Mechanisms of Behavioral Effects of Potentiated Morphine Forms

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Effects of morphine and its potentiated (homeopathic) form on rat behavior in an elevated plus-maze were studied. Combined application of potentiated and non-potentiated morphine enhanced the anxiolytic and sedative effects. Patch-clamp experiments on isolated *Helix pomatia* giant neurons revealed a blocking effect of potentiated morphine on μ-receptors.

Key Words: *morphine; potentiated forms; behavior; isolated neurons; membrane currents*

Homeopathic drug forms applied in combination with conventional doses of these agents can play an important role in preventing drug abuse when using narcotic analgetics [2]. It explains the necessity of studying the effects of homeopathic doses of such widely used drugs as morphine, which binds to endorphin receptors [8].

There is evidence on the effect of potentiated morphine on learning and self-stimulation reaction in animals, which can be mediated by changes in cerebral monoamines [2]. The antistress capacity of such preparations calls for further detailed investigation.

Our aim was to study the effects of potentiated morphine and its combined application with this drug in conventional doses on rat behavior in an elevated plus-maze (EPM), which is used in screening for anxiolytic drugs [7], and on reactions reinforced by low-intensity illumination.

MATERIALS AND METHODS

Series I of behavioral experiments was carried out on 20 male Wistar rats weighing 200-300 g, which were kept two per cage under standard vivarium conditions with a natural day-night cycle and food and water *ad libitum*. The rats were randomized into control and test groups (9 rats in each group).

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To study the behavioral dynamics, morphine hydrochloride was injected in doses of 3 and 2 mg/kg or applied in hundredth potencies 30 and 200 (M-30 and M-200, respectively) prepared as described previously [2] and containing 10^{-60} and 10^{-400} mass fractions. The drugs were injected intraperitoneally in 0.5 ml isotonic NaCl 15 min before behavioral tests. In experiments with combined application of the drugs, potentiated morphine was injected 10 min prior to injection of allopathic doses of the analgetic. The controls were injected with the corresponding volumes of saline.

EPM consisted of two open (5×10 cm) and two closed (50×10×40) arms with open ceiling. Both arms of each type were oppositely directed, and the ceiling was lifted to an altitude of 50 cm. A rat was placed in the center and the number of exits from closed arms and the duration of stay outside these arms were recorded for 5 min.

When a rat was tested with light stimuli, it was placed into a shuttle box (30×20×20) illuminated with a 40-W electric lamp. The rats were preliminary trained to turn the lamp off by means of transition from one half of the chamber to the other. Experimental session included no less than 15 presentations of light stimuli with maximal duration of 40 sec. If the stimulation provoked the motor reaction, illumination was turned off for 40 sec. The effect of pharmacological preparations was assessed by the latency of conditioned response [2].

In series II the membranotropic effects of morphine, M-30, and M-200 were studied on cultured neu-

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TABLE 1. Effect of Morphine (3 mg/kg) and Its Potentiated Forms on Rat Behavior in EPM and Response to Light Stimulation (*M±m*)

Experimental series	Number of exits from closed arms of EPM		Latency of reaction to light stimuli, sec	
	control	test	control	test
Morphine	1.37±0.51	6.12±1.86*	21.65±4.77	17.03±3.63
M-30	1.77±0.61	0.77±0.36	20.60±5.50	14.20±4.70
M-30+morphine	1.56±0.66	8.87±1.83*	20.52±5.41	10.76±2.46*
M-200	1.33±0.33	1.11±0.51	10.05±1.26	6.86±0.49*
M-200+morphine	1.75±1.00	5.80±2.85	13.15±3.45	12.44±2.69

Note. *$p<0.05$ compared to the control.

rons from *Helix Pomatia* subesophageal ganglionic complex. Neurons were obtained by treating the ganglia with 0.35% pronase (Sigma) for 1-1.5 h. Isolated cells were placed on glass bottom of a chamber for electrophysiological study. They were cultured in physiological saline without amino acids at 6-12°C.

Ionic currents were recorded under voltage clamp conditions from a patch of neuron membrane attached to a glass micropipette (internal diameter 8-10 μm) by 1-10 gPa sucking pressure.

The value of patch ionic currents were 100-500 pA. The membrane potential outside the pipette was not clamped. Clamping of potential in a small fragment of neuronal membrane (less than $^1/_{1000}$ of total membrane area) did not disturb generation of action potential in the non-clamped membrane area. The test agents were applied using a perfusion system.

As in the behavioral experiments, the effects of morphine alone (10 mM) or in combination with the potentiated alkaloid were studied. The control solution was the solvent (alcohol) used to prepare potentiated forms.

The results were statistically analyzed using Student's *t* test.

RESULTS

Morphine significantly enhanced motor activity of rats in EPM, while its homeopathic dilutions M-30 and M-200 produced no significant effect on the number of exits from plus-maze closed arms (Table 1).

Combined application of M-30 and morphine significantly enhanced motor activity to a greater extent than morphine alone (the difference between the mean number of exits was 2.75±1.00, $p<0.05$). Hence, M-30 potentiated the anxiolytic effect of morphine without producing intrinsic effect.

The latency of responses to light stimulation was not significantly affected by morphine (Table 1), while M-200 and M-30 significantly shortened it in comparison with the control (6.50±2.70 sec, $p<0.05$).

M-30 produced the same effect, when applied together with morphine (3 mg/kg). The activating effect of M-30 and M-200 agrees with previously reported acceleration of acquisition of the conditioned avoidance and with decrease in pain sensitivity thresholds by homeopathic morphine dilutions.

In the next experimental series successive presentations of light stimuli evoking motor responses included non-reinforced stimulus. Non-reinforcement activated motor activity of rats judging from the increased number of responses to the non-reinforced stimulus. Combined injections of morphine and M-30 or M-200 decreased the number of responses to the non-reinforced stimulus (Table 2), while injection of morphine alone was inefficient. Considering the increase in the number of responses to non-reinforced stimulus as the index of frustration induced by non-reinforcement, the used combinations of the drugs probably produced an antistressor effect. Presentation of the non-reinforced stimulus produced no significant effect on the latency of responses to the following reinforced stimuli both in control rats and in animals injected with 2 mg/kg morphine (Table 3). However, the latency of responses increased in rats treated with combination of M-30 or M-200 and usual dose of morphine. Therefore, the sedative effect was produced by combined application of morphine in homeopathic dilutions and morphine in the subsedative doses.

It can be hypothesized that this effect and accelerated acquisition of the avoidance response produced by morphine in homeopathic dilutions are mediated by μ-receptors, although new experiments are needed to clarify this point. Combined application of M-30 or M-200 with conventional doses of morphine seems to

TABLE 2. Number of Responses to Non-Reinforced Light Stimuli (*M±m*)

Experimental series	Control	Test
Morphine, 2 mg/kg	7.12±1.02	7.87±0.78
M-30+morphine	8.55±0.81	5.00±0.70**
M-200+morphine	7.22±0.87	4.11±0.99*

Note. Here and in Table 3: *$p<0.05$, **$p<0.01$ compared to the control.

TABLE 3. Effect of Non-Reinforcement on Latency of Response to Light Stimulation (sec, *M*±*m*)

Experimental series		Stimuli		
		1-4 before non-reinforcement	after non-reinforcement	
			1-4	5-8
Morphine, 2 mg/kg	control	5.72±0.92	5.84±0.83	4.49±0.89
	test	5.02±0.45	7.38±1.15	6.22±0.95
M-30+morphine	control	4.77±0.58	7.93±1.83	5.31±0.80
	test	6.98±1.68	12.30±2.48	12.88±2.28**
M-200+morphine	control	5.28±0.46	6.83±0.82	7.12±1.80
	test	6.52±0.80	12.60±2.25*	16.68±3.73*

potentiate the anxiolytic and sedative effect of morphine, which opens a possibility to decrease the doses and the incidence of side effects of narcotic analgetics.

The membrane study showed that morphine provoked generation of action potentials in neurons possessing no intrinsic activity. In active neuron it increased the frequency of discharge and the amplitude of inward currents (Fig. 1). These effects developed slowly over 10-15 min and peaked 30-40 min after morphine application (Fig. 1).

The effects of morphine were reversible: washout restored the initial neuronal activity after 35-40 min, while recovery of inward currents occurred later.

The perfusion system was stopped after 1 h, and M-200 was added to the chamber. In this case, neuronal activity and the amplitude of inward currents did not change. After 40-min exposure of a clamped neuron to potentiated alkaloid, the initial morphine solution was added to the chamber. It also produced no changes in neuronal activity and the amplitude of inward currents (Fig. 2).

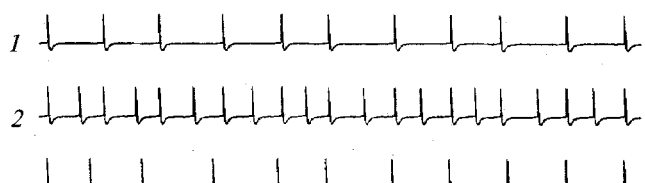

Fig. 1. Effect of morphine on electrical activity of isolated neuron. *1*) background activity before morphine application; *2*) after 30-min incubation with morphine; *3*) after 40-min incubation with M-200 followed by 30-min incubation with morphine and its potentiated form.

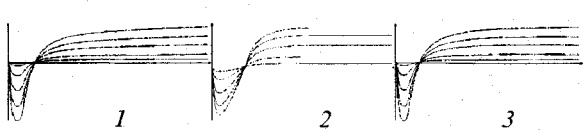

Fig. 2. Whole-cell ionic currents before application of morphine (*1*), after 30-min incubation with morphine (*2*), and after 40-min incubation with potentiated morphine followed by 30-min incubation with initial morphine solution with its potentiated form (*3*). Holding potential — 58 mV.

Isolated neurons were used as the model with a certain trigger level of intracellular reactions (ligand-receptor interaction on the outer membrane) and therefore characterized by a more definite and known set of effects of physiological doses of morphine, an agonist of opiate receptors. Neuronal opiate receptors are involved in the regulation of cAMP level in the cytoplasm via G-proteins [3-5]. Very slow changes in electrical activity after morphine application and washout attest to multistage interaction between opiate receptors and ionic channels.

The revealed similarity in the dynamics of basic (membrane) and behavioral phenomena suggests that potentiated morphine acts as a blocker of opiate receptors and modulates cytoplasmic level of cAMP in neurons. Therefore, it is reasonable to study the "bipathic" [2] and intrinsic properties of potentiated morphine in regimens used in the analysis of cAMP regulators and possibly by microchromatographic methods.

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