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Cyclopiazonic acid-induced changes in contractile activity of smooth muscle strips isolated from cat and guinea-pig stomach

Georgi V. Petkov *, Kiril K. Boev

Institute of Biophysics, Bulgarian Academy of Sciences, Acad. G. Bonchev Street, Block 21, 1113 Sofia, Bulgaria

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Abstract

The effects of cyclopiazonic acid (CPA), a specific inhibitor of sarcoplasmic reticulum Ca^{2+}-ATPase, on contractile activity of circular smooth muscle strips isolated from the antrum, corpus and fundus regions of the cat and guinea-pig stomach were studied. Contractile activity was recorded under isometric conditions, in organ baths. CPA, concentration dependently ($3 \cdot 10^{-7}$–$3 \cdot 10^{-5}$ M) increased the tone of the cat and guinea-pig gastric fundus and corpus as well as the amplitude of the phasic contractions of the cat corpus and antrum, affecting their frequency. CPA had a dual action on the phasic contractions of the guinea-pig antrum: an increase at low concentrations (up to 10^{-6} M) and inhibition at high concentrations (10^{-6}–$3 \cdot 10^{-5}$ M). Tetrodotoxin (10^{-6} M), atropine (10^{-6} M) and N^{ω}-nitro-L-arginine (10^{-4} M) did not change significantly the effects of CPA. Nifedipine completely inhibited the CPA-induced phasic contractions and partly inhibited the CPA-induced tonic contractions. The nitric oxide-releasing agents, sodium nitroprusside (10^{-3} M) and 3-morpholino-sydnonimine (10^{-3} M), completely inhibited the CPA-induced tonic and phasic contractions. CPA induced tonic contractions in the cat and guinea-pig gastric fundus precontracted by acetylcholine (10^{-5} M) and inhibited the acetylcholine (10^{-6} M)-induced phasic contractions in the guinea-pig gastric antrum and corpus. The results suggest multiple roles for sarcoplasmic reticulum Ca^{2+} stores and sarcoplasmic reticulum Ca^{2+}-ATPase in the shaping of spontaneous and evoked tonic and phasic contractions of the stomach, and highlight important species and tissue differences.

Keywords: Cyclopiazonic acid; Ca^{2+}-ATPase; Sarcoplasmic reticulum; Smooth muscle; Stomach; Contraction

1. Introduction

Recently, it has been shown that cyclopiazonic acid (CPA) is a very useful pharmacological tool for evaluation of the role of sarcoplasmic reticulum Ca^{2+}-ATPase in smooth muscle contraction, since it acts as a highly specific inhibitor of the sarcoplasmic reticulum Ca^{2+}-ATPase without affecting plasma membrane Ca^{2+}-ATPase (Seidler et al., 1989). The mycotoxin, CPA, an indole tetramic acid metabolite of *Aspergillus* and *Penicillium*, inhibits the Ca^{2+}-ATPase and Ca^{2+} transport activity of the sarcoplasmic reticulum in skeletal muscle (Seidler et al., 1989) and smooth muscles (Deng and Kwan, 1991; Bourreau et al., 1993; Uyama et al., 1992; Naganobu and Ito, 1994; Gonzalez De La Fuente et al., 1995). CPA appears to have a dual action on vascular smooth muscle, with contractile and relaxant effects depending on intactness of the endothelium (Zheng et al., 1994). It is suggested that the endothelium-dependent relaxation induced by CPA is due to Ca^{2+} release from the intracellular stores, resulting in activation of nitric oxide (NO) synthesis in the endothelial cells (Moritoki et al., 1994).

In previous studies, it was found that CPA (10^{-5} M) induces sustained tonic contraction in guinea-pig (Fusi et al., 1994) and cat (Petkov and Boev, 1996) gastric fundus, depending on the extracellular Ca^{2+} concentration and that a large part of this contraction is mediated by Ca^{2+} influx via L-type Ca^{2+} channels.

The aim of the present study was to examine the effects of CPA on the contractile activity of smooth muscles from the cat and guinea-pig gastric antrum, corpus and fundus. It was of interest to compare the effects of CPA in the different regions of the stomach as their spontaneous electrical and contractile activities are different. The smooth muscles of the antral region of the stomach manifest predominantly phasic contractions, those of the fundus are predominantly tonic, while the muscles of the corpus are

* Corresponding author. Tel.: (359-2) 7132130; fax: (359-2) 9712493; e-mail: gpetkov@iph.bio.acad.bg

characterized by both phasic and tonic contractions. Two different mechanisms of activation of the phasic and tonic contractions have been suggested (Boev et al., 1976). In addition, the cat antrum differs from the guinea-pig antrum with respect to the spontaneous electrical slow waves (Boev, 1972). The spontaneous plateau-type slow potentials in the cat antrum are always accompanied by phasic contractions. Unlike the plateau potentials in the cat antrum, the slow waves in the guinea-pig and rat antrum are of sinusoidal form with spike potentials superimposed on them (Boev, 1972). These spike potentials trigger phasic contractions.

Since non-adrenergic non-cholinergic neurotransmission, mediated by NO, is involved in the regulation of gastric smooth muscle activity (Lefebvre, 1995), we examined the effect of CPA in the presence of N^{ω}-nitro-L-arginine (L-NNA), an inhibitor of NO synthesis. We also studied the effects of two structurally different NO releasing agents, sodium nitroprusside and 3-morpholino-sydnonimine (SIN-1), on the action of CPA. The effect of CPA on acetylcholine-induced contractions was also evaluated.

2. Materials and methods

2.1. Tissue preparation

Male adult guinea-pigs weighing 250–350 g were stunned and exsanguinated. Male adult cats weighing 2.5–4.5 kg were anesthetized with α-chloralose (80 $mg \cdot kg^{-1}$ i.p.). Through a middline 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 (23–25°C). The stomach was opened along the longitudinal axis of the greater curvature, 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 from the antrum, corpus and fundus of cat and guinea-pig stomach, and the mucosal layer was removed. 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 (UL type, cap. ±10 g) 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. The bath solutions were thermostatically controlled (37°C) and continuously bubbled with a mixture of 95% O_2 and 5% CO_2 to achieve a pH of 7.4. There was a 90–120 min equilibration period. During this period the bath solution was changed every 15 min.

Concentration-response curves for the effect of CPA were obtained by cumulative application of the drug. Tetrodotoxin, L-NNA and atropine were added to the bath for at least 20 min before CPA.

2.3. Drugs

The drugs used were: acetylcholine, atropine, CPA, nifedipine, sodium nitroprusside, tetraethylammonium, tetrodotoxin, L-NNA (Sigma); SIN-1 (Cassella). All other compounds were of analytical grade. CPA was dissolved in dimethyl sulfoxide in a concentration of 10 mM as stock solution. L-NNA and nifedipine were dissolved in 65 mM HCl and ethanol, respectively. SIN-1 and sodium nitroprusside were prepared as aqueous solutions immediately before use. Dimethyl sulfoxide, HCl and ethanol in the concentrations used had no permanent effect on the contractility of the guinea-pig and cat gastric smooth muscle.

2.4. Statistics

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

3. Results

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

It has been shown that CPA at a concentration of 10^{-5} M induces sustained tonic contraction of the guinea-pig (Fusi et al., 1994) and cat (Petkov and Boev, 1996) gastric fundus and that this contraction has two components: one, nifedipine-sensitive, and the other, nifedipine-resistant. At the same concentration CPA induced changes in the spontaneous contractile activity of muscle strips isolated from different parts of guinea-pig and cat stomach (Fig. 1). In the cat gastric corpus CPA (10^{-5} M) increased both the tone and the amplitude of the phasic contractions, while in the guinea-pig corpus CPA (10^{-5} M) increased the tone but inhibited the phasic contractions. In cat and guinea-pig antrum strips, CPA (10^{-5} M) had an opposite effect: an increase of the amplitude and frequency of the phasic contractions of the cat antrum strips and complete inhibition of the phasic contractions of the guinea-pig antrum strips. CPA did not provoke phasic contractions in either cat or guinea-pig fundus, and did not induce tonic contractions in the antrum strips.

Cumulative concentration-response curves were obtained for the contractile effect of CPA ($3 \cdot 10^{-7}$–$3 \cdot 10^{-5}$ M) on the spontaneous tone of the cat (Fig. 2, left panel) and guinea-pig (Fig. 2, middle panel) gastric fundus and on the spontaneous phasic contractions of the cat gastric antrum (Fig. 2, right panel). The values of the maximal contractions caused by $3 \cdot 10^{-5}$ M CPA for cat fundus, guinea-pig fundus and cat antrum were $290 \pm 48\%$ ($n = 15$), $340 \pm 42\%$ ($n = 10$) and $732 \pm 93\%$ ($n = 10$), respectively. CPA ($3 \cdot 10^{-7}$–$3 \cdot 10^{-5}$ M) increased both the tone and the phasic contractions of the cat corpus in a concentration-dependent manner (data not shown). The cumulative application of CPA ($5 \cdot 10^{-7}$–$3 \cdot 10^{-5}$ M) to guinea-pig antrum had a dual effect. At concentrations up to 10^{-6} M CPA increased the amplitude and frequency of the phasic contractions (Fig. 3). In some antrum strips, the low concentrations of CPA ($5 \cdot 10^{-7}$–10^{-6} M) caused a transient decrease of the contraction amplitude 3–5 min after CPA administration, followed by an increase of the amplitude. At concentrations higher than 10^{-6} M the activating effect of CPA was transformed into an inhibitory one, i.e. the amplitude of the contractions decreased. CPA ($5 \cdot 10^{-7}$–10^{-6} M) concentration dependently increased the tone without significantly affecting the amplitude of the phasic contractions in the guinea-pig gastric corpus (Fig. 3). At concentrations above 10^{-6} M CPA further increased the tone but inhibited the phasic contractions. After complete inhibition of the phasic contractions by CPA ($3 \cdot 10^{-5}$ M) in the guinea-pig antrum and corpus, 10^{-2} M tetraethylammonium, a typical inhibitor of K^+ channels which indirectly activates the L-type Ca^{2+} channels, induced powerful phasic contractions with an amplitude greater than that of the spontaneous contractions (Fig. 3).

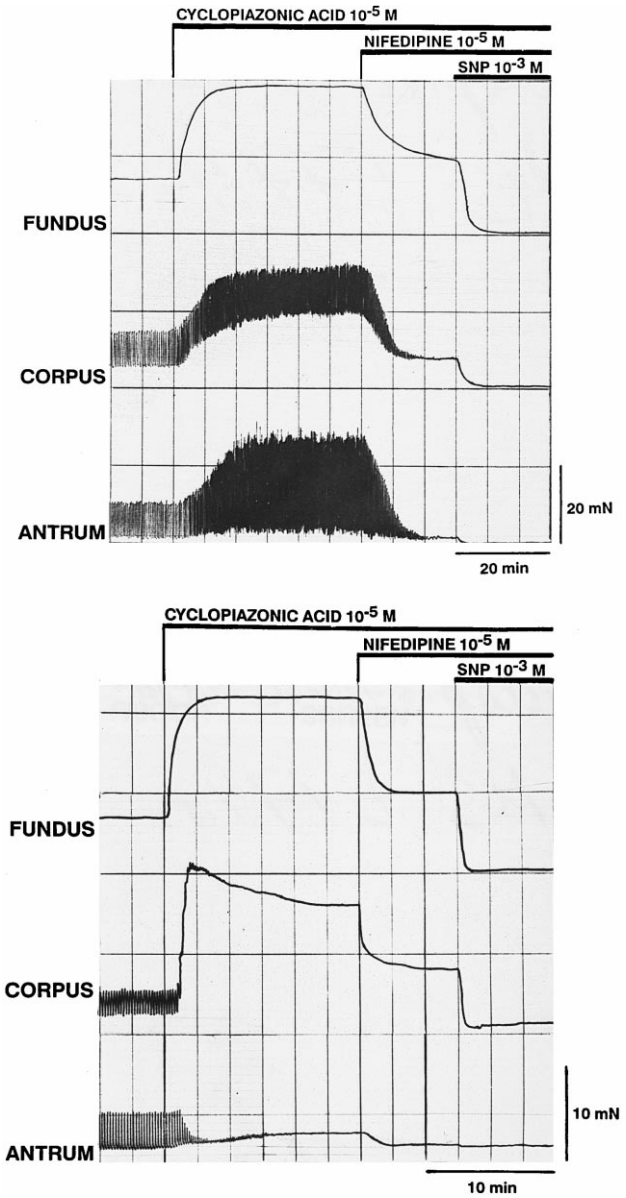

Fig. 1. Effects of 10^{-5} CPA on the contractile activity of smooth muscle strips isolated from three different parts of the cat (top panel) and guinea-pig (bottom panel) stomach. Upper traces: Typical changes in the muscle tension of fundus strips. CPA-induced tonic contractions of the cat and guinea-pig fundus were partially abolished by 10^{-5} M nifedipine and completely by 10^{-3} M sodium nitroprusside (SNP). Middle traces: CPA simultaneously increased the tone and the amplitude of the phasic contractions in the cat gastric corpus, but completely inhibited phasic contractions of the guinea-pig corpus. Nifedipine (10^{-5} M) partially abolished CPA-induced tonic contraction of the cat and guinea-pig corpus and completely inhibited the phasic contractions of cat corpus. The resting part of the tone was completely suppressed by 10^{-3} M sodium nitroprusside (SNP). Lower traces: CPA increased the amplitude of the phasic contractions in cat antrum strips, but completely inhibited the phasic contractions of the guinea-pig antrum. The CPA-induced phasic contractions in cat antrum were completely suppressed by 10^{-5} M nifedipine.

The effects of CPA in the guinea-pig stomach were completely reversible after washout. However, the effects of CPA on the cat stomach were only partly reversible even after prolonged washout.

3.2. Time course of the CPA effect

CPA induced sustained tonic contractions of the cat and guinea-pig stomach as well as sustained inhibition of the phasic contractions of the guinea-pig stomach.

The effect of CPA (10^{-5} M) on the spontaneous phasic contractions of the cat gastric corpus and antrum was biphasic. An increase in the amplitude and frequency was observed 15–20 min after CPA administration. Fifty to sixty minutes later a new steady state was reached which was characterized by a further increase of the amplitude and a marked decrease of the frequency (not shown).

3.3. Influence of tetrodotoxin, atropine and L-NNA on the CPA effect

Tetrodotoxin and atropine were used to study the possible involvement of nervous structures in the effect of CPA.

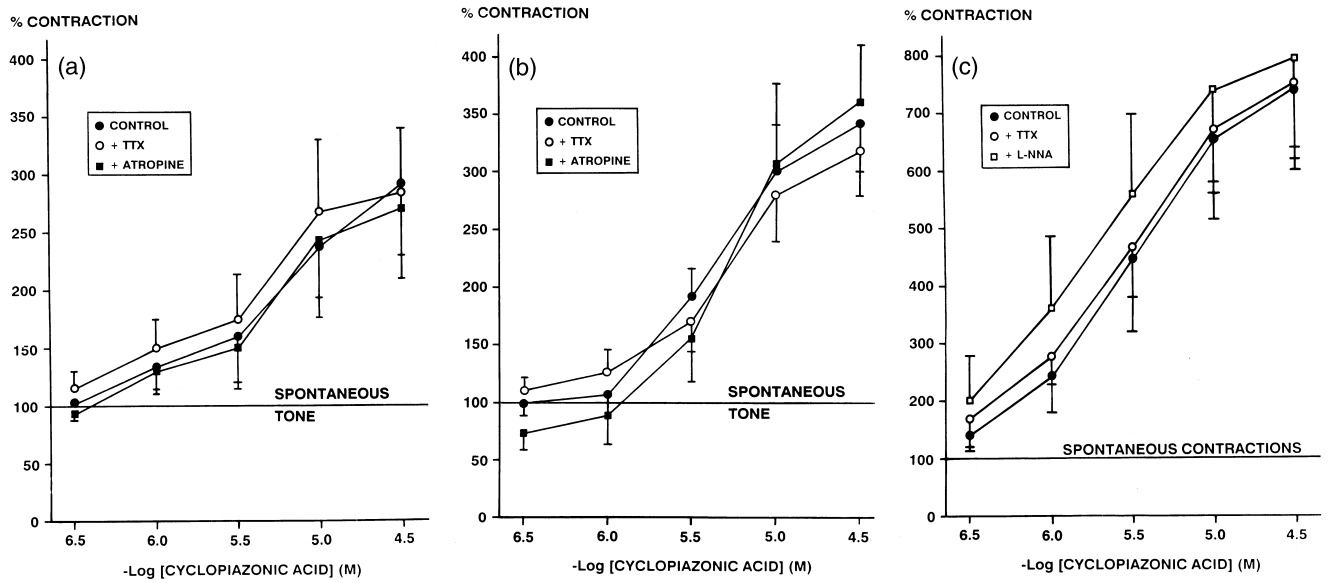

Fig. 2. Cumulative concentration-response curves for the contractile effect of CPA in gastric smooth muscle strips. Left panel: Cat gastric fundus. Control response, $n = 15$; in the presence of 10^{-6} M tetrodotoxin (TTX), $n = 14$, $P > 0.05$; in the presence of 10^{-6} M atropine, $n = 10$, $P > 0.05$. Middle panel: Guinea-pig gastric fundus. Control response, $n = 10$; in the presence of 10^{-6} M tetrodotoxin (TTX), $n = 8$, $P > 0.05$; in the presence of 10^{-6} M atropine, $n = 6$, $P > 0.05$. Right panel: Cat gastric antrum. Control response, $n = 10$; in the presence of 10^{-6} M tetrodotoxin (TTX), $n = 8$, $P > 0.05$; in the presence of 10^{-4} M L-NNA, $n = 6$, $P > 0.05$. Either the spontaneous tone or the amplitude of the spontaneous phasic contractions was taken to be 100%. Values are means ± S.E.M.

The concentration-response curves for the effect of CPA in the presence of 10^{-6} M tetrodotoxin or 10^{-6} M atropine did not differ significantly ($P > 0.05$) from the concentration-response curve obtained under control conditions (Fig. 2).

In another series of experiments, we used the NO synthase inhibitor, L-NNA, in order to find whether the excitatory responses to CPA could be affected by activation of NO synthesis, which might reflect an effect of the increased intracellular Ca^{2+} concentration ($[Ca^{2+}]_i$). In the

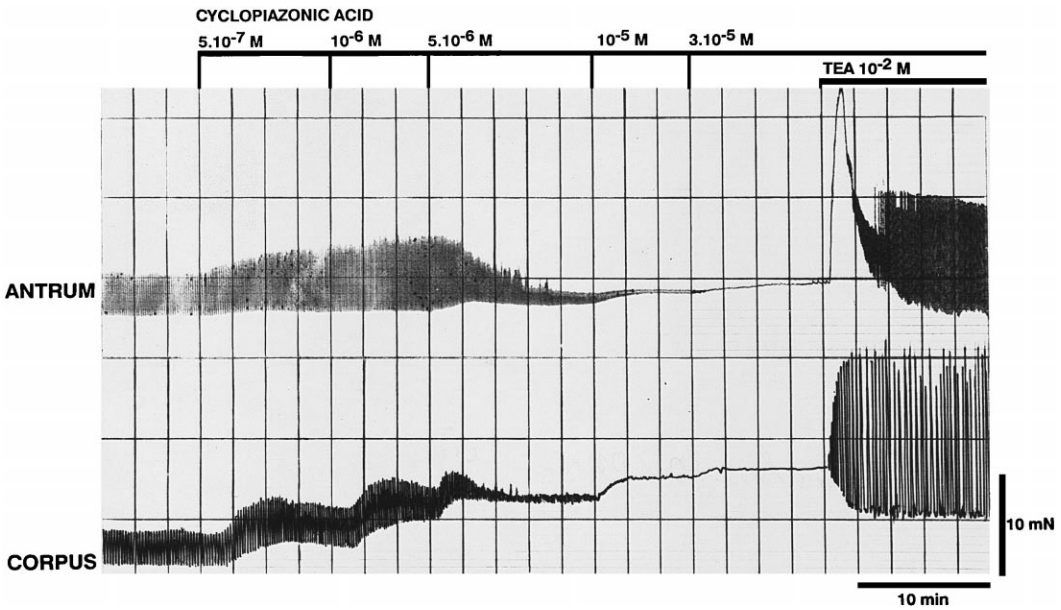

Fig. 3. Effect of cumulative application of CPA on contractile activity of guinea-pig gastric smooth muscle. Upper trace: The dual action of CPA in an antrum preparation. CPA (up to 10^{-6} M) increased the amplitude and frequency of the phasic contractions but in higher concentrations ($5 \cdot 10^{-6}$–$3 \cdot 10^{-5}$ M) decreased and completely suppressed the contractility. Lower trace: The effect of CPA in a corpus preparation. CPA concentration dependently increased the tone and, in concentrations higher than $5 \cdot 10^{-6}$ M, inhibited the phasic contractions. After complete inhibition of the phasic contractions in the antrum and corpus, 10^{-2} M tetraethylammonium (TEA) induced high-amplitude phasic contractions.

presence of 10^{-4} M L-NNA, the response to CPA was not significantly ($P > 0.05$) changed in the cat fundus (data not shown) and antrum (Fig. 2c) nor in the guinea-pig stomach (data not shown).

All this suggests that the effects of CPA are not mediated by the release of a neurotransmitter and that the effects should be considered myogenic ones. However, the possibility that endogenous substances not blocked by tetrodotoxin, atropine and L-NNA, could have affected the contractions after CPA should not be excluded.

3.4. Effects of nifedipine, sodium nitroprusside and SIN-1 on CPA-induced contractions

The involvement of L-type Ca^{2+} channels in the CPA-induced tonic and phasic contractions was investigated by using nifedipine, a selective inhibitor of these channels. It has been reported previously (Fusi et al., 1994; Petkov and Boev, 1996) that nifedipine at a supramaximal concentration of 10^{-5} M only partly suppresses the CPA-induced tonic contractions of fundus strips (Fig. 1). Nifedipine (10^{-5} M) partly suppressed the CPA-induced tone of the cat ($n = 6$) and guinea-pig ($n = 8$) corpus, but completely abolished the CPA-induced phasic contractions of the cat corpus ($n = 6$) and antrum ($n = 10$). In the presence of 10^{-5} M nifedipine CPA induced slow-developing, within 30–40 min, tonic contractions in the cat and guinea-pig fundus and corpus (an increase of up to 40–45% of the spontaneous tone, $n = 6$–8), but did not cause phasic contractions in the cat corpus and antrum.

Sodium nitroprusside (10^{-3} M) and SIN-1 (10^{-3} M) completely suppressed the CPA (10^{-5} M)-induced and spontaneous tone of the fundus and corpus. The same effect was observed in the presence of 10^{-5} M nifedipine (Fig. 1). Sodium nitroprusside (10^{-3} M) and SIN-1 (10^{-3} M) also completely suppressed the CPA (10^{-5} M)-induced phasic contractions of the cat corpus and antrum, even after a prolonged (3 h) preincubation of the muscle with CPA.

3.5. Effects of CPA on acetylcholine-induced contractions

Acetylcholine has been shown to induced sustained tonic contractions of the fundus, phasic contractions of the antrum, and both tonic and phasic contractions of the corpus (Boev et al., 1976). In fundus strips precontracted with a supramaximal concentration of acetylcholine (10^{-5} M), CPA (10^{-5} M) further potentiated the tone (by $45 \pm 9\%$, $n = 8$, $P < 0.01$) of the cat fundus and (by $38 \pm 11\%$, $n = 6$, $P < 0.05$) of the guinea-pig fundus. In guinea-pig antrum strips ($n = 6$), CPA (10^{-5} M) completely inhibited the acetylcholine (10^{-6} M)-induced phasic contractions. In guinea-pig corpus strips ($n = 9$), CPA (10^{-5} M) inhibited the acetylcholine (10^{-6} M)-induced phasic contractions and increased the tone (Fig. 4). After complete inhibition of the phasic contractions of the guinea-pig antrum ($n = 4$) and corpus ($n = 6$) by 10^{-5} M CPA, acetylcholine (10^{-6} M) failed to restore the phasic contractions, but induced a tonic contraction in the corpus preparations, which was atropine sensitive, i.e. it was completely abolished by 10^{-6} M atropine. In the presence of 10^{-5} M CPA, acetylcholine (10^{-6} M) was able to induce tonic contraction in cat ($n = 6$) and guinea-pig ($n = 6$) fundus.

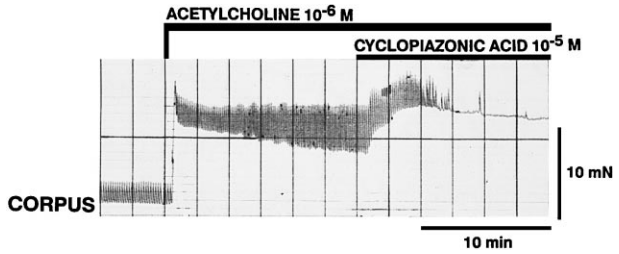

Fig. 4. The effect of CPA (10^{-5} M) on acetylcholine-induced contractions in a guinea-pig corpus preparation. CPA completely inhibited acetylcholine-induced phasic contractions but further increased the tone.

4. Discussion

The present study showed that CPA affected both the tone and the phasic contractions of the guinea-pig and the cat stomach. CPA increased the spontaneous tone of the guinea-pig and the cat gastric fundus and corpus as well as that of the human gastric fundus (Petkov, unpublished observations). CPA also increased the maximal contraction occurring after acetylcholine administration to fundus strips.

According to the 'superficial buffer barrier' hypothesis (Van Breemen et al., 1995), 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. This Ca^{2+} is extruded out of the cell via plasma membrane Ca^{2+} ATPase and Na^{+}/Ca^{2+} exchanger through a vectorial Ca^{2+} release from the sarcoplasmic reticulum toward the plasma membrane. Inhibition of the sarcoplasmic reticulum Ca^{2+} ATPase by CPA would interrupt this process, leading to an enhanced contractile response. Confirming this view are the observations that thapsigargin, another inhibitor of sarcoplasmic reticulum Ca^{2+}-ATPase, increases the tone of the guinea-pig (Duridanova et al., 1995) and the cat (Petkov and Boev, 1996) gastric fundus. CPA elevates $[Ca^{2+}]_i$ in smooth muscle (Uyama et al., 1993; Naganobu and Ito, 1994; Munro and Wendt, 1994) and induces tonic contractions of smooth muscle of the tonic type such as aorta (Deng and Kwan, 1991; Zheng et al., 1994; Gonzalez De La Fuente et al., 1995), urinary bladder (Munro and Wendt, 1994) and anococcygeus muscle (Gibson et al., 1994). CPA also affects the excitation-contraction coupling mechanism and causes depolarization in the phasic smooth muscle of the ureter (Maggi et al., 1995) and ileum (Uyama et al., 1993). 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. In ileal smooth muscle, acetylcholine induces a smaller Ca^{2+} release from the Ca^{2+} store in the presence of CPA than in the controls (Uyama et al., 1993). CPA interrupts the contraction of smooth muscles by preventing refilling of the Ca^{2+} store (Bourreau et al., 1993; Uyama et al., 1993). In antrum smooth muscles, methoxyverapamil (D600) and dihydropyridines inhibit spontaneous and acetylcholine-induced contractions (Boev et al., 1976; Ozaki et al., 1993). Unlike the CPA-induced tonic contractions of the fundus and corpus, the CPA-induced phasic contractions of the cat antrum and corpus depend exclusively on Ca^{2+} influx through L-type Ca^{2+} channels. However, the Ca^{2+} channel antagonists are unable to completely suppress the spontaneous tone of the cat (Boev et al., 1976) and guinea-pig (Duridanova et al., 1995) fundus. Nifedipine also fails to significantly affect CPA-induced tonic contractions in other smooth muscles of the tonic type (Gibson et al., 1994; Gonzalez De La Fuente et al., 1995).

There was an essential difference between the effects of CPA in cat and guinea-pig antrum: potentiation of the phasic contractions of the cat antrum and complete inhibition of the guinea-pig antrum. This may have been due to the different characteristics of sarcoplasmic reticulum Ca^{2+} stores and also of Ca^{2+} influx and Ca^{2+} efflux through the plasma membrane during stimulation (Uyama et al., 1993). The present data do not exclude the existence of a CPA-insensitive Ca^{2+} store in the cat antrum. The possibility of direct refilling of the Ca^{2+} store through an L-type Ca^{2+} channel from the extracellular space has been demonstrated in some types of smooth muscles (Bourreau et al., 1993; Gagov et al., 1994).

Ca^{2+}-induced Ca^{2+} release has been demonstrated in spike generating smooth muscles (Iino, 1989). In these smooth muscles the Ca^{2+}-induced Ca^{2+} release is triggered by Ca^{2+} entry during the spike action potential. According to Iino (1989), Ca^{2+}-induced Ca^{2+} release cannot play a primary role in triggering a physiological contraction, but is important as a modulating factor in the Ca^{2+}-activated K^+ channel opening. CPA enhances smooth muscle excitability (Uyama et al., 1993) and inhibits K^+ current without directly blocking the K^+ channel (Suzuki et al., 1992). Based on contractility studies, other authors (Omote and Mizusawa, 1994) suggest that, instead of inhibiting, CPA increases $[Ca^{2+}]_i$ and activates Ca^{2+}-activated K^+ channels. The differences in the effects of CPA in cat and guinea-pig antrum are very likely due to differences in the control over the membrane ion channels by the superficially located Ca^{2+} stores.

CPA does not affect the Ca^{2+} sensitivity of the contractile myofilaments in smooth muscles (Uyama et al., 1992; Gonzalez De La Fuente et al., 1995). It seems unlikely that CPA decreases Ca^{2+} sensitivity in guinea-pig antrum and corpus, since, after CPA treatment, tetraethylammonium restored the phasic contractions whose amplitude was greater than that of the spontaneous contractions. Moreover, CPA inhibited the spontaneous and acetylcholine-induced phasic contractions of the guinea-pig corpus but increased the tone, thus favouring the 'superficial buffer barrier' hypothesis.

The possibility that CPA causes the release of NO in the stomach should not be excluded, but the CPA-induced changes in the contractility of guinea-pig and cat gastric smooth muscles appear not to be mediated by NO. It is well known that sodium nitroprusside and SIN-1 metabolize to NO in the smooth muscle cells and activate soluble guanylate cyclase, increasing the level of guanosine 3′,5′-cyclic monophosphate (cyclic GMP), which in turn activates the cyclic GMP-dependent protein kinase (Kerwin and Heller, 1994). One of the substrates of the cyclic GMP-dependent protein kinase is phospholamban, and its phosphorylation stimulates the sarcoplasmic reticulum Ca^{2+}-ATPase activity and Ca^{2+} uptake (Raeymaekers et al., 1988). Sodium nitroprusside and SIN-1 completely inhibit the spontaneous electrical and contractile activities of guinea-pig and cat gastric smooth muscles (Boev et al., 1976; Petkov et al., 1994; Duridanova et al., 1995). These compounds completely antagonized the contractile effects of CPA. It has been reported that sodium nitroprusside and SIN-1 increase the outward K^+ current in esophageal smooth muscle and that CPA reduces this effect (Jury et al., 1996). Inhibition of sarcoplasmic reticulum Ca^{2+}-ATPase by thapsigargin in guinea-pig gastric fundus decreases the sodium nitroprusside- and cyclic GMP analogue-induced activation of Ca^{2+}-dependent K^+ current (Duridanova et al., 1995). All these findings suggest that the inhibitor of the sarcoplasmic reticulum Ca^{2+}-ATPase CPA and its activators, i.e. NO-releasing agents and cyclic GMP, interact competitively for the target, the sarcoplasmic reticulum Ca^{2+}-ATPase.

In conclusion, CPA by inhibiting sarcoplasmic reticulum Ca^{2+}-ATPase and preventing the refilling of sarcoplasmic reticulum Ca^{2+} stores modulates the contractile activity of the cat and guinea-pig gastric smooth muscle. The sarcoplasmic reticulum Ca^{2+}-ATPase and sarcoplasmic reticulum Ca^{2+} stores (i) contribute to spontaneous and agonist-evoked phasic and tonic contractions, and (ii) contribute differently to these processes in the different regions of the stomach in the same species, and in the identical tissue from different species.

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