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Title page

Stimulatory Action of Itopride Hydrochloride on Colonic Motor Activity *In Vitro* and *In Vivo*.

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Running title page

a) Itopride stimulated colonic motility *in vitro* and *in vivo*.

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ABSTRACT

We investigated the effects of itopride hydrochloride (itopride, N-[4-[2-(dimethylamino)ethoxy]benzyl]-3,4-dimethoxybenzamide hydrochloride), a gastroprokinetic agent, on the colonic motor activity *in vitro* and *in vivo*, in comparison with benzamides, cisapride hydrate (cisapride) and mosapride citrate (mosapride). Itopride stimulated both peristaltic and segmental motility induced by applying intraluminal pressure to the isolated guinea pig colon. Though cisapride and mosapride enhanced the segmental motility, they markedly reduced the peristaltic motility. In conscious dogs with implanted strain gauge force transducers, itopride stimulated contractile activity in the gastrointestinal tract from the stomach to the colon. Cisapride stimulated contractile activity in the gastric antrum, ileum and ascending-colon. Mosapride stimulated contractile activity only in the gastric antrum and ileum. In guinea pigs and rats, itopride accelerated colonic luminal transit. On the other hand, cisapride and mosapride failed to enhance colonic transit. These results demonstrate that itopride has a stimulatory action on colonic peristalsis, propelling colonic luminal contents, different from that of cisapride and mosapride. Therefore, itopride may be a useful drug for the treatment of functional bowel disorders such as functional constipation.

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Itopride hydrochloride (itopride) and mosapride citrate (mosapride) have been used as a gastroprokinetic agent in some countries, including Japan, for the symptomatic treatment of functional dyspepsia. Delayed gastric emptying is considered one of causes of functional dyspepsia. Itopride was reported to enhance gastric emptying in dogs, rats and human, and stimulated canine gastrointestinal motility (Iwanaga et al., 1990, 1991; Harasawa and Miwa, 1993). Mosapride was also reported to stimulate upper gastrointestinal motility *in vivo* and *in vitro* (Mine et al., 1997). On the other hand, cisapride hydrate (cisapride) was reported to stimulate motor activity not only in the stomach but also in the colon in conscious dogs (Yoshida et al., 1991). Though cisapride was only prescribed for the treatment of reflux oesophagitis or heartburn in the USA, cisapride was thought to be useful in improving patients with constipation as well as functional dyspepsia, gastroparesis and gastric stasis (McCallum et al., 1988). Recently it became problematic to use cisapride for the treatment of patients with gastrointestinal disorders because of its side effects, namely heart rhythm disturbances including QT prolongation, syncope and serious ventricular arrhythmias such as torsades de pointes (Barbey et al., 2000). Under this situation, new gastroprointestinal prokinetic agents are required for the remedy of functional bowel disorders such as functional constipation. A novel 5-HT_4 receptor agent, tegaserod was recently accepted in the USA as an agent for the female irritable bowel syndrome (IBS) patients complaining of constipation. However, there are little useful data showing the stimulatory action of clinically available gastroprokinetics on colonic

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motility.

Therefore we investigated the effects of itopride on colonic motility and *in vitro* and *in vivo* transit in comparison with cisapride and mosapride, so as to determine the possibility of using itopride as a remedy for functional bowel disorders as well as functional dyspepsia.

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Materials and Methods

Animals and housing conditions. Male Hartley guinea pigs (Japan SLC, Inc., Hamamatsu, Japan) weighing 329 to 804 g, beagle dogs of either sex (Oriental Yeast Co., Ltd., Tokyo, Japan) weighing 7 to 14 kg and male Sprague-Dawley rats (Charles River Japan Inc., Hino, Japan) weighing 213 to 281 g were used. Before the experimental phase, animals were housed under standard controlled environmental conditions at 20-26ºC and 30-70% humidity, with 12-hour light/dark cycles and food and water available *ad libitum*. Guinea pigs and rats were allowed at least 1 week to acclimate to the laboratory conditions before the experiments were performed, and dogs were allowed at least 2 weeks. Animals with normal appetite and stool consistency were used in the experiments. All experiments were conducted in accordance with the guideline established by the Hokuriku Seiyaku Co., Ltd. Animal Care and Use Committee.

Contractile activity in isolated guinea pig colon. Guinea pigs were stunned by a blow on the head and exsanguinated. 8-cm segments of proximal colon in the distance of 5 cm from the ileo-cecal junction and distal colon in the distance of 5 cm from the anus were dissected and the luminal contents were washed out. The preparations were horizontally mounted in a 50 ml organ bath containing Krebs solution (composition in mM: NaCl 118, KCl 4.7, KH_2PO_4 1.2, $MgSO_4$ 1.2,

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$CaCl_2$ 2.6, $NaHCO_3$ 25.0, glucose 11.1) bubbled with a mixture of 95% O_2 and 5% CO_2. Spontaneous contractions were evoked according to a modified method of Bülbring and Lin (1958).

The oral and aboral ends of the colonic segment were fastened over to the respective tubes in the organ bath. The oral end was connected to a syringe pump (STC-525, TERUMO, Tokyo, Japan). The intraluminal pressure was measured at the aboral side by connecting to a pressure transducer (TP-400T, Nihon Kohden Co., Tokyo, Japan) and recorded in the computer system. The intraluminal pressure was applied to the colon by infusing Krebs solution into the colon with the use of the syringe pump at the speed of 2 ml/min. When long-lasting peristalsis was triggered as the visible contractions composed of peristaltic and segmental contractions, intraluminal pressure was considered to reach the threshold pressure, and then the infusion was stopped to maintain the threshold pressure. The mean threshold pressure was about 3 cmH_2O. Macroscopically, high and low amplitude contractions could be seen representing peristaltic and segmental motility respectively. After this regular peristalsis was confirmed in the preparations, a single concentration of test drug was applied to the serosal side of the segment. For quantitative analysis, the contractions whose amplitudes were greater than 50 or were 10-50 percentage of the highest contractions observed for the 15 minutes before application of the drugs were defined as peristaltic contractions or segmental contractions respectively. The number of each contraction was counted using computer software (Eight Star, Star Medical, Tokyo, Japan), and the increase in frequency was expressed as the

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percentage change of the frequency over the 15-minute period after application of the drug compared with that in the pre-application period.

Contractile activity in conscious dogs. Dogs were deprived of food 18 hours prior to surgery with free access to water. Under general anesthesia with a mixture of nitrous oxide, oxygen and enflurane (Ethrane, Abbott Laboratories, Chicago, IL), eight strain gauge force transducers (W13 × D8 mm, F-12IS, Star Medical) were implanted into the serosal side of the gastric body, gastric antrum, duodenum, upper- and middle-jejunum, ileum and ascending- and descending-colon, in a direction which made it possible to measure circular muscle contractions. The sites of the implantation of the gastric body, gastric antrum, duodenum, upper- and middle-jejunum, ileum and ascending- and descending-colon were 5-cm distal to the cardia, 5-cm distal to the pylorus, 7-cm distal to the pylorus, 50-cm distal to the pylorus, in the middle of small intestine, 15-cm proximal to the cecum, 10-cm distal to the cecum and nearest to the anus respectively. The free end of the transducer was brought out through a skin incision between the scapulae and protected with a jacket. A silicon tube (OD, 2.0 mm, Kaneka Medix Corporation, Osaka, Japan) was inserted into the external jugular vein and sutured onto the adjacent skin as a route for the intravenous injection of the test drugs. The animals were allowed to recover for at least 10 days after this surgery before the commencement of the experiments.

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The free end of the strain gauge force transducer was connected to an amplifier (FA-01, Star Medical) and contractile activity was recorded on a pen-writing recorder (WR-3701, GRAPHTEC, Tokyo, Japan) and simultaneously stored in a computer for quantitative analysis. The test drugs or solvent were given intravenously via the indwelling silicon tube after postprandial gastrointestinal motility became stable (more than 2 hours after feeding). Drug was administered only once per day. Data for each contractile motor activity were stored continuously and then analyzed by means of a gastrointestinal motility measuring system (FA-01, FS-08M and FB-01, Star Medical). The motor index was calculated by integrating the area under the contractile wave over every 30-minute period and expressed as the percentage change from the pre-administration period.

Colonic luminal transit in guinea pigs and rats. Colonic luminal transit in guinea pigs and rats was examined with a slight modification of the reported method (Ueda et al., 1969). Each animal was anesthetized with pentobarbital sodium at 50 mg/kg, i.p. and the cecum was exposed by laparotomy. A polyethylene tube (OD, 1.2 mm, Natsume Seisakusho Co., Ltd., Tokyo, Japan) was inserted from the small incision in the cecum to the beginning of the colon. The other end of the tube came out through the back. The animals were allowed to adapt to individual cages for more than 3 days.

Animals were deprived of food overnight before experiment. Test drugs were given orally. A

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marker (barium sulfate, 60% w/v, 0.5 ml/animal) was administered through the colonic cannula 30 minutes later for the guinea pigs but immediately for the rats. The guinea pigs and rats were euthanized by cervical dislocation at 30 and 60 minutes respectively after administration of the marker. The colon was removed, and the length from the colo-cecal junction to the front traveling edge of the barium sulfate was measured. Colonic luminal transit was expressed as the percentage distance traversed to the total length of the colon.

Gastric emptying in rats. A solution of 0.05% (w/v) phenol red in aqueous carboxymethyl cellulose (4.5% w/v) was used as a test meal. After an 18 hours fast, test drug was administered orally and then the test meal was given 30 minutes later. Each rat was sacrificed at 15 minutes after the test meal administration, and the stomach was removed immediately.

The removed stomach containing residual phenol red solution was incised in 20 ml distilled water and shaken for 10 minutes. The pieces of stomach were rinsed and discarded, and then the recovered phenol red solution was made up to a total volume of 40 ml with distilled water. The recovered phenol red solution was centrifuged at 3000 rpm for 10 minutes, and three milliliters of the supernatant was added to 2 ml of 1 M NaOH to develop the color. The absorbance at 558 nm wave length of the solution was measured with a spectrophotometer (U-2000, Hitachi, Tokyo, Japan).

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The gastric emptying (G.E.) for each rat was calculated according to the following formula:

G.E.(%)={1-(Amount of residual phenol red recovered 15 minutes after test meal administration)/(Average amount of phenol red present in the stomach immediately after test meal administration)}×100

Drugs. Itopride, cisapride and mosapride were synthesized by Hokuriku Seiyaku Co., Ltd (Katsuyama, Japan). Barium sulfate and phenol red were purchased from Wako Pure Chemical Industries (Osaka, Japan). Carboxymethyl cellulose was from Nakalai Tesque, Inc. (Kyoto, Japan).

Itopride was dissolved in saline or distilled water. Cisapride and mosapride were dissolved in a solution containing 1% lactic acid.

Statistical analyses. All results are presented as means ± S.E.M. Statistical analysis of the *in vivo* data was performed with Williams' multiple range test. For the *in vitro* experiments, Student's t-test was used to test the significance of any differences. Probability values less than 0.05 were considered statistically significant.

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Results

Effects on peristaltic and segmental motility in the isolated guinea pig colon. Stable contractions were induced by applying the threshold pressure to the isolated guinea pig colon. Figure 1 shows the typical effects of itopride, cisapride and mosapride at 10 μM on the colonic contractions. Contractions with higher amplitude were considered to be peristaltic contractions that started from the oral end and migrated to the aboral end. Contractions with lower amplitude and higher frequency were considered to be segmental contractions that did not migrate. Itopride significantly increased the frequency of peristaltic and segmental contractions in the proximal and distal colon in a concentration dependent manner (Fig. 1A and 2). The amplitude of peristaltic contractions was not changed by itopride (Fig. 1A). On the other hand, cisapride and mosapride reduced the frequency of peristaltic contractions in the proximal and distal colon (Fig. 1B, 1C and 2). The inhibitory effects were significant at 10 μM. With respect to segmental motility, itopride significantly increased the frequency of contractions with enhancement of amplitude (Fig. 1A and 2B). Itopride and mosapride produced similar responses in the proximal and distal colon (Fig. 1A, 1C and 2B) but cisapride exerted distinct effects on segmental motility between the proximal and distal colon. Cisapride increased the frequency of segmental contraction in the distal colon only at the highest concentration of 10 μM. In contrast, cisapride significantly increased the frequency of

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segmental contraction in the proximal colon up to 1 μM but significantly decreased the frequency at 10 μM (Fig. 1B and 2B).

Effects on gastrointestinal motility in conscious dogs. Figure 3 shows the stimulatory effects of itopride (10 mg/kg, i.v.), cisapride (0.3 mg/kg, i.v.) and mosapride (3 mg/kg, i.v.) on the postprandial gastrointestinal motor activity in conscious dogs. As shown in figure 3A and 4A, itopride dose-dependently stimulated gastrointestinal motility from the stomach through the colon, although the stimulatory effect on the small intestine was weaker than for other regions. Itopride enhanced contractile activities in the gastric antrum, duodenum and upper -jejunum significantly at 3 mg/kg (Fig. 4A). In the middle -jejunum, ileum, ascending -colon and descending -colon, itopride stimulated contractile activities significantly at 10 mg/kg (Fig. 4A). Cisapride enhanced antral, ileal and colonic motility significantly at 0.03, 0.1 and 0.3 mg/kg respectively (Fig. 3B and 4B). On the other hand, mosapride did not enhance colonic motility up to 3 mg/kg though it significantly stimulated antral and ileal motility at 1 and 3 mg/kg respectively (Fig. 3C and 4C). In addition, itopride produced giant migrating contractions, which were high amplitude, rapidly migrating contractions which propelled colonic contents to the rectum (Sarna et al., 1984; Karaus and Sarna, 1987), followed by defecation in some dogs at 10 mg/kg, but this was not seen with cisapride nor mosapride. Throughout this experiment, no behavioral changes were observed.

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Colonic transit in guinea pigs and rats, and gastric emptying in rats. Itopride accelerated colonic transit dose-dependently in both guinea pigs and rats and significant acceleration was observed at 10 mg/kg, p.o. in both animals (Fig. 5A and 6A). However cisapride did not affect colonic transit significantly up to 10 mg/kg in guinea pigs and rats (Fig. 5B and 6B) nor did mosapride in guinea pigs (Fig. 5C). Moreover, mosapride slightly delayed colonic transit in rats, the delay being statistically significant at a dose of 1 mg/kg and above (Fig. 6C).

In rats, itopride, cisapride and mosapride all enhanced gastric emptying dose-dependently (Fig. 7), statistically significant effects being observed at doses of 10, 1 and 1 mg/kg respectively. Therefore, itopride exerted stimulatory effects on gastric emptying and colonic transit at the same dose while on the other hand, cisapride and mosapride were found not to accelerate colonic transit at 10 mg/kg, p.o., a dose that was 10 times higher than that required to accelerate gastric emptying.

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Discussion

Itopride has anti-acetylcholinesterase (AChE) activity as well as dopamine D_2 (D_2) receptor antagonist activity and is used for the symptomatic treatment of functional dyspepsia (Iwanaga et al., 1990, 1994). It is well established that the M_3 receptor exists on the smooth muscle layer throughout the whole gut and that acetylcholine released from the enteric nerve endings stimulates the contraction of smooth muscle through the M_3 receptor. Therefore, we predicted that itopride might have a colonic prokinetic action but there were only a few data with respect to the effects of itopride on colonic motility. In this study, we evaluated the effects *in vitro* and *in vivo*, in comparison with the benzamide derivatives, cisapride and mosapride, which both have a gastroprokinetic action with a 5-HT_4 receptor agonist effect.

The *in vitro* study exhibited that cisapride and mosapride increased segmental motility but inhibited the peristaltic motility in the isolated guinea pig colon. Buchheit and Buhl (1991, 1992, 1993) reported that benzamides induced the inhibition of circular muscle contraction and an increase in longitudinal muscle activity via 5-HT_4 receptor activation. Peristalsis is known to consist of the circular muscle contraction at oral side and the relaxation at aboral side in the intestine. Although longitudinal muscle contraction during the preparatory phase of peristalsis precedes circular muscle contraction, the main propulsive drive in the peristaltic reflex comes from the aborally-directed

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contraction of the circular muscle during the emptying phase (Kosterlitz et al., 1956). Therefore the peristalsis requires not only coordination between contraction and relaxation in circular muscle but also coordination between circular muscle and longitudinal muscle. Cisapride and mosapride might collapse the coordinated peristalsis composed of peristaltic and segmental motility due to their relaxant effects on the smooth muscles. This could be responsible for their failure to have a stimulatory action on colonic transit in guinea pigs and rats. Cisapride and mosapride both decreased peristaltic motility in the isolated guinea pig colon, but mosapride increased segmental motility in a concentration-dependent manner. Enhancement of segmental motility not accompanied by peristaltic motility may interfere with colonic transit *in vivo*. Stimulatory effect of mosapride on the segmental motility seems stronger than that of cisapride and this explains that mosapride did not accelerate colonic transit. In guinea pigs, cisapride tended to accelerate colonic transit despite the *in vitro* inhibitory effect on the peristaltic motility. The stimulatory effect of cisapride on the colonic transit may be ascribed to the enhancement of gastric emptying and intestinal motility. On the other hand, itopride enhanced both the peristaltic and segmental motility in the isolated guinea pig colon. It is true that stimulatory effects on the isolated gastrointestinal motility do not always produce the propulsion of the luminal contents, but it was confirmed that the stimulatory effects of itopride successfully increased colonic transit in guinea pigs and rats.

In vivo studies using dogs and rats indicate gastrointestinal region selectivity of the prokinetic

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action of each agent. As a result, cisapride and mosapride selectively stimulated upper gastrointestinal motility, when compared with itopride. In conscious dogs, cisapride significantly stimulated antral motility and the effective dose for antral motility was 10 times less than that for colonic motility. Furthermore, mosapride failed to stimulate colonic contractions. Our findings of the selectivity of cisapride and mosapride for the stomach are consistent with the study by Mine at al (1997). Gastrointestinal region selectivity for cisapride and mosapride was also demonstrated in rats. Cisapride and mosapride had no stimulatory effect on the colonic transit at the dose that was effective to enhance the gastric emptying. On the contrary, itopride had stimulatory effects on all sites of the canine gastrointestinal tract from the stomach through the colon. The effective dose of itopride stimulating the canine colon was not more than three times greater than that stimulating antral motility. In addition, itopride enhanced gastric emptying and colonic transit at the same dose, 10 mg/kg, in rats.

Though stimulation of gastrointestinal motility by itopride is ascribed to activation of the cholinergic drive based on D_2 receptor blocking and anti-AChE activity (Iwanaga et al., 1990, 1994), the stimulatory action on colonic motility seems mainly due to anti-AChE activity. Since gastrointestinal smooth muscle is directly stimulated by ACh through the activation of the M_3 receptor irrespective of the gastrointestinal site and animal species, it is apparent that itopride can stimulate colonic contractions as well as antral contractions in all species. On the other hand,

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benzamides, such as cisapride and mosapride, stimulate gastrointestinal motility via activation of the 5-HT_4 receptor (Mine et al., 1997; Yoshida et al., 1991, 1993). The 5-HT_4 receptor is located on smooth muscle and the excitatory neurons, which mediate relaxation and contractions respectively. With regard to stomach and colon, Sakurai-Yamashita et al. (1999) reported that there are some differences in the 5-HT_4 receptor density between smooth muscle and the excitatory neurons. It can be interpreted by the difference of 5-HT_4 receptor localization between stomach and colon that cisapride and mosapride both stimulated gastric motility at lower doses than colonic motility. Moreover, the different effects of cisapride and mosapride on colonic motility might be explained by a distinct receptor binding affinity. Though mosapride exhibits no affinity for the D_2, adrenaline α_1 (α_1), adrenaline α_2, 5-HT_1 and 5-HT_2 receptors except for the strong affinity for the 5-HT_4 receptor, cisapride possesses affinity for the D_2, 5-HT_2, α_1 and muscarinic receptors as well as the 5-HT_4 receptor (Yoshida et al., 1989; Karasawa et al., 1990; Briejer et al., 1995). Recently, a 5-HT4 receptor partial agonist, tegaserod was reported to stimulate colonic motility in dogs (Appel et al., 1996; Nguyen et al., 1997) and accelerate propulsion in isolated guinea pig colon (Jin et al., 1999). It still remains unclear but the difference of the effects on the colon among tegaserod, cisapride and mosapride might be related to the heterogeneity of 5-HT_4 receptors between the stomach and the colon reported by Gerald et al. (1995). Differently from these benzamides, itopride had little affinity for 5-HT_4 receptors in guinea pigs striatal membranes (pIC50 < 4) (Kakiuchi et al., 1997)

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and it seems unlikely that itopride stimulates colonic motility through the activation of 5-HT_4 receptor.

Stimulation of gastrointestinal motility by itopride is ascribed to the activation of the cholinergic drive based on D_2 receptor blocking and anti-AChE activity (Iwanaga et al., 1990, 1994) but the stimulatory action on colonic motility seems mainly due to anti-AChE activity. D_2 receptor agonists, domperidone and metoclopramide are also accepted as gastrprokinetic agents. Domperidone is a selective D_2 receptor antagonist and metoclopramide possesses 5-HT_4 receptor agonistic activity as well as D_2 receptor antagonistic activity. Though domperidone was reported to enhance the gastric motor activity but not to stimulate small intestinal and colonic motor activity in conscious dogs (Miyashita et al., 1991).

A potent anti-AChE inhibitor, neostigmine is well known to improve postoperative ileus and it is worth while to notice that such old drug is recently prescribed in patients with acute colonic pseudo-obstruction. However, the use of neostigmine is limited because of its side effects. Basically, neostigmine dose-dependently and significantly enhanced the antral and colinc motor activity in dogs at 30 and 100 μg/kg, i.v. but tended to increase blood pressure and to suppressrespiration (Kishibayashi et al., 1994). Besides, dogs often collapsed when neostigmine was intravenously administered at 1000 μg/kg (Iwanaga et al., 1990). Itopride did not cause any adverse effects such as salivation, snivel, vomiting and diarrhea based on anti-AChE action

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throughout our experiments. The inhibitory action of itopride on AChE was 100 times stronger than that on butyrylcholinesterase (BuChE), whereas the inhibitory action of neostigmine on AChE was 10 times stronger than that on BuChE (Iwanaga et al., 1994). The selectivity on AChE seems a cause of the differences of safety window. Several studies on proarrhythmic potential of itopride demonstrate that itopride is devoid of QT prolongation at least at the present dose levels (Kakiuchi et al., 1997). Furthermore, itopride was reported not to penetrate into the blood brain barrier (Yamada et al., 1994).

In conclusion, it is anticipated that itopride could be of value as a safe and feasible alternative to other existing prokinetic agents for the treatment of functional bowel disorders such as functional constipation and constipation-dominant irritable bowel syndrome, without an excessive increase in dose.

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Legends for figures

Fig. 1. Typical tracings of the effects of itopride (A), cisapride (B) and mosapride (C) on contractile activity in the isolated guinea pig colon. Contractions were traced by measuring the intraluminal pressure in the colon. Itopride, cisapride and mosapride were applied to the serosal side at the point indicated by the arrow.

Fig. 2. Effects of itopride, cisapride and mosapride on the frequency of peristaltic (A) and segmental contractions (B) in the isolated guinea pig colon. The contractions whose amplitudes were over 50% and 10-50% of the maximum contraction over the 15 minutes before drug administration were regarded as peristaltic and segmental contractions respectively. The increase in frequency was calculated as the percent change over 15 minutes after the administration versus that before it. Each column represents the mean ± S.E.M. of 5 separate experiments. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ compared with control (vehicle).

Fig. 3. Typical tracings of the effects of itopride (A), cisapride (B) and mosapride (C) on gastrointestinal motility in conscious dogs during the digestive state. The gastrointestinal motor activity of conscious dogs was measured by means of strain gauge force transducer chronically

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implanted in a direction to measure circular muscle contractions. Itopride, cisapride and mosapride were administered intravenously at the point indicated by the arrow during the postprandial period.

Fig. 4. Effects of itopride (A), cisapride (B) and mosapride (C) on the gastrointestinal motility in conscious dogs during postprandial period. The motor index was calculated by integrating the area between the contractile wave and baseline over the 30-minute period before and after drug administration and expressed as the percentage change. Each column represents the mean ± S.E.M. of 6-8 dogs. * $p < 0.05$, ** $p < 0.01$ compared with control (vehicle).

Fig. 5. Effects of itopride (A), cisapride (B) and mosapride (C) on colonic transit in guinea pigs. Colonic transit was calculated as the percentage of the distance traveled by an administered intracolonic marker relative to the total length of the colon for 30 minutes after marker administration. Drugs were given orally 30 minutes before administration of the marker. Each column represents the mean ± S.E.M. of 10 guinea pigs. ** $p < 0.01$ compared with control (vehicle).

Fig. 6. Effects of itopride (A), cisapride (B) and mosapride (C) on colonic transit in rats. Colonic transit was calculated as the percentage of the distance traveled by an administered intracolonic

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marker relative to the total length of the colon for 60 minutes after administration of the marker. Drugs were given orally just before administration of the marker. Each column represents the mean ± S.E.M. of 10-12 rats. * $p < 0.05$, ** $p < 0.01$ compared with control (vehicle).

Fig. 7. Effects of itopride (A), cisapride (B) and mosapride (C) on gastric emptying in rats. Gastric emptying was measured by the phenol red method. 0.05% phenol red was orally given at 30 minutes after oral drug administration. Gastric emptying was assayed at 15 minutes after the administration of phenol red. Each column represents the mean ± S.E.M. of 8-11 rats. * $p < 0.05$, ** $p < 0.01$ compared with control (vehicle).

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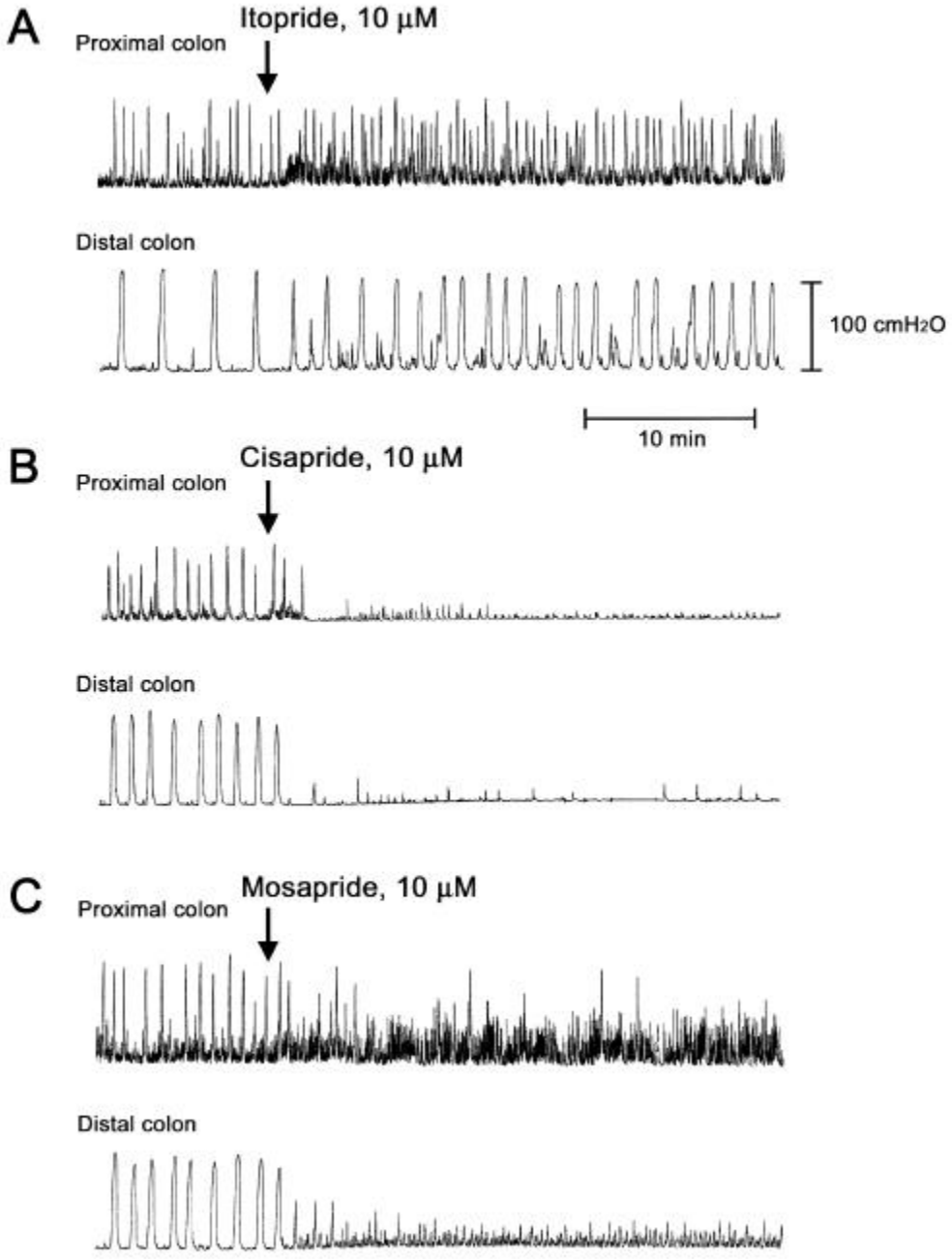

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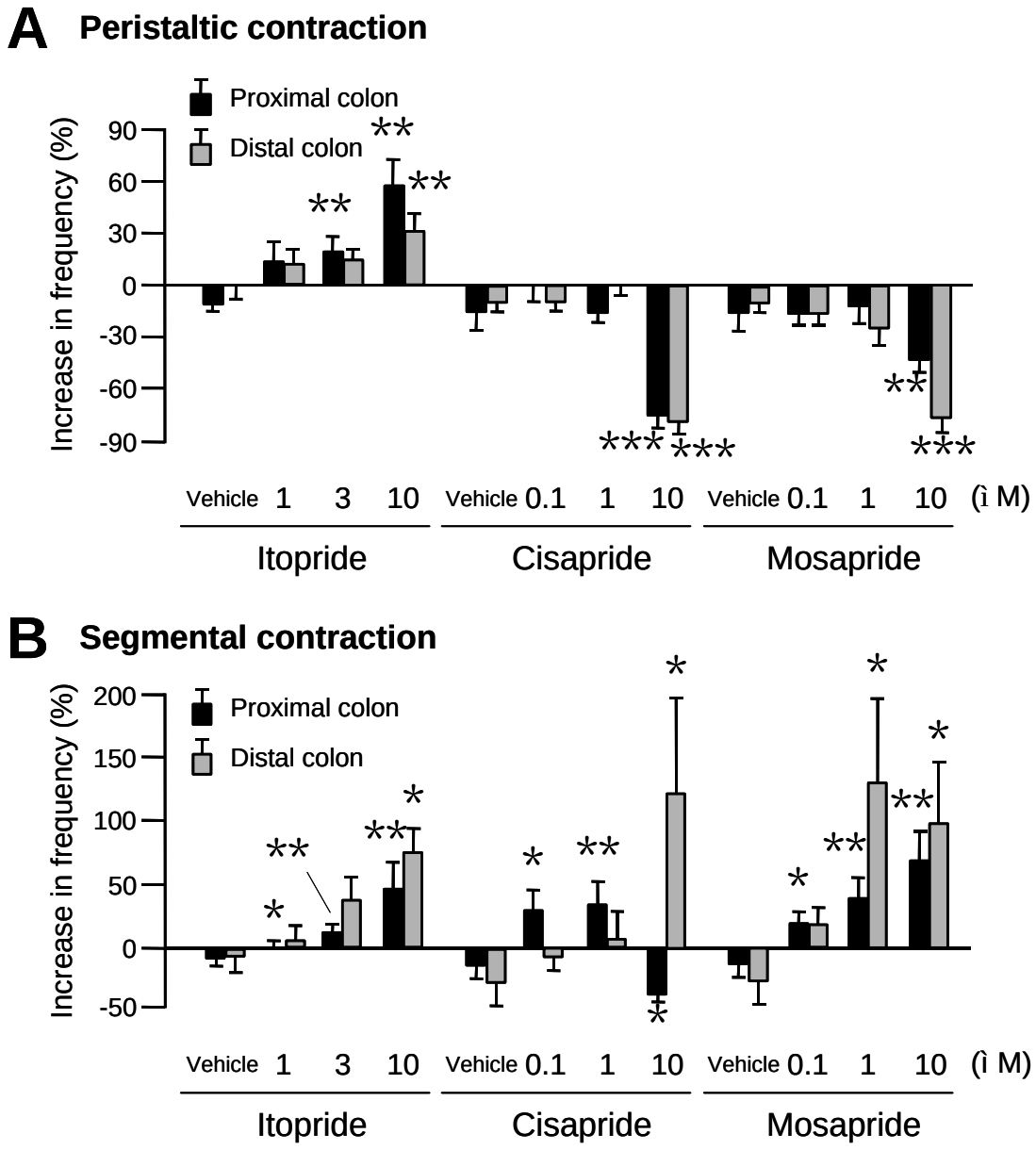

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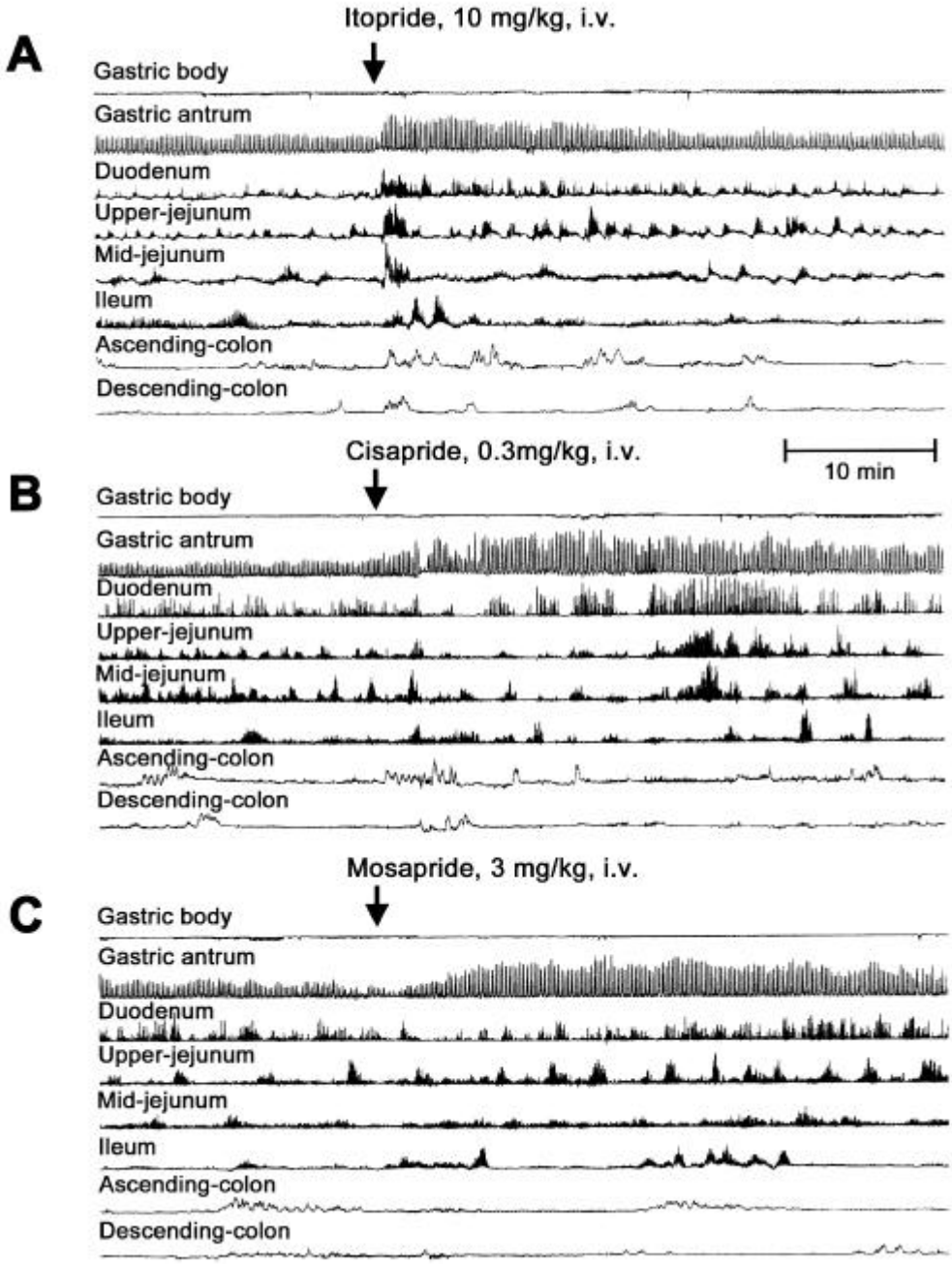

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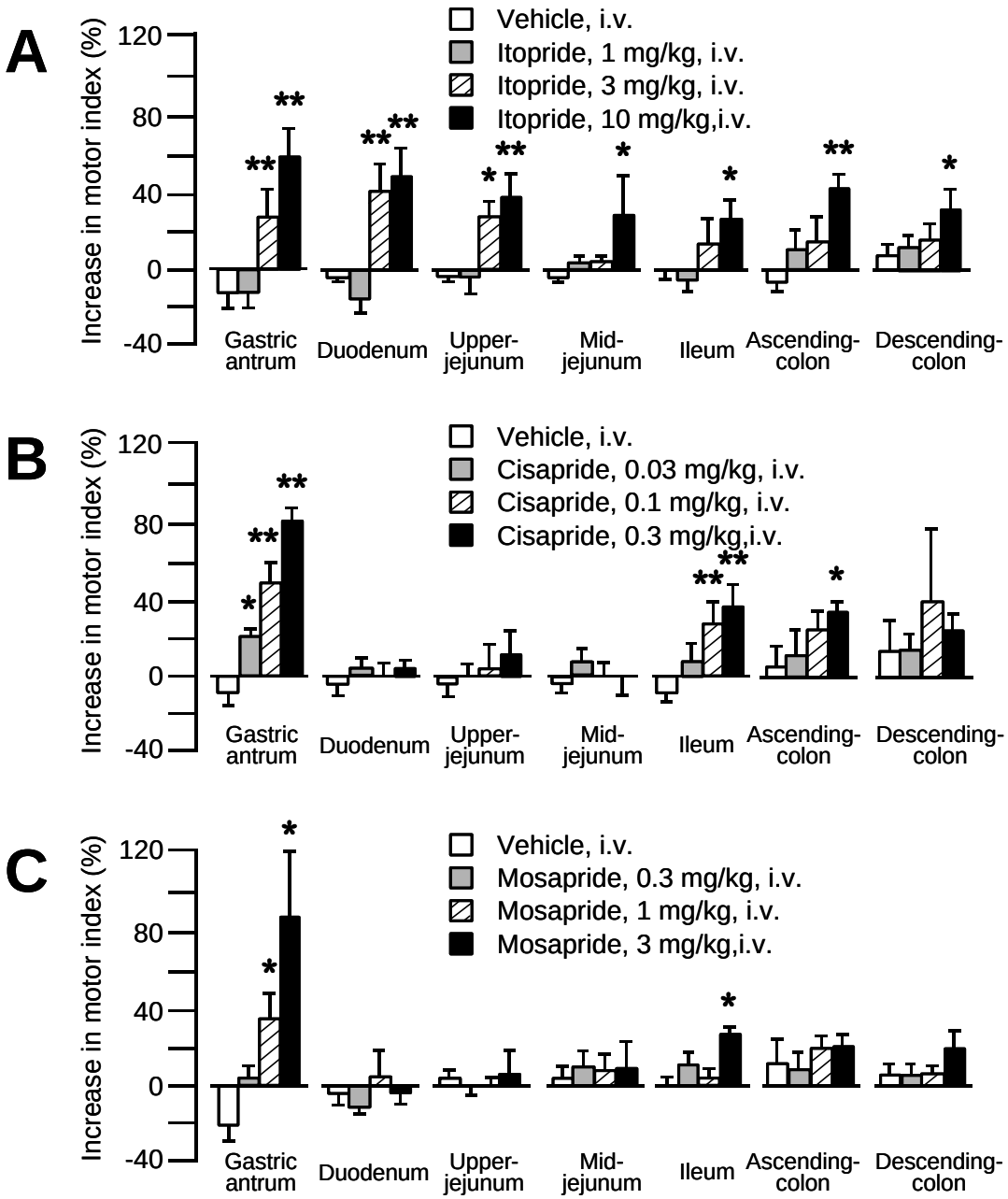

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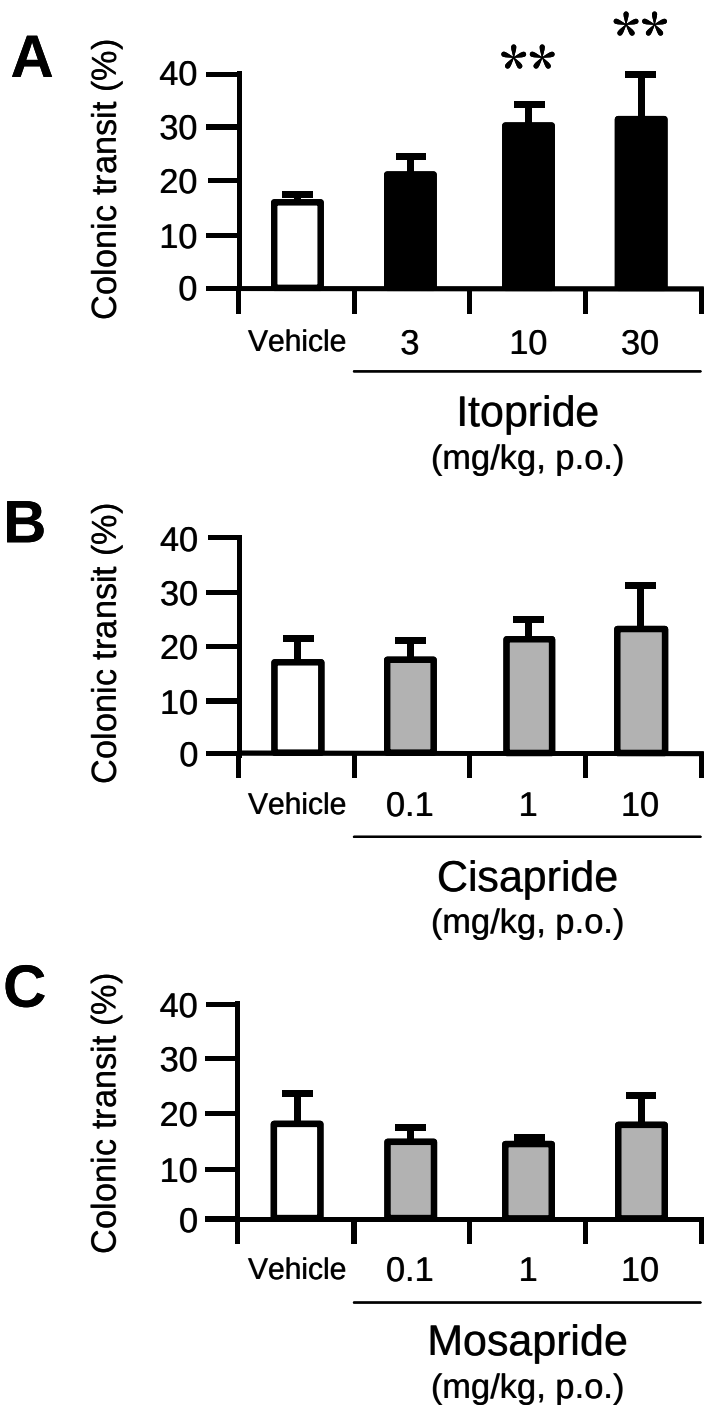

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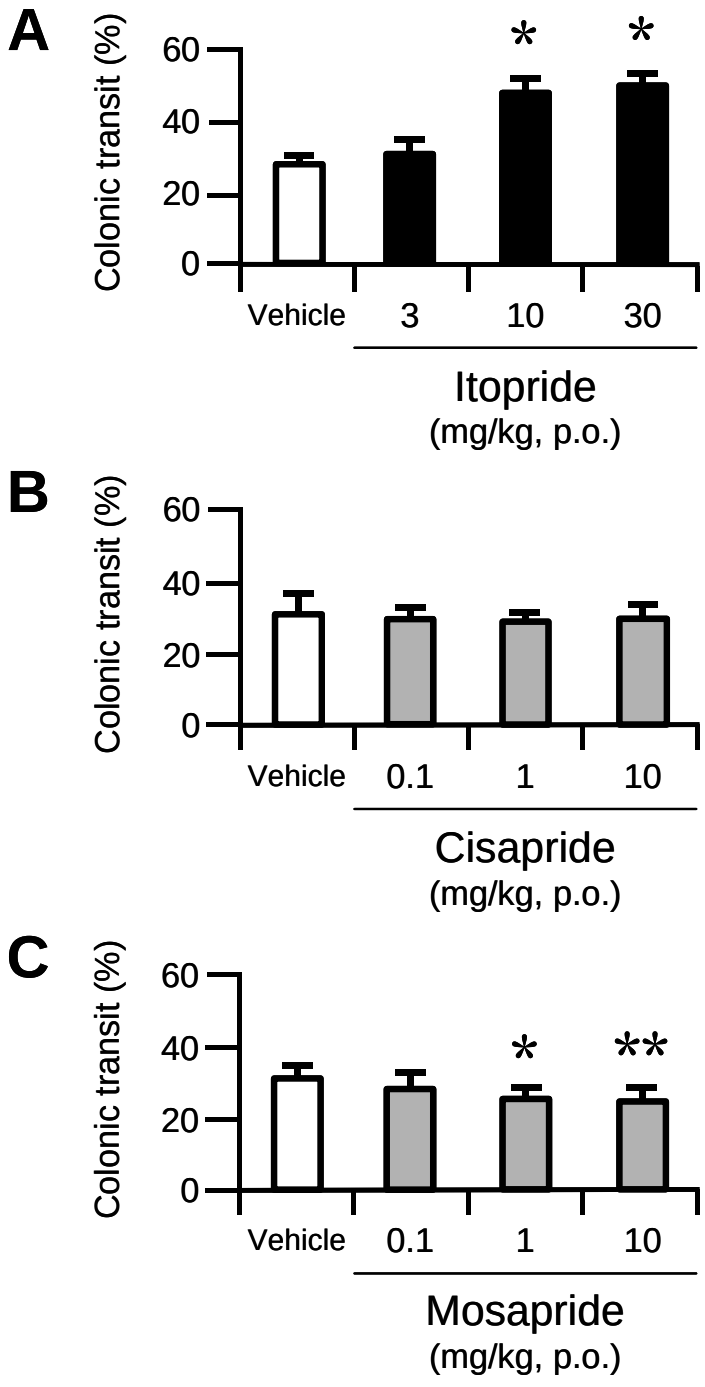
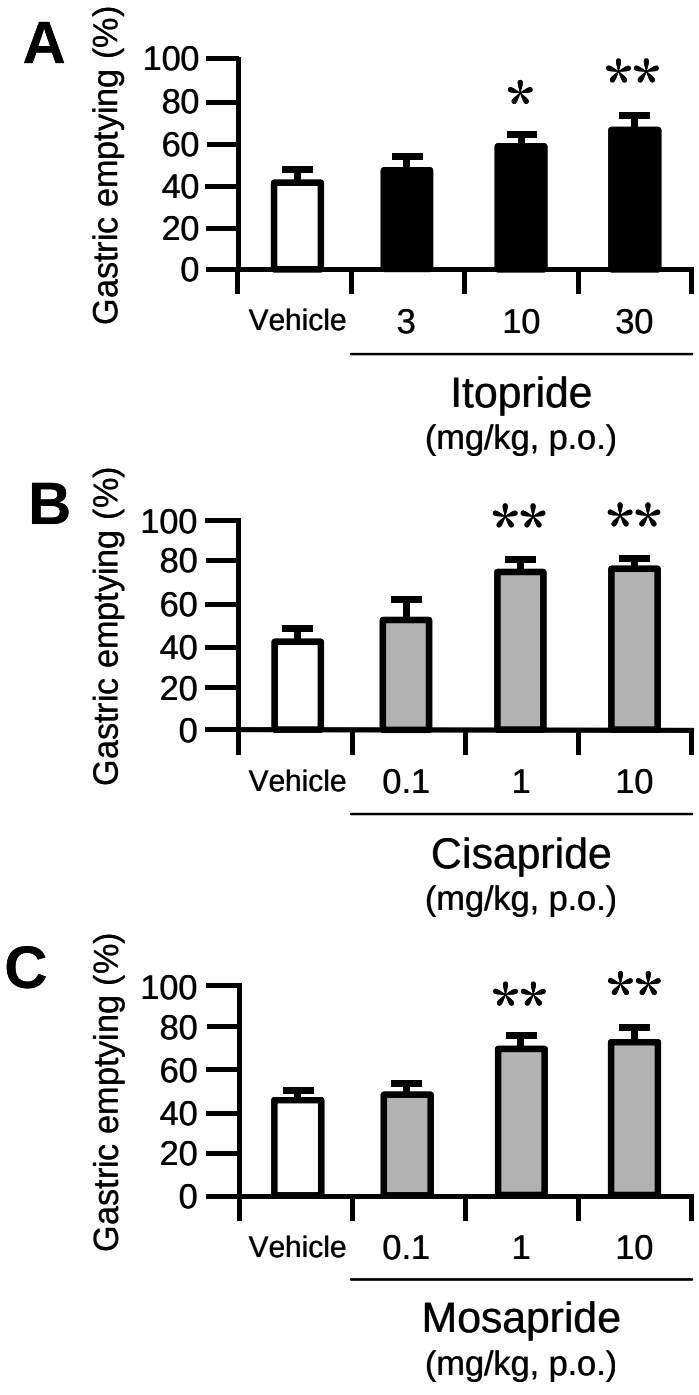
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