Chronic Cocaine Injections Attenuate Behavioral Response of κ-Opioid Receptors to U-50,488H Agonist

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Chronic injections of cocaine (20 mg/kg daily for 10 days) increase activity and decrease anxiety in male C57Bl/6j mice in comparison with animals chronically injected with normal saline. U-50,488H (κ-opioid receptor agonist; 2.5 mg/kg) produced an anxiolytic effect in animals preinjected with normal saline and had no effect in animals chronically injected with cocaine. Presumably, chronic activation of dopaminergic systems caused by cocaine injections is paralleled by desensitization of k-opioid receptor system.

Key Words: *κ-opioid receptors; dopaminergic systems; cocaine; U-50,488H*; *anxiety*

Dopaminergic and opioidergic systems of the brain are involved in the regulation of emotions and social behavior [9,10]. Positive experience gained by male mice in agonistic confrontations for 10 days is associated with activation of the cerebral dopaminergic systems [1]. It was also shown that the response to injection of κ-opioid receptor agonist U-50,488H is determined by social status of the mice (experience of victories or defeats) [3]. This preparation (2.5 mg/kg) caused an anxiolytic effect only in males with repeated experience of social defeats. The authors claim that permanent activation of the opioid systems, presumably due to activation of the cerebral dopaminergic systems, leads to desensitization of κ-opioid receptors in winners [3].

In order to verify this hypothesis, another approach was used: chronic injections of cocaine activating dopaminergic neurotransmission [2,5-7] associated with changes in the κ-opioid receptor system [8,9].

In the present study we, using an elevated plus-maze, evaluated the sensitivity of κ-opioid receptors to their agonist U-50,488H in male mice, whose dopaminergic systems were chronically activated by 10-day treatment with cocaine.

MATERIALS AND METHODS

Experiments were carried out on adult male C57Bl/6j mice. The animals were kept under standard vivarium conditions at 12:12 h light:dark regimen with free access to standard fodder and water. The animals (aged 12 weeks) were kept in cages (4 per cage) for 25 days before the experiment for adaptation. All manipulations with the animals were carried out in accordance with international regulations for handling animals (European Communities Council Directive 86/609/EEC).

The tests were carried out in an elevated plus-maze [4]. The room was illuminated with a red lamp placed above the maze. The following parameters were determined over 5 min: number of entries into open arms, into the center, and into closed arms (presented as absolute values and percentage of total number of entries and exits); total time spent in open arms, in the center, and in closed arms (presented as absolute values and percentage of total time of testing); total number of entries/exits; number of peepings under the maze and number of peeping out of closed arms; num-

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ber of passages from one closed arm into another. In addition to these parameters, the time spent in closed, open arms, and in the center, divided by the number of the corresponding entries (mean duration per entry), was evaluated. The maze was thoroughly washed and dried after each animal.

Before testing half of animals (n=8-9) were intraperitoneally injected with cocaine in a daily dose of 20 mg/kg (10 ml/kg) at 9.00-10.00. Other animals received injections of normal saline. During the first 5 days of injections the animals lived in groups, after 5 days in individual cages. On day 11 half of males in each group received an acute injection of U-50,488H (trans-3,4-dichloro-N-methyl-N-(2,1-pyrrolidinyl)-cyclohexyl-benzeneacetamide, Sigma Chemical Co) in the most effective dose (2.5 mg/kg subcutaneously) [3], while other mice in both groups were injected with normal saline 30 min before maze testing.

Behavioral data were evaluated using ANOVA analysis of variances with evaluation of the effects of factors "chronic injection" (cocaine, saline) and "acute injection" (U-50,488H, saline) with subsequent paired comparison of the groups using Student's t test.

RESULTS

Chronic treatment with cocaine increased the number of entries into closed arms ($F(1.28)=7.4$, $p=0.01$), center ($F(1.28)=8.4$, $p<0.01$, and open arms ($F(1.28)=5.2$, $p<0.05$), and the total number of entries/exits ($F(1.28)=8.7$, $p<0.01$), which attests to higher activity of animals (Table 1). The total time spent in the open arms (absolute value and percentage) also increased after chronic cocaine treatment: $F(1.28)=4.7$, $p<0.05$, and ($F(1.28)=4.7$, $p<0.05$, respectively; for closed arms these parameters decreased: $F(1.28)=3.9$, $p=0.05$, and $F(1.28)=3.9$, $p=0.05$. This attests to decreased anxiety of animals. After chronic cocaine treatment the mean time spent in closed arms per entry decreased ($F(1.28)=9.8$, $p<0.01$), similarly as the number of peepings out ($F(1.28)=6.8$, $p=0.01$).

Acute injection of U-50,488H prolonged the mean duration of stay in open arms during one entry only for the group chronically injected with normal saline (factors interaction $F(1.28)=6.6$, $p=0.01$). This suggests that U-50,488H decreased anxiety in these animals, but not in mice chronically treated with cocaine. The agent also led to an increase in the number of peepings under the maze ($F(1.28)=6.5$, $p<0.05$).

TABLE 1. Effects of U-50,488H on the Behavior of Male Mice in an Elevated Plus-Maze after Chronic Cocaine Injections ($M\pm m$)

Parameter		Normal saline		Cocaine	
		acute injection of saline	acute injection of U-50,488H	acute injection of saline	acute injection of U-50,488H
Entries into closed arms, number	**aa**	12.22±0.91	13.25±0.96	14.50±0.91	15.78±0.76
Entries into closed arms, %		35.56±1.57	36.67±1.46	33.85±1.07	35.56±1.64
Time spent in closed arms, sec	**a**	166.16±8.94	159.95±6.84	145.94±11.8	144.02±8.29
Time spent in closed arms, %	**a**	55.29±2.99	53.21±2.27	48.57±3.92	47.93±2.76
Mean time spent in closed arms, sec	**aa**	14.24±1.27	12.30±0.52	10.59±1.48	9.27±0.70
Entries into the center, number	**aa**	17.44±1.57	18.13±1.11	21.75±1.45	22.44±1.65
Entries into the center, %		50.00±0.00	50.15±0.38	50.49±0.25	49.47±0.31
Time spent in the center, sec		107.07±7.51	102.93±5.06	107.83±5.93	115.57±5.11
Time spent in the center, %		35.63±2.49	34.24±1.69	35.89±1.98	38.46±1.70
Mean time spent in the center, sec		6.42±0.62	5.92±0.63	5.05±0.30	5.45±0.56
Entries into open arms, number	**a**	5.22±0.89	4.75±0.56	6.88±0.83	7.11±1.07
Entries into open arms, %		14.44±1.57	13.19±1.35	15.65±1.14	14.97±1.62
Time spent in open arms, sec	**a**	27.29±3.52	37.70±3.16	46.71±7.56	40.87±5.50
Time spent in open arms, %	**a**	9.08±1.17	12.54±1.05	15.55±2.52	13.60±1.83
Mean time spent in open arms, sec	**aabb**	5.57±0.55	8.32±0.74*	6.58±0.37	6.24±0.67
Total number of entries/exits	**aa**	34.89±3.15	36.13±2.14	43.13±2.94	45.33±3.27
Peepings, number	**b**	12.67±1.38	16.63±1.51	12.25±1.67	17.33±2.31
Peeping out, number	**aa**	5.44±1.27	4.13±0.48	1.75±0.88	2.89±0.87
Passages, number		5.89±1.14	5.50±0.78	6.50±0.98	6.78±0.46

Note. **a**≤0.05, **aa**≤0.01: effect of chronic treatment; **b**≤0.05, **bb**≤0.01: effect of acute treatment; **aabb**≤0.01: interaction of factors (ANOVA). *$p<0.05$ compared to acute injection of normal saline (Student's t test).

Hence, mice chronically treated with cocaine for 10 days exhibited higher activity and lower anxiety in the elevated plus-maze in comparison with animals receiving normal saline. After cocaine the number of peepings out from closed arm (risk evaluation index) decreased. Acute injection of U-50,488H produced an anxiolytic effect in mice chronically treated with saline and prolonged of the mean time spent in open arms during one entry. This effect was described previously [3]. However, the κ-opioid receptor system became less sensitive to the agonist after chronic treatment of animals with cocaine. This could be due to activation of the cerebral dopaminergic systems, resulting from cocaine treatment [2,5-7]. No anxiolytic effect of U-50,488H was observed in aggressive animals with experience of repeated victories [3], in which dopaminergic systems of the brain were activated [1]. Hence, chronic activation of dopaminergic systems by repeated injections of cocaine or repeated positive experience of agonistic confrontations induces desensitization of the κ-opioid receptor system.

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