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Effect of a selective 5-HT_3 receptor agonist on gastric motility in fasted and fed dogs

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Abstract

The effect of *m*-chlorophenylbiguanide, a selective 5-HT_3 receptor agonist, on gastric antral motility was investigated in conscious dogs with a force transducer implanted chronically. *m*-Chlorophenylbiguanide (0.1–1 mg/kg i.v.) dose dependently enhanced antral motility in the fasted state, and the amplitude of *m*-chlorophenylbiguanide (1 mg/kg i.v.)-induced antral contractions reached the level of natural phase III contractions. In contrast, *m*-chlorophenylbiguanide reduced the amplitude of antral contractions in the fed state. A selective 5-HT_3 receptor antagonist, ramosetron (0.0003–0.03 mg/kg i.v.), inhibited both effects of *m*-chlorophenylbiguanide. *m*-Chlorophenylbiguanide (1 mg/kg i.v.)-induced contractions were inhibited by atropine (0.03 or 0.1 mg/kg i.v.). These results indicate that pharmacological activation of 5-HT_3 receptors has opposite effects on canine gastric antral motility in the fasted and in the fed state, being stimulatory and inhibitory, respectively. The stimulatory effect seems to be mediated mainly via the release of acetylcholine.

Keywords: 5-HT_3 receptor; Gastric motility; *m*-Chlorophenylbiguanide; Ramosetron; (Force transducer)

1. Introduction

The 5-HT_3 receptor is located exclusively on neuronal tissues. It has been found in both the brain and periphery, and has been reported to be involved in various body function including gastrointestinal motility. Gastric motility in dogs and humans is clearly divided into two states, namely the fasted state, which is characterized by alternation of periods of quiescent (phase I of the migrating motor complex) and of active (phase II and phase III of the migrating motor complex) motility, and the fed state, which is characterized by continual regular low-amplitude contractions in the antrum (Itoh et al., 1977; Sarna, 1985). It has been reported that selective 5-HT_3 receptor antagonists inhibit the occurrence of gastric phase III contractions of the migrating motor complex in the fasted state in dogs (Itoh et al., 1991; Yoshida et al., 1991) and humans (Wilmer et al., 1993), suggesting that 5-HT_3 receptors play an important role in the initiation of gastric phase III contractions of the migrating motor complex in the fasted state in these species. On the other hand, selective 5-HT_3 receptor antagonists have been reported to have no effect on gastric motility in the fed state in dogs (Itoh et al., 1991; Yoshida et al., 1991), suggesting that 5-HT_3 receptors have no or only a minor role in the regulation of physiological gastric motility in the fed state.

In spite of the abundance of studies of 5-HT_3 receptor antagonists, little is known about the effect of 5-HT_3 receptor agonists on gastric motility. We therefore felt it necessary to investigate the effect of activation of 5-HT_3 receptors, using a selective 5-HT_3 receptor agonist, on gastric motility in both the fasted and fed states. In the present study, we investigated the effect of *m*-chlorophenylbiguanide, a selective and potent 5-HT_3 receptor agonist (Higgins et al., 1993; Kilpatrick et al., 1990; Van Wijngaarden et al., 1990), on canine gastric antral motility in both the fasted and fed states. The involvement of 5-HT_3 receptors therein was confirmed with ramosetron, a selective and highly potent 5-HT_3 receptor antagonist (Ito et al., 1995; Miyata et al., 1991, 1995).

2. Materials and methods

2.1. Preparation of animals

Eight male beagle dogs weighing 9–12 kg were used. The animals were fasted for 18 h before surgery with free

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access to water. Under halothane (Fluothane, Takeda Chemical Industries, Osaka, Japan) anesthesia, a strain gauge force transducer (F-12IS, Star Medical, Tokyo, Japan) was sutured to the gastric antrum (3 cm proximal to the pyloric ring) to measure circular muscle contractions. The free end of the transducer was brought subcutaneously to a skin incision made between the scapulae and protected with a jacket (FPJ-12, Star Medical). The animals were allowed to recover for at least 14 days after surgery before the commencement of experiments.

2.2. Recordings

The dogs were fasted for 18 h with free access to water before each experiment. During the experiments, each animal stood on a Pavlov stand supported by a nylon-mesh sling. The free end of the strain gauge force transducer was connected to a gastrointestinal motility measuring system (ESC-820A; Star Medical) via connecting cables. Gastric antral motor activity from the transducer was measured continuously with the measuring system and recorded and analyzed by means of a computer system (ESC-820C; Star Medical). Antral motility was quantified by determining a motility index which was calculated with the computer system. The motility index was equivalent to the integrated area between the contractile wave and baseline during a certain fixed period.

2.3. Experimental procedure

The pattern of gastric antral motility in each dog was confirmed to be similar to that reported by Itoh et al. (1977). We found that, in the fasted state, phase III contractions of the migrating motor complex occurred at regular intervals (90–120 min) followed by a period of quiescent motility, whereas in the fed state, regular low-amplitude contractions continued for at least 4 h after feeding in all the dogs.

All drugs were dissolved in saline and injected, in a volume of 0.3 ml/kg, into the saphenous leg vein over 30 s at a constant rate. All drug doses are in terms of the free base. In the fasted state, just after the termination of phase III contractions, motility indices for 7 sequential 10-min periods were measured. *m*-Chlorophenylbiguanide was given after measurement of the first motility index. In the antagonist study, antagonists were given just after the termination of the phase III contractions (corresponding to 10 min before *m*-chlorophenylbiguanide administration). Canned food (Dog meal of Hokuetsu, 20 g/kg body weight; Hokeutsu Foods Lab., Niigata, Japan) was given to switch gastrointestinal motility from the fasted to the fed state. In the fed state, motility indices for 7 sequential 10-min periods were measured from 110–140 min after feeding. *m*-Chlorophenylbiguanide was given after measurement of the first motility index (corresponding to 2–2.5 h after food) and antagonists were given 10 min before *m*-chlorophenylbiguanide.

All experiments were conducted with paired control (vehicle treatment) and drug treatment observations for each dog. Each animal was used for experiments at an interval of 3 days or more. All experiments were performed according to the regulations of the Animal Ethical Committee of Yamanouchi Pharmaceutical.

2.4. Statistics

All results are presented as the means ± S.E.M. Statistical analysis of the data was performed with Student's *t*-test for paired data. Probability values of < 0.05 were considered significant.

2.5. Drugs

m-Chlorophenylbiguanide and ramosetron hydrochloride were synthesized at Yamanouchi Pharmaceutical (Tsukuba, Ibaraki, Japan). Atropine sulfate was purchased from Sigma (St. Louis, MO, USA).

3. Results

3.1. Effect of m-chlorophenylbiguanide on gastric antral motility in the fasted state

m-Chlorophenylbiguanide (0.1–1 mg/kg i.v.) dose dependently stimulated gastric antral motility in the fasted state. The contractile response was maintained over the observation period (for 60 min after administration). The

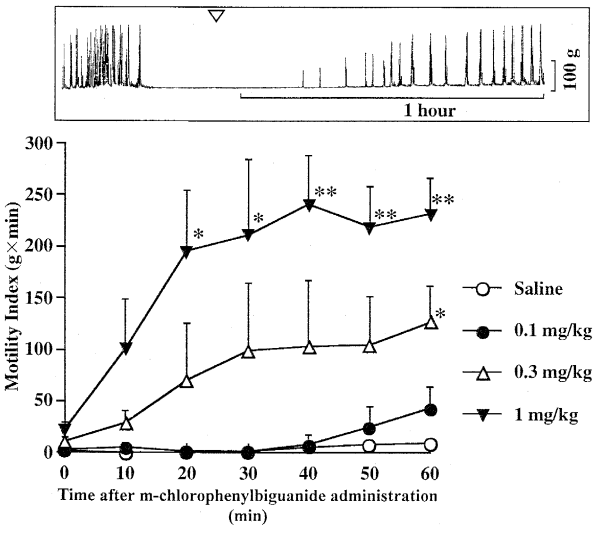

Fig. 1. The effect of intravenously administered *m*-chlorophenylbiguanide on canine gastric antral motility in the fasted state. Just after the termination of phase III contractions, motility indices for 7 sequential 10-min periods were measured. The drug was given after the measurement of the first motility index. Each point represents the means with S.E.M. for four dogs. $^{*}P < 0.05$; $^{**}P < 0.01$, significant differences from saline treatment. Inset: A typical effect of *m*-chlorophenylbiguanide at 1 mg/kg i.v. The drug was administered at the point indicated by an open triangle.

amplitude of most *m*-chlorophenylbiguanide (1 mg/kg i.v.)-induced contractions reached the level of natural phase III contractions (Fig. 1). Hyperpnea occurred in all animals when *m*-chlorophenylbiguanide was given at 1 mg/kg i.v. This response occurred immediately after the start of the injection and ended within 1 min. No other behavioral change was observed after *m*-chlorophenylbiguanide application.

3.2. Effect of antagonists on m-chlorophenylbiguanide-induced contractions in the fasted state

Ramosetron at doses of 0.0003–0.03 mg/kg i.v. dose dependently inhibited *m*-chlorophenylbiguanide (1 mg/kg i.v.)-induced contractions (Fig. 2A). In the presence of ramosetron (0.03 mg/kg i.v.), no contractions occurred during the observation period after *m*-chlorophenylbiguanide (1 mg/kg i.v.) administration. Ramosetron at 0.003 mg/kg i.v. or more completely inhibited the occurrence of hyperpnea caused by *m*-chlorophenylbiguanide. As shown in Fig. 2B, atropine (0.03 and 0.1 mg/kg i.v.) strongly inhibited *m*-chlorophenylbiguanide (1 mg/kg i.v.)-induced contractions. However, low-amplitude contractions occurred sporadically even in the presence of atropine (0.03 or 0.1 mg/kg i.v.). It was confirmed that atropine at 0.03 mg/kg i.v. completely inhibited the contractile response to a gastroprokinetic drug, renzapride at 0.1 mg/kg i.v. (data not shown).

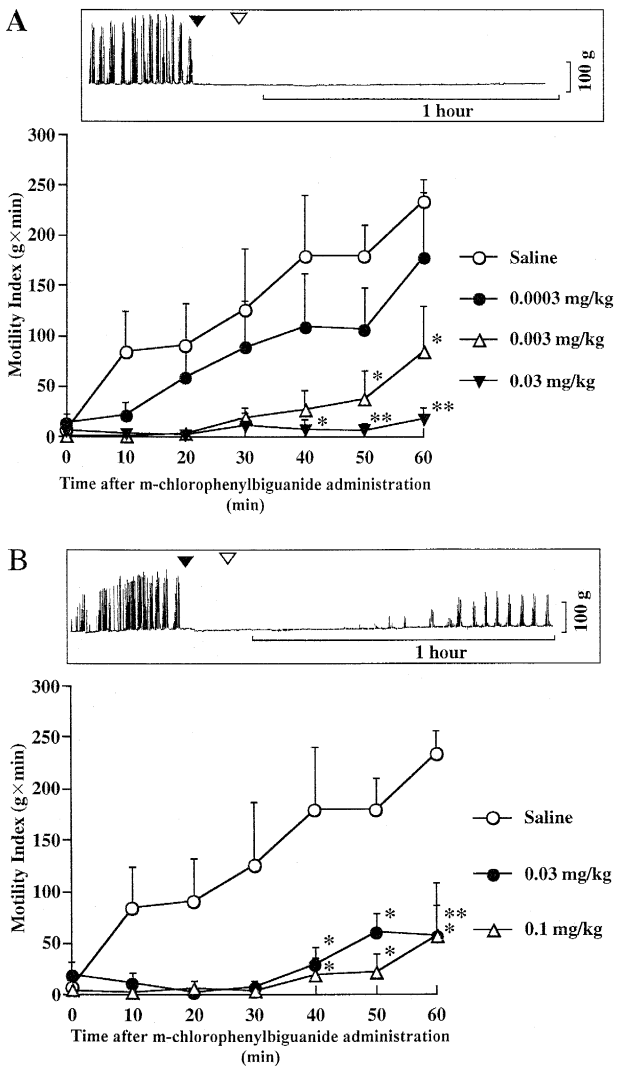

Fig. 2. Effects of ramosetron (A) and atropine (B) on *m*-chlorophenylbiguanide (1 mg/kg i.v.)-induced contractions in the fasted state. Antagonists were intravenously given 10 min before *m*-chlorophenylbiguanide administration. Each point represents the means with S.E.M. for four dogs. * $P < 0.05$; ** $P < 0.01$, significant differences from saline treatment. Insets in A and B show typical effects of ramosetron (0.03 mg/kg i.v.) and atropine (0.03 mg/kg i.v.), respectively. Antagonists and *m*-chlorophenylbiguanide were administered at the points indicated by the closed and open triangle, respectively.

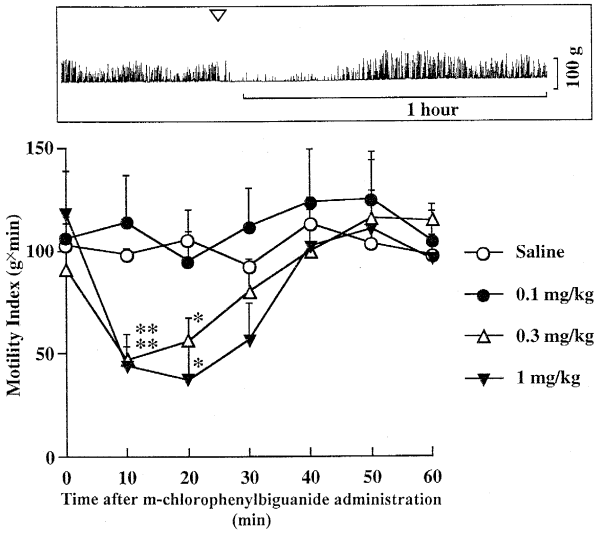

Fig. 3. The effect of intravenously administered *m*-chlorophenylbiguanide on canine gastric antral motility in the fed state. Motility indices for 7 sequential 10-min periods were measured from 110–140 min after canned food was given. Drugs were given just after the measurement of the first motility index (corresponding to 2–2.5 h after food). Each point represents the means with S.E.M. for four dogs. * $P < 0.05$; ** $P < 0.01$, significant differences from saline treatment. Inset: A typical effect of *m*-chlorophenylbiguanide at 1 mg/kg i.v. The drug was administered at the point indicated by the open triangle.

3.3. Effect of m-chlorophenylbiguanide on gastric antral motility in the fed state

m-Chlorophenylbiguanide (0.1–1 mg/kg i.v.) dose dependently reduced the amplitude of gastric antral contractions in the fed state. The inhibition of gastric motility by *m*-chlorophenylbiguanide (1 mg/kg i.v.) persisted for about 30 min after administration (Fig. 3).

3.4. Effect of ramosetron on m-chlorophenylbiguanide-induced inhibition of gastric antral motility in the fed state

Ramosetron reversed *m*-chlorophenylbiguanide (1 mg/kg i.v.)-induced inhibition of gastric antral motility in the fed state (Fig. 4). The effect of ramosetron was dose-dependent (0.0003–0.03 mg/kg i.v.) and the dose range

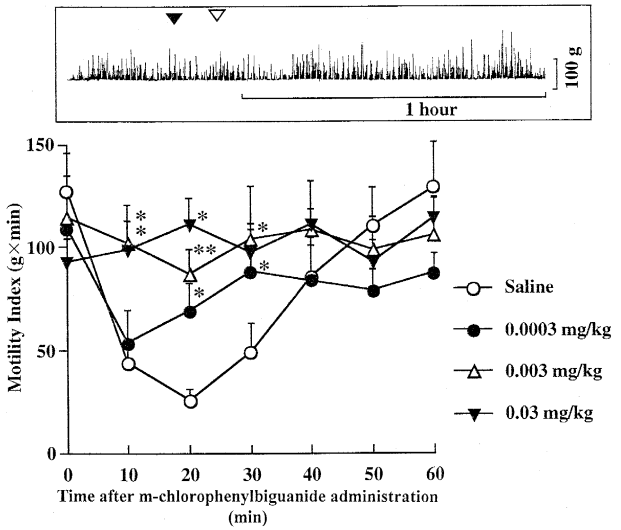

Fig. 4. The effect of ramosetron on *m*-chlorophenylbiguanide (1 mg/kg i.v.)-induced inhibition of motility in the fed state. Ramosetron was intravenously given 10 min before *m*-chlorophenylbiguanide administration. Each point represents the means with S.E.M. for four dogs. * $P < 0.05$; ** $P < 0.01$, significant differences from saline treatment. Inset: A typical effect of ramosetron (0.03 mg/kg i.v.). Ramosetron and *m*-chlorophenylbiguanide were administered at the points indicated by the closed and open triangle, respectively.

was almost the same as that required to inhibit *m*-chlorophenylbiguanide (1 mg/kg i.v.)-induced contractions in the fasted state.

4. Discussion

In the present study, *m*-chlorophenylbiguanide and ramosetron were used to pharmacologically activate and block 5-HT_3 receptors, respectively. *m*-Chlorophenylbiguanide has been reported to show high affinity for 5-HT_3 receptors of mammalian species (Hoyer et al., 1994), and has been shown to induce 5-HT_3 receptor-mediated biological responses, including depolarization in the rat vagus nerve, the von Bezold-Jarisch reflex in anaesthetized cats (Kilpatrick et al., 1990), emesis in ferrets (Kamato et al., 1993) and a transient inward current in whole-cell voltage-clamped N1E-115 neuroblastoma cells (Sepulveda et al., 1991). In contrast, this compound shows negligible pharmacological action at other 5-HT receptor subtypes (Higgins et al., 1993; Kilpatrick et al., 1990; Van Wijngaarden et al., 1990). Ramosetron has been reported to exert potent 5-HT_3 receptor blocking activity in the isolated guinea-pig colon and in anesthetized rats but to interact slightly with other 5-HT and a wide range of non-5-HT receptors (Ito et al., 1995; Miyata et al., 1991, 1995). In the light of these findings, *m*-chlorophenylbiguanide and ramosetron are considered to be suitable compounds with which to selectively activate and block 5-HT_3 receptors, respectively.

The results of the present study showed that *m*-chlorophenylbiguanide has opposite effects in the fasted and in the fed state, being stimulatory and inhibitory, respectively. Both stimulatory and inhibitory effects were completely inhibited by ramosetron, indicating that they were actually mediated via activation of 5-HT_3 receptors. This finding means that the activation of 5-HT_3 receptors has a double-sided potential capability for regulation of gastric motility. The outcome of the activation seems to depend on the state of gastric motility. It is possible that a stimulatory component appears when the activation occurs in the phase I period of the fasted state, during which gastric motility is not tonically stimulated by endogenous factors. Feeding changes the gastric motility pattern from that in the fasted state to that of the fed state, during which gastric motility seems to be tonically stimulated by feeding-induced factors, such as hormonal factors and mechanical stimulation of the gastric wall by contents. An inhibitory component of the capability may be unmasked in this latter state.

It has been shown that a selective 5-HT_3 receptor antagonist, ondansetron, inhibits the initiation of high-amplitude (natural phase III) contractions in the fasted state in dogs and humans (Itoh et al., 1991; Wilmer et al., 1993; Yoshida et al., 1991). The induction of high-amplitude contractions by 5-HT_3 receptor activation in the fasted state, first shown in the present study, is consistent with this evidence. These data on the effects of 5-HT_3 receptor agonists and antagonists on gastric antral motility strongly indicate that 5-HT_3 receptors play a key role in the induction of high-amplitude gastric contractions in the fasted state. Because ondansetron has been reported to have no effect on gastric motility in the fed state in dogs (Itoh et al., 1991; Yoshida et al., 1991), 5-HT_3 receptors may play only a minor role in the physiological regulation of gastric motility in the fed state. However, the present study was the first to show that pharmacological activation of 5-HT_3 receptors leads to inhibition of gastric motility in the fed state. It has been reported that the activation of 5-HT_3 receptors is involved in the delay of gastric emptying induced by a trichotecene mycotoxin (Fioramonti et al., 1993). Our results may concern an extraordinary activation of 5-HT_3 receptors in such special situations.

Because 5-HT_3 receptors are not found on non-neural tissues, they seem not to finally mediate gastric smooth muscle contractions induced by *m*-chlorophenylbiguanide. It is possible that *m*-chlorophenylbiguanide stimulates or inhibits gastric smooth muscle contractions not directly but indirectly, via the release of stimulatory and inhibitory transmitter(s). It is known that the activation of 5-HT_3 receptors induces the release of various transmitters (Bogers et al., 1991; Fox and Morton, 1990; Ramirez et al., 1994). The stimulatory effect of *m*-chlorophenylbiguanide was strongly inhibited by pretreatment with atropine in the present study, suggesting that acetylcholine is a main transmitter mediating the *m*-chlorophenylbiguanide-induced contractions in the fasted state. Our results also suggest, however, that there is a small contribution of

another transmitter to this effect because a small number of contractions with low amplitude persisted even in the presence of an adequate dose of atropine. Because atropine strongly inhibited basal motility in the fed state (data not shown), it is less likely that acetylcholine mediates the inhibitory effect of *m*-chlorophenylbiguanide in the fed state. The transmitters mediating the inhibitory effect of *m*-chlorophenylbiguanide remain to be elucidated.

The putative 5-HT_{1P} receptor is located in the mammalian intestinal neurons where it seems to mediate slow excitatory postsynaptic potentials of the cell body of myenteric and submucous plexus neurons induced by neurally released 5-HT (Mawe et al., 1986). Thus, stimulation of this receptor may cause the release of other enteric excitatory or inhibitory neurotransmitters. The affinities of *m*-chlorophenylbiguanide and ramosetron for this receptor have not been determined so far. Therefore, it cannot be completely excluded that this receptor is involved in the *m*-chlorophenylbiguanide-induced responses. However, the possibility is made less likely by the fact that ramosetron was reported to have no effect on slow excitatory postsynaptic potentials induced by 5-HT in enteric neurons (Ito and Tamura, 1995). If the effect of *m*-chlorophenylbiguanide on gastric motility was mediated through the production of enteric slow excitatory postsynaptic potentials, such an effect must not have been inhibited by ramosetron.

The results of the present study indicate that pharmacological activation of 5-HT_3 receptors has opposite effects on canine gastric antral motility in the fasted and in the fed state, being stimulatory and inhibitory, respectively. The stimulatory effect seems to be mediated mainly via the release of acetylcholine. The 5-HT_3 receptors are located on various neuronal tissues, such as the central nervous system, pre- and postganglionic autonomic neurons and neurons of the sensory and enteric nervous systems (Hoyer et al., 1994). It remains to be elucidated which of these 5-HT_3 receptors mediates the effect of *m*-chlorophenylbiguanide.

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