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Radish Extract Stimulates Motility of the Intestine via the Muscarinic Receptors

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Abstract

The effects of radish (*Brassica oleraceae*, Cruciferae) on gastrointestinal motility were examined using rat intestinal segments with myenteric plexus in-vitro and measuring the intestinal transit of charcoal in-vivo. Radish extract ($10\,\mu g\,mL^{-1}$ to $2\,mg\,mL^{-1}$) caused a dose-dependent increase in contractions of the duodenum, jejunum and ileum, and $1\,mg\,mL^{-1}$ was the maximum effective dose. The largest contraction by the extract was found in ileal segments. The extract-induced ($0{\cdot}5\,mg\,mL^{-1}$) ileal contraction was remarkably inhibited by pretreatment of segments with atropine (10^{-7} M) for 10 min, but not by hexamethonium (0·5 mM). Moreover, antagonists of the muscarinic receptor reduced the radish-induced ileal contraction by a different ratio. The rank order of inhibitory effects was 4-diphenylacetoxy-*N*-methyl-(2-chloroethyl)-piperidine methiodide (90·5% of control) > tropicamide (67·4%) > pirenzepine (42·8%) > methoctramine (16·7%). Oral administration of radish extract ($300-500\,mg\,kg^{-1}$ body weight) to mice remarkably improved the intestinal transit of charcoal, and this was significantly attenuated by co-administration of atropine ($50\,mg\,kg^{-1}$). Taken together, these results suggest that radish extract stimulates gastrointestinal motility through activation of muscarinic pathways.

In the gastrointestinal tract, the acetylcholine receptors, metabotropic muscarinic and ionotropic nicotinic receptors, play an important physiological role in the stimulation of spontaneous in-vivo phasic and tonic contractions in the fasting and postprandial states (Wood 1984; Shi and Sarna 1997). These receptors are composed of multiple subtypes with different pharmacological characteristics in the mammalian gastrointestinal tract (Buckley & Burnstock 1986; Caulfield & Birdsall 1998).

Several studies have shown that radish (*Brassica oleracea*, Cruciferae) contains a number of secondary plant metabolites that can exert protective effects on chemically induced carcinogenesis in animals and tumour growth and formation in humans (Bradfield & Bjeldanes 1991; Witte et al 1996). In folk remedies, it is also widely known that radish has a pharmacological activity that improves indigestion and constipation. However, no evidence is available to demonstrate this traditional acceptance. In this regard, this study examined the effects of radish on the spasmogenic activity of the gastrointestinal tract using isolated intestinal segments in-vitro and measurement of the intestinal transit of charcoal in-vivo.

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Materials and Methods

Rats and mice

Male Sprague-Dawley rats, 150–180 g, were used for examining gastrointestinal contractility in-vitro. ICR mice, 15–20 g, were used to study intestinal transit charcoal in-vivo. The study was begun after acclimatization of mice for 1 week under a temperature of $23 \pm 2^{\circ}C$, relative humidity 55–60% and a 12-h dark–light cycle. Food was withheld for 12 h before the experiment, but there was free access to drinking water. Each mouse was placed in a separate cage at the beginning of the experiments.

Preparation of radish extract

Radish root was obtained directly from the cultivating district (Bongdong, Chonbuk) in Korea, and

authenticated by Dr Sung Oh Yoo, Wonkwang University. Roots were washed with distilled water to remove soil and other contaminants. Radish extracts were prepared using a juice extractor and filtered using 3M filter paper. Filtrates were lyophilized in a freeze drier (Labconco, Preezone) and kept at 4°C until use. The yield of material was about 4·3% (weight/volume) of filtrates.

Gastrointestinal contractility

Gastrointestinal contractility was measured using a modification of the methods previously described (Aube et al 1996; Kitazawa et al 1996). Acetylcholine was used as a positive control to demonstrate the effects of radish extract on the motility of the rat intestine. Rats were killed by decapitation, and the proximal duodenum, jejunum and ileum were removed. Approximately 2-cm long longitudinal segments were mounted in a 5-mL water-jacketed tissue bath. The tissue was maintained at 37°C in a Krebs/bicarbonate solution (pH 7·4) which contained (mM): 128 NaCl; 4·5 KCl; 2·5 $CaCl_2$; 1·18 $MgSO_4$; 1·18 KH_2PO_4; 25·0 $NaHCO_3$; 5·5 D-glucose. The solution was aerated with a mixture of 95% O_2 and 5% CO_2. The tissues were allowed to equilibrate for at least 30 min. The experiments were performed under a resting tension of 1 g. The contractile activity of segments was recorded using a Grass FT03 force displacement transducer coupled to a polygraph (Grass 7E).

Small intestinal transit

The method of Mascolo et al (1994) and Taniyama et al (1991) was generally followed with a slight modification. In order to examine the competitive effect of atropine on radish-induced gastrointestinal transit, atropine (50 mg kg^{-1}) was subcutaneously injected 5 min before treatment with the radish extract. Radish extract (300 or 500 mg kg^{-1}) was orally administered 20 min before treatment of a marker (0·2 mL per mouse, 10% charcoal suspension in 5% gum arabic). Fifteen minutes after treatment of the marker, the mice were killed and the whole region of small intestine was removed. The distance travelled by the marker was measured and expressed as a percentage of the total length of the small intestine from the pylorus and ileum.

Chemicals and reagents

Acetylcholine, atropine and hexamethonium were obtained from Sigma Chemicals (St Louis, MO, USA). Pirenzepine, methoctramine, 4-diphenylacetoxy-*N*(2-chloriethyl)-piperidine hydrochloride (4-DAMP) and tropicamide were purchased from Research Biochemical Inc. (Natick, MA). All other chemicals were of the highest grade from commercial sources.

Statistical analysis

Values obtained in this study were expressed as mean ± s.e.m. Statistical comparison was made by one-factor analysis of variance and the Student–Newman–Keuls procedure. $P < 0.05$ was considered significant.

Results

Effect of radish extract on intestinal segments

As shown in Figure 1A, radish extracts (0·5 mg mL^{-1}), in a similar way to acetylcholine, caused a tonic contraction of the duodenum, jejunum and ileum. Contraction reached maximum levels within 10 s of adding extract to an organ bath, followed by a gradual decline to the resting level. The highest response to the extract in the three regions tested was in the ileal segment.

Acetylcholine (10^{-9}–10^{-5} M) and radish extract (0·01–2 mg mL^{-1}) produced a contraction of ileal segments in a dose-dependent manner (Figures 1B and C), and similar dose–response curves were also observed in the duodenum and jejunum (data not shown). The maximum effective doses of acetylcholine and radish extract were found to be 10^{-6} M and 1 mg mL^{-1} respectively. The ED_{50} values of acetylcholine and radish extract were estimated to be approximately 10^{-7} M and approximately 200 μg mL^{-1} respectively.

Effect of antagonists on radish extract-induced ileal contraction

To clarify a possible mechanism involved in the radish extract-induced intestinal contraction, muscarinic and nicotinic receptor antagonists were used. Both acetylcholine (5×10^{-7} M)- and radish extract (0·5 mg mL^{-1})-induced ileal contractions were dramatically inhibited by pretreatment of segments with atropine (10^{-7} M) for 10 min, but not by 0·5 mM of hexamethonium (Figure 2). Additionally, both acetylcholine (5×10^{-7} M)- and radish extract (0·5 mg mL^{-1})-induced ileal contractions were dose-dependently inhibited by pretreatment of segments with atropine (10^{-9}–10^{-7} M) for 10 min (data not shown). These results suggest that radish extract-induced ileal contraction might be mainly mediated by the activation of muscarinic receptors.

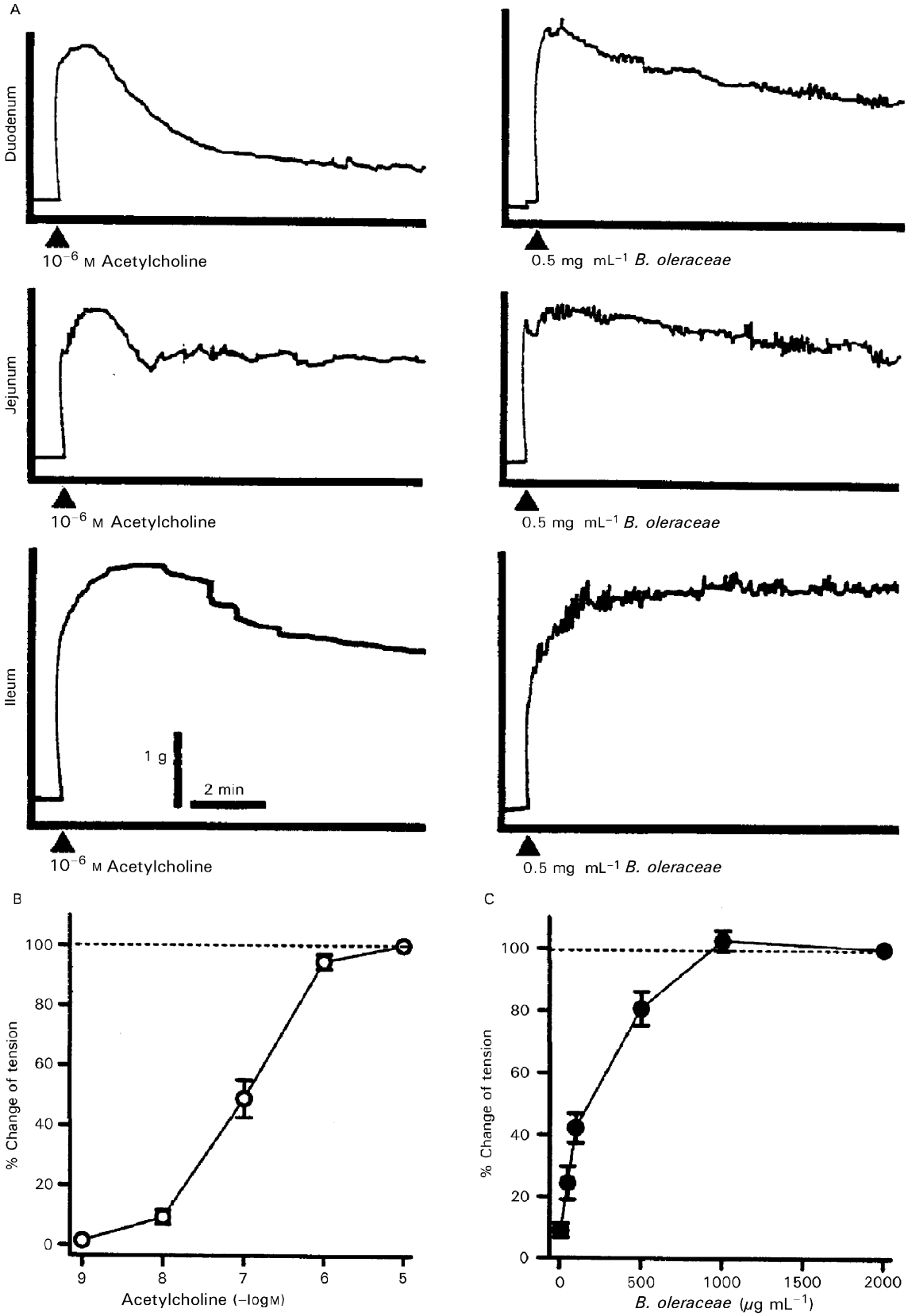

Figure 1. Effects of acetylcholine and radish extract on rat intestinal contraction. After intestinal segments were allowed to equilibrate for at least 30 min, acetylcholine or radish extract was applied to a 5 mL organ bath. A. Typical records showing the tonic contractions induced by ACh (10^{-6} M) and radish extract (0·5 mg mL^{-1}). B and C. Dose–response effect of acetylcholine and radish extract on the contraction of ileal segments, respectively. Results are expressed as percentage changes of the contraction caused by the maximum dose of each drug. Each point represents the mean ± s.e.m. from six to eight rats.

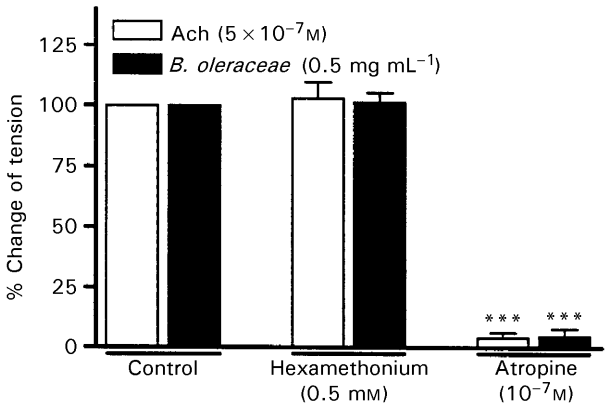

Figure 2. Effects of cholinergic antagonists on acetylcholine and radish extract-induced rat ileal contractions. Ileal segments were pretreated with each antagonist for 10 min at 37°C, followed by addition of acetylcholine (5×10^{-7} M) and radish extract (0·5 mg mL^{-1}). Each point represents the mean ± s.e.m. from six to eight rats. ***$P < 0.001$ versus control.

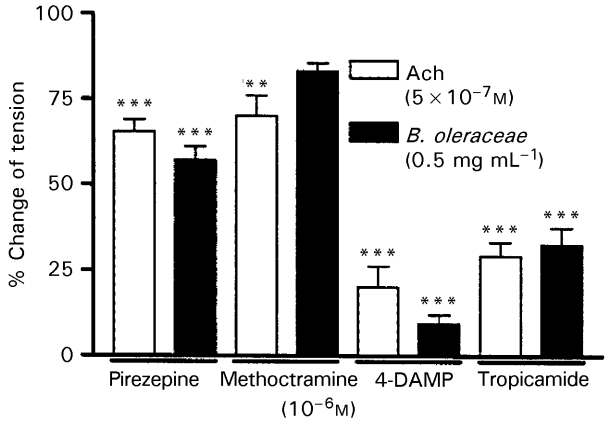

Figure 3. Effects of muscarinic receptor antagonists on acetylcholine and radish extract-induced rat ileal contractions. Ileal segments were pretreated with each antagonist (10^{-6} M) for 10 min at 37°C, followed by treatment with acetylcholine (5×10^{-7} M) or radish extract (0·5 mg mL^{-1}). Results are expressed as percentage changes of agonist alone. Each point represents the mean ± s.e.m. from six to eight rats. **$P < 0.01$, ***$P < 0.001$ versus agonist alone.

Effect of muscarinic receptor antagonists on radish extract-induced ileal contraction

We also tested the involvement of the muscarinic mechanism in radish extract-induced ileal contractions using muscarinic antagonists (Eglen et al 1996; Caulfield & Birdsall 1998; Stadelmann et al 1998; Shi et al 1999). As shown in Figure 3, the radish extract (0·5 mg mL^{-1})-induced ileal contractions were significantly inhibited by pretreatment of segments with 10^{-6} M of pirenzepine (M_1), 4-DAMP (M_3) and tropicamide (M_4) for 10 min ($P < 0.01$–0.001). A similar phenomenon, except for methoctramine (M_2), was observed in the acetylcholine (5×10^{-7} M)-induced ileal contractions. The rank order of percent inhibition was 4-DAMP (90·5% of control) > tropicamide (67·4%) > pirenzepine (42·8%) > methoctramine (16·7%).

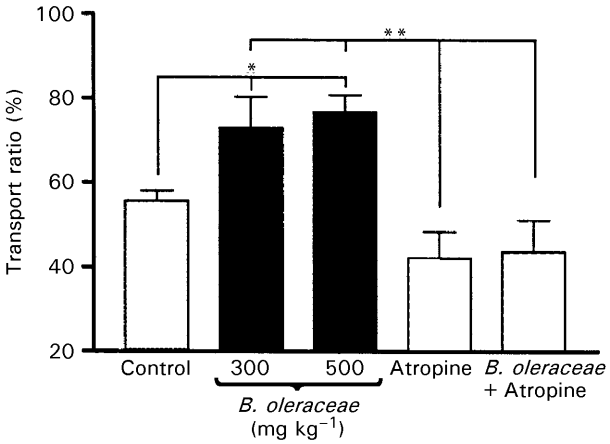

Figure 4. Effect of radish extract on the intestinal transit of charcoal in mice. Atropine (50 mg kg^{-1} body weight) was subcutaneously injected 5 min before treatment of radish extract. Thirty minutes after oral administration of radish extract, 0·2 mL of 10% charcoal suspension in 5% gum arabic was orally administered to each mouse. Each point represents the mean ± s.e.m. from six mice. *$P < 0.05$, **$P < 0.01$.

Effect of radish extract on gastrointestinal transit

To evaluate the pharmacological properties of radish extract on gastrointestinal motility in-vivo, we examined the effects of radish extract on the intestinal transit of charcoal using mice (Figure 4). In contrast to the traversed distance of marker in control rats (55·7 ± 2·42% of the total length of the intestine), radish extract (300 and 500 mg kg^{-1} body weight) significantly increased the transit of marker through the small intestine ($P < 0.05$). The increase of intestinal transit of charcoal by extract was remarkably reduced by co-administration of atropine (50 mg kg^{-1}). These results show the oral effect of radish extract on intestinal motility in-vivo.

Discussion

This study examined the spasmogenic effect of radish extract in relation to the cholinomimetic mechanism both in-vitro and in-vivo. Radish extract produced a gastrointestinal contraction, which was inhibited by muscarinic antagonists. Oral administration of radish extract exhibited increasing effects on the intestinal transit of charcoal, and these were significantly attenuated by atropine. This study therefore provides meaningful information suggesting that stimulation of radish extract-induced intestinal motility might be medi-

ated by activation of a muscarinic mechanism in-vitro and in-vivo.

In the alimentary tract, cholinomimetic mechanisms related to the excitatory actions of acetylcholine may principally be involved in the regulation of intestinal motility (Eglen et al 1996; Caulfield & Birdsall 1998) therefore in this study the potency of intestinal contraction by radish extracts was compared with that induced by acetylcholine, which was employed as a positive control to demonstrate the spasmogenic activity of the gastrointestinal tract. As shown in Figure 1, radish extract conspicuously produced intestinal contraction, and this was comparable to the acetylcholine-induced intestinal contraction. Based on previous suggestions (Eglen et al 1996; Caulfield & Birdsall 1998) and our results, it is conceivable that cholinomimetic mechanisms may be participating in the regulation of spasmogenic activity mediated by radish extracts in the gastrointestinal tract.

Recently, a relative distribution of muscarinic (Stadelmann et al 1998) and nicotinic (Delbro & Lange 1997; Nakamura et al 1998) receptor subtypes has been reported in the alimentary tract of rats. It is true that the paucity of highly selective antagonists has impeded the unambiguous identification of cholinergic receptor subtypes mediating many important responses. However, the cholinergic blockades used in this study are generally accepted as a useful tool for demonstrating the pharmacological and physiological functions of cholinergic receptor subtypes in the gastrointestinal tract (Kitazawa et al 1996; Shi & Sarna 1997; Nakamura et al 1998; Stadelmann et al 1998). Atropine conspicuously inhibited the acetylcholine and radish extract-induced ileal contractions, but these contractions were unaffected by the nicotinic receptor blocker hexamethonium (Delbro & Lange 1997). These results suggest that muscarinic synapses are important factors in mediating the stimulation of radish extract-induced gastrointestinal contraction in rats. Additionally, 4-DAMP (M_3 blockade) and tropicamide (M_4 blockade) greatly inhibited both the acetylcholine and radish extract-induced ileal contractions, whereas the inhibitory effects of pirenzepine (M_1 blockade) and methoctramine (M_2 blockade) on these contractions were relatively low. These results might be explained by previous data suggesting that contraction of intestinal smooth muscle is mainly regulated in response to the muscarinic M_3 receptor, even though this receptor is only a small percentage of the total muscarinic receptor population (Wood 1984; Eglen et al 1996). We could therefore consider cholinergic muscarinic receptors to be involved in radish extract-induced gastrointestinal contractions.

Although intestinal transit of charcoal is not a quantitative method in the sense that radioisotopic methods are (Purdon & Bass 1973), it is widely used as a useful visible marker to estimate gastrointestinal motility in-vivo (Gaginella et al 1994; Ishii et al 1994). In this study, we observed that the leading front of charcoal progressed further in the radish extract-treated rats and mice than in the controls, and atropine significantly blocked this response. This might be due to an effect of radish extract on the stomach and/or gastrointestinal tract through activation of muscarinic mechanisms. This hypothesis could be explained by previous evidence indicating that post-junctional muscarinic receptor subtypes play an important role in regulating physiological excitation in gastrointestinal fundic strips from several species (Spero 1978; Eglen et al 1996; Thomas & Ehlert 1996). Based on the results obtained here, although no direct information is available to explain the active component(s) of radish extracts involved in the activation of gastrointestinal motility, we consider that the active principal component might be a non-ionized molecule and/or a substance that can be absorbed in the gastrointestinal tract. In conclusion, these findings suggest that radish extract stimulates the spasmodic activity of the gastrointestinal tract through regulation of post-junctional muscarinic receptors in-vitro and in-vivo. Based on our knowledge, this may be the first evidence to demonstrate a pharmacological property of radish for the regulation of gastrointestinal motility.

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References

Aube, A. C., Blottiere, H. M., Scarpignato, C., Cherbut, C., Roze, C., Galmiche, J. P. (1996) Inhibition of acetylcholine induced intestinal motility by interleukin 1b in the rat. Gut 39: 470–474

Bradfield, C. A., Bjeldanes, L. F. (1991) Modification of carcinogen metabolism by indolylic autolysis products of *Brassica oleraceae*. Adv. Exp. Med. Biol. 289: 153–163

Buckley, N. J., Burnstock, G. (1986) Autoradiographic localization of peripheral M_1 muscarinic receptors using [^{3}H]pirenzepine. Brain Res. 375: 83–91

Caulfield, M. P., Birdsall, N. J. M. (1998) International union of pharmacology. XVII. Classification of muscarinic acetylcholine receptor. Pharmacol. Rev. 50: 279–290

Delbro, D. S., Lange, S. (1997) Effect of ganglionic blocking compounds on in-vivo fluid secretion in the rat small intestine. J. Pharm. Pharmacol. 49: 1109–1113

Eglen, R. M., Hegde, S. S., Watson, N. (1996) Muscarinic receptor subtypes and smooth muscle function. Pharmacol. Rev. 48: 531–565

Gaginella, T. S., Mascolo, N., Izzo, A. A., Autore, G., Capasso, F. (1994) Nitric oxide as a mediator of bisacodyl and phenolphthalein laxative action: induction of nitric oxide synthase. J. Pharmacol. Exp. Ther. 270: 1239–1245

Ishii, Y., Tanizawa, H., Takino, Y. (1994) Studies of aloe. IV. Mechanism of cathartic effect. (3). Biol. Pharm. Bull. 17: 495–497

Kitazawa, T., Kikui, S., Taneike, T., Ohaga, A. (1996) Does motilin stimulate the gastrointestinal motility of the pig? In-vitro study using smooth muscle strips and dispersed muscle cells. Gen. Pharmacol. 27: 655–664

Mascolo, N., Izzo, A. A., Autore, G., Barbato, F., Capasso, F. (1994) Nitric oxide and caster oil-induced diarrhea. J. Pharmacol. Exp. Ther. 268: 291–295

Nakamura, K., Takahashi, T., Taniuchi, M., Hsu, C. X., Owyang, C. (1998) Nicotinic receptor mediates nitric oxide synthase expression in the rat gastric myenteric plexus. J. Clin. Invest. 101: 1479–1489

Purdon, R. A., Bass, P. (1973) Gastric and intestinal transit in rats measured by a radioactive test meal. Gastroenterology 64: 968–976

Shi, X. Z., Sarna, S. K. (1997) Inflammatory modulation of muscarinic receptor activation in canine ileal circular muscle cells. Gastroenterology 112: 864–874

Shi, H., Wang, H., Wang, Z. (1999) Identification and characterization of multiple subtypes of muscarinic acetylcholine receptors and their physiological functions in canine hearts. J. Pharmacol. Exp. Ther. 55: 497–507

Spero, L. (1978) Atropine blockade of cholinergic drugs on rabbit stomach muscle. Can. J. Physiol. Pharmacol. 56: 873–876

Stadelmann, A. M., Walgenbach-Telford, S., Telford, G. L., Koch, T. R. (1998) Distribution of muscarinic receptor subtypes in rat small intestine. J. Surg. Res. 80: 320–325

Taniyama, K., Nakayama, S., Takeda, K., Matsuyama, S., Shirakawa, J., Sano, I., Tanaka, C. (1991) Cisapride stimulates motility of the intestine via the 5-hydroxytrytamine receptor. J. Pharmacol. Exp. Ther. 258: 1098–1104

Thomas, E. A., Ehlert, F. J. (1996) Involvement of the M_2 muscarinic receptor in contractions of guinea-pig trachea, guinea-pig esophagus and rat fundus. Biochem. Pharmacol. 51: 779–788

Witte, J. S., Longnecker, M. P., Bird, C. L., Lee, E. R., Frankl, H. D., Haile, R. W. (1996) Relation of vegetable, fruit, and grain consumption to colorectal adenomatous polyps. Am. J. Epidemiol. 144: 1015–1025

Wood, J. D. (1984) Enteric neurophysiology. Am. J. Physiol. 247: G585–G598