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Sustained contraction produced by caffeine after ryanodine treatment in the circular muscle of the guinea-pig gastric antrum and rabbit portal vein

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1 Caffeine inhibited spontaneous mechanical activity at 0.3–1 mM, but produced a tonic contraction at concentrations higher than 3 mM in the circular muscle of the guinea-pig gastric antrum. In the circular muscle of the rabbit portal vein, caffeine at concentrations higher than 1 mM produced an early phasic contraction followed by a small tonic component. The caffeine-induced contraction was abolished by removal of the external Ca^{2+} more rapidly in the gastric antrum than the portal vein.

2 When the preparations were pretreated with ryanodine (1 μM) a sustained contraction developed on wash-out of caffeine (10 mM) both in the gastric antrum and portal vein. This contraction was not affected by nicardipine (3 μM) or verapamil (3 μM), but was readily abolished by removal of the external Ca^{2+} or by addition of cobalt (1 mM). Spontaneous electrical activity, the slow wave, in gastric muscles was blocked in the presence of 10 mM caffeine, but reappeared during the sustained contraction.

3 Both the contractions induced directly by caffeine and those produced following caffeine wash-out after ryanodine treatment were accompanied by a maintained increase in intracellular Ca^{2+} concentration measured with fura-2.

4 The presence or absence of Ca^{2+} during the application of ryanodine did not affect the ability of caffeine to initiate sustained contractions, provided Ca^{2+} was present during the exposure to caffeine.

5 It is concluded that caffeine can induce a sustained contraction after ryanodine treatment both in the guinea-pig gastric antrum and rabbit portal vein, by activating a Ca^{2+} influx pathway insensitive to organic Ca^{2+} channel blockers. No clear evidence was obtained for involvement of the Ca^{2+} influx pathway activated through depletion of intracellular Ca^{2+} stores. A hypothesis is proposed that the plasma membrane of these preparations is similar to the sarcoplasmic reticulum membrane in that Ca^{2+} permeability can be increased almost irreversibly by a combination of caffeine and ryanodine in the presence of the external Ca^{2+}.

Keywords: Caffeine; ryanodine; smooth muscle; Ca influx; guinea-pig gastric antrum; rabbit portal vein

Introduction

Caffeine is known to produce a contraction by releasing Ca^{2+} from intracellular stores in various vascular and intestinal smooth muscles (Itoh *et al.*, 1981; 1982; Iino, 1989). The effect of caffeine, however, may differ in different smooth muscles or experimental conditions. In myometrium, caffeine has only an inhibitory effect (Osa, 1973; Savineau & Mironneau, 1990) and in some intestinal smooth muscles, both contractile and relaxant action of caffeine have been reported (Osa, 1973; Ito *et al.*, 1974).

The circular muscle of the guinea-pig gastric antrum has spontaneous rhythmic activity, but the mechanism underlying this activity is not known. It is possible that intracellular Ca^{2+} release may be involved in some way in the generation of this activity. Since caffeine can release Ca^{2+} from the intracellular stores probably by potentiating the Ca^{2+}-induced Ca^{2+} release mechanism (Iino, 1989) and ryanodine is considered to unlock the Ca^{2+}-induced Ca^{2+} release channels, thereby resulting in depletion of the stores (Iino *et al.*, 1988; Ganitkevich & Isenberg, 1993), we examined whether caffeine and ryanodine modified the spontaneous activity. During these experiments, caffeine was found to produce a very prolonged contraction when preparations were pretreated with ryanodine. In the present experiments, some of the properties of this sustained contraction were analysed. Since caffeine-induced tonic contractions in gastric muscle were quickly abolished by removal of the external Ca^{2+}, we compared this muscle with rabbit portal vein in which caffeine induced phasic contractions which were relatively resistant to Ca^{2+} removal.

Methods

For gastric muscle, Hartley guinea-pigs (250–350 g) of either sex were killed by stunning and bleeding and the stomach was excised. After careful removal of the mucosa under a binocular microscope, circular muscle strips (approximately 1 × 5 mm) were separated from the longitudinal muscle layer, after dissecting out from the antral region. For portal vein, rabbits (2–2.5 kg) of either sex were killed by bleeding under anaesthesia with pentobarbitone (100 mg kg^{-1}). Circular muscle strips (approximately 1 × 5 mm) were obtained after carefully removing connective tissue. Mechanical activity was recorded simultaneously from the muscle strips of stomach and the portal vein with strain gauges in the same small chamber (0.3 ml) in which each preparation was mounted horizontally. In some experiments only gastric muscles were used. The chamber was perfused at a constant rate of 3 ml min^{-1} with physiological solution prewarmed to 35°C.

The normal solution contained (mM): NaCl 127, $KHCO_3$ 6, $CaCl_2$ 2.4, $MgCl_2$ 1.2, glucose 12, tris-hydroxymethyl-aminomethane (Tris) 10 (pH adjusted to 7.4 at 35°C with HCl). When caffeine was added, NaCl was substituted to maintain the osmolarity. In some experiments, atropine (1 μM), phenoxybenzamine (1 μM), N^G-nitro-L-arginine (50 μM), and

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indomethacin (1 μM) were added, to block contributions of acetylcholine, noradrenaline, nitric oxide, and prostaglandins, respectively, but no fundamental differences were found.

Intracellular Ca^{2+} concentration ($[Ca^{2+}]_i$) was also measured from a small muscle strip (1 × 5 mm) which was mounted in a chamber (0.2 ml) fixed on a stage of an inverted microscope (IMT-2, Olympus Optical Co., Japan) and one end was connected to an isometric strain gauge to record tension development. The chamber was perfused at a rate of 3 ml min^{-1} with physiological solution prewarmed at 35°C, except for a period of fura-2 AM loading. The preparation was exposed to physiological solution containing 10 μM fura-2 AM for 1 h and the measurement of fura-2 signals was started about 20 min after restarting superfusion to remove external fura-2 AM. Fluorescence signals (510 nm) excited at 340 and 380 nm wavelength were collected at 3 s intervals with an image processor (ARGUS-50, Hamamatsu Photonics, Japan). The ratio of fluorescence intensities at 340 and 380 nm ($R_{340/380}$) was used to estimate changes in $[Ca^{2+}]_i$. The size of spot where the fluorescent signals were detected was about 60 μm square. In these experiments, physostigmine (10 μM), atropine (1 μM), and probenecid (20 μM) were added throughout to inhibit the esterase activity of the tissues and to improve the maintenance of intracellular fura-2 (Watanabe *et al.*, 1992).

The membrane potential was measured only in the gastric preparations, by the conventional microelectrode technique, as previously described (Katayama *et al.*, 1993). In these experiments, mechanical activities were also recorded simultaneously with a strain gauge.

The chemical agents used were obtained from Sigma (St. Louis, U.S.A.), except nicardipine (Wako Pure Chemical, Japan), fura-2-AM (Dojin, Japan) and phenoxybenzamine (Tokyo Chemical, Japan). Numerical data were expressed as means ± s.e.mean.

Results

Circular muscle strips of the guinea-pig gastric antrum have spontaneous activity, whereas those of the rabbit portal vein are usually quiescent, although some preparations produce irregular rhythmic activity at the beginning of experiments. As shown in Figure 1, caffeine inhibited the activity of gastric muscles at less than 1 mM, but produced a tonic contraction at concentrations higher than 3 mM. In the portal vein, caffeine produced a transient contraction followed by a small tonic contraction at 1 mM concentration or higher.

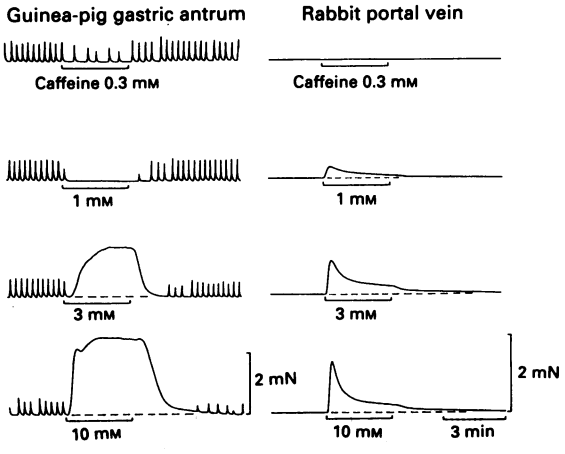

Figure 1 Simultaneous recording of tension responses to caffeine in the circular muscle strips of guinea-pig gastric antrum (left) and rabbit portal vein (right). Gastric muscle shows spontaneous phasic contractions which were inhibited by caffeine. Increasing concentration of caffeine was applied at 20 min intervals. Caffeine produced concentration-dependent contractions in both tissues.

Caffeine-induced contractions quickly disappeared in Ca^{2+}-free solution and after more than 3 min no contraction could be observed in the gastric muscle ($n = 6$) (Figure 2b), whereas in the portal vein, the decrease in contractions induced by Ca^{2+} removal was slow and was reduced to 37 ± 8% ($n = 6$) of the control after 5 min exposure to Ca^{2+}-free solution (Figure 2d). When 2.4 mM Ca^{2+} was reintroduced in the presence of 10 mM caffeine, a tonic contraction reappeared in gastric muscle (Figure 2b), but not in the portal vein (Figure 2d). Application of 1 mM cobalt had effects very similar to Ca^{2+} removal, but caffeine-induced contractions were not significantly affected by 3 μM verapamil or 3 μM nicardipine either in the gastric muscle or portal vein (not shown).

When ryanodine (1 μM) was applied for 2–5 min during caffeine-induced contractions, no effect was observed until caffeine was washed out, but a slow sustained contraction developed following removal of caffeine both in the gastric muscle and portal vein (Figure 2e,f). This contraction lasted for more than 1 h and the maximum tension developed was 237 ± 32% of the level of the caffeine-induced contraction after 3 min application in gastric muscle ($n = 16$) and 326 ± 45% in portal vein ($n = 14$). The sustained contraction was not affected by nicardipine (3 μM, Figure 2e,f) or verapamil (3 μM, not shown), but was reversibly abolished by removal of the external Ca^{2+} (Figure 2e,f) or by application of cobalt (1 mM, not shown), suggesting involvement of Ca^{2+} influx through a pathway insensitive to organic Ca^{2+} channel inhibitors. In the portal vein, relaxation caused by Ca^{2+} removal was slow and sometimes incomplete in 5 min (e.g. Figure 5f), but this was not studied further. Subsequent reapplication of caffeine (10 mM) during the sustained contraction reduced the tension almost to the level before caffeine wash-out (not shown).

In the stomach, some rhythmic activity could be observed during the sustained contraction, although there was no such activity in the presence of caffeine. Other xanthine derivatives, such as theophylline (1–10 mM) and isobutylmethylxanthine (1–100 μM) did not produce any contraction, but inhibited spontaneous rhythmic activity, before and also after treatment with ryanodine (1 μM).

Figure 3 shows changes in intracellular Ca^{2+} concentration ($[Ca^{2+}]_i$) estimated by fura-2 fluorescence signal, recorded simultaneously with mechanical activity in the guinea-pig

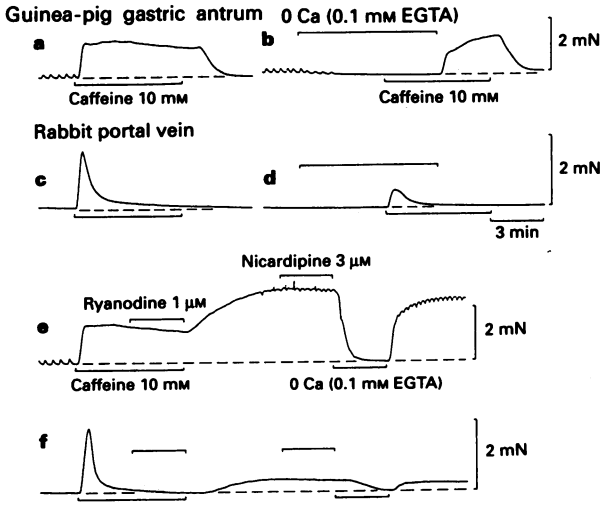

Figure 2 Simultaneous recording of mechanical responses to caffeine and ryanodine in muscle strips of guinea-pig stomach (a,b,e) and rabbit portal vein (c,d,f). (a and c) Control responses to caffeine (10 mM); (b and d) caffeine application 5 min after exposure to Ca^{2+}-free solution containing 0.1 mM EGTA and reapplication of 2.4 mM Ca^{2+} in the presence of caffeine; and (e and f) sustained contraction developed on wash-out of caffeine and ryanodine, and the lack of effect of nicardipine and reversible relaxation by removal of the external Ca^{2+}. Interval of caffeine application was 20 min.

gastric muscle. Caffeine (10 mM) produced a sustained increase in $[Ca^{2+}]_i$. In this experiment ryanodine (1 μM) was applied for 4 min and washed out for 2 min before the second caffeine application. Ryanodine did not produce any clear effect during the application, but a sustained contraction was produced following the second application of caffeine, as shown in Figure 3. There was a close correlation between changes in $[Ca^{2+}]_i$ and tension development, except that the rate of increase in $[Ca^{2+}]_i$ caused by caffeine was faster than that of tension development. When the external Ca^{2+} was removed during the sustained contraction, tension and $[Ca^{2+}]_i$ both decreased rapidly suggesting the sustained contraction is supported by continuous Ca^{2+} influx. Regular rhythmic $[Ca^{2+}]_i$ changes corresponding to rhythmic contractions could not be observed in this preparation due to a poor signal-noise ratio. Similar results showing a long-lasting increase in $[Ca^{2+}]_i$ during the sustained contraction following caffeine wash-out were obtained in three preparations of gastric antrum and two preparations of portal vein examined.

It is possible that an increase in $[Ca^{2+}]_i$, rather than caffeine itself, triggers a standard contraction after a ryanodine treatment. Another possibility is that an increase in $[Ca^{2+}]_i$ as well as adenosine 3':5'-cyclic monophosphate (cyclic AMP) is a prerequisite for the sustained contraction, since caffeine is expected to increase cyclic AMP due to inhibition of phosphodiesterase. Therefore, contractions produced by carbachol alone or together with forskolin, an adenylase cyclase activator, were compared with caffeine-induced contractions in gastric muscle, as shown in Figure 4. The experiment was carried out in the continuous presence of verapamil and indomethacin (1 μM) to inhibit phasic contractions produced by carbachol (CCh) and a possible involvement of endogenous prostaglandins, respectively. Both CCh (1 μM) alone and CCh plus forskolin (1 μM) failed to produce a sustained contraction after treatment with ryanodine (1 μM) for 5 min (Figure 4c,d). Caffeine (10 μM) applied 34 min after ryanodine treatment was still able to initiate a sustained contraction which was partially reversed by forskolin (1 μM) (Figure 4e).

Caffeine was still effective in initiating the sustained contraction even when ryanodine was applied in Ca^{2+}-free solution and also when caffeine was applied more than 1 h after removal of ryanodine. In the experiment shown in Figure 5, the preparations were treated with ryanodine for 5 min in

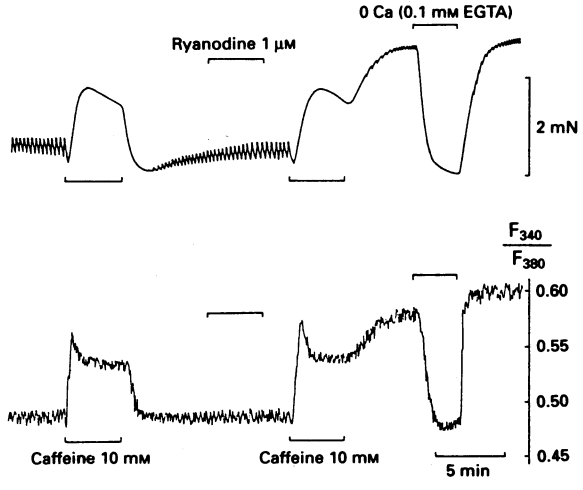

Figure 3 Simultaneous recording of fura-2 signals (lower) and mechanical response (upper trace) obtained from a gastric muscle strip. Fura-2 signal was expressed by the ratio of fluorescence intensities activated by 340 and 380 nm wavelengths (F_{340}/F_{380}). Caffeine application is indicated by horizontal bars underneath, and ryanodine application and removal of Ca^{2+} are shown by bars above the records.

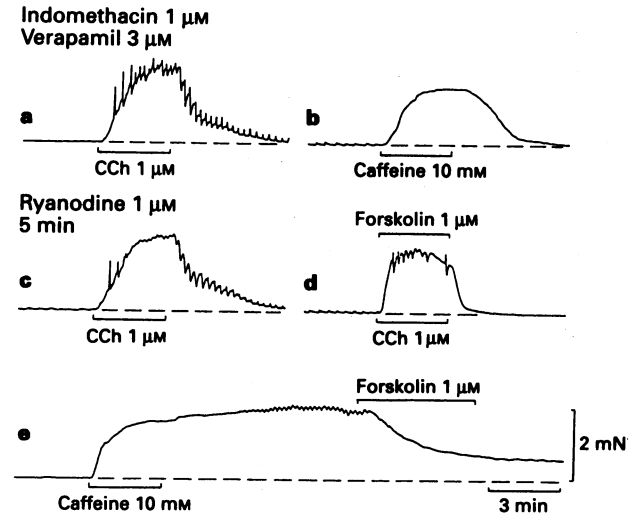

Figure 4 Contractions produced by carbachol (CCh) and caffeine before (a,b) and after ryanodine treatment (c,d,e) in gastric muscle. Indomethacin (1 μM) and verapamil (3 μM) were present throughout the experiment to inhibit phasic contractions. Ryanodine (1 μM) was applied between (b) and (c) for 5 min and CCh (1 μM) was applied 5 min after ryanodine wash-out (c). In (d) CCh was applied together with forskolin (1 μM). Note that caffeine applied 35 min after ryanodine wash-out initiated a sustained contraction (e), but not by CCh (c) or CCh plus forskolin (d). Forskolin partially inhibited the sustained contraction (e). CCh or caffeine was applied at 12 min intervals except for (c) in which CCh was applied at 15 min interval.

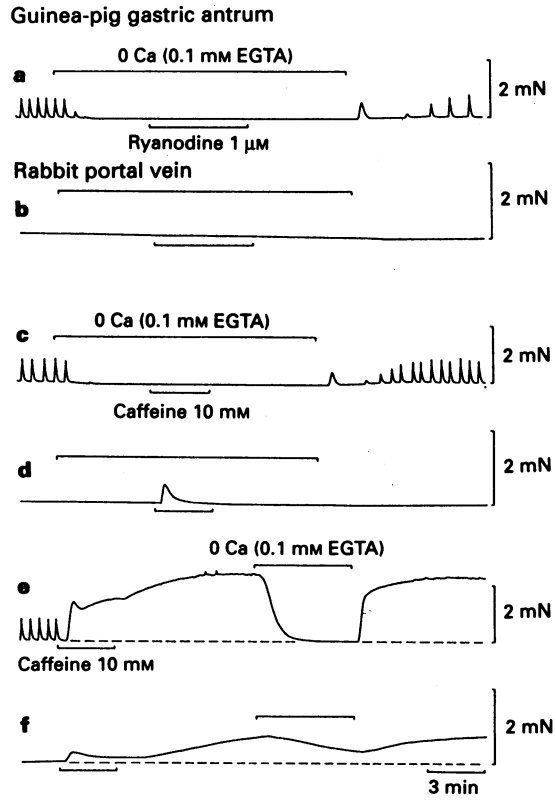

Figure 5 Simultaneous record of mechanical response in muscle strips of stomach (a,c,e) and portal vein (b,d,f). (a and b) Ryanodine application in Ca^{2+}-free solution as indicated; (c and d) caffeine application in Ca^{2+}-free solution. Note that ryanodine pretreatment in Ca^{2+}-free solution was still effective in producing the sustained contraction on caffeine wash-out in the presence (e,f), but not in the absence of Ca^{2+} (c,d); (e) and (f) also show effect of Ca^{2+} removal during the sustained contraction.

Ca^{2+}-free solution. On reapplication of 2.4 mM Ca^{2+}, a transient contraction was produced in gastric muscle (Figure 5a), but no clear response was observed in the portal vein (Figure 5b). The gastric preparation shown in this figure produced a transient contraction on Ca^{2+} reapplication, but in 2 out of 4 preparations no such contraction was observed. When caffeine was applied, 30 min after ryanodine wash-out, in the absence of Ca^{2+}, no contraction was produced in gastric muscle (Figure 5c) and a transient contraction in portal vein (Figure 5d), as observed without ryanodine-pretreatment (Figure 2b,d). The second application of caffeine, 58 min after ryanodine wash-out, in the presence of Ca^{2+} produced a typical sustained Ca^{2+}-sensitive contraction (Figure 5e,f).

It appears that the presence of external Ca^{2+} is necessary for caffeine to evoke a sustained contraction after ryanodine treatment. The ability of intracellular Ca^{2+} to evoke the sustained contraction was examined by use of caffeine (10 mM) and CCh (100 μM) in the absence of the external Ca^{2+} (Figure 6). CCh (100 μM) is considered to produce a contraction by releasing Ca^{2+} from intracellular stores in this muscle (Parekh & Brading, 1991). As shown in Figure 6b, caffeine applied together with CCh produced a transient contraction in Ca^{2+}-free solution, but on reapplication of 2.4 mM Ca^{2+} only large phasic contractions, but no sustained contraction, could be observed. Caffeine applied 10 min after Ca^{2+} reapplication produced a typical sustained contraction (Figure 6c). These results suggest that the presence of extracellular Ca^{2+} (or Ca^{2+} influx) is necessary for the caffeine-induced sustained contraction following exposure to ryanodine.

In muscle strips of the gastric antrum, ryanodine did not affect either the resting tension or the frequency of spontaneous activity at 1 μM, but when the concentration was increased to more than 3 μM a tonic contraction slowly developed with a small increase in frequency. The increase in frequency of spontaneous activity was $17 \pm 3\%$ ($n = 6$) 10 min after 5 min treatment with 10 μM ryanodine. When the basal tone was increased the size of rhythmic contractions became reduced.

Figures 7 and 8 show simultaneous recordings from gastric strips of mechanical and electrical activity recorded with intracellular microelectrodes. Caffeine (10 mM) produced a tonic contraction and blocked rhythmic mechanical and electrical activity, the slow wave, with membrane depolarization (Figure 7a). Ryanodine (1 μM) had no noticeable effects on mechanical activity and slow wave on its own, but a sustained contraction was produced after a subsequent application of caffeine. In the preparation shown in Figure 7, in which caffeine produced a relatively large depolarization (9.5 mV), the membrane stayed depolarized, the amplitude of the slow wave was reduced, and its frequency was slightly increased during the sustained contraction on which small ripples were superimposed (Figure 7b). Although successful recordings were difficult, similar results were obtained in four other preparations, a mean value of depolarization being 8.6 ± 2.2 mV ($n = 5$) and the resting potential (the most negative value between slow waves) -66 ± 4 mV. In two additional preparations, however, membrane depolarization produced by caffeine (10 mM) was relatively small (3–4 mV) and no clear changed in membrane potential and slow wave were observed during the sustained contraction, except that the frequency during the recovery from the caffeine effect was slightly reduced (Figure 8b). The reason for the difference was not clear, but inhomogeneity of electrical properties within the preparation is unlikely, because the mechanical activity recorded from the whole preparation correlated well with the pattern of electrical response recorded with a microeletrode.

Discussion

There is a clear difference in caffeine-induced contractions between guinea-pig gastric muscle and rabbit portal vein, the former being of a tonic and the latter of a phasic type. This may be due to the fact that the contraction in portal vein depends more on intracellular Ca^{2+} release and that in gast-

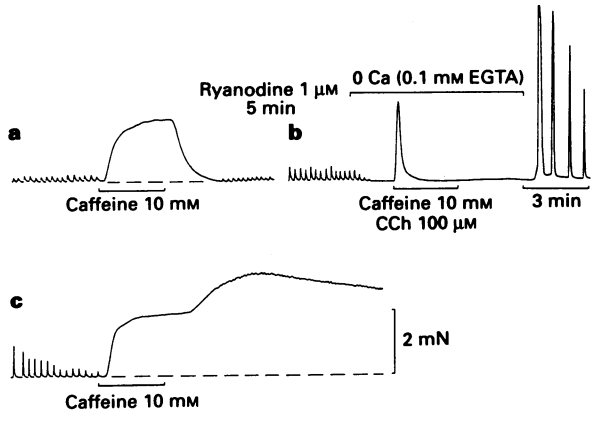

Figure 6 Contractions produced by simultaneous application of carbachol (CCh) and caffeine in the absence of Ca^{2+} (b) and by caffeine in the presence of Ca^{2+} (c) after ryanodine treatment in gastric muscle. (a) Control response to 10 mM caffeine; (b) CCh (100 μM) and caffeine (10 mM) were applied 7 min after ryanodine wash-out and 2 min after exposure to Ca^{2+}-free solution containing 0.1 mM EGTA. (c) Caffeine was applied 25 min after ryanodine treatment.

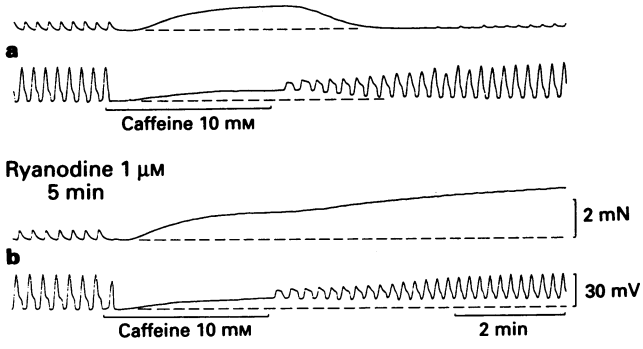

Figure 7 Simultaneous recording of mechanical activity (upper) and intracellular membrane potential (lower trace) in a gastric muscle strip. Electrical activity was obtained from the same cell and interval between records (a) and (b) is 12 min. Ryanodine (1 μM) was applied for 5 min, 10 min after wash-out of caffeine shown in (a).

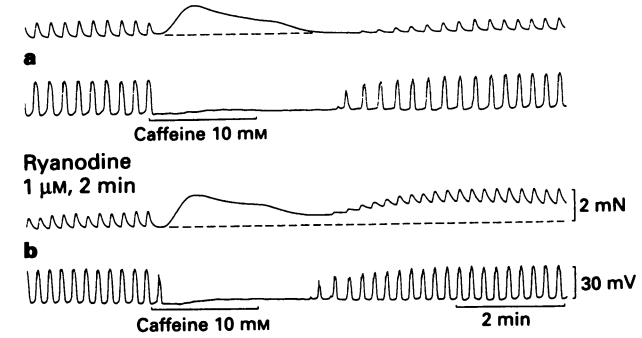

Figure 8 Similar records to Figure 7 in a gastric muscle strip obtained from a different guinea-pig. In this experiment, ryanodine was applied for only 2 min. Note that in this preparation, the sustained contraction, produced by caffeine wash-out after ryanodine pretreatment, was not accompanied by any clear change in membrane potential (the most negative value between slow waves).

ric muscle more on Ca^{2+} influx. This idea is supported by the observations that caffeine-induced contraction is more resistant to removal of Ca^{2+} from the external medium in the portal vein than in gastric muscle and that in gastric muscle the contraction can be readily restored by reapplication of Ca^{2+} in the presence of caffeine.

It has been reported in guinea-pig portal vein, pulmonary artery, and taenia caeci (Iino *et al.*, 1988) and coronary myocytes (Ganitkevich & Isenberg, 1993), that pretreatment with 1–30 μM ryanodine for a few minutes abolishes the caffeine-induced increase in $[Ca^{2+}]_i$, probably by depleting Ca^{2+} in the stores releasable by caffeine. Similarly, caffeine-induced contractions in rat aorta and mesenteric artery are inhibited by ryanodine pretreatment (Shima & Blaustein, 1992). In the present experiments, however, pretreatment with 1–10 μM ryanodine for 1–5 min did not essentially affect the caffeine-induced contraction, but resulted in a sustained contraction accompanied by a maintained high $[Ca^{2+}]_i$ level subsequent to caffeine removal both in gastric muscle and portal vein. The difference could be due either to the failure to deplete the store in the guinea-pig gastric antrum and rabbit portal vein or to a different action of caffeine and ryanodine in these preparations.

The sustained contraction initiated by removal of caffeine, following exposure to ryanodine is dependent on the presence of the external Ca^{2+}, suggesting that continuous Ca^{2+} influx is necessary. The Ca^{2+} influx pathway responsible for the sustained contraction was not clarified in the present experiments, but voltage-gated L-type channels do not seem to be involved because verapamil or nicardipine had no effect on the contraction. Although involvement of other types of voltage-gated Ca^{2+} channels insensitive to organic Ca^{2+} channel blockers cannot be completely disregarded, this is rather unlikely because the sustained contraction can be observed with little change in membrane potential and slow wave activity (Figure 8). It is known that some Ca^{2+} influx pathway in the plasma membrane can be activated when intracellular Ca^{2+} stores are depleted (Putney, 1990). In the present experiments, however, no clear evidence for such a pathway was obtained. Caffeine application in Ca^{2+}-free medium is expected to deplete Ca^{2+} stores, but on Ca^{2+} reapplication no contraction or only a very small transient contraction can be evoked with or without ryanodine pretreatment (Figure 5). Furthermore, since the caffeine-induced contraction was readily abolished by removal of the external Ca^{2+}, there was no clear evidence for Ca^{2+} release with caffeine in the gastric muscle. The Ca^{2+} influx pathway responsible for the sustained contraction is likely to be blocked by intracellular cyclic AMP, because the contraction is inhibited by caffeine and forskolin, which are known to increase cyclic AMP.

The presence or absence of external Ca^{2+} during exposure to ryanodine does not appear to affect the ability of caffeine to initiate a sustained contraction. Caffeine (in the presence of Ca^{2+}) applied about 1 h after ryanodine treatment in the absence of the external Ca^{2+} could still produce a typical sustained contraction on wash-out. On the other hand, caffeine applied in Ca^{2+}-free solution after pretreatment with ryanodine failed to produce a sustained contraction when Ca^{2+} was reapplied (Figure 5). Furthermore, this is also true even if a transient contraction is evoked by simultaneous application of CCh in Ca^{2+}-free solution. The idea that ryanodine acts on the Ca^{2+}-induced Ca^{2+} release channels of intracellular stores only when they are open (Iino *et al.*, 1988) is not sustained by the results in the present experiments. The present results suggest that ryanodine is nearly irreversibly bound at some sites controlling Ca^{2+} influx through the plasma membrane which can be activated by caffeine only when Ca^{2+} is present in the external medium. High $[Ca^{2+}]_i$ is not a sufficient condition to produce the sustained contraction, because carbachol which produced contractions of a similar size to caffeine-induced contractions could not produce a sustained contraction after ryanodine pretreatment. Excess K^+ also failed to evoke a sustained contraction (unpublished observations). In conclusion the plasma membrane of the circular muscles of the guinea-pig gastric antrum and rabbit portal vein appear to have properties similar to the sarcoplasmic reticulum, as previously considered (Ito & Kuriyama, 1971), so that Ca^{2+} permeability of the plasma membrane may be increased directly by caffeine and following an exposure to ryanodine this pathway may be kept open, once activated by caffeine, in the presence of Ca^{2+}.

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References

GANITKEVICH, V.Ya. & ISENBERG, G. (1993). Ca^{2+} entry through Na^+-Ca^{2+} exchange can trigger Ca^{2+} release from Ca^{2+} stores in Na^+-loaded guinea-pig coronary myocytes. *J. Physiol.*, **468**, 225–243.

IINO, M. (1989). Calcium-induced calcium release mechanism in guinea-pig taenia caeci. *J. Gen. Physiol.*, **94**, 363–383.

IINO, M., KOBAYASHI, T. & ENDO, M. (1988). Use of ryanodine for functional removal of the calcium store in smooth muscle cells of the guinea-pig. *Biochem. Biophys. Res. Commun.*, **152**, 417–422.

ITO, Y. & KURIYAMA, H. (1971). Caffeine and excitation-contraction coupling in the guinea-pig taenia coli. *J. Gen. Physiol.*, **57**, 448–463.

ITO, Y., OSA, T. & KURIYAMA, H. (1974). Topical differences of caffeine action on the smooth muscle cells of the guinea-pig alimentary canal. *Jpn. J. Physiol.*, **24**, 217–232.

ITOH, T., KAJIWARA, M., KITAMURA, K. & KURIYAMA, H. (1982). Roles of stored calcium on the mechanical response evoked in smooth muscle cells of the porcine coronary artery. *J. Physiol.*, **322**, 107–125.

ITOH, T., KURIYAMA, H. & SUZUKI, H. (1981). Excitation-contraction coupling in smooth muscle cells of the guinea-pig mesenteric artery. *J. Physiol.*, **321**, 513–535.

KATAYAMA, N., HUANG, S.-M., TOMITA, T. & BRADING, A.F. (1993). Effects of cromakalim on the electrical slow wave in the circular muscle of guinea-pig gastric antrum. *Br. J. Pharmacol.*, **109**, 1097–1100.

OSA, T. (1973). The inhibitory action of caffeine on the smooth muscle of mouse myometrium and guinea pig ileum. *Jpn. J. Physiol.*, **23**, 199–216.

PUTNEY, J.W. (1990). Capacitative calcium entry revisited. *Cell Calcium*, **11**, 611–624.

SAVINEAU, J.-P. & MIRONNEAU, J. (1990). Caffeine acting on pregnant rat myometrium: analysis of its relaxant action and its failure to release Ca^{2+} from intracellular stores. *Br. J. Pharmacol.*, **99**, 261–266.

SHIMA, H. & BLAUSTEIN, M.P. (1992). Modulation of evoked contractions in rat arteries by ryanodine, thapsigargin, and cyclopiazonic acid. *Circ. Res.*, **70**, 968–977.

WATANABE, C., YAMAMOTO, H., HIRANO, K., KOBAYASHI, S. & KANAIDE, H. (1992). Mechanisms of caffeine-induced contraction and relaxation of rat aortic smooth muscle. *J. Physiol.*, **456**, 193–213.

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