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The Contribution of Dopamine Autoreceptors to the Reactivating Effect of Opioid Antagonists

N. I. Dubrovina

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The dopamine-opioid interaction is analyzed in amnesia and forgetfulness with the use of passive avoidance conditioning in experiments on mice. Naloxone or ICI174.864 administration restored the conditioned response in both learning situations against the background of saline. Pretreatment with (+)3PPP eliminated the reactivating effects of μ- and δ-opiate receptor blockade in the case of amnesia. The activation of dopamine autoreceptors in forgetfulness disrupted the memory restoration induced by ICI174.864, but not that by naloxone. The findings attest that reactivation of an amnestied memory trace by opioid receptor blockade depends on dopaminergic system functioning, whereas in the case of forgetfulness dopamine-opioid interactions are probably determined by the functional heterogeneity of the μ- and δ-opiate receptors and diminished contribution of the dopamine system to the process.

Key Words: *amnesia; forgetfulness; opioid antagonists; dopamine autoreceptors*

The activation of the dopaminergic (DA) system and blockade of the μ- and δ-opiate receptors help counteract amnesia [1,7,11]. Investigations of DA and opioid receptor interaction in behavioral reactions studied in detail during analysis of the reinforcing system of the brain and motor activity [5,9], are now being pursued intensively. DA-receptor blockade effectively changes the modifying effect of the μ-, δ-, and κ-receptor agonists on habituation [4]. The role of DA-opioid interaction in recalling the memory trace is virtually unknown, although the modulating effects of neuropeptides on learning depending on the functional state of monoaminergic systems have been examined [3,6].

Laboratory of Memory Regulation Mechanisms, Institute of Physiology, Siberian Division of the Russian Academy of Medical Sciences, Novosibirsk. (Presented by V. A. Trufakin, Member of the Russian Academy of Medical Sciences)

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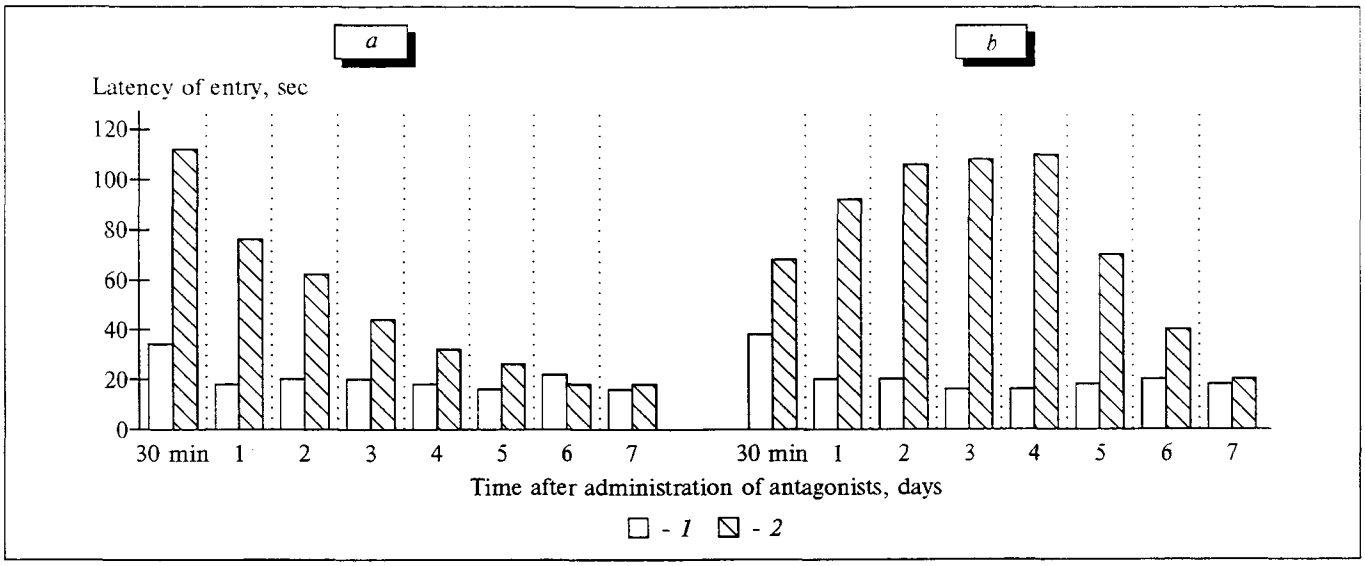

Fig. 1. Changes of the effects of opioid antagonists on the restoration of CPAR as a function of preactivation of DA receptors in amnesia. *1*) development of amnesia against the background of (+)3PPP at 2 mg/kg; *2*) development of amnesia against the background of physiological saline. *a*) administration on the 2nd day after conditioning and amnestic action of ICI174.864 at 3 mg/kg; *b*) administration of naloxone at 2 mg/kg.

The present study was undertaken to identify the mechanisms of DA-opioid interaction in memory. The effect of μ- and δ-opiate receptor blockade on restoration of the conditioned passive avoidance response (CPAR) in amnesia and forgetfulness was assessed with preactivation of DA autoreceptors.

MATERIALS AND METHODS

Experiments were carried out on male BALB/c mice weighing 17-23 g. CPAR was elaborated in animals routinely in an experimental chamber with two compartments, namely safe light and dangerous dark [1]. ON the day of conditioning a mouse entering the dark compartment received electrodermal stimulation with a current of 1 mA for 2 sec. The amnestic procedure consisted in leaving the animal in the dangerous compartment for 5 min immediately after electrodermal stimulation [1]. Spontaneous forgetting was achieved in the interval between the first test performed 1 day after conditioning and the second performed 21 days later. After the first test the animals with the latency of entry of 180 sec were transferred to vivarium cages, where spontaneous forgetting occurred. Mice used in the next experiment exhibited a latency of entry less than 45 sec 21 days later.

DA receptors were preactivated by the selective agonist (+)3PPP (Astra Lakemedel) at 2 mg/

TABLE 1. Dynamics of Latency of Entry in Learning, Amnesia, and Forgetfulness ($M \pm m$)

Group of mice	Number of animals	Period of testing, days									
		0	1	2	3	4	5	6	7	8	9
Physiological saline + conditioning	22	15±1	150±12	134±19	122±20	111±20	114±20	115±19	79±17	65±15	44±11
(+)3PPP + conditioning	17	16±2	139±17	124±17	130±15	109±21	104±19	110±19	84±15	60±14	52±13
Physiological saline + amnesia (1)	13	15±2	16±2	17±3	16±2	15±1	16±3	15±2	16±1	16±2	16±3
(+)3PPP + amnesia (2)	12	21±2	21±2	18±2	18±3	21±4	15±1	20±4	20±2	16±2	16±1
		0	1	21	22	23	24	25	26	27	
Physiological saline + forgetfulness (7)	9	16±2	180±0	16±3	17±3	17±2	14±2	18±3	17±3	17±2	
(+)3PPP + forgetfulness (8)	11	17±3	180±0	18±2	20±4	21±4	16±2	15±1	16±2	15±2	

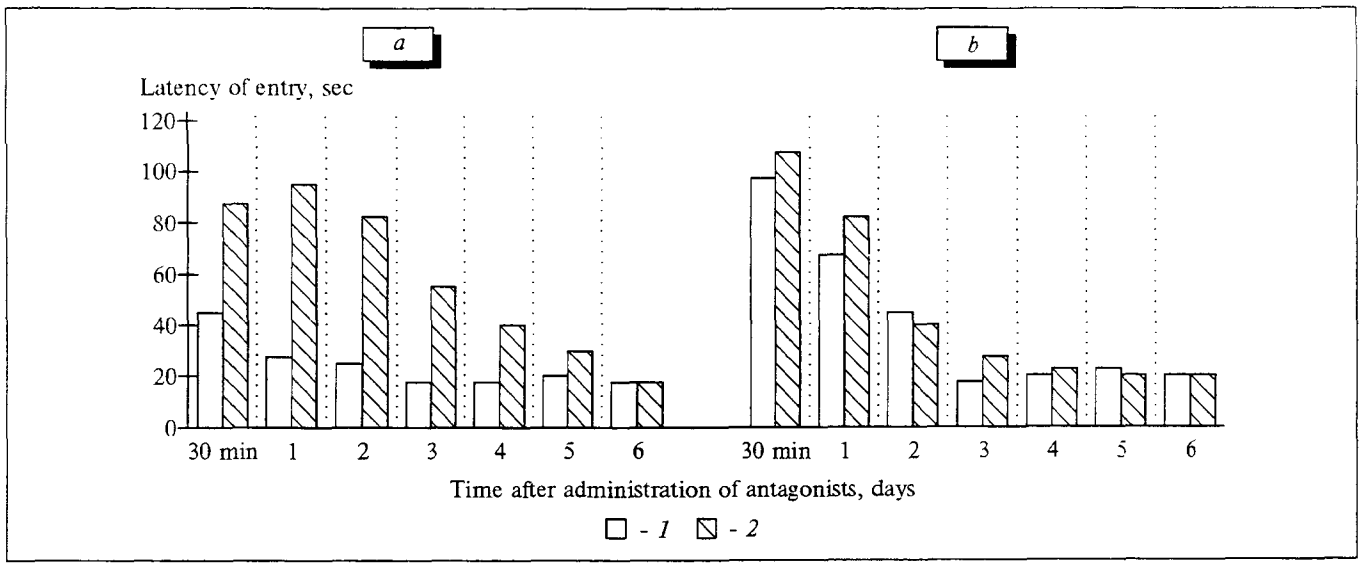

Fig. 2. Reactivating effects of μ− and δ−opioid receptor blockade as a function of DA autoreceptor preactivation. *1*) administration of (+)3PPP at 2 mg/kg 30 min prior to conditioning; *2*) administration of physiological saline. *a*) administration of ICI174.864 at 3 mg/kg on the 22nd day after conditioning; *b*) administration of naloxone at 2 mg/kg.

kg 30 min prior to conditioning (the series with amnesia) or before conditioning and the amnestic procedure (the series with psychogenic amnesia). The main μ-opioid-receptor antagonist naloxone (Sigma) at 2 mg/kg and the selective δ-receptor antagonist ICI174.864 (Imperial Chemical Industries) at 3 mg/kg were administered to mice before testing on the 2nd (amnesia series) or on the 22nd (forgetfulness series) day. Control mice received physiological saline. All preparations were injected i.p. in a volume of 0.2 ml per mouse. The groups of mice used were as follows: the 1st group was control for amnesia (13 mice), the 2nd was control for amnesia against the background of (+)3PPP (12 mice), the 3rd, amnesia against the background of physiological saline + naloxone (13 mice), the 4th, amnesia against the background of (+)3PPP + naloxone (15 mice), the 5th, amnesia (physiological saline) + ICI174.864 (18 mice), the 6th, amnesia against the background of (+)3PPP + ICI174.864 (15 mice), the 7th, control of forgetfulness (9 mice), the 8th , control of forgetfulness against the background of (+)3PPP (11 mice), the 9th, forgetfulness (physiological saline) + naloxone (15 mice), the 10th, forgetfulness against the background of (+)3PPP + naloxone (13 mice), the 11th, forgetfulness (physiological saline) + ICI174.864 (15 mice), and the 12th, forgetfulness against the background of (+)3PPP + ICI174.864 (14 mice). Findings were processed statistically using two-factor Anova software with subsequent analysis of multiple comparisons after Scheffe. The first factor used was the group factor, and the second the time factor.

RESULTS

Preactivation of DA autoreceptors did not affect either learning or the development of amnesia and forgetfulness (Table 1). There were no marked differences in the latencies periods of entry in corresponding groups of mice treated before conditioning and before conditioning + amnestic procedure, physiological saline, or (+)3PPP at 2 mg/kg. Thus, spontaneous restoration of CPAR does not occur in amnesia and forgetfulness.

The analysis of results obtained for administration of μ- and δ-receptor antagonists before testing showed that the preactivation of DA autoreceptors markedly changed the nature of the reactivating effects of these drugs. As is evident from Fig. 1, ICI174.864 (3 mg/kg) and naloxone (2 mg/kg) promote an increase of the latency of entry in amnesia, developing against the background of physiological saline, attesting to the restoration of CPAR. The preliminary administration of (+)3PPP blocks the reactivating effects of opioid antagonists. The results of Anova analysis listed in Table 2 point to the existence of a high degree of significance in groups of mice treated with naloxone and ICI174.864 in two situations of amnesia development (3rd and 4th and 5th and 6th groups). Subsequent scheduled comparisons on testing days showed that neither naloxone nor ICI174.864 against the background of (+)3PPP effectively restores CPAR, and therefore there are no reliable differences in the latency of entry between the groups with these drugs and the corresponding control of amnesia (2nd and 4th and 2nd and

TABLE 2. Anova Analysis of Effectiveness of Naloxone and ICI174.864 in Amnesia and Forgetfulness

	Group			
	1 and 3	1 and 5	2 and 4	2 and 6
Group factor	F(1.24) = 107.09 $p<0.0001$	F(1.29) = 47.09 $p<0.0001$	F(1.25) = 0.04 $p=0.82$	F(1.25) = 0.15 $p=0.70$
Time factor	F(7.168) = 8.48 $p<0.0001$	F(7.203) = 12.98 $p<0.0001$	F(7.175) = 1.24 $p=0.28$	F(7.175) = 1.56 $p=0.15$
Interaction of factors	F(7.168) = 8.06 $p<0.0001$	F(7.203) = 13.38 $p<0.0001$	F(7.175) = 1.47 $p=0.18$	F(7.175) = 2.03 $p=0.06$
	Group			
	5 and 6	3 and 4	9 and 7	7 and 11
Group factor	F(1.31) = 31.0 $p<0.0001$	F(1.26) = 90.49 $p<0.0001$	F(1.22) = 28.77 $p<0.0001$	F(1.22) = 56.89 $p<0.0001$
Time factor	F(7.217) = 16.51 $p<0.0001$	F(7.182) = 7.06 $p<0.0001$	F(7.134) = 12.93 $p<0.0001$	F(7.134) = 7.02 $p<0.0001$
Interaction of factors	F(7.217) = 8.25 $p<0.0001$	F(7.134) = 7.02 $p<0.0001$	F(7.134) = 11.34 $p<0.0001$	F(7.134) = 11.34 $p<0.0002$
	Group			
	8 and 10	8 and 12	9 and 10	11 and 12
Group factor	F(1.22) = 15.83 $p=0.0009$	F(1.23) = 3.17 $p=0.08$	F(1.23) = 3.17 $p=0.19$	F(1.27) = 19.68 $p=0.0003$
Time factor	F(7.154) = 7.68 $p<0.0001$	F(7.161) = 1.67 $p=0.12$	F(7.161) = 1.67 $p<0.001$	F(7.161) = 1.67 $p<0.0001$
Interaction of factors	F(7.154) = 6.24 $p<0.0001$	F(7.161) = 1.67 $p=0.48$	F(7.161) = 1.67 $p=0.46$	F(7.161) = 1.67 $p=0.0002$

6th). At the same time, the groups of mice developing amnesia against the background of physiological saline and (+)3PPP followed by the administration of naloxone (3rd and 4th) differed 30 min and 1 day later ($F(1.26)=10.48$, $p=0.003$ and $F(1.26)=20.29$, $p<0.0003$, respectively), while on the 7th day no differences were noted ($F(1.26)=$ $=1.09$, $p=0.31$). The same pattern of significances was found when the groups with ICI174.864 (5th and 6th) were compared.

The preactivation of DA autoreceptors in forgetfulness only blocked the efficiency of ICI174.864 (2 mg/kg) administered on the 22nd day after learning (Fig. 2). Anova software analysis of this group and the corresponding control (8th and 12th) points to the absence of significance in the group factor, time factor, and interaction of factors (Table 2), whereas naloxone (3 mg/kg) remained effective (8th and 10th). The preservation of the naloxone effect and blockage of the ICI174.864 effect in forgetfulness against the background of (+)3PPP was then confirmed in multiple comparisons of the groups of mice treated with the preparations in two situations (9 and 10, 11 and 12). There were no reliable differences between groups 9 and 10 on any day of testing (Fig. 2). The comparison of ICI174.864-treated mice (11 and 12) revealed reliable differences till the 5th day, namely $F(1.27)=3.89$, $p<0.05$ at 30 min, $F(1.27)=13.51$, $p<0.001$ after 1 day, and $F(1.27)=3.26$, $p=0.079$ after 5 days.

The findings attest that the restoration of CPAR in amnesia caused by the blockade of μ- and δ-opiate receptors is predicated upon the normal functioning of the DA system. The effect of (+)3PPP activation of DA autoreceptors on opioid receptors and on the improvement of CPAR recall for administration of naloxone and ICI174.864 may be realized via two mechanisms. First, the lowered dopamine synthesis and release due to the activation of DA autoreceptors may be followed by increased release of endogenous opioid peptides during the formation of the memory trace, which deepens amnesia. What this means is that in health the DA afferents produce tonic inhibition of transmitter synthesis in enkephalinergic neurons in terminal regions of the mesolimbic-cortical DA system. This is confirmed by biochemical and behavioral investigations of locomotor activity, where electrolytic destruction and pharmacological

blockade of the system resulted in the intensification of enkephalinergic transmission [8].

The second possible way may be realized via modification of the sensitivity not only of DA postsynaptic receptors, but also of opioid receptors in learning against the background of enhanced activity of DA autoreceptors, which results in discoordination of these receptors. The feasibility of such a mechanism is undoubted, because just a single administration of DA-receptor agonist causes a long-term change of DA-receptor binding properties [12].

In the reactivation of a forgotten memory trace the opioid interaction is more complicated and probably related to the lowered contribution of the DA system in the process [2] as well as to the functional heterogeneity of the μ- and δ-opioid receptors in mediating different behavioral reactions [10].

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