Exacerbation of Indomethacin-Induced Gastric Ulceration by Systemically Administered GABA in Rats: Possible Involvement of Peripheral GABA Receptors

Shuichi HARA, Satoshi YANAGIHARA, Kazunori SATOH and Shigeo MORIOKA
Pharmacological Research Laboratory, Sato Pharmaceutical Co., Ltd.,
1468 Hazama, Hachioji, Tokyo 193, Japan
Accepted April 14, 1988

Abstract—Effects of systemically administered γ-aminobutyric acid (GABA) on indomethacin-induced gastric ulceration were studied in rats. Orally administered GABA significantly exacerbated the ulceration in a dose-dependent manner, although GABA per se had no ulcerogenic activity. The exacerbation was restored by GABA receptor antagonists, bicuculline methiodide, picrotoxin and pentylene-tetrazol. Pretreatment with atropine sulfate antagonized the exacerbating effect of GABA on indomethacin-induced ulceration. 3-Amino-1-propanesulfonic acid, but not glycine, taurine or β-alanine, mimicked the effect of GABA on the ulceration, which was inhibited by picrotoxin. Muscimol and (−)-baclofen could not potentiate the ulceration. However, sodium pentobarbital and diazepam caused synergistic exacerbation of the ulcer when combined with GABA. Since it is known that systemically administered GABA cannot penetrate into the brain, these results suggest that systemically administered GABA may stimulate the cholinergic transmission mediating the activation of peripheral GABA receptors, resulting in the exacerbation of indomethacin-induced ulceration.

Bhargava et al. (1) demonstrated that intracerebroventricularly injected γ-aminobutyric acid (GABA) and muscimol caused marked reduction of the stress-induced gastric ulceration which was sensitive to the GABA antagonists, bicuculline and picrotoxin. This is supported by the observation that centrally given bicuculline enhanced the gastric motility which was restored by the injection of muscimol into the same area (2). It has also been reported that systemic administration of GABA alleviates the chronic ulceration evoked by acetic acid (3) as well as the stress-induced ulceration (4). In addition, Lloyd et al. (3) demonstrated that systemic administration of GABA mimetics protected the ulceration induced by restraint stress, phenylbutazone, ethanol and pyloric ligation, although baclofen had complicated effects on these ulcer models. These findings suggest that GABA and its agonists possess antiulcer activity when administered systematically as well as centrally, although complicated results were reported in the experimental ulcer models when the synthesis and metabolism of intrinsic GABA were inhibited by semicarbazide and aminooxyacetic acid, respectively (5). However, an in vitro study revealed the induction of gastrin release and the reduction of somatostatin release by GABA which could be antagonized by bicuculline (6). In addition, other evidence obtained in vitro demonstrated that GABA enhanced gastric acid output and motility (7), indicating that GABA would stimulate gastric functions. Thus, it seems that systemic administration of GABA alleviates gastric ulcers in spite of its activating effects on the gastric functions.

In the present study, however, we demonstrated the potentiation of indomethacin-induced gastric ulceration by systemically administered GABA and discuss the possible role(s) of peripheral GABA receptors in the
Materials and Methods

Animals: Male Sprague-Dawley rats, weighing 170–230 g, were maintained in an air-conditioned (22–24°C) room and a 12 hr light-dark cycle. Animals were allowed access to food and water ad libitum except food was withheld for 24 hr before the administration of indomethacin. After the drug administration, starvation was continued and water was withheld until the time of killing.

Drugs: Indomethacin (IDM), picrotoxin (PIX), bicuculline methiodide (BIC), pentylentetrazol (PTZ), muscimol and atropine sulfate (ATR) were purchased from Sigma Chemical Co., St. Louis, MO, U.S.A. \textit{\alpha}-Aminobutyric acid (GABA), glycine (Gly), taurine (Tau) and sodium pentobarbital were from Tokyo Kasei Kogyo Co., Tokyo, Japan. \(\beta\)-Alanine (\(\beta\)-Ala) and diazepam were from Wako Pure Chemical Co., Tokyo, Japan. 3-Amino-1-propanesulfonic acid (3-APS) was obtained from Janssen Chimica, Beerse, Belgium. (-)-Baclofen hydrochloride was kindly given to us by Dr. H. Schr"{o}ter and Dr. K. Scheibli, Ciba-Geigy, Ltd., Basel, Switzerland.

Drug treatments: IDM was suspended in 5% arabic gum solution at a dose of 20 mg/kg and was administered immediately before the treatment with GABA which was dissolved in distilled water. Both IDM and GABA were orally given, unless otherwise indicated. Gly, Tau, \(\beta\)-Ala, 3-APS, muscimol and (-)-baclofen were dissolved in distilled water and orally administered immediately after IDM. BIC (2 mg/kg), PIX (2 mg/kg), PTZ (20 mg/kg), diazepam (0.1 mg/kg), sodium pentobarbital (5 mg/kg) and ATR (1 mg/kg) were injected intraperitoneally. BIC, PIX and PTZ were administered immediately, 1 hr and 2 hr after IDM. Diazepam and sodium pentobarbital were given immediately after IDM. ATR was pretreated 30 min before IDM. Drugs given intraperitoneally were dissolved in isotonic saline except diazepam which was suspended in 5% arabic gum solution. The dosage volume of all drugs was 5 ml/kg. When drugs were omitted, the corresponding vehicles were administered.

Determination of ulcer index in rat stomach: Determination of the ulcer index in rat stomach was performed by the procedure previously reported by Okabe et al. (8), with minor modification. Rats were killed 3 or 7 hr after the treatment with indomethacin, except for in the time course study (Fig. 2). The stomach was removed, inflated by the injection of 8 ml of 2% formalin solution, and then immersed in the solution. After the fixation with formalin solution, the stomach was incised along the greater curvature and carefully rinsed with tap water. The length (mm) of all lesions was measured and the sum of the length was regarded as an ulcer index, indicating the severity of the ulceration.

Statistical analyses: Student's \(t\)-test was used for the comparison between two groups. When the antagonistic or synergistic effects were analyzed, two-way analysis of variance was employed.

Results

Exacerbation of IDM-induced ulceration by GABA: The indices of IDM-induced gastric ulcer 7 hr after the drug were increased in parallel with increasing doses of GABA, and they were significantly increased at the doses of 300 and 500 mg/kg of GABA as compared with those without GABA (Fig. 1). When
GABA alone at doses of 300 and 500 mg/kg was administered orally, the ulcer indices (0.4±0.1 and 0.2±0.1, respectively) were comparable to those in the animals treated with the vehicle alone (0.3±0.2). Orally given GABA significantly increased the ulcer index from 1.9±0.6 to 11.4±1.8 at 7 hr after intraperitoneal injection of IDM. Furthermore, the index induced by oral administration of IDM was also enhanced from 9.8±2.4 to 23.3±3.0 by GABA given intraperitoneally. These results would rule out the participation of chemical interaction between IDM and GABA by simultaneous oral administration or direct irritation of the stomach by GABA in the potentiation of the ulceration. Figure 2 shows the time course study of the effect of GABA (300 mg/kg) on IDM-induced ulceration. In contrast to the fact that the index in the rat treated with IDM and the vehicle of GABA had reached the plateau level 5 hr after IDM, that in combination with GABA was further increased.

**Effects of PIX, BIC and PTZ on the potentiation of IDM-induced ulceration by GABA:** Figure 3 (A–C) shows the effects of three GABA antagonists, PIX, BIC and PTZ, on the exacerbation of IDM-induced gastric ulcer by GABA (300 mg/kg). In these experiments, the animals received each antagonist immediately, at 1 hr and at 2 hr after the administration of IDM in order to obtain a continuous blockade of the GABA-ergic system, which was referred to as the prolonged effect of GABA (Fig. 2) and brief duration of efficacy of PIX as well as PTZ (9). These three antagonists completely abolished the exacerbation of IDM-induced ulceration by GABA, although they *per se* did not change the IDM-induced ulcer index without GABA.

**Effects of Gly, Tau, β-Ala and 3-APS on IDM-induced ulceration:** Oral administrations of Gly, Tau and β-Ala at doses of 218.4, 364.1 and 259.2 mg/kg, which corresponded to
300 mg/kg of GABA on a molar basis, did not significantly change the index of IDM-induced ulcer 3 hr after the administration of the drug (Fig. 4).

On the other hand, when 3-APS at a dose of 404.9 mg/kg, corresponding to 300 mg/kg of GABA on a molar basis, was administered, IDM-induced ulceration was significantly exacerbated, and this potentiation was antagonized by PIX (Fig. 5).

**Effects of muscimol and (-)-baclofen on IDM-induced ulceration:** Although muscimol did not alter the ulceration at doses up to 2 mg/kg, it significantly decreased the ulcer index at 5 mg/kg (Table 1). (-)-Baclofen at doses of 0.5 and 5 mg/kg failed to affect the ulceration (Table 1).

**Synergistic exacerbation of GABA in combination with sodium pentobarbital or diazepam on IDM-induced ulceration:** Although oral administration of GABA at a dose of 20 mg/kg was not effective on IDM-induced ulceration, the ulcer index was increased when combined with sodium pentobarbital which did not show any change in IDM-induced ulceration (Table 2). Statistical analysis using analysis of variance revealed that the increment of the index was syner-

---

**Table 1. Effects of muscimol and (-)-baclofen on the ulcer index after the administration of IDM in rats**

<table>
<thead>
<tr>
<th>Dose (mg/kg)</th>
<th>Muscimol</th>
<th>(-)-Baclofen</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>4.7±0.4 (16)</td>
<td>5.2±0.7 (6)</td>
</tr>
<tr>
<td>0.5</td>
<td>4.2±0.9 (5)</td>
<td>5.6±1.1 (6)</td>
</tr>
<tr>
<td>1.0</td>
<td>5.9±0.7 (5)</td>
<td>—</td>
</tr>
<tr>
<td>1.5</td>
<td>7.1±3.0 (6)</td>
<td>—</td>
</tr>
<tr>
<td>2.0</td>
<td>5.6±0.7 (6)</td>
<td>—</td>
</tr>
<tr>
<td>5.0</td>
<td>2.8±0.5* (5)</td>
<td>4.3±0.3 (6)</td>
</tr>
</tbody>
</table>

The ulcer index was determined 3 hr after IDM and was expressed as the mean±S.E. Figures in parentheses represent the number of animals used. * indicates significant (P<0.05) difference from the group without muscimol.
Another synergistic potentiation was found by the combination with diazepam (Table 2).

**Effect of the pretreatment with ATR on the exacerbation of IDM-induced ulceration by GABA:** As shown in Fig. 6, pretreatment with ATR at a dose of 1 mg/kg significantly decreased the ulcer index after the administration of IDM as previously reported by Kasuya et al. (10). ATR pretreatment completely eliminated the aggravating effect of GABA on indomethacin-induced ulceration.

**Table 2.** Effects of GABA in combination with sodium pentobarbital or diazepam on IDM-induced gastric ulceration in rats

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Ulcer index</th>
<th>%</th>
<th>n</th>
</tr>
</thead>
<tbody>
<tr>
<td>IDM</td>
<td>3.5±0.3</td>
<td>100</td>
<td>6</td>
</tr>
<tr>
<td>IDM +GABA</td>
<td>4.5±1.0</td>
<td>130</td>
<td>6</td>
</tr>
<tr>
<td>IDM +Pentobarbital</td>
<td>3.8±0.4</td>
<td>104</td>
<td>6</td>
</tr>
<tr>
<td>IDM +GABA + Pentobarbital</td>
<td>9.3±1.8</td>
<td>266</td>
<td>6</td>
</tr>
<tr>
<td>IDM</td>
<td>4.4±0.5</td>
<td>100</td>
<td>12</td>
</tr>
<tr>
<td>IDM +GABA</td>
<td>5.7±1.0</td>
<td>130</td>
<td>12</td>
</tr>
<tr>
<td>IDM +Diazepam</td>
<td>4.8±0.4</td>
<td>109</td>
<td>12</td>
</tr>
<tr>
<td>IDM +GABA + Diazepam</td>
<td>9.9±1.2</td>
<td>255</td>
<td>12</td>
</tr>
</tbody>
</table>

The ulcer index was determined 3 hr after IDM and was expressed as the mean±S.E. Interactions by analysis of variance: GABA×Pentobarbital, F(1,20)=4.46, P<0.05; GABA×Diazepam, F(1,44)=5.07, P<0.05.

**Fig. 6.** Effect of ATR on the exacerbation of IDM-induced ulceration by GABA. GABA was administered at a dose of 300 mg/kg. The ulcer index was determined 3 hr after IDM. Each column with vertical bar represents the mean±S.E. obtained from eight animals. * and ** indicate significant (P<0.05 and P<0.01, respectively) difference, as compared with the group treated with IDM and the vehicles. Interaction between GABA and ATR: F(1,28)=21.30, P<0.01.

**Discussion**

It has been suggested that GABA has the ability to alleviate the chronic ulcer model elicited by acetic acid when injected intramuscularly (3). Minano et al. (4) also reported the reduction of stress-induced gastric ulcer by GABA administered orally as well as intraperitoneally in guinea pigs. In contrast, the present study demonstrated that oral administration of GABA could increase the index of IDM-induced ulcer in a dose-dependent manner (Fig. 1). However, GABA itself at the doses used had no ulcerogenic activity, which was in agreement with the report of Lloyd et al. (3). A time course study on the indices of IDM-induced ulcer showed that the ulcer index had been significantly increased 3 hr after IDM by GABA treatment. The enhancement was further continued until 7 hr after the dose in contrast to the fact that the ulcer index induced by IDM, but without GABA, had reached a plateau at 5 hr (Fig. 2).

As shown in Fig. 3, PIX, BIC and PTZ could abolish the aggravating effect of GABA on IDM-induced ulceration although they per se did not alter the ulceration. It was elucidated that bicuculline antagonizes GABA-ergic transmission by direct binding to GABA®
receptor and PIX reduces GABA-ergic activity through blockade of chloride-ionophore coupled with GABA_4 receptor (11, 12). Although a relationship between PTZ-induced convolution and the central monoaminergic systems has been documented (13–16), it was recently demonstrated that the convulsant potencies of PTZ and its derivatives were closely related to the binding abilities to the benzodiazepine-GABA receptor-ionophore complex (17).

Although putative glycinergic agonists such as Gly, Tau and β-Ala (18) were challenged at the doses corresponding to 300 mg/kg of GABA on a molar basis, they could not significantly change the ulceration caused by IDM (Fig. 4). In contrast, 3-APS, a GABA_4 receptor agonist (19–22), significantly exacerbated IDM-induced gastric ulcer (Fig. 5). This potentiated ulceration was restored to the level of the group treated with IDM without GABA by PIX (Fig. 5). However, both muscimol and (-)-baclofen, agonists of GABA_4 and GABA_6 receptors, respectively, failed to enhance IDM-induced ulceration (Table 1).

On the other hand, the exacerbating effect of GABA on IDM-induced ulceration could be synergistically potentiated by the combined treatment with sodium pentobarbital or diazepam (Table 2), both which potentiate the transmitter output by mediating the activations of chloride-ionophore and benzodiazepine receptors, respectively, coupled with GABA_4 receptor in the central nervous system (11, 12).

These findings suggest that the stimulation of GABA_4 receptors could be associated with the exacerbation of IDM-induced ulceration in rats. In addition, it seems that the administration of GABA may activate the cholinergic system, since the effect of GABA disappeared by the pretreatment with an anticholinergic agent, ATR (Fig. 6).

Evidence has been provided that the GABA-ergic neural system is distributed in the peripheral nervous system, particularly in the myenteric plexus (23–26), where activations of GABA_4 and GABA_6 receptors elicit contraction and relaxation, respectively, in the intestinal muscle by modulating the cholinergic transmission (19, 21, 22). As for the actions of GABA on the stomach, Tsai et al. (7) reported that GABA and muscimol, but not baclofen, caused acid secretion and contraction in vitro which could be inhibited by bicuculline and scopolamine. Harty and Franklin (6) demonstrated the induction of gastrin release and the reduction of somatostatin release after the addition of GABA to an antral mucosal fragment, which disappeared in the presence of bicuculline. In addition, the induction of gastrin release was inhibited and potentiated by atropine and eserine, respectively (27). These suggest the existence of GABA receptors in the stomach which might activate gastric functions via cholinergic stimulation. In contrast to the reports on in vitro studies, it was suggested that the central GABA-ergic system would play an inhibitory role in the gastric functions because central administration of GABA not only restored the stimulation of gastric motility produced by bicuculline (2) but also protected against stress-induced ulcer (1). Therefore, it seems that the peripheral GABA-ergic system may play different roles from the central one in the regulation of the gastric functions; i.e., the activation of the former and the latter may lead to excitatory and inhibitory effects, respectively. This might be interpreted as muscimol poorly mimicking the exacerbation of IDM-induced ulceration by GABA, although it remains unclear whether the central effects after systemic administration of muscimol are specifically mediated by the GABA-ergic system (28).

In contrast, it is well elucidated that systemically administered GABA poorly penetrates the blood-brain barrier both in normal and aminooxyacetic acid-treated animals (29, 30). Furthermore, Zeneroli et al. (31) reported that the uptake of exogeneous GABA to the brain was not changed even in the rats treated with galactosamine which caused an enhanced permeability of the blood-brain barrier.

From these reports, it is concluded that systemically administered GABA may stimulate the cholinergic system mediated through the activation of peripheral GABA_4 receptor, resulting in the potentiation of IDM-induced ulceration. From this study, we can not interpret the discrepancy between the present
results and the previous reports indicating the protective effects of GABA in the ulcer models (3, 4). However, it might be due to the difference of the ulcer models since complicated effects of the GABA-ergic system on the experimental ulcer models have been reported (5). In addition, the physiological role(s) of the GABA-ergic system on the gastric functions remains unclear since the antagonists per se could not cause any changes in MDMA-induced ulceration, which is similar to the report of Harty and Franklin (6) that bicuculline did not change gastric response despite the antagonism against the effects of exogenous GABA.

Acknowledgments: We would like to thank Miss Ayako Kubota and Miss Reiko Watanabe for technical assistance.

References


23 Jessen, K.R., Mirsky, R., Dennison, M.E. and Burnstock, G.: GABA may be a neurotransmitter in the vertebrate peripheral nervous system. Nature 281, 71-74 (1979)


