ARTÍCULO ORIGINAL

Effects of picrotoxin administration on sexual behavior of male rats

Efectos de la administración de picrotoxin sobre el comportamiento sexual en ratas machos

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ABSTRACT

The effects of several doses of picrotoxin on sexual behavior of inexperienced male rats were examined. The lowest doses tested (0.5 and 0.75 mg kg^{-1}) did not alter the sexual behavioral parameters, whereas the higher doses (1.5 and 2.0 mg kg^{-1}) increased latencies for the first mount and intromission and reduced the number of mounts and intromissions and the total number of mounts. None of the picrotoxin doses significantly modified the mount frequency or the copulatory efficiency. The sexual activity index decreased after administration of 1.5 and 2.0 mg kg^{-1} picrotoxin. The inhibitory effects of the larger doses of picrotoxin on male sexual behavior may be a consequence of both a drug interference with motor function and a dose-dependent picrotoxin-induced stress influence.

Key words: GABA, male sexual behavior, picrotoxin, sexual stimulation, sexual inhibition.

RESUMEN

Fueron evaluados los efectos de varias dosis de picrotoxina sobre el comportamiento sexual de ratas macho. La dosis más baja (0.5 mg kg^{-1}) no alteró, la dosis intermedia (0.75 mg kg^{-1}) facilitó algunas de las variables del comportamiento sexual (disminuyó las latencias para la primera monta, primera intromisión y eyaculación), mientras que las dosis más altas (1.5 and 2.0 mg kg^{-1}) aumentaron las latencias para la primera monta e intromisión y redujeron el número total de montas e intromisiones. Ninguna de las dosis de picrotoxina estudiadas modificó significativamente la frecuencia de monta o la eficiencia copulatoria. El índice de actividad sexual disminuyó después de la administración de 1.5 o 2.0 mg kg^{-1} de picrotoxina. Los efectos inhibitorios de las dosis más altas de picrotoxina sobre el comportamiento sexual de los machos pueden ser consecuencia, tanto de la interferencia con la función motora como de la influencia del estrés inducido por la picrotoxina.

Palabras Clave: GABA, comportamiento sexual masculino, picrotoxina, estimulación sexual, inhibición sexual.

INTRODUCTION

Male sexual behavior is one of the many types of behavior modified by GABA (Paredes and Agmo, 1992). This neurotransmitter is believed to inhibit male copulatory behavior and the whole erectile response in particular. Stimulation of $GABA_A$ receptors in the middle preoptic area (MPOA) decreases the number of animals showing mounts, intromissions, and ejaculations, while the blockade of $GABA_A$ receptors dramatically decreases the postejaculatory interval (Fernández-Guasti *et al.*,

1990) and facilitates reinstatement of copulation with testosterone treatment after castration. Stimulation of $GABA_B$ sites in the lumbosacral spinal cord inhibited *ex copula* penile erection (Bitran and Hull, 1987).

MPOA injection of drugs that induce elevation in GABAergic function such as the potent GABA agonist, muscimol, or of an inhibitor of GABA degradation, inhibits male sexual behavior, while similar treatment with GABA antagonists such as picrotoxin or (+) bicuculline methiodide, or with 3-MPA, a GABA synthesis inhibitor, facilitates the expression of this behavior (Fernández-Guasti *et al.*, 1986a, 1986b). In contrast, Agmo and Paredes (1985) and Agmo and Fernández (1991) reported that picrotoxin at subconvulsive doses inhibits male sexual behavior, whereas no effect was obtained with bicuculline. These contradictory results might be caused by differences in dose or in the experimental design. The present study was designed to examine the dose-response effects of picrotoxin administration on sexual behavior of male rats.

METHODS

Inexperienced male and female Wistar rats from our own colony, weighing 250-270 g and about 100 days of age were used. The animals were housed in polypropylene cages (32 x 40 x 18 cm) under controlled temperature (22-24^{0}C), with free access to food and water. Animals were maintained on a 12 hour inverted light-dark cycle, lights on 10 p.m., for at least 21 days before the experiments. The animals used in this study were maintained in accordance to the guidelines of the Committee on Care and Use of Laboratory Animal Resources, National Research Council, U.S.A. Sixty one animals were divided into 6 groups of 9-12 animals each. The groups received a single injection of saline or 0.5, 0.75, 1.0, 1.5 or 2.0 mg kg^{-1}, picrotoxin s.c., 40 min before the behavioral test. All sexual behavior tests were held for 4 to 8 hours after the beginning of the dark period. The sexual behavior was observed as described previously (Felicio *et al.*, 1989; Chiavegatto *et al.* 1989). Male rats were allowed to mount ovariectomized females rendered sexually receptive with exogenous estradiol (50 μg kg^{-1}, sc, 54 h before the tests) and progesterone (2 mg kg^{-1}, sc, 6 h before tests). Rats were observed for the presence of mounts during the first 5 minutes and the presence of ejaculation within 15 minutes. The frequency and latencies for mounts, intromissions and ejaculation were observed. Also, copulatory efficiency (quotient = number of intromissions until first ejaculation/total number of mounts until first ejaculation times 100), sexual activity index (SAI), i.e. log (mount latency $^{-1}$ x 15) + log (intromission latency $^{-1}$ x 15) + log (ejaculation latency^{-1} x 15) + (number of mounts + number of intromissions)$^{1/2}$ + (4 if the animal ejaculated within 15 min. of observation or 0 if the animal did not ejaculate), intromission frequency/min (IF, i.e. number of intromissions/latency for first ejaculation) and mount frequency/min (MF, i.e. number of mounts/latency for first ejaculation) were calculated. Data were analyzed by ANOVA followed by the Dunnet test, with the level of significance set at $p<0.05$.

RESULTS

The two highest doses (1.5 and 2.0 mg kg^{-1}) induced significant increases in latency parameters (Figure 1). Significant variations in the latencies for the first mount and first intromission were observed. Post-hoc tests comparing picrotoxin data with the control group indicated that the groups injected with the 1.5 and 2.0 mg kg^{-1} doses were different from the control group. No significant differences were observed between the control and experimental groups for ejaculatory or postejaculatory mount latencies.

A significant effect of picrotoxin on the number of unsuccessful mounts, number of intromissions and on the total mounts (Table 1) was observed. The post-hoc test comparing picrotoxin data with control data showed that the 1.5 and 2.0 mg kg^{-1} doses reduced the number of uncessful mounts while only the 2.0 mg kg^{-1} dose reduced both number of intromissions and of total mounts. No significant differences were observed between groups for the percentage of ejaculations.

No significant differences were observed in mount frequency, intromission frequency or copulatory efficiency between the control and picrotoxin-treated animals (Table 2). A numerical decrease in the percentage of animals which ejaculated was observed after dosing with 1.5 and 2.0 mg kg^{-1} although statistical analysis did not show significant differences. Moreover, were observed a significant variation in the sexual activity index and the post-hoc test indicated that 2.0 mg kg^{-1} picrotoxin reduced this index (Table 2).

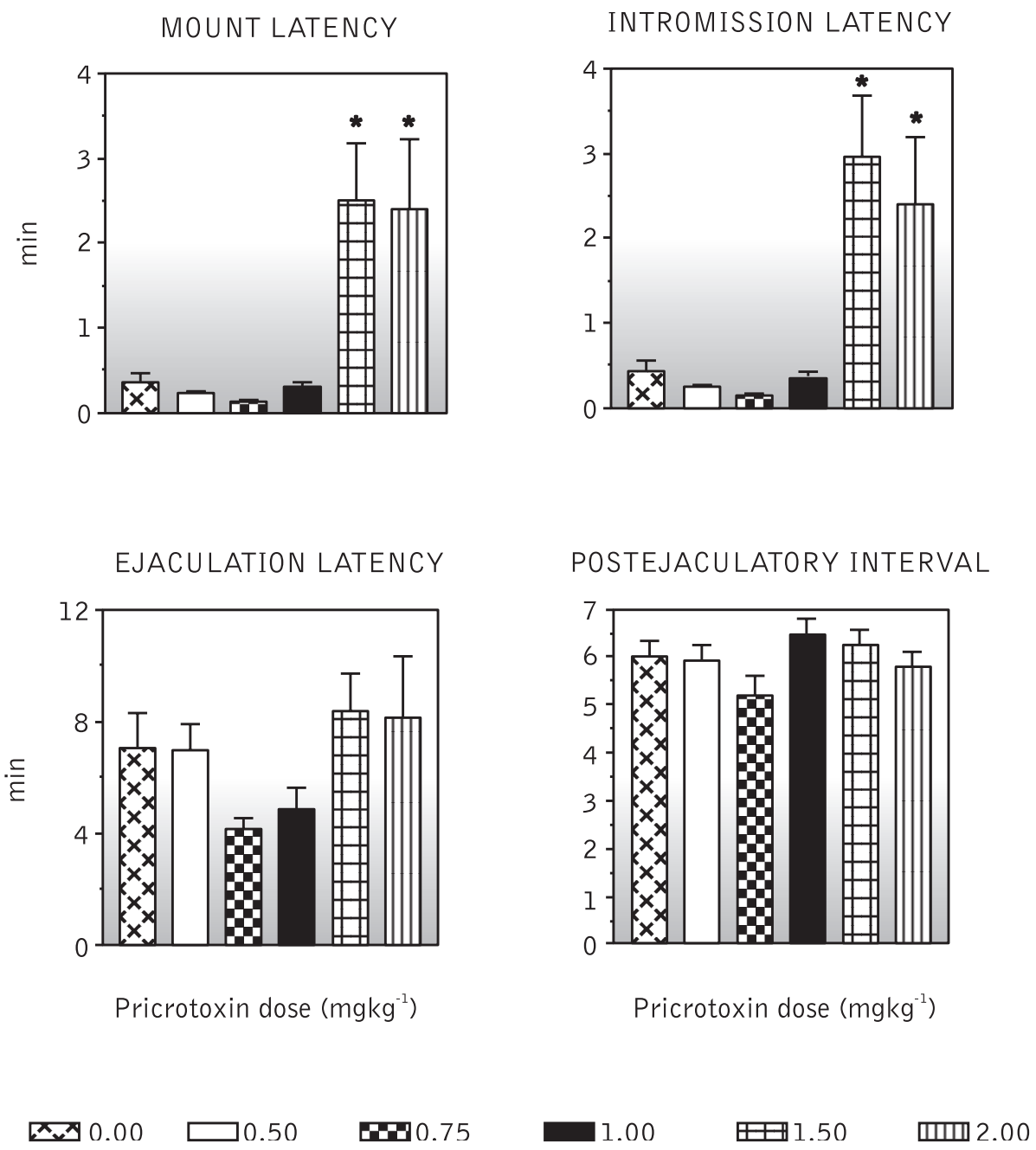

FIGURE 1. Effects of picrotoxin administration (0.0, 0.5, 0.75, 1.0, 1.5 or 2.0 mg kg^{-1}) on mount, intromission, ejaculatory latencies and postejaculatory interval. Data are presented as means ± SEM. *$p<0.05$ compared to the 0.0 group - ANOVA followed by the Dunnett test.

TABLE 1. Effects of picrotoxin administration (0.0, 0.5, 0.75, 1.0, or 2.0 mg kg^{-1}) on number of mounts and intromissions until the first ejaculation and the total number of mounts (TM). Data are presented as means ± SEM. n = number of animals.

PARAMETERS OF SEXUAL BEHAVIOR	DOSES (mg kg^{-1})					
	0.0	0.5	0.75	1.0	1.5	2.0
	(n = 11)	(n = 10)	(n = 12)	(n = 9)	(n = 10)	(n = 9)
Number of mounts	6.7± 2.8	4.3± 0.7	2.8± 0.9	3.1± 0.7	1.1± 0.5*	0.4± 0.6*
Number of intromissions	9.8± 0.8	9.1± 0.6	9.8± 0.5	10.9± 1.3	9.8± 1.2	4.6± 1.6
Total number of mounts	16.6± 3.0	13.4± 0.9	12.6± 1.0	14.0± 1.3	10.9± 1.4	5.0 ± 1.8*

*p<0.05 compared to the 0.0 group – ANOVA followed by Dunn's test

TABLE 2. Effects of picrotoxin administration (0.0, 0.5, 0.75, 1.0, or 2.0 mg kg^{-1}) on mount frequency, intromission frequency/min, copulatory efficiency and sexual activity index. Data are presented as means ± SEM. n = number of animals.

PARAMETERS OF SEXUAL BEHAVOR	DOSES (mg kg^{-1})					
	0.0	0.5	0.75	1.0	1.5	2.0
	(n = 11)	(n = 10)	(n = 12)	(n = 9)	(n = 10)	(n = 9)
Mounts frequency/min	2.6 ± 0.3	2.3 ± 0.3	3.2 ± 0.2	3.2 ± 0.3	1.7 ± 0.3	2.5 ± 0.9
Intromission frequency/min	1.8 ± 0.2	1.5 ± 0.2	2.6 ± 1.2	2.5 ± 0.3	1.6 ± 0.2	2.3 ± 0.9
Copulatory efficiency	0.7 ± 0.1	0.7 ± 0.1	0.8 ± 0.1	0.8 ± 0.1	0.7 ± 0.1	0.6 ± 0.1
Sexual activity index	11.1±0.6	11.7±0.2	12.2±0.2	11.6±0.2	8.2±1.4	6.4±1.9*
Percentage of animals that ejaculated	91	100	100	100	80	55

* p<0.05 compared to the 0.0 group – ANOVA followed by Dunn's test.

DISCUSSION

The present results show that gabaergic inhibition by picrotoxin modifies sexual behavior of male rats in a dose-dependent manner. The 1.5 and 2.0 mg kg^{-1} doses increased the mount and intromission latencies and decreased the number of both mounts and intromissions while the dose range of 0.5 to 1.0 mg kg^{-1} picrotoxin did not alter any of the sexual behavior parameters evaluated. These results are in accordance with other reports showing an inhibitory effect of picrotoxin at subconvulsive doses on male sexual behavior, whereas no effect was obtained with bicuculline (Agmo and Fernández, 1989; 1991).

The decrease in sexual activity index caused by 1.5 and 2.0 mg kg^{-1} doses might be a result of a decreased intensity of sexual behavior and/or motor function. This index represents the appetitive and consumatory aspects of sexual behavior and a decrease reflects interference with the libido and/or with the performance. Previous reports have suggested that some gabaergic drugs affect sexual behavior only indirectly, via an impairment of motor execution (Agmo *et al.*, 1987; Paredes and Agmo, 1989) Recently, Paredes *et al.* (1997) also suggested that changes in gabaergic neurotransmission reduced the sensitivity to environmental stimuli, thereby inhibiting sexual and drinking behavior in a nonspecific way.

The present findings do not support the hypothesis that a decrease in motor function is the only factor responsible for the reduction of male sexual behavior. In fact, no differences were observed in intromission frequencies, previously reported as a neuromotor coefficient (Soulairac, 1963), after administration of all picrotoxin doses. The higher doses of picrotoxin (1.5 and 2.0 mg kg^{-1}) decreased the first mount and intromission latencies, suggesting an effect on sexual motivation drives (Paredes *et al.*, 1993).

Stressful situations elicit adaptive responses in organisms in an attempt to re-establish homeostasis. The adaptive response to stress seems to depend on the type (physical or emotional), intensity and duration (acute or chronic) of the stimulus, as well as on the

characteristics and physiological state of the organism (De Wied, 1980). Physical or emotional stress is a profound disruptive factor for reproductive function (Johnson *et al.,* 1992). The endocrine response to stress activates the hypothalamic-pituitary-adrenocortical system but also the hypothalamic-pituitary-gonadal system and other neuroendocrine axes. Some evidence from empirical and clinical data suggests that certain levels of anxiety may result in premature ejaculation, while high anxiety levels result in complete inhibition of this behavior. Conversely, the administration of diazepam may result in an inhibition of rat sexual behavior (Fernández-Guasti *et al.,* 1990).

Several reports indicate that subconvulsive doses of central stimulating drugs have anxiogenic effects on adult animals and humans. File and Lister (1984) showed that subconvulsive doses of picrotoxin induce several behavioral signs of anxiety correlated with increased corticosterone levels. Moreover, injections of $GABA_A$ receptor antagonists such as picrotoxin and bicuculline into the dorsomedial hypothalamus of rats elicit a wide range of physiological responses, i.e., an increase in heart rate and in plasma catecholamine levels, in locomotion activity and anxiogenic behavioral like effects, as well as in conflict, in the elevated plus maze and social interaction tests (File and Lister, 1984).

In a recent article we reported that perinatal exposure to a subconvulsive dose of picrotoxin interferes with the sexually dimorphic behaviors of male rats, measured in open-field and social interaction tests (Silva *et al.,* 1997). The offspring of female rats exposed to a subconvulsive dose of picrotoxin (0.75 mg kg) on day 18 of pregnancy, immediately after parturition and daily during the first 5 days of lactation, after reaching adult age showed a lack of the classical sexual dimorphic response in the open field; in the anxiety test, exposed male rats showed a behavioral response similar to that of female control rats. These data suggest that perinatal exposure to picrotoxin may interfere with normal male masculinization, rather than increasing anxiety in male rats.

The present results showing that gabaergic inhibition modifies sexual behavior of male rats could be a consequence of stress induced by picrotoxin. In addition, the slight facilitatory effect of picrotoxin might be similar to the effects induced by stress on copulatory behavior which involves dopaminergic mesolimbic activation (Herman *et al.,* 1982), mainly in the motivational component (Pfaus *et al.*, 1990; Hull *et al.,* 1995; Moses *et al.,* 1995). On the other hand, the reduction of the sexual behavior observed after the highest picrotoxin doses may be related to the actions of the drug elicited by several hormones secreted during stressful situations, such as corticotropin-releasing factor, beta-endorphin and glucocorticoids, on hypothalamic-pituitary-gonadal axis function (Doerr and Pirke, 1976; Rivier *et al.*, 1986; McLusky *et al.*, 1988). The infusion of Corticotrophin-releasing Factor (CRF) into the third ventricle of sexually experienced male rats elicited a suppression of sexual performance (Sirinathsinghji, 1987). However, in the present study we observed that low doses of picrotoxin only produced a mild facilitatory effect, a result quite different from that reported by Fernández-Guasti *et al.* (1986 a, 1986b). This fact might be the consequence of the different routes of administration employed.

The present study shows that the GABA antagonist picrotoxin alters male sexual behavior according to the intensity of receptor blockage. The higher doses tested reduced this behavior either by an alteration in motor aspects or a decrease in the motivational state of the rat. Although low doses of the $GABA_A$ antagonist induced a mild facilitatory effect, it is possible that different levels of stress might be responsible for these modifications.

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