

Spontaneous Tone in Different Types of Longitudinal Muscle Preparations of Guinea Pig Ileum

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ABSTRACT—Spontaneous tone of longitudinal muscle in guinea pig ileum was investigated in three types of preparations, intact segment preparation, segment preparation deprived of the mucosal layer and longitudinal strip preparation. Tone was defined as the sustained contraction that was lost by 10^{-7} M atropine in the isotonicity recorded manner. The magnitudes of tone were constant for at least 3 hr in the two types of segment preparations. Contractions in response to 10^{-6} M acetylcholine, which were induced 9 times with an interval of 20 min between each induction, were almost identical throughout the period. In the longitudinal strip preparation, on the other hand, the tone gradually decayed and was eventually lost, while the amplitudes of acetylcholine-induced contractions were reciprocally increased. The tone in the intact segment preparation was reduced to 19% of the control by tetrodotoxin (3×10^{-7} M), to 51% by indomethacin (3×10^{-6} M) and to 26% by N^6 -cyclopentyladenosine (10^{-7} M), but was not affected by AA-861 (3×10^{-6} M) or CP-96,345 (3×10^{-7} M). In the three types of preparations, the dose-response curves for acetylcholine were alike with similar EC_{50} s. These results suggest that the tone of longitudinal muscle was mainly induced due to neural activity in the myenteric plexus of guinea pig ileum and that sensitivity to acetylcholine was not affected by the neural activity.

Keywords: Longitudinal muscle, Tone, Segment, Strip, Ileum (guinea pig)

Smooth muscles in various regions of the gastrointestinal tract are spontaneously active in vitro. The spontaneous activity is generally characterized as tone and rhythmically occurring contraction. It has been suggested that neurogenic (1–3) and myogenic mechanisms (4) are responsible for these activities. Autacoids such as prostaglandins, in addition, may also be involved in maintaining tone (5, 6).

Guinea pig ileum is one of the most frequently used gastrointestinal tissues in pharmacological studies for assessing the contractile action of drugs. It is well known that the longitudinal muscle of the ileum is spontaneously active, possessing tone with repetitive phasic contractions superimposed on it. The present study was carried out to investigate whether the tone of the longitudinal muscle of guinea pig ileum is influenced depending on the type of preparation, since available information for this issue is scarce.

MATERIALS AND METHODS

Three types of preparations

Male guinea pigs weighing 350 to 550 g (Sankyo Labo

Service Co. Inc., Tokyo) were killed by a blow on the head. The ileum was excised 10 cm proximal to the ileocecal junction, and then it was placed in a cold Krebs solution of the following composition: 140 mM NaCl, 6 mM KCl, 2 mM $CaCl_2$, 1 mM $MgCl_2$, 1 mM NaH_2PO_4 , 5 mM $NaHCO_3$ and 11 mM glucose. Intact segment preparations (approximately 3 cm) contained the mucosa and muscularis mucosa. Segment preparations deprived of the mucosal layer were made as follows: An approximately 4-cm segment was stretched on a glass rod with the mucosa outside, and a short incision was made through the muscularis mucosa at one edge of the segment. The mucosal layer, that is, mucosa and muscularis mucosa, was removed by grabbing the mucosal fringes at this end toward the other end. After that, the segment was returned to the normal state, i.e., serosa outside. In both types of segment preparations, a vinyl tube (5 mm in length, 3 mm O.D.) was inserted into one end of the segment and into the other end was inserted a stainless tube (10 mm in length, 2.5 mm O.D.) that was fixed to a tissue holder, thus allowing the lumen of the segment to be bathed with Krebs solution in an organ bath. Longitudi-

nal strip preparations (approximately 2×30 mm) were cut out from the ileal sheet that was made by opening a 4- to 5-cm segment along the mesenteric attachment. After the mucosa and muscularis mucosa were removed, the ends of the strip were tied with thread. Each preparation was vertically set under the load of 0.8 and 0.3 g for the two types of segment preparations and strip preparations, respectively, in an organ bath containing 30 ml Krebs solution. The Krebs solution in the organ bath was continuously bubbled with air and the temperature was maintained at 35°C . Preparations were equilibrated for 1 hr before exposure to the drugs.

Methods of recording tone and contraction

The tone and acetylcholine-induced contraction were recorded in an isotonic manner with 8-fold magnification on smoked paper. In all experiments, 10^{-7} M atropine was applied at the end of the experiment to relax the longitudinal muscle and determine the magnitude of tone. In 85% of the intact segment preparations (50 out of 59 preparations), the tone was immediately lowered by atropine, reached its lowest level and stayed there; in 15% of the preparations (9 out of 59), the tone, after reaching the lowest level, rose slightly and was maintained at a slightly higher level than the lowest one. In either case, the level of the maximal relaxation was defined as the zero level. During the experiments for investigating maintenance of tone, contractions in response to 10^{-6} M acetylcholine were induced for 9 times with an interval of 20 min between each induction. Magnitudes of tone and contraction were presented as a percent of the distance from the zero level, which was determined with atropine at the termination of the experiments, to the peak of contraction induced by the 1st application of 10^{-6} M acetylcholine. In some experiments for assessing the effects of various drugs on the tone, magnitudes of tone in the presence of the drugs were presented as a percent of the initial tone before exposure to the drugs. When the dose-response curves for acetylcholine were determined, each preparation was exposed to 10^{-6} M acetylcholine for 3 times, and then contractions were induced by increasing the concentrations of acetylcholine in a non-cumulative way at an interval of 15 min between the applications. Their amplitudes were determined as heights from the spontaneous tone level, not from the level of zero induced by atropine, to the peak of contractions; and they were expressed as a percent of the maximal amplitude in response to acetylcholine. EC_{50} with 95% confidence limits was calculated by a linear regression analysis for the responses ranging between 20% and 80% of the maximum.

Drugs

Drugs used in the present study were as follows:

Tetrodotoxin, indomethacin and N^6 -cyclopentyladenosine (Sigma Chemical Co., St. Louis, MO, USA); atropine sulfate, nicotine tartrate and AA-861 (Wako Pure Chemicals, Osaka); acetylcholine chloride (Dai-ichi Seiyaku, Tokyo), CP-96,345 (Pfizer Inc., Groton, CT, USA) and nifedipine (Yodogawa Seiyaku, Osaka).

Statistical analyses

Results are expressed as means \pm S.E.M. One-way analysis of variances was used for comparison of multiple means, followed by Dunnett's test where appropriate. Student's *t*-test was used for comparing two means. A difference was regarded significant when $P < 0.05$.

RESULTS

Tone maintenance and acetylcholine-induced contraction in three types of preparations

Maintenance of tone and contractile response to 10^{-6} M acetylcholine in three types of preparations was examined throughout the course of the experiments (180 min). Figure 1 shows that in the segment preparations, whether or not the mucosal layer was intact or removed, the tone and acetylcholine-induced contraction were maintained at almost constant levels. Magnitudes of tone were around 30% in both types of segment preparations and those of acetylcholine-induced contraction were around 70%. In the strip preparation, on the other hand, the tone was initially high (45%), but it was gradually reduced and was nearly abolished in 80 min after the start of the experiment (Fig. 1). Since the peak levels of acetylcholine-induced contraction were not changed throughout the period in the strip preparation, their magnitudes were increased in a reciprocal manner.

The occurrence of spontaneous phasic contractions lasting for at least 2 hr was observed in all intact segment preparations ($n=10$). Their amplitudes were 2–5% of that induced by 10^{-6} M acetylcholine. The frequency of spontaneous contractions was not measured in the present study because of slow speed of recording. In the segment preparations deprived of the mucosal layer, spontaneous phasic contractions developed repeatedly for at least 2 hr in 2 preparations out of 10. Four strip preparations out of 6 preparations showed spontaneous phasic contractions after the 1-hr equilibration period, but the phasic contractions faded during the course of the following experiment period.

Effects of drugs on the tone in intact segment preparation

In the intact segment preparation, tone was significantly reduced by 3×10^{-7} M tetrodotoxin, 10^{-7} M N^6 -cyclopentyladenosine (CPA), an adenosine A_1 -receptor agonist, and 3×10^{-6} M indomethacin (Fig. 2). Acetyl-

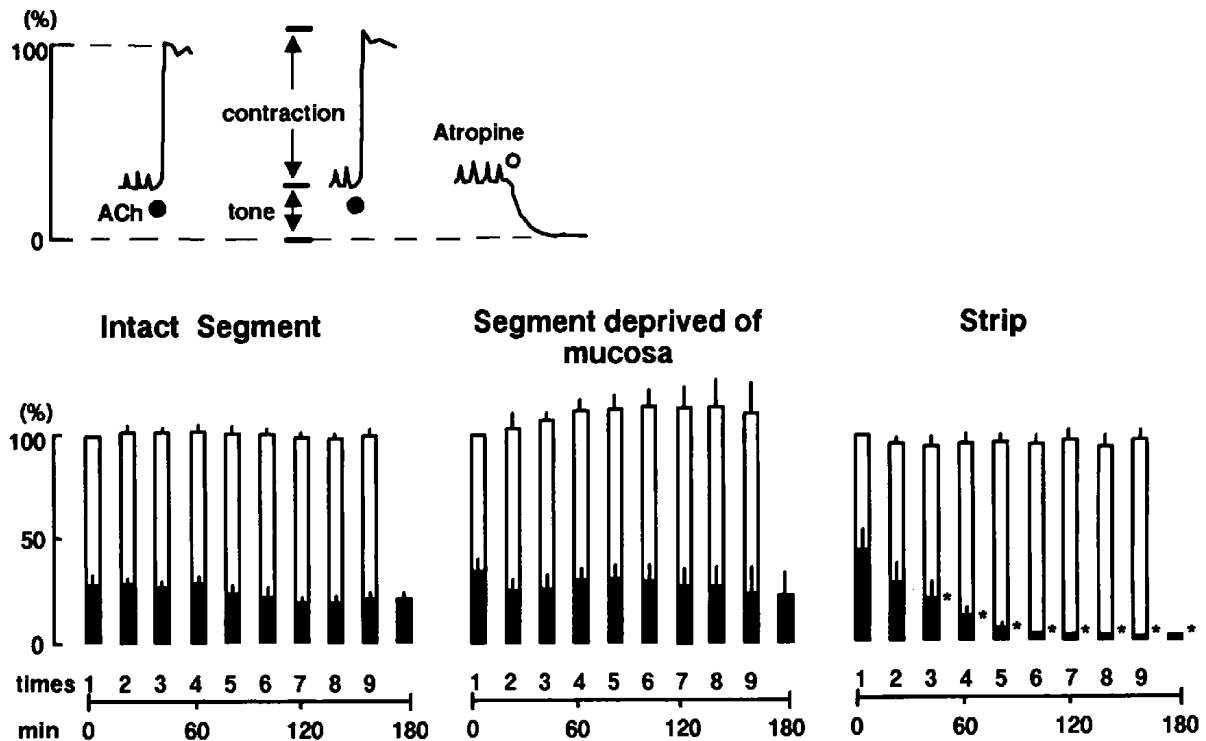


Fig. 1. Magnitudes of tone and 10^{-6} M acetylcholine-induced contractions in the intact and mucosal layer-deprived segment preparations and longitudinal strip preparation of longitudinal muscle of guinea pig ileum. The upper panel shows a scheme for quantifying the tone and acetylcholine-induced contraction. The tone induced by 10^{-7} M atropine added at the end of the experiments is taken as 0%, and the peak of acetylcholine-induced contraction applied for the first time is taken as 100%. In the lower panel, the magnitude of tone and acetylcholine-induced contraction, presented as a percent of the standard height, are shown in the filled and open portion of the column, respectively. The abscissa shows the number of acetylcholine application shown together with the period after the start of the experiments. Vertical bars present standard errors of the mean. The number of preparations tested are 7, 5 and 6 for the intact preparation, mucosal layer-deprived segment preparation and strip preparation, respectively. Asterisks show a significant difference from the initial tone level ($P < 0.05$).

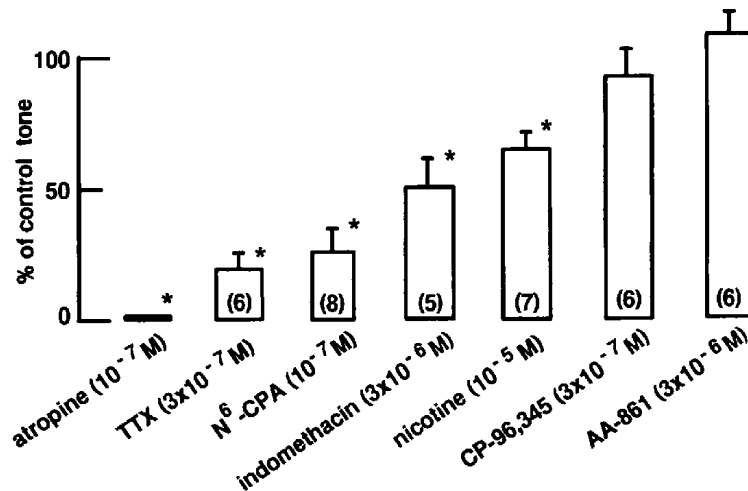


Fig. 2. Effects of drugs on the tone level in the intact segment preparation of guinea pig ileum. The ordinate presents the magnitude of the tone in the presence of drugs, represented as a percent of the initial tone magnitude determined as a relaxation induced by atropine (10^{-7} M). Vertical bars present standard errors of the mean. Asterisks show significant differences from the control tone level ($P < 0.05$). The number of preparations tested are given in parentheses.

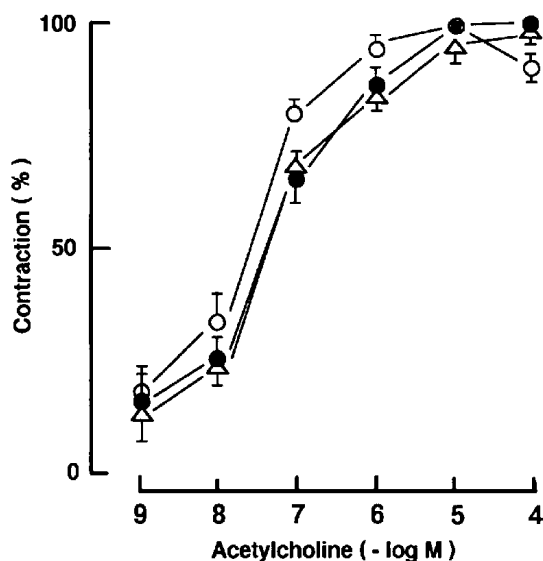


Fig. 3. Dose-response curves for acetylcholine in the intact segment (○), mucosal layer-deprived segment (●) and longitudinal strip preparation (△) of guinea pig ileum. Magnitudes of contractions are shown as a percent of the maximal response. Vertical bars show standard errors of the mean. The number of preparations tested are 6, 5 and 4 for the intact preparation, mucosal layer-deprived segment preparation and strip preparation, respectively.

choline (10^{-6} M)-induced contractions, however, were not inhibited by indomethacin or CPA; their magnitudes were rather increased (115% of the control for indomethacin and 119% for CPA), since the peak levels of acetylcholine-induced contractions were not affected by the two drugs. Nicotine (10^{-5} M) produced phasic contraction followed by sustained relaxation, the level of which was lower than the tone level prior to the addition of nicotine. Neither CP-96,345 (3×10^{-7} M) nor AA-861 (3×10^{-6} M) affected the tone in the segment preparation. When 10^{-7} M nifedipine was applied in the presence of 10^{-7} M atropine, slight further relaxation was induced ($12.1 \pm 4.6\%$ of that induced by atropine, $P < 0.05$, $n = 6$).

Dose-response curves for acetylcholine in three types of preparations

Whether or not the sensitivity of longitudinal muscle to acetylcholine was different in the three types of preparations was examined. As shown in Fig. 3, the dose-response curves for acetylcholine in the three types of preparations were similar, and there was no significant difference among EC_{50} values; they were 1.6×10^{-8} M (4.0×10^{-9} – 6.3×10^{-8} M, $n = 6$) for the intact segment preparation (95% confidence limits in parentheses), 3.6×10^{-8} M (1.0×10^{-8} – 1.6×10^{-7} M, $n = 5$) for the segment preparation deprived of the mucosal layer and 4.0×10^{-8} M (8.0×10^{-9} – 1.6×10^{-7} M, $n = 4$) for the strip preparation.

DISCUSSION

Spontaneous activity of many gastrointestinal tissues is characterized as inherent tone and repetitively developing phasic contraction. The aim of the present study was to investigate the maintenance of the tone in the longitudinal muscle of guinea pig ileum in three types of preparations. Although different development of spontaneous phasic contraction was observed in this study, this spontaneous activity was not analyzed in detail because of the difficulty in quantifying phasic contractions with the present recording methods.

When tone was recorded under isotonic conditions, its magnitude may be variable according to the definition of tone. If tone was defined as a quantity that was lost by omitting Ca^{2+} in the Krebs solution or by smooth muscle relaxants, its magnitude may be different from that determined in the present study. The tone defined as the magnitude sensitive to 10^{-7} M atropine implies that the tone is mainly induced by endogenous acetylcholine spontaneously released from neurons in the myenteric plexus. Spontaneous release of acetylcholine was shown to occur during the resting condition in guinea pig ileum (1). The present definition of tone appears to exclude possible myogenic mechanisms being responsible for tone. However, since nifedipine at 10^{-7} M relaxed the longitudinal muscle only slightly in the presence of 10^{-7} M atropine (by 12% further increase), myogenic mechanisms may not largely contribute to producing the tone in the longitudinal muscle of guinea pig ileum. As for smooth muscle relaxants, our preliminary results showed that papaverine at 10^{-5} M produced transient contraction immediately after the application, followed by repeatedly occurring phasic contractions with a slight reduction in the tone (observed in all of 4 preparations). This fact may indicate that papaverine may not be used as a relaxant in the longitudinal muscle of guinea pig ileum.

It has been proposed that longitudinal muscle is passively elongated by contraction of circular muscle in the small intestine (7, 8). Hence, tone in longitudinal muscle may be affected by contractile activity of the circular muscle in the segment preparation. We previously reported that the circular muscle in the guinea pig ileum exhibited spontaneous phasic contractions of small amplitude (less than 0.1 g) in the intact segment preparation (9). It therefore seems possible that drugs which induce contraction or increase the spontaneous activity in circular muscle cause elongation of the longitudinal muscle and thereby reduce the tone of longitudinal muscle. Involvement of such a mechanism seems likely for the effects of indomethacin as discussed later.

Tone was reduced in the segment preparation by drugs that inhibit neural functions. Tetrodotoxin and N^6 -

cyclopentyladenosine, an adenosine A_1 -receptor agonist, greatly decreased the tone by around 80% of the atropine-induced reduction. Activation of adenosine A_1 -receptors has been reported to inhibit the release of acetylcholine and tachykinin from myenteric neurons in the guinea pig ileum (10–12). The present results indicate that the motor neurons impinging on smooth muscle cells are spontaneously firing and releasing neurotransmitters continuously. Partial loss of tone by nicotine may indicate that neuro-neuronal interaction is in part involved in spontaneous firing in the motor neurons. Acetylcholine may largely be responsible for inducing the tone, and the role of tachykinin may be minor, since CP-96,345, an antagonist for NK_1 tachykinin receptors, did not significantly affect the tone.

Indomethacin inhibited the tone by approximately a half. In the rabbit small intestine, prostaglandins such as the E series were suggested to be the main substance responsible for the inherent tone, accounting for about 70–80% (6). Although the contribution of prostaglandins in the guinea pig ileum does not seem to be as large as that in the rabbit ileum, it is likely that prostaglandins may play a role in sustaining the tone either by itself or in concert with the neural mechanisms. It has been demonstrated that prostaglandin E_2 has a direct stimulatory action on the longitudinal smooth muscle cells and also increases the release of acetylcholine from the myenteric neurons in the guinea pig ileum (13–17). Furthermore, indomethacin may affect the tone of the longitudinal muscle indirectly by way of its action on circular muscle. Prostaglandin E_2 has been shown to inhibit the contraction of circular muscle of guinea pig ileum by activation of EP_2 receptors (17). Hence, the decrease of prostaglandin E_2 production by indomethacin may enhance the spontaneous phasic contraction in the circular muscle, which elongates the longitudinal muscle, causing reduction in longitudinal muscle tone. Involvement of leukotrienes may be negated in the light of no significant effect of AA-861, a 5-lipoxygenase inhibitor.

The tone in the longitudinal muscle was maintained at a constant level for at least 3 hr in the two types of segment preparations. In the strip preparation, the tone was relatively high at the start of the experiment in comparison with the segment preparations, and it was gradually lost. Previously, we reported that in the circular muscle of guinea pig ileum, substance P and neurokinin B, which were thought to release acetylcholine from myenteric neurons, produced large contractions in the segment preparation, but only produced small contractions in the circular strip preparation (18). We surmised that the small contraction in the circular strip preparation was due to poor preservation of intact neurons in the myenteric plexus, since it seemed most likely that neurons and con-

nections between them were much more damaged resulting from incision in longitudinal and circumferential orientation in this type of preparation. Loss of longitudinal muscle tone in the strip preparation, shown in the present study, may be accounted for by impairment of neural activity in the myenteric plexus. The initial high tone in the strip preparation is thought to be due to transient anomalous activity of myenteric neurons ensuing from injury caused by the preparing treatment. In support of this, our preliminary results showed that when strip preparations were made from the ileum cold-stored for a night, they had no tone at the completion of a 1-hr equilibration. Removal of the mucosal layer in the strip preparation cannot be responsible for the reduced tone, since this treatment did not affect the tone in the segment preparation.

Regardless of the different levels of the tone in the segment and strip preparations, the dose-response curves for acetylcholine were similar in the three types of preparations. This result, although predictable in view of the direct action of acetylcholine on the smooth muscle cells, clearly showed that sensitivity of the longitudinal muscle to acetylcholine was independent of neural activity in the myenteric plexus in the guinea pig ileum. However, this does not imply that the effects of neurotropic drugs will also be the same in the three types of preparations. The results shown in the present study may be useful in making an appropriate preparation of guinea pig ileum according to the purpose of a specific experiment.

REFERENCES

- 1 Paton WDM and Zar MA: The origin of acetylcholine released from guinea pig intestine and longitudinal muscle strip. *J Physiol (Lond)* **194**, 13–33 (1968)
- 2 Szurszewski JH: Modulation of smooth muscle by nervous activity: A review and a hypothesis. *Fed Proc* **36**, 2456–2461 (1977)
- 3 Neya T, Mizutani M and Nakayama S: Enteric opioid neurons modulate the basal tone of the isolated puppy ileum. *Can J Physiol Pharmacol* **65**, 1934–1936 (1986)
- 4 Prosser CL and Sperelakis N: Transmission in ganglion-free circular muscle from the cat intestine. *Am J Physiol* **187**, 536–545 (1956)
- 5 Bennett A, Eley KG and Stockley HL: The effects of prostaglandins on guinea-pig isolated intestine and their possible contribution to muscle activity and tone. *Br J Pharmacol* **54**, 197–204 (1975)
- 6 Ferreira SH, Herman AG and Vane JR: Prostaglandin production by rabbit isolated jejunum and its relationship to the inherent tone of the preparation. *Br J Pharmacol* **56**, 469–477 (1976)
- 7 Wood JD and Perkins WE: Mechanical interaction between longitudinal and circular axes of the small intestine. *Am J Physiol* **218**, 762–768 (1970)
- 8 Sarna SK: Gastrointestinal longitudinal muscle contractions.

- Am J Physiol **265**, G156–G164 (1993)
- 9 Suzuki N, Mizuno K and Gomi Y: Neurogenic "off" contractions are mediated by NK₂-receptors in the circular muscle of guinea pig ileum. *Jpn J Pharmacol* **64**, 213–216 (1994)
 - 10 Shinozuka K, Maeda T and Hayashi E: Effects of adenosine on ⁴⁵Ca uptake and [³H]acetylcholine release in synaptosomal preparation from guinea-pig ileum myenteric plexus. *Eur J Pharmacol* **113**, 417–424 (1985)
 - 11 Katsuragi T, Shirakabe K, Ogawa S, Soejima O and Furukawa T: Involvement of dihydropyridine-sensitive Ca²⁺ channel in adenosine-evoked inhibition of acetylcholine release from guinea pig ileal preparation. *J Neurochem* **55**, 363–369 (1990)
 - 12 Briad RM, McDonald TJ, Brodin E and Cook MA: Adenosine A₁ receptors mediate inhibition of tachykinin release from perfused enteric nerve endings. *Am J Physiol* **262**, G525–G531 (1992)
 - 13 Kadlec C, Masek K and Seferna I: A modulatory role of prostaglandins in contractions of the guinea-pig ileum. *Br J Pharmacol* **51**, 565–570 (1974)
 - 14 Gustafsson L, Hedqvist P and Lundgren G: Pre- and postjunctional effects of prostaglandin E₂, prostaglandin synthetase inhibitors and atropine on cholinergic neurotransmission in guinea pig ileum and bovine iris. *Acta Physiol Scand* **110**, 401–411 (1980)
 - 15 Takeuchi T, Okuda M and Yagasaki O: The differential contribution of endogenous prostaglandins to the release of acetylcholine from the myenteric plexus of the guinea-pig ileum. *Br J Pharmacol* **102**, 381–385 (1991)
 - 16 Lawrence RA, Jones RL and Wilson NH: Characterization of receptors involved in the direct and indirect actions of prostaglandins E and I on the guinea-pig ileum. *Br J Pharmacol* **105**, 271–278 (1992)
 - 17 Botella A, Delvaux M, Fioramonti J, Frexinos J and Bueno L: Stimulatory (EP₁ and EP₃) and inhibitory (EP₂) prostaglandin E₂ receptors in isolated ileal smooth muscle cells. *Eur J Pharmacol* **237**, 131–137 (1993)
 - 18 Suzuki N, Mizuno K and Gomi Y: Tachykinin-induced contractions in the circular muscle of guinea pig ileum. *Jpn J Pharmacol* **65**, 233–240 (1994)