A$ receptor does not produce dramatic differences in erectile responsiveness, as does stimulation of the $GABA_B$ receptor.

The finding that copulation prior to penile reflex testing attenuated the effects of baclofen (except at the highest dose tested) is puzzling in view of the dramatic increase of GABA in the cisternal CSF following ejaculation (30). Presumably, the penile reflex tests that we conducted following the postejaculatory interval were carried out at a time when GABA levels in the CSF were much elevated (30). Accordingly, one would predict that baclofen would have been more, rather than less, effective in inhibiting penile reflexes following copulation. Perhaps regional differences in CSF concentrations of GABA after ejaculation underlie these observations.

The inhibitory effects of baclofen on penile reflexes are not likely to reflect a nonspecific reaction to the drug. The negligible effect of baclofen on copulatory behavior, at doses which disrupted ex copula penile responses, is evidence that the drug treatment did not produce a general state of malaise or discomfort. Deficits in copulatory behavior have been observed following the systemic administration of baclofen, but only at doses which resulted in motoric incoordination (28).

The relation between the effect of baclofen on ex copula penile responses and penile events during copulation re-

mains to be determined. All animals in our studies showed motor patterns associated with intromission and ejaculation, but it is not known whether males had normal erections upon intromission. Intense penile erections are a prerequisite for proper placement of the ejaculatory plug against the vaginal cervix. This in turn facilitates sperm transport into the uterine horns [see (15,33) for references and further discussion]. Thus it is possible that baclofen-treated males may exhibit normal motoric patterns associated with copulatory activity and even gain vaginal insertion and ejaculation (21), but that their fertility is compromised. Studies addressing this possibility are currently under way.

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REFERENCES

1. Ågmo, A.; Paredes, R. GABAergic drugs and sexual behavior in the male rat. Eur. J. Pharmacol. 112:371–378; 1985.
2. Barfield, R. J.; Auerbach, P.; Geyer, L. A.; McIntosh, T. K. Ultrasonic vocalizations in rat sexual behavior. Am. Zool. 19:469–480; 1979.
3. Benson, G. S. Male sexual function: erection, emission, and ejaculation. In: Knobil, E.; Neill, J. D., eds. The physiology of reproduction. vol. 1. New York: Raven Press; 1988:1121–1141.
4. Bitran, D.; Hull, E. M. Pharmacological analysis of male rat sexual behavior. Neurosci. Biobehav. Rev. 11:365–389; 1987.
5. Bowery, N. G.; Hudson, A. L.; Price, G. W. $GABA_A$ and $GABA_B$ receptor site distribution in the rat central nervous system. Neuroscience 20:365–383; 1987.
6. Breedlove, S. M.; Arnold, A. P. Hormone accumulation in a sexually dimorphic motor nucleus of the rat spinal cord. Science 227:564–566; 1980.
7. Carati, C. J.; Creed, K. E.; Keogh, E. J. Autonomic control of penile erection in the dog. J. Physiol. 384:525–538; 1987.
8. Curtis, D. R.; Game, C. J. A.; Johnston, G. A. R.; McCullogh, R. M. Central effects of β-(*p*-chlorophenyl)-gamma-aminobutyric acid. Brain Res. 70:493–499; 1974.
9. Curtis, D. R.; Lodge, D.; Bornstein, J. C.; Peet, M. J. Selective effects of (−)-baclofen on spinal synaptic transmission in the cat. Exp. Brain Res. 42:158–170; 1981.
10. Davidoff, R. A.; Hackman, J. C. GABA: Presynaptic actions. In: Rogawski, M. A.; Baker, J. L., eds. Neurotransmitter action in the vertebrate nervous system. New York: Plenum Press; 1985:3–31.
11. Fernández-Guasti, A.; Larsson, K.; Beyer, C. GABAergic control of masculine sexual behavior. Pharmacol. Biochem. Behav. 24:1065–1070; 1986.
12. Fernández-Guasti, A.; Larsson, K.; Vega-Sanabria, J. Depression of postejaculatory ultrasonic vocalization by (+)-bicuculline. Behav. Brain Res. 19:35–39; 1986.
13. Giotti, A.; Luzzi, S.; Spagnesi, S.; Ziletti, L. GABAA and GABAB receptor-mediated effects in guinea pig ileum. Br. J. Pharmacol. 79:855–862; 1983.
14. Hancock, M. B.; Peveto, C. A. Preganglionic neurons in the sacral spinal cord of the rat: An HRP study. Neurosci. Lett. 11:1–5; 1979.
15. Hart, B. L. Role of testosterone secretion and penile reflexes in sexual behavior and sperm competition in male rats: A theoretical contribution. Physiol. Behav. 31:823–827; 1983.
16. Hart, B. L.; Melese-d'Hospital, P. Y. Penile mechanisms and the role of the striated penile muscles in penile reflexes. Physiol. Behav. 31:807–813; 1983.
17. Holmes, G. M.; Holmes, D. G.; Sachs, B. D. An IBM-PC based data collection system for recording rodent sexual behavior and for general event recording. Physiol. Behav. 44:825–828; 1988.
18. Krogsgaard-Larsen, P. THIP, a specific and clinically active GABA agonist. Neuropharmacology 23:837–838; 1984.
19. Krogsgaard-Larsen, P.; Christensen, A. V. GABA agonists and antagonists. Annu. Rep. Med. Chem. 15:41–50; 1980.
20. Kurtz, R. G.; Santos, R. Supraspinal influences on the penile reflexes of the male rat. A comparison of the effects of copulation, spinal transection, and cortical spreading depression. Horm. Behav. 12:73–94; 1979.
21. Leipheimer, R. E.; Sachs, B. D. GABAergic regulation of penile reflexes and copulation in rats. Physiol. Behav. 42:351–357; 1988.
22. Lumia, A. R.; Sachs, B. D.; Meisel, R. L. Sexual reflexes in male rats: Restoration by ejaculation following suppression by penile sheath removal. Physiol. Behav. 23:273–277; 1979.
23. Magoul, R.; Oteniente, B.; Geffard, M.; Calas, A. Anatomical distribution and ultrastructural organization of the GABAergic system in the rat spinal cord. An immunocytochemical study using anti-GABA antibodies. Neuroscience 20:1001–1009; 1987.
24. Nadelhaft, I.; Booth, A. M. The location and morphology of preganglionic neurons and the distribution of visceral afferents from the rat pelvic nerve: A horseradish peroxidase study. J. Comp. Neurol. 226:238–245; 1984.
25. Nakahiro, M.; Saito, K.; Yamada, I.; Yoshida, H. Antagonistic effect of delta-aminovaleric acid on bicuculline-insensitive gamma-amino butyric acid B (GABA B) sites in the rat's brain. Neurosci. Lett. 57:263–266; 1985.
26. Núñez, R.; Gross, G.; Sachs, B. D. Origin and central projections of rat dorsal penile nerve: Possible direct projection to autonomic and somatic neurons by primary afferents of non-muscle origin. J. Comp. Neurol. 247:417–429; 1986.
27. O'Hanlon, J. K.; Sachs, B. D. Penile reflexes in rats after different numbers of ejaculations. Behav. Neural Biol. 29:338–348; 1980.
28. Paredes, R.; Ågmo, A. Stereospecific actions of baclofen on sexual behavior, locomotor activity and motor execution. 19th Conference on Reproductive Behavior. Tlaxcala, Mexico, 1987.
29. Purinton, P. T.; Fletcher, T. F.; Bradley, W. E. Gross and light microscopic features of the pelvic plexus in the rat. Anat. Rec. 175:697–706; 1973.
30. Qureshi, G. A.; Södersten, P. Sexual activity alters the concentration of amino acids in the cerebralspinal fluid of male rats. Neurosci. Lett. 70:374–378; 1986.
31. Roberts, E. GABA: The road to neurotransmitter status. In: Olsen, R. W.; Venter, J. C., eds. Benzodiazepine/GABA receptors and chloride channels: Structural and functional properties. New York: Alan R. Liss; 1986:1–39.
32. Sachs, B. D. Role of striated penile muscles in penile reflexes, copulation, and induction of pregnancy in the rat. J. Reprod. Fertil. 66:433–443; 1982.

33. Sachs, B. D. Potency and fertility. Hormonal and mechanical causes and effects of penile actions of rats. In: Balthazart, J.; Pröve, E.; Gilles, R., eds. Hormones and behaviour in higher vertebrates. Berlin: Springler-Verlag; 1983:86–110.
34. Sachs, B. D.; Garinello, L. D. Interaction between penile reflexes and copulation in male rats. J. Comp. Physiol. Psychol. 92:759–767; 1978.
35. Sachs, B. D.; Garinello, L. D. Hypothetical spinal pacemaker regulating penile reflexes in rats: evidence from transection of spinal cord and dorsal penile nerves. J. Comp. Physiol. Psychol. 94:530–535; 1980.
36. Sachs, B. D.; Leipheimer, R. E. EMG activity of the bulbocavernosus muscle of the rat during copulation and penile reflexes ex copula. 18th Annual Conference on Reproductive Behavior, Montreal, Canada,1986.
37. Sawynok, J. GABAergic mechanisms of analgesia: An update. Pharmacol. Biochem. Behav. 26:463–474; 1987.
38. Schrøder, H. D. Organization of the motoneurons innervating the pelvic muscles of the male rat. J. Comp. Neurol. 192:567–587; 1980.
39. Sjöstrand, N. O.; Klinge, E. Principal mechanisms controlling penile retraction and protrusion in rabbits. Acta Physiol. Scand. 106:199–214; 1979.
40. Stefanick, M. L.; Smith, E. R.; Davidson, J. M. Penile reflexes in intact rats following anesthetization of the penis and ejaculation. Physiol. Behav. 31:63–65; 1983.
41. Yaksh, T.; Rudy, T. Chronic catheterization of the spinal subarachnoid space. Physiol. Behav. 17:1031–1036; 1976.