Peripheral Antinociceptive Action of Morphine and the Synergistic Interaction with Lamotrigine

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Background: Lamotrigine inhibits glutamate release through the preferential blockade of voltage-dependent Na^+ channels. In contrast, morphine reduces release of excitatory amino acids through the activation of opioid receptors and also inhibits tetrodotoxin-resistant Na^+ channels on peripheral afferent neurons. The current study was designed to investigate the antinociceptive effects of locally administered morphine and lamotrigine. The interaction between morphine and lamotrigine at the periphery was also examined.

Methods: Morphine, lamotrigine, or a combination of morphine and lamotrigine was administered locally to female Wistar rats, and the antinociceptive effect was determined in the formalin test. Isobolographic analyses were used to define the nature of the functional interactions between morphine and lamotrigine.

Results: Peripheral administration of either morphine or lamotrigine produced a dose-related antinociceptive effect. Isobolographic analyses revealed that peripheral morphine and lamotrigine interacted synergistically in the formalin test.

Conclusions: The study shows a functional interaction between lamotrigine and morphine at the peripheral level.

LAMOTRIGINE is an anticonvulsant drug suggested to be an effective analgesic in the treatment of pain in rats. Either oral or intrathecal administration of lamotrigine produces a dose-dependent antinociception in rat experimental models of acute and chronic pain.[1,2] Studies on neuropathic pain showed that this drug could reverse cold allodynia, but not tactile allodynia, and it also reduced the development of neuropathic pain.[3] Clinical studies have also shown that lamotrigine is able to reduce neuropathic pain after oral administration.[4–7] However, other studies have failed to find an analgesic effect in neuropathic pain.[8] The antinociceptive effect of lamotrigine has been attributed to the blockade of voltage-dependent Na^+ channels with the inhibition of glutamate release.[9]

Unpublished observations have shown that a combination of lamotrigine and the opiate dipipanone did not increase analgesia induced by the opiate in humans.

However, recent data indicate that lamotrigine significantly increases morphine analgesia.[10] Therefore, the current study was designed to assess the peripheral antinociceptive effect of lamotrigine and morphine and their possible synergistic interaction by isobolographic analyses.

Materials and Methods

Animals

All experiments were conducted in accordance with the "Guidelines on Ethical Standards for Investigation of Experimental Pain in Animals."[11] In addition, the study was approved by the Institutional Animal Care and Use Committee (Centro de Investigación y de Estudios Avanzados del Instituto Politécnico Nacional, Granjas Coapa, Mexico City, Mexico). Female Wistar rats aged 6–7 weeks (weight range, 160–180 g) from our own breeding facilities were used in this study. Animals had free access to food and drinking water before the experiments.

Measurement of Antinociceptive Activity

Antinociception was assessed using the formalin test. Rats were placed in open Plexiglass observation chambers for 30 min to allow them to accommodate to their surroundings, then they were removed for formalin administration. Fifty microliters of diluted formalin (1%) was injected subcutaneously into the dorsal surface of the right hind paw with a 30-gauge needle. Animals were then returned to the chambers, and nociceptive behavior was observed immediately after formalin injection. Mirrors were placed to enable unhindered observation. Nociceptive behavior was quantified as the number of flinches of the injected paw during 1-min periods every 5 min up to 60 min after injection.[12] Flinching was readily discriminated and was characterized as rapid and brief withdrawal or flexing of the injected paw. Formalin-induced flinching behavior is biphasic. The initial acute phase (0–10 min) is followed by a relatively short quiescent period, which is then followed by a prolonged tonic response (15–60 min). At the end of the experiment, the rats were killed in a carbon dioxide chamber.

Drugs

Lamotrigine was a gift of GlaxoSmithKline (Mexico City, Mexico). Morphine–HCl was obtained from Secretaría de Salud (Mexico City, Mexico). Both morphine and lamotrigine were dissolved in saline.

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Table 1. Doses Used in the Study of the Interaction between Morphine and Lamotrigine in the Formalin Test

Morphine Alone	Lamotrigine Alone	Morphine in the Combination	Lamotrigine in the Combination	Total Dose in the Combination
1.25	0	0.01	4.0	4.06
2.5	50	0.03	8.1	8.13
5	100	0.06	16.1	16.16
10	200	0.13	32.3	32.43
20	400	0.26	64.6	64.86

Doses are in μg/paw.

Study Design

Rats received a subcutaneous injection (50 μl) in the dorsal surface of the right hind paw of saline or increasing doses of either morphine (1.25, 2.5, 5, 10, and 20 μg), lamotrigine (50, 100, 200, and 400 μg), or the morphine–lamotrigine combination (table 1) 20 min before formalin injection at the same paw (ipsilateral). To assess if the antinociceptive effect of drugs was caused by a local action, formalin was administered in one paw, and the greatest dose of the tested drugs was administered in the contralateral paw (subcutaneously). Doses were selected on the basis of previous pilot studies in our model.[13] The observer was unaware of the treatment in each animal. Rats in all groups were tested for possible side effects such as reduction of righting, stepping, and corneal and pinna reflexes before and after drug treatment.

Data Analysis and Statistics

All results are presented as mean $\pm$ SEM for at least six animals per group. Curves were constructed plotting the number of flinches as a function of time. The area under the number of flinches against time curves was calculated by the trapezoidal rule.[14] Dose–response curves for each compound tested were established based on the percent maximum possible effect (expressed as percent antinociception) calculated from area under the curve of phase 2 of each individual rat:

% Antinociception

$$= ((\text{vehicle-post-compound})/\text{vehicle}) \times 100.$$

For evaluation of the interaction between peripheral morphine and peripheral lamotrigine, isobolograms were constructed using doses producing 30% maximum possible effect (ED_{30}) values obtained when the drugs were administered alone or combined. We used ED_{30} instead of ED_{50} values because neither drug was able to reach more than 50% of antinociception in our model.[15] The construction of the dose–response curves and the determination of ED_{30} values were computed. To perform the isobolographic analysis, lamotrigine and morphine were administered in combination as fixed ratios of equieffective ED_{30} dose for each drug (lamotrigine: morphine = 1:1). The ED_{30} values ($\pm$ SEM) for morphine and lamotrigine alone were plotted on the x- and y-axes, respectively, and the theoretical additive point

was calculated according to Tallarida *et al.*[14] From the dose–response curve of the combined drugs, the ED_{30} value of the total dose of the combination was calculated. Statistical significance between the theoretical additive point and the experimentally derived ED_{30} value was evaluated using the Student *t* test. An experimental ED_{30} significantly less than the theoretical additive ED_{30} ($P < 0.05$) was considered to indicate a synergistic interaction between morphine and lamotrigine.

Results

Peripheral Antinociceptive Effect of Morphine and Lamotrigine

Formalin administration produced a typical pattern of flinching behavior. The first phase started immediately after administration of formalin and then diminished gradually in approximately 10 min. The second phase started at 15 min and lasted until 1 h.[12] Ipsilateral, but not contralateral, local administration of morphine or lamotrigine produced a dose-dependent reduction in the flinching behavior, otherwise observed after formalin injection (fig. 1). Both drugs significantly reduced the number of flinches during phase 2 ($P < 0.05$). In contrast, they had a small but significant effect on phase 1 (fig. 1). The maximal observed effect, assessed as percent antinociception, was approximately 45% in both treatments, and higher doses were not able to further increase the antinociceptive effect. However, morphine doses to block formalin-induced nociceptive behavior were remarkably greater than lamotrigine (fig. 1). No differences in the measured reflexes were observed before and after drug treatment in either group, control or treated.

Effect of the Combination of Peripheral Morphine and Lamotrigine

Peripheral coadministration of morphine and lamotrigine induced dose-dependent increases in the percent antinociception (fig. 2). The antinociceptive effect of the combination was observed mainly in the second phase of the test. ED_{30} values of morphine, lamotrigine, and the combination were 2.92 (0.30 SEM), 126.76 (30.6 SEM), and 17.69 (2.9 SEM), respectively. As shown in figure 3, the experimentally derived ED_{30} ($\pm$ SEM) in the isobologram is below the theoretically additive

Fig. 1. Local antinociceptive effect of morphine (*A* and *B*) and lamotrigine (*C* and *D*) during the first and second phase of the formalin test. Rats were pretreated with morphine or lamotrigine into either the right or left (CL) paw, before formalin injection. Data are expressed as the percent antinociception. Data are the mean ± SEM for six animals. *Significantly different from saline ($P < 0.05$), as determined by analysis of variance followed by the Tukey test.
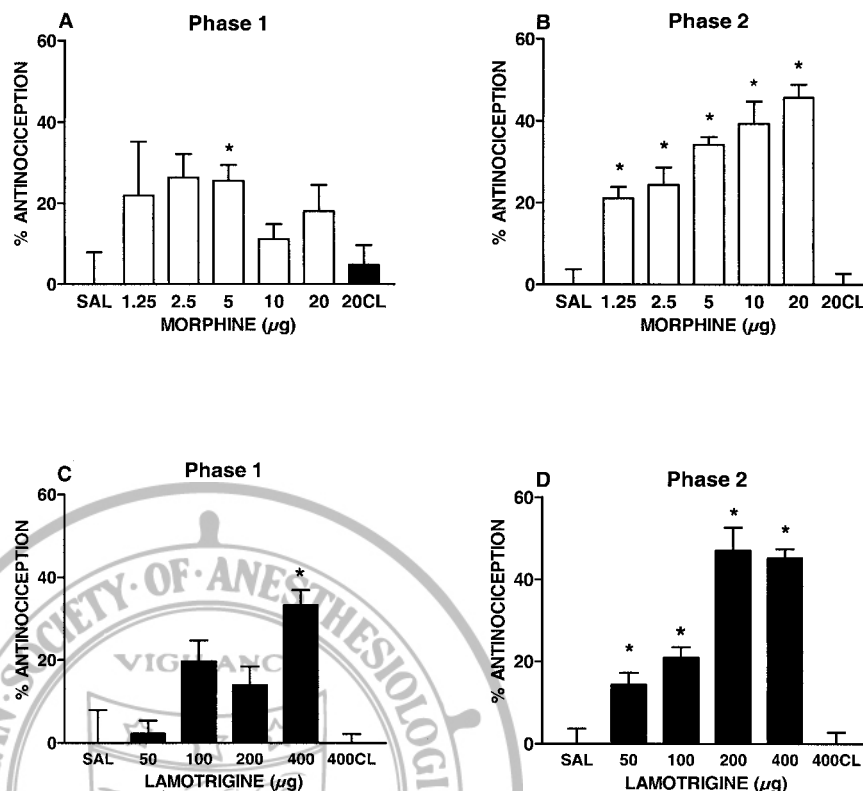
dose line, indicating a significant synergistic interaction between morphine and lamotrigine in the formalin test ($P = 0.016$).

Discussion

The current study demonstrated that peripherally administered lamotrigine produced a dose-dependent antinociception during the second phase of the formalin test. We also demonstrated that peripherally coadministered morphine and lamotrigine have a synergistic antinociceptive interaction against noxious stimuli produced by 1% formalin. The antinociceptive effect of lamotrigine has been demonstrated in acute and chronic pain models.[1-3] However, there are no reports on the antinociception of this drug after local administration. In this study we were able to observe dose-related antinociception after local administration of lamotrigine without any side effect, suggesting the possibility of using this drug subcutaneously to reduce inflammatory pain. Because lamotrigine is a glutamate release inhibitor, our data indicate that lamotrigine could be reducing the pronociceptive actions of glutamate in the periphery. On the other hand, local administration of morphine produced dose-related antinociception, 10–20 μg being the highest dose used. This result is similar to that reported for local administration of morphine[16-17] and confirms previous observations about the ability of mor-

phine to produce antinociception in the formalin test after peripheral injection.

Coadministration of lamotrigine and morphine produced a significantly synergistic interaction. The mechanism of this antinociceptive interaction remains to be elucidated. The mechanism of the synergy observed could be a result of the different sites of action of lamotrigine and morphine. In addition to their participation in nociceptive transmission in the spinal cord, there is evidence that *N*-methyl-D-aspartate (NMDA) receptors also play an important role in sensory transduction in the periphery. Some evidence indicates that nociception and inflammation caused by formalin injection[18] induces the release of peripheral glutamate in the rat, probably from the peripheral terminal of the primary afferent C fibers[19] or from macrophages.[20] In addition, NMDA receptors have been localized on sensory axons in the skin,[21] and intraplantar injection of NMDA produces nociception, which is attenuated after local injection of NMDA receptor antagonists.[22,23] Moreover, glutamate antagonists are able to diminish in a dose-dependent manner glutamate-induced nociceptive behaviors at the periphery.[24] Because lamotrigine is a glutamate release inhibitor,[9,25,26] it is likely that reduction in glutamate release at the site of injection could be responsible of the antinociceptive effect observed after administration of this drug alone or combined with morphine. It can be suggested that reduction in glutamate release could avoid primary afferent activity, which would reduce cen-
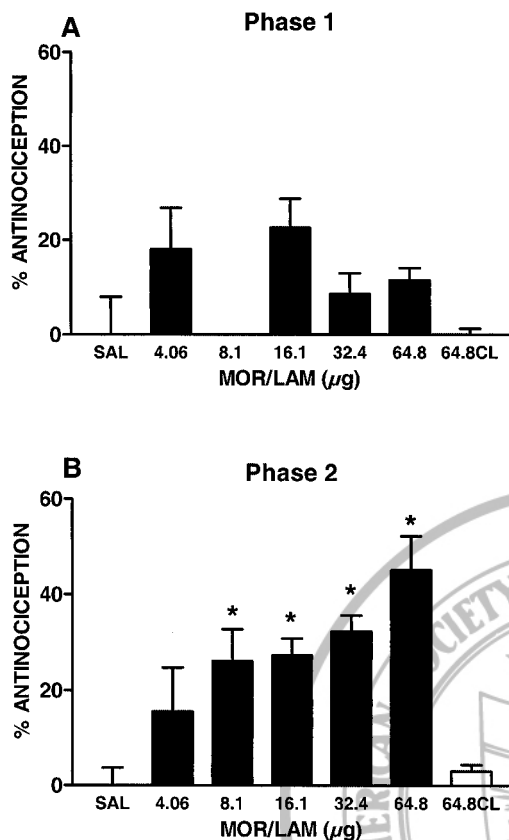
Fig. 2. Antinociceptive effect of the lamotrigine–morphine (MOR/LAM) combination during phase 1 (*A*) and 2 (*B*) of the formalin test. Rats were pretreated with combination into either the right or left (CL) paw, before formalin injection. Data are expressed as the percent antinociception. Data are the mean ± SEM for six animals. *Significantly different from the saline group (*P* < 0.05), as determined by analysis of variance followed by the Tukey test.
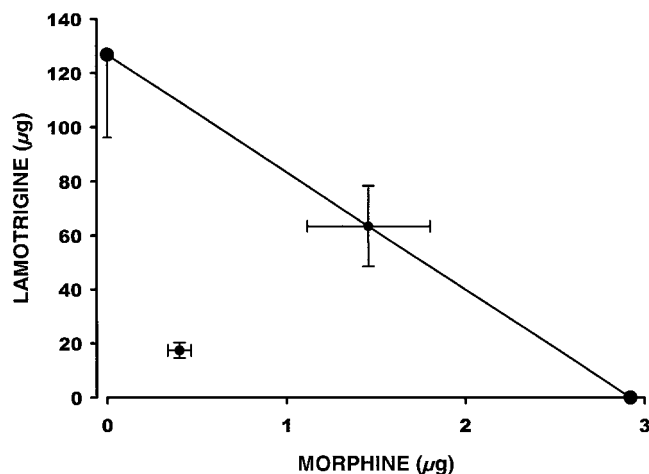

Fig. 3. Isobologram showing the peripheral interaction of lamotrigine and morphine in the formalin test. Horizontal and vertical bars indicate SEM. The oblique line between the x- and y-axes is the theoretical additive line. The point in the middle of this line is the theoretical additive point calculated from the separate ED_{30} values. The experimental point lies far below the additive line, indicating a significant synergism (*P* = 0.016).

tral sensitization of dorsal cells or phase 2 of the formalin test.[27,28] However, it is also possible that the lamotrigine effect could be a result of voltage-dependent Na^+ channel blockade.

There are now a number of studies that indicate that peripheral opioid receptors play an important role in the control of peripheral sensitization. Opioid receptors are present at the primary afferent neurons,[29,30] and local administration of μ and κ, but not δ agonists has been reported to suppress spontaneous activity observed after inflammation. Peripherally applied μ-opioid receptor agonists produce antinociception in several nociceptive tests.[16–18,31–35] The activation of μ-opioid receptors may produce several effects. First, μ-opioid receptor agonists act to inhibit activation of adenylyl cyclase and tetrodotoxin-resistant Na^+ channels on peripheral afferent neurons produced by inflammatory mediators such as prostaglandin E_2 and serotonin.[36] Second, they may also inhibit release of substance P and calcitonin gene-related peptide from primary afferent neurons.[37] Third, they may open adenosine triphosphate–sensitive K^+ channels *via* Gi proteins,[38] resulting in hyperpolarization, reduction in fir-

ing of the primary afferent neuron, and antinociception.[39] All of these effects could act to produce a morphine-induced peripheral antinociceptive effect. In addition, these effects could be significantly increased by glutamate release inhibition or voltage-dependent Na^+ channel blockade produced by lamotrigine.

N-methyl-D-aspartate receptor antagonists, administered either systemically[40–44] or intrathecally,[45–47] increase antinociception induced by morphine on thermal and chemical nociceptive tests. However, there are no data about peripheral coadministration of these drugs. Because NMDA and opioid receptors are present at the primary afferent neuron, a synergistic interaction between NMDA receptor antagonists or glutamate release inhibitors and opioid receptor agonists can be predicted. In our study, the glutamate release inhibitor lamotrigine significantly produced antinociception and increased the antinociceptive action of morphine in the formalin test, confirming that peripherally administered NMDA receptor antagonists and opioid receptor agonists indeed have a functional synergistic interaction. Clinical evidence has shown that this synergistic interaction also seems to be produced in humans.[10,48]

In summary, morphine and lamotrigine produced peripheral antinociception in the rat formalin test. Isobolographic analysis indicates a functional interaction between the glutamate release and voltage-activated sodium channel blocker (lamotrigine) and opioid (morphine) agonist at the peripheral level.

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