The Interaction Between Gabapentin and Amitriptyline in the Rat Formalin Test After Systemic Administration

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We examined the effects of systemically administered gabapentin on flinching and biting/licking behaviors produced by 2.5% formalin in the rat, compared these with those of amitriptyline, and determined the effects of combinations of gabapentin with amitriptyline. Gabapentin produced a dose-related inhibition of Phase 2, but not Phase 1, flinching and biting/licking behaviors. In contrast, amitriptyline produced an increase in Phase 2 flinching behaviors while simultaneously decreasing biting/licking behaviors. Fifty percent effective dose (ED$_{50}$) values against biting/licking behaviors were 22.9 ± 1.3 mg/kg and 8.5 ± 1.3 mg/kg for gabapentin and amitriptyline, respectively. Combinations of increasing fractional increments of ED$_{50}$ doses of gabapentin and amitriptyline produced an additive effect against biting/licking behaviors, as revealed by isobolographic analysis. These increments had no effect on flinching behaviors except at the ED$_{25}$ + ED$_{25}$ doses, at which flinching was increased, again revealing additivity between the two drugs. Flinching behaviors in rats do not reflect the analgesic properties of systemically administered amitriptyline observed in humans and may not be useful for predicting an effect of combinations of drugs with amitriptyline. Biting/licking behaviors do reflect analgesic properties for both drugs and may be more useful in this regard.

A variety of animal test models have been developed to examine various aspects of chronic pain. Chronic pain is not simply continuing or persistent acute pain; it involves adaptive changes in pain signaling in primary afferent neurons, in the spinal cord, and at supraspinal sites, and it can exhibit distinct neurochemical features (1–4). The pharmacological treatment of chronic pain is not as effective as the treatment of acute pain, because these underlying changes alter the sensitivity of the system to traditional analgesics (nonsteroidal antiinflammatory drugs and opioids), and chronic pain therapy has come to rely on a group of drugs classified as adjuvants (e.g., antidepressants, anticonvulsants, local anesthetics, and α-adrenergics) (5,6). These drugs have other primary pharmacological indications, but they can modify aspects of the changes that occur with chronic pain and can lead to pain relief. Adjuvants, however, generally do not produce complete pain relief, and their actions can be limited by adverse effects. One potential strategy for minimizing side effects is to use combinations of drugs (7,8). If a drug combination produces synergistic effects, minimal doses could be used, and this would minimize adverse effects. However, if two drugs simply exhibit additive effects, but their side effect profiles are different and noninteracting, this could still serve to augment pain relief and minimize side effects.

Amitriptyline (an antidepressant) and gabapentin (an anticonvulsant) represent different classes of adjuvants used for the treatment of chronic pain. Amitriptyline is widely used, and its efficacy has been established with metaanalysis and systematic approaches (9,10). Gabapentin is used to treat neuropathic pain in particular (11). Both drugs exhibit a comparable efficacy in diabetic neuropathy when compared directly in a single study (12) and when number-needed-to-treat scores are generated (6). Although these drugs produce a comparable incidence of side effects, the pattern of these effects differs somewhat (12).

The formalin test is a model of continuing pain involving continued activation of sensory afferents, inflammation, and central sensitization (13,14). Activation of excitatory amino acid receptors in the spinal cord contributes to central sensitization and chronic pain, and the formalin test has proved useful for revealing the pharmacology of this system (14,15). Both amitriptyline (16) and gabapentin (17,18) may modify aspects of excitatory amino acid function in the spinal...
cord to produce analgesia. We recently reported the analgesic properties of amitriptyline in the rat formalin test after systemic administration (19). In this study, we determined the analgesic properties of gabapentin after systemic administration in the rat formalin model and compared these with those produced by amitriptyline. We also examined the effects of combinations of gabapentin with amitriptyline to determine whether these two adjuvants could produce additive or synergistic effects. Gabapentin has been examined in combination with morphine (20), ibuprofen (21), clonidine (22), and a non-N-methyl-D-aspartate excitatory amino acid receptor antagonist (23) in various pain models, whereas amitriptyline has been administered in combination with opioids, neostigmine, and clonidine (24,25). There have been no reports on the effects of the combination of gabapentin with amitriptyline in any model, but the effects of this combination in the formalin model are of particular interest because of its potential relevance to chronic pain. Results of this study have been presented in abstract form (26).

**Methods**

Experiments were conducted with male Sprague-Dawley rats (125–150 g; Charles River, Quebec, Canada). Rats were housed in pairs with a 12:12-h light/dark cycle at 22°C, with free access to food and water. All experiments were approved by the University Committee on Laboratory Animals, and experiments were conducted in accordance with International Association for the Study of Pain and the Canadian Council on Animal Care standards.

Formalin 2.5% was administered in a volume of 50 μL by subcutaneous injection into the dorsal surface of the hindpaw at Time 0. Gabapentin and drug combinations were administered systemically as a 20-min intraperitoneal (IP) pretreatment in a volume of 5 mL/kg. Each behavioral testing session was preceded by a 20-min acclimatization to the 28 × 28 × 28-cm Plexiglas observation chamber. After injection of formalin, rats were returned to the chamber. Flinching behaviors (lifting, shaking, or rippling of the haunch, with episodes scoring as a single event) were recorded as a cumulative number of events, and biting/licking behaviors were recorded as time spent exhibiting that behavior, for 60 min after formalin injection (Phase 1 = 0–12 min; Phase 2 = 16–60 min). Two rats in adjacent chambers were observed at a time, with observations occurring in alternate 2-min bins. Values were not corrected for time, so scores represent approximately half of the total behaviors. Statistics were performed on the time-course and dose-response data by using analysis of variance followed by the Student-Newman-Keuls test for multiple groups and time courses.

The dose of drug required to produce 50% of the maximum possible decrease in behavior (ED50) was obtained for each drug by using linear regression. Subsequently, dose-response information for the concurrent administration of both drugs was obtained by using constant dose ratios of the respective ED50 value for each drug (i.e., ED50 + ED50, ED37.5 + ED37.5, ED25 + ED25, and ED12.5 + ED12.5). The ED50 value for the drug combination was calculated by linear regression and isobolographic analysis, as previously described (27,28). The theoretical and actual ED50 values for the drug combination were compared by using Student’s t-tests.

Gabapentin was supplied by Parke Davis (Ann Arbor, MI). Amitriptyline hydrochloride and formalin were purchased from the Sigma Chemical Co. (St. Louis, MO).

**Results**

The IP injection of gabapentin 10–50 mg/kg before 2.5% formalin produced a reduction in both flinching and biting/licking behaviors, particularly in the later part of the second phase of activity (Figs. 1 and 2). Phase 1 behaviors were unaltered. A larger dose (70 mg/kg) produced sedation (somnolence and flaccidity) (data not shown). Amitriptyline augments flinching but decreases biting/licking behaviors (Fig. 2), and these actions were expressed uniformly throughout the second phase of activity. Regression analysis revealed an ED50 of 22.9 ± 1.3 mg/kg for gabapentin and 8.5 ± 1.3 mg/kg for amitriptyline for Phase 1 biting/licking responses. Combinations of fixed increments of ED50 values for the two drugs had no significant effect on flinching behaviors except at the ED25 + ED25 doses, at which an augmentation in flinching was observed (Fig. 3A). However, these combinations exhibited an additive effect against biting/licking behaviors (Fig. 3B). This was verified by isobolographic analysis, with no significant difference between theoretical and actual ED50 values for the drug combination (Fig. 4).

**Discussion**

This study demonstrates that systemic administration of gabapentin in rats inhibits both flinching and biting/licking behaviors produced by formalin. Other studies have previously noted the efficacy of gabapentin in the formalin test when given both systemically (21) and spinaly (29–31). The suppression of behaviors was observed in Phase 2, but not in Phase 1, as noted in each of those reports, and was prominent in the later part of Phase 2, as also noted previously (21). The observation that spinal gabapentin is less
potent when administered after the formalin and preferentially suppresses Fos-like immunoreactivity induced by larger concentrations of formalin suggests that gabapentin acts on elements of central sensitization that are recruited during the second phase of activity (31). Gabapentin also produces analgesic properties in other inflammatory pain tests and nerve injury models that are considered to be relevant models of chronic pain in humans (11). Although the specific mechanisms by which gabapentin produces analgesia are not resolved, a spinal component of action is clearly involved. Thus, gabapentin is not only active in behavioral models after spinal application, but it also inhibits the formalin-evoked expression of Fos-like immunoreactivity in the spinal cord (31) and affects excitatory transmission mediated by glutamate in the superficial spinal cord (17,18). Gabapentin binds to the $\alpha_2\delta$ subunit of voltage-dependent Ca$^{2+}$ channels (32) and inhibits Ca$^{2+}$ influx and neurotransmitter release at central sites (33,34), and these actions also could contribute to analgesia. Even though gabapentin interacts with specific heterodimers of $\gamma$-aminobutyric acid type B receptors (35), a selective antagonist for this receptor did not block antihyperalgesic effects of gabapentin (36).

In contrast to gabapentin, systemic administration of amitriptyline augments flinching behaviors while simultaneously inhibiting biting/licking behaviors; the mechanisms underlying this duality of action are considered elsewhere (19). Multiple mechanisms, including inhibition of biogenic amine reuptake, interactions with opioid and adenosine systems, and interactions with N-methyl-D-aspartate receptors and ion channels, are thought to contribute to antinociception.

Figure 1. Time course of suppression of 2.5% formalin-evoked (A) flinching and (B) biting/licking behaviors produced by systemic (intraperitoneal) pretreatment with gabapentin (GP). Values depict mean $\pm$ SEM for six per group; ns indicates $P > 0.05$ compared with the Saline-Pretreated group.

Figure 2. Comparison of the effects of systemically-administered gabapentin (GP) and amitriptyline (AMI) in the 2.5% formalin (FOR) test against Phase 2 (A) flinching and (B) biting/licking behaviors. Data for amitriptyline redrawn (19) with permission. Lines were fit by linear regression analysis of individual groups. *$P < 0.05$ compared with the Saline-Pretreated group; $n = 6$ per group.
resulting from systemic administration of amitriptyline (37,38).

Although the details of the mechanisms by which gabapentin and amitriptyline produce analgesia are not precisely defined, they represent two distinct classes of drugs and seem to interact with different endogenous pain regulatory systems. Thus, it was initially anticipated that these two drugs might recruit parallel or sequential mechanisms in pain transmission pathways and exhibit a synergistic analgesia. However, when gabapentin and amitriptyline were combined in fixed fractional proportions, an additive effect was obtained with both behaviors. Biting/licking behaviors were reduced in a dose-dependent manner with fixed-dose increments of ED₅₀ values, and isobolographic analysis indicated a strictly additive effect. The drug combination also produced an additive effect on flinching behaviors, even though effects of the two individual drugs on this behavior differed qualitatively. Thus, at the ED₂₅ + ED₂₅ dose, there was an increase in flinching responses reflecting an earlier expression of the facilitating effect of amitriptyline, whereas at larger doses, flinches were no longer augmented, indicating that some degree of suppression of enhanced behaviors was now occurring because of the activity of gabapentin.

Given that gabapentin and amitriptyline both produce pain relief in humans (9–11), biting/licking behaviors seem to be a more appropriate end point to consider in the formalin model for amitriptyline combinations. Whether the additive analgesia observed with this behavior also manifests clinically as an additive pain relief remains to be determined in a controlled and systematic manner. Before such a study, it would be prudent to determine (a) the effects of gabapentin and amitriptyline combinations in other preclinical models of chronic pain (there are more chronic models of inflammation and nerve injury in which both gabapentin and amitriptyline exhibit analgesic activity), (b) whether there is an interaction with adverse effects (although sedation was not quantitated or examined systematically in this study, no sedation was noted in the combination groups), and (c) the effects of chronic administration of these two drugs (it
is not entirely clear whether drug effects that require chronic administration paradigms represent a pharmacokinetic or a pharmacodynamic mechanism).

The differential effects of gabapentin and amitriptyline on flinching behaviors in the formalin test raise the question of the usefulness of this outcome score for predicting outcomes in clinical paradigms. The flinch response is, to some extent, a spinally-mediated response, because it can be elicited in chronically-spinalized animals (39). However, some supraspinal integration, modulation, or both are involved as well, because acute spinalization can reduce flinch responses (40,41). The fact that a drug that is widely used to treat chronic pain (amitriptyline) actually enhances flinching behaviors indicates that the sole use of this particular indicator as a measure of pain in the formalin test may not be appropriate for determining analgesic activity. A MEDLINE search of the literature for 1997–1998 revealed that 43% of studies that used the formalin test used flinching as a single measure, and it was argued that multiple behavioral measures should be used with the formalin test (42). The results of this study support that conclusion.

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References