Effect of Zaldaride Maleate, an Antidiarrheal Compound, on Visceral Pain Reflex Induced by Small Intestinal Distention in Anesthetized Rats

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ABSTRACT—Using distention of the small intestine as a visceral pain model, we investigated the effect of zaldaride maleate (ZAL), a selective inhibitor of calmodulin, on the depressor response. In pentobarbital-anesthetized rats, small intestine distention was induced by rapid application of intraluminal pressures of 40 cmH₂O causing a reflex fall in arterial blood pressure. The depressor response to intestinal distention was abolished by intraperitoneal administration of capsaicin (5 mg/rat), which depletes neuropeptides such as substance P from the sensory neurons, on the mesenteric stalk and by neonatal pretreatment with capsaicin (50 mg/kg, s.c.). Morphine (20 mg/kg, s.c.) reduced the depressor response following intestinal distention. At doses of 3 mg/kg (i.v.) and higher, ZAL significantly reduced depressor response. The effect of morphine was reversed by naloxone (5 mg/kg, i.v.); the effect of ZAL was not affected. These results suggest that ZAL helps reduce the visceral pain induced by noxious stimulus and that the antinociceptive effect of ZAL is not mediated by opioid receptors.

Keywords: Zaldaride maleate, Visceral pain, Calmodulin, Intestinal distention

Irritable bowel syndrome (IBS) is widely accepted as a gastrointestinal dysfunction. The principal clinical signs of IBS are diarrhea and/or constipation with abdominal pain. In patients with IBS, abdominal pain induced by the distention of the colon occurs at lower sensory thresholds than that in healthy volunteers (1). The distention of the duodenum (2), jejunum (3), ileum (4) or the colon (5) in anesthetized rats causes a decrease in arterial blood pressure. These cardiovascular responses are inhibited by morphine, an opioid analgesic. It is considered that the depressor response following intestinal distention is a visceral pain response. The depressor response to intestinal distention in anesthetized rats is useful for evaluating the action of compounds on visceral pain because this animal model quantifies the effects of the compounds for visceral nociception or visceral hyperalgesia.

Zaldaride maleate (ZAL), 1,3-dihydro-1-[1-[(4-methyl-4H,6H-pyrrolo[1,2-a][4,1]-benzoxazepine-4-yl)methyl]-4-piperinyl]-2H-benzimidazol-2-one-maleate, is a highly selective and potent inhibitor of calmodulin (CaM) (6), and it has been reported to alleviate secretory diarrhea without compromising gastrointestinal propulsion in mice and rats. In addition, at the doses studied, ZAL has little adverse effect on the central nervous system (7, 8). ZAL has been shown to reduce the severity and duration of traveler’s diarrhea (9, 10). Loperamide hydrochloride, an antidiarrheal drug, has been shown to have an antinociceptive effect on acetic acid-induced writhing in mice (11). Whether ZAL reduces the abdominal pain accompanied by the inflammatory and functional bowel disorders such as IBS, nonulcer dyspepsia and noncardiac chest pain remains unclear. In the present study, we examined the effect of ZAL on the depressor response induced by the noxious distention of the small intestine as a visceral pain in pentobarbital-anesthetized rats.

MATERIALS AND METHODS

Experimental animals

Male Sprague-Dawley rats (Charles River, Atsugi) weighing 250–450 g were used in the present study. They were housed in a controlled environment at a temperature of 22–24°C and humidity of 50–60% with light from 7:00 a.m. to 7:00 p.m. Commercial rat chow and water were given ad libitum prior to the study. The present study was conducted in compliance with the Guiding
Principles for the Care and Use of Laboratory Animals approved by The Japanese Pharmacological Society, and the experimental protocols were approved by the Ethical Committee of the Pharmaceutical Research Institute, Kyowa Hakko Kogyo Co., Ltd.

Materials

The following compounds were used: ZAL (Novartis Consumer Health, Inc., Nyon, Switzerland); atropine sulfate (Nacalai Tesque, Co., Inc., Kyoto); phentolamine (Sigma Chemical Co., Inc., St. Louis, MO, USA); morphine hydrochloride (Shionogi & Co., Ltd., Tokyo); sodium pentobarbital (Diichi Seiyaku Co., Ltd., Osaka); capsaicin, naloxone, papaverine and trifluoperazine dimaleate (Wako Pure Chemical Industries Co., Ltd., Osaka). Morphine was dissolved in a saline solution. Capsaicin was suspended in a saline solution of 10 vol% ethanol and 10 vol% Tween 80, and other reagents were dissolved in a saline solution consisting of 10 vol% dimethylsulfoxide.

Systemic capsaicin desensitization

Newborn rats were pretreated with capsaicin (50 mg/kg, s.c.) on the second day of life, as previously described (12). Control animals were pretreated with equal volumes of saline solution containing 10 vol% ethanol and 10 vol% Tween 80.

Methods

The visceral pain reflex was evaluated using a model based on a previously described method (3). Rats were fasted 18–20 h before the experiments, but were offered drinking water ad libitum. Animals were anesthetized by sodium pentobarbital (60 mg/kg, i.p.), and the small intestine was exposed by a midline incision of the abdomen. A 10-cm ligature of the small intestine was performed beginning 5 cm from the pylorus. The distal end was cannulated with tubing connected to a bottle containing Tyrode solution that was kept 40 cm above the animal. The Tyrode solution was maintained at 37°C, and its composition was as follows: 136.9 mM NaCl, 2.7 mM KCl, 1.8 mM CaCl₂, 1.0 mM MgCl₂, 0.4 mM Na₂HPO₄, 11.9 mM NaHCO₃ and 5.6 mM glucose. During the experiments, rats were maintained at 37°C with a heating pad. The right jugular vein and left carotid artery were catheterized for injecting the test compounds and monitoring the arterial blood pressure, respectively. The trachea was cannulated to maintain a clear airway. Anesthesia was maintained by intravenous injection of sodium pentobarbital (1 mg/kg) as needed.

After a 30-min equilibration period, the intraluminal pressure was rapidly increased by opening the inflow valve to the Tyrode solution, and 30 s later, intraluminal pressure was reduced to normal by opening the outflow valve. This procedure was repeated at 10-min intervals until a constant depressor response was obtained. Rats were rejected from the experiments if the depressor response was not obtained or was too weak. The depressor response to intestinal distention was measured at 30 min after administering the compounds and also at 45 min when naloxone was administered at 35 min. Control animals were administered saline solution of 10 vol% dimethylsulfoxide.

Capsaicin (5 mg/rat) was administered intraperitoneally to the rat, and the abdomen was carefully closed using forceps. Thirty minutes later, the depressor response to intestinal distention was measured. Control animals were administered saline solution of 10 vol% ethanol and 10 vol% Tween 80.

In the capsaicin- or control-vehicle-pretreated neonatal rats, the depressor response to intestinal distention was measured at 30 min after the experimental procedure. The antinociceptive effect of the test compounds was shown as follows:

\[
\% \text{ Nociceptive response} = \frac{\text{depressor response after compound or vehicle administration (mmHg)}}{\text{depressor response before compound or vehicle administration (mmHg)}} \times 100
\]

Statistical analyses

Each value is expressed as the mean ± S.E.M. for the groups of experiments. Statistical significance between the control vehicle group and the test compound group was analyzed by Student's t-test, paired t-test or the one-way analysis of variance (one-way ANOVA) followed by the Dunnett's test. Significance between the prevalue and postvalue of naloxone was analyzed by Student's t-test. A P-value less than 0.05 was considered statistically significant.

RESULTS

In the preliminary experiments, the depressor response induced by small intestine distention was evoked intraluminally, which was dependent on intraluminal pressure (Fig. 1). The increase in intraluminal pressure (40 cm H₂O) was followed by a fall in arterial blood pressure to 31 ± 1 mmHg (mean ± S.E.M. of 3 animals) (Fig. 1).

Effect of capsaicin on depressor response induced by intestinal distention

In pentobarbital-anesthetized rats pretreated neonatally with capsaicin (50 mg/kg, s.c.), a slight rise in arterial blood pressure induced by the intestine distention was observed; in contrast, the intestinal distention in the vehicle-treated rats evoked a drop in arterial blood pres-
Fig. 1. Relation between the increase in intraluminal pressure and the magnitude of the depressor response in anesthetized rats. Each value represents the mean of 3 experiments. ●: the depressor response to intestinal distention. ○: intraluminal pressure.

Fig. 2. Effect of capsaicin on depressor or nociceptive response induced by small intestine distention in pentobarbital-anesthetized rats. Each column with a bar represents the mean ± S.E.M. of 5 animals. Upper: capsaicin (50 mg/kg, s.c.) was administered neonatally to rats. Lower: capsaicin (5 mg/rat) was administered intraperitoneally. **P < 0.01: statistically significant vs the control vehicle group (Student’s t-test).

Effects of phenotolamine, atropine and papaverine on depressor response induced by intestinal distention

Phenotolamine (1 mg/kg, i.v.) and atropine (5 mg/kg, i.v.) slightly reduced the depressor response induced by intestinal distention (Fig. 3). Papaverine (3 mg/kg, i.v.) slightly increased the depressor response to intestinal distention (Fig. 3). Phenotolamine (1 mg/kg, i.v.) significantly decreased the basal blood pressure, whereas atropine (5 mg/kg, i.v.), papaverine (3 mg/kg, i.v.) and vehicle had no effect on it (Table 1).

Effect of ZAL on depressor response induced by intestinal distention

At doses of 3 mg/kg (i.v.) and higher, ZAL significantly reduced the depressor response to intestinal distention (Fig. 4). The depressor response induced by intestinal distention was significantly reduced by trifluoperazine at a dose of 10 mg/kg (i.v.) (Fig. 4).

ZAL (3 and 10 mg/kg, i.v.) significantly decreased basal blood pressure (Table 1). Trifluoperazine (10 mg/kg, i.v.) also significantly decreased basal blood pressure (Fig. 4).
Table 1. Effect of each compound on basal arterial blood pressure in anesthetized rats

<table>
<thead>
<tr>
<th>Compounds</th>
<th>mg/kg (i.v.)</th>
<th>N</th>
<th>Basal blood pressure (mmHg)</th>
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<tbody>
<tr>
<td></td>
<td></td>
<td>Pre</td>
<td>Post</td>
</tr>
<tr>
<td>Vehicle</td>
<td>6</td>
<td>105±4</td>
<td>110±5</td>
</tr>
<tr>
<td>Atropine</td>
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<tr>
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<td>6</td>
<td>112±6</td>
</tr>
<tr>
<td>Papaverine</td>
<td>3</td>
<td>5</td>
<td>113±3</td>
</tr>
<tr>
<td>Vehicle</td>
<td>11</td>
<td>107±4</td>
<td>110±3</td>
</tr>
<tr>
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<td>5</td>
<td>115±9</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>6</td>
<td>112±6</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>5</td>
<td>124±5</td>
</tr>
<tr>
<td></td>
<td>6</td>
<td>5</td>
<td>110±5</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>5</td>
<td>121±4</td>
</tr>
</tbody>
</table>

Each value represents the mean±S.E.M. for each group of experiments. Pre: basal arterial blood pressure before administration of each compound. Post: basal arterial blood pressure 30 min after administration of each compound. *P<0.05, **P<0.01: statistically significant vs the basal arterial blood pressure before administration (Paired t-test). ZAL: zaldaride maleate. N: number of animals.

Fig. 4. Effect of ZAL on nociceptive response induced by small intestine distention in pentobarbital-anesthetized rats. Each column with a bar represents the mean±S.E.M. of 5 to 11 animals. *P<0.05, **P<0.01: statistically significant vs the control vehicle group (one-way ANOVA + Dunnett’s test or Student’s t-test). ZAL: zaldaride maleate. TFP: trifluoperazine.

Fig. 5. Effect of naloxone on depressor response induced by small intestine distention after treatment with ZAL and morphine in pentobarbital-anesthetized rats. Each column with a bar represents the mean±S.E.M. of 5 or 6 animals. **P<0.01: statistically significant vs a 15-min trial (paired t-test). ZAL: zaldaride maleate.

Table 2. Effects of ZAL, naloxone and morphine on basal blood pressure in anesthetized rats

<table>
<thead>
<tr>
<th></th>
<th>Basal blood pressure (mmHg)</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>0 min</td>
</tr>
<tr>
<td>Morphine</td>
<td>Vehicle 6</td>
</tr>
<tr>
<td></td>
<td>Naloxone 6</td>
</tr>
<tr>
<td>ZAL</td>
<td>Vehicle 5</td>
</tr>
<tr>
<td></td>
<td>Naloxone 5</td>
</tr>
</tbody>
</table>

Each value represents the mean±S.E.M. for each group of experiments. 0 min: basal arterial blood pressure before morphine or ZAL administration. 30 min: basal arterial blood pressure before naloxone or the vehicle administration, 45 min: basal arterial blood pressure 15 min after naloxone or the vehicle administration, ZAL: zaldaride maleate, N: number of animals.

Effect of naloxone on depressor response induced by intestinal distention after treatment with ZAL and morphine

The depressor response to intestinal distention was changed to a rise in blood pressure after administration of morphine (20 mg/kg, s.c.), and the response was reversed to the basal level by the administration of naloxone (5 mg/kg, i.v.) (Fig. 5). ZAL (6 mg/kg, i.v.) significantly reduced the depressor response induced by intestinal distention, and this reduction was not affected by naloxone.
(5 mg/kg, i.v.) administration (Fig. 5).

Morphine (20 mg/kg, s.c.), naloxone (5 mg/kg, i.v.) and the vehicle did not affect basal blood pressure (Table 2).

DISCUSSION

The depressor response following distention of the intestine was alleviated by the administration of capsaicin. Treatment with capsaicin evokes the transient release of neuropeptides such as substance P and neurokinin A from the sensory neurons due to the increase in Ca\(^{2+}\) influx into the neuronal cells (13). However, capsaicin causes the depletion of neuropeptides from the sensory neurons and destroys the unmelinated C-fiber and small-diameter myelinated \(\delta\)-fiber sensory neurons due to the excessively sustained Ca\(^{2+}\) influx into the neuronal cells (14–15). Capsaicin also elicits the desensitization of neurons due to the stimulation of the Ca\(^{2+}\)-dependent phosphatase calcineurin activity (13, 16–17). These findings confirm that the depressor response to intestinal distention is mediated by sensory neurons.

In our present study, atropine did not affect the depressor response induced by intestinal distention. It is well-known that acetylcholine decreases arterial blood pressure induced by nitric oxide release from visceral epithelial cells. It is believed that the depressor response to intestinal distention does not relate to acetylcholine release from the efferent cholinergic neurons; that is, cholinergic mechanisms are not associated with the depressor response.

Phentolamine and papaverine also did not inhibit the depressor response to intestinal distention. Papaverine, which has a high affinity for the intestinal tissue, relaxes smooth muscle. These results suggest that alpha-adrenergic mechanisms and intestinal smooth muscle relaxation do not relate to the depressor response.

Morphine, a nonselective opioid agonist, is traditionally used for severe nociception. In the present study, the antinociceptive effect of morphine was reversed by naloxone, an opioid receptor antagonist. The antidiarrheal drug loperamide, an opioid \(\mu\)- and \(\delta\)-agonist (18), has less central side effect. Loperamide also reduced the depressor response to intestinal distention, and the effect of loperamide was reversed by naloxone (N. Aikawa and K. Ohmori, unpublished observation). Opioid receptors, opioid \(\mu\)-, \(\kappa\)- and \(\delta\)-subtypes (19), exist peripherally and centrally. In the dorsal horn of the spinal cord, opioid receptors localize the afferent sensory neuron endings. The activation of opioid receptor is related to the release of neuropeptides from the sensory neurons (20). The stimulation of opioid receptors inhibits adenylate cyclase activity and Ca\(^{2+}\) channel opening, and it also activates the K\(^{+}\) channels (21). The activation of peripheral opioid \(\mu\)- and \(\kappa\)-receptors displays antinociceptive effects against the visceral pain reflex to colonic distention in anesthetized rats (22–24). This suggests that morphine inhibits the depressor response to small intestine distention due to the inhibition of the release of neuropeptides from the sensory neurons mediated by peripheral and central opioid receptors. Naloxone at low doses block the opioid \(\mu\)-receptors. At the high doses used in this study, naloxone may antagonize all opioid receptors and could reverse the morphine-induced effects.

The antinociceptive effect of ZAL, unlike that of morphine, was not reversed by naloxone. ZAL is a highly selective and potent inhibitor of CaM (6). It is believed that the antinociceptive effect of ZAL is induced by the inhibition of CaM activity and is not mediated by opioid receptors.

ZAL inhibited the depressor response following intestinal distention. The intestinal distention induced by increasing intraluminal pressure evokes the release of prostaglandins from the intestinal mucosa and 5-hydroxytryptamine (5-HT) release from the enterochromaffin cells of the intestinal tract (25–28). Prostaglandins and 5-HT are recognized as important aalgic mediators. The biosynthesis of prostaglandin from cell membrane phospholipids is regulated by CaM (29–30). Prostaglandin receptors are distributed throughout the body and localize with high density in the dorsal horn of the spinal cord (31). Due to the increase in the intracellular Ca\(^{2+}\) and cAMP levels within the afferent sensory neuronal cells, prostaglandins such as prostaglandin (PG)\(E_2\) and \(I_2\) stimulate the neurotransmitters from afferent sensory neurons mediated by the EP and IP prostanoid receptors (32). The activation of prostaglandin receptors stimulates adenylate cyclase activity, which is modulated by CaM (33–34). The anti-PGE\(_2\) monoclonal antibody 2B5 and indomethacin, a nonselective inhibitor of cyclooxygenase types I and II, prevent carrageenan-induced paw hyperalgesia in rats (35). These findings suggest that ZAL reduces the depressor response to intestinal distention due to the inhibition of the prostaglandin biosynthesis and adenylate cyclase activity.

5-HT release from enterochromaffin cells is regulated by both Ca\(^{2+}\) and cyclic nucleotides (36) and is modulated in part by the enteric cholinergic neurons (37). Adenylate cyclase activity is regulated by CaM (33, 34), and acetylcholine release from the cholinergic neurons is also regulated by CaM (38). 5-HT\(_3\) receptor antagonists such as granisetron, ondansetron and tropisetron, like opioid agonists, have been reported to inhibit the depressor response to intestinal distention (2, 39). The activation of 5-HT\(_3\) receptors located on the peripheral afferent sensory nerves causes the release of neuropeptides by the increase
in intracellular Ca\(^{2+}\) levels. An increase in intracellular Ca\(^{2+}\) stimulates the production of prostaglandins from the membrane phospholipids, which are regulated by CaM (30, 33, 34). This suggests that ZAL inhibits the depressor response due to the reduction of 5-HT release from the enterochromaffin cells, which is induced by cyclic nucleotide synthesis and the inhibition of intracellular Ca\(^{2+}\) effects such as prostaglandin production.

Histamine is also an analgesic mediator, whose production is regulated by CaM (40). Constitutive nitric oxide synthase activity is regulated by CaM (41). Nitric oxide induces hyperalgesia in sensory neurons and enhances the release of neuropeptides from neuron terminals. The antinociceptive effect of ZAL may be mediated by reducing the production of histamine and nitric oxide.

The reference compound trifluoperazine is a widely used CaM inhibitor and potent neuroleptic. In our present study, trifluoperazine also inhibited the depressor response to intestinal distention, probably due to its peripheral and central effects.

In conclusion, ZAL may alleviate visceral pain in humans having inflammatory and functional bowel disorders such as IBS, nonulcer dyspepsia and noncardiac chest pain, and it definitely reduces the hyperalgesia of afferent sensory neurons to the noxious stimuli.

REFERENCES

4 Clark SL and Smith TW: Opiate-induced inhibition of the visceral distention reflex by peripheral and central mechanisms. Naunyn Schmiedebergs Arch Pharmacol 330, 179–183 (1985)
40 Bergstrand H and Lundquist B: Human basophil histamine release is differently affected by inhibitors of calmodulin, diacylglycerol kinase and peptidyl prolyl cis-trans isomerase in a secretagogue specific manner. Allergy 47, 353–361 (1992)
41 Schini VB and Vanhoutte PM: Inhibitors of calmodulin impair the constitutive but not the inducible nitric oxide synthase activity in the rat aorta. J Pharmacol Exp Ther 261, 553–559 (1992)