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PROPERTIES OF CALCIUM CHANNELS IN GUINEA-PIG GASTRIC MYOCYTES

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SUMMARY

1. The inward membrane current in enzymatically dispersed guinea-pig gastric myocytes was studied using whole-cell voltage clamp technique.

2. Only one inward membrane current was found in gastric myocytes which was identified as the Ca^{2+} current based on its inhibition by Ni^{2+}, Cd^{2+} and Co^{2+}, its dependence on $[Ca^{2+}]_o$, and its insensitivity to variations of $[Na^+]_o$.

3. Ca^{2+} current activated at -20 mV, peaked around $+10$ mV and was markedly enhanced when the holding potential was increased from -40 to -90 mV. The enhancement of I_{Ca} at negative holding potentials did not alter the activation threshold of I_{Ca}. When Ba^{2+} was substituted for Ca^{2+}, I_{Ba} was similarly enhanced at more negative potentials.

4. In cells where internal Ca^{2+} was buffered with 10 mM-EGTA, the time course of inactivation was fitted with two exponentials, with time constants: $\tau_f = 53{\cdot}4 \pm 18{\cdot}1$ ms and $\tau_s = 175{\cdot}2 \pm 46{\cdot}1$ ms. When Ba^{2+} was the charge carrier through the channel, the time course of inactivation could be fitted often by only one exponential which approximated τ_s for inactivation of I_{Ca}. The voltage dependence of steady-state inactivation of Ca^{2+} channels was not significantly altered when Ba^{2+} was the charge carrier.

5. Using different buffering systems (EGTA, EDTA and citrate), we found that citrate maintained the I_{Ca} and slowed inactivation more effectively than the other buffers tested. Because the calculated change in $[Ca^{2+}]_i$ did not differ significantly between buffer systems, we speculate that suppression of inactivation by citrate is related to increased accessability of the buffer to cytoplasmic Ca^{2+} near the Ca^{2+} channel. Changes in $[Mg^{2+}]_i$ affected peak I_{Ca} but not the kinetics of inactivation indicating that $[Mg^{2+}]_i$ may regulate the steady-state inactivation or the availability of the Ca^{2+} channels.

6. The divalent selectivity of the Ca^{2+} channel had the following sequence: $Ba^{2+} > Ca^{2+} \geqslant Sr^{2+} \ggg Mg^{2+}$. In very low extracellular Ca^{2+} ($< 10^{-7}$ M), the Ca^{2+} channel conducted Na^+.

7. Increasing $[H^+]_o$ appeared to differentially affect peak and maintained components of I_{Ca}. At pH $< 6{\cdot}5$, the maintained component of I_{Ca} was suppressed more than the peak component indicating possible time- and voltage-dependent inhibition of I_{Ca} by protons.

8. Nifedipine, D600 and diltiazem inhibited I_{Ca} in a voltage-dependent manner. The order of potency for inhibition of peak I_{Ca} was nifedipine $\approx$ D600 $\gg$ diltiazem. Diltiazem, unlike the other two drugs, enhanced the inactivation of I_{Ca}, even when peak I_{Ca}, was not affected suggesting either a time- and voltage-dependent block of the open channel or possible existence of more than one population of Ca^{2+} channels with different drug sensitivities.

9. At much lower concentration (100 nM) diltiazem enhanced the peak but not the maintained component of the Ca^{2+} current. A similar agonistic effect was also recorded for D600.

10. The Ca^{2+} channel in the guinea-pig gastric myocyte is similar to Ca^{2+} channels of other tissues in its activation, ionic selectivity and inactivation. The inactivation of the Ca^{2+} channel is dependent both on voltage and $[Ca^{2+}]_i$. Although some of our data support the possible presence of more than one population of Ca^{2+} channels, we could provide definitive proof for only one channel type.

INTRODUCTION

Calcium channels are ubiquitous, having been described in most excitable tissues (Hagiwara & Byerly, 1981; Tsien, 1983). Variations in calcium channel function and characteristics may, however, depend on the specific cell type. These variations may be described by differences in activation and inactivation kinetics, the ionic selectivity, and the pharmacological sensitivity of the calcium channel.

Application of the patch clamp technique to gastrointestinal smooth muscle cells has provided evidence for the presence of Ca^{2+} channels in rabbit jejunum and ileum, guinea-pig ileum and taenia caeci, and guinea-pig and toad stomach (Walsh & Singer, 1981; Benham, Bolton & Lang, 1984; Mitra & Morad, 1985*a*, *b*; Droogmans & Callewaert, 1986; Ganitkevich, Shuba & Smirnov, 1986; Ohya, Terada, Kitamura & Kuriyama, 1986). Some studies show the voltage-dependent calcium channel to be similar in its characteristics to those of other tissues in that they also conduct Ba^{2+} and are blocked by Mn^{2+}, Co^{2+} and Cd^{2+}. However, other properties of the calcium channel appear to vary in different visceral myocytes. Peak activation of calcium channel current, for instance, has been shown to occur between -20 and 0 mV in guinea-pig ileum (Droogmans & Callewaert, 1986) but at more positive potentials in rabbit ileum (Ohya *et al.* 1986), guinea-pig taenia caeci (Ganitkevich *et al.* 1986), and toad stomach (Walsh & Singer, 1981).

Inactivation kinetics of calcium current have also been shown to be much longer in cells for taenia coli (Ganitkevich *et al.* 1986) when compared to rabbit ileum (Ohya *et al.* 1986) or toad stomach (Walsh & Singer, 1981). The mechanism of inactivation of the Ca^{2+} channel has also been suggested to be different in various tissues. In guinea-pig taenia coli, calcium-induced inactivation appears to be predominant (Ganitkevich, Shuba & Smirnov, 1987), whereas in toad stomach, voltage-dependent inactivation seems to be more important (Walsh & Singer, 1981, 1987). In some studies, two time constants have been shown to describe the inactivation of I_{Ca} but these constants may differ anywhere from tens to hundreds of milliseconds, depending on the myocyte studied (Droogmans & Callewaert, 1986; Ganitkevich *et al.* 1986; Ohya *et al.* 1986). Although substitution of Ba^{2+} for Ca^{2+} changes

inactivation from a two to one time constant process in rabbit ileal cells (Ohya *et al.* 1986), two time constants appear still to be present for the inactivation of Ba^{2+} current in guinea-pig ileum myocytes (Droogmans & Callewaert, 1986).

Organic calcium channel antagonists have been shown to block the calcium current in some of the studies reported. Verapamil blocks I_{Ca} in taenia caeci (Ganitkevich *et al.* 1986) and nisoldipine inhibits I_{Ca} in rabbit ileal muscle (Ohya *et al.* 1986). No comparison of the effectiveness of different antagonists on visceral smooth muscle cells from different species and gastrointestinal sites have been reported.

In this study, we atempted to characterize fully the calcium current in enzymatically isolated guinea-pig gastric myocytes so as to provide a basis for comparison with the calcium channel of other visceral myocytes. The inactivation of the calcium current was not only analysed in terms of its kinetics, voltage and cationic specificity, but also in response to different intracellular Ca^{2+} buffers used. We found that organic calcium channel antagonists may serve as calcium channel agonists or antagonists depending on the concentration of drug used.

METHODS

Cell preparation. Cells were isolated according to a procedure previously described (Mitra & Morad, 1985*a*, *b*). Briefly, guinea-pigs weighing 250–400 g were injected initially with heparin sodium solution (10 units/g, I.P.; LyphoMed, Rosemont, IL, USA). Ten minutes following the heparin injection, animals were anaesthetized using sodium nembutal (50–75 mg, I.P.; Abbott Laboratories, North Chicago, IL, USA). A complete gastrectomy was performed and the animal was immediately killed. The stomach was oxygenated in Ca^{2+}-free Tyrode solution (pH 7·20, 40 °C). Ca^{2+}-free Tyrode solution of the same composition, oxygenated and heated (40 °C) in a Langendorff column (height, 1 m) was perfused under pressure through the layers of a portion of the gastric body wall through a 22 gauge polyethylene catheter placed into the submucosal space. Following 20 min of Ca^{2+}-free solution, the stomach was perfused with the enzyme-containing solution. The stomach was then washed free of enzyme with 0·1 mM-Ca^{2+} Tyrode solution. Portions of the circular and longitudinal muscle layers were gently teased off with a forceps and cells were dispersed in 5 ml of 0·1 mM-Ca^{2+} Tyrode solution by gentle agitation with a Pasteur pipette.

Morphology of isolated gastric myocytes. Enzymatic digestion of the corpus yielded about 60% calcium-tolerant myocytes. Cells were approximately 150–300 μm in length and 3–5 μm in width. Healthy cells were characterized by a smooth membrane contour with tapered ends in a relaxed state. The cell shape was generally curvy in appearance. The nucleus was centrally located and easily defined using phase contrast microscopy. Cells with this appearance typically permitted gigaseal formation on whole-cell clamping and had well-defined time-dependent inward and outward currents and a small leak current. Other cells in which gigaseal formation was difficult or unstable were characterized by a rough membrane contour with acute angulations along the length of the cell, a shorter cell length, and granular appearance of the cytoplasm. Such cells were not used in this study.

Whole-cell voltage clamping. Isolated cells suspended in an acrylic cell chamber and perfused with a 5 mM-Ca^{2+} Tyrode (control) solution were voltage clamped using the whole-cell voltage clamp technique (Hamill, Marty, Neher, Sakmann & Sigworth, 1981). Cells were monitored through an inverted microscope (Nikon Daiphot, Garden City, NY, USA) on a video screen (USI International, Taiwan, ROC). Patch pipettes (tip diameter, 1–2 μm) were pulled from WPI Kwik-fil capillaries (outside diameter, 1·5 mm; WPI New Haven, CT, USA) using a Flaming-Brown Micropipette puller (Sutter Instrument Co., San Rafael, CA, USA). Pipette resistances ranged from 5 to 10 MΩ. A clamp amplifier (Dagan model 8900) fitted with a 100 MΩ headstage was used for voltage clamp experiments. Series resistance compensation was used to the point of ringing. Data were recorded on a Tektronix storage oscilloscope and recorded on Polaroid film. Most experiments from which data were collected were from cells which had leak currents that were less than 5 pA with 10 mV

hyperpolarization, were very small when compared to measured Ca^{2+} currents, and did not change under control situations and after experimental manipulation. In the few experiments from which data were derived in which a change in leak current had occurred, leakage subtraction was performed. When data from such an experiment was used, the finding was confirmed in cells which had small or negligible leak current. Additionally, only experiments in which bracketing of the data could be successfully achieved were used in the quantitative analysis of the data. Experiments shown in the figures were conducted using one myocyte only except where indicated in the figure legends. Pulse stimulation was maintained at a frequency of 0·05 Hz to minimize the Ca^{2+} current run-down. At this pulse rate, stable currents could often be maintained for 30–60 min.

TABLE 1. Composition of internal solution (in mM)

	Potassium gluconate	KCl	NaCl	Mg ATP	Na_2 ATP	HEPES	EGTA	EDTA	$CaCl_2$	CsCl	Caesium citrate
A	70	70	10	5	—	10	10	—	1	—	—
B	—	—	10	5	—	10	10	—	1	100	—
C	—	—	10	5	—	10	10	—	1	70	30
D	—	—	10	5	—	10	10	—	1	10	90
E	—	—	10	5	—	10	10	10	1	100	—
F	—	—	10	—	5	10	10	4	1	100	—

Solutions and drugs used. Ca^{2+}-free Tyrode solution contained (in mM): NaCl, 136; KCl, 5·4; $MgCl_2$, 1; NaH_2PO_4, 0·5; HEPES buffer, 10. Enzyme solution included Ca^{2+}-free Tyrode solution with collagenase I (1·8 mg/ml) and protease (0·6 mg/ml) both from Sigma (St Louis, MO, USA). The compositions of the internal solutions is shown in Table 1. Calcium channel blockers nifedipine, diltiazem and D600 were dissolved at the desired concentrations in the appropriate external solutions.

Statistical analysis. Data are expressed as means ± S.D. and were analysed by standard *t* test where appropriate.

RESULTS

Inward and outward time-dependent currents

Depolarizing pulses in KCl-dialysed cells activated an inward current followed by an outward current (Fig. 1, inset). The inward current activated at −20 to −30 mV, peaked around 0 mV and was difficult to measure at potentials positive to +40 mV (Fig. 1, graph). The maximum peak inward current seen in this preparation was 400 pA but the mean was 175·5 pA (n = 10 cells). The inward current was followed by an outward current at potentials positive to −30 mV. The outward current increased in amplitude and activated more rapidly with more positive pulses (up to +60 mV tested, Fig. 1). The outward current slowly relaxed during the depolarizing pulse. It peaked at about 70 ms into the clamp pulse (inset, Fig. 1) and relaxed over 3·0–4·0 s similar to those described for toad gastric myocytes (Walsh & Singer, 1981, 1987).

Calcium channels carry the inward current

When Cs^+ or *N*-methylglucamine was substituted for K^+ in the internal solution, the outward current was markedly suppressed but the activation kinetics of the inward current did not change significantly. The inactivation of the inward current on the other hand appeared largely dependent on the nature of the substituted cations. Under these conditions, an increase in the peak of the inward current (343·5

vs. 175·5 pA, $n = 10$ cells each, Table 2) was often noted, suggesting that the outward current was activated early in the course of inward current reducing the net inward current. The inward current activated near −30 mV, was maximum around 0 mV, and had very small values at potentials positive to +50 mV (Fig. 2).

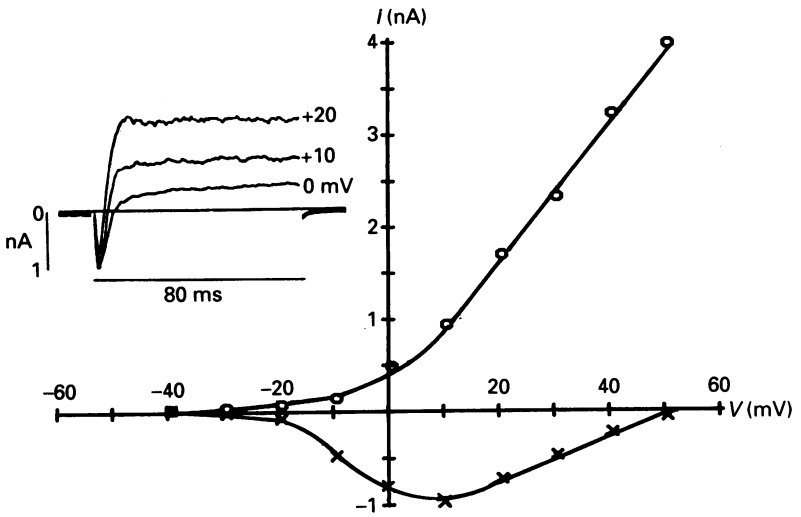

Fig. 1. Current–voltage relation for peak inward (×) and peak outward current (○). Inset: membrane currents recorded during short depolarizing pulses (potentials indicated at the corresponding traces) from a holding potential of −80 mV. Pipette solution: 70 mM-KCl, 70 mM-potassium gluconate (internal solution A). Bathing solution: 5 mM-Ca^{2+} Tyrode solution.

The inward current was thought to flow only through Ca^{2+} channels because of the following findings. (1) The current amplitude and kinetics were not affected in the presence of 10 μm-TTX, known to block the conventional voltage-gated Na^+ channels. (2) Substitution of either TEA or *N*-methylglucamine for external Na^+ did not affect the peak inward current or its activation kinetics suggesting that the inward current is not carried by a TTX-insensitive Na^+ channel. (3) Increasing $[Ca^{2+}]_o$ increased (Fig. 2) and reducing $[Ca^{2+}]_o$ reduced the peak inward current. (4) The inward current was specifically blocked by 5 mM-Ni^{2+}, Co^{2+} and Cd^{2+}, known to block I_{Ca} in other tissues. The concentration of Ni^{2+} necessary for 50% inhibition of peak I_{Ca} was about 500 μm.

Inactivation of I_{Ca} in gastric myocytes

Progressive decrease of the holding potentials to potentials positive to −80 mV decreased the Ca^{2+} current activated with test depolarizing pulses to 0 mV. Figure 3 compares the voltage dependence of the inactivation of the Ca^{2+} channel when the channel carries Ca^{2+} or Ba^{2+}. Irrespective of the charge carrier, the current completely inactivated at potentials positive to −50 mV with half-maximal inactivation around −65 mV. In some experiments, current was still seen at holding potentials as positive as −10 mV but in most experiments, current was completely

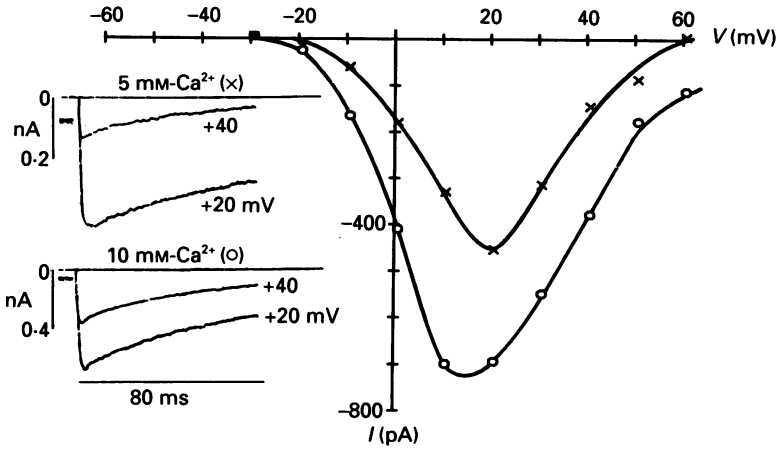

Fig. 2. Current–voltage relation for peak inward current in 5 mM (×) and 10 mM (○) $[Ca^{2+}]_o$. Inset: membrane currents recorded during depolarizing pulses to +20 and +40 mV from a holding potential of −60 mV in 5 mM (top traces) and 10 mM (bottom traces) $[Ca^{2+}]_o$. Pipette solution: 90 mM-caesium citrate (internal solution D).

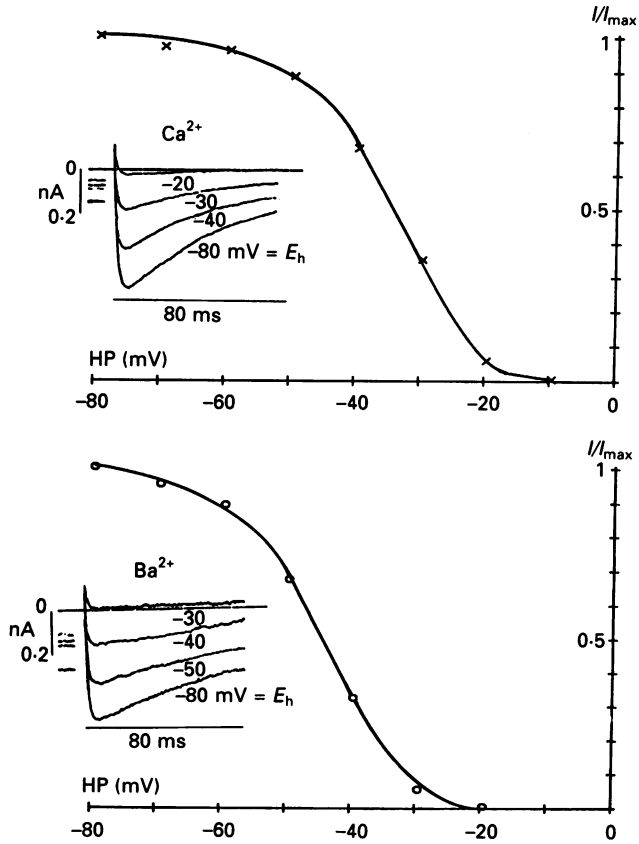

Fig. 3. Steady-state inactivation of Ca^{2+} (×) and Ba^{2+} (○) currents as a function of membrane potential. Steady-state inactivation was determined by holding the potential at various levels, followed by a depolarizing step to 0 mV. The peak amplitude of the inward currents induced by this procedure (shown in insets) were normalized and plotted as a function of holding potential. Pipette solution: 100 mM-CsCl (internal solution C). Bathing solution: 5 mM-Ca^{2+} or Ba^{2+} Tyrode solution.

inactivated at −30 to −20 mV ($n = 7$). Thus, although Ba^{2+} altered the kinetics of inactivation of the Ca^{2+} channel (see Fig. 9), it did not alter its voltage dependence suggesting that steady-state voltage-dependent inactivation is an inherent property of the channel.

When peak I_{Ca} was compared at holding potentials of −80 and −40 mV, the amplitude of the peak current decreased with a less negative holding potential (Fig. 4). The relative decrease in I_{Ca} was approximately the same at all potentials tested.

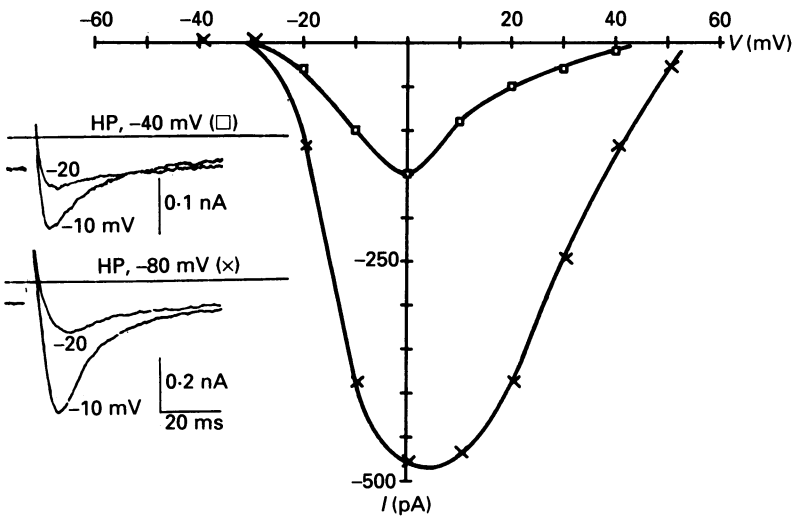

Fig. 4. Effect of holding potential on I_{Ca}. Current–voltage relations for currents measured from cell at holding potential, $E_h = -40$ mV (□) and −80 mV (×). Insets: I_{Ca} recorded during depolarizing steps (potentials indicated at the corresponding traces) from a holding potential of −40 mV (top) and −80 mV (bottom). Pipette solution: 100 mM-CsCl (internal solution B). Bathing solution: 5 mM-Ca^{2+} Tyrode solution.

Large negative holding potentials did not expose a hump in the current-voltage relation nor were the inactivation kinetics of I_{Ca} altered when the holding potential was changed from −40 to −80 mV. These findings suggest that a large fraction of the Ca^{2+} channels are inactivated at −40 mV, and does not provide critical evidence for the existence of more than one type of Ca^{2+} channel (Carbone & Lux, 1984*a*, *b*; Bean, 1985; Nilius, Hess, Lansman & Tsien, 1985; Mitra & Morad, 1986).

Time course of inactivation of I_{Ca} in gastric myocytes

Inactivation of Ca^{2+} current occurs in two phases. Two single exponentials describe reliably the time course of the inactivation. At +10 mV, where I_{Ca} reaches maximum, the time constants (τ) of the two exponentials were $\tau_f = 53{\cdot}4 \pm 18{\cdot}1$ ms and $\tau_s = 129{\cdot}3 \pm 51{\cdot}6$ ms. Figure 5 compares the kinetics of inactivation of the Ca^{2+} channel at two different holding potentials (E_h). At E_h values of −80 and −40 mV, the values of τ_f and τ_s were similar despite a large difference in peak I_{Ca} ($n = 5$). These findings are consistent with the experiment of Figs 3 and 4 and support the idea of the existence of a single population of Ca^{2+} channels.

Figure 6 compares the voltage dependence of kinetics of inactivation of the Ca^{2+}

channel. At 0 mV (Fig. 6*B*), the time constants of inactivation were $\tau_f = 28{\cdot}6$ ms and $\tau_s = 111{\cdot}1$ ms. At more positive or negative potentials (−20 and +20 mV, Fig. 6*A* and *C*), τ_f and τ_s were more difficult to differentiate. Figure 6*D* compares the voltage dependence of I_{Ca} and τ_f. The fast time constant has a U-shaped voltage dependence,

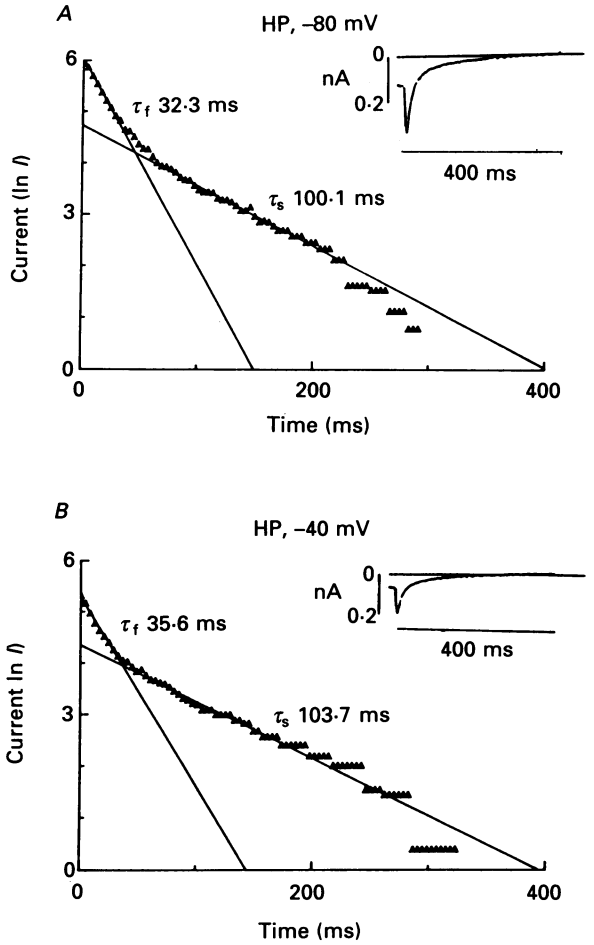

Fig. 5. Inactivation time course of I_{Ca} at different holding potentials. Semilogarithmic plot of I_{Ca} (shown in insets) elicited by a depolarizing pulse from a holding potential of −80 mV (*A*) or −40 mV (*B*) to +10 mV. Inactivation could be fitted with the sum of two exponentials with nearly identical time constants. The parameters of the slow (τ_s) and fast (τ_f) exponential components are indicated. Pipette solution: 100 mM-CsCl (internal solution B). Bathing solution: 5 mM-Ca^{2+} Tyrode solution.

with a minimum around 0 mV. This value corresponds closely to where maximum Ca^{2+} current occurs, suggesting that a process related to influx of Ca^{2+} may contribute to this phase of inactivation. In contrast, the slow time constant did not show a strict U-shaped dependence on membrane potential (not plotted). Although, the smallest τ_s values were seen around where I_{Ca} peaked (Fig. 6*A–C*), at more positive potentials, τ_s deviated from the U-shaped dependence. These findings

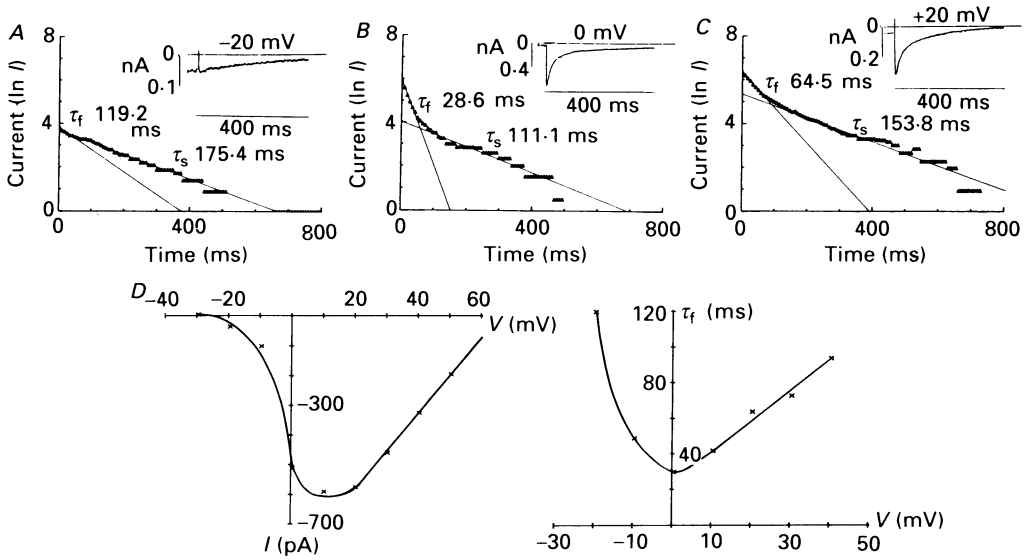

Fig. 6. Inactivation time course of I_{Ca} during different voltage steps. Semilogarithmic plots of I_{Ca} (shown in insets) elicited by a depolarizing pulse from a holding potential of $E_h = -80$ mV to -20 (*A*), 0 (*B*) and $+20$ mV (*C*). Inactivation could be fitted with the sum of two exponentials (time constants indicated). *D*, voltage dependence of peak I_{Ca} (left panel) and time constant of fast component (right panel, τ_f). Pipette solution: 100 mM-CsCl (internal solution B). Bathing solution: 5 mM-Ca^{2+} Tyrode solution.

TABLE 2. Effect of different intracellular Ca^{2+} buffering systems on peak I_{Ca} (I_{max})

	Internal solution				
				Citrate	
	KCl	CsCl	EDTA (4 mM)	30 mM	90 mM
I_{max} (mean ± S.D.)	203·7 ± 170·0	474·0 ± 231·3	579·0 ± 80·65	781·7 ± 356·3	854·7 ± 391·79
n	15‡	15*†‡	5	12*	15†

* $P = 0{\cdot}014$; † $P < 0{\cdot}000$; ‡ $P = 0{\cdot}001$.

suggest that more than one mechanism may mediate the inactivation of the Ca^{2+} channel.

Inactivation of I_{Ca} vs. intracellular Ca^{2+} buffers

Since intracellular Ca^{2+} and Mg^{2+} are known to regulate the Ca^{2+} channel (Eckert & Chad, 1984; White & Hartzell, 1988), we compared the effect of a number of divalent cation buffering systems on the magnitude of I_{Ca} and its inactivation kinetics. When EDTA (4 mM) or citrate (30 or 90 mM) was added to the standard dialysing internal solution containing 10 mM-EGTA, peak current increased from 474·0 to 579·0, 781·7 and 854·7 pA, respectively (Table 2). These findings suggested that Ca^{2+} and/or Mg^{2+} may modulate I_{Ca} in gastric myocytes. To help determine

if intracellular Mg^{2+} regulates the Ca^{2+} channel (White & Hartzell, 1988; Agus, Kelepouris, Dukes & Morad, 1989), I_{Ca} was first compared in cells dialysed with 10 mM-EGTA and 5 mM-MgATP, 10 mM-EGTA and 5 mM-NaATP or 10 mM-EGTA, 5 mM-MgATP and 20 mM-$MgCl_2$. When NaATP was substituted for MgATP ($n = 5$), no difference was seen in either the peak of I_{Ca} or its kinetics of early inactivation. However, addition of 20 mM-$MgCl_2$ to the dialysing internal solution completely

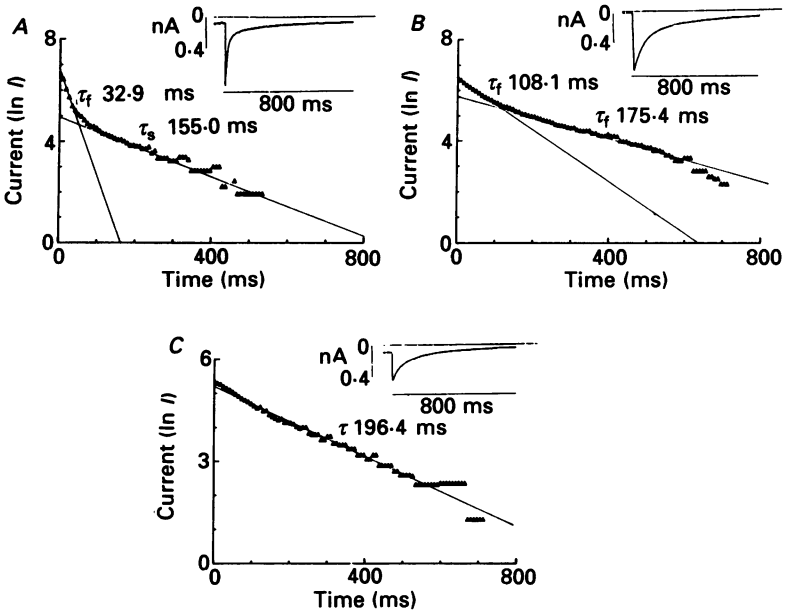

Fig. 7. Effect of intracellular citrate on inactivation time course of I_{Ca}. I_{Ca} (shown in insets) was elicited by a depolarizing pulse from −80 to +10 mV and its time course of inactivation was fitted with the sum of two exponentials. *A*, 0 mM-citrate (internal solution B) B, 30 mM-citrate (internal solution C). *C*, 90 mM-citrate (internal solution D). Bathing solution 5 mM-Ca-Tyrode solution. Three separate cells used.

inhibited I_{Ca} ($n = 5$). The reduction of I_{Ca} in high intracellular Mg^{2+} suggests that Mg^{2+} may regulate the inactivation of the Ca^{2+} channel. The lack of significant effect on omitting Mg^{2+} from the dialysing solution is likely to be related to well-buffered $[Mg^{2+}]_i$ in the presence of 5 mM-ATP.

Because citrate has been shown to slow down the kinetics of I_{Ca} more effectively than EGTA in guinea-pig atria cardioballs (Becham & Pott, 1985), the effects of two different concentrations of citrate in the presence of 10 mM-EGTA on the inactivation of the calcium current were compared with those cells dialysed with EGTA alone. Fifteen similarly prepared cells were divided into three groups of five each. Each group was dialysed with either the 10 mM-EGTA alone or 30 or 90 mM-citrate added. Figure 7 shows the time course of inactivation of the three dialysing solutions in three different cells. In a cell dialysed with EGTA and CsCl (Fig. 7*A*), τ_f was approximately five times more rapid than τ_s (32·9 *vs.* 155·0 ms). In the cell dialysed with EGTA and 30 mM caesium citrate (Fig. 8*B*), both τ_f and τ_s were larger when compared to the myocyte dialysed with EGTA and CsCl, with citrate increasing

primarily τ_f (3-fold). When gastric myocytes were dialysed with 90 mM caesium citrate (Fig. 7*C*), both τ_f and τ_s were further increased compared to cells dialysed with either 10 mM-EGTA and CsCl or 30 mM-citrate. In fact, with 90 mM-citrate τ_f became so slow as to make it indistinguishable from τ_s in three of five cells tested, i.e. the time course of inactivation could be approximated by a single exponential. Table 3 shows the means for τ_f and τ_s in fifteen different cells, each group of five cells

TABLE 3. Effect of different intracellular Ca^{2+} buffers on inactivation time constants

	Internal solutions		
	Control	Citrate (30 mM)	Citrate (90 mM)
τ_f (ms)	53·4 ± 18·1*†	90·1 ± 35·3*†	143·5 ± 46·7†‡
τ_s (ms)	175·2 ± 46·1	213·2 ± 33·1	257·3 ± 166·1

$n = 5$ for each group; * $P = 0{\cdot}06$; † $P = 0{\cdot}004$; ‡ $P = 0{\cdot}05$.

dialysed with one of the three buffer systems. The data suggests that addition of 30 and 90 mM-citrate to the internal solution caused slowing of τ_f by a factor of 2- to 3-fold compared to cells dialysed with EGTA alone. Although τ_s was also prolonged, the effect was less pronounced.

To determine whether the effect of citrate on the inactivation of Ca^{2+} current was caused by possible citrate-induced buffering of $[Mg^{2+}]$, the effect of solutions containing 4 mM-EDTA in addition to EGTA was examined. This concentration of EDTA reduced the $[Mg^{2+}]_i$ from 550 to 40 nM (calculated by iterative computer program, CaBuf) without a significant change of $[Ca^{2+}]_i$. Although such a dialysing solution consistently enhanced peak I_{Ca} (Table 2) it did not alter the kinetics of inactivation of I_{Ca} significantly ($n = 3$). These results suggest that early inactivation of the Ca^{2+} current is largely regulated by $[Ca^{2+}]_i$. $[Mg^{2+}]_i$, on the other hand, may be involved in steady-state availability of functional Ca^{2+} channels.

Permeation of other cations through the Ca^{2+} channel

To examine the effect of Ca^{2+}-induced inactivation of I_{Ca}, we studied the permeation of Ba^{2+} through the Ca^{2+} channel. Figure 8 shows that in the gastric myocyte Ba^{2+} permeates the Ca^{2+} channel better than Ca^{2+} at all potentials tested ($n = 4$). Ba^{2+} also prolongs the time course of inactivation (Fig. 8, insets). Figure 8*B* compares the time course of inactivation of Ca^{2+} channel when Ca^{2+} and Ba^{2+} are the charge carriers through the channel in a cell dialysed with EGTA. As shown previously, when Ca^{2+} was the charge carrier, a fast (τ_f) and a slow (τ_s) time constant could well describe the inactivation kinetics of I_{Ca}. When Ba^{2+} was substituted for Ca^{2+}, one time constant was almost sufficient to describe the inactivation of the calcium channel similar to rabbit ileal cells (Ohya *et al.* 1986). The slow time constant of inactivation measured in the presence of Ba^{2+} was similar to that of τ_s measured when Ca^{2+} permeates the channel (135·1 ms, I_{Ca} *vs.* 144·7 ms, I_{Ba}). These results further suggest that τ_f but not τ_s is primarily regulated by $[Ca^{2+}]_i$. Thus, the rapid inactivation phase of I_{Ca} maybe determined primarily by local elevations of $[Ca^{2+}]_i$ caused by the rapid influx of Ca^{2+} through the Ca^{2+} channel.

Sr^{2+} similarly permeates through the Ca^{2+} channel. When Ca^{2+} was substituted mol/mol by Sr^{2+}, the maximum measured I_{Sr} approximated I_{Ca} (Fig. 8*A*, $n = 4$ cells). Similar to I_{Ba}, I_{Sr} also prolonged the inactivation of the inward current when compared to I_{Ca}. In contrast to Ba^{2+} and Sr^{2+}, Mg^{2+} did not appear to permeate

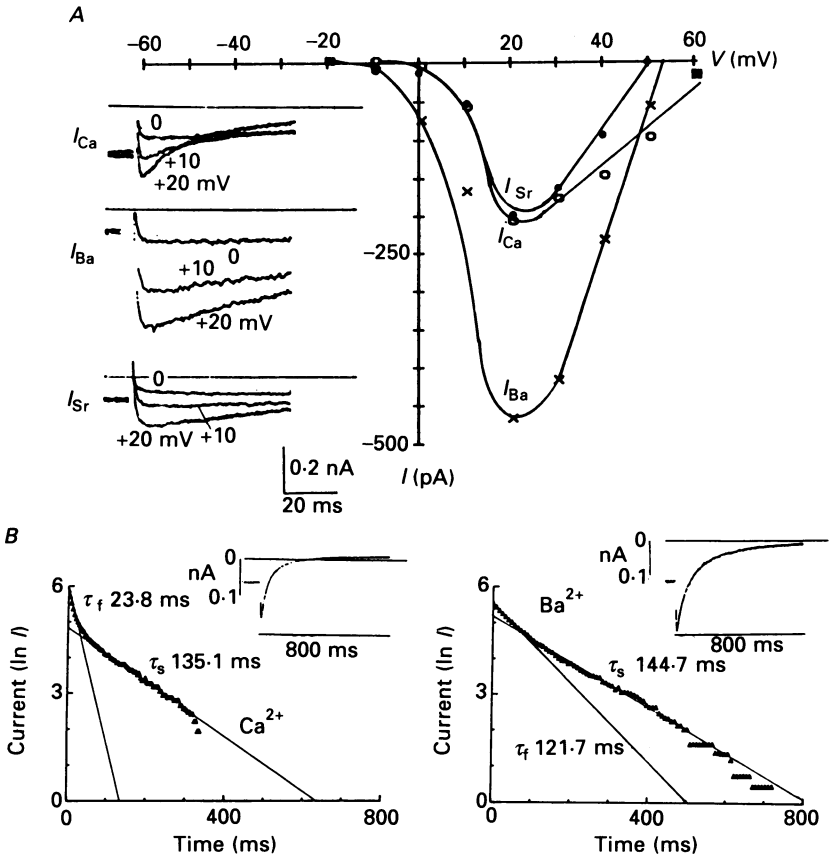

Fig. 8. Permeation of Ba^{2+} and Sr^{2+} through the Ca^{2+} channel. *A*, current–voltage relation of peak inward current in the presence of 5 mM $[Ca^{2+}]_o$ (○), 5 mM $[Ba^{2+}]_o$ (×) and 5 mM $[Sr^{2+}]_o$ (●). Insets: inward Ca^{2+} (top), Ba^{2+} (middle) and Sr^{2+} (bottom) currents recorded during depolarizations (potentials indicated at corresponding traces) from −60 mV. *B*, time course of inactivation of I_{Ca} (left) and I_{Ba} (right). When external Ca^{2+} is replaced with Ba^{2+}, the fast component of inactivation is indistinguishable from the slow component. Pipette solution: 100 mM-CsCl (internal solution B). *A* and *B* are from two cells.

through the Ca^{2+} channel when Mg^{2+} was substituted for Ca^{2+} in the external solutions in concentrations as high as 20 mM. Thus, the Ca^{2+} channel allows permeation of other divalents in the order of $Ba^{2+} > Ca^{2+} \approx Sr^{2+} \gg Mg^{2+}$.

Na^+ also permeates through the Ca^{2+} channel when extracellular Ca^{2+} was reduced below micromolar concentrations. In gastric myocytes which were perfused with Ca^{2+}-free, Mg^{2+}-free 4 mM-EGTA Tyrode solution, a large Na^+ current was recorded. Removal of Ca^{2+} led to initial decrease in Ca^{2+} current, but after approximately 2 min, an inward current appeared which reached steady-state values after

approximately 4 min. Slightly more negative holding potentials were required for full activation of the Na^+ current through the Ca^{2+} channel (Bulbring & Tomita, 1970; Jmari, Mironneau & Mironneau, 1987). Figure 9 shows that Na^+ current through the Ca^{2+} channel was slightly larger in amplitude than I_{Ca} (Hess, Lansman & Tsien, 1986; Jmari *et al.* 1987) and the voltage dependence of its activation was

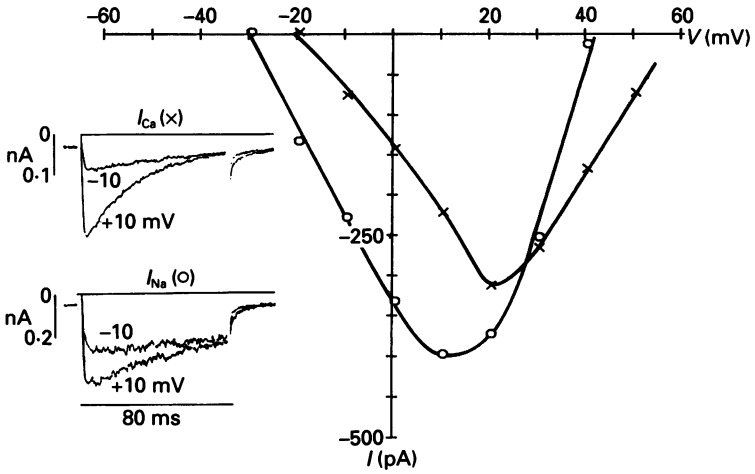

Fig. 9. Permeation of Na^+ through the Ca^{2+} channel. Current–voltage relation of peak inward current in the presence (5 mM; ×) and absence (○) of external Ca^{2+}. Insets: membrane currents recorded during depolarizing pulses (potentials indicated at corresponding traces) from $E_h = -60$ mV in the presence (top) and absence (bottom) of external Ca^{2+}. Pipette solution: 100 mM-CsCl (internal solution B). Ca^{2+}-free Tyrode solution was obtained by adding 4 mM-EGTA to nominal Ca^{2+}-free medium.

shifted by 10–20 mV to more negative potentials (Ohya *et al.* 1986; Jmari *et al.* 1987). The measured reversal potential for Na^+ current through the Ca^{2+} channel approximated the predicted Nernst potential, $E_{Na} \approx +45$ mV ($[Na^+]_o = 140$ mM and $[Na^+]_i = 20$ mM). The inactivation kinetics of I_{Na} were also slower when compared to I_{Ca} (Fig. 9, inset), consistent with the idea that a component of the inactivation of the Ca^{2+} channel was regulated by a Ca^{2+}-induced process.

pH dependence of I_{Ca} in gastric myocytes

Changing the extracellular pH alters the I_{Ca} significantly in different cell types (Iijima, Ciani & Hagiwara, 1986; Prod'hom, Pietrobon & Hess, 1987; Davies, Lux & Morad, 1988). Figure 10 demonstrates that when the extracellular pH was increased from 7·2 to 8·0 in a gastric myocyte, the amplitude of I_{Ca} increased but the inactivation kinetics remained unchanged (insets left and graph). Acidification of the external solution to pH 6·0 inhibited I_{Ca} (bottom inset left and graph). Between pH 8 and 6, the peak I_{Ca} decreased linearly with decreasing pH (inset right). Generally, I_{Ca} inactivated more rapidly at pH 6 than at higher pH values (Fig. 10, inset). The pH induced enhanced kinetics of inactivation were often observed at pH < 6·5 (n = 3). Although both the peak and maintained I_{Ca} were suppressed at the high external

proton concentration, the maintained current appeared to be completely suppressed. These findings suggest that protons induce an open-channel block of Ca^{2+} channels (i.e. enhancing their inactivation) or that a subpopulation of Ca^{2+} channels, representing the maintained portion of I_{Ca}, might be more sensitive to proton block.

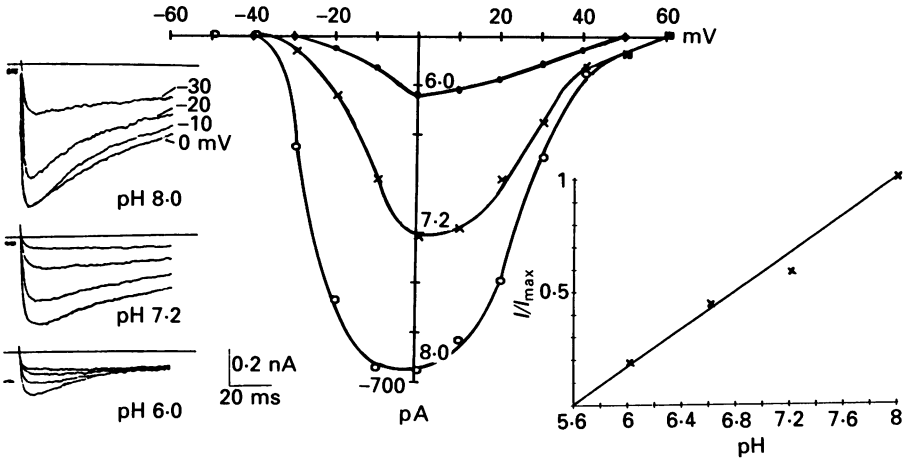

Fig. 10. Effect of pH_o on I_{Ca}. Current–voltage relations of peak inward current at pH 8·0 (○), 7·2 (×) and 6·0 (●). Left inset: I_{Ca} recorded during depolarizing steps (potentials indicated at the corresponding traces) from a holding potential of −60 mV at pH 8·0 (top traces), 7·2 (middle traces) and 6·0 (bottom traces). Right inset: I/I_{max} as a function of pH_o. I_{max} was defined as peak amplitude of I_{Ca} recorded at pH 8·0 during a depolarization from −60 to 0 mV. I is the peak amplitude of I_{Ca} recorded during a depolarizing step from −60 to 0 mV. Pipette solution: 90 mM-caesium citrate (internal solution D). Bathing solution: 5 mM-Ca^{2+} Tyrode solution.

Effects of Ca^{2+} channel antagonists on I_{Ca}

The effects of nifedipine, D600 and diltiazem on gastric myocytes were studied in caesium citrate-dialysed cells. All three drugs inhibited the calcium current in a voltage-dependent manner. Table 4 and Figs 11–13 compare the effect of these agents at E_h values of −80 and −40 mV. Nifedipine (Fig. 11) and D600 (Fig. 12) in 100 nM concentrations completely suppressed calcium current at $E_h = -40$ mV but only nifedipine inhibited the Ca^{2+} current significantly when E_h was −80 mV (compare Fig. 11 with Fig. 12). At this same dose, diltiazem failed to inhibit I_{Ca} at either of the holding potentials tested. Perfusion of 500 nM-diltiazem partially inhibited I_{Ca} (Fig. 13) but when compared to D600 or nifedipine, complete inhibition of I_{Ca} by diltiazem did not occur until 10-fold higher concentrations were used (1·0 μm). Even at 1·0 μm concentrations, complete inhibition occurred only with $E_h = -40$ mV as compared to 50% inhibition at $E_h = -80$ mV. Thus, at $E_h = -40$ mV, the order of potency for inhibition of I_{Ca} was nifedepine = D600 ⩾ diltiazem, but at $E_h = -80$ mV the order was nifedipine > D600 ⩾ diltiazem.

Figure 13*A* (inset) also demonstrates that perfusion with 500 nM-diltiazem caused a faster inactivation of I_{Ca}. At $E_h = -40$ mV where peak I_{Ca} was only partially inhibited by diltiazem, I_{Ca} measured at the end of the depolarizing step (80 ms) was

almost completely suppressed (Fig. 13*A*). At $E_h = -80$ mV, where peak I_{Ca} was not significantly affected by 500 nM-diltiazem, I_{Ca} measured at 80 ms was also markedly suppressed. This same effect on I_{Ca} at 80 ms was also seen with perfusion of 1 μm-diltiazem (data not shown). This finding suggested that either diltiazem blocks the open channel in a time-dependent manner or that a second population of Ca^{2+}

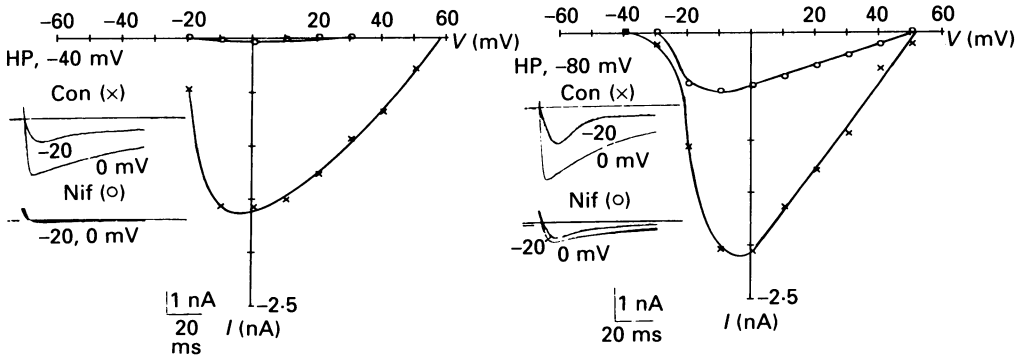

Fig. 11. Effect of nifedipine on I_{Ca}. Effect of 100 nM-nifedipine on I_{Ca} elicited by depolarizing voltage clamp steps from $E_h = -40$ mV (left panel) or -80 mV (right panel). Current–voltage relations of peak I_{Ca} in control Tyrode solution (×) and in the presence of 100 μM-nifedipine (○). Pipette solution: 90 mM-caesium citrate (internal solution D).

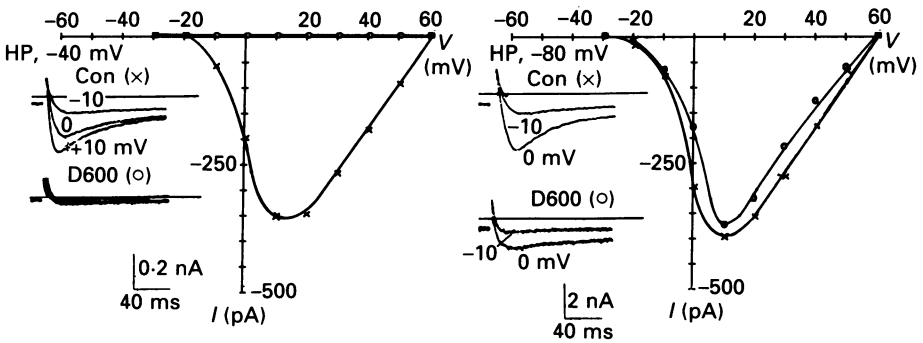

Fig. 12. Effect of D600 on I_{Ca}. Effect of 100 nM-D600 on I_{Ca} elicited by depolarizing voltage clamp steps from $E_h = -40$ mV (left panel) or -80 mV (right panel). Pipette solution: 90 mM-caesium citrate (internal solution D).

channels was being preferentially inhibited by diltiazem. This differential block of maintained and peak I_{Ca} was not seen with D600 or nifedipine suggesting that this property was a unique effect of diltiazem on the gastric myocyte Ca^{2+} channel.

At a drug perfusion rate of approximately 2 ml/min, and measuring I_{Ca} at 20 s intervals, diltiazem and D600 inhibited I_{Ca} at around 5 min. This is consistent with the use-dependent block of the drug previously described for verapamil in taenia caeci (Ganitkevich *et al.* 1986). In contrast, nifedipine inhibited the peak I_{Ca} within 1 min of the drug application.

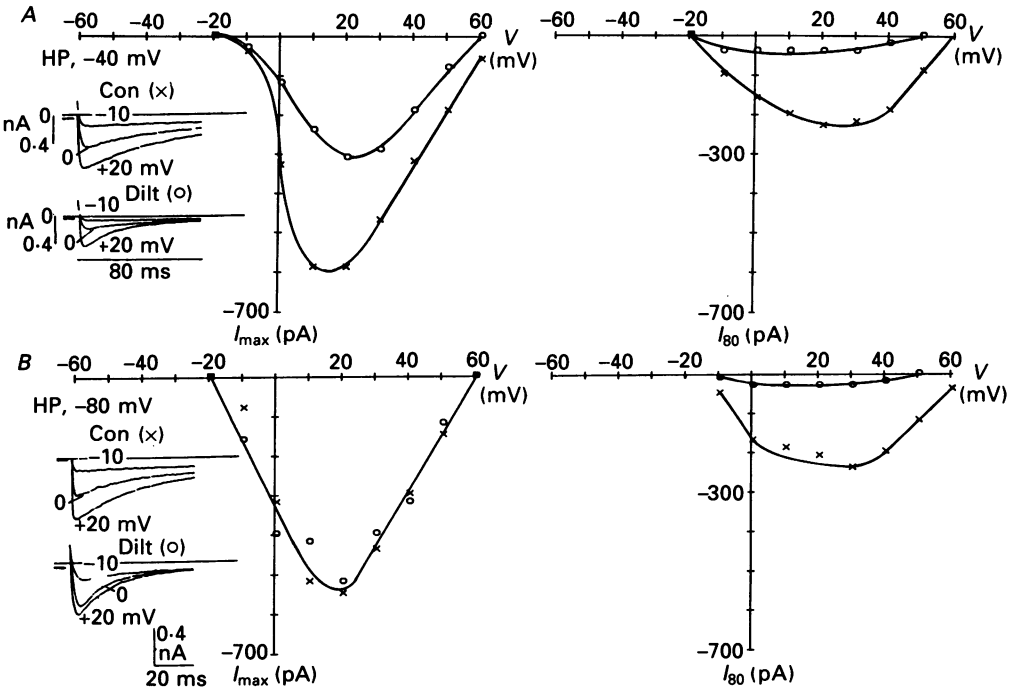

Fig. 13. Antagonistic effect of diltiazem on I_{Ca}. Effect of 500 nM-diltiazem on I_{Ca} elicited by depolarizing voltage clamp steps from $E_h = -40$ (*A*) or -80 mV (*B*). Current–voltage relations are shown for peak I_{Ca} (left, I_{max}) and of the current measured after 80 ms (right, I_{80}) in control Tyrode solution (×) and in the presence of 500 nM-diltiazem (○). Pipette solution: 90 mM-caesium citrate (internal solution D).

TABLE 4. Effect of calcium channel antagonists (M) on calcium current (% inhibition)

	10^{-9}	10^{-7}	5×10^{-7}	10^{-6}	10^{-5}
Nifedipine					
−40	0	95	100	100	100
−80	0	75	100	100	100
D600					
−40	0	100	100	100	100
−80	0	5	—	50	100
Diltiazem					
−40	0	+	50	100	100
−80	0	+	0	50	50

+, agonistic effect; —, not performed.

Both D600 and diltiazem in addition to their antagonistic effect had also an agonistic effect on the calcium current. Figure 14 shows that at 100 nM-diltiazem causes an increase of I_{Ca} at all potentials tested. This effect occurred at both −40 and −80 mV holding potentials. The greatest agonist effect seemed to occur at potentials negative to where maximum I_{Ca} was measured. Furthermore, when currents were analysed at peak and at the end of the depolarizing pulse (80 ms), peak current was selectively enhanced when compared to final current. Specifically, at $E_h = -40$ mV,

I_{Ca} at 80 ms was unchanged or slightly decreased by 100 nM-diltiazem while the peak current (5–10 ms) was strongly enhanced (Fig. 14*A*). At $E_h = -80$ mV the enhancement of peak I_{Ca} was stronger even when I_{Ca} at 80 ms was unchanged or suppressed (Fig. 14*B*). The agonistic effect of diltiazem was most prominent at negative potentials while the antagonistic effect predominated at the positive potentials. Perfusion of a gastric myocyte with 100 nM-D600 also caused an initial

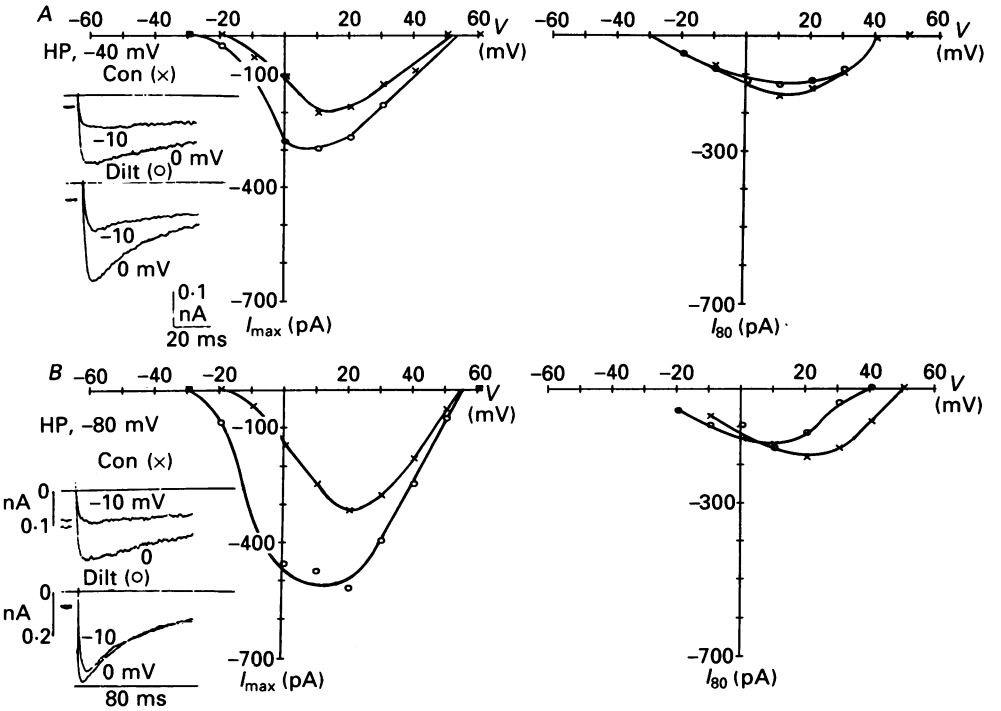

Fig. 14. Agonistic effect of diltiazem on I_{Ca}. Effect of 100 nM-diltiazem on I_{Ca} recorded by depolarizing voltage clamp steps from $E_h = -40$ mV (*A*) or -80 mV (*B*). Current–voltage relations are shown for peak I_{Ca} (left, I_{max}) and the current measured after 80 ms (right, I_{80}) in control solution (×) and in the presence of 100 nM-diltiazem (○). Pipette solution: 90 mM-caesium citrate (internal solution D).

increase in calcium current. This effect occurred within 2 min of addition of the drug and lasted for approximately 1 min after which progressive inhibition of the I_{Ca} occurred. This transient agonistic effect was seen in three of five cells tested with D600. A concentration at which D600 showed only the agonistic effect was not easily identifiable.

DISCUSSION

The main finding of this report is that in guinea-pig gastric myocytes, the inward current is carried only by the voltage-gated Ca^{2+} channel. Although the channel activates around -20 mV, holding potentials of -80 to -90 mV were necessary for full activation of I_{Ca} and complete removal of the inactivation. Ca^{2+} channels of

gastric myocytes are similar to cardiac Ca^{2+} channels in respect to their ionic selectivity, drug sensitivity and voltage and time dependence (Lee & Tsien, 1983; Hess, Lansman & Tsien, 1986; Lansman, Hess & Tsien, 1986).

Ca^{2+} channels of guinea-pig gastric myocytes have many properties in common with those of other gastrointestinal myocytes studied (toad stomach, Walsh & Singer, 1981; guinea-pig taenia caeci, Ganitkevich *et al.* 1986; rabbit ileum, Ohya *et al.* 1986; and guinea-pig ileum, Droogmans & Callewaert, 1986). I_{Ca} in guinea-pig gastric myocytes activated at approximately −20 mV, peaked around 0 mV and was not seen at potentials positive to +60 mV similar to the guinea-pig ileum (Droogmans & Callewaert, 1986), taenia caeci (Ganitkevich *et al.* 1986, 1987), rabbit ileum (Ohya *et al.* 1986) and jejunum (Benham, Bolton & Lang, 1984). When outward K^+ current was suppressed by substituting Cs^+ or *N*-methylglucamine for K^+ in the internal dialysing solution, the apparent reversal potential for the Ca^{2+} current shifted to more positive potentials.

Are there more than one type of Ca^{2+} channels in gastric myocytes?

In the guinea-pig gastric myocytes, we could not clearly identify the presence of more than one type of Ca^{2+} channel, as has been seen in other excitable cell types (Carbone & Lux, 1984*a*, *b*; Armstrong & Matteson, 1985; Bean, 1985; Fedulova, Kostyuk & Veselovsky, 1985; Nilius *et al.* 1985; Nowycky, Fox & Tsien, 1985; Mitra & Morad, 1986; Benham, Hess & Tsien, 1987). Evidence against the presence of more than one type of Ca^{2+} channel is: (1) more-negative holding potentials did not alter the threshold for activation of I_{Ca} even though the current was markedly enhanced; (2) changes in the holding potential did not alter the kinetics of inactivation of I_{Ca} (Figs 3, 4 and 5) as seen with T- and L-type channels in cardiac and DRG neurones (Carbone & Lux, 1984*a*, *b*; Mitra & Morad, 1986); and (3) nifedipine did not preferentially block I_{Ca} activated at different potentials as is the case in cardiac myocytes (Mitra & Morad, 1986).

In support of the possibility of the existence of more than one type of Ca^{2+} channel we found that (1) two exponentials were necessary to describe the time course of inactivation of I_{Ca}; (2) proton concentrations larger than pH > 6·5 resulted in faster inactivation of I_{Ca} appearing as a preferential block of the maintained component (see insets, Fig. 10); (3) diltiazem at concentrations which did not affect the peak component of I_{Ca} strongly suppressed the maintained component of I_{Ca} (Fig. 13*C* and *D*); and (4) at concentrations where diltiazem induced only an agonistic effect, the peak component of I_{Ca} was selectively increased without significant effect on the maintained component (Fig. 14). It is tempting to speculate that diltiazem may enhance a T-type Ca^{2+} channel and block the L-type channels in gastric myocytes. It should be noted, however, that the selective inhibitory effect of diltiazem and protons on the slowly inactivating component of I_{Ca} may be secondary to a time- and voltage-dependent interaction of these agents with the Ca^{2+} channel. Two Ca^{2+} channel types have been reported in guinea-pig taenia coli myocytes (Yoshino, Someya, Nishio & Yabu, 1988). Both channel types appear to be activated at the same voltage (−40 mV) and required addition of Bay K 8644 to help distinguish them. Unlike our results (Fig. 11), however, 100 nM-nifedipine selectively blocked the high-threshold Ca^{2+} channel (L) in taenia coli myocytes (Ganitkevich

et al. 1986). Thus, our results did not yield definitive evidence for the existence of more than one type of Ca^{2+} channel in gastric myocytes.

Kinetics of inactivation of I_{Ca}

Inactivation of the calcium current was defined by two time constants similar to taenia caeci (Ganitkevich *et al.* 1986), guinea-pig ileum (Droogmans & Callewaert, 1986) and rabbit ileum (Ohya *et al.* 1986). The τ_f in gastric myocytes was similar to that of taenia caeci (Ganitkevich *et al.* 1987) but slower than both ileal preparations reported (Ohya *et al.* 1986; Droogmans & Callewaert, 1986). The τ_s, however, was similar to the ileal cells but was 2–3 times faster than that of taenia caeci. Similar to taenia caeci, in gastric myocytes the inactivation process was in part calcium dependent (Figs 6–8), consistent with other smooth muscles (Jmari *et al.* 1986). In gastric myocytes, τ_f appears primarily affected by $[Ca^{2+}]_i$. This conclusion is based on the finding that: (1) τ_f appears to correlate to the magnitude of I_{Ca} (Fig. 6); (2) prolongation of τ_f with the addition of citrate to the dialysing solution (Fig. 7, Table 3); (3) prolongation of τ_f by using Ba^{2+} or Na^+ as charge carriers through the Ca^{2+} channel (Figs 8 and 9). It should be noted that an increase in I_{Ca} with more negative holding potentials (Figs 3, 4, 11–14) without a change in τ_f might argue against τ_f dependence on $[Ca^{2+}]_i$. This may be explained, however, if the increase in I_{Ca} resulted from an increase in the number of open channels (Droogmans & Callewaert, 1986) rather than an increased conductance of the unitary channel, i.e. there would be no increase in the number of Ca^{2+} ions crossing each individual channel. Furthermore, even if an increase in conductance of the unitary channel were to occur, the effect on the inactivation time course would probably be minimized by the use of an internal solution with a high Ca^{2+}-buffering capacity (10 mM-EGTA).

Although the citrate effect on the inactivation of I_{Ca} was most probably mediated through increased buffering of $[Ca^{2+}]_i$, direct measurement of $[Ca^{2+}]_i$ under these conditions was not available. Further, since 10 mM-EGTA reduces the $[Ca^{2+}]_i$ to very low baseline concentration (10^{-8} M, calculated by iterative computer program, CaBuf), it is unlikely that the addition of citrate to such a solution further reduces the resting $[Ca^{2+}]_i$. When 30 or 90 mM-citrate was added to the EGTA buffered intracellular solution, the influx of Ca^{2+} during a 10 ms I_{Ca} (500 pA, into a myocyte of 150 μm length and 5·0 μm diameter) was reduced from 8·47 to 6·22 to 4·82 μM, respectively. This calculation shows that the change in $[Ca^{2+}]_i$ was not by itself sufficient to account for the marked slowing of the inactivation of I_{Ca} in the presence of high concentrations of citrate (Fig. 7). It may be that citrate enhances the Ca^{2+}-buffering capacity of a confined volume of myoplasm near the mouth of the calcium channel. This possibility might also explain why others have found that increasing the $[EGTA]_i$, which may not have easy access to the channel mouth, did not significantly affect the inactivation of I_{Ca} (Ganitkevich *et al.* 1986). The conclusion that τ_f was Ca^{2+} and not Mg^{2+} dependent was based on the lack of significant effect of the addition of 4 mM-EDTA on inactivation of I_{Ca} in EGTA-dialysed cells. Because such a dialysing solution increased peak I_{Ca} (Table 2), $[Mg^{2+}]_i$ may be involved in the steady-state regulation of the availability of Ca^{2+} channels (White & Hartzell, 1988; Agus *et al.* 1989).

Whether the slow inactivation phase of I_{Ca} (τ_s) represents a Ca^{2+}-dependent or a

voltage-dependent process is unclear from our studies. In support of a Ca^{2+}-dependent process we found: (1) increased $[Ca^{2+}]_i$ buffering with citrate slightly prolonged τ_s (Table 3); (2) the shortest τ_s occurred at peak I_{Ca}; (3) there was no clear voltage dependence (in contrast to guinea-pig taenia caeci where inactivation was faster at potentials positive to +20 mV; Ganitkevich *et al.* 1987). On the other hand, in support of a voltage dependent inactivation: (1) Ba^{2+} did not significantly prolong τ_s (in contrast to guinea-pig ileum, Droogmans & Callewaert, 1986; see Fig. 8*B*); (2) inactivation still occurred with Ba^{2+}, Sr^{2+} and Na^+. Thus, although other studies in smooth muscle suggest that the slow phase of inactivation may be voltage dependent, our studies do not differentiate critically between the two possibilities.

Ionic selectivity of the Ca^{2+} channel

The ionic selectivity of the gastric myocyte calcium channel was also similar to the calcium channels in other smooth muscle cells and excitable cell types (Hagiwara & Byerly, 1981). We found the ionic permeability of the channel for Ba^{2+} to be greater than Ca^{2+} (Fig. 8). This finding is similar to that reported in guinea-pig and rabbit ileum, and guinea-pig taenia caeci (Droogmans & Callewaert, 1986; Ganitkevich *et al.* 1986; Ohya *et al.* 1986) and other types of smooth muscle (Jmari *et al.* 1986, in rat uterine smooth muscle). The calcium channel was also able to conduct Sr^{2+} but not Mg^{2+}. The permeability of Sr^{2+} equalled that of Ca^{2+} which is different from that reported for rabbit ileum (Ohya *et al.* 1986). This is consistent with the relative permeability of cations through the Ca^{2+} channel in other cell types (Hagiwara & Byerly, 1981). Finally, the ability of the Ca^{2+} channel to conduct Na^+ when $[Ca^{2+}]_o$ was reduced to less than micromolar concentrations (Fig. 9) is similar to that reported in rabbit ileum (Ohya *et al.* 1986) and uterine smooth muscle (Jmari *et al.* 1986). Thus, these findings suggest that within the gastrointestinal tract, calcium channels, although generally similar, may have subtle differences in their cationic permeability.

Ca^{2+} channel antagonists

The response of the guinea-pig gastric Ca^{2+} channel to organic Ca^{2+} channel blockers was similar to results described in other excitable cells but with some unique differences (Figs 11–13). Their voltage-dependent and, in some cases, use-dependent block are similar to that previously described in other smooth muscle cells (Terada, Kitamura & Kuriyama, 1987) and excitable tissues (Lee & Tsien, 1983; Tung & Morad, 1983; Uehara & Hume, 1985). The specific sensitivity of the gastric Ca^{2+} channel to calcium channel antagonists varied when compared to myocytes isolated from different gastrointestinal sites used in other studies. In myocytes from taenia caeci (Ganitkevich *et al.* 1986) and rabbit ileum (Ohya, Terada, Kitamura & Kuriyama, 1987), inward current was still seen in the presence of 5 and 3 μM-verapamil, respectively, whereas in our study, no I_{Ca} was seen in concentrations of D600 greater than 0·5 μM at either −40 or −80 mV holding potentials tested. In rabbit ileal myocytes, 1 nM-nisoldipine partially inhibited I_{Ca} (Ohya *et al.* 1986) whereas in our study, this concentration of nifedipine had no effect on I_{Ca}. The relative potency of antagonists on Ca^{2+} current also differed. In rabbit ileal myocytes, verapamil and diltiazem were similarly potent in blocking the Ca^{2+} channel, whereas in our study, diltiazem was a much weaker antagonist when

compared to D600. Thus, differences in species and/or gastrointestinal cell types may determine in part the response of the Ca^{2+} channel to Ca^{2+} antagonists.

Diltiazem and D600 also showed agonistic properties on the calcium current at low drug concentrations (Fig. 14). An agonist effect of these calcium channel modulators has been reported in frog atrial myocytes (Uehara & Hume, 1985), dorsal root ganglia cells (Scott & Dolphin, 1987) and guinea-pig ventricular myocytes (Brown, Kunze & Yatani, 1986). The mechanism for the action of these drugs in our experiments is yet unclear. Interaction with G-binding protein was suggested in dorsal root ganglion neurones for all three antagonists studied here (Scott & Dolphin, 1987). If this mechanism also applies to the action of D600 in gastric myocytes, it would be distinct from the mechanism of Ca^{2+} channel blockade which may occur at the outer surface of the membrane in visceral smooth muscle (Ohya *et al.* 1987). In guinea-pig ventricular myocytes, the agonist–antagonist effect of nitrendipine on Ca^{2+} current was dependent on membrane potential (Brown *et al* 1986). In our studies, diltiazem enhanced I_{Ca} at the more negative holding potential tested (-80 mV) but without as much potential dependence as seen in ventricular myocytes. Whether this potential-dependent mechanism applies also to the effect of different classes of Ca^{2+} channel antagonists on the visceral smooth muscle cells is not as yet clear.

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