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Effect of a new Colonic Prokinetic Compound, T-1815, on Gastrointestinal Motility in Anesthetized and Conscious Fasted Dogs

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Abstract

Effect of T-1815, a new colonic prokinetic compound, on gastrointestinal motility was studied in anesthetized and conscious dogs fasted for 24 hr before experiment. In anesthetized dogs, intravenous injection of T-1815 in doses of 0.3-3.0 mg/kg caused a biphasic effect on the gastric motility, a slight decrease followed by a slight increase. While the compound elicited only an increase in motility of the duodenum, jejunum and colon. In the colon, high-amplitude contractions were observed in 2 out of 5 animals at 1 mg/kg, i.v. of T-1815 and 4 out of 5 animals at 3 mg/kg. Bethanechol at 0.01 mg/kg, i.v. produced only a potentiation of the motility in all of the sites, but never induced high-amplitude contractions in the colon. During the interdigestive state in conscious dogs, intravenous T-1815 at 1 and 3 mg/kg caused contractions similar to the interdigestive phase III contractions at the stomach and duodenum in only 2 out of 7 experiments, and colonic motility was slightly depressed at 3 mg/kg. Oral administration of T-1815 at 30 and 50 mg/kg did not elicit the phase III-like contractions but produced persistent contractions at the stomach and duodenum in 2 out of 4 conscious animals during the interdigestive state. In the proximal and middle colon, high-amplitude contractions were observed in 5 out of 7 animals by 10-50 mg/kg, p.o. of T-1815. From the above results, it is concluded that the pharmacological effect of T-1815 on gastrointestinal motility is different from that of the cholinergic agonist. In addition, T-1815 seems to have a characteristic to induce high-amplitude contractions which are known to be closely related to defecation.

Key words: gastrointestinal motility, anesthetized dog, conscious dog, high-amplitude contractions, T-1815

Introduction

T-1815 is a new colonic prokinetic compound which reverses the delay of colonic propulsion induced by clonidine and loperamide in mice and rats, and causes neither diarrhea nor tolerance (Yamada and Onoda, 1992; Yamada and Onoda, 1993). It was of interest to clarify mechanisms of the action of the compound on the motility of the gastrointestinal tract, especially of the colon, which might be involved in its strong colonic propulsive activity. In the present study, effects of T-1815 on the gastrointestinal motility was studied in anesthetized and

conscious dogs.

Methods

1. Anesthetized dogs

Five (4 male and 1 female) adult dogs, weighing 11-13 kg, were used. After fasting for 24 hr, the animals were anesthetized with 30 mg/kg, i.v. pentobarbital sodium, the abdominal cavity was opened and strain gauge force transducer (F-12IS, Star Medical Inc.) each was sutured on the serosal surface of the gastric body (10-15 cm proximal to the pylorus), gastric antrum (3-5 cm to the pylorus), duodenum (10-15 cm distal to the pylorus), jejunum (midportion of the small intestine), proximal colon (ascending, 5-7 cm distal to the ileo-cecal junction) and distal colon (descending, 15-20 cm to the ileo-cecal junction). Contractile activity of the circular muscle was recorded on a Linearcorder (WR-3101, Graphtec) via strain amplifiers (8M-52, NEC-San'ei). Continuous infusion of pentobarbital at the i.v. dose of 4 mg/kg/hr was kept during the experiment. In each animal, i.v. administration of bethanechol at 0.01 mg/kg was repeated twice or three times to ascertain approximately constant responses, and then T-1815 (0.3, 1.0 and 3.0 mg/kg) was administered i.v. in a dose-increasing manner at intervals of 30 to 40 min. Tracing of the contractions from 10 min before to 30 min after administration of the drug was divided into 10 min-intervals and the area under the curve (AUC) in each 10 min-division was measured by a digitizer (Mitablet-II, Graphtec). Effect of the test drug was determined by expressing the AUC value as percent of the value before administration of the drug.

2. Conscious dogs

Seven (5 male and 2 female) adult dogs, weighing 18-20 kg, were used.

Implantation of the strain gauge force transducers (F-12IS, Star Medical Inc.) was made under pentobarbital anesthesia (30 mg/kg, i.v.). In 5 animals, the transducers were sutured on the serosal surface of the gastric antrum (3-5 cm proximal to the pylorus), duodenum (10-15 cm distal to the pylorus) and middle (transverse) colon. In the other 2 dogs, the transducers were sutured on the proximal (ascending), middle (transverse) and distal (descending) site of the colon. At the beginning of the experiment, the lead wires of the transducers were connected to strain amplifiers (8M-52, NEC-San'ei). Contractile activity of the circular smooth muscle was recorded on a Linearcorder (WR-3701, Graphtec), and also on a data recorder (DFR-3315, Sony Magnescale) in order to replay them later. On days without an experiment, the animals were fed once a day in the morning. They usually had the habit of defecation in their cages within 2 hr after feeding. Experiments were started about 2 weeks after the surgery and the animals were fasted for about 24 hr before experiment so that they were in the interdigestive state (Yamada *et al.*, 1982). Recording was continued for 6-10 hrs on the day of experiment, and repeated at intervals of 5 days or more in each animal.

Other details were described previously (Yamada *et al.*, 1982; Yamada *et al.*, 1983).

3. Drugs

T-1815, bethanechol chloride and pentobarbital sodium were synthetized in Tanabe Seiyaku. T-1815 was administered i) intravenously, in a physiological saline solution,

through the cannula inserted into the cephalic vein, or ii) orally in a gelatin capsule (J.P. No. 00).

Results

1. *Anesthetized dogs*

A bolus injection of bethanechol at the i.v. dose of 0.01 mg/kg potentiated the spontaneous-

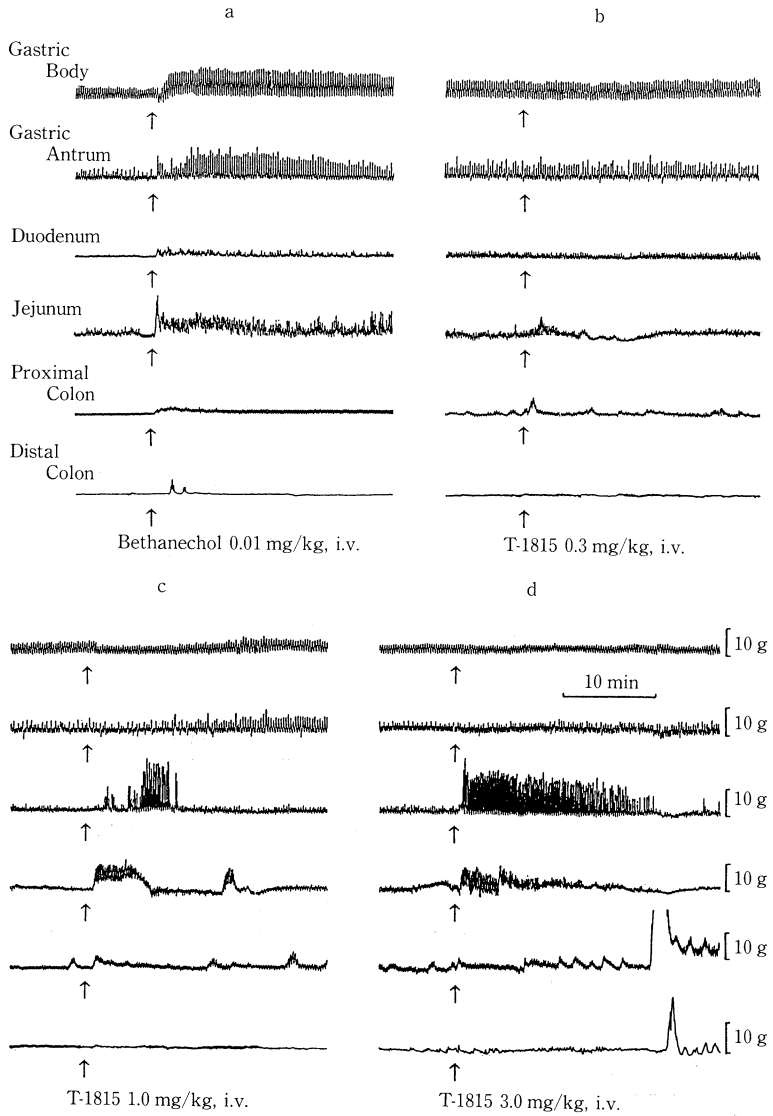

Fig. 1. A typical record of the effects of bethanechol and T-1815 on gastrointestinal motility in an anesthetized dog.
Note that high-amplitude contractions occurred at the proximal and distal colon about 20 min after administration of T-1815 at 3.0 mg/kg, i.v. (**d**).

ly occurring contractions at all sites of the gastrointestinal tract recorded in anesthetized dogs (Fig. 1-a). However, T-1815 at intravenous doses of 0.3-3.0 mg/kg caused divergent motility effects between the stomach and intestine. In the gastric body and antrum, T-1815 caused both a slight decrease in amplitude and a fall in tone followed by a slight increase in amplitude of the spontaneous contractions (Fig. 1, 2). In the small and large intestine, T-1815 caused only a potentiating effect (an increase in amplitude and/or in tone of the contractions, Fig. 1 and 2).

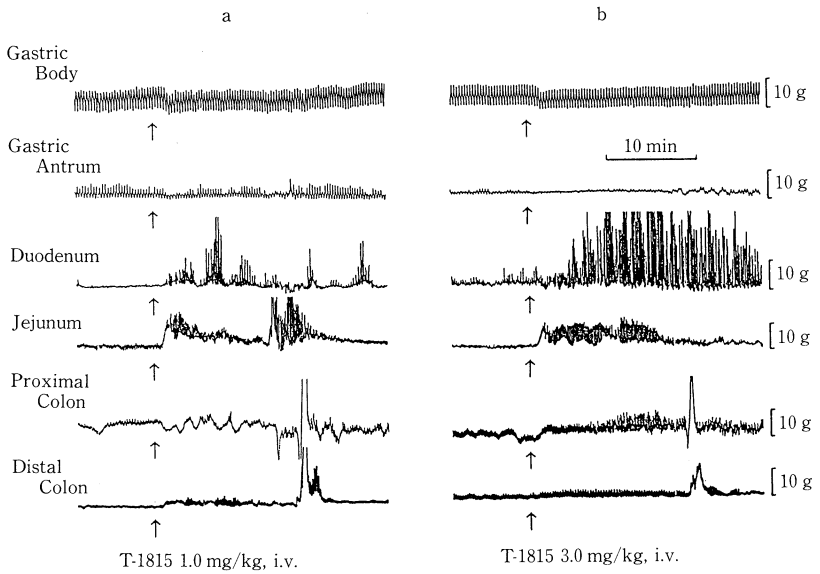

Fig. 2. A record of the effect of T-1815 on gastrointestinal motility in an anesthetized dog. Note that T-1815 at 1.0 and 3.0 mg/kg, i.v. caused high-amplitude contractions at the proximal and distal colon.

Table 1. Effects of bethanechol and T-1815 on gastrointestinal motility in anesthetized dogs

Drug / Dose / Site	Bethanechol	T-1815		
	0.01 mg/kg, i.v.	0.3 mg/kg, i.v.	1.0 mg/kg, i.v.	3.0 mg/kg, i.v.
Gastric body	160.7± 34.1	106.3± 9.3	111.2± 7.0	(98.3±11.0) 104.8± 9.9
Gastric antrum	416.3±128.7	(96.3± 2.3) 113.5± 6.6	(97.6±12.3) 106.2± 7.6	(88.3±15.0) 110.5± 5.2
Duodenum	161.1± 19.4	193.1±63.2	213.0±42.3	289.7±48.2
Jejunum	169.1± 32.6	103.3± 4.4	179.4±13.0	225.5±27.5
Proximal colon	184.5± 24.6	118.2± 8.4	174.6±27.8	196.3±85.5
Distal colon	176.4± 40.9	146.1±23.8	165.6±33.2	148.2±31.3

Area under the tracing was measured.
Numerals show the percentage of the pre-dosing value which is taken as 100.
( ): Inhibitory response to T-1815

The effects of bethanechol and T-1815 on the motility (the AUC of the contractions) in the stomach and small intestine are summarized in Table 1. The percent values of AUC in all the sites recorded after administration of 0.01 mg/kg, i.v. of bethanechol were over 100%, which means that bethanechol caused only contractile responses, especially in the gastric antrum. However, T-1815 at the i.v. doses of 0.3 to 3 mg/kg caused only small, but biphasic effects on the AUC in the gastric body and antrum. In the duodenum and jejunum, however, T-1815

Table 2. Colonic high-amplitude contractions induced by intravenous administration of T-1815 in anesthetized dogs

Drug	Dose (mg/kg, i.v.)	No. of Exp.	No. of HAC observed	Latency (min)	Time lag (sec)
Bethanechol	0.01	5	0	—	—
T-1815	0.3	5	0	—	—
	1.0	5	2	4.9, 16.0	13, 19
	3.0	5	4	3.9-20.9	17-22

HAC: High-Amplitude Contractions. —: HAC were not observed.
Latency: Time period between administration of T-1815 and onset of HAC
Time lag: Time difference of onset of HAC between proximal and distal colon

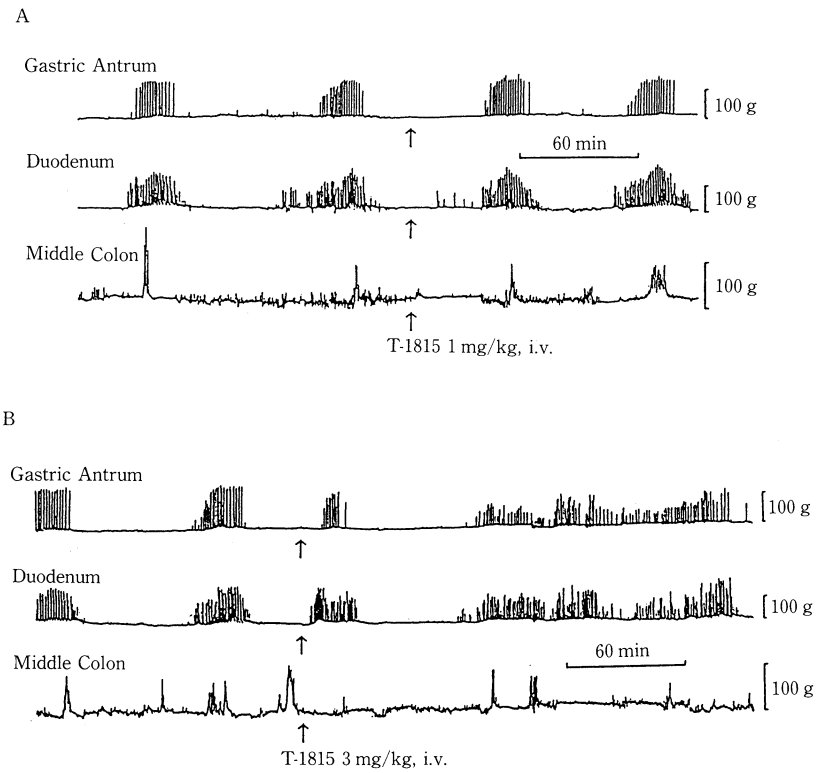

Fig. 3. Records of the effect of T-1815 at 1 mg/kg, i.v. (A) and 3 mg/kg, i.v. (B) on motility of the gastric antrum, duodenum and middle colon in conscious dogs.

produced a dose-dependent increase in AUC. The dose of 3 mg/kg T-1815 produced clearly larger AUC values of contractions than 0.01 mg/kg bethanechol. In the colon, T-1815 at 1 and 3 mg/kg, i.v. elicited abrupt increases in colonic contractions (high-amplitude contractions) with latencies of 3.9 to 20.9 min after the administration (Fig. 1, 2). The colonic high-amplitude contractions were observed in 2 out of 5 animals tested at the dose of 1 mg/kg, i.v., and 4 out of 5 at 3 mg/kg, i.v. (Table 2). The high-amplitude contractions in the distal colon were recorded with a time lag of 13 to 22 sec following those in the proximal colon.

2. Conscious dogs

i) Intravenous administration of T-1815

T-1815 was administered intravenously to conscious dogs 20-30 min after cessation of the interdigestive phase III contractions at the stomach. There were no clear responses to the compound at 1 mg/kg in 3 out of 4 animals (Fig. 3-A), although one animal showed contractions

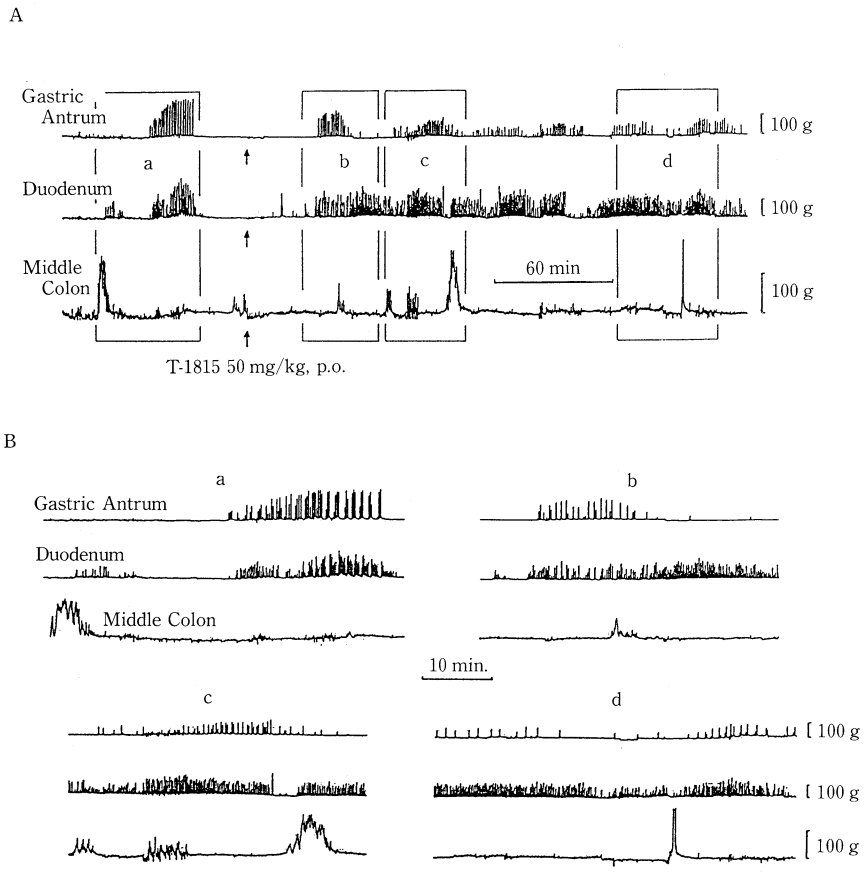

Fig. 4. Records of the effect of T-1815 at 50 mg/kg, p.o. on motility of the gastric antrum, duodenum and middle colon in a conscious dog.
B: Expanded records of A. Note that high-amplitude contractions (off scale) occurred in the colon in **d**.

similar to the interdigestive phase III contractions in the stomach and duodenum. At 3 mg/kg, the phase III-like contractions followed by persistent contractions with relatively low amplitudes were observed in one out of 3 animals (Fig. 3-B). In the colon, T-1815 at 3 mg/kg caused a slight depression of the motility in 2 out of 3 animals (Fig. 3-B).

ii) Oral administration of T-1815

Effect of oral administration of T-1815 during the phase I of the interdigestive state was examined at doses of 30 mg/kg (N=2) and 50 mg/kg (N=2). These dosages caused persistent contractions in 1 out of 2 experiments at the stomach and duodenum (Fig. 4). In the middle colon, the high-amplitude contractions were recorded at the doses of 30 (N=1) and 50 (N=1) mg/kg of T-1815 (Fig. 4-d, Table 3-A).

In order to ascertain where the high-amplitude contractions occur, experiments were performed with two dogs in which transducers were implanted on the proximal, middle and distal sites of the colon. The high-amplitude contractions were observed at the proximal colon in all of the three experiments after oral administration of T-1815 at 10 and 30 mg/kg (Fig. 5, Table 3-B). The contractions migrated to the middle colon in only one experiment. In addition, there were no high-amplitude contractions recorded in the distal colon.

No defecations were observed during the experiments with conscious animals. Moreover, no high-amplitude contractions were recorded in the animals during the control experiment (Table 3).

Discussion

In anesthetized dogs, T-1815 caused a potentiating effect on the spontaneous contractions

Table 3. Colonic high-amplitude contractions induced by oral administration of T-1815 in consicious dogs

A. Transducer was implanted on the middle colon only

Drug	Control		T-1815 30 mg/kg, p.o.		T-1815 50 mg/kg, p.o.	
Dog	Site	Latency	Site	Latency	Site	Latency
A	NR	NR	Middle	210 min	NR	NR
B	NR	NR	NR	NR	—	—
C	NR	NR	—	—	Middle	223 min

B. Transducers were implanted on the proximal, middle and distal colon

Drug	Control		T-1815 10 mg/kg, p.o.		T-1815 30 mg/kg, p.o.	
Dog	Site	Latency	Site	Latency	Site	Latency
D	NR	NR	Proximal & Middle	113 min	—	—
E	NR	NR	Proximal	209 min	Proximal	85 min

NR: High-amplitude contractions were not recorded. —: Not Tested
Site: The site of the colon where high-amplitude contractions occurred
Latency: Time period between administration of T-1815 and onset of high-amplitude contractions

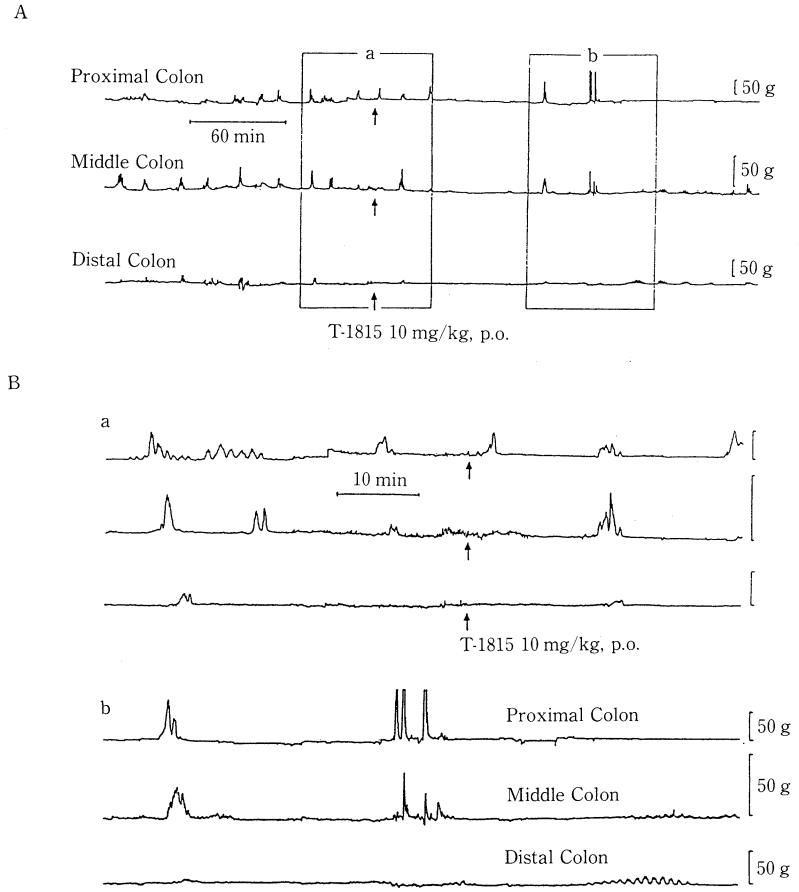

Fig. 5. Example rocord of the effect of T-1815 at 10 mg/kg, p.o. on colonic motility in a conscious dog.
B: Expanded records of A. Note that three high-amplitude contractions were recorded in the proximal colon (b). The first contraction migrated to the middle colon but the other two contractions faded out.

of the small and large intestine, although the compound showed a biphasic effect (a slight decrease followed by an increase) on the contractions of the stomach. In addition, T-1815 at 1 and 3 mg/kg, i.v. produced high-amplitude contractions in the colon. The cholinergic agonist bethanechol never caused high-amplitude contractions in the colon though it produced only contractile responses in all of the sites examined. Thus, the pharmacological property of T-1815 is probably different from that of cholinergic agonists.

Karaus and Sarna (1987) have found that, in conscious dogs, the high-amplitude contractions which migrate to the distal colon precede the spontaneous and chemically induced defecation, and they called these contractions 'giant migrating contractions'. A similarly unique motility of the colon was also observed in humans by Narducci *et al.* (1987) and Moreno-Osset *et al.* (1989). The latter authors demonstrated that the high-amplitude contractions propel the bolus caudad in the colon.

Development of the colonic high-amplitude contractions induced by intravenous T-1815 in anesthetized dogs had a time lag of 13 to 22 sec between the proximal and distal site, suggesting that the contractions migrate caudad.

In conscious dogs, intravenous T-1815 at 1 and 3 mg/kg caused no clear effects on the motility of the gastric antrum, duodenum and colon in the majority of the animals examined. T-1815 developed contractions similar to the interdigestive phase III contractions in only 2 animals out of 7, and did not cause such a clear depression of the contractions in the stomach and such a conspicuous occurrence of high-amplitude contractions in the colon as observed in anesthetized dogs.

The reason why the intravenous T-1815 did not cause high-amplitude contractions in conscious dogs at the same doses as in anesthetized animals is unclear, but some explanations are possible. First, the animals used in the present study were fasted for 24 hr before experiment, and they usually finished defecation within 2 hr after feeding. Therefore, contents (feces) in the colon to be propelled caudad might be scarce during the experiment. It may be difficult to cause defecation under such conditions even if T-1815 had a strong colonic propulsive activity. Some reflex activities which exist in conscious animals may play a role not to cause extraordinal defecation. Second, T-1815 has a unique property that, in mice, it causes a remarkable prokinetic effect when colonic propulsion is disturbed (delayed) by chemical agents, despite it induces only a weak effect in naive (untreated) animals (Yamada and Onoda, 1993). Since pentobarbital inhibits the gastric emptying and small intestinal transit (Borella and Lippmann, 1980), the anesthetic will possibly delay the colonic propulsion. It, therefore, is suggested that, under such an anesthetic condition, T-1815 can cause the high-amplitude contractions which is known to play an important role to cause defecation (Karaus and Sarna, 1987).

One of the two animals in which the transducer was implanted on only the midportion of the colon showed development of the high-amplitude contractions after oral administration of 30 mg/kg T-1815. While, in all of the 3 experiments using dogs with transducers implanted on the three sites of the colon, i.e., the proximal, middle and distal colon, T-1815 at relatively low oral doses (10 and 30 mg/kg) produced high-amplitude contractions at the proximal site, and the contractions at the middle colon were observed in only one experiment. Furtheremore, there were no high-amplitude contractions in the distal colon. Therefore, T-1815 appears to have a characteristic to cause high-amplitude contractions in the proximal site of the colon rather than in the distal sites, and it may not cause the contractions to migrate to the distal site by the reason mentioned above. Sarna *et al.* (1987) have also reported that, in conscious dogs, such high-amplitude (giant) contractions can easily occur at the proximal region of the colon, and the majority of the proximally originated contractions migrate caudad before defecation.

In the present study, there were no defecation in conscious dogs despite that the high-amplitude contractions occurred. This is probably due to the emptied colon after defecation and 24-hr fasting.

Further studies are necessary to define the effect of T-1815 on the gastrointestinal motility during the digestive state or under disturbed colonic propulsion.

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