Behavioral Effects of Potentiated Antibodies to Morphine and μ-Opioid Receptors during Withdrawal Syndrome

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We studied the effects of potentiated antibodies to morphine and μ-opioid receptors on animal behavior in the forced swimming test and reaction of light avoidance after morphine withdrawal. Antibodies to morphine normalized behavioral characteristics in the forced swimming test, while antibodies to μ-opioid receptors reduced dark preference in animals.

Key Words: *potentiated antibodies; morphine; μ-opioid receptors; withdrawal syndrome; behavior*

The search for preparations preventing the development of drug dependence after the use of psychotropic agents (*e.g.*, morphine and its derivatives) for medical and non-medical purposes attracts much attention. Combination therapy with homeopathic preparations and medicinal agents in standard doses holds much promise in this respect [2].

Recently, endomorphines, i.e. endogenous ligands of μ-opioid receptors, were discovered [4], the effects of potentiated morphine (in homeopathic doses) on learning and self-stimulation reaction in experimental animals were studied [2], and modulation of the effects standard morphine dose by potentiated morphine was demonstrated [1,3]. Here we studied the effects of potentiated antibodies to morphine (PAB-M) and μ-receptors (PAB-μR) on experimental opiate dependence.

MATERIALS AND METHODS

Experiments were performed on 50 male Wistar rats weighing 180-220 g (Nursery of the Institute of Cytology and Genetics). The animals were kept in a vivarium in 40×25×20-cm cages (2 rats per cage) under natural light/dark regimen with free access to water and food. The animals were randomly divided into control and experimental groups.

Morphine hydrochloride in increasing concentrations (20-60 mg/kg) was injected intraperitoneally for 5 days for modeling morphine dependence. Control animals received physiological saline. After the last injection PAB-M and PAB-μR were added to a drinking bowl for 3 days (1/3 fluid volume). Behavioral tests were performed on days 1 (pronounced somatic symptoms of abstinence [5]) and 5 after the last injection of morphine (disappearance of symptoms).

The reaction of rats to illumination conditions was studied in a shuttle box equipped with a 40-W lamp and detectors of animal position. The animals can switch off the lamp by transition form one compartment of the chamber to another. Twelve light stimuli with the maximum duration of 40 sec were presented at 40-sec intervals [1]. This test allows evaluation of locomotor activity (this parameter increases on day 1 after morphine withdrawal [11]) and anxiety (correlates with avoidance of illuminated areas [10]).

Morphine modulates rat behavior in the forced swimming test [6]. In our experiments this test was used in the early period after withdrawal, which received little attention in previous studies. We used a cylindrical tank (20 cm in diameter). Contact sensors were fixed on walls at heights of 0, 1, 2, and 3 mm above the water surface. We recorded 20 indications per sec, which surpassed the frequency of waves generated during swimming. Automatic recording allowed us not only to measure the duration of immobility, but also to evaluate the intensity of swimming movements by the number and height of waves above the water surface. Locomotor activity was estimated by changes in the water level and expressed in arbitrary units. The period of immobility corresponded to the absence of waves above the water surface during 5-min swimming.

The results were analyzed by Student's *t* test.

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RESULTS

The latency of reactions to light stimuli progressively increased from the 1st to 12th presentation (Table 1). In animals receiving morphine alone the latency of reactions to the first 3 presentation decreased on day 1 after withdrawal. These changes became more pronounced 5 days after morphine withdrawal. The effect can be explained by learning during the first trial on day 1 after morphine withdrawal. It should be noted that learning against the background of withdrawal syndrome was more effective than in the control.

In animals receiving PAB-M after morphine withdrawal the reaction to light stimuli was similar (Table 1). In rats receiving PAB-μR the latency of reactions on day 1 after morphine withdrawal was longer than in animals treated with morphine alone. On day 5 after morphine withdrawal these changes became more pronounced (Table 1): behavioral parameter in animals receiving PAB-μR did not differ from the control. Thus, this preparation reduces the increased reactivity to light stimuli produced by morphine withdrawal.

In the forced swimming test the duration of immobility decreased, while the intensity of swimming movements increased 1 day after morphine withdrawal (Table 2). These changes probably reflect enhanced total and motor excitability of rats [11]. On day 5 after morphine withdrawal the differences between the control rats and animals receiving morphine disappeared. These results suggest that the increased reactivity during forced swimming correlates with the severity of somatic symptoms of abstinence, which decreases 5 days after morphine withdrawal [5].

The behavioral characteristics of rats receiving PAB-μR did not differ from the control. Administration of PAB-M after morphine withdrawal abolished its effect on day 1, which manifested in decreased activity of morphine-treated rats. On day 5 after morphine withdrawal the differences between various groups disappeared (Table 2).

It seems that the development of abstinence syndrome not only modulates animal reaction to various stimuli (excitability, self-stimulation rate [8]), but also affects memory processes (judging from the results of forced swimming test and reactions of animals to light stimuli after repeated learning in the shuttle box). It can be hypothesized that PAB-M decrease enhanced reactivity to exogenous factors and, probably, endogenous signals responsible for physical discomfort during withdrawal syndrome.

Increased anxiety is the major component of withdrawal syndrome. However, studies in the plus-maze do not necessarily reveal these changes [6]. At the same time increased avoidance of illuminated places

TABLE 1. Latency of Reaction to Light Stimuli (sec, $M \pm m$)

Period after withdrawal; presentation		Control	Morphine		
			without correction	+PAB-M	+PAB-μR
Day 1	1-3	80.5±11.5	52.3±7.1**	47.2±5.1**	88.8±11.6^{++}
	4-6	88.4±7.5	94.5±7.0	82.6±6.3	89.5±12.1
	7-9	100.9±9.4	106.3±4.5	108.2±5.9	106.1±7.4
	10-12	110.5±5.2	109.2±5.9	107.0±4.1	107.8±8.2
Day 5	1-3	92.8±9.6	64.6±7.2**	63.6±10.2	104.8±5.2^{+}
	4-6	115.2±3.1	81.2±6.7*	90.2±9.7**	105.2±5.4^{++}
	7-9	111.9±5.3	91.7±6.3**	92.2±8.5	107.4±5.4
	10-12	115.8±4.1	98.9±7.5	106.2±6.7	114.7±3.2

Note. *p<0.001 and **p<0.05 compared to the control; ^{+}p<0.001 and ^{++}p<0.05 compared to morphine without correction.

TABLE 2. Activity and Period of Immobility in the Forced Swimming Test ($M \pm m$)

Parameter, time after withdrawal		Control	Morphine		
			without correction	+PAB-M	+PAB-μR
Activity, arb. units	day 1	469±26	600±24*	528±27^{+}	584±31**
	day 5	354±27	398±34	420±30	348±28
Time, sec	day 1	131.3±10.0	102.4±8.6**	115.5±12.8	115.6±10.5
	day 5	166.5±10.2	153.3±10.6	150.9±14.4	172.4±12.3

Note. *p<0.01 and **p<0.05 compared to the control; ^{+}p<0.05 compared to morphine without correction.

is considered as a result of influence of anxiogenic factors [7]. In light of this shortening of the latency of reactions to light stimuli reflects increased anxiety during learning. Therefore, PAB-μR attenuate or eliminate symptoms of anxiety associated with morphine withdrawal. This problem requires further investigations.

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