Cyclic GMP-induced Activation of Potassium Currents by Sarcoplasmic Reticulum Ca^{2+}Pump-dependent Mechanism

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Abstract. In voltage-clamped single smooth muscle cells from the circular layer of the guinea-pig gastric fundus NO-liberating substance or an analogue of cyclic 3′,5′-guanosine monophosphate (cGMP) increased or decreased the outward K^+ current amplitudes depending on the Ca^{2+} buffering capacity of the intracellular medium. In a high EGTA-containing pipette solution dibutyryl-cGMP or sodium nitroprusside (SNP) attenuated both the fast and the late K^+ current components. In pipette solution with lower Ca^{2+}-buffering capacity these drugs caused a sustained increase of K^+ current amplitudes, which was effectively antagonized by thapsigargin, an inhibitor of Ca^{2+}-ATPase in the sarcoplasmic reticulum (SR). Our data suggest that, in gastric fundus smooth muscles, NO-liberating substances and cGMP analogues contribute to the activation of a Ca^{2+}-release mechanism from the cell bulk, i.e. the myoplasm surrounding the contractile filaments, towards the plasma membrane, crossing the SR Ca^{2+}-stores. Thus, a decreased intracellular free calcium concentration ($[Ca^{2+}]$) is coupled with an elevation of subplasmalemmal calcium, which in turn causes cell membrane hyperpolarization. The latter is a consequence of the opening of tetraethylammonium-sensitive Ca^{2+}-activated K^+ channels and leads to sustained smooth muscle relaxation, most characteristic for gastric fundus preparations.

Key words: Gastric fundus — Nitric oxide — Calcium stores — Sodium nitroprusside — Cyclic 3′,5′-guanosine monophosphate — Thapsigargin

Introduction

Smooth muscles of the gastric fundus spontaneously develop tonic contractions, consisting of two components: a verapamil – (I_{Ca}) – sensitive and a verapamil-insensitive (Boev et al. 1976). Direct electrical stimulation (sucrose-gap technique) partly reduces the tone, even under supramaximal hyperpolarizing currents, to the same level as do L-type Ca^{2+} channels blockers, while NO-liberating substances

such as sodium nitroprusside abolish the entire muscle tonus, i.e. both the potential (I_{Ca}) dependent and the potential independent components.

NO-liberating substances as well as different cGMP analogues, known to relax smooth muscle preparations, have been supposed to act via activation of K^+ currents in different gastrointestinal (Thornbury et al. 1991; Cayabyab and Daniel 1994) and vascular (Williams et al. 1988; Gurney 1994) smooth muscles. NO-dependent relaxation (Lefebvre et al. 1992) and hyperpolarization (Spassov, unpublished data) have been observed in guinea-pig gastric fundus as well. On the other hand, it is well known that NO-donors, such as sodium nitroprusside and 3-morpholino-syndonimine-hydrochloride (SIN-1) exert their relaxing action via activation of soluble guanylyl cyclase resulting in an increase of intracellular cGMP content (for review see Schmidt et al. 1993). The cGMP-dependent relaxation is associated with a lowering of free Ca^{2+} concentration in the surroundings of the contractile apparatus (Karaki et al. 1988). This hypothesis assumes cGMP-induced increase of Ca^{2+} uptake into sarcoplasmic reticulum (SR) Ca^{2+}-stores via activation of SR Ca^{2+}-ATPases (Raeymaekers et al. 1988; Cornwell et al. 1991).

The mechanism by which NO increases potassium currents and relaxes smooth muscle cells is still subject of discussion (Sanders and Ward 1992; Gurney 1994). There are two possible explanations of this phenomenon: 1) Direct phosphorylation of K^+ channels by the cGMP-dependent protein kinase, which results in their activation, and 2) cGMP-dependent protein kinase-induced phosphorylation of a target involved in the regulation of intracellular Ca^{2+} movements.

In 1989, van Breemen and Saida proposed a mechanism for superficial Ca^{2+} buffering in resting smooth muscle cells. According to their assumption, calcium is pumped from deep myoplasm into SR Ca^{2+} stores and is released preferentially towards the plasmalemma. This flux of Ca^{2+} ions directed to the plasma membrane has been termed *vectorial calcium release* (Chen and van Breemen 1992). The latter provides a negative feed-back regulation of plasma membrane excitability through activation of Ca^{2+}-sensitive K^+ channels, which activation leads to plasma membrane hyperpolarization. Stehno-Bittel and Sturek (1992) have strongly supported the above hypothesis showing that in some vascular smooth muscles the preloaded sarcoplasmic reticulum Ca^{2+} stores spontaneously release Ca^{2+} mainly towards the plasma membrane. Together with the data about the existence of a close contact between the plasmalemma and the membranes of the intracellular calcium stores (Somlyo et al. 1985; van Breemen and Saida 1989; Stehno-Bittel and Sturek 1992; Missiaen et al. 1992), this data suggests that in some smooth muscle cells a sustained subplasmalemmal increase in Ca^{2+} concentration ($[Ca^{2+}]$) may be directly coupled with the Ca^{2+} release from submembrane Ca^{2+} stores.

The aim of the present study was to clarify the mechanism of NO- and cGMP-induced increase of K^+ current amplitudes in single smooth muscle cells isolated from the circular layer of the guinea pig gastric fundus.

Materials and Methods

Organ bath smooth muscle preparations for isometric recording of tension

Smooth muscle strips, 15 mm in length, dissected from the circular layer of the guinea-pig gastric fundus, were mounted in organ baths of 10 cm^3 volume, filled with modified Krebs solution, containing (in mmol/l): Na^+ 137.5; K^+ 6; Mg^{2+} 1.2; Ca^{2+} 2.5; Cl^- 124.6; $H_2PO_4^-$ 1.2; HCO_3^{2-} 25; glucose 11; bubbled with 95% O_2 and 5% CO_2 to achieve pH of 7.2 at 37 °C. The strips were stretched under a tension of 10 mN in calcium free Krebs' solution to estimate the zero level tone. After 20 min the bath solution was changed to a Ca^{2+}-containing Krebs' solution and the strips were left to equilibrate for 60–90 min until a stable spontaneous muscle tone developed.

Whole cell patch-clamp experiments

Experiments were performed on single smooth muscle cells, freshly isolated from the circular layer of the guinea-pig gastric fundus. The animals weighed 300–400 g. Isolated muscle strips were placed in physiological salt solution (PSS) (see below) and cut into small pieces. The pieces were then transferred into Ca^{2+}-free PSS, prewarmed to 37 °C and containing 1 g/l collagenase (type 1A, SIGMA), 0.5 g/l soybean trypsin inhibitor and 1.5 g/l bovine serum albumin. After 55–70 min of incubation at 37 °C the enzyme was carefully washed from the pieces with 20 ml prewarmed Ca^{2+}-free PSS. Single smooth muscle cells were then obtained by gentle agitation of the pieces with two Pasteur pipettes with different tip openings in 1 ml fresh modified "KB" solution (Isenberg and Klockner 1982) until the solution became cloudy. Cells were stored up to 12 h at 6 °C in this solution. Only fully relaxed cells were used. Cells which reduced their length by more than 35% or did not contract at all after the application of 10^{-7} mol/l acetylcholine were discarded, as well as those that did not relax after the acetylcholine-induced contraction. PSS and drugs diluted in it were perfused continuously to the recording chamber with the cells adhering to the glass bottom.

The whole-cell mode of the patch-clamp technique was employed. The patch electrodes were from borosilicate glass (Jencons) and, when filled with the internal solution, had resistances of approx. 2.5 MΩ. Membrane currents were recorded by an EPC-7 (List Electronics) amplifier. Current signals were recorded and further analyzed on an AT 286 PC through a TL-1 DMA (AXOPATCH) interface, and Square Wave Cell Tester, version 1.0, (George Shkodrov, Institute of Biophysics, Bulgarian Academy of Sciences), software.

Comparison of absolute values of K^+ current amplitudes measured in different cells is not very appropriate as there were considerable difference in the capacitive and also the visible membrane surface between single cells isolated from the circular layer of the gastric fundus smooth muscle. Therefore, we found it necessary to estimate the values of the current densities, expressed as $\iota_K = \mu A/cm^2$ of membrane surface (assuming a specific membrane capacitance of 1 $\mu F/cm^2$, see Hamill et al. 1981) and to plot it versus the potential applied in order to obtain comparable data for statistical analysis. In all experiments the holding potential (V_h) was −50 mV.

The physiological salt solution (PSS) used in the experiments was of the following composition (in mmol/l): 110 NaCl; 5.6 KCl; 10 HEPES; 20 taurine; 20 glucose; 1.2 $MgCl_2$; 1.8 $CaCl_2$; 5 Na-pyruvate; pH 7.4. The medium used for cell isolation consisted of 85 KCl; 30 KH_2PO_4; 5 $MgCl_2$; 20 taurine; 5 Na_2-ATP; 5 Na-pyruvate; 5 creatine; 5 oxalacetate; 1 g/l bovine serum albumin (pH 7.2). Internal solution I in the recording pipette contained: 105 KCl; 5 HEPES; 11 EGTA; 1 $CaCl_2$; 2 $MgCl_2$; 5 Na-pyruvate;

5 succinic acid; 5 oxalacetic acid; 1.5 Na_2-ATP; pH 7.2; pCa 8.3. Internal solution II contained: 122 KCl; 5 HEPES; 4 EGTA; 0.5 $CaCl_2$; 0.5 $MgCl_2$; 5 Na-pyruvate; 5 succinic acid; 5 oxalacetic acid; 1.5 Na_2-ATP; pH 7.2; pCa 7.4. The values of the intracellular solutions pCa were estimated according to a modified version of the program of Fabiato and Fabiato (1979). Cyclopiazonic acid, tetrodotoxin (citrate free), charybdotoxin, iberiotoxin, apamin, 4-aminopyridine, dibutyryl cyclic 3′5′-guanosine monophosphate (dibutyryl-cGMP), thapsigargin (products of SIGMA Chemical Co.), and tetraethylammonium, sodium nitroprusside (SNP) (products of SERVA), were added to the bath solution. All experiments were carried out at 31 ± 2 °C.

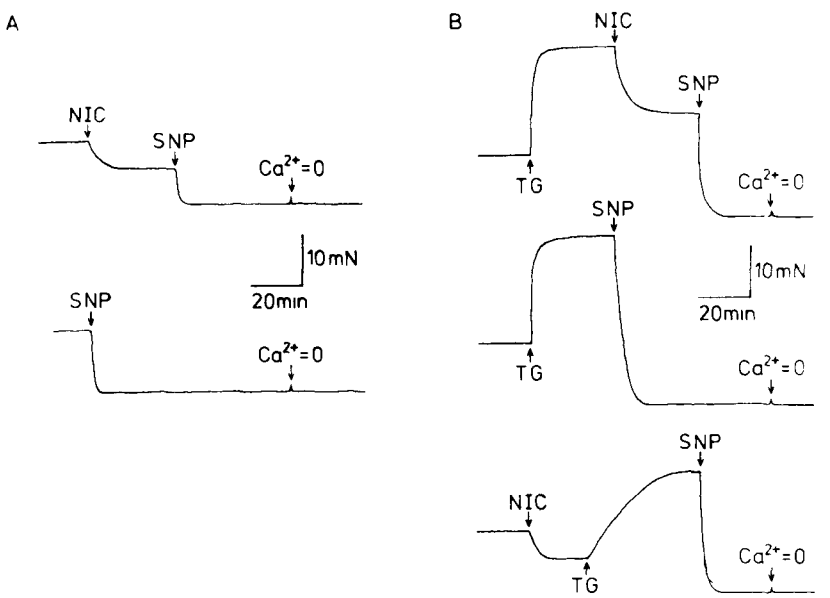

Figure 1. Original recordings of changes in tension of smooth muscle preparations from the circular layer of the guinea-pig gastric fundus. (*A*) Upper trace: $5 \cdot 10^{-5}$ mol/l nicardipine suppressed partly the spontaneous tone, and 10^{-6} mol/l sodium nitroprusside (SNP) led to a further decrease to the level of tension obtained in Ca^{2+} free solution (modified Krebs solution). Lower trace: total inhibition of spontaneous tonus (i.e. of the nicardipine-sensitive and insensitive components) by 10^{-6} mol/l SNP. (*B*) Thapsigargin-induced increase of spontaneous tonus. Upper trace: effect of 10^{-5} mol/l nicardipine. The rest of the tension was entirely inhibited by 10^{-5} mol/l SNP to the level of tension obtained in Ca^{2+} free solution (zero level). Middle trace: 10^{-5} mol/l SNP abolished both the spontaneous and the thapsigargin-induced tension. Lower trace: 10^{-6} mol/l thapsigargin evoked tonic contraction in the presence of $5 \cdot 10^{-5}$ mol/l nicardipine. Thapsigargin-induced contraction was also inhibited by 10^{-5} mol/l SNP to zero Ca^{2+} level. Tension and time scales apply to all traces. Experiments were performed in the presence of 10^{-6} mol/l tetrodotoxin in the organ bath.

Results

The L-type Ca^{2+} channel antagonist nicardipine partly decreased the spontaneous tone of smooth muscle strips even at $5 \cdot 10^{-5}$ mol/l, as shown in Fig. 1*A*, upper trace. Sodium nitroprusside (SNP), 10^{-6} mol/l, inhibited the rest of the tonus to zero (calcium free) level. SNP (10^{-6} mol/l) (Fig. 1*A*, lower trace) was able to entirely inhibit the spontaneous muscle tone of the gastric fundus strips. Our results showed that thapsigargin in a concentration range $10^{-8} - 10^{-6}$ mol/l increased the muscle tension to 271.7% ± 23.4% ($n = 8$) as related to the spontaneous tone (not shown). As illustrated in Fig. 1*B* (upper trace) thapsigargin-induced contraction was partly reduced by $5 \cdot 10^{-5}$ mol/l nicardipine and subsequently entirely inhibited by 10^{-5} mol/l SNP; thus thapsigargin-induced tonic contraction consisted of two components, one nicardipine-sensitive and an other nicardipine-resistant. On the other hand, thapsigargin itself (10^{-6} mol/l) elicited a nicardipine-resistant tonic contraction in $5 \cdot 10^{-5}$ mol/l nicardipine-containing bath solution.

Table 1. Current density, measured at pCa 8.3 in the pipette solution

V_M	$i_K = \mu A/cm^2$	Control	Nic[1]	Nic + SNP[2]	Nic + SNP + Thap[3]
−40 mV	i_{max}[4]	0.6 ± 0.1	0.6 ± 0.1	0.3 ± 0.05	0.3 ± 0.05
	i_{end}[5]	0.6 ± 0.1	0.6 ± 0.1	0.3 ± 0.05	0.3 ± 0.05
−20 mV	i_{max}	1.3 ± 0.2	0.2 ± 0.15	0.8 ± 0.15	0.7 ± 0.15
	i_{end}	1.2 ± 0.2	1.0 ± 0.15	0.5 ± 0.10	0.5 ± 0.10
0 mV	i_{max}	3.5 ± 0.3	3.3 ± 0.3	2.2 ± 0.25	2.3 ± 0.25
	i_{end}	3.0 ± 0.3	3.0 ± 0.3	1.6 ± 0.20	1.5 ± 0.20
+20 mV	i_{max}	6.6 ± 0.5	6.6 ± 0.5	3.6 ± 0.30	3.5 ± 0.30
	i_{end}	4.8 ± 0.4	5.0 ± 0.4	2.8 ± 0.30	3.0 ± 0.30
+40 mV	i_{max}	10.4 ± 0.6	10.0 ± 0.6	5.7 ± 0.40	5.5 ± 0.40
	i_{end}	8.0 ± 0.5	7.3 ± 0.5	4.3 ± 0.35	4.0 ± 0.35

[1] Nic – Nicardipine $3 \cdot 10^{-6}$ mol/l, currents were measured 6 min after the addition of the drug.
[2] Nic + SNP – sodium nitroprusside (10^{-6} mol/l) was added to the nicardipine-containing bath solution. Currents were measured 6 min after the addition of SNP.
[3] Nic + SNP + Thap – thapsigargin (10^{-6} mol/l) was added into the bath solution on the background of $3 \cdot 10^{-6}$ mol/l nicardipine and 10^{-6} mol/l sodium nitroprusside. Currents were measured 6 min after the addition of thapsigargin.
[4] i_{max} – current density estimated by the peak current amplitudes.
[5] i_{min} – current density estimated by the current amplitudes, measured at the end of the test pulse (400^{th} ms from the pulse onset).
Data are means ± S.E.M. for 8 cells

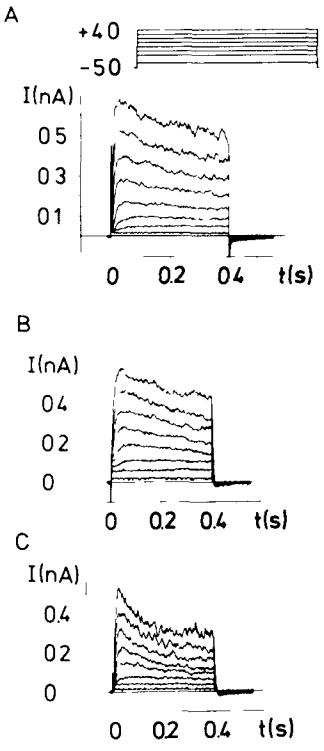

Figure 2. Typical depolarization-evoked current responses of cell, dialysed with pipette solution I (pCa 8.3) and measured 6 min after the addition of $3 \cdot 10^{-6}$ mol/l nicardipine to PSS (A), or of a cell, dialysed with pipette solution II (pCa 7.4) without addition of nicardipine to the bath (*B*). (*C*) Depolarization-evoked current responses of the same cell as in (*B*) after 20 min exposure to nicardipine-containing ($3 \cdot 10^{-6}$ mol/l) PSS. The standard depolarizing pulses protocol is shown in the right upper corner. $V_h = -50$ mV.

These results indicate that in the gastric fundus smooth muscle SNP inhibited both nicardipine-sensitive and nicardipine-resistant tonic contraction in control bath solution and in the presence of thapsigargin.

Dependence of NO- and cGMP-induced changes in I_K amplitudes on intracellular calcium concentration

Table 1 gives the voltage dependence of the K^+ current densities, estimated by the net current amplitudes obtained from cells dialysed with high EGTA-containing internal solution (internal solution I, pCa 8.3). Under these conditions the blockade of L-type Ca^{2+} channels with nicardipine ($3 \cdot 10^{-6}$ mol/l), i.e. the entire I_{Ca} in guinea-pig gastric fundus (Lammel et al. 1991), failed to affect the K^+ currents (Table 1, see also Fig. 2*A*), even after 20 min exposure of the cells to $3 \cdot 10^{-6}$ mol/l nicardipine. Subsequent addition of 10^{-6} mol/l SNP or 10^{-6} mol/l dibutyryl-cGMP reduced the amplitudes of both K^+ current components (Table 1).

With the less EGTA-containing pipette solution (internal solution II, pCa 7.4) the maximum amplitudes and the kinetics of K^+ current waveforms (Fig. 2*B*) were very similar to those obtained with high-EGTA containing internal solution. The prolonged exposure to nicardipine (30 min) could not affect significantly the

Table 2. Current density, measured at pCa 7.4 in the pipette solution

V_M	$i_K = \mu A/cm^2$	Control	Nic[1]	Nic + SNP[2]	Nic + SNP + Thap[3]
−40 mV	i_{max}[4]	0.5 ± 0.1	0.5 ± 0.1	3.0 ± 0.2	3.5 ± 0.3
	i_{end}[5]	0.5 ± 0.1	0.5 ± 0.1	3.0 ± 0.2	3.5 ± 0.3
−20 mV	i_{max}	1.4 ± 0.2	1.1 ± 0.15	12.6 ± 0.65	13.0 ± 0.7
	i_{end}	1.0 ± 0.2	0.8 ± 0.15	12.6 ± 0.65	13.0 ± 0.7
0 mV	i_{max}	3.7 ± 0.3	3.2 ± 0.3	25.0 ± 1.7	28.0 ± 1.8
	i_{end}	2.2 ± 0.3	2.2 ± 0.2	24.0 ± 1.7	28.0 ± 1.8
+20 mV	i_{max}	6.6 ± 0.4	6.0 ± 0.4	46.0 ± 2.4	48.0 ± 3.0
	i_{end}	5.3 ± 0.3	4.8 ± 0.3	44.0 ± 2.4	47.0 ± 3.0
+40 mV	i_{max}	10.5 ± 0.6	10.0 ± 0.5	62.0 ± 3.8	65.0 ± 4.0
	i_{end}	8.5 ± 0.5	6.3 ± 0.4	60.0 ± 3.8	65.0 ± 1.0

[1] Nic – Nicardipine ($3 \cdot 10^{-6}$ mol/l), currents were measured 6 min after the addition of the drug.
[2] Nic + SNP – sodium nitroprusside (10^{-6} mol/l) was added to the nicardipine-containing bath solution. Currents were measured 6 min after the addition of SNP.
[3] Nic + SNP + Thap – thapsigargin (10^{-6} mol/l) was added into the bath solution on the background of $3 \cdot 10^{-6}$ mol/l nicardipine and 10^{-6} mol/l sodium nitroprusside. Currents were measured 6 min after the addition of thapsigargin.
[4] i_{max} - current density estimated by the peak current amplitudes.
[5] i_{end} – current density estimated by the current amplitudes, measured at the end of the test pulse (400th ms from the pulse onset). Data are means ± S.E.M. of 7 cells.

peak amplitudes of K^+ current, which were found to depend mainly on an initial component with a fast kinetics. However, the stationary amplitudes of the slowly developing component (measured at 400 ms from the pulse onset) decreased after 20 min nicardipine treatment (Fig. 2*C*). Preliminary studies had shown that 400 ms lasting test pulses were sufficient to allow the slow component to reach its peak amplitude. Thus, the 400 ms lasting stimuli were further applied in this study in order to minimize the risk of cell damage due to sustained depolarization.

Further measurements under nicardipine were carried out after an 8 min exposure to "pure" PSS to load the sarcoplasmic reticulum Ca^{2+} stores (see Stehno-Bittel and Sturek 1992). Table 2 shows the voltage dependence of the current densities, estimated by the net K^+ currents expressed in cells dialysed with internal solution II (pCa 7.4). Under these conditions application into the bath of 10^{-6} mol/l SNP (Table 2), or 10^{-7} to 10^{-6} mol/l dibutyryl-cGMP at the background of $3 \cdot 10^{-6}$ mol/l nicardipine, resulted in an increase in amplitudes of the late K^+ cur-

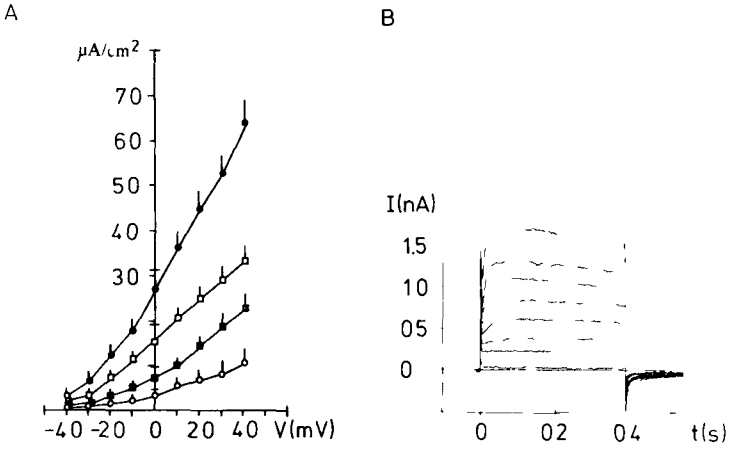

Figure 3. Effect of dibutyryl-cGMP on depolarization-evoked K^+ currents, obtained in $5 \cdot 10^{-6}$ mol/l nicardipine-containing PSS and pipette solution II (pCa 7.4). V_h = −50 mV. Dibutyryl-cGMP caused an increase of K^+ current amplitudes in a time- and concentration-dependent manner. (*A*) Potential dependence of current density, estimated by the peak K^+ current amplitudes (open circles) as compared to those obtained 5 min (closed squares) and 12 min (open squares) after the application of 10^{-7} mol/l dibutyryl-cGMP into the bath. Subsequent addition of dibutyryl-cGMP to a final concentration of 10^{-6} mol/l led to a further increase of K^+ current amplitudes, when measured 6 min later (closed circles). Data are means ± S.E.M. for 7 cells. (*B*) Typical K^+ current waveforms activated by depolarization pulses after 12 min of exposure to 10^{-7} mol/l dibutyryl-cGMP experimental solution. The same voltage protocol as in Fig. 2.

rent component Fig. 3*B*, which "covered" the fast-activating one in 1–3 min (not shown). The amplitudes of the net K^+ current continued to increase depending on the concentrations and on the time of exposure to dibutyryl-cGMP (Fig. 3*A*) or SNP (not shown).

According to these results the effects of cyclic GMP analogue and NO-liberating substance on K^+ currents depend on the intracellular Ca^{2+} concentration.

Does vectorial Ca^{2+} release exist in guinea-pig gastric smooth muscle cells?

The possibility for participation of sarcoplasmic reticulum (SR) Ca^{2+} pumps in the observed NO- and dibutyryl-cGMP-induced activation of K^+ currents was investigated. All experiments were performed under pCa 7.4 in the pipette solution (i.e. with pipette solution II).

In control bath solution, i.e. external solution without nicardipine, the SR Ca^{2+} pump inhibitor thapsigargin (Thastrup 1990) induced a significant increase of K^+ current amplitudes (Fig. 4*A*) depending on the number of test protocols, i.e. on the quantity of Ca^{2+} which entered the cell during the depolarization pulses.

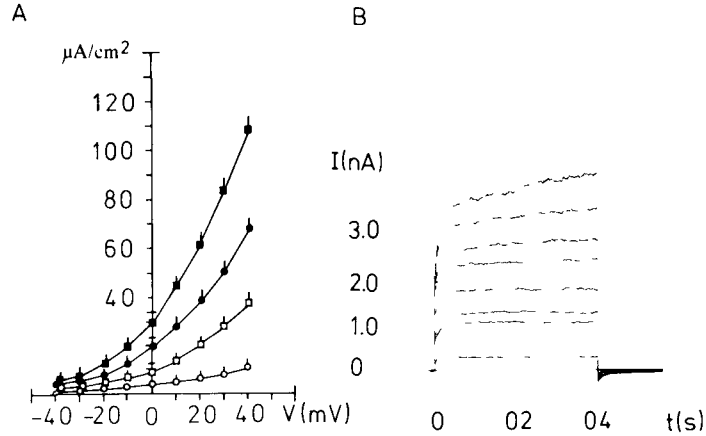

Figure 4. Time dependence of thapsigargin-evoked increase of K^+ currents. $V_h = -50$ mV. PSS outside and pipette solution II (pCa 7.4). (*A*) Voltage dependence of the current density estimated by the peak K^+ current amplitudes in PSS (open circles) and after addition of 10^{-6} mol/l thapsigargin to it. K^+ currents increased at 6 min, second test protocol (open squares), 9 min, third test protocol (closed circles), and 15 min, fifth test protocol (closed squares) after thapsigargin application. Data are means ± S.E.M. for 4 cells. (*B*) Depolarization-evoked K^+ current waveforms obtained after 9 min of exposure to 10^{-6} mol/l thapsigargin-containing PSS: K^+ current amplitudes continued to increase, and this was observable even during the pulse.

In the presence of thapsigargin K^+ current amplitudes continued to rise during the test pulses (Fig. 4*B*). Thapsigargin added to pure PSS enhanced the Ca^{2+}-activated K^+ current since Ca^{2+} stores located in the subplasmalemma cannot take up the entering calcium. However, in the presence of nicardipine thapsigargin failed to affect the amplitudes of K^+ currents (not shown). Subsequent addition of dibutyryl-cGMP or SNP had no significant effect on K^+ currents. Dibutyryl-cGMP in submicromolar concentrations remained ineffective, but 10^{-6} mol/l caused a slight increase of K^+ currents (Fig. 5*A*), which did not inactivate during the 400 ms lasting pulses (Fig. 5*B*). Thus, the application of the SR Ca^{2+} pumps blocking drug effectively abolished the cGMP- and NO-induced K^+ current activation in the presence of nicardipine, i.e. in the absence of Ca^{2+} influx.

The addition of selective K^+ channel blockers, charybdotoxin and iberiotoxin up to 10^{-5} mol/l, apamin (up to $5 \cdot 10^{-6}$ mol/l), glibenclamide (up to $5 \cdot 10^{-5}$ mol/l) and 4-aminopyridine (up to 1 mmol/l) were without any significant effect on K^+ current components: the fast inactivating and the sustained ones (not shown). However, tetraethylammonium (20 mmol/l) inhibited both these compo-

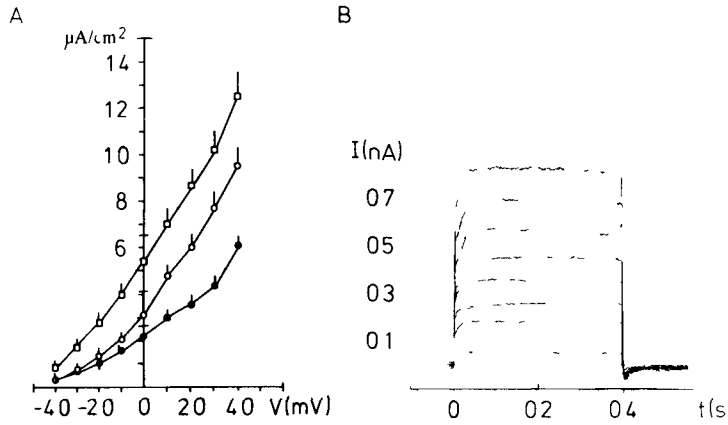

Figure 5. Effect of dibutyryl-cGMP on depolarization-evoked K^+ currents in $3 \cdot 10^{-6}$ mol/l nicardipine-containing PSS. Pipette solution II (pCa 7.4). $V_h = -50$ mV. (*A*) Voltage dependence of the current density estimated by the peak (open circles) and late (closed circles) K^+ current amplitudes as compared to the peak (open squares) and late (closed squares) K^+ currents obtained 12 min after the application of 10^{-6} mol/l thapsigargin into the bath. Under these conditions, 10^{-6} mol/l dibutyryl-cGMP caused significant increase of K^+ currents in 6 min (squares). Data are ± S.E.M. for 8 cells. (*B*) Depolarization-evoked K^+ current in a cell exposed to thapsigargin- and nicardipine-containing PSS as measured 6 min after the application of 10^{-6} mol/l dibutyryl-cGMP into the bath.

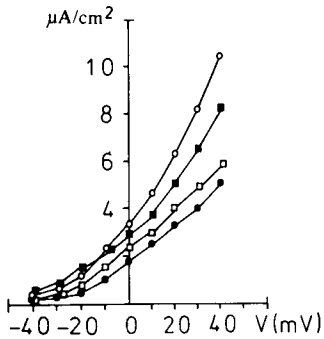

Figure 6. Effects of 20 mmol/l tetraethylammonium and 10^{-6} mol/l dibutyryl-cGMP on the whole-cell K^+ current, measured in $3 \cdot 10^{-6}$ mol/l nicardipine-containing PSS. Pipette solution II (pCa 7.4). $V_h = -50$ mV. Results are expressed as current densities of K^+ current activated by different voltage steps applied to the cells exposed to nicardipine-containing PSS (open circles), at 8 min of exposure to 20 mmol/l tetraethylammonium (closed circles), at the 8 min after subsequent addition of 10^{-7} mol/l dibutyryl-cGMP (open squares) or 10^{-6} mol/l dibutyryl-cGMP (closed squares). Error bars were omitted for clarity. Data are ± S.E.M. for 6 cells.

nents (Fig. 6). Subsequent application of dibutyryl-cGMP, even at 10^{-6} mol/l, could not restore the K^+ current amplitudes, and caused only a slight increase of the late I_K component.

Discussion

Under our experimental conditions the effects of cGMP-dependent protein kinase activators dibutyryl-cGMP and SNP (Schmidt et al. 1993) on K^+ currents were found to depend on the Ca^{2+} buffering capacity of pipette solutions used. With the high EGTA-containing pipette solution dibutyryl-cGMP and SNP produced a slight decrease in the amplitudes of both current components of K^+. With the pipette solution with the lower Ca^{2+}-buffering capacity the addition of cGMP-dependent protein kinase activators led to a sustained K^+ current increase, which could effectively be antagonized by pretreatment with a SR Ca^{2+}-ATPases blocker. As the experiments were performed in the presence of nicardipine used to stop Ca^{2+} entry (Lammel et al. 1991), the only source of second messenger Ca^{2+} for activation of Ca^{2+}-activated K^+ currents could be the SR Ca^{2+} stores. The observed effects of thapsigargin suggest that the dynamics of Ca^{2+} release from the stores is controlled by the activity of SR Ca^{2+}-ATPase, known as a conventional and specific target of cGMP-dependent protein kinase (Raeymaekers et al. 1988; Cornwell et al. 1991).

Having reached the subplasmalemmal space, Ca^{2+} can increase the open probability of Ca^{2+}-activated K^+-channels (McManus 1991), which in turn results in a shift of the cell membrane potential towards K^+ equilibrium, i.e. hyperpolarization of the cell membrane. The effectiveness and duration of thus released second messenger Ca^{2+} as an activator of K^+ conductivity depend on Ca^{2+} efflux capacity of the plasma membrane. This means that if the quantity of released Ca^{2+} exceeds the Ca^{2+} extrusion capacity, its concentration in the vicinity of K^+ channels will remain high over long periods of time. During this time Ca^{2+} influx is inhibited due to the plasma membrane hyperpolarization and Ca^{2+}-dependent inactivation of L-type Ca^{2+} channels (Yamamoto et al. 1989; Vogalis et al. 1992). Thus, the subplasmalemmal Ca^{2+}-enriched intracellular space acts as a superficial buffer barrier to Ca^{2+} entrance (see van Breemen and Saida 1989; Chen and van Breemen 1992).

In conclusion, the data obtained provide evidence that in some smooth muscles cells the activators of cGMP-dependent protein kinase can trigger a vectorial Ca^{2+} release in a direction opposite to that of the depolarization-evoked Ca^{2+} entry. This process results in a more or less sustained elevation of subplasmalemmal $[Ca^{2+}]$ and activation of Ca^{2+}-sensitive K^+ currents, and can be effectively antagonized by sarcoplasmic reticulum Ca^{2+}-ATPases blocking drugs. Thus, the relative decrease of free $[Ca^{2+}]$ near the contractile filaments seems to be coupled with a simultaneous elevation of $[Ca^{2+}]$ in the subplasmalemmal space. The later results in plasma membrane hyperpolarization due to the opening of tetraethylammonium-blockable Ca^{2+}-activated K^+-channels (related to nicardipine-sensitive tonus), while the former suppresses the voltage- (i.e. nicardipine) insensitive component of the tonic contraction. Acting together both these mechanisms may provide a sustained relaxation of the gastric fundus.

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