Neurogenic “Off” Contractions Are Mediated by NK₂-Receptors in the Circular Muscle of Guinea Pig Ileum

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ABSTRACT—In guinea pig ileal circular muscle, electrical stimulations by a train of 10–100 pulses (0.05-msec duration, 10 Hz) produced tetrodotoxin-sensitive “off” contractions that were initiated upon the termination of stimulations. Atropine at 10⁻⁶ M did not inhibit the “off” contractions. FK224 at 10⁻⁵ M, a dual antagonist for NK₁- and NK₂-receptors, but not 10⁻⁷ M CP-96,345, an antagonist for NK₁-receptors, almost abolished the “off” contractions in the presence of atropine. These results suggest that the neurogenic “off” contraction was mediated mainly by NK₂-receptors in the circular muscle of guinea pig ileum.

Keywords: NK₂-receptor, Circular muscle (guinea pig ileum), Neurogenic “off” contraction

It has been reported that in guinea pig ileal circular muscle, electrical stimulations produced two temporally distinguished neurogenic contractions: one is the “on” contraction that is induced immediately after the start of stimulation and is sustained during the stimulation period, and the other is the “off” contraction that is initiated at the termination of the stimulation (1, 2). Compared with the obvious function of the “on” contraction, it is not clear whether the “off” contraction may play a role under physiological conditions. However, development of the “off” contraction in response to electrical stimulation has long been known in in vitro experiments using isolated guinea pig ileum (3). Since there has been little study done on characterizing the “off” contraction, the present study was carried out to investigate the receptor type responsible for the “off” contraction in the ileal circular muscle of guinea pig.

Immediately after killing male guinea pigs (300–600 g) by a blow on the head, approximately 20 cm of the ileum was taken out at about 15-cm proximal to the ileocecal junction and then immersed in cold Krebs solution of the following composition: 120 mM NaCl, 2.0 mM CaCl₂, 1.0 mM MgCl₂, 1 mM NaH₂PO₄, 20.0 mM NaHCO₃, 5 mM HEPES and 11.0 mM glucose. Luminal chyme was washed out, and a segment of ileum (2–3 cm) without lymphoid tissues was dissected for immediate use. The remaining portion was stored in a refrigerator for up to 6 hr until later use.

Recording of contractions of the circular muscle was done by our recently reported method (4). In brief, an ileal segment was put on an aluminum tube holder with a small rectangular window opened in the middle portion, and the segment was horizontally set in an organ chamber containing Krebs solution (25 ml). Two silicon tubes were connected to the oral and anal end of the holder, respectively, for intraluminal infusion. A small hook was fastened onto the ileal surface over the window and was connected via thread to a force-displacement transducer (UL-10 GR; Shinkoh, Nagano). A resting tension of 0.2 g was applied, and the contractions were isometrically recorded. Krebs solution bathing and intraluminally perfusing the ileal segment was bubbled with 95% O₂ and 5% CO₂. Experiments were carried out at 35°C.

For electrical stimulation, one of a pair of silver wire electrodes, which were connected to an electric stimulator (SEN 3301; Nihon Kohden, Tokyo), was placed inside the lumen over the window and was connected via a force-displacement transducer to a force-displacement transducer (UL-10 GR; Shinkoh, Nagano). A resting tension of 0.2 g was applied, and the contractions were isometrically recorded. Krebs solution bathing and intraluminally perfusing the ileal segment was bubbled with 95% O₂ and 5% CO₂. Experiments were carried out at 35°C.

The drugs were applied to the bathing solution in the organ chamber, and their effects on the contractions were generally determined 15 min after the application.

In the ileal segment held in the current method, the circular muscle contracted spontaneously at a frequency of 16 to 20 times per min under continuous luminal perfusion. Their amplitudes were small (less than 0.1 g) and...
negligible in determining the electrical stimulation-induced contractions.

The top panel of Fig. 1A shows the actual traces of contractions in response to electrical stimulations of increasing number of pulses (1 to 100 pulses) delivered at a fixed frequency of 10 Hz. The "off" contraction was evoked at the termination of stimulations of more than 3 pulses. Although stimulations of 1 and 3 pulses evoked contractions apparently developing after the completion of stimulations, these contractions were not referred to as the "off" contraction, but rather as the "on" contraction, for the following two reasons: First, these contractions, which were monophasic with slow rising and decaying rate, were similar to the "on" contractions in response to the stimulations of 10 pulses. Secondly, these contractions were sensitive to atropine, as in the case of the "on" contractions induced by the stimulations of a larger number of pulses (see below). The "off" contractions in response to 10-pulse stimulations were evoked in 9 preparations out of 12, and the longer train of stimulations produced the "off" contractions in all the preparations tested.

In response to the stimulations of 10 and 30 pulses, the "off" contractions were usually evoked with relatively fast rising rate on the slowly decaying phase of the "on" contractions (top panel of Fig. 1A), being distinct from the "on" contractions. In 4 preparations, out of 12, however, the "off" contractions induced by 30-pulse stimulations were evoked following the rising phase of the "on" contractions; Hence, initiation of the "off" contraction was less clear. The maximal amplitudes of the "on" contractions in response to 100-pulse stimulations were as large as those of the "off" contractions, and initiation of the "off" contraction was recognized by a notch between the "on" and the "off" contraction (top panel of Fig. 1A). A few phasic contractions were evoked during the course of these long train of stimulations, and the "off" contractions occasionally waxed and waned before returning to the resting level. Both "on" and "off" contractions at all stimulations were abolished by 3 x 10^{-7} M tetrodotoxin (data not shown), indicating that the two contractions were neurogenic in nature.

As shown in the middle panel of Fig. 1A, the "on" contractions were almost abolished by 10^{-6} M atropine at all stimulation pulses, whereas the "off" contractions were evoked in response to the stimulations of more than 3 pulses in the presence of atropine. The "off" contractions in the presence of atropine were monophasic (middle panel of Fig. 1A). Their amplitudes were slightly smaller than those in the absence of atropine, although the difference was not significant (Fig. 2, A and B).

The bottom panel of Fig. 1A shows that the "off" contractions in the presence of atropine were strongly inhibited by 10^{-5} M FK224, a dual antagonist for NK1- and NK2-receptors (5, 6) at all pulses. In the presence of atropine and FK224, slight relaxation was induced during the course of the stimulations. Figure 2A shows the inhibitory effects of atropine and FK224 on the pulse number-"off" contraction relationship.

The "off" contraction was repeatedly and reproducibly evoked in response to electrical stimulation. The traces shown in Fig. 1B that were recorded under the slow paper
The "off" contractions were transiently inhibited, but the amplitudes re-

amplitude of the "off" contractions were presented as a percent of the "off" con-

the presence of atropine. As shown in Fig. 2B, 10^{-7} M CP-96,345, blocked the "off" contractions in the presence of atropine, (NK1 or NK2) is responsible for the inhibition by FK224

the presence of atropine. Since it has been indicated that CP-96,345 may act as an antagonist of L-type Ca^{2+} channels (9), effects of higher concentrations of CP-96,345 were not investigated in the present study. However, an assumption for substantial, if not total, blockade of NK1-receptors with 10^{-7} M CP-96,345 almost completely inhibited 10^{-7} M substance P-induced contrac-

atropine were dose-dependently inhibited by further treatment with 10^{-7} to 10^{-5} M FK224. At 10^{-3} M FK224, the atropine-resistant "off" contractions were almost abol-

To determine which subtype of tachykinin receptor (NK1 or NK2) is responsible for the inhibition by FK224 of the "off" contraction in the presence of atropine, effects of CP-96,345, an antagonist for the NK1-receptor (7), on the "off" contractions were investigated in the presence of atropine. As shown in Fig. 2B, 10^{-7} M CP-96,345 did not affect the "off" contractions in the presence of atropine.

The present study has shown that the "off" contraction was resistant to atropine and that the "off" contraction in the presence of atropine was inhibited by FK224, a dual antagonist for NK1- and NK2-receptors, but not by CP-96,345, an NK1-receptor antagonist. FK224 is a novel drug that possesses tachykinin-receptor antagonistic action as recently reported by Morimoto et al. (5, 8) and Murai et al. (6). FK224 dose-dependently inhibited the atropine-resistant "off" contractions in the dose range of 10^{-7} to 10^{-5} M, which was shown to competitively and specifically block NK1- and NK2-receptors in the rat duodenum and guinea pig ileum (6). FK224 at 10^{-3} M almost abolished the "off" contractions induced by 10- to 100-

their results and the present ones may be ascribed to two different experimental procedures employed in the two studies. One is: in the study by Bartho et al. (2), the effects of tachykinin-receptor antagonists were investigated in the presence of apamin, whereas in the present study, the effects of FK224 and CP-96,345 were assessed in the absence of apamin. In the presence of apamin, phasic contractions were repeatedly induced, which were resistant to CP-96,345 were not investigated in the present study. Furthermore, in light of the pA2 of 8.1 to 8.9 reported for CP-96,345 in blocking NK1-receptors in the longitudinal muscle of guinea pig ileum (10–12), it may be presumed that NK1-receptors were blocked to a large extent with 10^{-7} M CP-96,345 in the circular muscle of guinea pig ileum. Then the fact that FK224, but not CP-96,345, blocked the "off" contractions in the presence of atropine may suggest that the "off" contraction is mediat-
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Fig. 2. Effects of atropine and tachykinin antagonists on the "off" contractions in the guinea pig ileal circular muscle induced by electrical stimulations of 1-100 pulses (0.05 msec, 10 Hz). (A) Effects of 10^{-8} M atropine and its combination with 10^{-7} M FK224. The control (○) shows the responses in the absence of the two drugs; the "off" contractions for the 1 and 3-

atropine to 10^{-5} M FK224. The control (○), 10^{-6} M atropine plus 10^{-5} M FK224 (△). (B) Effects of 10^{-6} M atropine and its combination with 10^{-7} M CP-96,345. Control (○), 10^{-6} M atropine (○), 10^{-6} M atropine plus 10^{-7} M CP-96,345 (△). Amplitudes of the "off" contractions were presented as a percent of the "off" con-

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ed that about 50% of the preparations showed an irregular, low amplitude phasic activity that often waned and re-appeared during the course of the experiment. Such irregular contractions, however, were not observed in the segment preparations, although they showed rhythmic spontaneous contractions of small amplitudes throughout the experiment. Development of irregular contraction in the strip preparation seems to imply the enhanced excitability of this type of preparation. The inhibitory action of tachykinin antagonists may be counteracted by the enhanced excitability in the strip preparation. As described above for the seeming resistance to tachykinin antagonists in the case of apamin-treatment, fusion of an irregular phasic contraction and the “off” contraction may lead to reduced sensitivity of the contraction to tachykinin antagonists.

In summary, the present results using the ileal segment have indicated that the “off” contraction in response to electrical stimulation in the circular muscle of the guinea pig ileum is mediated mainly by NK2-receptors. The physiological role of the “off” contraction, however, remains to be studied.

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