Caffeine and Ryanodine May Act on the Plasma Membrane of the Circular Muscle at the Flexure Region in the Guinea-Pig Colon

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Abstract

The actions of caffeine and ryanodine on the spontaneous rhythmic activities in the guinea-pig colon were studied by mechanical tension recording. Caffeine reduced the amplitude of the spontaneous rhythmic activity at low concentration (0.3 mM-1 mM). At high concentration (3-10 mM), it induced a phasic transient contraction. The spontaneous rhythmic activity and a phasic contraction induced by caffeine, were blocked by verapamil (3 μM) or by removal of external Ca²⁺. Ryanodine affected neither resting tension nor frequency of spontaneous activity at 1 μM. However in the circular muscle strips pretreated with ryanodine, a sustained contraction was initiated after the removal of caffeine (10 mM). Continuous Ca²⁺ influx was necessary for spontaneous rhythmic activities and a phasic transient contraction, because it was abolished completely by the removal of external Ca²⁺. Verapamil (3 μM), a voltage gated L-type Ca²⁺ channels blocker, inhibited the spontaneous rhythmic activities and also inhibited phasic transit contraction followed by a sustained contraction induced by 10 mM caffeine. Our results suggest that caffeine may produce a sustained contraction by activating verapamil sensitive Ca²⁺ channel. In the muscle pretreated with both caffeine and ryanodine, continuous Ca²⁺ influx may occur also through verapamil sensitive pathway.

key words: caffeine, ryanodine, calcium influx, smooth muscle.

Introduction

The circular muscle at the mesenteric border of the flexure region in the guinea-pig colon produced 10-12 cycle/min regular spontaneous mechanical contractions. Various drugs were found to affect on this tissue (Kobayashi et al., 1996). It is well known that caffeine releases Ca²⁺ from the sarcoplasmic reticulum (Weber and Herz, 1968) and ryanodine is considered to act on the Ca²⁺ induced Ca²⁺ release channels (Fleischer et al., 1985; Naganobu et al., 1994). However, actions of caffeine and ryanodine differ among various types of smooth muscles and

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also depend on different experimental condition. For example in vascular smooth muscles (Itoh et al., 1981; Sato et al., 1988), caffeine produces a contraction in Ca\(^{2+}\) free solution, but it does not in the myometrium (Savineau and Mironneau, 1990) and in the guinea-pig gastric antrum (Chowdhury et al., 1995). On the other hand low concentration of ryanodine opens Ca\(^{2+}\) release channels, whereas, much higher concentration of ryanodine (300 \(\mu\)M) closes the Ca\(^{2+}\) release channels (Nagasaki et al., 1988). Moreover in the smooth muscle, ryanodine depletes Ca\(^{2+}\) stores by opening Ca\(^{2+}\) release channels. It makes the sarcoplasmic reticulum leaky to Ca\(^{2+}\) so that caffeine sensitive intracellular Ca\(^{2+}\) stores empty (Hwang et al., 1987, Iino et al., 1988, Shima et al., 1992).

We showed in the previous study that caffeine and ryanodine acted on the Ca\(^{2+}\) influx pathway of the plasma membrane rather than on the Ca\(^{2+}\) stores of the sarcoplasmic reticulum in the circular muscle of the guinea-pig gastric antrum (Chowdhury et al., 1995). Thus it seems useful to examine the effects of caffeine and ryanodine on the spontaneous rhythmic activity of the circular muscle at the flexure region in the guinea-pig colon. The present study analyzed the action of caffeine and ryanodine on this smooth muscle using mechanical tension recording.

**Methods**

**Tissue preparation and tension recording**

Hartly guinea-pig (250-350 g) of either sex were used. They were sacrificed by stunning and bleeding, and flexure regions of colons were isolated. After careful removal of the mucosa and submucosa from the segment, circular muscle strips including the submucosal surface (approximately 1×5 mm) were cut parallel to the muscle fibers. The tissue was mounted horizontally in a small chamber (0.3 ml). One end was fixed by a pin to the bottom of the chamber and the other end was tied to a strain gauge force transducer system. We used a transducer UL-10 GR (Minebea) and a flatbed recorder FBR-252 A (TOA). The chamber was perfused with a normal Krebs solution at a rate of 2 ml/min. Each figure presented in this paper is one of six records.

**Drugs and solutions**

Caffeine, ryanodine, verapamil, EGTA, atropine, \(N^0\)-nitro-L-arginine methyl ester and phenoxybenzamine were purchased from Sigma (St. Louis U.S.A). The normal Krebs solution contained (mM): NaCl 127, KHCO\(_3\) 6, CaCl\(_2\) 2.4, MgCl\(_2\) 1.2, Glucose 10, Tris-HCl 12 (pH adjusted to 7.4 at 35°C). When caffeine was added, NaCl was substituted to maintain osmolality. In some experiments atropine (1 \(\mu\)M), \(N^0\)-nitro-L-arginine methyl ester (50 \(\mu\)M) phenoxybenzamine (1 \(\mu\)M), indomethacin (1 \(\mu\)M), were added to inhibit contributions of neurotransmitters and autocoids, but no fundamental differences were found.

**Results**

In normal Krebs solution, the circular muscle at the flexure region of the guinea-pig colon showed spontaneous rhythmic contractions at regular frequencies ranging between 10-12 cycle/
Calcium influx by caffeine and ryanodine

Fig. 1. Effects of various concentrations of caffeine on the spontaneous rhythmic activities of the flexure region at the mesenteric border in the guinea-pig colon. Low concentrations of caffeine (0.3-1 mM) reduced spontaneous rhythmic activities (a, b). High doses of caffeine (3-10 mM) produced transient phasic contraction (c, d). Each concentration of caffeine was applied for 4 min (horizontal bar) followed by a 20 min interval.

min. Under the application of caffeine (0.3-1 mM), the amplitude of the spontaneous rhythmic activities were reduced (Fig. 1a, b). However, caffeine (3-10 mM) produced a phasic transient contraction and then relaxation to the resting level (Fig. 1c, d). After rinse with normal Krebs solution the spontaneous contractions were gradually restored.

When Ca** was removed from the physiological solution the spontaneous mechanical activity was disappeared within 3-4 min, and application of 10 mM caffeine did not produce a phasic transient contraction in Ca** free solution (Fig. 2b). When 2.4 mM Ca** was reintroduced in the presence of caffeine (10 mM), a partial restoration of contraction induced by caffeine occurred (Fig. 2b). The spontaneous mechanical activity reappeared in the presence of Ca**. Reapplication of caffeine (10 mM) induced a phasic contraction again after the restoration of spontaneous activity (Fig. 2c).
Fig. 2. Effects of caffeine (10 mM) on the spontaneous activity in the presence or absence of Ca\(^{2+}\). Comparing to a control responses (a), 10 mM caffeine did not produce phasic contraction in Ca\(^{2+}\) free solution containing 0.1 mM EGTA (b). Reapplication of 2.4 mM Ca\(^{2+}\) in the presence of caffeine (10 mM) induced incomplete phasic contraction (b). Caffeine (10 mM) induced contraction after restoration of spontaneous rhythmic activity (c). Note that the spontaneous rhythmic activities disappeared within 2-3 min after removal of Ca\(^{2+}\) and caffeine (10 mM) did not produce contraction under this condition.

The effect of caffeine was investigated with ryanodine (1 \(\mu\)M), which is known to unlock the Ca\(^{2+}\) induced Ca\(^{2+}\) release channels and depletes Ca\(^{2+}\) from the store sites. Ryanodine also did not change the frequency or amplitude of the spontaneous activity as shown in figure 3A. In a preparation pretreated with ryanodine for 5 min, contraction produced by 10 mM caffeine was nearly similar to that without ryanodine pretreatment (Fig. 3A a,b,c). However, a sustained contraction was initiated after removal of caffeine and continued for more than 1 hour (Fig. 3Ac). The sustained contraction returned gradually to the resting tension level indicating the removal of ryanodine from the binding site by washing with physiological solution (Nagasaki et al., 1988). Removal of external Ca\(^{2+}\) from the physiological solution during the sustained contraction reduced the muscle tension to the resting level and reapplication of 2.4 mM Ca\(^{2+}\) induced the sustained contraction (Fig. 3B).

In the presence of verapamil (3 \(\mu\)M), a voltage dependent L-type Ca\(^{2+}\) channels blocker, the spontaneous activity was completely abolished within 10-15 min (Fig. 4c). The phasic contraction induced by caffeine (10 mM) was also inhibited under the presence of verapamil. A sustained contraction which was produced by pretreatment of ryanodine (1 \(\mu\)M) was not initiated under this condition (Fig. 4b, c).
Fig. 3. **Panel A** Effects of caffeine and ryanodine on the flexure region of the guinea pig colon. Caffeine (10 mM) induced transient contraction (a), and spontaneous mechanical activities was not affected by 1 μM ryanodine for 5 min (b). However, a sustained contraction associated with spontaneous contractions developed after washout of 10 mM caffeine (c). Caffeine was applied 20 min after the exposure to 1 μM ryanodine.

**Panel B** Effect of Ca²⁺ free solution on the sustained contraction. Sustained contraction was initiated by 1 μM ryanodine-pretreatment after the removal of caffeine in ryanodine-pretreated muscle. It is same to the panel Ac. This contraction was reduced to the resting tension level by the application of Ca²⁺ free solution containing 0.1 mM EGTA. Then it returned to the previous level by readmission of 2.4 mM Ca²⁺ to the physiological solution.

**Discussion**

Although low concentration of caffeine (0.3–1 mM) significantly reduced the amplitude of
Fig. 4. Effects of caffeine and ryanodine in the presence of 3 μM verapamil. Caffeine (10 mM) induced contraction (a), and 1 μM ryanodine for 5 min did not affect spontaneous contractions (b). When 3 μM verapamil was added in the physiological solution the spontaneous activity was abolished within 10-15 min as shown in (c). Under this condition 10 mM caffeine did not induce contraction.

the spontaneous rhythmic activity, higher concentration (3-10 mM) induced phasic transient contraction in the circular muscle at the flexure region of the guinea-pig colon. The similar inhibitory action of caffeine has been reported in rat aortic smooth muscle (Sato et al., 1988) and in the cultured smooth muscle cells from the pregnant rat myometrium (Martin et al., 1989). In those cases caffeine inhibited Ca^{2+} influx through voltage dependent Ca^{2+} channels at the cell membrane.

It was also reported that caffeine induced contractions by a release of Ca^{2+} from the internal Ca^{2+} stores in the guinea-pig mesenteric artery (Itoh et al., 1981) and in rat aorta (Sato et al., 1988). Whereas in pregnant rat uterine smooth muscles (Savineau and Mironneau, 1990) there was not a caffeine sensitive Ca^{2+} release channels. This idea was suggested by the observation that caffeine (0.1-50 mM) induced a contraction neither in the presence nor absence
of extracellular Ca\(^{2+}\). On the contrary our results showed that caffeine induced phasic transient contraction under high concentration and it was abolished in Ca\(^{2+}\) free solution. This observation is consistent with the findings in guinea-pig gastric antrum (Chowdhury et al., 1995) in which contractions induced by caffeine depend on Ca\(^{2+}\) influx. In the present experiment under the presence of verapamil (3 \(\mu\)M), caffeine (10 mM) did not produce any contraction, though in the guinea-pig gastric antrum contraction produced by 10 mM caffeine was resistant to verapamil (Chowdhury et al., 1995). Thus it is likely that caffeine (10 mM) inducing-contraction in the flexure region is mainly due to the influx of Ca\(^{2+}\), because such contraction was abolished by removal of external Ca\(^{2+}\) and by Ca\(^{2+}\) channels blocker.

Several experiments suggest that ryanodine locks Ca\(^{2+}\) release channels in an open state (Fleischer et al., 1985; Rousseau et al., 1987) and abolishes function of caffeine sensitive internal Ca\(^{2+}\) stores (Sato et al., 1988; Iino et al., 1988). It is reported that in the rat mesenteric artery and aorta, pretreatment with ryanodine abolished contraction induced by caffeine (Shima et al., 1992). Although contraction induced by caffeine was not affected by the pretreatment with ryanodine (1-10 \(\mu\)M), a sustained contraction which was continued for more than 1 hour was initiated after the removal of caffeine in this study. This contraction was reversibly abolished by Ca\(^{2+}\) free solution. Those data are consistent with the results in the circular muscle of the guinea-pig gastric antrum and rabbit portal vein (Chowdhury et al., 1995) in which a continuous Ca\(^{2+}\) influx is necessary for the sustained contraction. Moreover, in our experiments sustained contraction was abolished under the presence of verapamil (3 \(\mu\)M) and this result differed from the guinea-pig gastric antrum where the sustained contraction was insensitive to verapamil (Chowdhury et al., 1995).

In conclusion, the present study indicates that caffeine and ryanodine act on the plasma membrane, and they activate a verapamil sensitive Ca\(^{2+}\) influx pathway which remains open for a period and produces a sustained contraction.

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**References**


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