EFFECT OF TRIMEBUTINE MALEATE ON BETHANECHOL-INDUCED CONTRACTIONS OF GASTROINTESTINAL TRACT IN CONSCIOUS AND ANAESTHETIZED DOGS

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Abstract—The effect of trimebutine maleate (TM-906) on bethanechol-induced contractions of the gastrointestinal tract in dogs was studied by means of chronically implanted force transducers, and it was compared with that of metoclopramide and atropine. During the period of motor quiescence in the fasted state of conscious dog, a bolus i.v. injection of 10 to 50 μg/kg bethanechol caused rhythmic contractions in the stomach and small intestine, in which an increase in basal tone was often accompanied in the distal ileum. The stomach responded to a less extent than the small intestine. Intravenous infusion of TM-906 at 3.0 mg/kg-hr for 20 to 60 min reduced the gastric contractions induced by 20 to 30 μg/kg bethanechol in five out of six conscious animals examined. The drug, however, enhanced the contractions of the duodenum and jejunum in a majority of the animals. Metoclopramide (1.8 mg/kg-hr) showed a tendency to potentiate the bethanechol-induced contractions in the stomach and small intestine, and atropine (0.06 mg/kg-hr) diminished them. In pentobarbital-Na anaesthetized dogs, effects of TM-906 on bethanechol-induced contractions resembled those in conscious dogs, inhibition in the stomach and potentiation in the small intestine.

Trimebutine maleate (2-dimethylamino-2-phenylbutyl 3,4,5-trimethoxybenzoate hydrogen maleate; TM-906) has been shown to improve inveterate symptoms such as abdominal pain, constipation, distention, flatulence and/or diarrhoea in patients with irritable bowel syndrome and others (1-3). The drug has been evaluated as effective as metoclopramide in relieving the symptoms (3).

The gastrointestinal motor activity of conscious dogs under a fasting condition is characterized by long lasting motor quiescence and cyclically recurring high-amplitude contractions (interdigestive contractions) (4, 5). In conscious dogs in a fasted state (6), intravenous infusion of TM-906 shortened the interval between the recurring interdigestive contractions in the stomach and small intestine, and caused a decrease in the number and duration of the interdigestive contractions of the gastric antrum. In some cases of fasting dogs, irregular fasted motor activity was observed, which consisted of weak contractions occurring persistently and randomly. In these dogs, a bolus i.v. injection of TM-906 depressed the contractions of the antrum. On the other hand, metoclopramide showed only enhancement on the fasted motor activity, and the effects were clearly different from those of TM-906.

Itoh et al. (7) postulated that one of the main factors disturbing the regularity of fasting motor activity was acid-secretion. In the present experiment, the contractile response to an acid-secreting cholinergic agent, bethanechol (8), and the effect of
TM-906 on it were studied in conscious dogs and, in part, anaesthetized dogs with chronically implanted force transducers.

Materials and Methods

Six mongrel dogs weighing 12.5 to 20.0 kg were used. Animals were anaesthetized with 30 mg/kg pentobarbital-Na i.v.; the abdominal cavity was opened and strain gauge force transducers (Implantable Force Transducer F-121S, Star Medical Inc.) were implanted on the serosal surface of the gastric body, gastric antrum, duodenum, jejunum and distal ileum, according to the method of Itch et al. (4) and Itoh (5). The transducer was sutured in a direction to record contraction of the circular smooth muscle layer. The dogs were allowed to recover for 12 to 17 days and were trained to lie quietly for many hours before the start of experiments.

Experiments in conscious dog: After 19 to 20 hr of fasting, when the gastrointestinal motor activity had changed from the fed state into the fasted state and the interdigestive contractions of the stomach had occurred once or twice (6), the dog was placed in a small (90 cm x 120 cm) experimental room where the animal was allowed to lie or move about to such a degree that lead wires or cannula were not damaged for 5 to 9 hr. The experiments were done at more than a 5 day interval in each animal. Contractile activity was recorded on an ink-writing oscillograph through strain amplifiers (6M-52 or 6M-53, San-ei Sokki) connected to the transducers. Other details were described previously (6).

There were cases where, 70 to 90 days after implantation, some of the implanted transducers became unresponsive to contraction because of the breaking down of the wire in the body or because of the increase in exuberant granulation or scar formation between transducer and the serosal surface.

Intravenous injection of bethanechol was repeated 20 to 30 min after the cessation of the cyclically occurring interdigestive contractions of the stomach (Fig. 2A and 2B). To determine the effect of test drugs, the response to bethanechol during i.v. infusion of a test drug was compared with the response before infusion.

Experiments in anaesthetized dog: The dogs with chronically implanted transducers were anaesthetized with pentobarbital-Na 30 mg/kg i.v. in the fasted state, and the effect of TM-906 on bethanechol-induced contractions was examined in a similar way as in conscious dogs.

Drugs: Drugs used were bethanechol hydrochloride (synthetized in Tanabe Seiyaku), TM-906 (synthetized in Tanabe Seiyaku), metoclopramide (Fujisawa Pharmaceutical) and atropine sulfate (Tanabe Seiyaku). Drugs were administered through the cannula inserted to the cephalic vein.

Results

1) Conscious dog

A) Response to a bolus i.v. injection of bethanechol: A bolus injection of bethanechol in doses of 10 to 50 µg/kg i.v. at 20 to 30 min after the cessation of the interdigestive contractions of the stomach (during the period of motor quiescence) elicited rhythmic contractions at the stomach and small intestine in a conscious dog. At the same time, salivation was observed. Each of the injections of bethanechol repeated after the interdigestive contractions caused similar responses (Fig. 1A), although the first response was sometimes smaller than the others. In the distal ileum, an increase in basal tone often accompanied the rhythmic contractions at the stomach and small intestine in a conscious dog. At the same time, salivation was observed. Each of the injections of bethanechol repeated after the interdigestive contractions caused similar responses (Fig. 1A), although the first response was sometimes smaller than the others. In the distal ileum, an increase in basal tone often accompanied the rhythmic contractions (Figs. 3A and 4a). In some animals, 10 to 20 µg/kg of bethanechol failed to elicit the contractions of the gastric antrum (Fig. 1B and Table 1). Longer latency between the injection and the onset
of contraction was needed in the stomach than in the small intestine, where the contraction by bethanechol developed immediately after the injection (Table 1). Moreover, the maximum amplitude of the bethanechol-induced contractions in the antrum was relatively smaller than in the small intestine as compared with the maximum amplitude of the interdigestive contractions (Fig. 4a and Table 1).

**B) Effect of TM-906 on bethanechol-induced contractions:** Intravenous infusion of 3.0 mg/kg-hr of TM-906 for 20 to 60 min showed different effects on the contractions of the stomach from those of the small intestine induced by bethanechol in a dose of 20 or 30 μg/kg (Fig. 2 and Table 2). In the gastric body and antrum, TM-906 inhibited the contractions in 5 out of 6 animals. On the other hand, as shown in Fig. 2B and 2C, TM-906 produced potentiation of the contractile force in the duodenum. At the small intestine, the potentiating effect was observed in half or more of the animals examined, though one animal showed inhibition in the jejunum and ileum (Table 2).

**C) Effect of metoclopramide:** Figure 3 shows examples of the effect of metoclopramide on bethanechol-induced contractions. Metoclopramide at 1.8 mg/kg-hr produced a potentiation of the amplitude and a prolongation of the development of the contractions in the stomach. The drug also showed a tendency to potentiate the contractions in the small intestine, and no inhibitory effects by metoclopramide were observed in any of the regions (Table 2). These effects persisted after termination of

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**Table 1. Contractile response to bethanechol in the conscious dog**

| Dose (μg/kg) | Gastric antrum | Duodenum | Jejunum | Ileum |
|--------------|----------------|-----------|---------|-------|
| **Latency (min)** | 4.33±1.73<sup>1</sup> | 0.13±0.13<sup>*</sup> | 0.25±0.25<sup>*</sup> | 0.22±0.22<sup>*</sup> |
| (n=6<sup>1</sup>) | (n=8) | (n=8) | (n=8) |
| **Amplitude** | 0.40±0.09 | 0.60±0.10<sup>1</sup> | 0.50±0.07<sup>1</sup> | 0.81±0.07<sup>1</sup> |
| **Latency (min)** | 4.40±1.03 | 0.00±0.00<sup>1</sup> | 0.50±0.29<sup>1</sup> | 0.26±0.25<sup>1</sup> |
| (n=5) | (n=4) | (n=8) | (n=4) |
| **Amplitude** | 0.38±0.03 | 0.50±0.29 | 0.48±0.14<sup>1</sup> | 0.95±0.16<sup>1</sup> |

Data were obtained from the first response of one or two experiments in six animals. Bethanechol was injected 20 to 30 min after cessation of interdigestive contractions of the gastric antrum. Means±S.E. are given. Latency: time between injection and onset of contraction. Amplitude: expressed as a value relative to that of the maximum amplitude of interdigestive contractions which is taken as 1. 1No contractions by bethanechol were observed at the gastric antrum in 2 out of 8 experiments, and these data were excluded. *P<0.05 compared to the gastric antrum.
Fig. 2. Examples of the effect of TM-906 (3.0 mg/kg-hr) on bethanechol-induced contractions in conscious dogs. A: Interdigestive contractions and contractile response to 20 μg/kg bethanechol i.v. The records shown in B (a to d) are the same as those in the frames of A (a to d) at a faster paper speed. C: Response to 20 μg/kg bethanechol before TM-906 infusion (a), during infusion of TM-906 (43 min after start of infusion (b) and 80 min after termination of infusion (c). TM-906 was infused for 50 min in B and C.

Table 2. Effect of TM-906, metoclopramide and atropine on bethanechol induced contraction

| Drug         | Dose   | Effect (a) | No. of experiments, effective/total |
|--------------|--------|------------|------------------------------------|
|              |        |            | Gastric antrum | Duodenum | Jejunum | Ileum |
| TM-906       | 3.0 mg/kg-hr | Potentiation | 1/6 | 4/5 | 3/5 | 2/4 |
|              |        | No effect  | 5/6 | 1/5 | 1/5 | 1/4 |
| Metoclopramide | 1.8 mg/kg-hr | Potentiation | 2/2 | 2/3 | 2/3 | 1/2 |
|              |        | No effect  | 1/3 | 1/3 | 1/3 | 1/2 |
| Atropine     | 0.06 mg/kg-hr | Potentiation | 2/2 | 2/2 | 2/2 | 2/2 |

a) Bethanechol at 20–30 μg/kg was administered i.v. 20–30 min after termination of interdigestive contraction in the gastric antrum.
b) Response to bethanechol during infusion of the test drug was compared with the control response.
infusion when metoclopramide was infused for 60 min (Fig. 3A).

D) Effect of atropine: An example of the effect of atropine infusion on the contractions by bethanechol is shown in Fig. 4. The drug in a dose of 0.06 mg/kg-hr depressed these contractions completely (Table 2). Moreover, infusion of atropine over 30 min suppressed the development of the interdigestive contractions.

2) Effect of TM-906 in anaesthetized dog

In a pentobarbital-Na anaesthetized dog, bethanechol at 20 to 30 μg/kg i.v. produced rhythmic contractions. The antrum responded to bethanechol immediately after injection (Fig. 5a); the latency between the injection

![Fig. 3. Example records of the effect of 1.8 mg/kg-hr metoclopramide on bethanechol-induced contractions in conscious dogs. Bethanechol was injected at 20 μg/kg i.v. in A and B. a: Control response. b: During metoclopramide infusion (45 and 32 min after start of infusion in A and B, respectively). c: 90 and 40 min after end of infusion in A and B, respectively. Metoclopramide was infused for 60 min in A and 30 min in B.](image)

![Fig. 4. Conscious dog. Interdigestive contractions and contractile response to 20 μg/kg bethanechol i.v. (a) and response to bethanechol during infusion of atropine at 0.06 mg/kg-hr (72 min after the start of infusion) (b).](image)
and the onset of contraction observed in the conscious state disappeared under the anaesthetized condition (cf. Fig. 3A).

TM-906 at 3.0 mg/kg-hr suppressed the bethanechol-induced contractions in the stomach and enhanced them in the duodenum, jejunum and ileum (Fig. 5). These effects were qualitatively similar to those observed in conscious dogs.

**Discussion**

In the present experiments with conscious dogs, TM-906, metoclopramide and atropine showed effects in three different ways on the gastrointestinal contractions induced by a cholinergic agent, bethanechol. An anticholinergic agent, atropine, inhibited the contractions in all the regions, whereas metoclopramide introduced enhancement of the contractions in the stomach and showed a tendency to potentiate those in the small intestine. Kowalewski and Kolodej (9) have shown that metoclopramide augments the action of acetylcholine using isolated canine stomach, and the present results support this.

The effect of infusion of metoclopramide for 60 min persisted 90 min after cessation of the infusion. In the previous study (6), the same dose of metoclopramide infused over 60 min enhanced the interdigestive contractions, and the effect persisted after the cessation of the infusion. Bateman and Davies (10) have shown that in dogs, the elimination half-life of metoclopramide in plasma after 0.14 to 0.83 mg/kg i.v. is 107.5 to 186.2 min, and this is irrespective of the doses. Consequently, the persistent effect of metoclopramide in our experiment may be introduced by a relatively high plasma concentration of the drug.

TM-906 showed diverse effects on different regions. The drug depressed the bethanechol-induced contractions in the stomach, and it enhanced the contractions in the duodenum in a majority of the animals. The result resembled that with pentobarbital-Na anaesthetized dogs. In the preceding study (6), a bolus i.v. infusion of TM-906 produced a transient suppression of the anomalous, continuous low-amplitude contractions occurring randomly observed in some of the fasting dogs. The anomalous motor activity

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**Fig. 5.** Anaesthetized dog (the same animals as in Fig. 3A). Bethanechol was injected at 20 µg/kg i.v. Response before TM-906 (a), during infusion of 3.0 mg/kg of TM-906 at 15 min (b) and 45 min (c) after the start of infusion, and at 25 min (d) and 55 min (e) after termination of infusion. TM-906 was infused for 60 min.
in the fasted state is considered to be caused partly by an increase in spontaneous secretion of acid in the stomach (7). The depressive effect of TM-906 on the contractions of the stomach induced by a strong secretagogue (bethanechol) might be related to suppression of the spontaneous increase in acid-secretion.

In the present experiment using conscious dogs, the stomach responded to bethanechol less sensitively than the small intestine. It is considered that the relatively long latency in the stomach is brought about via the central nervous system because it decreased in anaesthetized dogs (Fig. 5a). Burnstock and Costa (11) have described that inhibitory neurones in the stomach are controlled largely by extrinsic nerves; whereas, in the small intestine, they appear to be controlled solely by intramural nerves without extrinsic connections. Therefore, it is postulated that in conscious animals, there may be excitation by bethanechol of the centrally connected inhibitory control pathways to the stomach, the efferent impulses of which could cancel the peripheral events: for example, the development of the contractions by direct action of bethanechol on the smooth muscle cells.

The development of the contraction with some delay in the stomach still remains to be explained. Fujii and Takasugi (12) showed that in the denervated pouch of an anaesthetized dog, the weak contraction that developed 5 to 20 min after stimulation of the central cut end of the vagal or splanchnic nerve were abolished by an histamine H1-receptor antagonist, chlorpheniramine, and the release of histamine was mediated via endogenous gastrin.

Motility of the gastrointestinal tract will be explained with rather complicated nervous and/or hormonal control systems (13, 14). Further experiments are needed to elucidate the differential actions of TM-906, as well as bethanechol, on the stomach and small intestine.

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