Anxiolytic Effect of Dalargin on Rat Behavior in Vogel's Conflict Test and in Elevated Plus-Maze

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The anxiolytic effects of the δ-opioid receptor agonist dalargin revealed in two behavioral tests in outbred albino rats were blocked by naloxone. The effect of dalargin depended on the initial locomotor activity and was observed after central and peripheral administration of the drug. The μ-opioid receptor agonist DAGO produced no behavioral effects. It is suggested that the development of behavioral manifestations of anxiety and phobia is related to more than one opioid-dependent mechanism, which can explain the peculiarities of the anxiolytic effect of dalargin.

Key Words: *opioid peptides; anxiety; raised cruciform maze; Vogel's conflict test, motor activity*

Clinical and experimental data accumulated over the past years suggest that the opioid system is implicated in the development of some phobias and anxiety [1, 2, 5]. Our preliminary data indicate that the synthetic enkephalin analog dalargin which binds predominantly to δ-opioid receptors (OR) produces tranquilizing effects in patients with reactive neurotic disorders [2]. It has also been shown that dalargin modifies rat behavior in a shuttle box, which is possibly due to its anxiolytic effect [1]. On the other hand, the OR antagonist naloxone was shown to have a proconflict effect in the Vogel's conflict test (VCT) and to block the anxiolytic effects of diazepam [5].

Elucidation of the role of different OR in the development of anxiety and phobia with all their specific clinical manifestations will provide a pathogenetically justified approach to the improvement of their pharmacological therapy. The aim of the present study was to investigate the effects of dalargin and DAGO (μ-OR agonist) on the performance in VCT and in an elevated plus-maze (EPM).

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MATERIAL AND METHODS

The study was carried out on 237 outbred male albino rats weighing 210±30 g. The animals were purchased from the Stolbovaya breeding center and maintained at 21-23°C and 12:12 light-dark cycle (lights on at 7.00) with free access to food and water. The rats were allowed to habituate to these conditions for no less than 3 weeks.

Locomotor activity was tested in a computer-assisted Auto Track System (Columbus Instruments) as described previously [1]. On the basis of horizontal motor activity the rats were assigned to 3 groups: intact, control, and experimental.

The animals were given 5 intraperitoneal injections of saline, or dalargin, or DAGO (20 μg/kg in 0.2 ml, once a day). Naloxone (10 mg/kg intraperitoneally) was injected together with dalargin. Central administration of dalargin was performed as a single infusion in the left lateral brain ventricle through the hole previously drilled in the skull under ether and novocaine anesthesia according to the AP+0.5, L1, and H4 coordinates [3]. After surgery the animals were housed individually for 1 day with free access to water and food. On the next day the drug in a dose of

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0.05, 1, or 20 μg/kg in 2 μl was slowly (30 sec) infused in the ventricle to a depth of 4 mm. Four days before the behavioral tests, the rats were deprived of water but had free access to food.

Behavioral indices were recorded 30 min after the last intraperitoneal injection or 20 min after the intracerebroventricular infusion of the drug. The level of anxiety in VCT was assessed in an anxiometer (Columbus Instruments) by the number of spout lickings in the situation when every 20th licking was accompanied by light electric shock (0.13 mA, 50 Hz) applied to the spout for 2 sec. The number of punished lickings was counted during 3 min after the first one. During the next 3 min drinking motivation was assessed without punishment.

The EPM consisted of a central square platform (10×10 cm) connected to two open arms (45×10 cm) and two arms closed on both sides and open at the top (45×10×40 cm). The apparatus was raised to a height of 50 cm. The animals were placed in the center facing an open arm and observed for 5 min to determine the number of entries into the open arms and the time of staying there.

The data were treated by discriminant and cluster analyses and statistically evaluated with Student's *t* test.

RESULTS

Primary analysis of the data showed that neither physiological saline, nor DAGO and dalargin injected intraperitoneally to water-deprived animals affected their behavior in VCT. However, the cluster and discriminant analyses showed that the horizontal motor activity is an important classifying factor. On the basis of this parameter the whole population was divided into low-activity and high-activity groups. Dalargin increased the number of punished lickings in rats with low motor activity, having no effects on the behavior of high activity rats (Fig. 1). DAGO had no effect on the VCT-related behavior in both subgroups.

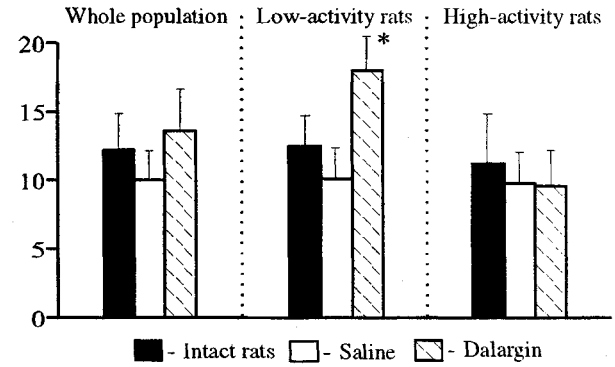

Fig. 1. Effect of dalargin on drinking behavior in Vogel's test in rats with different horizontal activity recorded by an Auto Track System. *$p < 0.05$ compared with saline. Each subgroup includes no less than 17 rats. Ordinate: number of punished lickings. Intact group consisted of water-deprived rats.

The effects of dalargin in VCT could be explained by increased drinking motivation in animals injected with δ-OR agonists [6] . However, under our experimental conditions none of the substances changed the

TABLE 1. Effect of Intraperitoneal Injection of Dalargin on Behavior of Water-Deprived Rats in Elevated Plus-Maze (*M*±*m*)

Group		Number of rats	Time spent in open arms	Number of entries into open arms
Intact	whole population	19	76.7±14.1	2.8±0.7
	high-activity rats	11	90.1±20.0	3.5±0.7
	low-activity rats	8	58.4±18.5	1.9±0.6
Saline	whole population	22	16.7±4.7***	0.8±0.2***
	high-activity rats	10	11.0±5.1**	0.4±0.2***
	low-activity rats	12	21.5±7.5*	1.1±0.3
Dalargin, 20 μg/kg				
	whole population	23	48.1±12.0*	1.8±0.4*
	high-activity rats	11	76.4±20.5**	3.0±0.7**
	low-activity rats	12	22.1±8.5+	0.8±0.3++
Dalargin 20 μg/kg+naloxone, 10 mg/kg				
	whole population	24	20.0±6.3*	0.6±0.2*
	high-activity rats	12	15.1±8.6**	0.5±0.2***
	low-activity rats	12	24.8±9.5	0.8±0.4

Note. Here and in Table 2: *$p<0.05$, **$p<0.01$, ***$p<0.001$ compared with the preceding group; +$p<0.05$, ++$p<0.01$ compared with rats with high locomotor activity.

TABLE 2. Effect of Intracerebroventricular Infusion of Dalargin on Rats Behavior in Evaluated Plus-Maze ($M \pm m$)

Group		Number of rats	Time spent in open arms	Number of entries into open arms
Intact	whole population	20	45.8±10.5	1.3±0.3
	high-activity rats	11	55.1±14.3	1.3±0.3
	low-activity rats	9	34.3±15.6	1.3±0.6
Saline	whole population	17	6.1±5.5**	0.2±0.1**
	high-activity rats	8	11.6±11.6*	0.3±0.3*
	low-activity rats	9	1.2±1.2*	0.1±0.1
Dalargin, 0.05 μg/kg				
	whole population	24	46.5±10.2**	1.2±0.3**
	high-activity rats	12	66.8±16.1*	1.5±0.4*
	low-activity rats	12	26.3±10.1*°	0.8±0.4
Dalargin, 1 μg/kg				
	whole population	22	31.7±8.8°	0.8±0.3°
	high-activity rats	8	36.5±16.7	0.5±0.2
	low-activity rats	14	28.9±10.5°	1.1±0.4
Dalargin, 20 μg/kg				
	whole population	10	21.0±21.0	0.1±0.1
	high-activity rats	5	26.3±26.3	0.1±0.1
	low-activity rats	5	0	0

Note. °$p<0.05$ compared with saline.

drinking motivation. Another explanation could be the analgetic effects of opioids, but the μ-receptor agonist DAGO which is a more efficient analgetic [4] than dalargin did not affect VCT performance. These data suggest that the effect of dalargin can be attributed to its anxiolytic activity.

Dalargin and DAGO had no effect on EPM performance in rats maintained under conditions of free access to water. At the same time, under conditions of water deprivation dalargin produced an anxiolytic effect, which can be blocked by naloxone. This effect was statistically significant for the whole population and for the rats with high locomotor activity: the number of entries and the time spent in open arm increased 7-fold (Table 1). DAGO did not affect the EPM behavior. It is of interest that the anxiolytic effect of dalargin manifested as the transition from active to passive avoidance strategy in a shuttle box was also typical of rats with high locomotor activity [1].

Central infusion of dalargin was also effective in the EPM. The most pronounced anxiolytic effect was produced by a minimal dose of 0.05 μg/kg, while in a dose of 20 μg/kg dalargin was effective in intraperitoneal injection and produced only insignificant effects in central administration (Table 2). These data suggest that the anxiolytic effect of dalargin results from its interaction with central δ-OR.

In this study we demonstated anxiolytic effect of the δ-OR agonist dalargin on rat behavior, which can be blocked by naloxone. Since this effect in VCT and the EPM was observed in rats with the initially different behavioral characteristics, we assumed that its realization involves more than one opioid-dependent mechanism.

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