Effects of Cisapride on Isolated Guinea Pig Colon

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Abstract—Effects of cisapride were studied on the whole segments and muscle strips isolated from the guinea pig ascending colon. In whole segment preparations, external application of cisapride (0.4–10 μM) produced relaxation and inhibited spontaneous and evoked intraluminal pressure changes. Intraluminal application (1–10 μM) increased the pressure response. In many circular muscle strips (18 preparations out of 20), cisapride (0.3–1 μM) increased the mechanical activity, particularly when the pH was lowered, associated with an increased spike activity. However, this action was relatively weak, and in some preparations (2 preparations out of 20), almost no effect was observed. At higher concentrations (more than 1–5 μM), cisapride reduced the muscle tone and nerve-mediated contractions. The cisapride effect could be demonstrated in the presence of atropine (0.5 μM) and serotonin (5 μM). It was concluded that cisapride has direct inhibitory as well as excitatory actions. There was a tendency for the excitatory action to appear at a lower concentration than for the inhibitory action.

Cisapride has been reported to increase gastro-intestinal motility. However, its action seems rather complex. The main action is considered to be due to an increase in acetylcholine release from myenteric neurons (1–3). In the guinea pig intestine, cisapride (10 μM) increased spontaneous release, whereas it inhibited the outflow of acetylcholine evoked by nerve stimulation (4). Cisapride antagonized serotonin effects on the myenteric neurons (5, 6). However, the relationship between this action and the mechanical potentiation of cisapride is not clear, because electrophysiological studies failed to show excitatory actions of cisapride on the neurons (6). In the guinea pig taenia caeci, cisapride has been shown to depolarize the membrane and to increase spontaneous spike activity. This is considered to result from a direct action on smooth muscle by inhibiting Ca-pump activity (7). It has also been reported that in the guinea pig colon, cisapride produced large contractions of the longitudinal muscle at 0.1–1 μM and that this effect was not blocked by tetrodotoxin and atropine (3, 8). In the present experiments, effects of cisapride were further examined using whole segments and longitudinal and circular muscle strips isolated from the guinea pig colon. The results suggest that cisapride exerts direct inhibitory effects on both circular and longitudinal muscles and weak direct excitatory effects mainly on the circular muscle, although a partial contribution of acetylcholine release cannot be neglected.

Materials and Methods

Guinea pigs (Hartley, 250–300 g) of either sex were stunned, then killed by exsanguination, and the colon (about 10 cm long) was removed at about 20 cm anal to the ileocaecal junction. For one type of experiment, the whole segment (about 7 cm long) was used to record isotonic longitudinal contraction and intraluminal pressure. This method was fundamentally similar to that described by Bulbring et al. (9), except that the responses were electrically recorded using an isotonic transducer (Nihon Kohden, TD 112S, with a load of 2–5 g) and a pressure transducer (Nihon Kohden, MPU-0.5A) on a potentiometric pen recorder (Graphtec Corp. SR 6221). The preparation was mounted in an organ bath (capacity: 50 ml) and superfused at a rate of 5 ml/min (35°C). The inside of the colon was separately perfused at a rate of
about 1 ml/min.

For another type of experiments, isolated muscle strips were used. After removing the mucosal layer, small pieces (approximately 2×7 mm) were dissected out in a circular or longitudinal direction of the colon for recording of isometric contraction. Occasionally, slightly larger pieces with mucosa attached were also used, but no difference was found in the effect of cisapride. The preparations were mounted vertically with a weight of about 0.2 g in a small chamber (3 ml capacity), through which solution flowed continuously at a constant rate of 3 ml/min. For both types of experiment, electrical transmural or field stimulation (0.5 msec pulses, 20 Hz) was applied for 1 sec at twice threshold intensity. The normal solution contained 118 mM NaCl, 6 mM KHCO₃, 2.4 mM CaCl₂, 1.2 mM MgCl₂, 11.8 mM glucose and 15 mM Tris-buffer. This solution was titrated to pH 7.4 with HCl and saturated with 100% oxygen. In some experiments, De Jalon solution (3) was used which contained: 154.1 mM NaCl, 5.6 mM KCl, 0.54 mM CaCl₂, 5.9 mM NaHCO₃ and 2.8 mM glucose, gassed with 95% O₂ and 5% CO₂. However, no fundamental difference was found between these two solutions.

The conventional microelectrode method was used for intracellular recording of membrane potential. To achieve stable penetration, mechanical activities were minimized by fixing the preparations in the bottom of a chamber (0.5 ml) with many fine pins. The chamber was continuously perfused at a rate of 2 ml/min with solutions pre-warmed at 35°C.

Drugs used were cisapride (cis-4-amino-5-chloro-N-(1-[3-(4-fluorophenoxy)propyl]-3-methoxy-4-piperidinyl)-2-methoxy-benzylamide monohydrate), tetrodotoxin, carbachol, atropine and serotonin. All drugs were obtained from Sigma, except cisapride (Janssen). Cisapride was dissolved in lactic acid (4%) to prepare a stock solution of 100 mM.

Results

Isolated segments of the colon were always spontaneously active. Electrical transmural stimulation elongated the preparation and increased the intraluminal pressure. Cisapride applied to the organ bath relaxed the preparation and inhibited the spontaneous and evoked increases in the intraluminal pressure (Fig. 1). In contrast to the previous report (3), no shortening of the preparation was observed in response to cisapride (0.01–10 μM), and the result was the same even when both ends of the preparation was kept open to eliminate pressure changes.

There was often a transient increase in spontaneous pressure changes before the inhibition developed, and this was followed by a prolonged increase in the activity when the cisapride concentration was less than 1 μM. However, when the concentration was increased to more than 1 μM, the inhibition became stronger and prolonged. Since effects of cisapride varied among the preparations and the recovery was generally very slow, quantitative analysis was difficult.

As shown in Fig. 2, effects of intraluminal application of cisapride differed from those of external application. Cisapride, applied internally, potentiated the pressure changes induced by electrical stimulation, and at higher than 5 μM, it also increased spontaneous pressure changes. On the other hand, when externally applied, cisapride produced inhibitory effects, particularly at a high concentration (10 μM).

The pressure response is considered to represent mainly the activity of the circular muscle. On the other hand, the elongation of the colon may be caused either by relaxation of longitudinal muscle or by contraction of circular muscle, or both, due to a nearly constant volume of the lumen. Therefore, effects of cisapride were examined on an isolated muscle strip dissected in a longitudinal or circular direction. They were both spontaneously active, but their pattern of activity varied in different preparations to some degree. Longitudinal muscle strips often produced a regular rhythmic activity. Figure 3 shows an example of cisapride effects on the longitudinal muscle strip. In most preparations (18 preparations out of 20), cisapride reduced the spontaneous contractions dose-dependently at higher than 1 μM and made contractions irregular. The effects were weaker than those observed in the whole prep-
In the preparations (2 preparations out of 20) which showed very irregular contractions of high frequency, cisapride weakly increased the contraction at 1 μM, but when the concentration was increased to more than 3 μM, it reduced the contraction. During the inhibition caused by a high concentration of cisapride, the activity was desynchronized and became continuous. These effects of cisapride were not affected by atropine (1 μM).

In the circular muscles, the mechanical activity was usually small and consisted of irregular rhythmic contractions. Low concentrations of cisapride (0.1–1 μM) slightly increased muscle tone and frequency of spontaneous activity in many preparations (8 preparations out of 12), but the degree of potentiation differed among the preparations, and no clear effect was observed in the remaining preparations. In contrast to cisapride, carbachol always produced a clear contraction, and the largest contraction observed...
with 1 μM cisapride was about 10% of the response to 0.1 μM carbachol. Figure 4 shows an example of the clear stimulating effects of cisapride. Lactate used as a solvent for cisapride did not produce any consistent effects. When the concentration of cisapride was increased to more than 5 μM, the muscle tone and size of spontaneous activity were reduced, although the frequency of activity was increased. In the following experiments, the cisapride effect was mainly analyzed in the circular muscle strips.

Figure 5 shows cisapride effects on electrical activities recorded from a piece of circular muscle with an intracellular microelectrode. The resting potential was between −55 and −60 mV. The action potentials in the circular muscle were irregular in amplitude and frequency, as shown in (a). Cisapride (1 μM) depolarized the membrane by about 5 mV, but due to unstable membrane potential, its precise estimation was difficult. The depolarization was accompanied with an increased frequency of spikes. The effect of cisapride recovered very gradually. Even at a concentration of 10 μM, which produced an inhibitory action on mechanical activity, cisapride depolarized the membrane and increased the frequency of spontaneous spike activity. As observed for the mechanical activity the high spike activity continued for more than 20 min after wash-out of cisapride.

In circular muscles, electrical stimulation produced a large phasic contraction, followed...
Fig. 3. Effects of cisapride (1–10 μM) on spontaneous contractions of a longitudinal muscle strip of guinea pig colon, in the absence (a–c) and in the presence (d–f) of atropine (0.5 μM). Cisapride reduced the amplitude of spontaneous contractions (continuous isometric recording).

by slow relaxation, as shown in Fig. 6. The response to field stimulation was considered to be nerve-mediated, because it was abolished by tetrodotoxin (0.5 μM). Cisapride (0.1–1 μM) potentiated the response to field stimulation in some preparations (6 preparations out of 12), but in others, it did not have any significant effect (3 preparations out of 12) or produced weak inhibitory effects (3 preparations out of 12), as shown in Fig. 6b. When the concentration was increased to more than 5 μM, cisapride always reduced the response.

Since the potentiating effect of cisapride on the circular muscle was variable from one preparation to another, we have examined whether the pH of the superfusing solution had any effect. At higher than pH 7.8, cisapride (1 μM) showed only a very weak or almost no excitatory effect (n=4). On the other hand, at a pH of about 7.0 (6.8–7.2), a clear excitatory effect was always observed (n=4). An example of the pH effect was shown in Fig. 7. When the solution was made alkaline, the muscle tone was increased, and the spontaneous activity and evoked contractions became small. The cisapride effect on the muscle tone recovered by lowering
Fig. 4. Effects of cisapride on spontaneous mechanical activity in circular muscle. Lactate, the solvent for cisapride, was first applied for 5 min before cisapride with the same concentration of lactate. Cisapride of increasing concentrations was applied at 40 min intervals. The broken line indicates the level of control basal tension.

Fig. 5. Effects of cisapride (1 μM) on electrical activity in the circular muscle, recorded continuously with an intracellular microelectrode. Cisapride was applied for 5 min, as indicated by the horizontal bar on the top of the record.

the pH to 7.0, but the recovery of spontaneous contractions was poor (c).

Serotonin (5 μM) increased the mechanical activity transiently, lasting only for 2 min (Fig. 8b). In the presence of serotonin, which might have desensitized the receptors, cisapride (1 μM), applied 12 min after serotonin, still produced an increase in muscle tone, but inhibited the response to electrical stimulation.

Atropine (0.5 μM) reduced the muscle tone
Fig. 6. Effects of cisapride on spontaneous and electrically evoked contractions in the circular muscle. Electrical field stimulation (0.5 msec pulses, 20 Hz for 2 sec) was applied every 6 min. At 0.1 μM, cisapride slightly increased muscle tone and the response to nerve stimulation (a). At 1 μM, excitatory action on spontaneous activity was increased, but electrically evoked responses were inhibited (b). At 10 μM, both responses were reversibly inhibited by cisapride (c, d).

and spontaneous mechanical activity. Contractions produced by electrical stimulation were also inhibited, but some contractile response always remained (Fig. 8c, d). The remaining responses were not affected by increasing the atropine concentration to 1 μM (not shown). The increase in muscle tone caused by cisapride was slightly less in the presence of atropine, but this reduction was much weaker compared with that of spontaneous or evoked activity.

Discussion

It has been reported that the guinea pig colon, vertically suspended, is not spontaneously active and that cisapride (5.4 nM–5.4 μM) produced a large shortening of the preparation (3). However, we failed to confirm these results. In the whole segments and also in the muscle strips, there was always spontaneous activity and cisapride (0.3–10 μM) produced relaxation, even in the same (De Jalon) solution as used by Schuurkes et al. (3). In the circular muscle, a small but clear excitatory action of cisapride can be demonstrated, particularly in an acidic solution (pH 6.8–7.2), although the action of cisapride was much weaker compared with carbachol. In the whole segment preparations, shortening of the longitudinal muscle may partly be antagonized by contraction of the circular muscle, but this effect does not seem to be significant, because the main action of cisapride on isolated longitudinal muscle strips is inhibitory. The reason for the difference between the results obtained by Schuurkes et al. (3) and the results of the present experiments is not clear.

Application of cisapride from the luminal side cases potentiation of both spontaneous and nerve-mediated contractions, at concentrations (1–10 μM) which have inhibitory actions when applied externally. This may be due to the fact that with luminal application, the concentration of cisapride in the muscle layers may not reach the concentration in the lumen, due to slow penetration from the mucosal side, in conjunction with efficient wash-out of cisapride with external perfusing solution. Therefore, with luminal application, selective activation of excitatory myenteric neurons by cisapride might be possible without interference of direct inhibitory action on the smooth muscle. Furthermore, myenteric nerves in the whole segment preparations may exert a stronger excitatory effect than that in isolated muscle strips.

The increased tone in circular muscle by cisapride has been considered to be due to an increase in acetylcholine release from cholinergic nerve fibers (4). However, since the excitatory effect of cisapride is not blocked by atropine, at least in the colon, some atropine-
resistant mechanism is involved in this action, as previously reported (3, 8). Serotonin does not seem to be responsible for the atropine-resistant potentiation, because the cisapride action is not essentially affected in the presence of 5 μM serotonin. Furthermore, since serotonin itself has an excitatory action on the guinea pig colon, the blockade of serotonin receptors by cisapride, as demonstrated by Neya et al. (5) and Nemeth et al. (6), would cause inhibition rather than excitation.

Mechanical potentiation by cisapride is associated with depolarization of the membrane and an increase in spike activity, as previously observed in the taenia caeci with the sucrose-gap method (7). It is very likely that this is due to a direct action of cisapride on the plasma membrane. At a high concentration (more than 5 μM), cisapride reduces the muscle tone and markedly inhibits the response to nerve stimulation. However, there is no electrical change which correlates with the mechanical inhibition. The depolarization of the membrane and the increase in spike activity caused by 10 nM cisapride are nearly the same as those observed with 1 μM cisapride. Therefore, cisapride seems to inhibit excitation-contraction coupling at excessive doses.

The results indicate that cisapride has both excitatory and inhibitory actions on the smooth muscle of the guinea pig colon, confirming the previous observation (8). This may be one of the reasons for the variability in the effect of cisapride. At low concentrations, the excitation is dominant, particularly in acidic solution (pH 6.8–7.2), whereas at high concentrations, the inhibition becomes relatively predominant. The cisapride action may also be related to the muscle tone. The inhibitory action seems potentiated at a high muscle tone, as demonstrated for the methacholine-induced contraction in the guinea pig ileum (3). Inversely, the excitatory action is more easily demonstrated at a low muscle tone, as observed in acidic solution. The excitatory action is probably much more long-lasting than the inhibitory action, and this may be the reason for the potentiation of spontaneous mechanical activity following removal of high concentrations of cisapride.

Fig. 7. Cisapride effects on the circular muscle in solutions of pH 7.0 and 7.8. Electrical field stimulation was applied at 6 min intervals. The pH was changed 20 min before the beginning of the records (b) and (c). For further explanations, see the text.
Fig. 8. Effects of serotonin and atropine on the cisapride action. Cisapride was applied in the absence (a) and in the presence (b) of serotonin (5 μM) as indicated by horizontal bars underneath. The same experiment was repeated in the presence of atropine (0.5 μM) on a different preparation (c, d).

References
1 Van Nueten, J.M., Van Daele, P.G.H., Reyntjens, A.J., Janssen, P.A.J., and Schuurkes, J.A.J.: Gastrointestinal motility stimulating properties of cisapride, a non-antidopaminergic non-cholinergic compound. In Gastrointestinal Motility, Edited by Roman, C., p. 513–520, MTP Press, Lancaster (1984)
2 Schuurkes, J.A.J., Akkermans, L.M.A. and Van Nueten, J.M.: Stimulating effects of cisapride on antroduodenal motility in the conscious dog. In Gastrointestinal Motility, Edited by Roman, C., p. 95–102, MTP Press, Lancaster (1984)
3 Schuurkes, J.A.J., Van Nueten, J.M., Van Daele, P.G.H., Reyntjens, A.J. and Janssen, P.A.J.: Motor-stimulating properties of cisapride on isolated gastrointestinal preparations of the guinea pig. J. Pharmacol. Exp. Ther. 234, 775–783 (1985)
4 Pfeuffer-Friederich, I. and Kilbinger, H.: Facilitation and inhibition by 5-hydroxytryptamine and R 51619 of acetylcholine release from guinea pig myenteric neurones. In Gastrointestinal Motility, Edited by Roman, C., p. 527–534, MTP Press, Lancaster (1984)
5 Neya, T., Itano, N., Mizutani, M., Yamasato, T., Takaki, M. and Nakayama, S.: The effect of cisapride on neural 5-HT receptors in guinea-pig isolated ileum. Eur. J. Pharmacol. 106, 221-222 (1985)

6 Nemeth, P.R., Ort, C.A., Zafirov, D.H. and Wood, J.D.: Interactions between serotonin and cisapride on myenteric neurons. Eur. J. Pharmacol. 108, 77-83 (1985)

7 Den Hertog, A. and Van den Akker, J.: The effect of cisapride on smooth muscle cells of guinea-pig taenia caeci. Eur. J. Pharmacol. 126, 31-35 (1986)

8 Nakayama, S., Neya, T., Yamasato, T., Takaki, M. and Itano, N.: Effects of cisapride on the motility of digestive tract in dogs and guinea pigs. Japan. J. Smooth Muscle Res. 21, 1-9 (1985)

9 Bübring, E., Crema, A. and Saxby, O.B.: A method for recording peristalsis in isolated intestine. Br. J. Pharmacol. 13, 440-443 (1965)