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Evaluation of Interaction between Gabapentin and Ibuprofen on the Formalin Test in Rats

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Background: **Gabapentin is active in the regulation of facilitated pain states evoked by tissue injury. The mechanism of this action is believed to be through a specific binding site, likely at the spinal level. Nonsteroidal antiinflammatory drugs have a comparable behavioral profile, although their actions are believed to be mediated by cyclooxygenase inhibition at the spinal level. This study was undertaken to determine the nature of the interaction of these two mechanistically distinct antihyperalgesic agents in rats in a model of facilitated processing, the formalin test.**

Methods: **The effects of intraperitoneal gabapentin and ibuprofen were examined on flinching behavior and cardiovascular response (mean arterial blood pressure [MABP] and heart rate measured in the tail artery) evoked by the injection of formalin (5%; 50 μl). Their interaction was characterized using an isobolographic analysis.**

Results: **Injection of formalin into the hind paw caused a biphasic flinching and parallel increases in MABP. Gabapentin and ibuprofen produced a limited effect on the flinching in phase 1, but both drugs produced dose-dependent suppression of the flinching observed during phase 2 (gabapentin ED_{50} = 88 mg/kg; ibuprofen ED_{50} = 19 mg/kg). Gabapentin similarly showed a dose-dependent suppression of the MABP and heart rate response only during phase 2; ibuprofen showed dose-dependent reduction of MABP response in both phases. The isobolographic analysis carried out using equipotent dose ratios in phase 2 revealed an additive interaction between the two drugs. Neither gabapentin nor ibuprofen affected the baseline cardiovascular measures.**

Conclusion: **Gabapentin and ibuprofen independently alter the facilitated state as measured by somatomotor and autonomic response. Together these agents interact in an additive fashion if delivered concurrently. This combination may prove useful in managing postinjury pain states in humans. (Key words: cyclooxygenase; *N*-methyl D-aspartate receptor; spinal; tissue injury.)**

GABAPENTIN is a γ-aminobutyric acid analog originally synthesized for its anticonvulsant actions.[1] Although neither the systemic nor the intrathecal injection of gabapentin has any effect upon acute thermal nociception, this agent reverses thermal hyperalgesia induced by the injection of substance P or thermal injury to the paw[2,3] and by the second phase of the formalin test[4,5] after systemic or intrathecal administration. The action of gabapentin is believed to be *via* a specific binding site.[6,7] Nonsteroidal antiinflammatory drugs have a comparable behavioral profile. Intrathecal nonsteroidal antiinflammatory drugs have little effect upon acute thermal nociception but reduce the thermal hyperalgesia induced by intrathecal substance P and the paw flinching during the second phase of the formalin.[8,9] These data support the hypothesis that the antihyperalgesic action of nonsteroidal antiinflammatory drugs is mediated by an inhibition of cyclooxygenase at the spinal level.

The aforementioned findings suggest that nonsteroidal antiinflammatory drugs and gabapentin may exert their actions mainly on the facilitated state that occurs secondary to the persistent afferent input generated by a local tissue injury. We sought to determine the nature of the interaction between these two antihyperalgesic agents, gabapentin and ibuprofen, in rats on a model of facilitated processing, the formalin test. Tissue injury states evoke not only somatomotor response but autonomic activity (*e.g.*, increased blood pressure). It has been shown that, like the somatomotor response, the autonomic response displays a biphasic time course, suggesting that the injury-driven autonomic activity is also subject to the facilitated processing believed to be characteristic of the second phase, which is typically noted after the injection of formalin into the paw. Accordingly, we hypothesized that this second-phase auto-

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nomic activity would be similarly sensitive to the actions of ibuprofen and gabapentin.

Materials and Methods

Animal Preparation

Experiments were conducted according to a protocol approved by the Institutional Animal Care Committee of the University of California, San Diego. Male Sprague–Dawley rats (300–325 g) were kept in group cages and maintained on a 12-h light/12-h dark cycle. Animals had free access to food and water at all times.

Drugs and Injection

The drugs used in this study were gabapentin (1-[aminomethyl] cyclohexanacetic acid; Neurontin, Parke-Davis, Ann Arbor, MI) and R/S-ibuprofen (courtesy of D. P. Bauer and C. W. Matthews, Ethyl Corporation, Baton Rouge, LA). Gabapentin was stored at 5°C in an opaque container and dissolved in 0.9% physiologic saline. Ibuprofen was dissolved in a 5% solution of 2-hydroxy-propyl-β-cyclodextrin (Research Biochemicals, Natick, MA). For intraperitoneal administration, both drugs and physiologic saline were injected in volumes of 3 ml/kg.

Hemodynamic Measurement

Each rat was briefly anesthetized with 2–3% halothane in oxygen-enriched room air. Intramedic polyethylene tubing (Clay Adams, Parsippany, NJ) tubing was inserted into the tail artery. The catheter was flushed with 0.5 ml heparinized saline. The rat was then removed from the anesthetic and placed in a restraint cylinder constructed from longitudinally oriented rods. The arterial line was connected to a pressure transducer that led to a polygraph (model 7, Grass Instrument, Quincy, MA) for continuous recording of blood pressure and heart rate.

Nociceptive Test

All rats recovered well from anesthesia. After acclimation for 30–40 min, 50 μl of 5% formalin solution was injected subcutaneously into the plantar surface of hind paw with a 30-gauge needle. Pain behavior was quantified by counting the incidences of spontaneous flinching or shaking of the injected paw. The flinches were counted for 1-min periods between 1 and 2 and 5 and 6 min, and then for 5-min intervals during the interval between 10 and 60 min. Two phases of spontaneous flinching of the injected paw were observed after formalin injection. Phase 1 and phase 2 were defined as from 0–9 and 10–60 min after formalin injection, respectively. Upon completion for the 60-min observation, the rat was killed with pentobarbitol sodium phenytoin sodium solution.

Experimental Paradigm

After tail-artery cannulation and after a 30–40-min interval of acclimation, baseline blood pressure and heart rate were measured. The animal was then entered into one of the drug-treatment groups.

Dose–Effect Studies for Gabapentin and Ibuprofen

The first series of experiments was performed to determine the time course and dose-dependency for flinching and cardiovascular response of intraperitoneally administered gabapentin and ibuprofen on the formalin test. Several doses of gabapentin (10, 30, 100, 300 mg/kg) and ibuprofen (3, 10, 30 mg/kg) were each examined to determine the dose that produced an ED_{50} one-paw–flinching response. Intraperitoneal administration of saline and two drugs was performed 30 min before formalin injection. Each rat was used for a single treatment.

Drug-interaction Studies

An isobolographic analysis was used to determine the nature of the drug interaction between gabapentin and ibuprofen. The method is based on comparison of dose combinations in which the dose combinations are made of doses of each of the two agents that are determined to be equipotent. Thus, from the dose–response curves of two agents alone, the respective ED_{50} values (effective dose resulting in a 50% reduction of control formalin response) are determined. Subsequently, a dose–response curve is obtained by concurrent delivery of the two drugs in a constant dose ratio based on the ED_{50} values of the single agent. Thus, separate groups received: gabapentin ED_{50} + ibuprofen ED_{50}; (gabapentin ED_{50} + ibuprofen ED_{50})/2; (gabapentin ED_{50} + ibuprofen ED_{50})/4; and (gabapentin ED_{50} + ibuprofen ED_{50})/8. From the dose–response curves of the combined drugs, the ED_{50} value of the combination was calculated, and these dose combinations were used for plotting the isobologram. In this experiment, the ED_{50} values were determined from the flinching data during phase 2 on the formalin test.

The isobologram was constructed as described previously.[10] In brief, the ED_{50} values of the single agents were plotted on the *x*- and *y*-axes, respectively. The

theoretically additive dose combination was calculated. From the variance of the total dose, individual variances for the agents in the mixture were obtained. Furthermore, to describe the magnitude of the interaction, a "total dose fraction value" was calculated according to the following formula:

$$\text{Total fraction value} = \frac{ED_{50} \text{ of drug 1 with drug 2}}{ED_{50} \text{ for drug 1 given alone}} + \frac{ED_{50} \text{ of drug 2 with drug 1}}{ED_{50} \text{ for drug 2 given alone}}$$

Total fraction values near 1 indicate additive interaction. Values less than 1 indicate synergistic interactions. Values greater than 1 indicate an antagonistic interaction.

Statistical Analysis

All data are expressed means and SEM. The time–response data are presented as the number of flinches or percentage change from the baseline mean arterial blood pressure (MABP) and heart rate (HR). Dose–response data are presented as the sum of flinches or the sum of the percentage changes of MABP and HR in each phase. To obtain the flinching ED_{50} during phase 2, the number of flinches were converted to percentage maximal possible effect (MPE):

$$\%\ \text{MPE} = \frac{\text{Sum of phase 1 (or 2) with drug}}{\text{Sum of control phase 1 (or 2)}} \times 100$$

Statistical analysis of dose–response data was done using the Jonckheere test. The dose–response lines were fitted using least-squares linear regression, and ED_{50} and its 95% confidence intervals were calculated according to Tallarida and Murray.[11] The difference between theoretic ED_{50} and experimental ED_{50} was compared with a *t* test. The baseline MABPs and HRs of the several drug groups were examined by one-way analysis of variance. The cardiovascular change induced by intraperitoneal ibuprofen and gabapentin alone was examined by paired *t* test or repeated-measures analysis of variance. Values of $P < 0.05$ were considered statistically significant.

Results

Baseline and Response to Formalin

The baseline MABP and HR prior to intraperitoneal injection of drugs were 103 ± 1 mmHg and 417 ± 2 beats/min, respectively (n = 93). Baseline MABP and HR in experimental groups did not differ ($P > 0.4$ and $P > 0.2$, respectively).

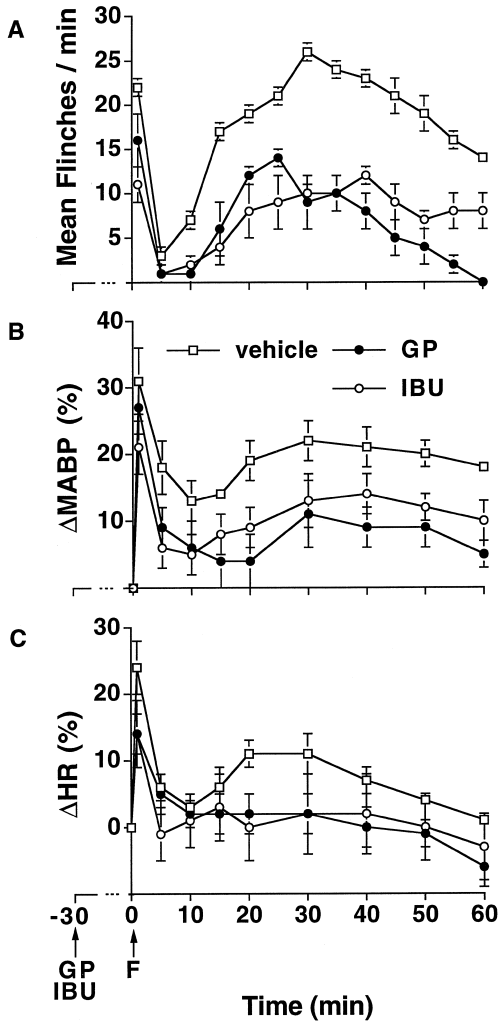

Fig. 1. Time–effect curve of intraperitoneal gabapentin (GP) 300 mg/kg and ibuprofen (IBU) 30 mg/kg for flinching (*A*), mean arterial blood pressure (MABP) (*B*), and heart rate (HR) (*C*) on the formalin test. GP and IBU were administered at T = −30 min and formalin (F) was injected subcutaneously at T = 0 min. Data are presented as number of flinches or percentage change from baseline MABP and HR. Each line represented the means ± SEM of 5–7 rats. Statistical analysis for phase 1 and phase 2 dose effects is presented in figure 2.

Subcutaneous injection of formalin into the hind paw caused a biphasic incidence of flinching of the injected paw and a biphasic increase in MABP and HR, as indicated in the time-versus-effect curve shown in figure 1.

Gabapentin and Ibuprofen Studies

Neither intraperitoneal gabapentin (300 mg/kg) nor ibuprofen (30 mg/kg), at the highest doses used, had any

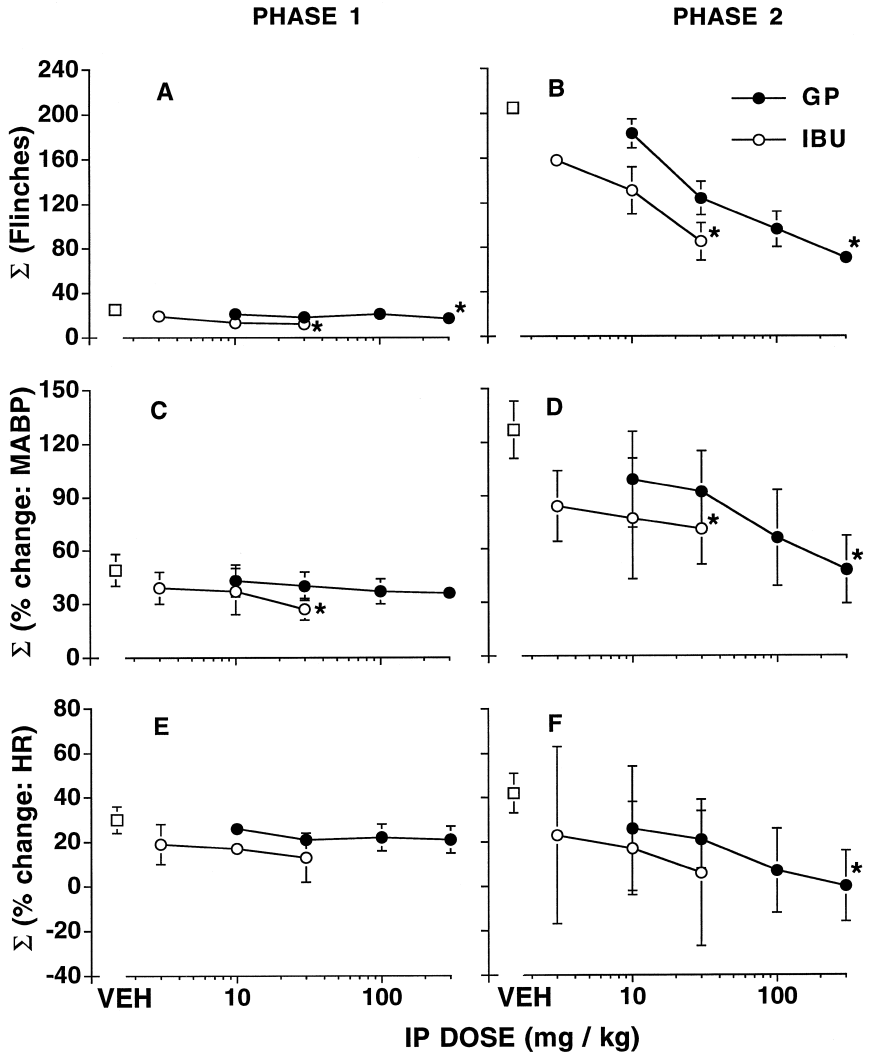

Fig. 2. Dose–response curve of intraperitoneal gabapentin (GP) and ibuprofen (IBU) for flinching (*A* and *B*), mean arterial blood pressure (MABP) (*C* and *D*), and heart rate (HR) (*E* and *F*) during phase 1 (*A*, *C*, and *E*) and phase 2 (*B*, *D*, and *F*) on the formalin test. Data are presented as the sum of flinches or the sum of percentage changes of MABP and HR. GP produced a dose-dependent suppression of the flinching and the magnitude of increasing cardiovascular response during phase 2; it had limited effect or no effect on the flinching and cardiovascular response in phase 1. The antinociceptive effect of IBU was similar to that of GP. IBU produced a dose-dependent reduction in the magnitude of MABP response in both phases; no effect was seen in HR response in either phase. Each point represents the means ± SEM of 5–7 rats. An asterisk represents dose-dependency ($P < 0.05$). VEH = vehicle.

effect on blood pressure or HR over the 60-min period after the two drugs were administered if given in the absence of formalin in the paw (data not shown).

Intraperitoneal administration of gabapentin and ibuprofen produced a limited (to approximately 69% and 47% MPE, respectively), but dose-dependent, reduction of the flinching during phase 1 on the formalin test. During phase 2, both drugs produced a dose-dependent suppression of the flinching (figs. 1 and 2). The ED_{50} values of gabapentin and ibuprofen alone for suppressing the flinching during phase 2 were 80 and 19 mg/kg, respectively (table 1). Thus, the calculated dose ratio for gabapentin and ibuprofen was 4.6:1.

Gabapentin showed a dose-dependent suppression of the magnitude of the increase in the MABP and HR response during phase 2 on the formalin test; no effect was seen on changes observed during phase 1. Ibuprofen showed a dose-dependent suppression of the MABP response in each phases. No change in the HR response was seen in either phase.

Drug-interaction Studies

The respective ED_{50} values of gabapentin and ibuprofen were 88 and 19 mg/kg intraperitoneal, respectively. Accordingly, gabapentin and ibuprofen were delivered to different groups of rats in fractions of the ED_{50} dose combination of 88 + 19 mg/kg ($ED_{50}s/2$ = 44 + 9.5 mg/kg; $ED_{50}s/4$ = 22 + 4.8 mg/kg; and $ED_{50}s/8$ = 11 + 2.4 mg/kg). Coadministration of gabapentin and ibuprofen produced a limited (to approximately 69% MPE), but dose-dependent, effect on phase 1, although the combination decreased the flinching otherwise observed dur-

Table 1. Flinching ED_{50} (mg/kg) and Slope with 95% Confidence Intervals of Intraperitoneal Agents

Agent	No. of Rats	ED_{50} (95% CI)	Slope (95% CI)
Gabapentin	20	88 (55–141)	−35 (−59 to 23)
Ibuprofen	16	19 (7–50)	−35 (−59 to 12)
Gabapentin*	21	53 (12–237)	−28 (−53 to 4)

ED_{50} = effective dose producing a 50% reduction of control formalin response; CI = confidence interval.

* This value is the ED_{50} for the dose of gabapentin in the dose mixture of gabapentin and ibuprofen. See text for details.

ing phase 2 in a dose-dependent fashion (fig. 3). The combination had no effect on the cardiovascular response in phase 1, but it showed a dose-dependent reduction of MABP and HR response during phase 2 on the formalin test. Isobolographic analysis conducted using the equipotent ratio in phase 2 revealed only an additive interaction between the two drugs (fig. 4). The experimental ED_{50} did not differ from the theoretic ED_{50}. Thus, the ED_{50} of the gabapentin–ibuprofen mixture was 53 mg/kg (table 1). Corresponding to this additive interaction, the dose fraction was 0.98.

Discussion

Mechanistic Components of the Response to Local Formalin

The injection of formalin into the paw results in an immediate increase in the activity of small slowly conducting afferents from the paw, and this increase is typically followed after approximately 10–15 min by a decline to a low, but not zero, level of ongoing activity.[12] Dorsal-horn wide dynamic range neurons after injection of formalin into the paw display elevated levels of activity corresponding to the high levels of first-phase afferent input and a second phase of activation that appears to be greater than anticipated, considering the diminished level of afferent input.[13] Behaviorally, the injection of formalin in the unanesthetized rat evokes a somatomotor response (flinching), the magnitude of which corresponds with the biphasic time course of the wide dynamic range activity.[14]

Mechanistically, examination of the spinal pharmacology of the formalin response suggests that there is an acute release of glutamate and substance P that, through their respective *N*-methyl D-aspartate and NK1 receptors, initiates a subsequent cascade that includes increases in intracellular calcium, activation of various kinases[15], and an enhanced release of agents such as prostaglandins.[16] These elements lead to a state of facilitated processing that underlies the second phase of the observed stimulus-evoked somatomotor response. With regard to the cardiovascular response, segmental small afferent input, as initiated by the injection of formalin into the paw, evokes a spinobulbospinal reflex that activates preganglionic sympathetic neurons.[17] This increased outflow evokes peripheral sympathetic nervous activity, which mediates the observed hypertension and tachycardia. The concurrent activation of the somatosympathetic re-

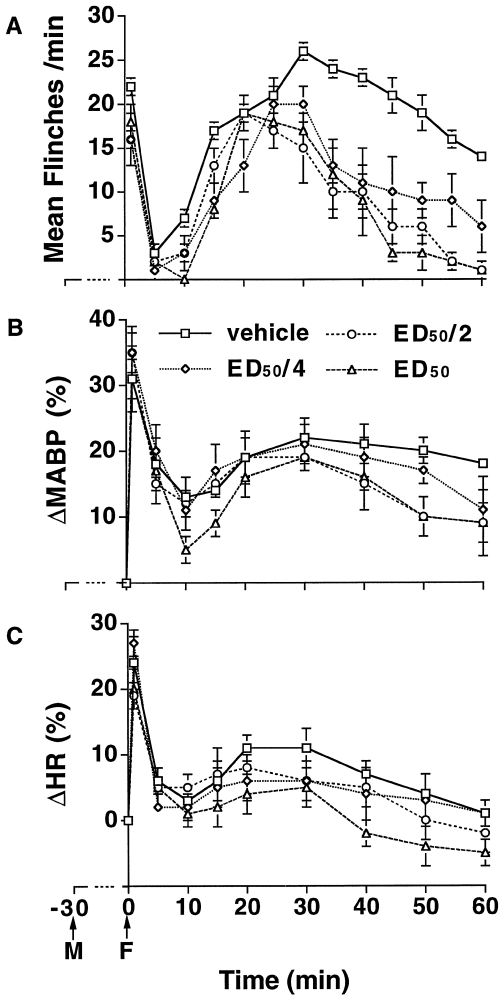

Fig. 3. Time–effect curve of the effects produced by the injection of the mixture (M) of gabapentin and ibuprofen for flinching (*A*), mean arterial blood pressure (MABP) (*B*), and heart rate (*C*) on the formalin test. The M was administered at T = −30 min and formalin (F) was injected subcutaneously at T = 0 min. Dose combinations of gabapentin + ibuprofen were: ED_{50} = 88 + 19 mg/kg; $ED_{50}/2$ = 44 + 9.5 mg/kg; $ED_{50}/4$ = 22 + 4.8 mg/kg. Data are presented as number of flinches or percentage change from baseline MABP and HR. Each line represents the means ± SEM of 5 or 6 rats.

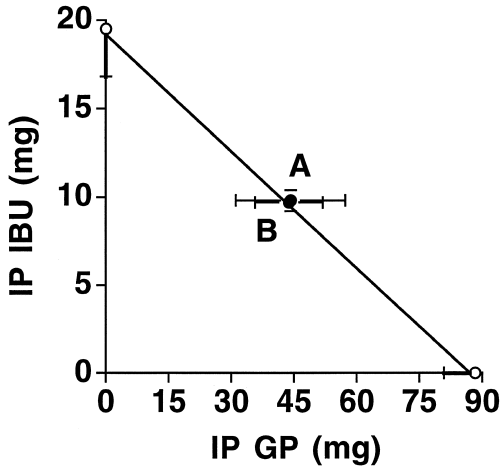

Fig. 4. Isobologram for drug interaction between gabapentin (GP) and ibuprofen (IBU) on the antinociceptive effect during phase 2 on the formalin test. The ED_{50} values for single agents are plotted on the *x*- and *y*-axes, respectively, and the heavy lines represent the SEM of ED_{50}. The experimental ED_{50} (*B*) was not significantly different from the theoretic ED_{50} (*A*), indicating an additive interaction.

flex by the formalin injection thus not surprisingly evokes a biphasic change in blood pressure.[18]

Mechanisms of Action of Gabapentin and Ibuprofen

Intraperitoneal administration of ibuprofen and gabapentin resulted in a significant suppression of the somatomotor flinching during phase 2 on the formalin test but had only a limited effect on phase 1. Both agents attenuated the somatosympathetic response without an effect upon resting cardiovascular indices.

The effects of ibuprofen are mediated by its ability to inhibit cyclooxygenase and accordingly block the synthesis and release of prostaglandins.[19] Although it is clear that ibuprofen may have a peripheral action (reflecting the local sensitization of peripheral nerve terminals by prostaglandins), previous work noted here has emphasized the role of persistent small afferent input to evoke a spinal sensitization mediated in part by the spinal release of prostaglandins. The spinal and systemic delivery of ibuprofen, as in the present studies, has been shown to block the formalin-evoked increase in release of spinal prostaglandins.[16] We thus believe that ibuprofen is exerting its antihyperalgesic action by blocking prostaglandin synthesis, at least at a spinal site.

The intrathecal and systemic delivery of gabapentin and its structural homologues have little effect upon acute nociceptive responses but diminish the hyperalgesic state evoked by tissue injury.[3–5,20] As with ibuprofen, because of the relative potency of the agent after intrathecal delivery and because the systemically delivered drug reverses models of centrally mediated hyperalgesia (*e.g.*, as evoked by intrathecal substance P or *N*-methyl D-aspartate),[2,9] it seems clear that the central action is of particular importance. At the membrane, current evidence supports the existence of a high-affinity site for the binding of gabapentin and its homologues. Importantly, this work indicates that the activity at the binding site parallels the antihyperalgesic activity of these agents after intrathecal delivery and correlates with binding to the $\alpha_2\delta$ subunit of voltage-sensitive calcium channels.[6,7] Although the potential importance of the binding to the $\alpha_2\delta$ subunit is clear, the importance of this binding to the biologic effects of the gabapentinoids is not established. There is, however, no known correspondence to other candidate sites such as for any excitatory amino acid or peptide receptor or as a direct inhibitor of prostaglandin synthesis.[1]

Drug Interaction

The parallel functional profiles of ibuprofen and gabapentin (*e.g.*, as antihyperalgesics with potential spinal action) led us to consider the characteristics of their interaction. The properties of a system that define a pharmacologic interaction between two classes of agents are likely complicated. As reviewed elsewhere, agents may interact by altering the kinetics of each other; at the membrane level, by acting on a common membrane to alter the actions of the other agent at its target site (*e.g.*, receptor or channel); or at a physiologic level, at which the separate drug systems interact with respect to a common endpoint (*e.g.*, hyperalgesia).[21] If fundamentally different mechanisms jointly contribute to the observed actions of two agents on a given endpoint, such as antihyperalgesia, a synergic interaction is considered likely. This synergy may be manifested by the production of a given effect by lower doses of either drug or an increased maximum achievable effect (*e.g.*, increased efficacy).

The isobolographic approach was chosen in the present study to define the nature of the interaction at a fixed dose combination. The ED_{50} value is typically chosen, as it falls by definition in the midrange of the maximum possible effect that could be achieved by either agent. In addition, in the case in which either drug alone fails to produce a complete blockade at the maximum usable dose (*e.g.*, a plateau effect), it is possible to determine if the drug combination can enhance the efficacy of the drug effect. The isobolographic approach

has been widely used to study the interaction of analgesic and anesthetic agents, and its implementation has been previously discussed.[22–24] Previous drug-interaction analysis using the formalin test has demonstrated synergy with several intrathecally delivered drug combinations including the spinal delivery of μ- and α_2-adrenergics [25], and ketorolac (cyclooxygenase inhibitor) with μ- or α_2-adrenergic agonists.[21] Accordingly, it is clear that a synergic interaction can be demonstrated with this test system. The essential observation in the present study was that the interaction between systemic gabapentin and ibuprofen in this model of formalin-evoked flinching does not differ from additivity. In addition, there was no increase in the maximum achievable effect. As noted, in previous work the delivery of several drug pairs has been demonstrated to be markedly synergistic. However, all drug interactions are not synergistic. Thus, in the formalin model, the concurrent intrathecal delivery of a cyclooxygenase inhibitor with κ-opioid or adenosine a_1-agonists also displayed additivity [21].

Several factors may govern the nature of a drug interaction. One important variable may be stimulus intensity. Previous work has shown that the synergy between morphine and a barbiturate in blocking the response to a strong pinch is defined by the intensity of the stimulus. At low intensities, there is a synergic interaction. At higher intensities, the interaction is additive or less.[26] Whether lower concentrations of formalin, which are believed to produce a milder stimulus (as defined by the number of flinches and the dose–effect relationships), would reveal synergy is an issue that should be addressed.

Clinical Significance

Limited effect on the flinching during the acute component of the postformalin response emphasizes that gabapentin and ibuprofen have a minimal effect on acute pain such as from surgical incision. Nevertheless, the ability of either drug to reduce the pain behavior and the autonomic response to a postinjury stimulus suggests that either drug may be useful in diminishing the anesthetic requirements. In recent work, we have shown that systemic 3-iso-butylgaba (a more potent gabapentin analog) has no minimum alveolar concentration–sparing effects in the rat in the face of an acute tail stimulus if tested with isoflurane, propofol, pentobarbital, or fentanyl (Mike Bogue and Tony Yaksh, unpublished data). On the other hand, the factors underlying the pain processing generated during and following surgery likely entail a component that represents facilitated processing. In this regard, agents that act only through antihyperalgesic mechanisms may well reduce the concentration of anesthetic or dose of adjuvant that would otherwise be required. The combination of gabapentin and a cyclooxygenase inhibitor may well have clinical virtue, as it serves at least to reduce by half the dose requirements of either agent. As the side-effect profile of each is distinct (*e.g.*, sedation *vs.* possible effects upon clotting and renal blood flow), the reduction of the respective dose by 50% to achieve a comparable degree of post–tissue injury pain relief is likely of practical benefit.

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