

EFFECTS OF ACUTE AND CHRONIC LITHIUM ON MORPHINE-INDUCED ANALGESIA IN MICE BY FORMALIN TEST

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Abstract - In this study, the effects of acute and chronic lithium on nociception induced by formalin and its interactions with morphine-induced analgesia were studied in mice. Formalin test was selected to study nociception. Subcutaneous injection of different doses of morphine (1.5, 3 and 6 mg/kg) induced analgesia in a dose dependent manner in both phases of the formalin test. Intraperitoneal administration of different doses of lithium (20, 40, 60 and 80 mg/kg) induced analgesia in late phase but did not show any significant effect in early phase. Acute administration of lithium (40, 80 mg/kg) increased analgesia induced by morphine (3 mg/kg) in both phases of formalin test. Pretreatment of the animals with chronic lithium (1200 mg/l) for 15 days induced antinociception and increased the analgesia induced by morphine in both phases of formalin test. It is concluded that administration of acute and chronic lithium can probably affect pain response in both phases of formalin test and interact with morphine-induced antinociception.

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INTRODUCTION

During the last decade, numerous studies have been carried out to explore the effects of acute and chronic lithium on the different methods of antinociception tests. A number of behavioural methods have been developed to study nociception in animals. Several of these tests, the tail flick test, the flinch-jump test, the pinch test and the hot plate test, measure the response to brief, noxious stimuli (1,2). The animals response in these tests is integrated at rather low levels in the central nervous system, probably giving information mainly about the pain threshold. The formalin test, on the other hand, measures the response to a long-lasting nociceptive stimulus and thus may have a closer resemblance to clinical pain (3,4). In this test, two types of pain

were postulated: a short-lasting pain caused by a direct effect on nociceptors followed by a long-lasting pain due to inflammation (5).

Lithium is an effective drug in the treatment of manic-depressive illness. Although the specific biochemical mechanisms responsible for the therapeutic efficacy of lithium are unknown, a variety of physiological processes are affected by this drug (6). Lithium has been shown to reduce phosphoinositide metabolism by inhibiting inositol monophosphatase (7). Some reports have also shown that lithium inhibits the effects of a number of adenylyl cyclase-linked receptors (8). The present study was carried out to examine the effects of acute and chronic lithium administration on morphine-induced analgesia by formalin test in mice.

MATERIALS AND METHODS

Animals

Male albino mice weighing 22-27 g were used in the experiments. The animals were housed in groups of 7 in conditions of constant temperature ($21 \pm 2^\circ\text{C}$) and light controlled room.

Drugs

These chemicals were used: morphine chloride and lithium chloride (Sigma, England). All drugs were dissolved in 0.9% saline to concentrations that allowed them to be administered in a volume of 10 mg/ml. Morphine was administered 30 min before formalin injection, and lithium chloride was administered 60 min before any injection.

Chronic lithium pretreated animals received

lithium chloride (1200 mg/l) for 15 days in drinking water.

Antinociception Recording

Mice were allowed to acclimatize for 30 minutes before any injection. Twenty-five microliters of formalin (0.05%) was injected subcutaneously into the dorsal surface of the right hind paw of the mouse by using a microsyringe with a 26-gauge needle. Immediately after formalin injection, animals were placed individually in glass cylinder (20 cm wide, 25 cm length) on a flat glass floor, and a mirror was arranged in a 45° angle under the cylinder to allow clear observation of the paws of the animals. The total time (seconds) spent licking and biting the injected paw during periods of 0-5 min and 20-30 min were measured as an indicator of pain.

Chronic Experiments

For chronic administration, lithium chloride at 1200 mg/l was dissolved in drinking water (tap water) and animals received lithium chloride for a period of 15 days. Control animals received tap water.

Determination of Serum Lithium Levels

Lithium measurements were made after 15 days of treatment. Animals were sacrificed by decapitation, and blood collected from the neck wound in to siliconized tubes. Serum was separated immediately by centrifugation in a microcentrifuge (Eppendorf, Germany) and diluted for estimation of lithium in distilled and deionized water. Lithium levels in serum samples were then determined using a Shimadzo AA-670 atomic absorption spectrophotometer (Shimadzo, Japan). Readings were made in triplicate at a wavelength of 670.8 nm. Peak height measurements were compared with values for standards of known concentration made up in similarly diluted serum.

Statistical Analysis

The data presented as the mean \pm SEM and was analyzed by one-way analysis of variance (ANOVA) followed by Newman-Keulz test. Differences with $p < 0.05$ were considered statistically significant.

RESULTS

Antinociception Induced by Different Doses of Morphine on Formalin Test in Mice

Antinociceptive effects of morphine on formalin test are shown in figure 1. ANOVA indicates a significant difference between animals treated subcutaneously with different doses of morphine in the early phase (1.5-6 mg/kg) and the late phase (1.5-6 mg/kg) with saline. Further analysis showed that morphine induced a dose dependent antinociception in the early and late phases.

Antinociception Induced by Different Doses of Lithium on Formalin Test in Mice

There is a significant difference between animals that were injected intraperitoneally by different doses of lithium chloride (20-80 mg/kg) 60 min before formalin injection with saline in the late phase (Fig. 2). Antinociception in the early phase was not significant.

Interaction of Acute Lithium Chloride With Morphine and Analgesia

Effects of lithium chloride on morphine-induced antinociception are shown in Table 1. Lithium chloride (40,80 mg/kg, i.p.) significantly increased the effects of morphine (3 mg/kg, s.c.) in the early and late phases. Other results of interaction of lithium with morphine are shown in Table 2.

Effects of Chronic Lithium Pretreatment on Nociception Induced by Formalin and Morphine-Induced Analgesia

Chronic lithium administration (1200 mg/l, 15

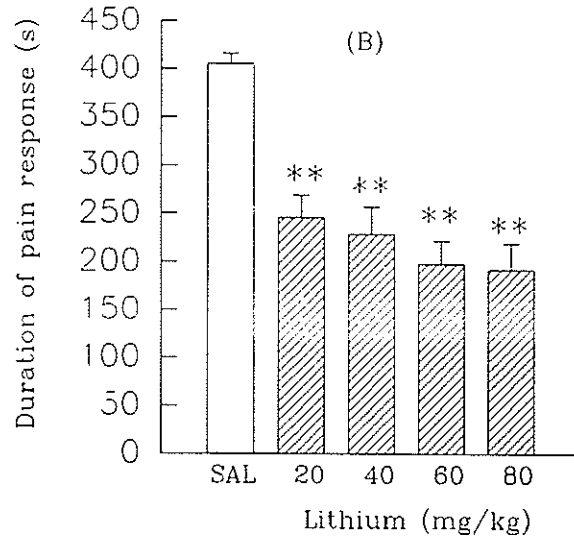
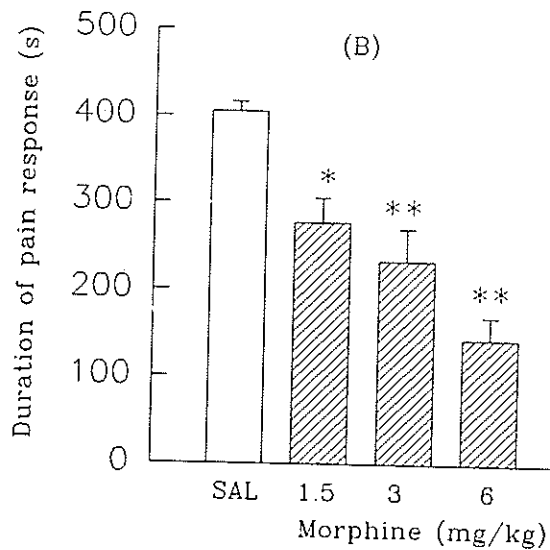
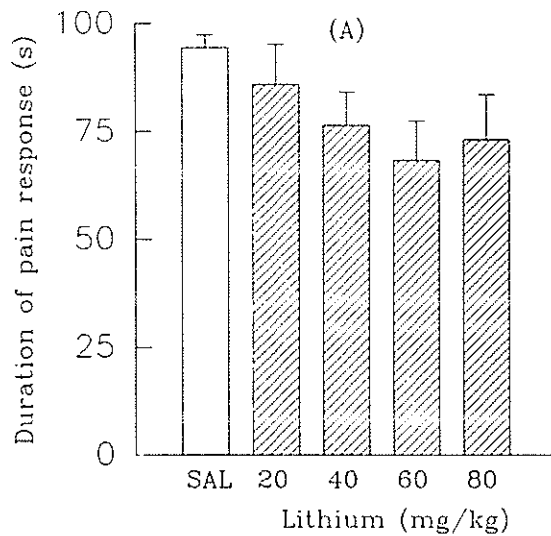
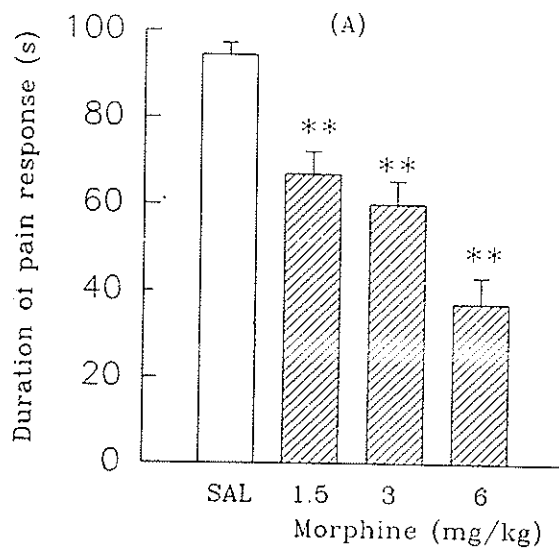


Fig 1. Effects of different doses of morphine on the formalin test in mice. Animals were administered S.C. either saline (2 ml/kg) or morphine (1.5, 3 and 6 mg/kg). Saline+morphine was injected 30 min before formalin administration. Antinociception was recorded (A) 0-5 (early phase) and (B) 20-30 min (late phase) after formalin injection. Each point is the mean±SEM of seven animals. *p<0.05, **p<0.01 different from saline control group.

Fig 2. Effects of different doses of lithium chloride on the formalin test in mice. Animals were administered i.p. either saline (5 ml/kg) or lithium chloride (20, 40, 60 and 80 mg/kg). Saline+lithium chloride was injected 60 min before formalin administration. Antinociception was recorded (A) 0-5 min (early phase) and (B) 20-30 min (late phase) after formalin injection. Each point is the mean±SEM of seven animals. *p<0.05, **p<0.01 different from saline control group.

Lithium and Morphine-Induced Analgesia

Table 1. Effects of different doses of lithium chloride on morphine (1.5, 3 mg/kg)-induced analgesia.

Treatment (mg/kg)	Early phase (sec)	Late phase (sec)
Morphine 1.5+Saline	67.00 ± 5.14	277.14 ± 28.32
Morphine 1.5+Li(20)	54.43 ± 12.03	269.29 ± 22.28
Morphine 1.5+Li(40)	59.64 ± 10.26	232.65 ± 16.32
Morphine 1.5+Li(80)	65.29 ± 3.73	186.86 ± 13.65
Morphine 3+Saline	60.14 ± 5.36	233.43 ± 36.90
Morphine 3+Li(20)	52.46 ± 6.48	198.24 ± 21.52
Morphine 3+Li(40)	15 ± 6.78	82.43 ± 20.58
Morphine 3+Li(80)	48.75 ± 1.57	125.71 ± 19.56

Animals were treated i.p. either saline (5mg/kg) or lithium chloride (20, 40 and 80 mg/kg) 60 min before morphine injection. Morphine (1.5, 3 mg/kg, s.c.) was injected 30 min before formalin administration. Antinociception was recorded 0-5 min (early phase) and 20-30 min (late phase) after formalin injection. Each point is the mean ± SEM of seven animals. *p < 0.05, **p < 0.01 different from respective control group.

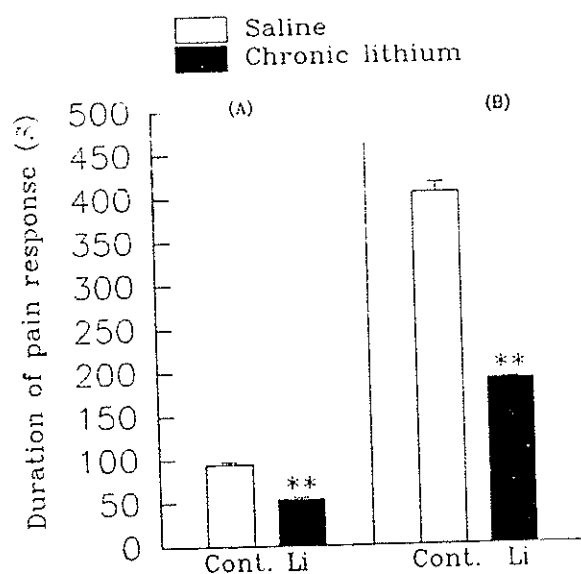


Fig. 3. Effects of chronic lithium on the formalin test in mice. Chronic lithium pretreated animals (■) received lithium chloride 1200 mg/l in drinking water for a 15 days period. Control group (□) received tap water. Antinociception was recorded (A) 0-5 min (early phase) and (B) 20-30 min (late phase) after formalin injection. Each point is the mean ± SEM of seven animals. *p < 0.05, **p < 0.01 different from control group.

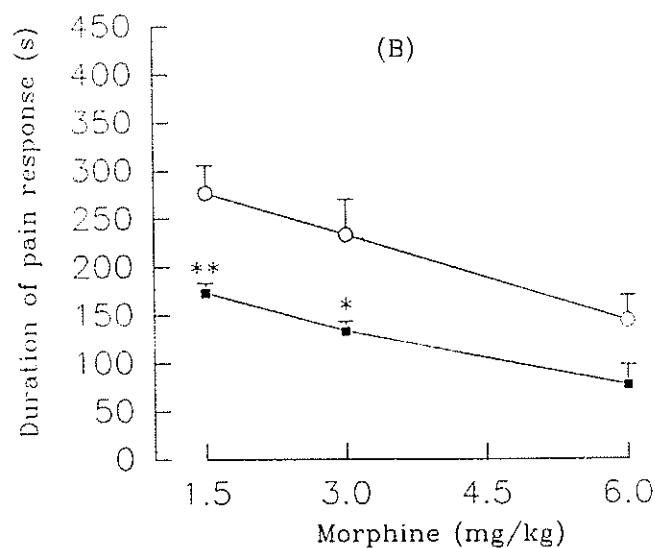
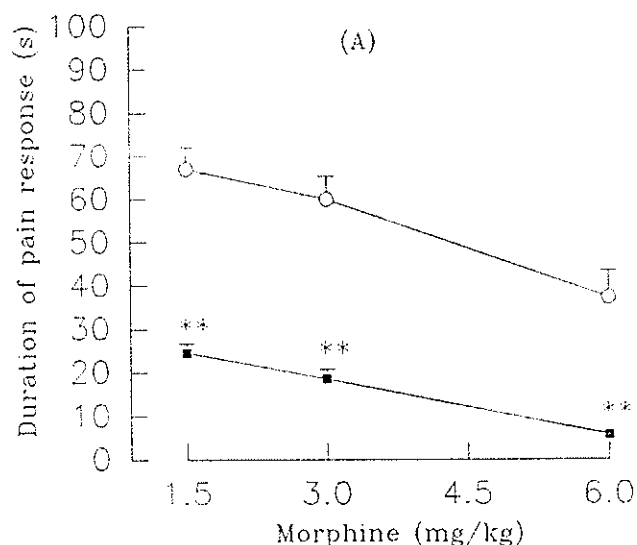


Fig. 4. Effects of morphine-induced antinociception in chronic lithium pretreated mice. Chronic lithium pretreated animals (■) received lithium chloride 1200 mg/l in drinking water for a 15 days period. Control mice received tap water (O). Morphine was injected s.c. 30 min before formalin injection. Antinociception was recorded (A) 0-5 min (early phase) and (B) 20-30 min (late phase) after formalin administration. Each point is the mean ± SEM of seven animals. *p < 0.05, **p < 0.01 different from respective control group.

days in drinking water) with serum level of 0.35 ± 0.02 mmol/l decreased significantly nociceptive effect of formalin in early (A) and late (B) phases (Fig. 3). Pretreatment of animals with chronic lithium (1200 mg/l, 15 days in drinking water), increased the antinociceptive effect of morphine (1.5 - 6 mg/kg, S.C.) in both early and late phases significantly (Fig. 4).

Serum lithium concentration

In this study, when lithium was given chronically, the serum concentration after 15 days was 0.35 ± 0.02 mmol/l (mean \pm SEM).

DISCUSSION

Metal ions can interfere with most biological processes and affect many kind of cellular activities. Although the effects of exposure to ion metals have long been recognized, relatively little is known regarding the cellular and subcellular mechanisms involved, especially in nerve membrane (9). Despite the successful and widespread use of lithium in controlling manic-depressive psychosis, it is not very clear how lithium might act within the CNS. Lithium could interfere with phosphatidyl inositol (PI) turnover and signal transduction indirectly by selectively inhibiting inositol monophosphatase and inducing inositol depletion. Inositol phosphate hydrolysis is the main source of intracellular inositol, which is involved in the synthesis of the precursor phosphate lipid required to generate second messengers such as inositoltriphosphate (IP3) and diacylglycerol (DAG) when agonist stimulates PI turnover in the CNS (10 - 13). In the present study, there were indications that acute and chronic lithium administration alter response to noxious stimuli. Acute administration of lithium alone did not show hypoalgesic effect in early phase but induced antinociception in late phase. Pretreatment of animals with chronic lithium caused significant antinociceptive effect in both phases. Previous

studies on analgesia indicated that the effect of lithium alone on responsiveness to painful stimuli have yielded inconsistent and sometimes contradictory results (14). In some studies, for example, both acute (15 - 17) and chronic (18, 19) administration of lithium produced analgesia in animals, whereas in others no analgesia (16, 20) has been reported. Our findings showed that the antinociceptive effect of acute lithium in late phase of formalin test is probably more than early phase but pretreatment with chronic lithium induced hypoalgesia in both phases. There is evidence that the analgesia is associated with a decrease in the content of calcium in animal brain tissue (21 - 26). Since lithium causes an alteration in phosphatidyl-inositides cascade by inhibiting the inositol-1-phosphatase and reduces the level of free inositol (27 - 29) this could interfere with resynthesis of PIP2 and prevent the formation of IP3 and DAG. Thus, the antinociceptive effect of acute and chronic lithium alone may be related to a decrease of intracellular calcium by inhibition of IP3 formation due to influencing the signalling mechanisms operating the phosphoinositides pathway. It is also suggested that the hypoalgesic effect of lithium may be calcium mediated and that the phosphatidylinositides cascade is probably more sensitive to the action of lithium in late phase than early phase after acute administration. In addition, lithium can enhance the biosynthesis (30, 31) or release (32, 33) of endogenous opioids in brain tissue. Other investigators also showed that lithium iontophoresis could inhibit the discharge of pain-excitation neurons, while the discharges of pain-inhibition neurons were activated (34). These findings appear to support the suggestion that endogenous opioid peptides and opioid receptors may be involved in the analgesic effect of lithium. The effects of lithium on the antinociceptive action of morphine was also studied in several methods such as tail flick and hot plate test (14, 19, 34, 35, 36). The results obtained in a number of studies were different

(14, 34, 35). Another major finding of the present study is that acute and chronic lithium administration can potentiate antinociceptive effects of morphine in both phases of formalin test. It seems that acute lithium administration caused synergistic effect with morphine in formalin test. Since the analgesic effect of morphine is associated with a decrease of intracellular calcium in brain tissue (25, 26) and lithium as well as morphine decreases the content of intracellular calcium via interaction with PI (28, 29, 37), therefore the interaction of lithium with morphine is probably calcium-mediated and it is suggested that there existed some similarity in the mechanisms of analgesia induced by lithium and morphine. Zhang and coworkers (36) also showed that the antagonistic effect of CCK-8 on ohmfentanyl-induced antinociception is regulated by lithium via interpretation with PI signal system. Our previous works identified that lithium can alter all the responses mediated by PI signalling (38, 39). The effects of lithium on the biosynthesis (31) or release (32, 33) of endogenous opioids is another possibility for the synergistic effects of lithium and morphine. The data obtained from animal experiments in this study support the suggestion that chronic lithium caused more synergistic effects than acute lithium on morphine-induced antinociception in formalin test. Considering the different effects of lithium, one may speculate that lithium might interfere with morphine-induced analgesia through nonspecific mechanisms. In conclusion our results showed the possible involvement of phosphoinositide signaling in the interaction of lithium and morphine on antinociception in both phases of formalin test.

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