Pharmacology Biochemistry & Behavior, Vol. 38, pp. 781–788. © Pergamon Press plc, 1991. Printed in the U.S.A. 0091-3057/91 $3.00 + .00

Benzodiazepine Receptor Ligands and Sexual Behavior in the Male Rat: The Role of GABAergic Mechanisms

ANDERS ÅGMO[1] AND HILDA FERNÁNDEZ

Department of Psychology, Universidad Anáhuac, Mexico City

Received 5 December 1989

ÅGMO, A. AND H. FERNÁNDEZ. *Benzodiazepine receptor ligands and sexual behavior in the male rat: The role of GABAergic mechanisms*. PHARMACOL BIOCHEM BEHAV **38**(4) 781–788, 1991.—Diazepam and chlordiazepoxide produced a dose-dependent inhibition of ambulatory activity, motor execution and sexual behavior. The benzodiazepine antagonist flumazenil had no effect on these behaviors, while the inverse agonist FG 7142 inhibited sexual behavior without affecting motor functions. The GABA antagonist bicuculline was ineffective in all behavioral paradigms, while picrotoxin inhibited all behaviours. Picrotoxin blocked the motor effects of low doses of the benzodiazepines, but not those of higher doses. Neither did this drug block the effects of benzodiazepines on sexual behavior. Bicuculline was unable to block the effects of benzodiazepines on all behaviors. FG 7142, in a low dose, inhibited the effects of diazepam and chlordiazepoxide on ambulatory activity, but not their effects on motor execution or sexual behavior. The effects of the benzodiazepines and picrotoxin on sexual behavior could be a consequence of the motor impairment produced by these drugs, since the doses required to affect these two behaviors were similar. However, the fact that picrotoxin could block the motor deficiencies induced by the benzodiazepines without restoring sexual behavior suggests that these behavioral actions of the drugs can be differentiated. While some evidence was obtained suggesting a role of GABA in the motor effects of benzodiazepines, no evidence could be found for a role of GABA in their effects on sexual behavior.

Benzodiazepines GABA Sexual behavior Ambulatory activity Motor execution

DURING the last few years, the GABAergic control of male sexual behavior has been the subject of several studies. It appears that the GABA-B receptor agonist baclofen inhibits that behavior independently of actions on motor systems (3). A detailed study of the effects of baclofen on sociosexual interaction showed that this drug specifically inhibits precopulatory behaviors (26). Low doses of baclofen also inhibit penile reflexes ex copula, without affecting sexual behavior (19).

The role of the GABA-A receptor is less clear. Although it has been shown that the GABA-A antagonist bicuculline reduces the postejaculatory and the interintromission interval after infusion into the medial preoptic area (11), GABA-A agonists, such as THIP or 3-aminopropanesulfonic acid or GABA transaminase inhibitors, do not have the opposite effect (3). Rather, they produce a nonspecific suppression of sexual behavior. It has even been suggested that the inhibitory effects on sexual behavior of this kind of drugs are a consequence of their motor actions (4).

Since benzodiazepines are believed to facilitate GABAergic neurotransmission through an interaction with GABA-A receptors [reviewed in (10, 25, 29)], it was considered of interest to study the effects of this class of compounds on male sexual behavior. The purpose of the present experiments, therefore, was to evaluate the role of the benzodiazepine binding site in the control of male rat sexual behavior, and the possible involvement of GABAergic mechanisms in the actions of benzodiazepines. The effects of several doses of the benzodiazepines diazepam and chlordiazepoxide on male rat sexual behavior were evaluated. These drugs were then combined with the GABA antagonists bicuculline and picrotoxin, for the purpose of determining the GABAergic involvement in their actions. Additionally, the effects of the GABA antagonists alone on sexual behavior were determined. The effects of an inverse benzodiazepine agonist were also evaluated. In order to determine the specificity of the actions of the drugs on sexual behavior, their effects on ambulatory activity and motor execution were also studied.

METHOD

Subjects

Male Wistar rats (300–400 g) from a local colony were housed under a reversed light/dark cycle (12/12 h) in a room with a constant temperature (22°C) and given commercial rat pellets and water ad lib.

Animals to be used in tests for sexual behavior were subjected

[1]Requests for reprints should be addressed to Anders Ågmo, Escuela de Psicologia, Universidad Anáhuac, Apdo. Postal 10-844, 11000 Mexico, D.F., Mexico.

to three preliminary mating tests of 30 min duration. Only animals that ejaculated at least once in these tests were included in the study. Since benzodiazepines affect testicular androgen production (31), and since changes in testosterone levels have been shown to have short-term effects on sexual behaviour (22), the animals were castrated under ether anesthesia and subcutaneously implanted with a 20 mm long Silastic capsule (0.062 in. i.d.; 0.125 in. o.d.; Dow Corning Corp.) filled with crystalline testosterone (Sigma). Such an implant has been shown to maintain sexual behavior similar to that of intact rats for several months (9). Experiments were initiated about one week after castration.

Males employed in tests of locomotor activity and motor execution were left intact. Unpublished data have shown that drug effects on these behaviors appear to be similar in intact and in castrated, testosterone-implanted males.

Females used in tests for sexual behavior were ovariectomized at least two weeks before use and subcutaneously injected with estradiol benzoate (25 μg/rat; Sigma) 52–56 h before tests and with progesterone (1 mg/rat; Aldrich) 4–6 h before tests. The steroids were dissolved in corn oil and injected in a volume of 0.2 ml/rat.

Procedure

Sexual behavior was evaluated in rectangular observation cages ($40 \times 60 \times 30$ cm high). The male was introduced into the cage where a receptive female had already been placed. The following parameters of sexual behavior were registered: *Mount latency*, time from introduction of the male until first mount with pelvic thrusting; *intromission latency*, time from introduction of the male until first mount with vaginal penetration; *ejaculation latency*, time from the first intromission until ejaculation; *postejaculatory interval*, time from ejaculation until the following intromission; *number of mounts* during the test or until ejaculation; *number of intromissions* during the test or until ejaculation. The mating test was ended at the end of the postejaculatory interval or 15 min after introduction of the male if no ejaculation had occurred. This test duration has been found adequate to detect drug effects on sexual behavior in several studies (1, 3, 4).

Ambulatory activity was quantified in a circular arena (diameter 60 cm) surrounded by a 37.5 cm high wall. Six photocells covered by infrared filters were located around the wall, 2.5 cm above the grid floor. The number of photobeam interruptions during the 10 min following introduction of the male constituted the measure of ambulatory activity. Before drug treatments, the animals were habituated to the activity cages during three 10-min sessions, separated by at least 48 h.

Motor execution was evaluated using a treadmill (rotarod). The animals were placed on a cylinder (diameter 16 cm) rotating at 11 rpm. Whenever an animal fell down, it was replaced on the cylinder. The number of falls during a 3-min test was counted. Before experiments, animals were trained to walk on the cylinder during a 15-min session. The animals that fell down more than 3 times during the last 5 min were eliminated.

All behavioral tests were performed between the 3rd and the 6th h of the dark period under dim white light. The procedures used have been described in detail before (1,4).

Drugs

Chlordiazepoxide (CDP in the tables; Roche de Mexico) was dissolved in distilled water, while diazepam and flumazenil (Hoffmann-La Roche) were suspended in distilled water to which two drops of Tween 80 had been added. The inverse agonist N-methyl β-carboline-3-carboxamide (FG 7142; Research Biochemicals) was suspended in the same way. Picrotoxin (Sigma) was dissolved in hot physiological saline. Bicuculline (Sigma) was dissolved in hot physiological saline to which one drop of glacial acetic acid had been added. The solution was then cooled and kept in ice until injection. All drugs were administered intraperitoneally in a volume of 1 ml/kg b.wt. (chlordiazepoxide, picrotoxin and bicuculline) or 2 ml/kg b.wt. (diazepam, flumazenil and FG 7142). The interval between drug injection and behavioral observation was 30 min for chlordiazepoxide, diazepam and flumazenil, 20 min for FG 7142 and 10 min for bicuculline and picrotoxin.

Control treatments always consisted of the appropriate vehicle, administered the same time before behavioral observation as the respective drug.

Experimental Design

In tests for sexual behavior, drugs were administered according to a latin square design. In that way, each animal received all the doses of a given drug, or all combinations of drugs in a given experiment. Upon all sessions, all doses of a drug, or all combinations of drugs, were administered to approximately equal numbers of animals. No subject was used in more than one experiment.

In tests of locomotor activity or motor execution, a counterbalanced design was used. On the first experimental session, half of the animals in the group were given drug and the other half the appropriate vehicle. The following session, treatments were reversed. No subject was used in more than one experiment.

Experimental sessions were separated by 7 days. This interval should be sufficient to avoid drug effects from the previous session being carried over. Separate groups of animals were used for sexual behavior, ambulatory activity and motor execution tests.

Statistical Analysis

Data from experiments on sexual behavior were evaluated with the following tests: The Cochran Q-test followed by the McNemar's test for the significance of changes, or the binomial test where appropriate, for the proportion of animals displaying mounts, intromissions and ejaculation; Friedman's two-way ANOVA followed by the Wilcoxon matched-pairs signed-ranks test for number of mounts and intromissions; Kruskal-Wallis ANOVA followed by the Mann-Whitney U-test for the latencies and the postejaculatory interval (these parameters were not always registered from all animals, making the use of tests for independent groups necessary). Motor execution was analyzed with the Wilcoxon test.

Nonparametric tests were used to analyze the sex behavior and motor execution data since the distribution deviated considerably from normality and error variances were not homogenous. Ambulatory activity was evaluated with the *t*-test for repeated measures because data appeared to be normally distributed. When several doses of the same drug was administered, the significance level for all *t*-tests was corrected with the Bonferroni procedure. All probabilities given are two-tailed.

RESULTS

Ambulatory Activity

As can be seen in Fig. 1, both benzodiazepines caused a dose-dependent reduction of locomotor activity. The antagonist flumazenil as well as the inverse agonist FG 7142 were without effect in the dose range employed. The GABA antagonist bicuculline was also without effect, while picrotoxin produced a dose-dependent reduction in locomotor activity, the lowest effective dose

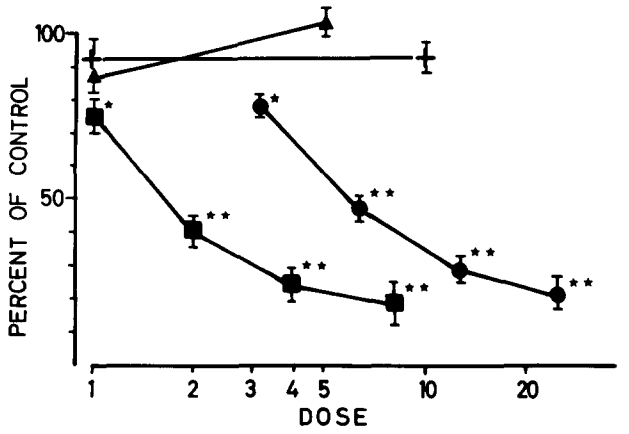

FIG. 1. Ambulatory activity in male rats treated with benzodiazepine agonists and antagonists. N = 9–11 per dose. Control activity counts varied between 131.5 ± 13.13 and 217.8 ± 28.87 (mean ± S.E.). Doses in mg/kg. ○, Chlordiazepoxide; □, diazepam; △, flumazenil; ×, FG 7142. ★, Different from vehicle, $p<0.05$, ★★, $p<0.01$.

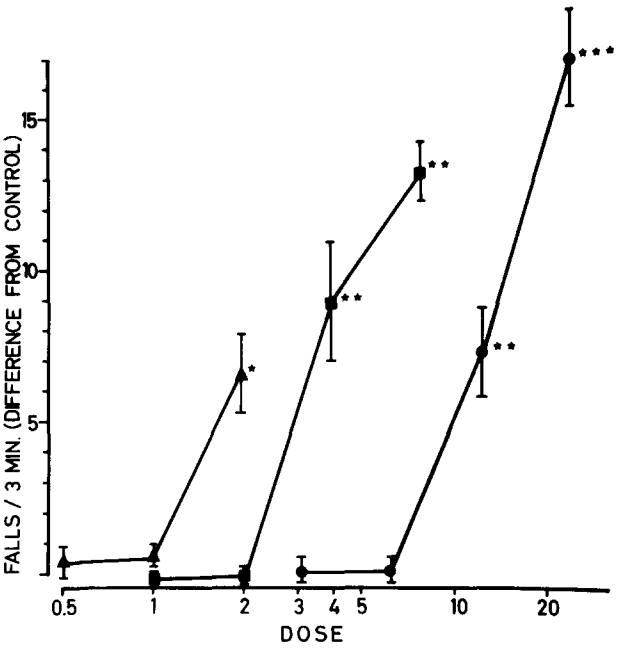

FIG. 2. Motor execution in male rats treated with chlordiazepoxide and diazepam, and with the GABA antagonist picrotoxin. N = 10 per dose. The number of falls in the vehicle-treated animals varied between 0 and 3. Doses in mg/kg. ○, Chlordiazepoxide; □, diazepam; △, picrotoxin. ★, Different from control, $p<0.05$, ★★, $p<0.01$, ★★★, $p<0.001$.

being 1 mg/kg (data not shown).

The inhibitory effect of both diazepam (1 mg/kg) and chlordiazepoxide (3.125 mg/kg) could be blocked by concurrent administration of picrotoxin (0.5 mg/kg), by flumazenil (10 mg/kg) and by FG 7142 (1 mg/kg), but not by bicuculline (2 mg/kg) (data not shown). An effort was then made to block the effects of chlordiazepoxide (6.25 mg/kg) with picrotoxin. Neither 0.5 mg/kg nor 1 mg/kg of the latter drug was effective (data not shown).

Motor Execution

The benzodiazepines impaired motor execution in a dose-dependent way, the minimum effective dose of diazepam being 4 mg/kg and of chlordiazepoxide 12.5 mg/kg. Picrotoxin also impaired motor execution when given in a dose of 2 mg/kg (Fig. 2).

TABLE 1

ANTAGONISM BY GABA AND BENZODIAZEPINE ANTAGONISTS OF THE EFFECTS OF CHLORDIAZEPOXIDE (12.5 mg/kg) AND DIAZEPAM (4 mg/kg) ON MOTOR EXECUTION

Treatment	Falls/3 min	Treatment	Falls/3 min
Vehicle + vehicle	1.0 ± 0.52	Vehicle + vehicle	3.4 ± 1.09
CDP + vehicle	8.7 ± 1.57‡	Diazepam + vehicle	12.5 ± 3.33†
Vehicle + vehicle	3.0 ± 1.18	Vehicle + vehicle	0.1 ± 0.1
CDP + picrotoxin 0.5	4.4 ± 1.28	Diazepam + picrotoxin 0.5	0.9 ± 0.26
Vehicle + vehicle	2.0 ± 0.96	Vehicle + vehicle	2.6 ± 0.82
CDP + picrotoxin 1	0.7 ± 0.21	Diazepam + picrotoxin 1	3.3 ± 1.54
Vehicle + vehicle	0.3 ± 0.24	Vehicle + vehicle	0.6 ± 0.34
CDP + bicuculline 1	7.6 ± 1.88†	Diazepam + bicuculline 1	8.9 ± 2.1†
Vehicle + vehicle	0.1 ± 0.1	Vehicle + vehicle	0.3 ± 0.24
CDP + bicuculline 2	7.8 ± 1.08†	Diazepam + bicuculline 2	4.0 ± 1.75*
Vehicle + vehicle	3.5 ± 1.20	Vehicle + vehicle	1.8 ± 0.36
CDP + flumazenil 10	4.3 ± 2.06	Diazepam + flumazenil 10	2.1 ± 0.48
Vehicle + vehicle	1.6 ± 1.19	Vehicle + vehicle	0.4 ± 0.31
CDP + FG 7142 1	5.9 ± 2.02*	Diazepam + FG 7142 1	4.2 ± 1.36*

*Different from vehicle + vehicle, $p<0.05$, †$p<0.01$, ‡$p<0.001$.
Ten animals per group. Data are means ± S.E. Doses in mg/kg.

TABLE 2

PARAMETERS OF SEXUAL BEHAVIOR IN MALE RATS TREATED WITH VARYING DOSES OF CHLORDIAZEPOXIDE

Behavior Parameter	Vehicle	CDP 3.125	CDP 6.25	CDP 12.5	CDP 25
Mount percentage	88	94	88	31†	31†
Intromission percentage	88	94	69	19†	6†
Ejaculation percentage	56	38	31	0†	6†
Mount latency[b]	0.6±0.10	1.5±0.62	2.1±0.88	4.1±1.43†	2.7±1.70
Intromission latency[b]	0.8±0.18	3.0±0.90	3.8±0.96†	10.2±2.51†	—[a]
Postejaculatory interval[b]	6.2±0.43	8.9±0.61*	7.5±0.51	—[a]	—[a]
Number of mounts	5.5±1.6	8.7±1.38	8.2±1.83	1.9±1.05*	0.8±0.39†
Number of intromissions	7.8±0.94	5.6±0.80	3.1±0.69†	0.3±0.20†	0.4±0.44†

*Different from vehicle, $p<0.05$, †$p<0.01$. [a]Data obtained from 0–1 animal. [b]Only animals that displayed the behaviors are included.

N = 16. Data are means ± S.E. Doses in mg/kg.

Bicuculline had no effect in doses up to 2 mg/kg. Higher doses were not tried, because of the high incidence of convulsions. FG 7142 and flumazenil were also ineffective in doses of 5 and 10 mg/kg, respectively (data not shown for these latter drugs).

The motor impairment produced by chlordiazepoxide (12.5 mg/kg) and by diazepam (4 mg/kg) was blocked by picrotoxin (0.5 or 1 mg/kg), by flumazenil (10 mg/kg) but not by bicuculline (1 or 2 mg/kg) nor by FG 7142 (1 mg/kg) (Table 1). The effects of higher doses of the benzodiazepines were not blocked by picrotoxin (data not shown).

Sexual Behavior

None of the treatments affected ejaculation latency. This pa-

TABLE 3

PARAMETERS OF SEXUAL BEHAVIOR IN MALE RATS TREATED WITH VARYING DOSES OF DIAZEPAM

Behavior Parameter	Vehicle	Diazepam 1	Diazepam 2	Diazepam 4	Diazepam 8
Mount percentage	94	94	75	25*	25[redacted]
Intromission percentage	81	69	56	19*	13*
Ejaculation percentage	56	44	31	6*	13*
Mount latency[b]	2.0±0.95	2.2±0.94	3.3±1.21	1.7±0.36	0.7±0.56
Intromission latency[b]	1.1±0.31	2.4±0.82	3.6±1.74	8.7±1.84	5.6±3.28
Postejaculatory interval[b]	6.4±0.78	6.8±0.36	8.0±1.09	—[a]	5.7±0.77
Number of mounts	7.1±1.08	9.4±1.73	5.0±1.05	2.2±1.25†	3.6±1.98†
Number of intromissions	7.8±1.39	5.2±1.20	3.4±1.04†	0.7±0.39†	0.6±0.43†

*Different from vehicle, $p<0.05$, †$p<0.01$. [a]Data obtained from 1 animal. [b]Only animals that displayed the behavior are included.

N = 16. Data are means ± S.E. Doses in mg/kg.

TABLE 4

SEXUAL BEHAVIOR IN MALE RATS TREATED WITH CHLORDIAZEPOXIDE 12.5 mg/kg IN COMBINATION WITH GABA AND BENZODIAZEPINE ANTAGONISTS

Behavior Parameter	Vehicle + Vehicle	CDP + Vehicle	CDP + Picrotoxin	CDP + Bicuculline	CDP + Flumazenil	CDP + FG 7142
Mount percentage	100	75	75	69	75	56*
Intromission percentage	88	25†	63	38†	63[b]	38*
Ejaculation percentage	56	19	6†	6†	31	13*
Mount latency[c]	1.6±0.56	3.7±1.01	3.1±0.93	2.0±0.73	2.5±1.15	2.4±1.02
Intromission latency[c]	1.5±0.42	4.5±1.23†	5.5±1.52†	2.0±1.13	2.1±0.92	7.4±1.99†
Postejaculatory interval[c]	5.7±0.46	13.4±2.57†	—[a]	—[a]	8.0±1.07	9.6±1.04*
Number of mounts	7.5±1.21	5.0±1.26	5.0±1.41	5.4±1.35	7.0±1.75	6.4±1.92
Number of intromissions	6.3±0.87	0.9±0.46†	1.1±0.38†	1.8±0.72†	3.4±0.84*[b]	1.2±0.44†

*Different from vehicle, $p<0.05$, †$p<0.01$. [a]Data obtained from 1 animal. [b]Different from CDP + vehicle, $p<0.05$. [c]Only animals that displayed the behavior are included.

N = 16. Data are means ± S.E. The doses of the antagonists were: picrotoxin, 1 mg/kg; bicuculline, 2 mg/kg; flumazenil, 10 mg/kg; FG 7142, 1 mg/kg.

rameter is therefore not further discussed nor shown in the tables.

Effects of chlordiazepoxide and diazepam. Both benzodiazepines inhibited sexual behavior in a dose-dependent fashion. The proportion of animals displaying mounts, intromissions and ejaculation was reduced after chlordiazepoxide 12.5 and 25 mg/kg, and after diazepam 4 and 8 mg/kg (Tables 2 and 3). An analysis of the parameters of sexual behaviour showed that chlordiazepoxide (3.125 mg/kg) prolonged the postejaculatory interval, and chlordiazepoxide (6.25 mg/kg) prolonged the intromission latency and reduced the number of intromissions. A higher dose (12.5

TABLE 5

SEXUAL BEHAVIOR IN MALE RATS TREATED WITH DIAZEPAM 4 mg/kg IN COMBINATION WITH GABA AND BENZODIAZEPINE ANTAGONISTS

Behavior Parameter	Vehicle + Vehicle	Diazepam + Vehicle	Diazepam + Picrotoxin	Diazepam + Bicuculline	Diazepam + Flumazenil	Diazepam + FG 7142
Mount percentage	69	39	31	15*	54	8†
Intromission percentage	62	31	15*	0†	39	0†
Ejaculation percentage	46	0*	0*	0*	23	23*
Mount latency[b]	1.6±0.49	5.0±2.10	1.7±0.57	0.8±0.04	1.8±0.57	—[a]
Intromission latency[b]	1.3±0.35	4.0±2.34	7.3±3.68	—[a]	3.6±1.88	—[a]
Postejaculatory interval[b]	8.9±1.92	—[a]	—[a]	—[a]	6.0±1.33	—[a]
Number of mounts	4.0±1.48	1.9±0.86	2.6±1.29	0.9±0.58	2.6±1.15	0.1±0.08*
Number of intromissions	3.9±1.10	0.8±0.43†	0.8±0.57*	0†	1.7±0.75	0†

*Different from vehicle, $p<0.05$, †$p<0.01$. [a]Data obtained from 0–1 animal. [b]Only animals that displayed the behaviors are included.

N = 13. Data are means ± S.E. The doses of the antagonists were the following: picrotoxin, 1 mg/kg; bicuculline, 2 mg/kg; flumazenil, 10 mg/kg; FG 7142, 1 mg/kg.

mg/kg) prolonged mount and intromission latencies and reduced the number of mounts and intromissions (Table 2). Diazepam had no effects on latencies, but 2 mg/kg reduced the number of intromissions, while higher doses reduced both the number of mounts and intromissions (Table 3).

Effects of bicuculline and picrotoxin. Bicuculline (1 and 2 mg/kg) lacked effect, while picrotoxin (2 mg/kg) reduced the proportion of animals displaying mounts and ejaculation, as well as the number of intromissions. Lower doses had no effects (data not shown).

Effects of flumazenil and FG 7142. The benzodiazepine antagonist flumazenil had no effect on sexual behavior in doses of 1 or 10 mg/kg. The inverse agonist FG 7142 had no effect in a dose of 1 mg/kg, but produced a considerable inhibition in a dose of 5 mg/kg. The proportion of animals displaying mounts, intromissions and ejaculation was reduced. Mount and intromission latencies were prolonged, while the number of mounts was reduced (data not shown).

Inhibition of the effects of chlordiazepoxide with GABA and benzodiazepine antagonists. An effort was made to block the slight inhibitory effect of chlordiazepoxide (6.25 mg/kg) with doses of GABA and benzodiazepine antagonists which lacked effects by themselves. It was found that the GABA antagonists inhibited the effects of this dose of chlordiazepoxide on 1 out of 3 affected parameters, while flumazenil and FG 7142 inhibited the effects on 2 out of 3 affected parameters (data not shown). Since these results were not very clearcut, although suggestive, it was decided to study the effects of the antagonists after a higher dose of chlordiazepoxide. In an additional experiment, 12.5 mg/kg of chlordiazepoxide was administered concurrently with the antagonists.

Table 4 summarizes the results of this experiment. Picrotoxin inhibited the effects of chlordiazepoxide on intromission percentage, but the ejaculation percentage, not significantly reduced after chlordiazepoxide + vehicle, was now reduced. Bicuculline inhibited the increase in intromission latency, but it reinforced the inhibitory effect of chlordiazepoxide on ejaculation percentage. Flumazenil blocked most of the inhibitory effects of chlordiazepoxide. Only the number of intromissions remained reduced, although it was significantly higher than after chlordiazepoxide + vehicle. FG 7142 did not block any of the effects of chlordiazepoxide.

Inhibition of the effects of diazepam with GABA and benzodiazepine antagonists. When the effects of a low dose of diazepam (2 mg/kg) were studied after concurrent administration of antagonists, very clear results emerged. Both picrotoxin and bicuculline not only failed to block the only significant effect produced by diazepam 2 mg/kg, a reduction of the number of intromissions, but their concurrent administration also produced a reduction in the number of mounts. Diazepam had no effect at all when combined with flumazenil, while diazepam + FG 7142 produced effects identical to those of diazepam + vehicle (data not shown).

A higher dose of diazepam (4 mg/kg) was then administered together with the antagonists. Again, picrotoxin and bicuculline seemed to reinforce the effects of diazepam rather than inhibit them. The same appeared to be the case with FG 7142. Flumazenil, however, blocked all effects of diazepam. Data are summarized in Table 5.

DISCUSSION

In agreement with previous studies in rodents and humans (7, 18, 23, 24, 38), benzodiazepines inhibited male sexual behavior. However, these drugs do not appear to be particularly potent inhibitors of that behavior. The doses required to produce a clearcut reduction of sexual behavior were four times those required to reduce ambulatory activity, and sufficiently high to produce deficiencies in motor execution. They are also well above those necessary for anticonflict effects. Using the Vogel procedure (40), we have found that 1 mg/kg of diazepam is an effective dose, as well as 2.5 mg/kg of chlordiazepoxide (Ågmo et al., unpublished observations). It is, therefore, not likely that the anxiolytic actions of benzodiazepines are of any importance for male sexual behavior. However, they could become of importance in stressful situations, as shown to be the case in stallions (20,21) and in talapoin monkeys (39). In these contexts, diazepam or midazolam were found to facilitate sexual interactions in rather low doses. The lack of facilitatory effects in rats could suggest that sexual interaction is not associated with stress in this species, at least under laboratory conditions.

In order to try to block the actions of benzodiazepines on sexual behavior with GABA antagonists, it was necessary to determine the effects of these latter agents when administered alone. We have previously reported bicuculline to be without effect in castrated animals maintained at a low sexual activity by weekly injections of testosterone propionate (3), while Fernandez-Guasti et al. (12) found that an infusion of bicuculline into the medial preoptic area stimulated sexual behavior in castrated rats shortly after injection of a high dose of testosterone. However, in the present studies, no stimulatory effect could be observed with bicuculline. Picrotoxin, on the other hand, reduced sexual activity. Since the dose required to observe this effect was such as to produce motor deficiencies, it is difficult to determine its specificity.

The benzodiazepine antagonist flumazenil was without effect on sexual behavior. This may suggest that possible endogenous benzodiazepine receptor ligands do not exert a tonic inhibition of this behavior. The inverse benzodiazepine agonist FG 7142 was found to have multiple effects on sexual activity, including increased mount and intromission latencies when administered in a dose of 5 mg/kg. Since this drug did not affect motor functions, it is probable that the effect is specific to sexual behavior. Another β-carboline, β-carboline carboxylic acid ethyl ester, has been found to inhibit sexual behavior in dominant talapoin monkeys (39), and the authors suggested that the anxiogenic action of the compound could be responsible for this effect. FG 7142 has been reported to be anxiogenic in several behavioral paradigms (8, 27, 37). It could, therefore, be supposed that its anxiogenic actions could be responsible for its inhibition of sexual behavior in the male rat. However, other treatments, such as electric shock to the skin (5,6) or frequent handling (18), which could be considered as generating anxiety, stimulate male rat sexual behavior instead of inhibiting it. Until further studies have been performed, it is not possible to determine the exact mechanism of action of FG 7142.

An additional difficulty in interpreting the effects of FG 7142 stems from the fact that its actions are similar to those of benzodiazepine agonists and of picrotoxin. It appears that most modifications of the GABA/benzodiazepine system impair sexual behavior. This can be due to multiple actions of these systems at multiple sites within the central nervous system. For example, it has been proposed that β-carbolines preferentially bind to the benzodiazepine type I receptor, while diazepam and chlordiazepoxide have similar affinities for both type I and type II receptors (36). Moreover, the distribution of the receptor types appears to be uneven (30). Differential actions at specific sites could perhaps explain the observation that FG 7142 has actions similar to those of diazepam and chlordiazepoxide.

In order to elucidate the exact functions of the benzodiazepines, it may be necessary to infuse drugs at specific brain sites and employ receptor-specific drugs. Until this has been done, it would be premature to speculate about the localization of the effects and

the involvement of particular benzodiazepine receptor types.

The role of GABA in the behavioral actions of benzodiazepines has been much discussed [for reviews see (33–35)]. The present data show that the effects of both diazepam and chlordiazepoxide on ambulatory activity and motor execution cannot be blocked by the GABA-A antagonist bicuculline. Picrotoxin, however, blocked the benzodiazepine actions on both ambulatory activity and motor execution. This is in agreement with previous reports (13). It is important to note, though, that the blocking capacity of picrotoxin is dependent on benzodiazepine dose. Complete inhibition of the effects of chlordiazepoxide (3.125 mg/kg) on ambulatory activity was observed with picrotoxin (0.5 mg/kg). The effects of a higher dose of chlordiazepoxide (6.25 mg/kg) could not be blocked by picrotoxin in any dose tried. The antagonism by picrotoxin of benzodiazepine actions on motor execution also seemed to be dose-dependent. Both 0.5 and 1 mg/kg blocked the effects of chlordiazepoxide (12.5 mg/kg) and of diazepam (4 mg/kg). However, the effects of chlordiazepoxide (25 mg/kg) and of diazepam (8 mg/kg) could not be blocked by picrotoxin. In order to evaluate a possible interaction between benzodiazepines and GABA it therefore seems necessary to use an extensive range of doses of both kinds of drugs.

The actions of the benzodiazepines on sexual behavior could be indirect, their primary effect being on motor execution. It has previously been proposed that sexual behavior is impaired whenever motor execution is impaired (4). However, when the benzodiazepines were administered together with picrotoxin in doses such that actions on motor execution were antagonized, the effects on sexual behavior persisted. It can, therefore, be proposed that the inhibitory actions of benzodiazepines on sexual behavior are not solely a consequence of motor deficiencies.

Even though the GABA antagonist picrotoxin blocked motor effects of the benzodiazepines, at least under some conditions, the drug was, as mentioned, unable to block their actions on sexual behavior. It could be argued, of course, that the dose of picrotoxin was not sufficiently large to effectively antagonize enhanced GABAergic neurotransmission. This is unlikely, however, since even half the dose used in studies of sexual behavior was sufficient to antagonize benzodiazepine actions on motor execution.

Rather than antagonizing the effects of the benzodiazepines on sexual behavior, the GABA antagonists seemed to reinforce them. There is no explanation available for this at present, and the importance of this observation can only be determined by further studies. Nevertheless, it was not possible to obtain any definite evidence for GABAergic mediation of benzodiazepine effects on sexual behavior. A similar conclusion was reached with regard to the hyperphagia induced by chlordiazepoxide (32). Indeed, it appears that GABA involvement in anticonvulsive and motor effects of benzodiazepines is probable (14), whereas the evidence is equivocal for other benzodiazepine actions (33,35). It is important to observe that not all GABA receptors are associated with benzodiazepines, and that not all benzodiazepine receptors are associated with GABA (15,16). This affords a structural basis for the proposal that benzodiazepine action on sexual behavior may be independent of GABAergic mechanisms. There is, indeed, quite a lot of evidence showing that several other actions of the benzodiazepines do not depend on GABA systems [reviewed in (28)]. The fact that the benzodiazepine antagonist flumazenil inhibited most effects of the benzodiazepines on motor functions and on sexual behavior provides evidence for a specific action on benzodiazepine receptors.

FG 7142 inhibited the actions of benzodiazepines on ambulatory activity, but not those on sexual behavior or motor execution. This can be due to the relatively low dose used in these experiments (1 mg/kg) in relation to the high doses of benzodiazepines. However, a higher dose could not reasonably be employed, since it had effects by its own on sexual behavior.

To summarize, the present data show that benzodiazepines inhibit sexual behavior in the male rat, and offers evidence suggesting that this inhibition is not only a consequence of motor impairment. While a role of GABA in the motor effects of the benzodiazepines could be demonstrated, no such evidence was found regarding their actions on sexual behavior.

ACKNOWLEDGEMENTS

Financial support was received from the Universidad Anahuac. Chlordiazepoxide was generously supplied by Dr. Juan Barrera of Roche de Mexico, and diazepam and flumazenil by Drs. H. Gutmann and E. Kyburz of F. Hoffmann-La Roche, Switzerland. The technical assistance of Mr. Ramon Perez and Ms. Maria Teresa Hernandez is gratefully acknowledged.

REFERENCES

1. Ågmo, A.; Fernandez, H. Dopamine and sexual behavior in the male rat: A reevaluation. J. Neural Transm. 77:21–37; 1989.
2. Ågmo, A.; Giordano, M. The locomotor-reducing effects of GABAergic drugs do not depend on the GABA-A receptor. Psychopharmacology (Berlin) 87:51–54; 1985.
3. Ågmo, A.; Paredes, R. GABAergic drugs and sexual behaviour in the male rat. Eur. J. Pharmacol. 112:371–378; 1985.
4. Ågmo, A.; Paredes, R.; Fernandez, H. Differential effects of GABA transaminase inhibitors on sexual behavior, locomotor activity, and motor execution. Pharmacol. Biochem. Behav. 28:47–52; 1987.
5. Barfield, R. J.; Sachs, B. D. Sexual behavior: Stimulation by painful electric shock to the skin in male rats. Science 161:392–394; 1968.
6. Barfield, R. J.; Sachs, B. D. Effect of shock on copulatory behavior in castrated male rats. Horm. Behav. 1:247–253; 1970.
7. Carter, C. S.; Daily, R. F.; Leaf, R. Effects of chlordiazepoxide, oxazepam, chlorpromazine, and d-amphetamine on sexual responses in male and female hamsters. Psychopharmacology (Berlin) 55:195–201; 1977.
8. Corda, M. G.; Blaker, W. D.; Mendelson, W. B.; Guidotti, A.; Costa, E. β-carbolines enhance shock-induced suppression of drinking in rats. Proc. Natl. Acad. Sci. USA 80:2072–2076; 1983.
9. Damassa, D. A.; Smith, E. R.; Tennent, B.; Davidson, J. M. The relationship between circulating testosterone levels and male sexual behavior in rats. Horm. Behav. 8:275–286; 1977.
10. Enna, S. J.; Mohler, H. γ-aminobutyric acid (GABA) receptors and their association with benzodiazepine binding sites. In: Meltzer, H. Y., ed. Psychopharmacology: The third generation of progress. New York: Raven Press; 1987:265–272.
11. Fernandez-Guasti, A.; Larsson, K.; Beyer, C. GABAergic control of masculine sexual behavior. Pharmacol. Biochem. Behav. 24:1065–1070; 1986.
12. Fernandez-Guasti, A.; Larsson, K.; Beyer, C. Effect of bicuculline on sexual activity in castrated male rats. Physiol. Behav. 36:235–237; 1986.
13. File, S. E. Chlordiazepoxide-induced ataxia, muscle relaxation and sedation in the rat: Effects of muscimol, picrotoxin and naloxone. Pharmacol. Biochem. Behav. 17:1165–1170; 1982.
14. Haefely, W. E. Behavioral and neuropharmacological aspects of drugs used in anxiety and related states. In: Lipton, M. A.; DiMascio, A.; Killam, K. F., eds. Psychopharmacology: A generation of progress. New York: Raven Press; 1978:1359–1374.
15. Johnston, G. A. R. Multiplicity of GABA receptors. In: Olsen, R. W.; Venter, J., eds. Benzodiazepine/GABA receptors and chloride channels: Structural and functional properties. New York: Alan R. Liss; 1986:57–71.
16. Johnston, G. A. R.; Skerritt, J. H. GABARINS and the nexus between GABA and benzodiazepine receptors. In: Bowery, N. G., ed.

Actions and interactions of GABA and benzodiazepines. New York: Raven Press; 1984:179–189.
17. Larsson, K. Non-specific stimulation and sexual behavior in the male rat. Behaviour 20:110–114; 1963.
18. Leavitt, F. I. Drug-induced modifications in sexual behavior and open field locomotion of male rats. Physiol. Behav. 4:677–683; 1969.
19. Leipheimer, R. E.; Sachs, B. D. GABAergic regulation of penile reflexes and copulation in male rats. Physiol. Behav. 42:351–357; 1988.
20. McDonnell, S. M.; Kenney, R. M.; Meckley, P. E.; Garcia, M. C. Conditioned suppression of sexual behavior in stallions and reversal with diazepam. Physiol. Behav. 34:951–956; 1985.
21. McDonnell, S. M.; Kenney, R. M.; Meckley, P. E.; Garcia, M. C. Novel environment suppression of stallion sexual behavior and effects of diazepam. Physiol. Behav. 37:503–505; 1986.
22. Malmnas, C. O. Short-latency effect of testosterone on copulatory behaviour and ejaculation in sexually experienced intact male rats. J. Reprod. Fertil. 51:351–354; 1977.
23. Martino, V.; Mas, M.; Davidson, J. M. Chlordiazepoxide facilitates erections and inhibits seminal emission in rats. Psychopharmacology (Berlin) 91:85–89; 1987.
24. Munjack, D. J.; Crocker, B. Alprazolam-induced ejaculatory inhibition. J. Clin. Psychopharmacol. 6:57–58; 1986.
25. Olsen, R. W. Drug interactions at the GABA receptor-ionophore complex. Annu. Rev. Pharmacol. Toxicol. 22:245–277; 1982.
26. Paredes, R.; Ågmo, A. Stereospecific actions of baclofen on sociosexual behavior, locomotor activity and motor execution. Psychopharmacology (Berlin) 97:358–364; 1989.
27. Petersen, E. N.; Jensen, L. H. Proconflict effect of benzodiazepine inverse agonists and other inhibitors of GABA function. Eur. J. Pharmacol. 103:91–97; 1984.
28. Polc, P. Electrophysiology of benzodiazepine receptor ligands: Multiple mechanisms and sites of action. Prog. Neurobiol. 31:349–423; 1988.
29. Richards, J. G.; Mohler, H. Benzodiazepine receptors. Neuropharmacology 23:233–242; 1984.
30. Richards, J. G.; Mohler, H.; Schoch, P.; Haring, P.; Takacs, B.; Stahli, Ch. The visualization of neuronal benzodiazepine receptors in the brain by autoradiography and immunohistochemistry. J. Receptor Res. 4:657–669; 1984.
31. Ritta, M. N.; Campos, M. B.; Calandra, R. S. Effects of GABA and benzodiazepines on testicular androgen production. Life Sci. 40:791–798; 1987.
32. Sanger, D. J. Chlordiazepoxide-induced hyperphagia in rats: lack of effect of GABA agonists and antagonists. Psychopharmacology (Berlin) 84:388–392; 1984.
33. Sanger, D. J. GABA and the behavioral effects of anxiolytic drugs. Life Sci. 36:1503–1513; 1985.
34. Sepinwall, J. Behavioral effects of antianxiety agents: Possible mechanisms of action. In: Seiden, L. S.; Balster, R. L., eds. Behavioral pharmacology: The current status. New York: Alan R. Liss; 1985: 181–203.
35. Shephard, R. A. Neurotransmitters, anxiety and benzodiazepines: A behavioral review. Neurosci. Biobehav. Rev. 10:449–461; 1986.
36. Sieghart, W. Benzodiazepine receptors: multiple receptors or multiple conformations? J. Neural Transm. 63:191–208; 1985.
37. Stutzmann, J. M.; Bohme, G. A.; Cochon, M.; Roux, M.; Blanchard, J. C. Proconflict and electrocorticographic effects of drugs modulating GABAergic neurotransmission. Psychopharmacology (Berlin) 91:74–79; 1987.
38. Uhde, T. W.; Tancer, M. E.; Shea, C. A. Sexual dysfunction related to alprazolam treatment of social phobia. Am. J. Psychiatry 145: 531–532; 1988.
39. Vellucci, S. V.; Herbert, J.; Keverne, E. B. The effect of midazolam and β-carboline carboxylic acid ethyl ester on behaviour, steroid hormones and central monoamine metabolites in social groups of talapoin monkeys. Psychopharmacology (Berlin) 90:367–372; 1986.
40. Vogel, H.; Beer, H.; Clody, D. A simple and reliable conflict procedure for testing antianxiety agents, Psychopharmacology (Berlin) 21:1–7; 1971.