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Gabapentin Blocks and Reverses Antinociceptive Morphine Tolerance in the Rat Paw-pressure and Tail-flick Tests

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OPIOID tolerance is a diminution of analgesic effect or need for a higher dose to maintain the original effect following chronic opioid exposure.[1] While its clinical importance is controversial,[2–5] studies of opioid tolerance have advanced knowledge about analgesic mechanisms. In common with nerve or tissue injury, chronic opioid administration causes spinal changes involving translocation and activation of protein kinase C and production of nitric oxide (NO).[6] Furthermore, mechanisms of opioid tolerance include *N*-methyl-D-aspartate (NMDA) receptor[6] and 2-amino-3-hydroxy-5-methyl-4-isoxazole-proprionic acid (AMPA)/kainate receptor[7] modulation, dynorphin activity,[8] calcitonin gene–related peptide activity,[9] and cyclooxygenase activity.[10] In addition to suppressing opioid tolerance, drugs that modulate the previously mentioned mechanisms (such as NMDA receptor antagonists,[11] AMPA/kainate receptor antagonists,[12] and cyclooxygenase inhibitors[13]) are also antihyperalgesic and/or antiallodynic. Gabapentin is a γ-aminobutyric acid (GABA) analog that reduces pain, hyperalgesia, and allodynia following tissue or nerve injury through several possible mechanisms.[14] Previous data suggest that the effects of gabapentin are naloxone insensitive, chronic gabapentin administration does not lead to gabapentin tolerance, and morphine tolerance does not influence gabapentin analgesia in the rat formalin test.[15] While previous preclinical investigations have evaluated gabapentin–opioid interactions,[16–18] the effect of gabapentin on opioid tolerance has not been studied. Thus, the goal of this investigation is to test the hypothesis that gabapentin prevents and reverses chronic opioid tolerance.

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Materials and Methods

Study Animals and Nociceptive Testing

All experiments used adult, male Sprague-Dawley rats (250–300 g, Charles River, St. Constant, QC, Canada). Procedures were in accordance with the Animals for Research Act, the Guidelines of the Canadian Council of Animal Care, and the Queen's University Animal Care Committee. The paw-pressure[19,20] and tail-flick[21,22] tests were used to evaluate the response of the animals to nociceptive stimuli.

Study 1: Acute Effects of Gabapentin on Morphine Antinociception

Single intraperitoneal doses of a) 7.5 mg/kg morphine, b) 150 mg/kg gabapentin, c) 300 mg/kg gabapentin, and d) a combination of 7.5 mg/kg morphine and 150 mg/kg gabapentin were studied using the paw-pressure and tail-flick tests in naïve rats. Testing was performed every 10 min after drug administration for the first hour and every 30 min for the following 2 h.

Study 2: Effects of Gabapentin on Development of Morphine Tolerance

Rats received intraperitoneal injections of 15 mg/kg morphine once daily for 7 days. This dose has been shown previously to produce tolerance over 7 days following initial maximal antinociception.[23] Testing was performed before and 30 min after drug administration. On day 8, cumulative dose-response curves were constructed, and the ED_{50} values of morphine were determined as described previously.[23] To obtain these curves, animals received increasing doses of morphine every 30 min, and testing followed 30 min after each drug injection. This protocol continued until maximal antinociception was obtained.

To evaluate the effect of gabapentin on development of morphine tolerance, gabapentin (150 mg/kg, intraperitoneal) was coinjected with morphine (15 mg/kg, intraperitoneal) 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 gabapentin on morphine tolerance, another study evaluated gabapentin coinjected with morphine for days 1–3 followed by daily morphine alone on days 4–7.

Study 3: Effects of Gabapentin on Established Morphine Tolerance

Morphine (15 mg/kg) was given once daily for 4 days to induce tolerance. On the following 3 days, gabapentin

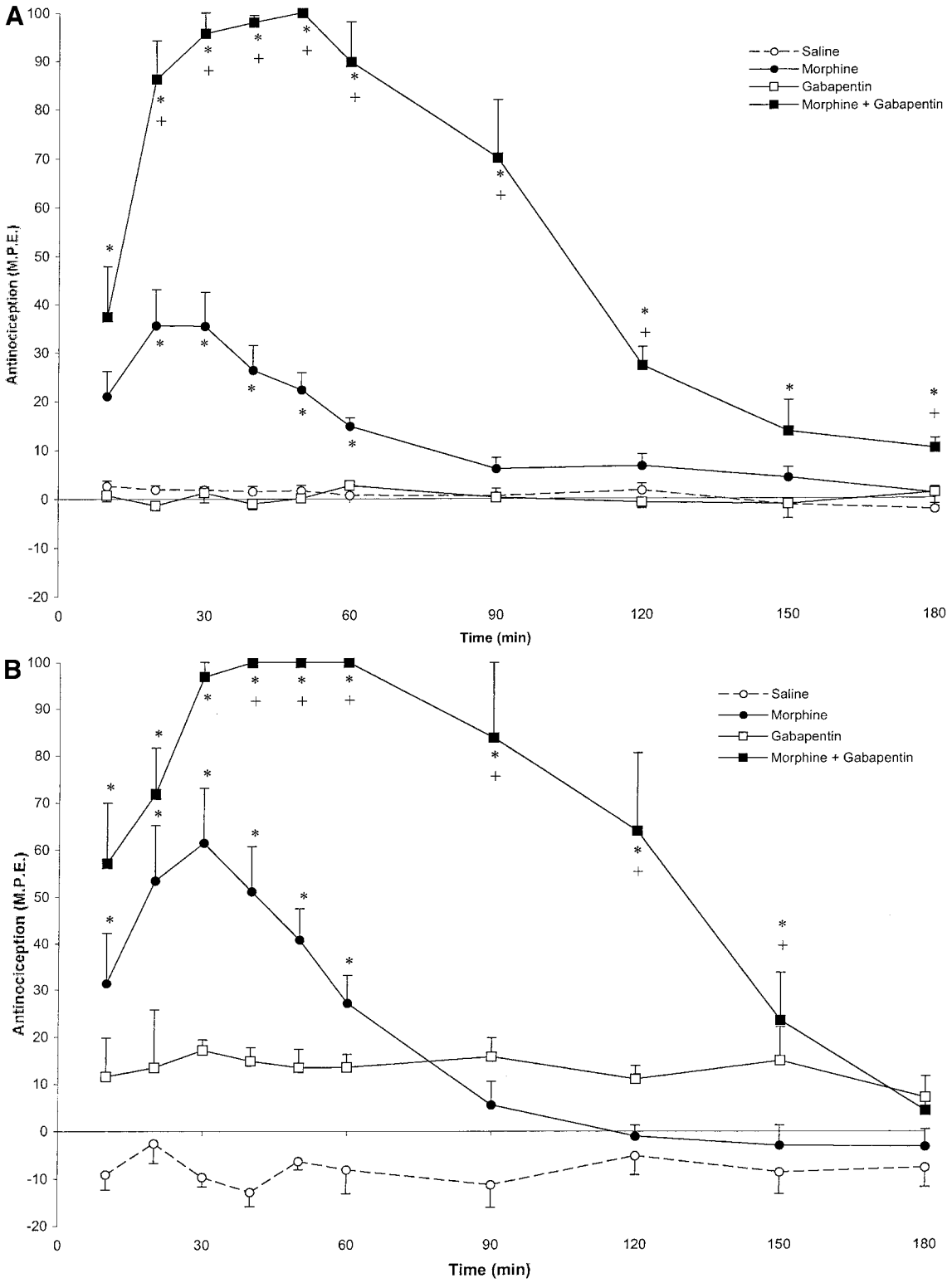

Fig. 1. (*A*) Acute paw-pressure responses (mean ± SEM) to saline, morphine, gabapentin, and morphine plus gabapentin. All doses of morphine and gabapentin are 7.5 mg/kg and 150 mg/kg, respectively. *$P < 0.05$ *versus* saline; +$P < 0.05$ *versus* morphine. (*B*) Acute tail-flick responses (mean ± SEM) to saline, morphine, gabapentin and morphine plus gabapentin. All doses of morphine and gabapentin are 7.5 mg/kg and 150 mg/kg, respectively. *$P < 0.05$ *versus* saline; +$P < 0.05$ *versus* morphine.

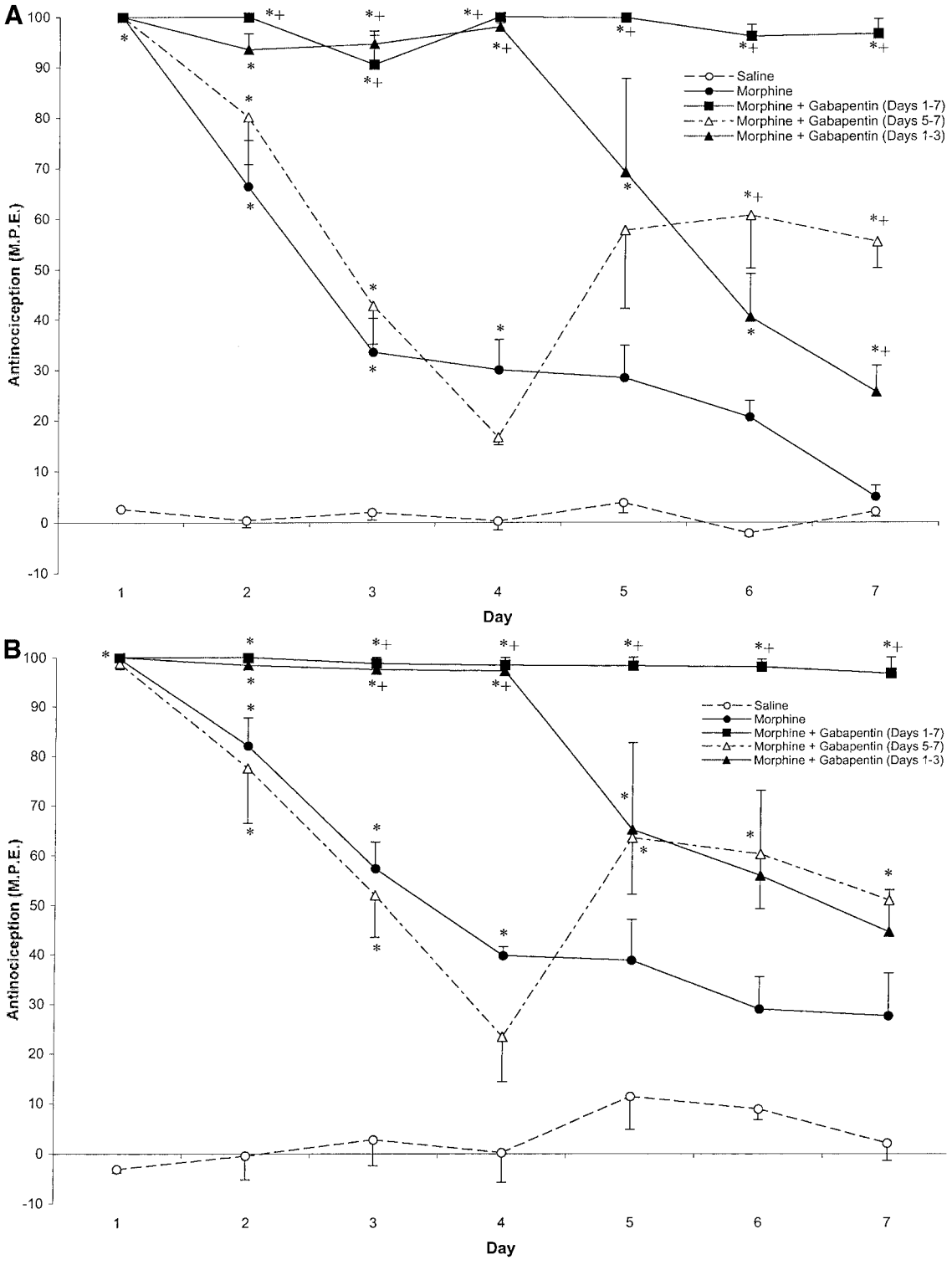

Fig. 2. (*A*) Paw-pressure responses (mean ± SEM) to chronic saline, morphine, and morphine plus gabapentin (gabapentin given for days 1–7; days 5–7; or days 1–3). All doses of morphine and gabapentin are 15 mg/kg and 150 mg/kg, respectively. *$P < 0.05$ *versus* saline; +$P < 0.05$ *versus* morphine. (*B*) Tail-flick responses (mean ± SEM) to chronic saline, morphine, and morphine plus gabapentin (gabapentin given on days 1–7; days 5–7; or days 1–3). All doses of morphine and gabapentin are 15 mg/kg and 150 mg/kg, respectively. *$P < 0.05$ *versus* saline; +$P < 0.05$ *versus* morphine.

Table 1. Effect of Gabapentin on the Development and Reversal of Systemic Morphine Tolerance

Chronic Treatment	ED_{50}, mg/kg	
	Tail-Flick	Paw-Pressure
Saline	5.5 ± 0.6*	9.0 ± 2.9*
Morphine	34.7 ± 5.3	30.8 ± 3.9
Morphine + gabapentin (1–7 days)	11.4 ± 3.1*	10.1 ± 1.4*
Morphine + gabapentin (5–7 days)	36.7 ± 9.3	11.3 ± 2.2*
Morphine + gabapentin (1–3 days)	18.4 ± 2.7	18.2 ± 6.2

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. ED_{50} values were derived from these curves.

* $P < 0.05$ compared to morphine alone.

(150 mg/kg) was introduced in combination with morphine. Morphine dose–response curves were generated on day 8, and acute morphine ED_{50} values were calculated.

Drugs

Morphine was obtained from BDH Pharmaceuticals (Toronto, ON, Canada) and gabapentin was obtained from Pfizer (Groton, CT). All drugs were dissolved in 0.9% saline.

Data Analysis

Tail-flick and paw-pressure values were converted to maximum percentage effect. All data are expressed as mean maximum percentage effect (± SEM). The ED_{50} 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

Study 1: Acute Effects of Gabapentin on Morphine Action

Submaximal doses of morphine (7.5 mg/kg) produced peak antinociception in both tail-flick and paw-pressure tests 30 min after administration. Gabapentin alone at doses of 150 mg/kg (figs. 1A, B) and 300 mg/kg (not shown) had no intrinsic effect in both tests. However, when given together, these doses of morphine and gabapentin resulted in maximal, and supra-additive, antinociception peaking 50 min after administration in the paw-pressure test and between 40 and 60 min after administration in the tail-flick test. The combination of gabapentin and morphine resulted in significantly larger responses than morphine alone, from 20 to 120 min for the paw-pressure test and from 40 to 150 min for the tail-flick test (figs. 1A, B). In both tests, responses returned to baseline by 150 to 180 min after injection. Visual inspection of treated animals revealed no signs of motor impairment.

Study 2: Effects of Gabapentin on Development of Morphine Tolerance

Administration of morphine (15 mg/kg) on day 1 produced maximal antinociception on day 1, which decreased to baseline levels by day 5. Coadministration of morphine with gabapentin (150 mg/kg) completely blocked the decrease in morphine effect throughout the entire 7-day period (figs. 2A, B). In a subsequent experiment, where gabapentin was coadministered with morphine only for days 1–3, maximal antinociception with morphine was still observed on day 4, but a subsequent decrease in effect was observed from days 5 to 7 (figs. 2A, B). Administration of morphine for 7 days significantly increased the ED_{50} value three- to sixfold more than that observed in saline-treated animals (table 1). Coadministration of gabapentin with morphine for the entire 7-day period resulted in ED_{50} values that were significantly lower than values for the morphine alone group (table 1).

Study 3: Effects of Gabapentin on Established Morphine Tolerance

In this study, morphine plus gabapentin 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 (figs. 2A, B). However, addition of gabapentin on days 5–7 resulted in a partial restoration of the morphine effect (figs. 2A, B) and significantly greater antinociception than for morphine alone on days 6 and 7 of the paw-pressure test. The ED_{50} value on day 8 for this treatment group was significantly lower than for that of morphine alone with the paw-pressure test but not the tail-flick test (table 1).

Discussion

This study shows, for the first time, that gabapentin inhibits development of antinociceptive tolerance to morphine. This is evident in sustained responses to morphine in the presence of gabapentin for 7 days, a leftward shift of the acute morphine dose–response curve, and a decrease in the acute morphine ED_{50} value compared to those of morphine tolerant animals. The tolerance to morphine, however, becomes apparent within 48 h of discontinuing gabapentin, indicating the need for continued gabapentin to maintain opioid potency. Finally, data from the paw-pressure test suggests that gabapentin can partially restore opioid potency in tolerant

rats. Taken together, these results support a role for gabapentin–opioid combinations or for the addition of gabapentin to opioids in the setting of tolerance.

Recent studies of gabapentin may explain its effects on opioid tolerance, which is mediated by L-glutamate action at spinal NMDA[6] and AMPA/kainate[7] receptors. Shimoyama *et al.*[24] demonstrated that gabapentin presynaptically inhibits glutamate transmission and Chizh *et al.*[25] showed that gabapentin antagonizes AMPA-evoked responses *in vivo*. Furthermore, a study in trigeminal nucleus slices showed that glutamate release activated by protein kinase C (also important in mediating opioid tolerance) is blocked by gabapentin.[26] Also, chronic morphine has been shown to increase spinal dynorphin expression, which can be pronociceptive[27] and, in this regard, Laughlin *et al.*[28] have demonstrated that gabapentin reduces dynorphin-induced allodynia. Dynorphin expression following chronic morphine exposure involves activation of descending pain facilitory systems,[8] suggesting the importance of supraspinal sites in the development of tolerance. In this regard, Andrews *et al.*[29] showed that gabapentin blocked morphine-induced "conditioned place preference" (a test of psychological dependence) as well as morphine-induced dopamine release from nucleus accumbens. Finally, the effects of gabapentin on tolerance may be related to its unique binding to the (α)2(δ) calcium channel subunit.[30,31] In this regard, a recent investigation by Luo *et al.*[32] has demonstrated upregulation of this subunit following nerve injury, a condition which shares some similarities with opioid tolerance.[6]

In certain situations, tolerance may limit opioid efficacy and an understanding of the underlying mechanisms may improve pain management. This study suggests that gabapentin augments the antinociceptive action of both acute and chronic morphine therapy. 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 gabapentin–opioid combinations may be useful.

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