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Compound 48/80, a histamine-depleting agent, blocks the protective effect of morphine against electroconvulsive shock in mice

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Abstract

We have shown that morphine has an anticonvulsive effect against maximal electroconvulsive shock (MES) in mice, and this effect is antagonized by histamine H_1-receptor antagonists. Brain histamine is localized both in neurons and in mast cells, and morphine is known to enhance the turnover of neuronal histamine and to release histamine from mast cells. In the present experiments, compound 48/80 was injected chronically (0.5 mg/kg on day 1, 1 mg/kg on day 2, 2 mg/kg on day 3, 3 mg/kg on day 4, and 4 mg/kg on day 5, twice daily, *ip*) to deplete mast cell contents. Morphine (0.001-10 mg/kg, *ip*; N = 20) produced a dose-dependent anticonvulsive effect against MES seizure in mice with non-depleted mast cells, whereas it did not exert any anticonvulsive effect in mice with depleted mast cells. These results indicate that morphine produces its anticonvulsive effect against maximal electroconvulsive shock in mice by liberating histamine from mast cells.

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It has been reported that some of the central effects of morphine are mediated by the histaminergic system (1-4). Brain histamine is localized in both neurons and mast cells (5-9). Acute morphine treatment is known to increase the turnover of neuronal histamine (10) and to release histamine from mast cells in peripheral tissues (11,12). As a result, there are two different possible ways for morphine to activate central histaminergic mechanisms. In a previous study, we showed that morphine has an anticonvulsive effect against maximal electroconvulsive shock (MES) in mice, and this effect was antagonized by histamine H_1-receptor antagonists (4).

In the present study we determined whether mast cell histamine content plays a major role in the anticonvulsive effect of morphine in mice.

Male albino mice (Eczacibasi-Turkey) weighing 25-30 g were used. The animals were housed at constant temperature (22 ± 1°C), with food and water *ad libitum*, on a 12-h light/dark cycle (lights on at 6:00 a.m., and off at 6:00 p.m.).

The experiments were approved by the "Center of Laboratory Animals-Animal Care Ethics Committee" of our institution.

Maximal electroshock seizures were in-

duced through ear clip electrodes by a current generator (Ugo Basile, ECT Unit, 7801). The mice were stimulated with 50 mA, 0.4-ms pulse width, 0.2-s duration, 60-Hz square wave current. This current, which was calculated in a previous study (4), produces maximal convulsive seizure in half of the animals (MES_{50}). Tonic hind-limb extension (THE) was accepted as maximal electroshock seizure. Mice which did not show THE were considered to be protected from MES.

The experiments were carried out on 12 groups of 20 mice each. All experiments were carried out from 2:00 to 5:00 p.m. The animals were used only once.

Group 1 received vehicle (0.9% NaCl solution, control group), and groups 2-6 received various doses of morphine (0.001-10 mg/kg, intraperitoneally (*ip*)) and were subjected to electroshock 1 h after the injections.

In groups 7-12, to deplete mast cell content, compound 48/80 was injected chronically as follows: 1st day 0.5 mg/kg, 2nd day 1 mg/kg, 3rd day 2 mg/kg, 4th day 3 mg/kg, and 5th day 4 mg/kg, *ip*, twice daily. On the 6th day, group 7 received vehicle (control) and groups 8-12 received morphine (0.001-10 mg/kg, *ip*) 1 h before being subjected to electroshock.

Morphine hydrochloride (Haver, Istanbul, Turkey) was diluted from commercial preparations. Compound 48/80 was purchased from Sigma Chemical Co., St. Louis, MO, USA. All chemicals were dissolved in isotonic NaCl and administered *ip* in a volume of 0.1 ml/10 g body weight. The control groups received only 0.1 ml/10 g vehicle *ip*.

Comparisons among groups were made by the chi-square test using Yates correction for continuity and the Fisher exact test when indicated.

Morphine produced a dose-dependent anticonvulsive effect against MES (Table 1) and completely protected against MES seizure at the doses of 1 and 10 mg/kg ($P<0.001$ *vs* vehicle, chi-square test).

There was no significant change in MES seizure percentage in mice submitted to mast cell depletion with compound 48/80. Morphine pretreatment did not protect these mast cell-depleted animals from MES (Table 1).

The brain histaminergic system is known to play important roles in some central morphine effects, i.e., morphine-stimulated locomotion (2), morphine antinociception (1), and morphine-induced hypothermia (3). In a previous experiment we showed that morphine has an anticonvulsive effect which is antagonized by histamine H_1-receptor antagonists and naloxone (4). Brain histamine is localized in both neurons and mast cells, and there is evidence that mast cell stores of histamine contribute significantly to the overall histamine content of brain (5-7). Acute morphine treatment is known to increase the turnover of neuronal histamine (10,13). Moreover, morphine is also known to release histamine from mast cells in peripheral tissues (11,12): therefore a morphine action

Table 1 - Effects of chronic treatment with compound 48/80 on the anticonvulsive effect of morphine against MES.

There were 20 mice in each group.

	% Seizure	P
Mice with non-depleted mast cells		
Vehicle	55	
Morphine (0.001 mg/kg, *ip*)	45	
Morphine (0.01 mg/kg, *ip*)	35	
Morphine (0.1 mg/kg, *ip*)	15	P<0.05 *vs* vehicle, chi-square test
Morphine (1 mg/kg, *ip*)	0	P<0.001 *vs* vehicle, chi-square test
Morphine (10 mg/kg, *ip*)	0	P<0.001 *vs* vehicle, chi-square test
Mice with depleted mast cells		
Vehicle	45	
Morphine (0.001 mg/kg, *ip*)	50	
Morphine (0.01 mg/kg, *ip*)	55	
Morphine (0.1 mg/kg, *ip*)	50	P<0.05 *vs* same dose of morphine alone in mice with non-depleted mast cells, chi-square test
Morphine (1 mg/kg, *ip*)	45	P<0.01 *vs* same dose of morphine alone in mice with non-depleted mast cells, Fisher exact test
Morphine (10 mg/kg, *ip*)	40	P<0.01 *vs* same dose of morphine alone in mice with non-depleted mast cells, Fisher exact test

on mast cells in the central nervous system should be taken into consideration.

It was reported that elevation of brain histamine concentrations by metoprine inhibits maximal hindleg extension after MES (14), and intracerebroventricular histamine injections or endogenous histamine release in mouse brain exert an anticonvulsive effect against electrically induced convulsions (15-17). By using histamine H_1- and H_3-receptor agonists and antagonists in electrically and pentylenetetrazole-induced convulsions, Yokoyama et al. (15-17) supported the hypothesis that the central histaminergic system is involved in the inhibition of seizures.

It was also reported that compound 48/80, which is a potent histamine liberator from mast cells, has a protective effect against hypoxia which is mediated by histamine H_1-receptors (18). Intracerebroventricular injection of compound 48/80 produces head and body shakes, paw tremor, grooming, unusual posture, sedation and catatonia, and decreases the histamine concentrations in almost all brain regions and the noradrenaline concentrations in the cerebellum, hypothalamus and medulla oblongata-pons, although the dopamine content was decreased only in the medulla oblongata-pons (6). Chronic treatment with compound 48/80 did not exert any convulsive or anticonvulsive effect in our study.

It has been shown that mast cell granule contents are depleted by chronic treatment with compound 48/80 (19). In the present experiment, we depleted mast cell histamine contents by chronic treatment with compound 48/80 using a slightly modified dose schedule compared to that used by Jaffery et al. (19).

Morphine has an anticonvulsive effect which is antagonized by histamine H_1-receptor antagonists (4). In the present study, morphine showed an anticonvulsive effect against MES in mice with non-depleted mast cells but not in mice with depleted mast cells. These results show that the anticonvulsive effect of morphine is mediated by histamine liberated from brain mast cells in mice. It should also be taken into consideration that mast cells also contain 5-hydroxytryptamine and some other endogenous mediators. Their roles in some central effects of morphine can also be investigated in similar experiments. We conclude that brain mast cell histamine content must be taken into consideration in the central effects of morphine, which are mediated by histaminergic mechanisms.

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