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BRIEF COMMUNICATION

Pharmacological Manipulation of Anxiety and Male Rat Sexual Behavior

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FERNÁNDEZ-GUASTI, A., G. ROLDÁN-ROLDÁN AND A. SALDÍVAR. *Pharmacological manipulation of anxiety and male rat sexual behavior*. PHARMACOL BIOCHEM BEHAV **35**(1) 263–267, 1990.—Several anxiolytic/anxiogenic treatments were evaluated on male rat sexual behavior. The anxiolytic drug diazepam (1.0 mg/kg) inhibited copulatory behavior as indicated by an increase in the number of mounts preceding ejaculation, prolongation of the ejaculation latency and the postejaculatory interval. These changes were not accompanied by alterations in motor coordination as tested on a treadmill apparatus. A lower dose of diazepam (0.5 mg/kg) did not affect the sexual behavior. The anxiogenic drug Zk 39106 (2 and 4 mg/kg) facilitated the copulatory behavior by reducing the number of intromissions preceding ejaculation. A higher dose of Zk 39106 (8 mg/kg) inhibited sexual behavior. The selective benzodiazepine antagonist, Ro 15-1788 (5 and 10 mg/kg), did not modify male sexual behavior, but effectively antagonized the effects of Zk 39106 and diazepam. The administration of Zk 39106 (2 mg/kg) reversed the inhibitory action of diazepam (1.0 mg/kg) on copulation; however, diazepam did not prevent the facilitatory effect of Zk 39106. The data are discussed in terms of the possible relationship existing between anxiety and masculine sexual behavior.

Diazepam Zk 39106 Ro 15-1788 Anxiolysis Anxiogenesis Male rat sexual behavior

THE role of anxiety on male sexual behavior has generally been neglected. Some evidence, mainly from empirical and clinical data, suggests that certain levels of anxiety may result in "ejaculatio praecox" (phenomenon that in animal research would be described as a facilitation of copulatory behavior, i.e., shortening of ejaculation latency), while high anxiety levels result in a complete inhibition of this behavior [cf. (27)]. Beach and Fowler (1) reported that "situational" anxiety facilitates sexual behavior by reducing the number of intromissions preceding ejaculation. Conversely, the administration of benzodiazepines may result in an inhibition of rat sexual behavior (22).

Recently, several pharmacological tools for investigating anxiety in the laboratory have been developed. At present, three main pharmacological classes of compounds, all acting at the benzodiazepine recognition site, have been established [cf. (15, 16, 26)]: A) Agonists at the benzodiazepine recognition site (e.g., diazepam, chlordiazepoxide, etc). The main behavioral action of these drugs is to reduce anxiety. B) Inverse agonists, a new group of compounds with actions exactly opposite to those of benzodiazepines, has been characterized. These drugs possess potent anxiogenic actions. Beta carbolines like DMCM, βCCM and Zk 39106 are typical of this group. C) Antagonists, a third group of drugs which have negligible effect on various animal anxiety tests, but block the action of both agonists and inverse agonists, have been established. Various imidazodiazepines like Ro 15-1788 represent this group.

The purpose of the present study was to analyze rat copulatory behavior under various anxiety states. Anxiety was modified by administering diazepam or the beta carboline Zk 39106 (previously named FG-7142). To ascertain whether the effects of these drugs was mediated through the benzodiazepine receptor site, an attempt to counteract their action by administering the selective antagonist Ro 15-1788 was made. Finally, to explore the possibility that diazepam effects were mediated via a nonspecific alteration of motor coordination, the action of this drug was evaluated on a treadmill test.

METHOD

Animals

Sexually experienced adult male Wistar rats (250–350 g body weight) were used. The animals were housed, seven per cage, in

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a room with controlled lighting (12 hr light:12 hr dark; lights on at 2200 hr). Throughout the experiments animals had ad lib access to water and Purina rat chow.

Sexual Behavior Observations

Observations of sexual behavior were begun two hours after the onset of darkness. The males were presented to a female brought into sexual receptivity by sequential treatment with estradiol valerianate (5 μg/rat) followed 44 hr later by progesterone (1.5 mg/rat) which was injected 4 hr before testing. The behavioral parameters registered were: number of mounts and intromissions preceding ejaculation, intromission and ejaculation latencies and postejaculatory interval [for definition of each behavioral component see (21)]. In cases where the drug treatment resulted in an extreme inhibition of sexual behavior, the data were expressed as the proportion of copulating animals in a 15-min period.

Drugs

Diazepam and Ro 15-1788 [ethyl-8-fluoro-5,6-dihydro-5-methyl-6-oxo-4H-imidazo (1,5a) (1,4) benzodiazepine-3-carboxylate] were kindly donated by Hoffmann-La Roche, México City, México. The beta carboline Zk 39106 (methyl-beta-carboline-3-carboxamide, FG-7142) was kindly donated by Schering Pharmaceuticals, Berlin, West Germany. Diazepam was dissolved in propylene glycol 40%, Ro 15-1788 was suspended in distilled water with a drop of Tween 80. Zk 39106 was suspended in distilled water with dimethyl sulphoxide (1.25%) and 150 μl Tween 80. Immediately before injection Ro 15-1788 and Zk 39106 were vigorously shaken to ensure a uniform suspension. All drugs were injected IP in a volume of 2.0 ml/kg. The observation latency was 30 min for diazepam and Ro-151788 and 10 min for Zk 39106.

Procedure

The first three series of experiments include the analysis of dose response curves for the drugs used in this study (for details see Table 1). Further series of experiments were made to analyze the interaction between the different drugs (see Figs. 1–3). In all cases, the different doses of the drug and the respective vehicle were administered according to a balanced Latin square design in such a way that each animal received all treatments. The treatment effect was evaluated using the Friedman's two-way analysis of variance followed by the Wilcoxon matched-pairs signed-ranks test (28). The proportion of copulating animals was evaluated using the Cochran's Q-test (28).

Treadmill Test

In this experiment a treadmill apparatus (diameter, 7 cm; rotating speed, 10 rpm) was used. All the animals were trained for 10 min for three consecutive days before drug administration. Vehicle or diazepam (0.5 or 1.0 mg/kg) were given according to a balanced Latin square design. The number of falls during a 5-min period was recorded. The data were analyzed using the Friedman two-way analysis of variance followed by the Wilcoxon matched-pairs signed-ranks test (28).

RESULTS

Effects of Diazepam on Copulatory Behavior and Motor Coordination

The effect of diazepam on masculine sexual behavior is shown in Table 1. Neither diazepam vehicle nor a low dose of diazepam (0.5 mg/kg) affected the sexual behavior. A high dose of diazepam (1.0 mg/kg) resulted in an inhibition of this behavior as indicated by a drastic increase in the number of mounts and a prolongation of the ejaculation latency and the postejaculatory interval. These changes cannot attributed to a general motor impairment, since at neither of the doses used did diazepam affect the motor coordination in a treadmill apparatus [median number of falls/5 min: control = 0; 0.5 mg/kg = 1; and 1.0 mg/kg = 1. Friedman two-way ANOVA, $\chi^2(2) = 2.21$, nonsignificant].

TABLE 1

EFFECT OF DIAZEPAM, Zk 39106 AND Ro 15-1788 ON MALE SEXUAL BEHAVIOR

Drug	Dose (mg/kg)	N	Behavioral Component M	I	EL	PEI
Diazepam	0	8	2	8	5.1	5.7
	0.5		2	7	5.7	6.1
	1.0		11‡	6	8.5	8.4*
$\chi^2(2)$			12.96	4.5	4.0	7.8
$p<$			0.01	N.S.	N.S.	N.S.
Zk 39106	0	17	2	9	5.3	5.8
	1		2	8	5.0	5.7
	2		1	5†	4.5	6.1
	4		1	7	5.7	6.8
$\chi^2(3)$			7.2	13.4	1.0	1.3
$p<$			N.S.	0.01	N.S.	N.S.
Ro 15-1788	0	11	1	8	2.7	5.4
	5		1	9	3.2	5.4
	10		1	7	3.4	5.7
$\chi^2(2)$			1.9	0.9	0.7	0.2
$p<$			N.S.	N.S.	N.S.	N.S.

Table denotes median values. M, mounts; I, intromissions; EL, ejaculation latency; and PEI, postejaculatory interval. The statistical comparisons were made using the Friedman two-way analysis of variance followed by the Wilcoxon matched-pairs signed-ranks test.

*$p<0.05$; †$p<0.02$; ‡$p<0.01$.

Effects of Zk 39106 on Copulatory Behavior

The effects of Zk 39106 on copulation are shown in Table 1. This drug showed a biphasic effect; thus at low doses (2 and 4 mg/kg) the drug produced a facilitatory action, while at higher doses (8 mg/kg) an inhibition of the behavior was found. The analysis of the proportion of copulating animals revealed that after the administration of vehicle, 1 or 2 mg/kg all the animals performed sexual behavior, while after the administration of 4 mg/kg a small proportion of animals (19%) did not show this behavior. However, at the highest dose level used (8 mg/kg), only 53% of the animals copulated. These data indicate that the reduction in the proportion of copulating animals is dose related (Cochran Q-test, $Q = 6.28$, $p<0.05$). A detailed analysis of the components of copulatory behavior after Zk 39106 is shown in Table 1. This analysis is based only on the data of those animals that ejaculated after the administration of 4 mg/kg ($n = 17$). Injection of 1 mg/kg did not alter sexual behavior compared with the vehicle-treated group. Interestingly, the injection of 2 and 4 mg/kg resulted in a slight, though very consistent, reduction in the number of intromissions preceding ejaculation.

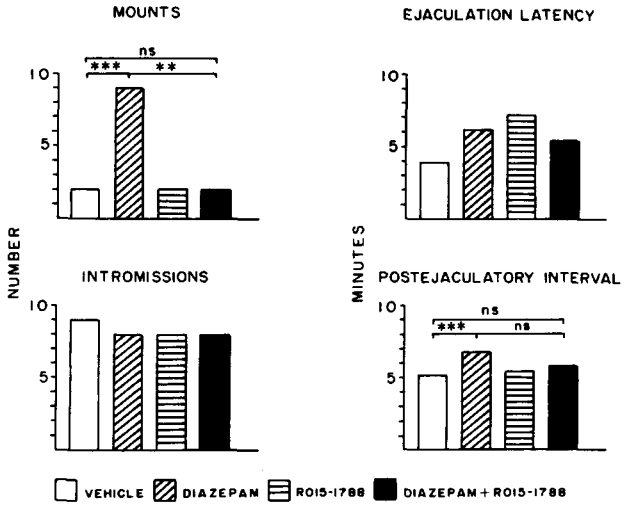

FIG. 1. Antagonism of diazepam effect by Ro 15-1788. Diazepam (1.0 mg/kg) and Ro 15-1788 (10 mg/kg) were administered 30 min before the observations. Figure shows median values based on the performance of 8 animals. Friedman two-way analysis of variance: Mounts, $\chi^2(3) = 15.68$, $p<0.01$; intromissions, $\chi^2(3) = 1.5$, n.s.; ejaculation latency, $\chi^2(3) = 5.25$, n.s.; and postejaculatory interval, $\chi^2(3) = 8.24$, $p<0.05$. Wilcoxon matched-pairs signed-ranks test, $*p<0.05$; $**p<0.02$; $***p<0.01$.

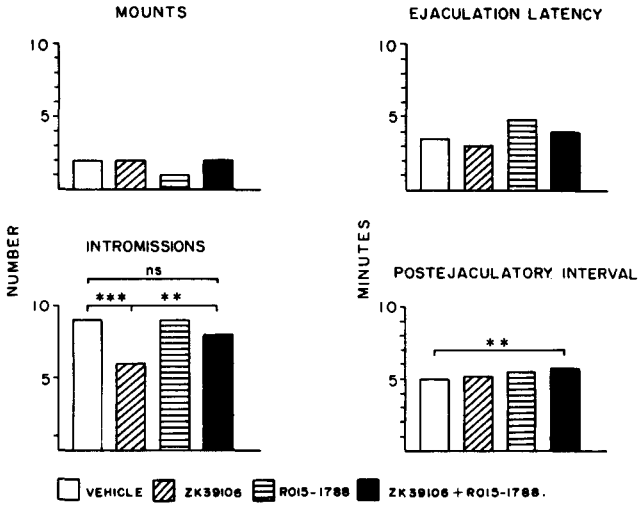

FIG. 2. Antagonism of Zk 39106 effect by Ro 15-1788. Zk 39106 (2 mg/kg) and Ro 15-1788 (10 mg/kg) were administered 10 and 30 min before the observations respectively. Figure shows median values based on the performance of 13 animals. Friedman two-way analysis of variance: Mounts, $\chi^2(3) = 2.21$, n.s.; intromissions, $\chi^2(3) = 15.20$, $p<0.01$; ejaculation latency, $\chi^2(3) = 4.84$, n.s.; and postejaculatory interval, $\chi^2(3) = 7.98$, $p<0.05$. Wilcoxon matched-pairs signed-ranks test, $*p<0.05$; $**p<0.02$; $***p<0.01$.

Effect of Ro 15-1788 on Copulatory Behavior

Table 1 also shows the effect of Ro 15-1788 (0, 5 and 10 mg/kg) on male sexual behavior. This drug did not modify this behavior at any dose tested.

Antagonism of Diazepam Effect by Ro 15-1788

Figure 1 shows the antagonistic effect of Ro 15-1788 on diazepam inhibition of male sexual behavior. In this experiment the administration of diazepam (1.0 mg/kg) resulted in an increased number of mounts and in a prolongation of the postejaculatory interval. Administration of Ro 15-1788 (10 mg/kg) did not affect the behavior, but effectively antagonized the inhibitory actions of diazepam on the above-mentioned parameters.

Antagonism of Zk 39106 Effect by Ro 15-1788

The antagonism by Ro 15-1788 on Zk 39106 effect on sexual behavior is shown in Fig. 2. In this experimental series 2 mg/kg of Zk 39106 produced a reduction in the number of intromissions. Treatments with Ro 15-788 (10 mg/kg) did not alter the copulatory behavior, but clearly counteracted the facilitatory effect of Zk 39106.

Reversal of Diazepam Effect by Zk 39106

Figure 3 presents the results of the combination of diazepam and Zk 39106 on male sexual behavior. Diazepam (1.0 mg/kg) administration resulted in an increase in the number of mounts and in a prolongation of the ejaculatory latency and postejaculatory interval. Additionally, the administration of Zk 39106 (2 mg/kg) caused a reduction in the number of intromissions preceding ejaculation. The combination of these two treatments resulted in a reversal of all the inhibitory actions induced by diazepam. In contrast, the effect of Zk 39106 on the number of intromissions was not modified by diazepam.

DISCUSSION

The present data indicate that diazepam produces an inhibition

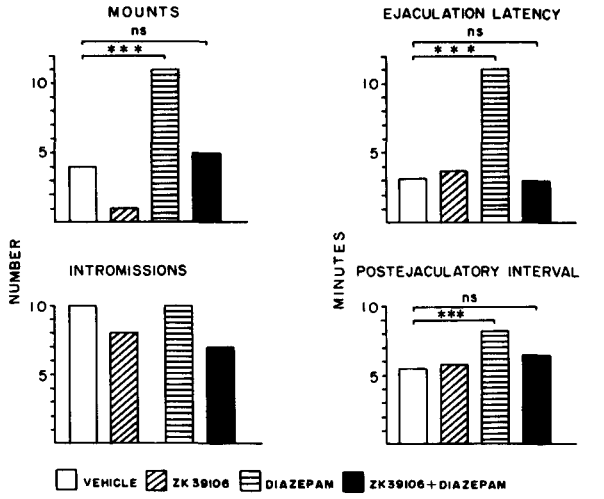

FIG. 3. Effect of the combined treatment with diazepam and Zk 39106. Diazepam (1.0 mg/kg) and Zk 39106 (2 mg/kg) were administered 30 and 10 min before the observations respectively. Figure shows median values based on the performance of 10 animals. Friedman two-way analysis of variance: Mounts, $\chi^2(3) = 17.93$, $p<0.001$; intromissions, $\chi^2(3) = 4.34$, n.s.; ejaculation latency, $\chi^2(3) = 9.48$, $p<0.05$; and postejaculatory interval, $\chi^2(3) = 9.72$, $p<0.05$. Wilcoxon matched-pairs signed-ranks test. $*p<0.05$; $**p<0.02$; $***p<0.01$.

of copulatory behavior by increasing the number of mounts, the ejaculatory latency and the postejaculatory interval. Conversely, presumably low levels of anxiety, induced by a low dose of Zk 39106 (12), resulted in a facilitation of copulatory behavior indicated by a reduction in the number of intromissions. In contrast, presumably high anxiety levels, induced by a high dose of Zk 39106 (12), resulted in a complete inhibition of the sexual behavior.

The inhibitory actions of diazepam on male rat sexual behavior are shared by other benzodiazepines. It has been reported (22) that chlordiazepoxide (3 mg/kg) produced a slight, but nonsignificant increase in the number of mounts and in the ejaculatory latency. Recently, we have found that larger doses of chlordiazepoxide (6 and 9 mg/kg) result in an increase of mounting. The actions of diazepam could be interpreted on the basis of nonspecific general motor impairment. However, the data showing that diazepam, at the dosages used, does not affect motor coordination as tested in a treadmill apparatus, do not support this interpretation. Additionally, the effectiveness of the specific central benzodiazepine antagonist Ro 15-1788 (17,25) in blocking the effects of diazepam, supports the hypothesis of central rather than peripheral mediation. Although a motor mechanism does not seem to be involved in the action of diazepam on copulatory behavior, further experiments will be necessary to completely exclude this possibility.

Interestingly, the inhibitory actions of diazepam are not restricted to the active phases of copulation, but it also affected the length of the postejaculatory interval. Although the precise mechanism of action of benzodiazepines is unknown, it is generally believed that these compounds act by increasing the GABAergic transmission (15, 16, 29). Therefore, the finding that diazepam prolongs the postejaculatory interval is consistent with previous reports showing that the GABAergic transmission exerts an inhibitory influence in the regulation of the length of this period (8,9). Another interpretation could be that prolonging the postejaculatory interval is related to the inability of animal to achieve intromission. This does not seem to be the case, since the postejaculatory intervals measured either to the first mount or to the first intromission were not significantly different (data not shown).

It has been demonstrated that the administration of the beta-carboline ZK 39106 (previously named FG 7142) induces anxiety clinically (4) and in several animal models [cf. (12)]. The facilitatory effect on masculine sexual behavior, manifested as a reduction in the number of intromissions, induced by low doses of Zk 39106 (2 and 4 mg/kg), may be interpreted on the basis of an anxiogenic action. Interestingly, similar findings have been previously reported: Beach in 1959 (1) showed that "situational" anxiety results in a slight reduction in the number of intromissions preceding ejaculation without modifying any other parameter. The present results are consistent with this observation and suggest that moderate levels of anxiety may result in a reduction in the number of intromissions.

The parallels between clinical and experimental sexual behavior research have to be cautiously considered. Clinical data have reported that moderate levels of anxiety result in "ejaculatio praecox" (13, 19, 27). This syndrome consists of an extreme reduction of the ejaculation latency associated with lower ejaculation threshold. In the analysis of rat masculine sexual behavior, it has been proposed that the ejaculation threshold is related to the number of intromissions preceding ejaculation (7). Although "ejaculatio praecox" is disruptive to normal human sexual behavior, it is worth noting that in animal research a reduction in ejaculation latency is considered facilitatory [cf. (21)]. Present results showing that low doses of the anxiogenic compound may facilitate sexual behavior are consistent with the clinical data. Furthermore, it has been established that high levels of anxiety may result in impotence associated with particular phobia (14,27). Present data showing that high doses of Zk 39106, presumably accompanied by high anxiety levels, completely inhibited the sexual behavior, are also consistent with the clinical findings.

Several binding (20), neurochemical (18), behavioral (3, 10, 11, 17) and electrophysiological (23) studies have shown that Ro 15-1788 counteracts the effects of benzodiazepines and beta carbolines. Furthermore, Ro 15-1788 has been considered to be a specific central benzodiazepine antagonist (17,25). The present results showing that Ro 15-1788 effectively antagonizes both the facilitatory and the inhibitory action of Zk 39106 and diazepam, respectively, indicate that these actions are mediated through central benzodiazepine receptors. It has been established that several beta-carbolines, including Zk 39106, share the property of reversing the effects of various benzodiazepines, including diazepam (5, 6, 23). Therefore, it is not surprising that Zk 39106 effectively counteracted the inhibitory actions of diazepam on copulatory behavior. Conversely, the capacity of benzodiazepines to counteract the actions of anxiogenics has been controversial (2, 11, 24). In the present study we failed to find a reversal of Zk 39106 actions by diazepam administration.

In summary, the present results indicate that pharmacologically induced changes in anxiety may alter the expression of male rat sexual behavior. Further experiments, using nonpharmacological methods to modify anxiety, should be undertaken in order to confirm the influence of anxiety on copulatory behavior.

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