Intrathecal lamotrigine blocks and reverses antinociceptive morphine tolerance in rats

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**Background:** Chronic administration of morphine leads to the development of tolerance. We investigated the effects of intrathecal lamotrigine on the spinal morphine tolerance in rats that are undergoing tail flick tests.

**Methods:** Sprague-Dawley rats were given intrathecal injections of saline 10 μl, lamotrigine 300 μg, morphine 15 μg or lamotrigine plus morphine combinations for 7 days (lamotrigine was given for days 1−7, days 1−3 or days 5−7). The acute and chronic nociceptive sensitivities were assessed using a tail flick test in which the distal 5 cm of the tail was dipped into warm water before and 30 minutes after the drug injection. With successive injections of morphine on day 8, a cumulative antinociceptive dose-response curve was constructed and the 50% effective dose (ED50) was calculated for each study group.

**Results:** The coinjection group of lamotrigine with morphine blocked the development of tolerance, as was shown by the preservation of morphine antinociception over 7 days and the concomitant decrease in the ED50 values on day 8, as compared with the morphine-alone group. Coinjection of lamotrigine blocked the development of morphine tolerance, as shown by the preservation of morphine antinociception over 7 days and the concomitant decrease in the ED50 values on day 8, as compared with the morphine-alone group.

**Conclusions:** This study suggests that lamotrigine augments the antinociceptive action of both acute and chronic morphine therapy, and it also attenuates the antinociceptive morphine tolerance in rats.

**Key Words:** Intrathecal, Lamotrigine, Morphine, Tail flick test, Tolerance.
at a dosage of 300 μg of intrathecal lamotrigine, but no severe motor impairment was found.) and intrathecal administration of lamotrigine produced a dose-dependent antiallodynic action in a rat model of spinal nerve ligation [14].

Furthermore, some reports suggest a role for lamotrigine in the therapy of pain from a variety of etiologies, including painful diabetic neuropathy [15], central poststroke pain [16], human immunodeficiency virus (HIV)-associated neuropathy [17], and trigeminal neuralgia [18]. However, the effect of lamotrigine on opioid tolerance has not been studied. Thus, the goal of this investigation is to test the hypothesis that lamotrigine prevents and reverses chronic opioid tolerance.

**MATERIALS AND METHODS**

**Study animals and nociceptive testing**

This study was performed under a protocol approved by the Animal Use and Care Committee. The experiments were conducted in male Sprague-Dawley rats (weight 200–250 g), which were housed individually in a temperature controlled vivarium and allowed to acclimate for 3 days in a 12/12-hour light/dark cycle.

For intrathecal drug administration, the rats were chronically implanted with catheters as previously described [19]. Intrathecally PE-10 tubing was passed caudally from the cistern magna to the spinal cord level of lumbar enlargement. The catheter was externalized through the skin. Proper location was confirmed by a temporary motor block of both hindlimbs after injection of 2% lidocaine 10 μL, followed by saline. Only animals with no evidence of neurologic deficit after the operation were studied. The drugs were given by using a microinjection syringe (Microliter™ #702, Hamilton Co., USA) over a 60-second interval in a volume of 10 μL, followed by a 10 μL flush. Investigators were blinded to drugs and doses used.

Acute nociceptive sensitivity was assessed using tail flick test. Reaction time (tail withdrawal latency), expressed as WL (withdrawal latency), in the test was determined by immersing the tail [20,21] into a slowly stirred water bath. To conduct the tests, rats were placed in a custom-made restrainer that held the body without restraining the head, paws and tail. The distal 5 cm of the tail dipped into warm water. When a withdrawal response occurred, the stimulus was terminated and the response latency was measured. The water temperature was adjusted to 50°C as this temperature produced an average baseline of WL of 2.4 ± 0.2 s in naive rats. Baseline measurements consisted of three trials of tail-flick latency at 3-min intertrial intervals. In the absence of a response up to 10 s (cut-off time), the trial was terminated to prevent tissue damage. The WL in the pain tests was converted to a percentage of maximum possible effect (%MPE) using following formula: %MPE = [(post-drug latency − baseline latency) / (cut-off time − baseline latency)] × 100%

For the determination of the time to peak effect and the 50% effective dose (ED50) estimated to produce 50% maximal possible effect (%MPE), the doses of morphine (0.3, 1, 3, and 10 μg) were intrathecally injected (n = 6 per subgroup).

**Study 1: Acute effects of lamotrigine on morphine antinociception**

Single intrathecal doses of 5 μg morphine, 100 μg lamotrigine, 300 μg lamotrigine, and a combination of 5 μg morphine and 300 μg lamotrigine were studied. Measurements were taken before and 15, 30, 45, 60, 90, 120, and 180 min after an intrathecal dose of the drug(s).

**Study 2: Effects of lamotrigine on development of morphine tolerance**

Rats of morphine group received intrathecal injections of 15 μg morphine once daily for 7 days. This dose has been shown previously to produce tolerance over 7 days following initial maximal antinociception [22]. Testing was performed before and 30 min after drug administration.

To evaluate the effects of lamotrigine on the development of morphine tolerance, lamotrigine 300 μg was intrathecally co-injected with morphine 15 μg once daily for 7 days. Testing was performed once daily and cumulative dose-response curves were generated on day 8. To characterize the offset of the effect of lamotrigine on morphine tolerance, another study evaluated lamotrigine co-injected with morphine for days 1–3 followed by daily morphine alone on days 4–7.

**Study 3: Effects of lamotrigine on established morphine tolerance**

Intrathecal morphine 15 μg was given once daily for 4 days to induce tolerance. On the following 3 days, intrathecal lamotrigine 300 μg was introduced in combination with morphine.

After study 2 and 3, on day 8, cumulative dose-response curves were constructed, and the ED50 values of morphine were determined. To obtain these curves, animals received increasing doses of morphine (3, 9, 21, 45, and 93 μg) for
morphine alone group and morphine plus lamotrigine (5–7 days) group every 30 min, and testing followed 30 min after each drug injection. For saline group, morphine plus lamotrigine (1–3 days) group, and morphine plus lamotrigine (1–7 days) group, animals received increasing doses of morphine (0.3, 0.9, 2.1, 4.5, 9.3 and 18.9 μg). This protocol continued until maximal antinociception was obtained.

Drugs

Morphine sulfate (MW = 668.76; Sigma, USA) was dissolved in 0.9% saline. Lamotrigine (MW = 256.09; Sigma, USA) was dissolved in dimethyl sulfoxide (DMSO, minimum 99.5%; Sigma) and diluted with 0.9% saline.

Data analysis

All data are expressed as mean maximum percentage effect (± SEM). The ED50 values were determined using nonlinear regression analysis. Statistical significance (P < 0.05) was determined using one-way ANOVA followed by a Dunnett post hoc test for multiple comparisons between groups.

RESULTS

Intrathecal morphine resulted in a dose-dependent antinociceptive effect (Fig. 1). The ED50 values and slopes (95% confidence intervals) are 3.8 (2.8–5.1) μg and 48.6 (38.9–58.3) for morphine.

Study 1: Acute effects of lamotrigine on morphine action

Submaximal doses of intrathecal morphine 5 μg produced peak antinociception (55.4 ± 4.3 %MPE) in tail-flick test 30 minutes after administration. Intrathecal lamotrigine alone at doses of 300 μg produced peak antinociception (32.0 ± 3.8 %MPE) 30 minutes after administration (Fig. 2). When given together, these doses of morphine and lamotrigine resulted in maximal, and supra-additive, antinociception (98.9 ± 0.9 %MPE) peaking between 30 and 45 min after administration. The combination of morphine and lamotrigine resulted in significantly larger responses than morphine alone (Fig. 2). In both tests, responses returned to baseline by 180 minutes after injection. Visual inspection of treated animals revealed no signs of motor impairment.

Study 2: Effects of lamotrigine on development of morphine tolerance

Administration of intrathecal morphine 15 μg, produced maximal antinociception on day 1 (99.7 ± 0.3 %MPE), which decreased to baseline levels by day 7. Coadministration of intrathecal morphine 15 μg with intrathecal lamotrigine 300 μg, completely blocked the decrease in morphine effect throughout

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Fig. 3. Tail-flick responses (mean ± SEM) to chronic saline, morphine, and morphine plus lamotrigine (lamotrigine given on days 1–7; days 5–7; or days 1–3) (n = 9 per subgroup). All doses of morphine and lamotrigine are 15 μg, and 300 μg, respectively. a)P < 0.05 versus saline, b)P < 0.05 versus morphine. I.T.: intrathecal.

Fig. 4. Effect of lamotrigine on the development and reversal of intrathecal morphine tolerance. Following the end of the 7-day chronic treatment period, cumulative dose-response curves to acute morphine were generated on day 8. ED50 values were derived from these curves (mean ± SEM). a)P < 0.05 compared to ED50 of morphine alone group (n = 9 per subgroup). I.T.: intrathecal.

Table 1. Effect of Lamotrigine on the Development and Reversal of Intrathecal Morphine Tolerance

<table>
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<tr>
<th>Chronic treatment groups</th>
<th>ED50 (μg)</th>
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| Saline                  | 5.3 ± 1.2
| Morphine 15 μg (days 1–7) | 26.3 ± 2.7 |
| Morphine 15 μg + Lamotrigine 300 μg (days 1–7) | 5.1 ± 0.9
| Morphine 15 μg + Lamotrigine 300 μg (days 1–3) | 7.6 ± 1.2
| Morphine 15 μg + Lamotrigine 300 μg (days 5–7) | 13.2 ± 1.9

Data shown as mean ± SEM. Following the end of the 7-day chronic treatment period, cumulative dose-response curves to acute morphine were generated on day 8. ED50 values were derived from these curves. a)P < 0.05 compared to morphine alone (n = 9 per subgroup).

Study 3: Effects of lamotrigine on established morphine tolerance

In this study, morphine plus lamotrigine were administered on days 5–7. Chronic administration of morphine alone on days 1–4 resulted in a decrease in antinociception similar to that observed previously (Fig. 3). However, addition of lamotrigine on days 5–7 resulted in a partial restoration of the morphine effect (Fig. 3) and significantly greater antinociception than for morphine alone on days 5–7. The ED50 value on day 8 for this treatment group was not significantly lower than for that of morphine alone group (Table 1, Fig. 4).

DISCUSSION

This study shows that lamotrigine inhibits development of antinociceptive tolerance to morphine. This is evident in sustained responses to morphine in the presence of lamotrigine for 7 days, a leftward shift of the acute morphine dose-response curve, and a decrease in the acute morphine ED50 value compared to those of morphine tolerant animals. The tolerance to morphine, however, becomes apparent within 48 h of discontinuing lamotrigine, indicating the need for continued lamotrigine to maintain opioid potency. Finally, it can be suggested...
that lamotrigine can partially restore morphine potency in tolerant rats. Taken together, these results support a role for lamotrigine-morphine combinations or for the addition of lamotrigine to morphine in the setting of tolerance.

Morphine tolerance is mediated by glutamate action at spinal NMDA [5] and AMPA/kainite [6] receptors. Interactions between NMDA and opioid receptors could occur in both directions [23]. Thus, any condition that results in activation of NMDA receptors within the CNS could modulate opioid receptors, causing reduced efficacy of opioid analgesia; conversely, repeated treatment with opioids could set up a condition mimicking ongoing nociceptive input through interactions between opioid and NMDA receptors [23,24]. Activation of AMPA receptors during chronic morphine treatment may contribute to analgesic tolerance. Because NMDA receptor antagonists are also effective in attenuating the development of morphine tolerance [25,26], it is suggested that activation of more than one excitatory amino acid receptor system mediates the development of morphine tolerance [7]. In addition, we can suggest that AMPA and NMDA receptors make similar but not identical contributions to the development of opioid tolerance [7].

Although there is no report about the anti-tolerance effect of intrathecal lamotrigine in any animal experiments, we could consider some mechanisms. Lamotrigine probably exerts its actions by blocking voltage-dependent sodium channels, thus stabilizing the presynaptic neuronal membrane and preventing the release of excitatory neurotransmitters, predominantly glutamate [27]. By this mechanism, intrathecally administered lamotrigine could prevent the activation of the glutamate receptors involved in pain transmission and central sensitization, i.e., AMPA, NMDA, and metabotropic receptors [28]. It is also reported that lamotrigine could block calcium currents, altering presynaptic release of glutamate and aspartate [29], and this action as an calcium channel blocker may be also, in part, related to the anti-opioid tolerance.

In conclusion, tolerance may limit opioid efficacy and an understanding of the underlying mechanisms may improve pain management in certain situations. This study suggests that lamotrigine augments the antinociceptive action of acute morphine therapy, and also attenuates antinociceptive morphine tolerance. Future studies are needed to further explain the sites and mechanisms of these actions. Also, clinical investigations are needed to identify specific settings and patient populations in which lamotrigine-opioid combinations may be useful.

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REFERENCES

13. Ma W, Du W, Eisenach JC. Intrathecal lidocaine reverses tactile