

Original Article

Effect of Lithium on Swim Stress-Induced Antinociception in Naive Mice and Mice with Subchronic Administration of Morphine or Swim Stress in Formalin Test

Soheila Fazli-Tabaeia PhD*, Navid Bazaz MD*, Azadeh Modirzadeh MD*, Avid Bazaz MD*, Amir Maghsoudi MD*, Mohammad-Reza Zarrindast PhD***,***,†

Background: Lithium has been shown to relieve mania and induce antinociception. In the present study, swim stress at 8°C induced antinociception in both phases of the formalin test. Intraperitoneal administration of lithium chloride (LiCl) (0.05, 0.25, and 0.5 mg/kg) also induced antinociception in both phases of the formalin test.

Methods: Antinociception was assessed by the formalin test method. Swim stress was achieved in the 8°C water in a container 5 cm in diameter and 20 cm tall filled with water to a depth of 11 cm.

Results: The drug (0.5 and 5 mg/kg) potentiated swim stress-induced antinociception in the second phase of the test. Repeated exposure to water swimming stress with a period of 40 sec, once daily for three days, in combination with lithium chloride did not alter stress-induced antinociception in either phases of the formalin test, when swim stress-induced antinociception was tested on the fourth day. Subchronic treatment with morphine (25 mg/kg), once daily for three days, in the presence or absence of lithium chloride (5 mg/kg) did not alter swim stress-induced antinociception, either, when swim stress-induced antinociception was tested on the fourth day.

Conclusion: It may be concluded that lithium chloride potentiates swim stress-induced antinociception, but the drug has no influence on the response of subchronic administration swim stress or morphine.

Archives of Iranian Medicine, Volume 11, Number 2, 2008: 166 – 172.

Keywords: Dextromethorphan • formalin test • mice • swim stress-induced antinociception • tolerance

Introduction

Water swimming stress (WSS) model, depending on the water temperature may induce antinociception through different mechanisms. This is an opioid-mediated type of response induced by the swim stress at low temperatures such as 4°C,^{1,2} and is reversed by

naloxone and other opioid receptor antagonists. Repeated cold water swim stress at 4°C, produces an opioid-mediated antinociception,^{1,2} and induces tolerance, which may be due to a reduction of opioid receptors in the brainstem, midbrain, and spinal cord regions.³

Lithium, on the other hand, is used in the treatment of manic-depressives and is reported to interact with the opioid system.⁴⁻⁶ Studies have suggested an important role for lithium in the treatment of drug addiction.^{7,8} Lithium inhibits morphine withdrawal signs in morphine-dependent mice,⁹ reduces the self-stimulation facilitated by morphine,¹⁰ and alters the morphine-induced analgesia in mice.¹¹ Morphine antinociception may be mediated through a lithium chloride (LiCl)-sensitive, IP₃-restorable pathway.¹² A cross-

Authors' affiliations: *Research Laboratory, Tehran Medical Unit, Azad University, **Department of Pharmacology and Iranian National Center for Addiction Studies, Tehran University of Medical Sciences, ***School of Cognitive Sciences, Institute for Studies in Theoretical Physics and Mathematics, †Institute for Cognitive Science Studies, Tehran, Iran.

Corresponding author and reprints: Mohammad-Reza Zarrindast PhD, Department of Pharmacology, Tehran University of Medical Sciences, Tehran, Iran. P.O. Box 13145-784, Tel: +98-216-611-2801, Fax: +98-216-640-2569, E-mail: zarinmr@ams.ac.ir.

Accepted for publication: 17 February 2007

tolerance between morphine and swim stress has also been shown.¹³ In the present study, the effect of LiCl on swim stress-induced antinociception (SIA) in the presence or absence of repeated exposure to water swim stress or repeated administration of morphine in the formalin test has been investigated.

Materials and Methods

Animals

Four hundred ninety male NMRI mice (25 – 30 g) from the Pasteur Institute of Iran (Tehran, Iran) were used for these experiments. The animals were housed ten per cage (43x30x15 cm) in a room maintained at 23±1°C with an alternating 12 hr light/dark cycle. Food and water were freely available expect during the experiments. Each mouse was used only once and was euthanized immediately after the experiment.

Subchronic administration of morphine

Morphine sulfate (25 mg/kg) was injected intraperitoneally (IP) once daily (at 10 a.m.) for three days to induce possible tolerance to morphine antinociception. Three doses of morphine sulfate (3, 6, and 9 mg/kg, IP) were also injected on the fourth day, 20 min before formalin injection to test antinociception by the opioid.

Water swimming stress

The mice were forced to swim in 8°C water (at 10 am) in a container 15 cm in diameter and 20 cm tall filled with water to a depth of 11 cm, for periods of 20, 40, and 60 sec, in order to induce antinociception, or for a period of 40 sec, once daily for three days in order to induce tolerance to antinociception induced by morphine and/or swim stress. After swimming, the mice were gently dried by patting the body with a towel for 2 min. Pain scores were recorded 15 min after WSS. Control groups were treated similarly, without being forced to swim.

Antinociceptive testing

Animals were allowed to acclimatize for 30 min before formalin injection. A dose of 40µL formalin (1%) was injected subcutaneously into the dorsal surface of the right hind paw of the mouse using a microsyringe (Hamilton Bonaduz AG, Switzerland) with a 27 gauge needle. Pain behavior

was recorded immediately after formalin injection. The behavior was that originally described by Dubuisson and Dennis,¹⁴ and reiterated by Abbott and colleagues,¹⁵ as 0=normal weight bearing on the injected paw, 1=limping during locomotion or resting the paw lightly on the floor, or elevation of the injected paw so that at most the nails touch the floor, 2=licking, and 3=biting or shaking the injected paw.

The animals were observed every 15 sec and pain scores between the first and fifth minutes (first phase; 0 – 60 scores) and 15th and 60th minutes (second phase; 0 – 540 scores) were recorded. Each mouse was observed by a separate observer who was unaware of the treatments and doses.

Drugs

Lithium chloride (LiCl; Merck Ltd., Germany) and morphine sulfate (Temad and Tolidaru, Iran) were used in these experiments. The drugs were injected IP in a dosage of 5 mL/kg, 20 min before formalin injection. The control groups received saline.

Drug treatment

The animals were treated as follows:

Swim stress-induced antinociception

Four groups of mice were selected to either be exposed to WSS with three durations (20, 40, and 60 sec) 15 min before formalin injection, or to serve as controls. Pain scores were recorded for a period of one hour after formalin injection.

Antinociceptive effect of LiCl

Four groups of mice received IP doses of either saline (5 mL/kg) or different doses of LiCl (0.05, 0.25, 0.5, and 5 mg/kg) 20 min before formalin injection. Pain scores were recorded immediately after formalin injection for a period of one hour.

Effect of LiCl on SIA in mice

Mice were injected with saline (5 mL/kg) or different doses of LiCl (0.5 and 5 mg/kg) 20 min before formalin injection and divided into four groups. Each group was divided into four subgroups, and they were either subjected to WSS with three different durations (20, 40, and 60 sec) 15 min before formalin injection or not. Pain scores were recorded for a period of one hour after formalin injection.

Effect of LiCl in mice that underwent repeated WSS

Mice received saline (5 mL/kg), LiCl (5 mg/kg), WSS (40 sec), or LiCl plus WSS, once daily for three days. They were divided into four groups. Each group was divided into four subgroups and they were either subjected to WSS with different durations (20, 40, and 60 sec) 15 min before formalin injection, or not, and pain scores were recorded for one hour after formalin injection.

Antinociceptive effect of swim stress in the animals that received subchronic treatment of morphine in the presence or absence of LiCl

In these experiments, three main groups of mice had received either morphine once daily for three days or not received morphine, with or without LiCl (5 mg/kg). Each group was divided into four subgroups, receiving either stress with different durations (20, 40, and 60 sec) 15 min before formalin injection or no stress at all. Pain scores were recorded immediately after formalin injection for a period of one hour.

Statistical analysis

Data were analyzed only for the early (5 min) and late (15 – 60 min) phases of the formalin test, using one-way analysis of variance ANOVA and two-tailed Newman-Keuls tests. Differences of $P < 0.05$ between experimental groups at each point were considered statistically significant.

Results

Swim stress-induced antinociception

Figure 1 shows the antinociceptive effect of swim stress. One-way ANOVA indicated a significant difference between the antinociception induced in controls and the three other groups of mice, in the first phase [$F(3,36)=14.3$, $P < 0.0001$], and the second phase [$F(3,36)=7.2$, $P < 0.001$] of the formalin test. Post hoc analysis showed that swim stress with different durations induced antinociception in both phases of the formalin test.

Antinociceptive effect of LiCl in mice

Figure 2 demonstrates the antinociceptive response of LiCl. One-way ANOVA indicated a significant difference between responses induced in the four groups of animals that received LiCl

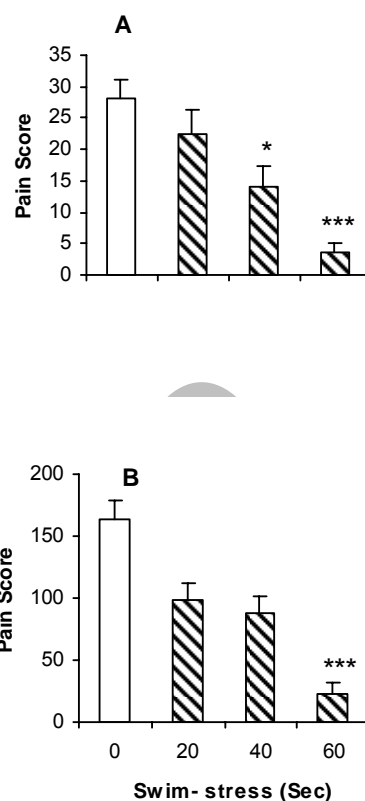


Figure 1. Swim stress-induced antinociception (SIA) in mice. Mice were forced to swim in water (8°C) for different durations (20, 40, and 60 sec). Pain scores were recorded in the first phase (A) or second phase (B) of the formalin test. Each point is the mean ± standard error of pain scores for 10 mice. *** $P < 0.01$, different from control group.

(0.05, 0.25, 0.5, and 5 mg/kg) and that of the control group in the first phase [$F(4,45)=7.9$, $P < 0.0001$], and in the second phase [$F(4,45)=3.5$, $P < 0.05$] of the formalin test. Post hoc analysis showed different doses of LiCl (0.05, 0.25, and 0.5 mg/kg) induced antinociception in both phases of the test.

Antinociceptive effect of swim stress in the presence or absence of LiCl

Figure 3 shows the effect of LiCl on swim SIA in mice. Two-way ANOVA indicated significantly different responses between animals that received a lower dose of LiCl (0.5 mg/kg) and the control group, in the first phase [$F(3,72)=5.9$, $P < 0.001$], but not in the second phase [$F(3,72)=0.92$, $P > 0.05$] of the formalin test. Analysis also showed a significantly different responses between animals

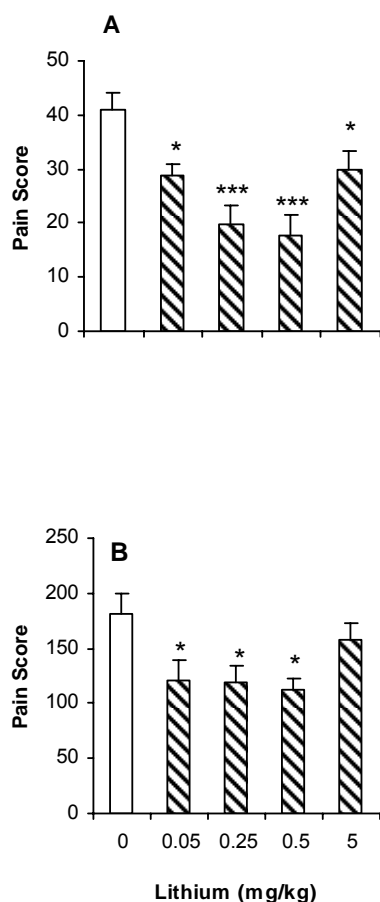


Figure 2. Antinociception induced by lithium chloride (LiCl) in mice. Animals were treated intraperitoneally with either saline (5 mL/kg) or different doses of LiCl (0.05, 0.25, 0.5, and 5 mg/kg) 20 min before formalin injection. Pain scores were recorded in the first phase (A) or second phase (B) of the formalin test. Each point is the mean±standard error of pain scores for 10 mice. * $P<0.05$, *** $P<0.001$, different from control group.

that received a higher dose of LiCl (5 mg/kg) and the control group, in the first phase [$F(3,72)=4.6$, $P<0.01$], but not in the second phase [$F(3,72)=0.91$, $P>0.05$] of the test.

Effects of subchronic LiCl and/or WSS on SIA

Figure 4 demonstrates the effect of subchronic LiCl, WSS, or LiCl plus WSS on SIA. Two-way ANOVA showed SIA was not altered in the presence of subchronic administration of LiCl (5 mg/kg) in the first phase [$F(3,72)=0.66$, $P>0.05$] or the second phase [$F(3,72)=1.8$, $P>0.05$] of the formalin test.

Analysis also indicated that SIA was not altered in the presence of subchronic administration of WSS (40 sec) in the first phase [$F(3,72)=1.1$,

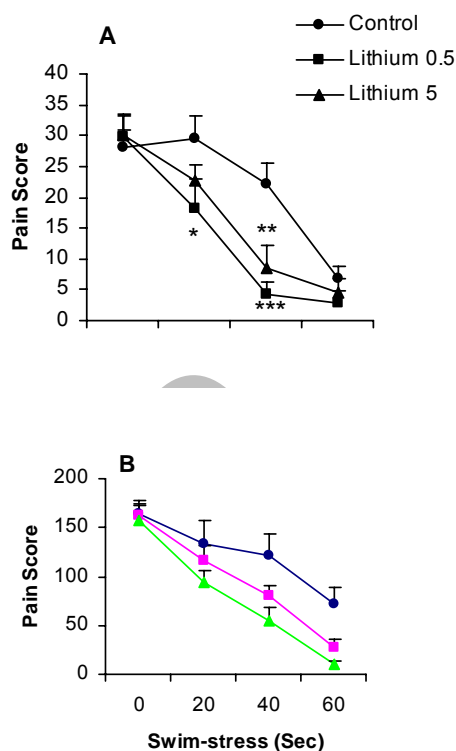


Figure 3. Effect of lithium chloride (LiCl) on swim stress of different durations in mice. Animals were injected with saline (5 mL/kg) or LiCl (0.5 and 5 mg/kg) 20 min and subjected to swim stress 5 min before formalin injection. Pain scores were recorded for one hour after formalin injection. Each point is the mean±standard error of pain scores for 10 mice. * $P<0.05$, *** $P<0.001$, different from control group.

$P>0.05$] or the second phase [$F(3,72)=0.83$, $P>0.05$] of the test. SIA was not altered in the presence of LiCl plus WSS in the first phase [$F(3,72)=2.0$, $P>0.05$] nor the second phase [$F(3,72)=1.8$, $P>0.05$] of the test.

Antinociceptive effect of swim stress in the mice which received subchronic morphine or morphine plus LiCl

Figure 5 shows the effect of subchronic morphine with or without LiCl on SIA. Two-way ANOVA showed SIA was not altered with subchronic administration of morphine in the first phase [$F(3,72)=1.4$, $P>0.05$], or the second phase [$F(3,72)=2.2$, $P>0.05$] of the formalin test. Analysis also showed that SIA was not altered in the mice that received morphine plus LiCl (5 mg/kg) in the first phase [$F(3,72)=1.1$, $P>0.05$] or the second phase [$F(3,72)=2.1$, $P>0.05$] of the formalin test.

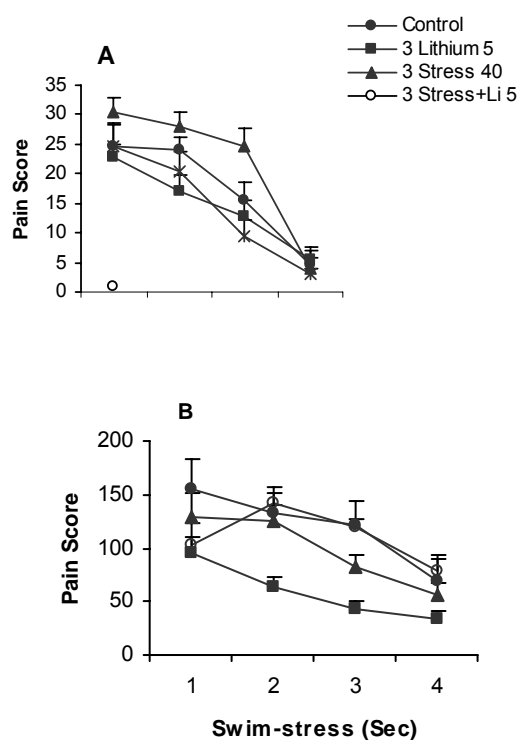


Figure 4. Antinociceptive effect of swim stress in the animals which were exposed to repeated water swimming stress (WSS) with or without LiCl. Mice were subjected to saline (●; 5 mL/kg), LiCl (■; 5 mg/kg), or WSS (▲; 40 sec) 15 min after saline or LiCl plus WSS (○) administration, once daily for three days. Control animals received no WSS. On the fourth day (test day), antinociception of swim stress of different durations (0, 20, 40, and 60 sec) were recorded in the first phase (A) or second phase (B) of the formalin test. Each point is the mean \pm standard error of pain scores of 10 mice.

Discussion

The formalin test is used widely in animal models for evaluating the antinociceptive effects of mild analgesic drugs.¹⁶ Formalin injections into the paw in mice produce a biphasic nociceptive response consisting of a transient early phase (acute pain) followed by a tonic late phase (chronic pain).^{17,18}

The present data show that 60 sec of WSS induced antinociception in both phases of the formalin test. This is in agreement with previous results of swim SIA.³ Furthermore, exposure to WSS at different temperatures may cause antinociception with different mechanisms. It has been shown that cold water swim stress at 4°C induces antinociception through endogenous

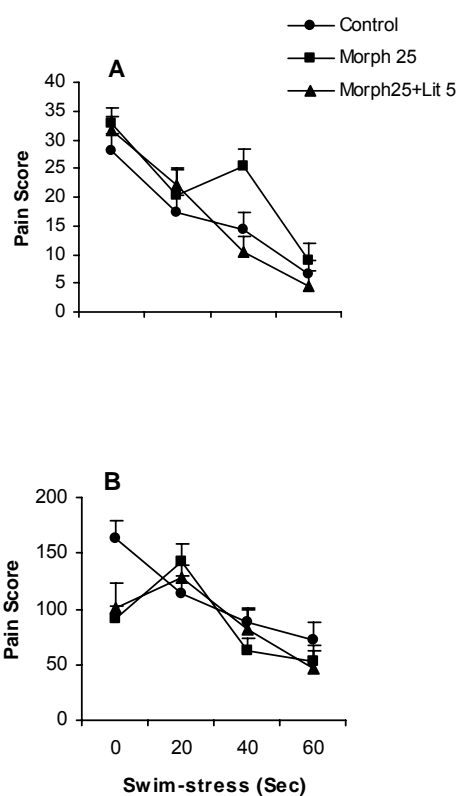


Figure 5. Antinociceptive effect of swim stress in the animals which received subchronic morphine injection, with or without LiCl. Mice were administered morphine (25 mg/kg) 5 min before saline (●) or LiCl (▲; 5 mg/kg) administration, once daily for three days. Control animals received no drug (○). On the test day, antinociception of swim stress (20, 40, and 60 sec) was recorded in the first phase (A) or second phase (B) of the formalin test. Each point is the mean \pm standard error of pain scores of 10 mice.

opioid peptides and the stimulation of $\delta 2$ -opioid receptors in the spinal cord.^{1,2} Since swim SIA was caused at low temperatures (8°C), it may be concluded that the opioid receptor mechanism is involved.

The present data also indicated that lithium induced antinociception in both phases of the formalin test. The analgesic effect of lithium has been shown previously in mice¹⁹ and rats,²⁰ in the hot-plate test. Lithium may increase biosynthesis,²¹⁻²³ or release of endogenous opioids in brain tissues.^{22,24} The drug has been proposed to relieve neuropathic pain in a rat model through intracellular phosphatidylinositol second messenger system in spinal cord neurons.²⁵ Therefore, the antinociceptive response of lithium may be mediated through such mechanisms.

Such mechanism of lithium and the suggestion

that swim stress antinociception in lower temperatures is mediated by an opioid mechanism,^{1,2} may support our data that swim stress antinociception in combination with lithium can be potentiated. However, there are data indicating that lithium may inhibit morphine analgesia.²⁶

Our present results indicated that repeated administration of lithium in combination with WSS was not able to induce tolerance to SIA. This may be due to the nature of the swim stress in this temperature, which does not induce tolerance to its response.

Our study also showed that lithium, in combination with a lower dose of morphine (25 mg/kg, once daily for three days) was not able to elicit tolerance to SIA. This may support the idea that stimulation of opioid receptors cannot cause tolerance to SIA. Furthermore, previous results have shown a cross-tolerance between morphine and swim SIA.¹³ However, in those experiments the temperature used for swim stress was 20°C.¹³ The N-methyl-D-aspartate (NMDA) receptor antagonist, dextromethorphan, potentiates water swim SIA at 8°C [unpublished data]. Therefore, it may be possible that the NMDA mechanisms have a role in lithium potentiation of swim SIA. To clarify the exact mechanism involved in the lithium response and its potentiation of swim SIA, more experiments may be required.

Acknowledgment

The authors wish to thank Dr. Touraj Nayer-Nouri for his assistance in the preparation of the manuscript.

References

- Mizoguchi H, Narita M, Kampine JP, Tseng LF. [met5]enkephalin and delta2-opioid receptors in the spinal cord are involved in the cold water swimming-induced antinociception in the mouse. *Life Sci.* 1997; **61**: PL81 – 86.
- Mizoguchi H, Narita M, Nagase H, Tseng LF. Antisense oligodeoxynucleotide to a delta-opioid receptor blocks the antinociception induced by cold water swimming. *Regul Pept.* 1995; **59**: 255 – 259.
- Kim KW, Choi SS, Woo RS, Suh HW. Development of antinociceptive tolerance and changes of opioid receptor ligand binding in central nervous system of the mouse forced to single and repeated swimming in the cold water. *Brain Res Bull.* 2003; **61**: 93 – 97.
- Prien RF, Kupfer DJ, Mansky PA, Small JG, Tuason VB, Voss CB, et al. Drug therapy in the prevention of recurrences in unipolar and bipolar affective disorders. Report of the NIMH collaborative study group comparing lithium carbonate, imipramine, and a lithium carbonate-imipramine combination. *Arch Gen Psychiatry.* 1984; **41**: 1096 – 1104.
- Nierenberg AA. Treatment choice after one antidepressant fails: a survey of northeastern psychiatrists. *J Clin Psychiatry.* 1991; **52**: 383 – 385.
- Nierenberg AA, Cole JO. Antidepressant adverse drug reactions. *J Clin Psychiatry.* 1991; **52 (suppl)**: 40 – 47.
- Jasinski DR, Nutt JG, Haertzen CA, Griffith JD, Bunney WE. Lithium: effects on subjective functioning and morphine-induced euphoria. *Science.* 1977; **195**: 582 – 584.
- Abrahamson JR. Use of lithium to control drug abuse. *Am J Psychiatry.* 1983; **140**: 1256.
- Dehpour AR, Samini M, Sharifzadeh M, Hasan-Mazandarani H. Effects of chronic lithium pretreatment on apomorphine-induced penile erection. *Gen Pharmacol.* 1995; **26**: 1015 – 1020.
- Liebman JM, Segal DS. Lithium differentially antagonizes self-stimulation facilitated by morphine and (+)-amphetamine. *Nature.* 1976; **260**: 161 – 163.
- Dehpour AR, Farsam H, Azizabadi-Farahani M. The effect of lithium on morphine-induced analgesia in mice. *Gen Pharmacol.* 1994; **25**: 1635 – 1641.
- Raffa RB, Martinez RP. Morphine antinociception is mediated through a lici-sensitive, ip3-restorable pathway. *Eur J Pharmacol.* 1992; **215**: 357 – 358.
- Fazli-Tabaei S, Yahyavi SH, Alagheband P, Samie HR, Safari S, Rastegar F, et al. Cross-tolerance between antinociception induced by swim-stress and morphine in formalin test. *Behav Pharmacol.* 2005; **16**: 613 – 619.
- Dubuisson D, Dennis SG. The formalin test: a quantitative study of the analgesic effects of morphine, meperidine, and brainstem stimulation in rats and cats. *Pain.* 1977; **4**: 161 – 174.
- Abbott FV, Franklin KB, Westbrook RF. The formalin test: scoring properties of the first and second phases of the pain response in rats. *Pain.* 1995; **60**: 91 – 102.
- Hunskar S, Fasmer OB, Hole K. Formalin test in mice: a useful technique for evaluating mild analgesics. *J Neurosci Methods.* 1985; **14**: 69 – 76.
- Vaccarino AL, Marek P, Sternberg W, Liebeskind JC. Nmda receptor antagonist mk-801 blocks non-opioid stress-induced analgesia in the formalin test. *Pain.* 1992; **50**: 119 – 123.
- Rosland JH, Tjolsen A, Maehle B, Hole K. The formalin test in mice: effect of formalin concentration. *Pain.* 1990; **42**: 235 – 242.
- Mannisto PT, Saarnivaara L. Effect of lithium on the analgesia caused by morphine and two antidepressants in mice. *Pharmacology.* 1972; **8**: 329 – 335.
- Yirmiya R, Lieblich I, Garcia J, Liebeskind JC. Analgesic properties of lithium. *Proc West Pharmacol Soc.* 1987; **30**: 303 – 306.
- Gillin JC, Hong JS, Yang HY, Costa E. [met5]enkephalin content in brain regions of rats treated with lithium. *Proc Natl Acad Sci U S A.* 1978; **75**: 2991 – 2993.
- Staunton DA, Deyo SN, Shoemaker WJ, Ettenberg A, Bloom FE. Effects of chronic lithium on enkephalin systems and pain responsiveness. *Life Sci.* 1982; **31**: 1837 – 1840.
- Sabol SL, Yoshikawa K, Hong JS. Regulation of

- methionine-enkephalin precursor messenger rna in rat striatum by haloperidol and lithium. *Biochem Biophys Res Commun.* 1983; **113**: 391 – 399.
- 24 Sivam SP, Smith DR, Takeuchi K, Hong JS. Lithium and haloperidol differentially alter the dynorphin a (1-8) and enkephalin levels in the neurointermediate lobe of rat pituitary. *Neuropeptides.* 1987; **10**: 291 – 298.
- 25 Shimizu T, Shibata M, Wakisaka S, Inoue T, Mashimo T, Yoshiya I. Intrathecal lithium reduces neuropathic pain responses in a rat model of peripheral neuropathy. *Pain.* 2000; **85**: 59 – 64.
- 26 Johnston IN, Westbrook RF. Inhibition of morphine analgesia by lithium: role of peripheral and central opioid receptors. *Behav Brain Res.* 2004; **151**: 151 – 158.

Archive of SID