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Control of the phasic and tonic contractions of guinea pig stomach by a ryanodine-sensitive Ca^{2+} store

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Abstract

In some smooth muscle cells, the rise in intracellular Ca^{2+} as a result of a Ca^{2+} influx via plasma membrane Ca^{2+} channels can activate a further increase in intracellular Ca^{2+} as a result of Ca^{2+} release from intracellular stores. This study examined the role of the Ca^{2+}-induced Ca^{2+} release from the ryanodine-sensitive intracellular Ca^{2+} stores in shaping the smooth muscle contractions of guinea pig stomach. The contractile activity of isolated muscle strips of the fundus, corpus and antrum region of the stomach was recorded under isometric conditions. Ryanodine, an activator of Ca^{2+}-induced Ca^{2+} release, concentration dependently (10^{-7}–3.10^{-5} M) increased the tone of fundus and corpus strips. Ryanodine had a dual action on the phasic contractions of the antrum and corpus: increase by the low concentrations (up to 10^{-6} M) and inhibition by the high concentrations (10^{-6}–3.10^{-5} M). Nifedipine (10^{-5} M) completely inhibited the ryanodine (10^{-6} M)-induced phasic contractions and only partly the ryanodine (3.10^{-5} M)-induced tonic contractions. In the presence of 10^{-5} M cyclopiazonic acid, a specific inhibitor of sarcoplasmic reticulum Ca^{2+}-ATPase, ryanodine (3.10^{-5} M) further increased the tone of the corpus and fundus strips. Ryanodine (3.10^{-5} M) induced tonic contractions in the fundus and corpus precontracted by acetylcholine (10^{-5} M), and inhibited the acetylcholine (10^{-6} M)-induced phasic contractions in the antrum and corpus. Ruthenium red, an inhibitor of Ca^{2+}-induced Ca^{2+} release, concentration dependently (10^{-6}–10^{-4} M) decreased the tone and amplitude of the phasic contractions. The data obtained provide evidence for the participation of a sarcoplasmic reticulum Ca^{2+}-induced Ca^{2+} release mechanism in shaping the tonic and phasic contractions of guinea pig stomach, and highlight important tissue differences.

Keywords: Ca^{2+} release; Sarcoplasmic reticulum; Ryanodine; Smooth muscle; Stomach; Ruthenium red

1. Introduction

The circular muscle of the guinea pig stomach has spontaneous contractile activity, but the mechanism underlying this activity is still unclear. Different stomach parts show essential differences in the character of their spontaneous contractile activity. The smooth muscles of the antral region of the stomach manifest predominantly phasic contractions, those of the fundus predominantly tonic, while the muscles of the corpus have both.

Two different mechanisms of activation have been suggested for the phasic and tonic contractions in the stomach (Boev et al., 1976). The guinea pig stomach fundus differs from the antrum with respect to spontaneous electrical activity (Boev, 1972; Boev et al., 1976; Tomita and Sakamoto, 1978). The fundus does not show spontaneous electrical activity and predominantly responds to external stimulation with spike-free tonic contractions. The antrum shows spontaneous electrical slow waves of sinusoidal form with superimposed spike potentials. These spike-potentials trigger phasic contractions.

Contractions of smooth muscle are regulated by the rise and fall of intracellular Ca^{2+} concentration, which could result from either Ca^{2+} influx into the cell from the extracellular space or Ca^{2+} release from an intracellular Ca^{2+} store, the sarcoplasmic reticulum (Somlyo and Somlyo, 1994; Karaki et al., 1997). In our previous studies we have found that both the spontaneous and agonist-induced tonic and phasic contractions of the guinea pig and cat gastric muscle depend on the activity of sarcoplasmic reticulum Ca^{2+}-ATPase and Ca^{2+} accumulation in the Ca^{2+} stores (Duridanova et al., 1995; Petkov and Boev,

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1996a,b). Moreover, the sarcoplasmic reticulum in the stomach plays a key role as Ca^{2+} sink for sustained smooth muscle relaxation (Petkov et al., 1994; Duridanova et al., 1995; Petkov et al., 1998). It is also possible that intracellular Ca^{2+} release from the sarcoplasmic reticulum is involved in shaping the spontaneous phasic and tonic contractions of the guinea pig stomach. Two types of Ca^{2+} release mechanism operate in sarcoplasmic reticulum Ca^{2+} stores of smooth muscle: one is the inositol 1,4,5-trisphosphate-induced Ca^{2+} release and the other is the Ca^{2+}-induced Ca^{2+} release (Iino et al., 1988, Iino, 1989; Somlyo and Somlyo, 1994; Karaki et al., 1997).

Iino (1989) was the first to show that a Ca^{2+}-induced Ca^{2+} release mechanism operates in the sarcoplasmic reticulum Ca^{2+} store in the intestinal smooth muscle. Recent reports on work with various types of smooth muscle preparations (for review see Karaki et al., 1997), including guinea pig gastric antrum (Chowdhury et al., 1995; Kim et al., 1997; Duridanova et al., 1997), gastric fundus of guinea pig (Duridanova et al., 1996) and cat (Petkov and Boev, 1998), reached controversial conclusions regarding the role of the Ca^{2+}-induced Ca^{2+} release mechanism in triggering contractions.

We now tested the hypothesis that Ca^{2+}-induced Ca^{2+} release from a ryanodine-sensitive Ca^{2+} store can regulate contractions of the guinea pig stomach by directly examining the effects of ryanodine and ruthenium red on smooth muscle contractile activity. Ryanodine, a plant alkaloid, has been shown to accelerate Ca^{2+}-induced Ca^{2+} release by binding to the sarcoplasmic reticulum Ca^{2+} release channels in the open state, and locking them open (Hwang and Van Breemen, 1987; Iino et al., 1988; Karaki et al., 1997). Ruthenium red, a polycationic dye, has been reported to inhibit the ryanodine-sensitive Ca^{2+} release channels of the sarcoplasmic reticulum (Zhang et al., 1993; Zucchi and Ronca-Testoni, 1997).

For functional removal of sarcoplasmic reticulum Ca^{2+} stores, ryanodine and ruthenium red were applied simultaneously with cyclopiazonic acid, a specific inhibitor of sarcoplasmic reticulum Ca^{2+}-ATPase (Seidler et al., 1989). In earlier study on guinea pig stomach (Petkov and Boev, 1996a), we have found that cyclopiazonic acid causes a stable increase in the tone of the fundus and corpus, and modifies the phasic contractions of the corpus and antrum due to the disruption of the 'buffer barrier' function of the sarcoplasmic reticulum (for review see Van Breemen et al., 1995).

2. Materials and methods

2.1. Tissue preparation

Male adult guinea pigs weighing 250–350 g were stunned and bled. Through a midline incision in the abdomen, the entire stomach was removed and immediately placed in a modified Ca^{2+}-containing physiological Krebs solution (composition in mM: 137.5 Na^+, 5.9 K^+, 2.5 Ca^{2+}, 1.2 Mg^{2+}, 134.2 Cl^-, 15.5 HCO_3^-, 1.2 $H_2PO_4^-$, 11.5 glucose) at room temperature (21–23°C). The stomach was opened along the longitudinal axis of the greater curvature then pinned flat in a Petri dish with the muscle side up and stretched to its in vivo length. It was carefully scraped free of fat and connective tissue.

2.2. Organ bath experiments

Circular smooth muscle strips (2 mm wide and 10 mm long) were cut out from the fundus, corpus and antrum regions of the stomach, removing the mucosal layer. The strips were then suspended vertically in 10-ml organ baths (two strips per bath). One end of each strip was anchored to the bottom of the bath and the other was connected to a force-displacement transducer (FT03, Grass) coupled to a pen recorder for isometric tension recording. The strips were suspended under 10 mN tension. These procedures were carried out in a Ca^{2+}-free Krebs solution, which was prepared like the Ca^{2+}-containing solution (see above) but with Ca^{2+} replaced by Na^+. Ten minutes later the bath solution was replaced by a Ca^{2+}-containing physiological Krebs solution to initiate contractions. These spontaneous contractions in the Ca^{2+}-containing solution are referred throughout as spontaneous phasic contractions or spontaneous tone. The bath solutions had thermostatically controlled temperature (37°C) and were continuously bubbled with 95% O_2–5% CO_2 to achieve a pH of 7.4. There was a 90 to 120-min equilibration period before stable spontaneous contractions (phasic or tonic) occurred. During this period the bath solution was changed every 15 min.

2.3. Drugs

The drugs used were: acetylcholine, cyclopiazonic acid, nifedipine, tetraethylammonium (Sigma); ryanodine (Calbiochem); ruthenium red (Merck). All other compounds were of analytical grade. Ryanodine and nifedipine were dissolved in ethanol. Cyclopiazonic acid was dissolved in dimethylsulfoxide. Dimethylsulfoxide and ethanol in the concentrations used had no permanent effect on the contractility of guinea pig gastric smooth muscles.

2.4. Statistics

All the responses are expressed in percentage, as the means ± S.E.M. for n, the number of preparations ($n/2$ = the number of animals). The amplitude of the spontaneous contractions was assumed to be 100%. The data were assessed for statistical significance using Student's t-test set at $P < 0.05$.

3. Results

3.1. Effect of ryanodine on the spontaneous contractions of guinea pig gastric fundus, corpus and antrum

To evaluate the role of Ca^{2+} release from the sarcoplasmic reticulum in maintenance of the spontaneous contractions of guinea pig gastric fundus, corpus and antrum, we first investigated the effect of ryanodine. Ryanodine applied at concentrations above 10^{-7} M concentration dependently increased the tone of fundus preparations, without provoking phasic contractions (Fig. 1). The value of the maximal tonic contraction of guinea pig fundus caused by 3.10^{-5} M ryanodine was $280 \pm 28\%$, of the spontaneous tone ($n = 8$; see also Fig. 3). The cumulative application of ryanodine (10^{-7}–3.10^{-5} M) to guinea pig antrum had a dual action depending on the concentration. At concentrations up to 10^{-6} M ryanodine increased the amplitude of the phasic contractions ($n = 10$; Fig. 1). At concentrations higher than 10^{-6} M the activating effect of ryanodine on the phasic contractions was transformed into an inhibitory one, i.e., the amplitude of the contractions decreased. However, ryanodine caused the appearance of tone in the antrum (Fig. 1). Ryanodine (10^{-7}–10^{-6} M) concentration dependently increased the tone and slightly increased the amplitude of the phasic contractions of the guinea pig gastric corpus ($n = 8$; Fig. 1). At concentrations above 10^{-6} M ryanodine further increased the tone but inhibited the phasic contractions. The ryanodine effect on the spontaneous contractions was sustained and persisted for a number of hours (recorded up to 4 h).

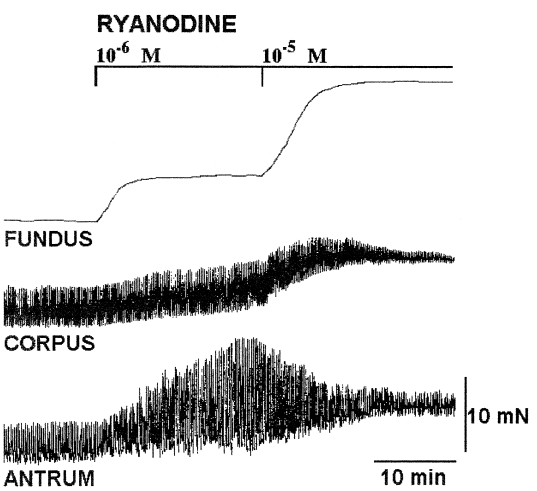

Fig. 1. Effect of cumulative application of ryanodine on the spontaneous contractile activity of smooth muscle strips isolated from three different parts of the guinea pig stomach. Upper trace: Ryanodine concentration dependently increased the spontaneous tone of a fundus preparation. Middle trace: The effect of ryanodine in a corpus preparation. Ryanodine concentration dependently increased the tone and, in concentrations higher than 10^{-5} M, inhibited the phasic contractions. Lower trace: The dual action of ryanodine on the phasic contractions in an antrum preparation. Ryanodine (10^{-6} M) increased the amplitude of the phasic contractions but in higher concentration (10^{-5} M) decreased the phasic contractions and provoked tone.

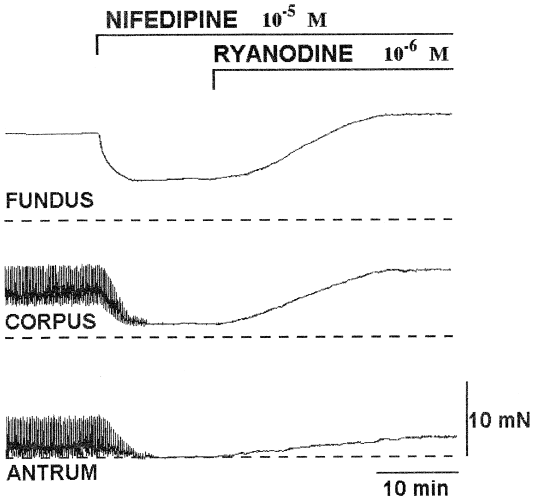

Fig. 2. Effect of nifedipine (10^{-5} M) and ryanodine (10^{-6} M) on the contractile activity of smooth muscle strips isolated from the fundus (upper trace), corpus (middle trace) and antrum (lower trace) of the guinea pig stomach. Nifedipine partly suppressed the spontaneous tone of the fundus and corpus and completely suppressed the phasic contractions of the corpus and antrum. After nifedipine, ryanodine induced tonic contractions in the fundus and corpus, and provoked a very slight tone in the antrum, but did not restore the phasic contractions in the corpus and antrum.

3.2. Effects of nifedipine and ryanodine

The involvement of L-type Ca^{2+} channels in the effect of ryanodine on the tonic and phasic contractions was investigated with nifedipine, a selective inhibitor of these channels. Nifedipine at a supramaximal concentration of 10^{-5} M only partly suppressed the ryanodine (3.10^{-5} M)-induced tonic contractions of fundus strips ($n = 8$). Nifedipine (10^{-5} M) partly suppressed the ryanodine (3.10^{-5} M)-induced tone of corpus strips ($n = 6$), but completely abolished the ryanodine (10^{-6} M)-induced phasic contractions of the corpus and antrum ($n = 6$–8). In the presence of 10^{-5} M nifedipine, ryanodine (10^{-6}–3.10^{-5} M) induced slow-developing, tonic contractions in the fundus and corpus, and provoked a very slight tone in the antrum (Fig. 2, $n = 6$). However, in the presence of 10^{-5} M nifedipine, ryanodine (10^{-6} M) did not induce phasic contractions in the corpus and antrum (Fig. 2, $n = 4$–6).

3.3. Effects of ryanodine and cyclopiazonic acid

The role of sarcoplasmic reticulum Ca^{2+} stores in the contractile activity of gastric smooth muscle could be evaluated after complete Ca^{2+} depletion, i.e., simultaneous activation of sarcoplasmic reticulum Ca^{2+} release and inhibition of Ca^{2+} uptake. In these experiments we therefore, investigated the effects of the combination of ryan-

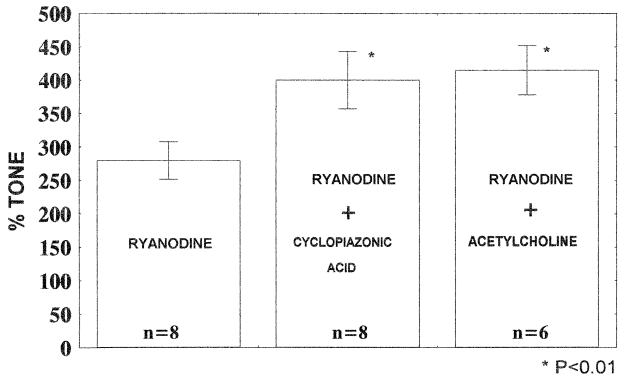

Fig. 3. Quantitative data showing the contractile effect of ryanodine (3.10^{-5} M); ryanodine (3.10^{-5} M) plus cyclopiazonic acid (10^{-5} M); ryanodine (3.10^{-5} M) plus acetylcholine (10^{-6} M) on guinea pig gastric fundus strips. The spontaneous tone was taken to be 100%. Values are means ± S.E.M.

odine and cyclopiazonic acid. In the presence of cyclopiazonic acid (10^{-5} M), ryanodine (3.10^{-5} M) further increased the tone of the corpus and fundus ($n = 4$–6). In the presence of 3.10^{-5} M ryanodine, cyclopiazonic acid (10^{-5} M) also increased the tone of the corpus and fundus ($n = 6$–8). In Fig. 3, the increase of the tone induced by 3.10^{-5} M ryanodine in fundus strips is compared to the increase induced by 3.10^{-5} M ryanodine and 10^{-5} M cyclopiazonic acid together.

3.4. Effects of ryanodine and acetylcholine

Acetylcholine induced sustained tonic contractions of the fundus, phasic contractions of the antrum, and both tonic and phasic contractions of the corpus. In order to understand whether ryanodine could modulate the contractions in response to acetylcholine which activates Ca^{2+} influx from the extracellular space and releases Ca^{2+} from the intracellular stores in the gastric smooth muscle, we evaluated the effect of ryanodine on acetylcholine-induced contractions.

In fundus strips precontracted with acetylcholine (10^{-6} M), ryanodine (3.10^{-5} M) further potentiated the tone, without provoking phasic contractions ($n = 6$). In antrum strips, ryanodine (3.10^{-5} M) completely inhibited the acetylcholine (10^{-6} M)-induced phasic contractions ($n = 4$). In corpus strips ryanodine (3.10^{-5} M) inhibited the acetylcholine (10^{-6} M)-induced phasic contractions and increased the tone ($n = 6$). After complete inhibition of the phasic contractions of the antrum ($n = 4$) and corpus ($n = 6$) by 3.10^{-5} M ryanodine, acetylcholine (10^{-6} M) failed to restore the phasic contractions, but induced a tonic contraction in the corpus preparations. In the presence of 3.10^{-5} M ryanodine, acetylcholine (10^{-6} M) was able to induce tonic contraction in the fundus ($n = 6$). In Fig. 3, the increase of the fundus tone induced by 3.10^{-5} M ryanodine is compared to the increase induced by 3.10^{-5} M ryanodine and 10^{-6} M acetylcholine together. These results suggest additive effects of ryanodine and acetylcholine on the guinea pig gastric smooth muscle.

3.5. Effect of ruthenium red

Ruthenium red was used to block the sarcoplasmic reticulum Ca^{2+} release channels. Added cumulatively (10^{-6}–3.10^{-5} M), ruthenium red decreased the amplitude and frequency of the spontaneous phasic contractions of antrum strips and at concentrations above 3.10^{-5} M was able to completely suppress contractility ($n = 8$; Fig. 4). In the corpus strips ruthenium red (10^{-6}–10^{-4} M) suppressed both spontaneous tone and phasic contractions ($n = 4$). Ruthenium red (10^{-6}–10^{-4} M) inhibited the spontaneous tone of fundus strips in a concentration-dependent manner ($n = 6$; Figs. 4 and 5A). In most of the fundus strips, ruthenium red (3.10^{-5}–10^{-4} M) caused a slight transient tonic contraction at the beginning of its administration, followed by sustained relaxation (Fig. 4).

Wash-out of ruthenium red followed by incubation of the muscle strips in a fresh Ca^{2+}-containing physiological solution successfully reversed the ruthenium red effect, resulting in the recurrence of spontaneous contractions. After complete inhibition of the spontaneous tonic and phasic contractions by ruthenium red (10^{-4} M) in the guinea pig fundus, corpus and antrum ($n = 6$–8), 10^{-2} M tetraethylammonium, a typical inhibitor of K^+ channels which indirectly activates the L-type Ca^{2+} channels, induced powerful phasic contractions (Fig. 4). The amplitude of tetraethylammonium-induced contractions was higher

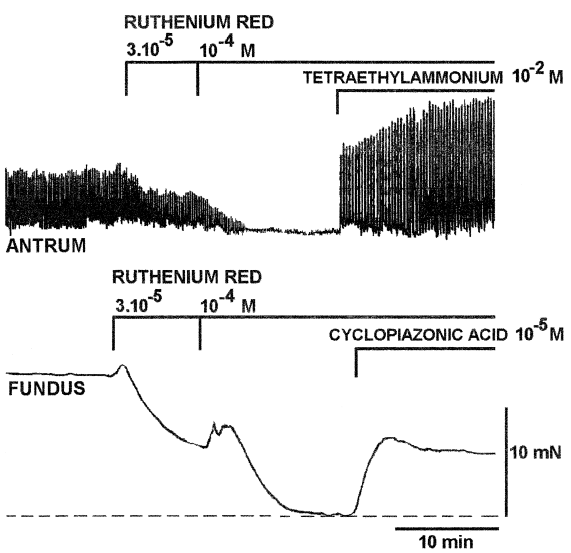

Fig. 4. Effect of cumulative application of ruthenium red on the spontaneous contractile activity of smooth muscle strips isolated from the antrum (upper trace) and fundus (lower trace) of the guinea pig stomach. After complete inhibition of the phasic contractions in the antrum by ruthenium red, 10^{-2} M tetraethylammonium caused the appearance of high-amplitude phasic contractions. After complete inhibition of the spontaneous tone of the fundus by ruthenium red, 10^{-5} M cyclopiazonic acid induced only a slight tonic contraction.

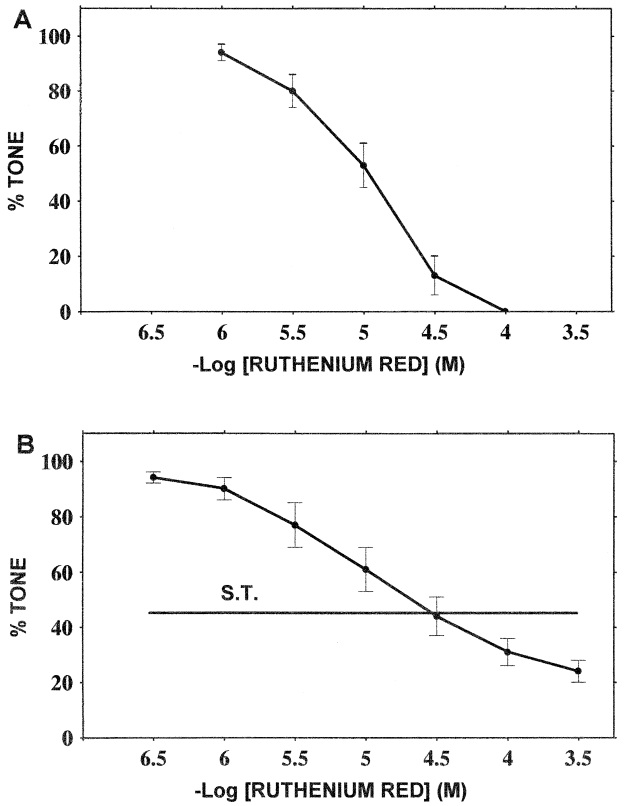

Fig. 5. Cumulative concentration-response curves: (A) for the relaxant effect of ruthenium red on the spontaneous tone of fundus strips and (B) for the relaxant effect of ruthenium red on cyclopiazonic acid-induced tone of fundus strips. In panel (A), the spontaneous tone was taken to be 100%. In panel (B), the cyclopiazonic acid (10^{-5} M)-induced tone together with the spontaneous tone was taken to be 100%. The line indicates the level of the spontaneous tone (S.T.). Values are means ± S.E.M.

than that of the spontaneous contractions and similar to that observed in control tissues not treated with ruthenium red.

These observations suggest that the ruthenium red effect was not associated with a decrease in Ca^{2+} sensitivity of the contractile myofilaments.

3.6. Effect of ruthenium red and cyclopiazonic acid

In order to block the sarcoplasmic reticulum Ca^{2+} release and Ca^{2+} uptake, we used cyclopiazonic acid and ruthenium red. As illustrated in Fig. 5B, in fundus strips contracted with 10^{-5} M cyclopiazonic acid, ruthenium red caused concentration-dependent relaxation, but did not completely suppress either cyclopiazonic acid-induced contraction or spontaneous tone ($n = 6$). On the other hand, after complete inhibition of the spontaneous tone by ruthenium red (10^{-4} M), 10^{-5} M cyclopiazonic acid induced a low-amplitude tonic contraction, which in no case returned to the level of the spontaneous tone ($n = 4$; Fig. 4). Following inhibition of the phasic contractions of antrum preparations by ruthenium red (10^{-4} M), cyclopiazonic acid (10^{-5} M) caused the appearance of a slight tone, without restoring the phasic contractions ($n = 6$). Thus, the simultaneous blockade of Ca^{2+} uptake and Ca^{2+} release led to inability of the sarcoplasmic reticulum to control smooth muscle contractility.

4. Discussion

According to Iino (1989), the Ca^{2+}-induced Ca^{2+} release cannot play a primary role in triggering a physiological contraction, but is important as a modulating factor for Ca^{2+}-activated K^+ channel opening. A more recent study on vascular smooth muscle (Knot et al., 1998) favours this hypothesis, i.e., that local Ca^{2+} release from a ryanodine-sensitive Ca^{2+} store, referred to as 'Ca^{2+} sparks' (Nelson et al., 1995) regulates myogenic tone solely through activation of Ca^{2+}-dependent K^+ channels. Our earlier studies have also shown that the Ca^{2+}-induced Ca^{2+} release can activate K^+ currents in smooth muscle cells from guinea pig gastric fundus (Duridanova et al., 1996) and antrum (Duridanova et al., 1997).

The present study, however, showed that the sarcoplasmic reticulum Ca^{2+} release from a ryanodine-sensitive Ca^{2+} store can contribute directly to the maintenance of spontaneous and agonist-induced contractions of the guinea pig gastric smooth muscle. We observed that ryanodine as well as ruthenium red affected both the spontaneous and evoked tonic and phasic contractions of the guinea pig stomach.

According to the 'Capacitative Ca^{2+} entry' model (Putney, 1990) depletion of the intracellular Ca^{2+} stores somehow provides a signal for activation of Ca^{2+} entry across the plasma membrane. We have suggested (Petkov and Boev, 1996b; Fusi et al., 1998) a possible involvement of capacitative Ca^{2+} entry as a source of Ca^{2+} for contraction of gastric fundus muscle. A more recent review (Gibson et al., 1998) suggests that capacitative Ca^{2+} entry through store-operated plasma membrane Ca^{2+} channels plays an important role in regulation of contraction in different types of smooth muscle. The 'Capacitative Ca^{2+} entry' model predicts that ryanodine should activate a Ca^{2+} influx pathway in guinea pig gastric smooth muscle cells, as suggested for anococcygeus muscle (Wayman et al., 1998). The relative contribution of capacitative Ca^{2+} entry to excitation–contraction coupling depends on the smooth muscle type and appears to be greatest in tonic smooth muscle (Gibson et al., 1998). This possibility is also supported by the results of the present study.

There was an essential difference between the effects of ryanodine on the phasic and on the tonic contractions. This may have resulted from differences in geometry and characteristics of sarcoplasmic reticulum Ca^{2+} stores in the tonic and the phasic smooth muscles (Nixon et al., 1994) and also in the Ca^{2+} influx and Ca^{2+} efflux through the

plasma membrane during stimulation (Boev et al., 1976, Bitar et al., 1986; Kim et al., 1997). It has been hypothesised that the plasma membrane of the guinea pig gastric antrum muscle is similar to the sarcoplasmic reticulum membrane (Chowdhury et al., 1995).

Ca^{2+}-induced Ca^{2+} release has been demonstrated for the first time in spike-generating smooth muscles (Iino, 1989). In these smooth muscles the sarcoplasmic reticulum Ca^{2+} release is triggered by Ca^{2+} entry during the spike action potential. In gastric antrum smooth muscles, the Ca^{2+} antagonists inhibit spontaneous and acetylcholine-induced phasic contractions (Boev et al., 1976; Ozaki et al., 1993). Unlike the ryanodine-induced tonic contractions of the fundus and corpus, the phasic contractions of the antrum and corpus induced by low concentrations of ryanodine (up to 10^{-6} M) depend exclusively on Ca^{2+} influx through L-type Ca^{2+} channels. However, L-type Ca^{2+} channel antagonists are also unable to completely suppress the spontaneous and acetylcholine-induced tone of the cat (Boev et al., 1976) and guinea pig (Duridanova et al., 1995; Petkov et al., 1998) fundus. We now found that, in the presence of nifedipine, the increase in muscle tone of the fundus and corpus caused by ryanodine was delayed, suggesting an important role of L-type Ca^{2+} channels in the effect of ryanodine, on the one hand, and the existence of a nifedipine-resistant part of the ryanodine-induced tonic contraction, on the other. This supports the assumption that the Ca^{2+} antagonist-resistant component of the tone at least partly results from the release of Ca^{2+} from a ryanodine-sensitive store. Furthermore, ruthenium red, used in our experiments as inhibitor of sarcoplasmic reticulum Ca^{2+} release channels, led to inhibition of the spontaneous contractions. It is suggested that, in cardiac muscle, ruthenium red could decrease the Ca^{2+} sensitivity of the contractile myofilaments (Tanaka et al., 1997). This could not be the case for guinea pig stomach, since addition of tetraethylammonium after ruthenium red-induced relaxation of the spontaneous contractions restored the contractions. However, an inhibitory effect of ruthenium red on the mitochondrial Ca^{2+} uptake in stomach smooth muscle (Drummond and Fay, 1996) should not be ruled out.

It is suggested that the source of Ca^{2+} responsible for the initial contraction of human and guinea pig gastric antrum in response to acetylcholine or some other agonists is intracellular (Bitar et al., 1986) but the extracellular Ca^{2+} is required for replenishment of the sarcoplasmic reticulum Ca^{2+} store. Unlike ryanodine, acetylcholine activates sarcoplasmic reticulum Ca^{2+} release through inositol 1,4,5-trisphosphate sensitive Ca^{2+} channels. The present results showed that ryanodine increased the maximal contraction after acetylcholine addition to fundus strips. Ryanodine also potentiated the tonic contraction seen when the sarcoplasmic reticulum Ca^{2+}-ATPase was blocked by its selective inhibitor, cyclopiazonic acid. Because Ca^{2+}-induced Ca^{2+} release depends on the intracellular Ca^{2+} concentration (Iino, 1989), acceleration of the Ca^{2+} release in the presence of acetylcholine or cyclopiazonic acid could be due to the increased Ca^{2+} in the cytoplasm. There is evidence that, in guinea pig gastric antrum, the global intracellular Ca^{2+} concentration is not significantly affected by the Ca^{2+}-induced Ca^{2+} release during depolarization (Kim et al., 1997).

Results of preliminary experiments on guinea pig and cat gastric smooth muscle preparations, using tetrodotoxin and atropine, suggest that the effects of ryanodine and cyclopiazonic acid are exerted directly on muscle rather than neurons (Petkov and Boev, 1996a,b, 1998; Fusi et al., 1998). However, the possibility should not be excluded that endogenous substances not blocked by tetrodotoxin and atropine could have affected the contractions after ryanodine and cyclopiazonic acid.

The present results could be interpreted in terms of the 'superficial buffer barrier' hypothesis (for review see Van Breemen et al., 1995). According to this hypothesis, part of the Ca^{2+} entering the smooth muscle cell through the plasma membrane is actively taken up by the sarcoplasmic reticulum Ca^{2+}-ATPase into Ca^{2+} stores, before it can reach the contractile myofilaments and activate contraction. Acceleration of sarcoplasmic reticulum Ca^{2+} release by ryanodine, or its inhibition by ruthenium red would interrupt this process, leading to relative changes in the contractile response.

It is well known that, in high concentrations, ryanodine can inhibit the Ca^{2+}-induced Ca^{2+} release channels instead of activating them (for review see Zucchi and Ronca-Testoni, 1997). However, it seems very unlikely that the inhibitory effect of ryanodine on the phasic contractions of guinea pig stomach, now reported, is due to the above reason. Ryanodine inhibited the spontaneous and acetylcholine-induced phasic contractions of the guinea pig corpus but increased the tone, thus favouring the 'superficial buffer barrier' hypothesis (Van Breemen et al., 1995). Furthermore, the effect of ryanodine on the phasic contractions of the guinea pig antrum and corpus resembles the effect of cyclopiazonic acid reported previously (Petkov and Boev, 1996a). All these findings indicate that functional intracellular Ca^{2+} stores are required for generation of the phasic contractions.

The present results suggest that the Ca^{2+}-induced Ca^{2+} release from a ryanodine-sensitive Ca^{2+} store plays a relatively important role in the shaping of spontaneous and evoked tonic and phasic contractions of the guinea-pig stomach.

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