Study of the analgesic, anti-inflammatory, and gastric effects of gabapentin

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ABSTRACT: Gabapentin, a drug used to treat neuropathic pain, was evaluated in models of acute nociceptive pain, in instances of haloperidol-induced catalepsy, carrageenan-induced paw edema, gastric lesions caused by indomethacin or ethanol, and gastric acid secretion in rats. Reaction time in a hot plate assay was delayed by gabapentin. The antinociceptive effect of the drug was produced with a dose of 12.5 mg/kg and a maximal increase in hot plate latency of 68% 1 h after drug administration was produced at 100 mg/kg. Gabapentin (25, 50 or 100 mg/kg) caused a significant rise in current threshold in a tail electrical stimulation test in mice, resulting in values of 20, 30, and 60.5% vs. control values, 1 h post-dosing. With the agent, the duration of paw licking following intraplantar capsaicin injection decreased in a dose-dependent manner. In contrast, gabapentin failed to have antinociceptive action in a mouse acetic-acid-induced writhing assay. The drug (12.5-50 mg/kg) increased the duration of catalepsy induced by haloperidol by 33.5, 47.4, and 53.2%, respectively. It had an anti-inflammatory effect at doses of 25 or 50 mg/kg. Gabapentin (12.5-50 mg/kg) reduced the number and severity of gastric mucosal lesions induced by subcutaneous indomethacin (20 mg/kg) or intragastric 96% ethanol, but at doses of 50 and 100 mg/kg it increased gastric acid secretion. In conclusion, gabapentin decreased thermal, electrical, and chemogenic pain but not visceral pain and had a gastric protective effect.

Keywords: Gabapentin, Pain, Gastric lesions, Mice, Rat

1. Introduction

Gabapentin, 1-(aminomethyl)cyclohexane acetic acid, is a structural analog of γ-aminobutyric acid (GABA), which was initially introduced in 1994 as an antiepileptic drug used especially to treat partial seizures. Despite the fact that gabapentin is structurally related to the neurotransmitter GABA, there is no conclusive evidence yet that gabapentin blocks GABA uptake or metabolism, that it binds to GABAA or GABAB receptors, or that it has any GABA-mimetic action (1,2).

In experimental animal models of mechanical hyperalgesia and mechanical/thermal allodynia, gabapentin has been reported to have a potent inhibitory effect (3-7). For example, the drug decreased tactile hypersensitivity and mechanical and cold hypersensitivity due to spinal cord compression in rats or due to paclitaxel- and vincristine-administration (8-10), it attenuated the second phase of nociceptive responses in a formalin test (11), it lessened mechanical hypersensitivity induced by intraplantar capsaicin (12), and it reduced mechanical hypersensitivity in a model of varicella zoster virus-associated hypersensitivity (13).

In humans, gabapentin has become increasingly popular as a treatment for chronic neuropathic pain. Clinical studies have shown that gabapentin is an effective analgesic in different types of neuropathic pain syndromes such as diabetic neuropathy (14), postherpetic neuralgia (15), trigeminal neuralgia (16), painful neuropathy resulting from HIV infection (17), cancer pain (18), fibromyalgia (19), pain following a burn injury (20), and complex regional pain syndromes (21). Although the exact molecular mechanism of action by which gabapentin reduces pain is not yet known, evidence suggests that the α2δ1 auxilliary subunit of voltage-gated calcium channels are the target for this drug's actions (22).

The aim of the present study was to investigate the effect of gabapentin on acute nociception and acute inflammation induced in rats by subplantar carrageenan injection. In addition, the effects of the compound on haloperidol-induced catalepsy, gastric acid secretion, and gastric mucosal damage caused by indomethacin or ethanol were evaluated.

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2. Materials and Methods

2.1. Animals

Sprague-Dawley strain rats weighing 120-130 g or Swiss albino mice weighing 20-25 g of body weight were used and housed under standardized conditions at the National Research Centre, Cairo, Egypt with free access to food and water. Experiments were performed between 900 and 1,500 h. All animal procedures were performed in accordance with the Ethics Committee of the National Research Centre and followed the recommendations for the proper care and use of laboratory animals (NIH publication No. 85-23, revised 1985). Equal-sized groups of 6 rats or 6 mice were used in all experiments. Gabapentin doses in this study were based upon human groups of 6 rats or 6 mice were used in all experiments. Gabapentin doses in this study were based upon human doses after converting to those for rats according to Paget and Barnes conversion tables (23).

2.2. Drugs and reagents

Gabapentin (Delta Pharma, Cairo, Egypt), carrageenan, capsaicin (Sigma-Aldrich, St Louis, MO, USA), indomethacin, and haloperidol (Kahira Pharm & Chem. IND Co., Cairo, Egypt) were used. Stock solutions of capsaicin (10 mg/mL) contained 10% ethanol, 10% Tween 80, and 80% saline solution. Analytical-grade glacial acetic acid (Sigma-Aldrich) was diluted with pyrogen-free saline to provide a 0.6% solution for i.p. injection. All drugs were dissolved in isotonic (0.9% NaCl) saline solution immediately before use, except for indomethacin, which was dissolved in a 5% solution of sodium bicarbonate.

2.3. Hot-plate assay

A hot-plate test was performed using an electronically controlled hotplate (Ugo Basile, Comerio, Italy) heated to 53°C (± 0.1°C). Each mouse was placed unrestrained on the hot plate for baseline measurement just prior to saline or drug administration. Different groups of mice (n = 6/group) were given gabapentin (12.5, 25, 50 or 100 mg/kg, 0.2 mL, intraperitoneally) or saline (control). Measurements were then taken 30 and 60 min after drug administration. The experimenter was blind to doses. Latency to licking a hind paw or jumping from the apparatus was recorded for the control and drug-treated groups. The cut-off time was 30 sec.

2.4. Tail electric stimulation test

Groups of mice (n = 6/group) were given gabapentin (12.5, 25, 50 or 100 mg/kg, i.p.) or saline (control). The minimum current required to elicit vocalization upon electrical stimulation of the tail was determined for the control and drug-treated groups 2 h post-treatment (24).

2.5. Capsaicin-induced hind paw licking

Gabapentin (12.5, 25, 50 or 100 mg/kg, i.p.) or saline was administered 60 min before injection of capsaicin (1.6 μg/paw; 25 μL) under the skin of the dorsal surface of the right hind paw. Observation started after capsaicin injection and lasted for 5 min. The time the animal spent licking the injected paw was determined using a stopwatch (25).

2.6. Acetic acid-induced writhing

Separate groups of 6 mice each were administered a vehicle (saline) or gabapentin 12.5, 25, 50 or 100 mg/kg; 0.2 mL, orally or intraperitoneally (i.p.). After 60 min of oral administration or 30 min of i.p. administration of gabapentin, mice received an i.p. injection of 0.6% acetic acid (0.2 mL) (26). The number of abdominal constrictions over 30 min following acetic acid injection was noted for the control and drug-treated groups.

2.7. Carrageenan-induced paw edema

Paw edema was induced by sub-plantar injection of 100 μL of 1% sterile λ-carrageenan in saline into the right hind paw of rats (27). The contralateral paw received an equal volume of saline. Paw volume was determined immediately before carrageenan injection and at selected times thereafter using a plethysmometer (Ugo Basile). The edema component of inflammation was quantified by measuring the paw volume (mL) at zero time (before carrageenan injection) and at 1, 2, 3, and 4 h after carrageenan injection; this value was then compared to the pre-injection value for each animal. Edema was expressed as a percentage change from control (pre-drug, zero time) values. The effect of systemic administration of gabapentin (25, 50 or 100 mg/kg, s.c., 0.2 mL, n = 6/group) given 30 min. before induction of inflammation by subplantar carrageenan was studied. The control group of carrageenan-treated rats received an equal volume of saline 30 min. before subplantar carrageenan injection (n = 6 each).

In addition, the effect of concomitantly administered gabapentin and indomethacin on edema formation was investigated. Different groups of rats (n = 6 each) were administered indomethacin (20 mg/kg, s.c.) alone or concomitantly administered gabapentin (100 or 200 mg/kg, i.p.) 30 min before subplantar carrageenan. The control group of carrageenan-treated rats received an equal volume of saline (0.2 mL) before subplantar carrageenan injection (n = 6).

Electrical stimulation of the tail was applied by means of the Pulse generator 57800-001 (Eugo Basil EXT Unit) (Frequency 50 pulse/sec, shock duration 2 sec).

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2.8. Rotarod testing

Motor performance in mice was measured as the latency to falling from an accelerating rotarod located over plates connected to an automatic counter (Ugo Basile). Mice were trained to remain on a rotating rod for 2 min as the rod rotated toward the animal. After the 2-min training period, the mice were administered a vehicle (saline) or gabapentin (50 or 100 mg/kg, i.p.) and 30 min later placed on the rotating rod as it accelerated from 4 to 40 rpm over 5 min; the time they were able to remain on the accelerating rod was noted (28). The cutoff time was 600 sec. The time was measured from the start of the acceleration period. The test was repeated 2 h after vehicle or drug injection. Six animals were used per dose and for the controls.

2.9. Haloperidol-induced catalepsy

Catalepsy, defined as a reduced ability to initiate movement and a failure to correct posture, was measured with a bar test. Mice were positioned so that their hindquarters were on the bench and their forelimbs rested on a 1 cm diameter horizontal bar 4 cm above the bench. The length of time the mouse maintained this position was recorded with a stopwatch for a maximum of 180 sec. This procedure was performed 30 min after haloperidol (2 mg/kg, i.p.) administration (29). Gabapentin (12.5, 25 or 50 mg/kg, i.p.) was concomitantly administered with haloperidol. Mice were judged to be cataleptic if they maintained this position for 30 sec or more.

2.10. Haloperidol-induced locomotor impairment

Mice were administered saline, haloperidol (2 mg/kg, i.p.), or haloperidol + gabapentin (25, 50 or 100 mg/kg, i.p.). Thirty min after treatment with drugs or the vehicle, mice were individually placed into a 40 cm³ activity monitor equipped with photoelectric detectors and the total number of horizontal beam interruptions (spontaneous locomotor activity) was counted over a 6 min period for each animal.

2.11. Gastric ulcerogenic studies

Gastric mucosal damage was induced in rats by administration of indomethacin (20 mg/kg, 0.2 mL, s.c.). The effect of gabapentin (12.5, 25 or 50 mg/kg, i.p.) administered at the time of indomethacin injection was studied. Food and water were provided ad libitum. In other experiments, the effect of gabapentin (12.5, 25 or 50 mg/kg, i.p.) on gastric damage caused by ethanol was evaluated. Rats fasted for 18 h but were allowed water ad libitum. They were administered either saline (control) or different doses of gabapentin 30 min prior to ethanol (96%, 1 mL, p.o.). Rats were sacrificed 24 h after indomethacin or 1 h after ethanol administration. Their stomachs were removed and opened along the greater curvature; the stomachs were then rinsed with saline, extended on a plastic board, and examined for mucosal lesions. The number and severity of mucosal lesions were noted and lesions were scaled as described by Mózsik et al. (30).

2.12. Gastric acid secretion studies

Investigations were carried out in a pylorus-ligated rat model. Pylorus ligation was done under light ether anesthesia in rats that had fasted for 18 h with access to water ad libitum. Care was taken so as not to interfere with the blood supply to the stomach or duodenum. The abdominal wall was closed in layers with silk sutures. Rats then received either saline (0.2 mL/rat, s.c., n = 6) (control) or different doses of gabapentin (50, 100, or 200 mg/kg, 0.2 mL/rat, s.c., n = 6/group). Rats were sacrificed 4 h later. Gastric acid output was determined by titration to pH 7.0 with 0.01 N NaOH and H⁺ output expressed as μEq/4 h.

2.13. Statistical analysis

Data are expressed as mean ± SE. Data were analyzed by one-way analysis of variance, followed by a Tukey’s multiple range test for post hoc comparison of group means. When there were only two groups, a two-tailed Student’s t test was used. For all tests, effects with a probability of p < 0.05 were considered to be significant.

3. Results

3.1. Anti-nociceptive effects of gabapentin

3.1.1. Hot-plate assay

The reaction time on the hot plate was delayed by gabapentin. Gabapentin at doses of 12.5-100 mg/kg significantly increased hot-plate latency in the mouse hot-plate test. The anti-nociceptive effect of the drug was produced with a 12.5 mg/kg and a maximal increase in hot-plate latency of 68% was noted 1 h after drug administration (Figure 1, Table 1).

3.1.2. Tail electric stimulation test

Gabapentin (25, 50 or 100 mg/kg) produced a significant rise in electrical current threshold in the tail stimulation test in rats; this rise was 20, 30, and 60.5% vs. control values, 1 h post-drug (Table 2).

3.1.3. Capsaicin-induced hind paw licking

The duration of paw licking following intraplantar capsaicin injection was reduced by gabapentin in...
Table 1. Anti-nociceptive activity of gabapentin in the hot-plate test in mice

<table>
<thead>
<tr>
<th>Drugs</th>
<th>Latencies (sec) for nociceptive reaction after</th>
<th>% change</th>
<th>0 min (baseline)</th>
<th>30 min</th>
<th>1 h</th>
<th>30 min 1 h</th>
</tr>
</thead>
<tbody>
<tr>
<td>Saline</td>
<td>11.2 ± 0.66</td>
<td></td>
<td>12.65 ± 0.61</td>
<td>12.7 ± 1.2</td>
<td></td>
<td></td>
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<tr>
<td>Gabapentin</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>12.5 mg/kg</td>
<td>10.6 ± 0.90</td>
<td></td>
<td>13.0 ± 0.8</td>
<td>13.8 ± 1.0*</td>
<td>22.6</td>
<td>30.2</td>
</tr>
<tr>
<td>25 mg/kg</td>
<td>10.4 ± 0.59</td>
<td></td>
<td>13.1 ± 0.91*</td>
<td>15.8 ± 1.1*</td>
<td>26.0</td>
<td>51.9</td>
</tr>
<tr>
<td>50 mg/kg</td>
<td>10.5 ± 0.65</td>
<td></td>
<td>13.9 ± 1.1*</td>
<td>16.35 ± 1.0*</td>
<td>32.0</td>
<td>55.7</td>
</tr>
<tr>
<td>100 mg/kg</td>
<td>11.1 ± 0.8</td>
<td></td>
<td>17.15 ± 1.2*</td>
<td>18.65 ± 1.0*</td>
<td>54.5</td>
<td>68.0</td>
</tr>
</tbody>
</table>

Shown are baseline (0 time) and drug-induced (30 min and 1 h measurements) latencies (in seconds) for the nociceptive reaction. Data are expressed as means and S.E.M. (*n = 6/group). Asterisks indicate a significant increase in nociceptive latencies (*p < 0.05, **p < 0.01) compared to the baseline level of nociceptive reaction (Student’s t-test).

Table 2. Anti-nociceptive activity of gabapentin in the tail electric stimulation test in mice

<table>
<thead>
<tr>
<th>Drugs</th>
<th>Electric current threshold (μA)</th>
<th>% change</th>
<th>0 min (baseline)</th>
<th>30 min</th>
<th>1 h</th>
<th>30 min 1 h</th>
</tr>
</thead>
<tbody>
<tr>
<td>Saline</td>
<td>200.0 ± 6.0</td>
<td></td>
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<td></td>
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<tr>
<td>Gabapentin</td>
<td></td>
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<td></td>
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<tr>
<td>12.5 mg/kg</td>
<td>221.3 ± 18.3</td>
<td>10.6</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>25 mg/kg</td>
<td>240.3 ± 16.1*</td>
<td>20.2</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>50 mg/kg</td>
<td>260.0 ± 21.0</td>
<td>30.0</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>100 mg/kg</td>
<td>320.0 ± 20.0*</td>
<td>60.0</td>
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</tbody>
</table>

Shown are control and drug-induced changes for the nociceptive reaction. Data are expressed as means ± SE. (*n = 6/group). Significant rise in electrical current threshold (μA) (p < 0.05) compared to the saline control group (One-way ANOVA, Duncan test).

3.2. Anti-inflammatory effects of gabapentin

The administration of gabapentin inhibited carrageenan-induced paw edema (two-way ANOVA; treatment effect: F3,80 = 57.1; p < 0.001; time effect: F3,80 = 42.6; p < 0.001). Edema was significantly inhibited by all doses of gabapentin at all measured times (-26.2, -42.6, -34.6, and -34.9% for 25 mg/kg gabapentin vs. -26.2, -41.1, -36.6, and -35.6% for 50 mg/kg gabapentin and -16.7, -25, -22.1, and -22.2% for 100 mg/kg gabapentin at 1, 2, 3, and 4 h post-carrageenan, respectively) (Figure 3).

The group treated with the lower dose of the drug (25 mg/kg) exhibited significant suppression of edema compared to the group treated with 50 mg/kg 2 h post-carrageenan and compared to the group treated with 100 mg/kg at 2 h and 3 h after carrageenan injection, respectively (Figure 3). Therefore, the effect of concomitantly administered gabapentin at high doses of 100 or 200 mg/kg and indomethacin was examined for a possible modulating effect on indomethacin’s anti-inflammatory effect.

Concomitant administration of gabapentin with indomethacin suppressed paw edema at all measured times (-36.7, -42.4, -43.7, and -44.4% for indomethacin vs. -33.4, -32.3, -38.4, and -37.6% for indomethacin + 100 mg/kg gabapentin and -35.5, -39.2, -39, and -36.1% for indomethacin + 200 mg/kg gabapentin at 1, 2, 3, and 4 h, respectively) (Figure 4). Two-way ANOVA revealed a significant main effect of treatment (F3,80...
= 81.3; p < 0.001) and time (F3,80 = 37.6; p < 0.001).
Post hoc comparisons showed significant inhibition of edema formation by indomethacin or indomethacin + gabapentin at all measured times.

3.3. Rotarod testing

Gabapentin did not produce any significant changes in mice with regard to rotarod performance (data not shown).

3.4. Effect of gabapentin on the duration of haloperidol-induced catalepsy

Haloperidol administered i.p. at a dose of 2 mg/kg produced a significant cataleptic response. The duration of haloperidol-induced catalepsy significantly increased 33.5, 47.4, and 53.2% as a result of 12.5, 25, and 50 mg/kg of gabapentin, respectively (Figure 5).

3.5. Effect of gabapentin on haloperidol-induced locomotor impairment

Spontaneous motor activity was markedly and significantly reduced in haloperidol-treated mice. Treatment with gabapentin resulted in a further reduction in motor activity (Figure 6).

3.6. Effect of gabapentin on gastric mucosal lesions caused by indomethacin or ethanol

Gabapentin (12.5-100 mg/kg) administered at the time of indomethacin injection or 30 min prior to ethanol (96%) prevented the development of gastric lesions caused by either ulcerogen in a dose-dependent manner (Figures. 7-10).

3.7. Effect of gabapentin on gastric acid secretion

Gabapentin (50-200 mg/kg) administered at the time of pylorus ligation increased gastric acid secretion; this effect was most marked with doses of 50 and 100 mg/kg (Table 4).
The drug increased the nociceptive threshold and resulted in antinociceptive effects in different acute pain models. Data from the present study indicates that the systemic administration of gabapentin on acute nociceptive pain. The aim of the present study was to investigate the effect of gabapentin neuronal sensitization and nerve injury. The antiallodynic effects of gabapentin in models involving cyclophosphamide-induced cystitis in mice, a model of visceral pain.

4. Discussion

Studies have demonstrated the analgesic and antiallodynic effects of gabapentin in models involving neuronal sensitization and nerve injury. The aim of the present study was to investigate the effect of gabapentin on acute nociceptive pain. Data from the present study indicates that the systemic administration of gabapentin resulted in antinociceptive effects in different acute pain models. The drug increased the nociceptive threshold to thermal or electrical stimuli. In addition, chemogenic pain behavior induced by capsaicin injection in mice was markedly reduced by gabapentin. Other researchers have noted a reduction in mechanical hypersensitivity induced by intraplantar capsaicin with gabapentin (31). At doses that caused effective anti-nociception, the drug did not impair mouse performance as evaluated by the rotarod test, thus ruling out the confounding influence of a possible sedative effect. In the writhing test in mice, a widely used model of visceral inflammatory pain that involves the local release of prostacyclin (32), gabapentin administered via oral or systemic routes failed to alter the number of abdominal constrictions induced by acetic acid injection into the peritoneal cavity. Gabapentin (56 mg/kg) also had no effect on cyclophosphamide-induced cystitis in mice, a model of visceral pain (33). Other investigators, however, have reported a reduction in the number of writhes as a result of gabapentin (10-70 mg/kg, p.o.) (34).

Gabapentin is thought to act at supraspinal and intraspinal sites to induce antinociceptive responses.
Gabapentin has been shown to modulate brain c-Fos expression in surgical paw incision and to attenuate acute morphine-induced c-Fos expression in the rat striatum (37,38). The activation of brain areas involved in nociceptive processing indicates a supraspinal site of action for gabapentin (36). In the present study, the drug increased latency in hotplate tests, which are reported to detect antinociception mediated primarily by supraspinal mechanisms (39). The mechanism of the analgesic action of gabapentin is not known, but evidence suggest that the α2δ1 auxiliary subunit of voltage-gated calcium channels serves the target for the drug’s actions (22), but acute inhibition of calcium currents by the drug is either very minor or absent (40). The analgesic effects of gabapentin might also involve inhibition of spinal release of substance P and CGRP (41) or of glutamate (42). There is also evidence to suggest that the antihyperalgesic and antiallodynic effects of gabapentin are mediated substantially by the descending noradrenergic system, resulting in the activation of spinal α2-adrenergic receptors (35).

In the present study, gabapentin also reduced the inflammatory edematogenic response to subplantar carrageenan injection, with the lower doses of 25 and 50 mg/kg being more effective than the higher dose of 100 mg/kg. The effect of gabapentin was less than that of indomethacin. Gabapentin at high doses, however, is unlikely to affect the anti-inflammatory effect of non-steroidal anti-inflammatory drugs e.g., indomethacin.

Catalepsy occurs following high dopamine D2 receptor blockade by a typical antipsychotic drug like haloperidol (29). Haloperidol-induced catalepsy is a behavioral predictor of susceptibility to extrapyramidal symptoms (43). In the present study, gabapentin increased the duration of catalepsy in a dose-related manner. In patients with advanced Parkinsonism, improvement (though non-significant) of rigidity, bradykinesia, and tremors was noted (44). Other researchers have found that gabapentin improved Parkinsonian symptoms and motor response following levodopa, although this improvement was not reflected in the daily motor status of patients. Levodopa-induced dyskinesias remained unchanged (45). Improvement in antipsychotic-induced akathisia upon treatment with gabapentin has also been reported (46). In patients on gabapentin treatment, somnolence and dizziness are observed side effects (47), but there are recent reports of dyskinesia (48) or even hemichorea (49) caused by gabapentin, and this issue warrants further study. Since there is no clear evidence that gabapentin has GABA-mimetic action (1,2), gabapentin’s effects on extrapyramidal motor symptoms are unlikely to be mediated via the GABA neurotransmitter system. Spinal cholinergic activation was reported after oral administration of gabapentin in rats (50). Since muscarinic acetylcholine receptor antagonists may contribute to the reduction of catalepsy (43), gabapentin’s effect on striatal cholinergic or other neurotransmitter systems may account for the accentuation of haloperidol catalepsy following gabapentin in the present study.

Findings of the present study also indicate that gabapentin has a gastric protective effect. Acute gastric mucosal lesions induced by indomethacin or ethanol in the rat decreased in a dose-dependent manner as a result of concomitant administration. Gabapentin increased gastric acid secretion in pylorus-ligated rats, indicating that its gastric mucosal protective properties are unlikely to include an effect on gastric acid.

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