Effects of CP-96,345, a Novel Non-Peptide Antagonist of NK₁ Receptor, on the Peristalsis in Isolated Guinea Pig Ileum

Nagao Suzuki and Yasuo Gomi

Department of Pharmacology, Faculty of Pharmaceutical Sciences, Kanazawa University, Kanazawa 920, Japan

Received January 9, 1992 Accepted February 17, 1992

ABSTRACT—CP-96,345, a novel non-peptide antagonist of the NK₁ receptor, at 10⁻⁸–10⁻⁶ M decreased the frequency of peristalsis and reduced peristalsis-associated longitudinal muscle contractions in isolated guinea pig ileum. In the presence of 10⁻⁶ M CP-96,345, further addition of 10⁻⁶ M atropine blocked the peristalsis. When 10⁻⁶ M atropine was first applied, more than half of the preparations developed atropine-resistant peristalsis. CP-96,345 at 10⁻⁶ M blocked the atropine-resistant peristalsis. These results are consistent with the view that substance P is involved in the peristalsis in guinea pig ileum.

It is accepted that acetylcholine is the most important excitatory motor transmitter for the contraction of longitudinal and circular smooth muscle in the peristaltic reflex of the small intestine. A number of studies have shown that muscarinic antagonists such as atropine and scopolamine strongly impaired small intestinal peristalsis (1). However, there are many reports demonstrating that peristalsis persisted after the blockade of muscarinic receptors in isolated guinea pig ileum (2, 3). Hence, it has been suggested that motor transmitters other than acetylcholine are also involved in the contractile responses of the intestinal peristalsis. Accumulating evidence has indicated that substance P is a non-cholinergic neurotransmitter of the motor neuron or the interneuron responsible for the atropine-resistant peristalsis in guinea pig ileum (2).

Recently, CP-96,345, a non-peptide agent, has been found to possess a potent antagonistic action against a subtype of substance P receptor, NK₁ (4). In the present study, we investigated the effects of CP-96,345 on the peristalsis in the absence or presence of atropine to elucidate the role of substance P in the peristaltic reflex of guinea pig ileum.

Male guinea pigs (300–500 g) were killed by a blow on the head, and 20-cm segments of ileum approximately 15 cm proximal to the ileocecal junction were taken. After the mesentery was carefully removed, a portion of ileum (3–4 cm) without lymphoid tissues was horizontally held in a 40-ml organ chamber. Continuous peristalsis was induced according to a modified method of Bülbring and Lin (5), in principle, by radial stretching of the intestinal wall due to the filling of the lumen with Krebs solution. As parameters of peristalsis, longitudinal muscle contractions and intraluminal pressure were recorded.

The oral and anal ends of the ileum were connected to the inflow silicon tube and outflow glass cannula, respectively. The inflow silicon tube was looped to allow the preparation to change its length. At the connected point of the oral end, one end of a 5-cm stainless rod was tied, and the other end was tied...
to a strain-gauge (Shinkoh, Japan) to assess the longitudinal muscle contraction. The contractions recorded under this condition, hence, are quasi-isotonic, although they were expressed as the degree of force (g) in the figures. The outflow cannula was constructed from a "|" shaped glass tube. The bottom end, bent at right angle, was connected to the anal end of the ileal preparation. The top end was connected to a pressure transducer (Nihon Kohden, Japan) to record the intraluminal pressure, and the horizontal end was used for the outlet of perfusate. The position of the outlet was held 2 cm above the preparation to load the pressure on the ileum.

After completion of the preparation setup, intraluminal perfusion was started at the rate of 0.3–0.4 ml/min, which induced the peristalsis approximately twice per min. The bathing solution in the chamber contained 3 × 10⁻¹⁰ M pirenzepine to enhance the peristalsis (6) and was continuously exchanged at the rate of 2 ml/min. When the effects of CP-96,345 or atropine were investigated, the drugs were added to the organ chamber and the bathing perfusion solution was switched to the drug-containing one. Experiments were done at 35°C and the perfusion solutions were prewarmed to 35°C.

The Krebs solution had the following composition: 120 mM NaCl, 2.0 mM CaCl₂, 1.0 mM MgCl₂, 20.0 mM NaHCO₃, 5.0 mM HEPES, 1.0 mM NaH₂PO₄, and 14.0 mM glucose; the Krebs solution was bubbled with 95% O₂ and 5% CO₂.

Drugs used were pirenzepine dihydrochloride (Sigma, USA), atropine sulfate (Wako Pure Chemicals, Japan) and CP-96,345 (Pfizer, Inc., USA).

By 60 min after the start of intraluminal perfusion, peristalsis became stable and continued thereafter with nearly constant frequency, magnitudes of the longitudinal contraction and expelling pressure (Figs. 1 and 2). Peristaltic reflex proceeded with the progressive shortening of the longitudinal muscle followed by the aborally propagating constriction of the circular muscle, which was accompanied by the relaxation of the longitudinal muscle. At the threshold pressure, constriction of the circular muscle was initiated at the oral end and propagated to the anal end, ejecting the intraluminal fluid.

Application of CP-96,345 (10⁻⁸–10⁻⁶ M) slightly inhibited the longitudinal muscle contractions and reduced the frequency of peristalsis (Fig. 1A). At 10⁻⁶ M CP-96,345, the longitudinal muscle contractions were reduced to 71.9 ± 2.1% of that prior to the addition of CP-96,345 (P < 0.05, analyzed with the paired t-test, mean ± S.E., n = 26), and the frequency was decreased from 15.2 ± 0.7 to 13.5 ± 0.6 times/10 min (P < 0.05). However, in none of the preparations tested was peristalsis blocked after the addition of 10⁻⁶ M CP-96,345.

The peristalsis in the presence of CP-96,345 was blocked by further addition of 10⁻⁶ M atropine. Figure 1B shows that 10⁻⁶ M atropine added subsequently to CP-96,345 abolished almost all the ileal contractile activity. Similar results were observed in 19 preparations out of 26; in these ileal preparations, the intraluminal perfusate dripped out from the anal end drop by drop. In the remaining 7 preparations, weak irregular segmental contractions which poorly ejected the intraluminal fluid were retained.

Conversely, when 10⁻⁶ M atropine was first applied, atropine-resistant peristalsis developed in more than half of the preparations tested (16 out of 24 preparations). Representative recordings of atropine-resistant peristalsis are shown in Fig. 2. The remaining 8 preparations either failed to develop any contractile activity (n = 3) or showed segmental contractions (n = 5). Atropine-resistant peristalsis developed shortly after the addition of atropine, within 10 min in most of the preparations, and lasted for at least 30 min (Fig. 2A). Longitudinal muscle contractions were immediately reduced after the addition of atropine and then partially restored to 42.5 ± 2.5% of that prior to the application of atropine (P < 0.05, mean ± S.E., n = 16). Except for the early unstable period, the frequency of atropine-
resistant peristalsis was much lower than that of the ordinary peristalsis; the frequency decreased from 14.4 ± 0.9 in the absence of atropine to 10.2 ± 0.6 times/10 min in the presence of atropine (P < 0.05, n = 16). Atropine raised the threshold pressure to initiate the ejection, which may account for the decreased frequency of the peristalsis.

CP-96,345 at 10^{-6} M blocked the atropine-resistant peristalsis in the 16 preparations which developed the atropine-resistant peristalsis; The contractile responses were abolished in 13 preparations (Fig. 2B). In the residual 3 preparations, where no peristaltic reflex occurred, weak segmental constrictions were retained, as described earlier for the effects of atropine in combination with CP-96,345.

CP-96,345 is a newly found non-peptide antagonist of the NK1 subtype of the substance P receptor, and it is viewed as a useful tool for studying the physiological function of
substance P (4). Lecci et al. have recently reported that CP-96,345 at concentrations up to $5 \times 10^{-7}$ M competitively antagonized the contracting action of substance P methylester in the guinea pig ileum, although CP-96,345 at more than this concentration produced unspecific depression (7). In our separate experiments, CP-96,345 at $10^{-6}$ M slightly inhibited the $10^{-5}$ M methacholine-induced contractions by 17% in the longitudinal strip of the guinea pig ileum, but did not inhibit the contractions in the circular strip.

The present study demonstrated that CP-96,345 at concentrations ranging from $10^{-8}$ M to $10^{-6}$ M inhibited the peristalsis in isolated guinea pig ileum. CP-96,345 at $10^{-6}$ M reduced the contraction of longitudinal muscle and decreased the frequency of peristalsis in the absence of atropine; however, CP-96,345 by itself did not block the peristalsis. Additional application of $10^{-8}$ M atropine blocked the peristalsis that was occurring in the presence of $10^{-6}$ M CP-96,345. When $10^{-6}$ M CP-96,345 was applied to the ileal preparations in the presence of atropine, CP-96,345 blocked the atropine-resistant peristalsis. These results may confirm the previous study indicating that acetylcholine and substance P are involved in the peristalsis of isolated guinea pig ileum (2, 3). It has been reported that NK1 receptor-mediated contractions are largely myogenic in the guinea pig ileum (8). Hence, CP-96,345 may inhibit the peristalsis by blocking the postsynaptic NK1 receptor in the smooth muscle cells, although there remains a possibility that the inhibitory action of CP-96,345 on peristalsis may not entirely be due to the antagonist action on the NK1 receptor, but partly due to its nonspecific action.

It is apparent that contribution of substance P is different between the ordinary and atropine-resistant peristalsis. For ordinary peristalsis, substance P is not essential, since in none of the preparations was the peristalsis abolished by CP-96,345. On the other hand, substance P seems crucial for the atropine-resistant peristalsis, since CP-96,345 blocked this peristalsis in all the preparations. Similar results with respect to the action of substance P in the two types of ileal peristalsis have been reported by other investigators using spantide or receptor desensitization to block the substance P receptor (2, 3).

The different role of substance P in the two types of peristalsis may, as suggested by Holzer (3), indicate that multiple neural pathways with different transmitters are responsible for the peristalsis and that by adaptive interactions between these pathways, the pathway including substance P takes over part of the function played by acetylcholine after blockade of the muscarinic receptors. However, the present study showed that atropine-resistant peristalsis took place shortly after the addition of atropine, probably too fast for the adaptive conversion of neuronal pathways to occur. Furthermore, release of substance P in association with the peristaltic reflex was observed in the absence of atropine (9). From these facts, an alternative conjecture may be deduced that the action of substance P is supplementary in ordinary peristalsis, but becomes functionally important and plays a major role under the blockade of the muscarinic action of acetylcholine.

Acknowledgments

Authors are grateful to Pfizer, Inc. for the gift of CP-96,345.

REFERENCES

1 Kosterlitz, H.W. and Lees, G.M.: Pharmacological analysis of intrinsic intestinal reflexes. Pharmacol. Rev. 16, 301–339 (1964)
2 Bartho, L. and Holzer, P.: Search for a physiological role of substance P in gastrointestinal motility. Neuroscience 16, 1–32 (1985)
3 Holzer, P.: Ascending enteric reflex: multiple neurotransmitters systems and interactions. Am. J. Physiol. 256, (Gastrointest. Liver Physiol. 19) G540–G545 (1989)
4 Snider, R.M., Constantine, J.W., Lowe, J.A., III, Longo, K.P., Lebel, W.S., Woody, H.A. et al.: A potent nonpeptide antagonist of the substance P (NK1) receptor. Science 251, 435–437 (1991)
5 Bülbirin, E. and Lin, R.C.Y.: The effect of intraluminal application of 5-hydroxytryptamine and 5-
hydroxytryptophan on peristalsis; the local production of 5-HT and its release in relation to intralu- minal pressure and propulsive activity. J. Physiol. (Lond.) 140, 381–407 (1958)

6 Schwörer, H. and Kilbinger, H.: Enhancement of guinea-pig intestinal peristalsis by blockade of muscarinic M1-receptors. Br. J. Pharmacol. 93, 715–720 (1988)

7 Lecci, A., Giuliani, S., Patacchini, R., Viti, G. and Maggi, C.A.: Role of NK1 tachykinin receptors in thermonociception: effect of (±)-CP-96,345, a non-peptide substance P antagonist, on the hot plate test in mice. Neurosci. Lett. 129, 299–302 (1991)

8 Maggi, C.A., Patacchini, R., Giachetti, A. and Meli, A.: Tachykinin receptors in the circular muscle of the guinea-pig ileum. Br. J. Pharmacol. 101, 996–1000 (1990)

9 Donnerer, J., Barthó, L., Holzer, P. and Lembeck, F.: Intestinal peristalsis associated with release of immunoreactive substance P. Neuroscience 11, 913–918 (1984)