Tetrahydroberberine, an Isoquinoline Alkaloid Isolated from Corydalis Tuber, Enhances Gastrointestinal Motor Function

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ABSTRACT

Because delayed gastric emptying and impaired gastric accommodation are regarded as pathophysiological mechanisms underlying functional dyspepsia (FD), prokinetics and fundic relaxants have been suggested as a new treatment for FD. We isolated tetrahydroberberine (THB), an isoquinoline alkaloid (5,8,13,13a-tetrahydro-9,10-dimethoxy-6H-benzol[g]-1,3-benzodioxolo[5,6-a]quinolizine) from Corydalis tuber, and found that it has micro-molar affinity for dopamine D2 (pKᵢ = 6.08) and 5-HT₁A (pKᵢ = 5.38) receptors but moderate to no affinity for other relevant serotonin receptors (i.e., 5-HT₁B, 5-HT₁D, 5-HT₂a, and 5-HT₄; pKᵢ < 5.00). Oral administration of THB not only resulted in significantly accelerated gastric emptying of normal rats in a bell-shaped relationship, with a maximal efficacy at a dose of 30 mg/kg, but also restored the delayed gastric emptying caused by apomorphine, which might be mediated by an antidopaminergic effect. Data from electromyography indicated enhanced motor function of the upper gastrointestinal tract by THB, which occurred through strengthening contractility and shortening the contraction interval. Furthermore, in rats stressed by repeated restraint, a significantly higher shift in the pressure-volume curve by THB (10 μg/kg, p < 0.05), which was inhibited by [O-methyl-3H]-N-[2-(4-(2-methoxyphenyl)-1-piperazinyl)(ethyl)]-N-(2-pyridinyl)cyclohexanecarboxamide trihydrochloride (WAY-100635), a 5-HT₁A antagonist, and N⁴-nitro-L-arginine methyl ester, a nitric-oxide synthase inhibitor but not a vasoactive intestinal peptide antagonist, was observed. Oral administration of THB resulted in a drastic increase of gastric accommodation in Beagle dogs. Area under the volume versus time curve was increased significantly by THB (30 μg/kg, p < 0.01) and comparable with that of sumatriptan (3 mg/kg), a potent fundic relaxant. Taken together, our data suggested that THB, with D2 receptor antagonist and 5-HT₁A receptor agonist properties, has significant potential as a therapeutic for treatment of FD.

Introduction

Functional dyspepsia (FD) is one of the main syndromes associated with gastrointestinal motor dysfunction (Tack, 2007). Delayed gastric emptying and impaired gastric accommodation are known to contribute to clinical manifestations, such as postprandial fullness, early satiation, epigastric pain, and burning sensation (Talley et al., 2006). Currently, prokinetics and fundic relaxants appear to be the drugs of choice for the treatment of FD (Tack, 2008; Brun and Kuo, 2010). Prokinetics stimulate smooth muscle contractions, leading to enhanced gastric emptying and acceleration of both small and large intestinal transits (Karamanolis and Tack, 2006; Tack, 2008). Fundic relaxants target impaired gastric accommodation of the upper gastrointestinal tract (Kindt and Tack, 2006; Tack, 2008). One of the major categories of prokinetic drugs is antidopaminergic agents (Tonini et al., 2004). The gastrointestinal tract actually has the ability to produce a substantial amount of dopamine. Enteric dopamine has been known to mediate inhibition of gut motility and decrease antroduodenal coordination in gut muscle, thereby inhibiting acetylcholine release from the cholinergic nerve by the activation of the neuronal dopamine D₂ receptor...
Domperidone, a D₂ receptor antagonist, has been reported to induce an increase in gastric muscle contraction in guinea pigs (Reddymasu et al., 2007). Ito-pride is a benzamide derivative antagonizing the D₂ dopamine receptor, which has demonstrated acceleration effects on gastrointestinal motility and gastric emptying in both animals and humans (Tsubouchi et al., 2003; Holtmann et al., 2006). Another significant category of prokinetic drugs is serotoninergic agonists (Tonini and Pace, 2006). The main effects relevant to gastrointestinal motility are mediated by 5-HT₄ receptors (De Maeyer et al., 2008). In an effort to determine the active component(s), we performed binding assays for various receptors controlling gastrointestinal motor functions using several compounds isolated from Corydalis tuber, was found to be bound to both dopamine D₂ and 5-HT₁A receptors but not other relevant serotonin receptors. In this study, we show that THB works not only as a prokinetic but also a fundic relaxant in animal models and that it has potential as a therapeutic for gastrointestinal motor dysfunctions such as FD.

**Materials and Methods**

**Identification of THB from Corydalis Tuber.** Tubers of *Corydalis yanhusuo* (10 kg) were extracted with 50% ethanol twice at room temperature. The ethanol extract (250 g) was suspended in distilled water (7.2 liters), followed by successive partitioning with n-hexane, trichloromethane, ethyl acetate, and n-butanol, yielding 10, 30, 4, and 26 g, respectively. The CHCl₃ soluble fraction (28 g) was subjected to silica gel column chromatography (230–400 mesh, 600 g) and eluted with trichloromethane/methanol [15:1 (3.0 liters) and 5:1 (3.0 liters)] to afford seven fractions [F1, 15:1, 1.0 liter; F2, 15:1, 1.0 liter; F3, 15:1, 1.0 liter; F4, 5:1, 0.5 liters; F5, 5:1, 0.5 liters; F6, 5:1, 1.0 liter; F7, 5:1, 1.0 liter]. F1 (3.5 g) and F2 (10.0 g) were mixed and subjected to silica gel column chromatography (230–400 mesh, 250 g, n-hexane/ethyl acetate, 3:1) to give seven subfractions [SF1–SF7 (each 1.0 liter)]. SF3 (350 mg) was purified by preparative high-performance liquid chromatography using a solvent of n-hexane/CHCl₃/ethyl acetate (6:3:5) at a flow rate of 2.0 ml/min [Apollo Silica 5-μm column; 250 × 10 mm; 5-μm particle size (Alltech, Nicholasville, KY); Shodex refractive index detector (Shodex, New York, NY)] to obtain THB (30 mg, 783 μg). Other chemicals and reagents used were of analytical or reagent grade.

**Radioligand Competition Receptor Binding Assays.** Frozen Chinese hamster ovary (CHO) K1 and human embryonic kidney 293 membranes containing cloned human recombinant serotonin 5-HT₁A or 5-HT₃ or dopamine D₁ receptors were purchased from PerkinElmer Life and Analytical Sciences (Waltham, MA). G proteins and receptors used were of analytical or reagent grade.

![Fig. 1. Structure of THB.](image-url)
were conducted at Ricerca Biosciences (Taipei, Taiwan) and Cal-
Chow; Purina, St. Louis, MO). All of the experimental procedures were conducted according to the principles enunciated in the Guide for the Care and Use of Laboratory Animals (Institute of Laboratory Animal Resources, 1996) and Dong-A Pharmaceutical.

**Animals.** Male Sprague-Dawley rats (200–220 g) were purchased from Orient Bio, Inc. (Gapyeung, South Korea) and had free access to water and a standard pellet diet. Rats were kept in an environmentally controlled room in groups of one to two animals before experiments.

**Gastric Emptying.** Gastric emptying was measured according to the method of Ozaki and Sukamto (1999). Male Sprague-Dawley rats (200–220 g) were fasted for 24 h before the start of all of the experiments; the animals then were orally administered the test drugs (THB or conventional prokinetics as positive controls at the indicated doses) or 3% (w/v) hydroxypropyl methylcellulose as a vehicle. Beagle dogs were purchased from Central Laboratory Animal, Inc. (Seoul, South Korea), individually housed in single, air-conditioned boxes, and given dog food in pellet form (Purina Dog Chow; Purina, St. Louis, MO). All of the experimental procedures were conducted according to the principles enunciated in the Guide for the Care and Use of Laboratory Animals (Institute of Laboratory Animal Resources, 1996) and Dong-A Pharmaceutical.

**Electromyography Study.** Male Sprague-Dawley rats (10–12 weeks old and weighing 350–450 g) were used. Animals were prepared surgically for chronic electromyography (EMG) under aseptic conditions and under general anesthesia with tiletamine/zolazepam (12.5 mg/kg i.m.). Three pairs of electrodes (silver, 0.005 in. bare, 0.007 in. coated diameter; A-M Systems, Carlsborg, WA) were implanted into the muscular wall of the antrum and the small intestine 5 and 15 cm distal to the pylorus, as described previously (Rukebusch and Fioramonti, 1975). Electrodes were fixed and fixed to the back of the animal to allow free movement in the cage. After the surgical procedure, rats were treated with antibiotic (penicillin G) for 48 h. Electrode position was verified during necropsy. EMG recordings started 7 days after surgery, when rats had recovered completely and exhibited clear migrating motor complex (MMC). Rats were placed in a plastic cage for 4 h per day for 4 days. Rats were fasted for 24 h before the start of the experiment (water was available ad libitum).

At the beginning of the experiment, rats were sedated with a single dose of ketamine (50 mg/kg i.m.; Yulan Corporation). Thereafter, a dose of 25 mg/kg was administered intramuscularly every hour to maintain sedation. During the experiment, the animals always were positioned lying down on their right sides. Ketamine sedation allowed the rats to tolerate swallowing of the finely folded polyethylene balloon that was adherent to the polyethylene tube and intubation of the tube during the experiment while spontaneous breathing was preserved. The bag was positioned in the stomach via a guide wire. A heating pad was used throughout the experiments to maintain the body temperature of the animal at 37°C. Gastric pressure–volume relationships were studied using a gastric barostat (Distender Series II; G&J Electronics, Toronto, ON, Canada). The system consists of an ultrathin polyethylene balloon (10-ml maximal capacity; Mui Scientific, Mississauga, ON, Canada), which was finely folded and can be inserted through the mouth into the proximal stomach, and has infinite compliance at the volumes used for distention of the rat stomach. A polyethylene balloon was adhered to a single lumen polyethylene tube (1.2-mm diameter; Natsume, Tokyo, Japan). The tube was connected to a pressure recording port and an air inflation port on a computer-driven barostat for recording of volume changes while the pressure was kept constant. Before the start of the experiment, the balloon was connected to the barostat, and the intrabag pressure was raised to 10 mm Hg. Monitoring of the constant intrabag volume ensured that there was no leak (Janssen et al., 2004). Drugs were administered after the intragastric volume maintained a stable baseline value during a period of at least 10 min at the constant pressure used. A single dose of THB was administered per experiment. Different doses (1, 3, 10, 30, or 100 μg/kg i.v.) of THB were divided randomly over different experiments. The doses of the drugs (WAY-100635, l-NMMA, l-arginine, and VIP antagonist) used for mechanism studies were chosen based on previous publications (Takahashi and Owyang, 1997; Zhou et al., 2008).

**Canine Gastric Accommodation.** Experiments were performed on four adult female Beagle dogs (7–9 kg b.wt.). Dogs were trained to stand quietly in a sling without sedation. Experiments were performed on conscious dogs after allowing at least 15 days for recovery after surgery. Before each experimental session, the dogs were fasted for at least 18 h; water was available ad libitum. Between consecutive experimental sessions with the same animal, a washout period

### TABLE 1

Experimental conditions for the determination of affinities at native and recombinant receptors in vitro

<table>
<thead>
<tr>
<th>Receptor</th>
<th>Source</th>
<th>Radioligand</th>
<th>( K_d ) (nM)</th>
<th>Non-specific (nM)</th>
<th>Buffer*</th>
<th>Included Time and Temperature</th>
</tr>
</thead>
<tbody>
<tr>
<td>5-HT(_A)</td>
<td>CHO K1</td>
<td>(^{[3]H})8-OH-DPAT (0.25)</td>
<td>0.29</td>
<td>Metergoline (10)</td>
<td>A</td>
<td>60 min, 27°C</td>
</tr>
<tr>
<td>5-HT(_B)</td>
<td>Sprague-Dawley cerebral cortex</td>
<td>(^{[3]H})Cyanopindolol (0.01)</td>
<td>0.19</td>
<td>Serotonin (10)</td>
<td>B</td>
<td>90 min, 37°C</td>
</tr>
<tr>
<td>5-HT(_T)</td>
<td>Human embryonic kidney 293</td>
<td>(^{[3]H})GR65630 (0.55)</td>
<td>0.57</td>
<td>MDL-72222 (10)</td>
<td>C</td>
<td>60 min, 25°C</td>
</tr>
<tr>
<td>5-HT(_T)</td>
<td>Hartley guinea pig striatum</td>
<td>(^{[3]H})GR113808 (0.70)</td>
<td>0.14</td>
<td>Serotonin (30)</td>
<td>D</td>
<td>30 min, 25°C</td>
</tr>
<tr>
<td>THB</td>
<td>CHO K1</td>
<td>(^{[3]H})Iodosopiperone (0.27)</td>
<td>0.12</td>
<td>Haloperidol (5)</td>
<td>E</td>
<td>120 min, 25°C</td>
</tr>
</tbody>
</table>

* Buffer A, 50 mM Tris-HCl (pH 7.4), 10 mM MgSO\(_4\), 0.5 mM EDTA, and 0.1% ascorbic acid; buffer B, 50 mM Tris-HCl (pH 7.4), 154 mM NaCl, 10 μM pargyline, and 30 μM isoprenaline; buffer C, 50 mM Tris-HCl (pH 7.5), 5 mM MgCl\(_2\), and 1 mM EDTA; buffer D, 50 mM Tris-HCl (pH 7.4); buffer E, 50 mM Tris-HCl (pH 7.4), 120 mM NaCl, 5 mM KCl, 5 mM MgCl\(_2\), and 1 mM EDTA.
of at least 72 h was allowed. Dogs were observed throughout the experiment, and any sign of discomfort or anomalous behavior was noted. The gastric cannula was opened, and after verification that the stomach did not contain any food residues, the bag of the barostat was introduced into the proximal stomach (position of the bag checked radiographically). Before and at the end of the in vivo tests, the bag was checked for air leaks by increasing the pressure to 20 mm Hg (Chen et al., 2009). To determine the influence of 5-HT1 receptor antagonists in the mediation of canine gastric accommodation induced by THB, WAY-100635 (0.1 mg/kg) was tested versus 30 µg/kg THB and administered intravenously 10 min before THB.

Data Analysis. Results were expressed as mean ± S.E.M. Differences in the data were evaluated using paired t test for the comparison of two groups or one-way analysis of variance (ANOVA) followed by Dunnett's test for multiple comparisons. A difference was considered significant if p < 0.05.

Results

Identification and Characterization of THB. In an effort to determine active component(s) from DA-9701, we have fractionated the ethanol extract of Corydalis tuber according to polarity and purified several compounds by column chromatography, as described under Materials and Methods. We have screened and chased the affinities of the compounds for relevant receptors involved in the control of gastrointestinal motor function, which included various serotonin receptors and dopamine D2 receptor, by radioligand competition binding assays, according to the conditions presented in Table 1. We found that a compound known as SF-2 has micromolar affinities for dopamine D2 receptor (pKi = 6.08) and 5-HT1A receptor (pKi = 5.38) but moderate to no affinity for other relevant serotonin receptors (i.e., 5-HT1B, 5-HT1D, 5-HT3, and 5-HT4; pKi < 5.00), as presented in Table 2. SF-2 showed a similar affinity for D2 receptor to that of domperidone compared with values reported previously (Freedman et al., 1994). In terms of 5-HT1A receptor affinity, SF-2 showed 132 times lower affinity than that of buspirone (pKi = 7.50). The structure of SF-2 was identified by NMR and mass spectroscopy. Spectroscopic data, including 1H NMR and 13C NMR, are as below. Yellow powder; m.p. 167°C; fast atom bombardment mass spectroscopy, m/z 340 [M + H]+; 1H NMR (500 MHz, CDCl3), δ 6.87 (1H, d, J = 8.5 Hz, H-12), 6.80 (1H, d, J = 8.5 Hz, H-11), 6.74 (1H, s, H-1), 6.60 (1H, s, H-4), 6.08 (2H, s, -OCH2O-), 4.27 (1H, d, J = 15.5 Hz, H-8), 3.86 (6H, s, OCH3-9, OCH3-10), 3.58 (2H, d, J = 15.5 Hz, H-8, H-14), 3.25 (1H, m, H-6), 3.22 (1H, m, H-13), 3.14 (1H, m, H-5), 2.86 (1H, dd, J = 15.0, 12.0 Hz, H-13), 2.68 (1H, m, H-6), 2.65 (1H, m, H-5); 13C-NMR (125 MHz, CDCl3), δ 150.2 (C-10), 146.1 (C-2), 145.8 (C-3), 145.0 (C-9), 130.6 (C-14a), 128.5 (C-8a), 127.7 (C-4a), 127.6 (C-12a), 123.8 (C-12), 110.9 (C-11), 108.3 (C-4), 105.4 (C-1), 101.0 (-OCH2O-), 59.9 (OCH3-9), 59.5 (C-14), 55.8 (OCH3-10), 53.8 (C-8), 51.3 (C-6), 36.3 (C-13), 29.4 (C-5). Spectral data for THB were determined to be identical to that of the source material obtained from Trademax (Shanghai, China). SF-2 is identified as THB, an isoquinoline alkaloid. The chemical structure is shown in Fig. 1. To the best of our knowledge, there have been no reports on serotonin receptors, although it has been known as an antidopaminergic agent (Niwa et al., 1991). On the basis of the above data, we performed testing to determine whether THB has potential as a prokinetic as well as a fundic relaxing agent.

Effects of THB on Gastric Emptying. To estimate the prokinetic effects of THB, we performed gastric emptying experiments using semisolid meals. In normal rats, compared with the control group, THB induced significant acceleration of gastric emptying. As shown in Fig. 2A, data from the control group indicated that only 28.1 ± 1.1% was emptied; however, the decrease in residual meal by THB was significant. The effect was a bell-shaped relationship, with a maximal efficacy at the dose of 30 µg/kg. The residual percentage of the meal was 50.3 ± 1.2% (p < 0.05), and the maximal effect of THB was comparable with that achieved with cisapride at a dose of 10 mg/kg p.o. (54.8 ± 1.5, n = 8, p < 0.05). We next used delayed models of gastric emptying to determine whether THB, known to function as a D2 receptor antagonist (Wu and Jin, 1996, 1997), is capable of correcting abnormally depressed gastric emptying. Apomorphine has inhibitory effects on gastric emptying through its action as a dopamine agonist (Blancquaert et al., 1982). Administration of apomorphine (0.05 mg/kg s.c.) resulted in a marked delay of gastric emptying of a semisolid meal by approximately 50% compared with that of naive rats (37.8 ± 2.4 versus 68.9 ± 7.4). Delayed gastric emptying was restored by THB. At doses of 10 and 100 µg/kg, the gastric emptying rates were 48.8 ± 5.2 (p < 0.05) and 55.9 ± 3.7 (p < 0.01), respectively. The effect of 100 µg/kg THB was comparable with that of cisapride at a dose of 30 mg/kg (Fig. 2B). These results suggested that THB could induce acceleration of gastric emptying under normal conditions as well as apomorphine-induced abnormally delayed gastric emptying.

![Image](https://via.placeholder.com/150)

TABLE 2

<table>
<thead>
<tr>
<th>Receptor</th>
<th>THB*</th>
<th>Cisapride†</th>
<th>Mosapride‡</th>
<th>Sumatriptan§</th>
<th>Buspirone¶</th>
<th>WAY-100635∥</th>
<th>GR127935**</th>
</tr>
</thead>
<tbody>
<tr>
<td>5-HT1A</td>
<td>5.38</td>
<td>&lt;6.00</td>
<td>&lt;5.00</td>
<td>6.43</td>
<td>7.50</td>
<td>9.05</td>
<td>7.58</td>
</tr>
<tr>
<td>5-HT1B</td>
<td>&lt;5.00</td>
<td>&lt;6.00</td>
<td>&lt;5.00</td>
<td>7.60</td>
<td>&lt;5.00</td>
<td>5.88</td>
<td>9.18</td>
</tr>
<tr>
<td>5-HT1D</td>
<td>N.D.</td>
<td>&lt;5.00</td>
<td>N.D.</td>
<td>7.92</td>
<td>&lt;5.00</td>
<td>6.48</td>
<td>8.41</td>
</tr>
<tr>
<td>5-HT3</td>
<td>&lt;5.00</td>
<td>&lt;6.00</td>
<td>&lt;6.00</td>
<td>N.D.</td>
<td>N.D.</td>
<td>N.D.</td>
<td>N.D.</td>
</tr>
<tr>
<td>5-HT4</td>
<td>&lt;5.00</td>
<td>7.40</td>
<td>7.01</td>
<td>N.D.</td>
<td>N.D.</td>
<td>N.D.</td>
<td>N.D.</td>
</tr>
<tr>
<td>D2</td>
<td>6.08</td>
<td>3.43</td>
<td>&lt;4.00</td>
<td>N.D.</td>
<td>N.D.</td>
<td>N.D.</td>
<td>N.D.</td>
</tr>
</tbody>
</table>

N.D., not determined.
* In this study.
† Yoshikawa et al., 1998.
‡ Kakigami et al., 1998.
§ Lyeon et al., 1996.
¶ Koeck et al., 1998.
∥ Gommeren et al., 1998.
** Gommeren et al., 1998.
Effects of THB on Motor Activity in the Upper Gastrointestinal Tract. To further evaluate the question of whether THB enhances gastric motor function, we measured contractility using EMG. Rats received surgical implantation with 41 pairs of electrodes in the antrum, duodenum, and jejunum. The effects of THB on gastrointestinal contractile activity upon intravenous injection under fasting conditions are presented in Fig. 3. Control rats injected with saline showed a typical pattern of motor activity; however, injection of THB (10 μg/kg i.v.) resulted in significant enhancement of contractile force in the gastrointestinal tract, and an overall increase in the motor activity index was observed. As shown in Fig. 3A, peak count was increased significantly by THB, not only in the gastric antrum but also the duodenum and jejunum, compared with that of the control. Amplitude and integral areas showed a significant increase, as shown in Fig. 3, B and C, respectively, indicating that THB induced stronger contractility compared with that of the control. The effects were prominent in the jejunum, resulting in approximately 2-fold increases, compared with that of the control. Furthermore, the interval of MMC was measured from the end of one activity to the end of the next one. The MMC occurred at regular intervals of approximately 10.1 ± 1.3 min in the control group. Injections of THB resulted in significant shortening of the interval between the MMCs in the duodenum (7.3 ± 1.1 min, p < 0.05) and jejunum (7.4 ± 0.4 min, p < 0.01), although significant effects were not observed in the antrum (Fig. 3D). These results suggested that THB could enhance gastric motor function in the upper gastrointestinal tract through strengthening contractility and shortening the contraction interval.

Fundic Relaxing Effects of THB. Because DA-9701 not only enhanced gastric accommodation in Beagle dogs, we also determined the binding affinity of THB to the 5HT<sub>1A</sub> receptor (Table 2) and attempted to determine whether THB has the ability to perform fundic relaxant activities, using two animal models. We first used rats with impaired gastric compliance by repeated restraint stress. Rats were restrained in small plastic bags for 4 h per day for 4 consecutive days; balloons connected with a barostat then were inserted into the stomach for the measurement of the volume-pressure relationship. A significant shift (p < 0.05) of the pressure-volume curve was observed in stressed rats toward the lower volume compared with that of control rats, indicating im-

Fig. 2. Effects of THB on gastric emptying. A, in normal rats, after 24 h of fasting, animals (n = 8 for each group) were orally administered THB and cisapride (Cisa) at the indicated doses or 3% hydroxypropyl methylcellulose as a vehicle (Con). B, effects of THB on delayed gastric emptying. In an apomorphine-induced delay model, animals were orally administered THB and itopride (Ito) at the indicated doses or 3% hydroxypropyl methylcellulose as a vehicle (Con) and were injected simultaneously with apomorphine (0.05 mg/kg s.c.). Naive animals were not injected with apomorphine and were orally administered the vehicle. Percentage gastric emptying was calculated as described under Materials and Methods. *, p < 0.05; **, p < 0.01 versus control. †, p < 0.01 versus normal (one-way ANOVA with post hoc Dunnett’s test).

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Fundic Relaxing Effects of THB. Because DA-9701 not only enhanced gastric accommodation in Beagle dogs, we also determined the binding affinity of THB to the 5HT<sub>1A</sub> receptor (Table 2) and attempted to determine whether THB has the ability to perform fundic relaxant activities, using two animal models. We first used rats with impaired gastric compliance by repeated restraint stress. Rats were restrained in small plastic bags for 4 h per day for 4 consecutive days; balloons connected with a barostat then were inserted into the stomach for the measurement of the volume-pressure relationship. A significant shift (p < 0.05) of the pressure-volume curve was observed in stressed rats toward the lower volume compared with that of control rats, indicating im-

Fig. 3. Effects of THB on gastrointestinal motor activity in conscious rats. THB (10 μg/kg i.v.) versus control ( intravenous saline) on the different EMG parameters (A, peak count; B, amplitude; C, integral area; D, interval of MMC) from the rat gastric antrum at 5 mm proximal to the pylorus, the duodenum, and the jejunum, respectively, at 5 and 15 cm distal to the pylorus. *, p < 0.05; **, p < 0.01 versus control (one-way ANOVA with post hoc Dunnett’s test).
paired gastric compliance, resulting in a significantly lower maximal gastric volume (data not shown). Intravenous administration of THB after maintenance of the intragastric volume at a stable baseline value resulted in a significantly higher shift in the pressure–volume curve in rats treated with the doses of 10 μg/kg (Fig. 4C) and 30 μg/kg (Fig. 4D), whereas doses below 10 and 100 μg/kg showed no significant effects. At ½P\text{max}, the gastric volume was 9.6 ± 0.8 ml (rats treated with a dose of 10 μg/kg, \( p < 0.05 \)) versus 7.6 ± 0.8 ml (control), and in rats treated with a dose of 30 μg/kg, the value was 9.5 ± 1.0 ml (\( p < 0.05 \)) versus 6.8 ± 0.9 ml (control). The maximum delta volume (approximately 2 ml) at ½P\text{max} was obtained in rats treated with 10 μg/kg THB (Fig. 4F). In addition, an effort to understand the mode of action for gastric relaxation, we conducted an antagonist study. WAY-100635, a 5HT\textsubscript{1A} receptor antagonist, was chosen based on data from the receptor binding assay of THB to the 5HT\textsubscript{1A} receptor, and, additionally, other antagonists of signaling molecules, including NO and VIP, also were used (Desai et al., 1991). WAY-100635 was administered (0.1 mg/kg i.v.) 10 min before the administration of THB (10 or 30 μg/kg i.v.) at doses presenting significant efficacy. THB induced an increase of gastric volume that was inhibited significantly by WAY-100635. The delta volume at ½P\text{max} was lowered to 0.02 ml (Fig. 5). Administration of L-NAME (10 mg/kg), a NO synthase inhibitor, also resulted in significant inhibition of delta volume at ½P\text{max} (0.2 ± 0.1 versus 1.5 ± 0.3 ml, \( p < 0.05 \)). In addition, to exclude nonspecific effects of L-NAME, we used L-arginine, a NO precursor. When L-arginine (100 μM/kg) was administered, L-NAME restored the gastric volume to the levels induced by THB, indicating involvement of NO. However, administration of the VIP antagonist (30 nmol/kg) did not result in any changes in gastric volume induced by THB. As shown in Fig. 5, the delta volume at ½P\text{max} was 1.0 ± 0.3 ml. We then assessed the effects of THB on gastric accommodation by oral administration in Beagle dogs surgically implanted with a barostat. Postprandial gastric volume was evaluated in three time intervals, 10 to 35, 35 to 60, and 10 to 60 min, after each meal (Fig. 6A). In dogs who received 30 μg/kg THB, postprandial volume was significantly higher than that of the control for each time interval (\( p < 0.01 \)), which was comparable with those of sumatriptan (3 mg/kg i.v.), a potent fundic relaxant. Area under the volume versus time curve was increased significantly in dogs treated with THB (Fig. 6B). Taken together, these data suggested the potential of THB as a fundic relaxant for an increase of gastric accommodation.

**Discussion**

This study began from DA-9701, an herb-based gastropokinetic agent formulated with Pharbitis semen and Corydalis tuber (Lee et al., 2008). A phase III clinical trial of DA-9701 for FD has been completed recently, and we are anticipating DA-9701 as a novel natural medicine for treatment of FD in South Korea. In this study, we provided several pieces of evidence to show that THB, an isoquinoline alkaloid isolated from Corydalis tuber, is one of the active component(s) responsible for gastropokinetic and fundic relaxing activity. THB has been known as an antidopaminergic agent; however, for the first time, we recognized THB as an agonist for the 5-HT\textsubscript{1A} receptor and explored the possibility of its possible use for the treatment of FD. We demonstrated that THB stimulates gastric emptying and gastrointestinal contractility as well as enhances gastric accommodation in conscious animals, which might be mediated by dopamine D\textsubscript{2} and/or serotonin 5-HT\textsubscript{1A} receptors. These findings suggested that THB may have potential as a novel agent in the treatment of FD associated with gastric motor dysfunction.
The extent of dopaminergic innervation and its roles in the gut are not completely understood; however, dopamine is known to cause potent inhibition of motility, reduced lower esophageal sphincter tone, reduced gastric tone and intragastric pressure, and decreased antral-duodenal gastrointestinal motility. Stimulation of peripheral dopamine D_2 receptor located on the postganglionic nerves causes a decrease in acetylcholine release from parasympathetic nerves. Indeed, after worldwide withdrawal of cisapride due to cardiac safety issues, antidopaminergic agents have been exploited clinically for the management of gastrointestinal motor dysfunction. Several antidopaminergic agents, including domperidone, metoclopramide, and itopride, are used primarily for the treatment of motility disorders of the upper digestive tract, such as FD, and some agents seem to be beneficial for the improvement of symptoms. However, the toxicity issue is still under consideration. Itopride at a dose of 100 mg/kg and metoclopramide at a dose of 10 mg/kg were reported to produce reduced activity, tremor, and abnormal behavior in dogs (Koizumi et al., 1992). Therefore, the development of safer and more effective antidopaminergic agents for the treatment of gastric functional disorders definitely is needed. THB was found to enhance gastric emptying in a bell-shaped, dose-dependent manner, and the maximal effective dose was 30 μg/kg, whereas cisapride, a conventional prokinetic used as a control in this experiment, showed an effect comparable with that at a much higher concentration (10 mg/kg), indicating that THB has an effect superior to that of conventional drugs.

Data from a delayed gastric emptying model using apomorphine together with a dopamine D_2 receptor binding assay indicated that THB might propel gastric emptying through dopaminergic antagonism. The effects, which were equivalent to those of itopride at a dose of 30 mg/kg, were prominent at a dose of 10 μg/kg. In addition, intravenous injection of THB resulted in significantly increased gastrointestinal motility. The dose of 10 μg/kg THB was sufficient for a significant increase of contractility from stomach to jejunum. Taken together, THB appears to be a new antidopaminergic prokinetic.

Both intravenous injection and oral administration of THB resulted in significantly increased gastric accommodation in restraint-stressed rats and conscious dogs, respectively. It has been accepted currently that fundic relaxation can be achieved by agonists for 5-HT1 receptor subtypes, including the 5-HT1A and/or 5-HT1B/D receptors. Activation of the 5-HT1A receptor causes a release of NO for the relaxation of the gastric fundus and a decrease of gastric fundus tone (Desai et al., 1991; Coulie et al., 1999). Sumatriptan, a 5-HT1B/D receptor agonist, has been reported to induce fast and profound relaxation of the proximal stomach in both canine models and human studies (Tack et al., 2000); however, its cost and mode of administration appear to be unsuitable for chronic treatment of FD. The nonselective 5-HT1A receptor agonist buspirone reduces fundic tone, enhances meal-induced relaxation, and enhances gastric accommodation and gastric emptying in humans in a dose-dependent manner; however, due to its central side effects, it is not suitable for routine clinical use for the treatment of FD (Koizumi et al., 1992). THB not only binds the 5-HT1A receptor but also induces a significant increase of gastric volume (at a dose of 10 μg/kg) in rats stressed by restraint. The relaxing effects were blocked almost completely by WAY-100635, a 5-HT1A receptor antagonist, and we also observed that treatment with a NO synthase inhibitor resulted in significant suppression of the THB-induced increase in gastric volume, which was reversed by the NO donor, whereas the VIP antagonist was not able to affect the gastric volume increase by THB. In addition, we recognized that oral administration of THB resulted in significantly increased postprandial gastric volume in conscious dogs and that the effects at a dose of 30 μg/kg were comparable with those of sumatriptan (3 mg/kg). On the basis of our data, THB is an orally available as well as injectable fundic relaxant acting on the 5-HT1A receptor. For safety, we performed testing to determine whether THB can induce ataxia, a serious side effect of the 5-HT1A receptor agonist, using the rotarod assay (Millan et al., 1996). THB (0.3 mg/kg, i.v.) did not show any rotarod performance 30 min after the administration, whereas buspirone (3 mg/kg i.v.) produced a significant decrease in rotarod performance (data not shown). Taken together, our study suggested that THB has significant potential as a therapeutic for FD for the enhancement of gastric motor function. Although the question of whether and/or how dual activities on dopamine and serotonin by THB work synergistically or co-operatively on gastric emptying and gastric accommodation remains to be elucidated, considering the superior effects of THB to the conventional drugs and its safety, further research for its development as medicine clearly is needed.
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