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Quinidine blockade of the carbachol-activated nonselective cationic current in guinea-pig gastric myocytes

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1 In guinea-pig gastric myocytes isolated from the antral circular layer, stimulation of muscarinic receptors by carbachol (CCh) induces a cationic current (I_{CCh}) which is known as the main mechanism of depolarization induced by muscarinic stimulation.

2 We tested the effects of a number of ion channel blockers on I_{CCh} and focused upon quinidine which was a highly potent blocker. Externally applied quinidine suppressed I_{CCh} ($IC_{50}=0.25$ μM) in a reversible and voltage-dependent manner. Applied internally, quinidine was about 100 times less potent than when applied externally. Persistent activation of G-protein by GTPγS also induced a cationic current similar to I_{CCh} and this current was also blocked by quinidine. 4-Aminopyridine and tetraethylammonium also suppressed I_{CCh} in a dose-dependent manner ($IC_{50}=3.3$ mM and 4.1 mM, respectively).

3 Pretreatment with quinidine (2 μM) selectively blocked the acetylcholine (ACh)-induced depolarization which was recorded in the multicellular tissues by a conventional intracellular microelectrode technique.

4 Voltage-dependent K-currents were also suppressed by quinidine but in a higher concentration range ($IC_{50}=3$ μM). Quinidine, 10 μM, decreased the amplitude of the voltage-dependent Ca current to only a small extent (15% decrease at 0 mV). Quinidine, 2 μM, also suppressed only a minute proportion of the Ca-activated K current (11.1% decrease at 45 mV).

5 From these experiments, it is concluded that some organic agents known as K channel blockers are able to block the CCh-activated cation channel in a non-specific manner and among them, quinidine can be used as an effective blocker for I_{CCh} in guinea-pig gastric myocytes.

Keywords: Cationic current; muscarinic stimulation; quinidine; gastric myocyte

Introduction

Stimulation of muscarinic receptors by acetylcholine (ACh) or carbachol (CCh) induces nonselective cationic currents (I_{CCh}) in mammalian gastrointestinal myocytes and it is suggested that these cationic currents are responsible for depolarization or excitatory junction potentials induced by ACh (Benham *et al.*, 1985; Inoue *et al.*, 1987; Vogalis & Sanders, 1990). Although the ionic nature and voltage-dependence of ACh- or CCh-activated cationic currents have been investigated in various gastrointestinal smooth muscles (Inoue & Isenberg, 1990a,b,c; Vogalis & Sanders, 1990), reports of the effect of blocking agents on these currents are limited. Cd^{2+} and other divalent metal ions have been shown to have direct blocking activity for CCh- or ACh-activated cation channels (Inoue, 1991). An inhibitory effect of the organic agent, quinine (0.5 mM) on CCh-activated current in colonic myocyte was briefly shown (Lee *et al.*, 1993). Chen *et al.* (1993) have also reported that K channel blockers, a Ca-releasing agent (caffeine), and diphenylamine-2-carboxylate (DPC) derivatives exerted nonspecific blocking effects on CCh-activated cation channels in ileal myocytes. Nonspecific blocking effects of K channel blockers (quinine, 4-aminopyridine) or a Na channel blocker (amiloride) have been observed in Ca^{2+}-activated nonselective cation channels (NSC channels) of an insulinoma cell line (Sturgass *et al.*, 1987) or colon cell (Gögelein & Capek, 1990).

In guinea-pig gastric myocytes, we recorded CCh-activated cationic currents and also tested a number of organic agents used as well-known blockers for other ion channels on I_{CCh}. In this process, we found that several agents known as K channel blockers can also inhibit this I_{CCh}. We mainly focused upon the effects of quinidine as this agent was a highly effective blocker for I_{CCh}.

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Methods

Cell isolation

Guinea-pigs of either sex, weighing 300-350 g, were exsanguinated after stunning. The stomach was isolated and cut in the longitudinal direction along the lesser curvature in phosphate-buffered Tyrode solution. The antral part of stomach was cut and the mucosal layer was separated from the muscle layers. The circular muscle layer was dissected from the longitudinal layer with fine scissors and cut into small segments (2 x 3 mm). These segments were incubated in a medium modified from the Kraft-Brühe (K-B) medium (Isenberg & Klöckner, 1982) for 30 min at 4°C. Then, they were transferred into nominal Ca^{2+}-free physiological salt solution (PSS) containing 0.1% collagenase (Böhringer Mannheim or Wako), 0.05% dithiothreitol, 0.1% trypsin inhibitor and 0.2% bovine serum albumin and incubated for 15-25 min at 35°C. After digestion, the supernatant was discarded and softened muscle segments were transferred again into the modified K-B medium and single cells were dispersed by gentle agitation with a wide-bored glass pipette. Isolated gastric myocytes were kept in the modified K-B medium at 4°C until use. All experiments were carried out within 12 h of harvesting cells and performed at room temperature.

Measurement of membrane currents

Isolated cells were transferred to a small chamber (400 μl) on the stage of an inverted microscope (IMT-2, Olympus, Japan). The chamber was perfused with physiological salt solution (PSS, 2-3 ml min^{-1}). Glass pipettes with a resistance of 2-4 MΩ were used to make a gigaseal of 5-10 GΩ. Standard whole cell patch clamp techniques were used (Hamill *et al.*, 1981). The cell membrane capacitance was measured by using a ramp pulse (1V s^{-1}) from a holding potential of -60 mV and the value was used in Figure 2c.

An Axopatch-1C patch-clamp amplifier (Axon Instruments, Burlingame, U.S.A.) was used to record membrane currents and command pulses were applied using an IBM-compatible AT computer and pCLAMP software v.5.51 (Axon Instrument, Burlingame, U.S.A.). The data were filtered at 5 kHz and displayed on a digital oscilloscope (PM 3350, Phillips, Netherlands), a computer monitor, and a pen recorder (Recorder 220, Gould, Cleveland, U.S.A.).

Intracellular recording of the electrical activity

Muscle strips (2–3 mm wide, 10–12 mm long) from the proximal part of the antrum were cut parallel to the circular fibres, and mounted on a silicon rubber in a 2 ml horizontal chamber. The strip was pinned out at one end with tiny pins and the other end was connected to a force transducer. The strip was constantly perfused at a rate of 2–3 ml min^{-1} with CO_2/bicarbonate-buffered Tyrode solution. Electrical activity was recorded by means of glass microelectrodes filled with 3 M KCl (tip resistance of 40–80 MΩ) and drawn by a chart recorder (MX-6, Device Ltd, Britain).

Solutions

Phosphate-buffered Tyrode solution contained (in mM): NaCl 147, KCl 4, $MgCl_2$ 1, $CaCl_2$ 1.8, NaH_2PO_4 0.42, Na_2HPO_4 1.81, glucose 5.5, pH 7.3. CO_2/bicarbonate buffered-Tyrode solution contained (in mM): NaCl 116, KCl 5.4, $CaCl_2$ 1.5, $MgCl_2$ 1, $NaHCO_3$ 24, glucose 5 (pH 7.3–7.4, bubbled with 5% CO_2: 95% O_2). PSS contained (in mM): NaCl 135, KCl 5, $MgCl_2$ 1, $CaCl_2$ 1.8mM, glucose 5, HEPES (N-[2-hydroxyethyl]piperazine-*N'*-[2-ethanesulphonic acid]) 5, and the pH was adjusted to 7.3 by Tris. $CaCl_2$ was simply omitted in the ca-free PSS. Na^+ and K^+ were replaced by Cs^+ throughout the experiments recording CCh-activated current. Modified K-B solution contained (in mM): L-glutamate 50, KCl 50, taurine 20, KH_2PO_4 20, $MgCl_2$ 3, glucose 10, HEPES 10, EGTA (ethyleneglycol-*bis*(β-aminoethyl ether)-*N,N,N',N'*-tetraacetic acid) 0.5 and pH was adjusted to 7.3 with KOH. The pipette solution consisted of (in mM): CsCl 135, MgATP 5, Tris-GTP 0.5, di-Tris-creatine phosphate 2.5, Na_2 creatine phosphate 2.5, $MgCl_2$ 1, HEPES 5, EGTA 0.5; the pH was adjusted to 7.3 with Tris. 20 mM of CsCl in the pipette solution was replaced with TEA-Cl for Ca^{2+} channel current recording in order to block K currents completely. To record voltage-activated K current, the pipette solution was composed of (in mM): K-aspartate 100, MgATP 5, di-Tris-creatine phosphate 5, KCl 20, $MgCl_2$ 1, EGTA 10, HEPES 5; the pH was adjusted to 7.3 with KOH.

Drugs

Drugs used were carbachol, quinidine, tetraethylammonium (TEA), 4-aminopyridine (4-AP), niflumic acid, apamin, tetrodotoxin, glibenclamide, and GTPγS (all from Sigma). Niflumic acid was dissolved in dimethylsulphoxide and glibenclamide was dissolved in alcohol with alkaline pH and diluted. All other drugs were dissolved in distilled water and diluted in the experimental solution. pH was readjusted to 7.35 after addition of 4-AP to the bath solution.

Results

CCh-activated current (I_{CCh}) in gastric myocytes

CsCl-rich solutions were used both in the pipette and bath to record the CCh-activated current. As the membrane potential was consistently held at −20 mV, 50 μM CCh (the concentration producing an almost saturating response) induced I_{CCh} (Figure 1a). Externally applied CCh activated an inward current which reversed around 0 mV (Figure 1c). The reversal potential was not changed notably when we replaced external Cs^+ with Na^+, Li^+, K^+, or Rb^+, which indicates a non-selective channel permeability with respect to monovalent alkaline cations. The reversal potentials for 140 mM external Na^+, Li^+, K^+ or Rb^+ relative to 135 mM internal Cs^+ were 0.4±0.7 (mean±s.e., n=22), −4.8±0.2 (n=5), 3.5±0.6

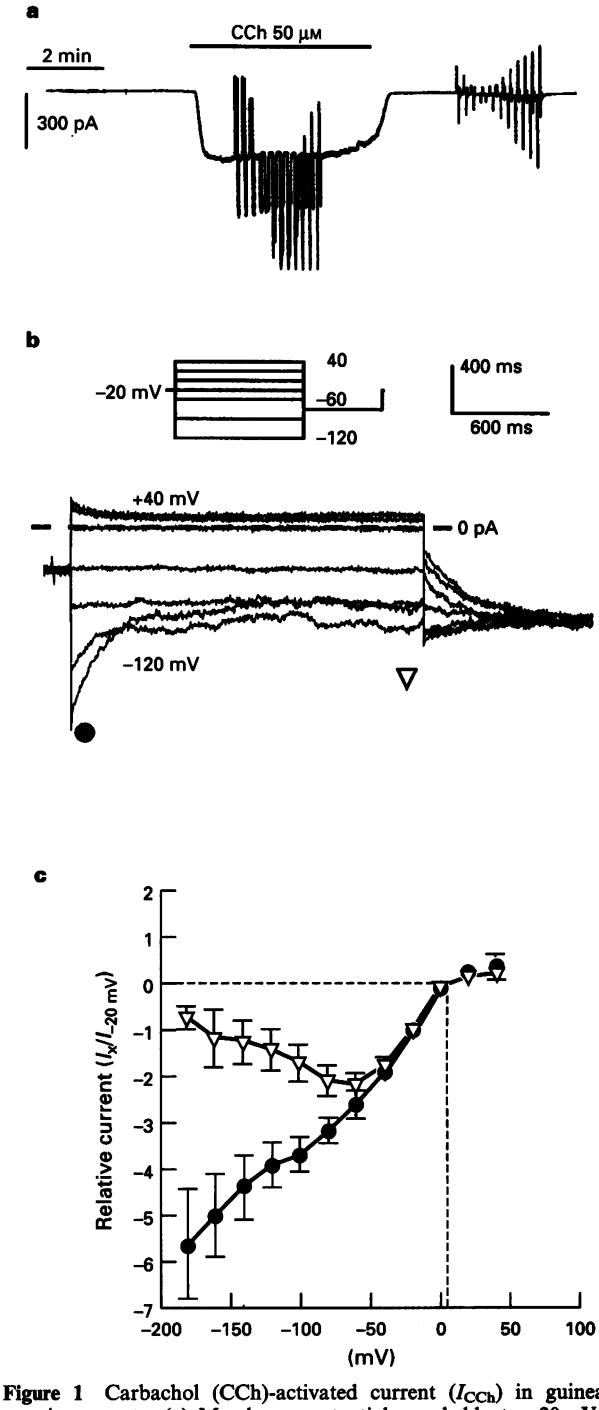

Figure 1 Carbachol (CCh)-activated current (I_{CCh}) in guinea-pig gastric myocyte. (a) Membrane potential was held at −20 mV and depolarizing or hyperpolarizing step pulses were applied in the presence and absence of CCh. Slowly declining inward current was induced by CCh. (b) Current responses at various potentials are traced as difference currents. Initial peak currents (●) relaxed to steady state values (▽) at negative potential range. (c) The current-voltage relationships of I_{CCh} are plotted (mean±s.e., n=8). The steady-state current values (▽) decrease as membrane becomes more hyperpolarized while the initial peak values (●) show linear *I-V* relationship. Numbers of vertical intercepts are current values normalized relative to the absolute value of inward current at −20 mV of each cell.

($n=4$) and 3.1 ± 0.6 ($n=9$), respectively. Permeability ratios were calculated from the measured reversal potentials (E_{rev}) using the modified Goldman-Hodgkin-Katz equation for biionic conditions (equation 1).

$$P_A/P_B = [B]_i/[A]_o \exp(E_{rev}F/RT) \quad \text{(equation 1)}$$

The relative permeability ratio was 1.1 : 1.1 : 1 : 0.98 : 0.80 for Rb^+: K^+: Cs^+: Na^+: Li^+. An anionic component seemed not to be present as there was a negligible shift of the reversal potential of I_{CCh} when extracellular concentrations of chloride ion were changed by isethionate. The reversal potentials for CsCl and caesium isethionate were 1.1 ± 0.6 ($n=4$) and 0.6 ± 1.4 ($n=4$), respectively (t test, $P>0.05$).

I_{CCh} recorded in this study showed rather sustained responses, and oscillations of cationic currents reported in guinea-pig ileal myocytes (Komori *et al.*, 1993) were not observed. This difference may have resulted from the concentration of Ca-buffering agent; our Ca-buffering agent used in the pipette solution ($[EGTA]_i = 0.5$ mM) was higher than that used by Komori *et al.* (1993) ($[EGTA]_i = 0.05$ mM).

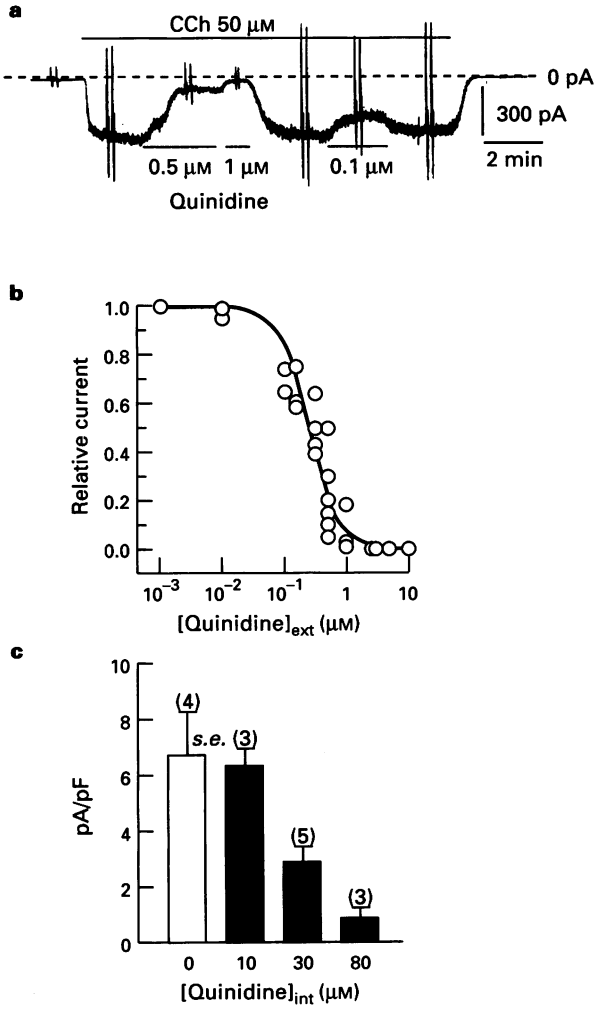

Figure 2 Effects of quinidine on carbachol (CCh)-induced current (I_{CCh}). (a) I_{CCh} was reversibly suppressed by submicromolar concentrations of quinidine. (b) Dose-dependence of this inhibitory effect is shown and fitted using non-linear regression equation ($1/\{1+([\text{quinidine}]/IC_{50})^n\}$). The half-inhibitory dose (IC_{50}) was 0.25 μM and coefficient (n) was 1.8 (obtained from 20 different cells). (c) Quinidine was added at various concentrations (10, 30, 80 μM) in the pipette solution and the inward currents induced by 50 μM of CCh were normalized to the membrane capacitance measured and compared (mean ± s.e., $n=3-5$; open column; control; solid column: pipettes containing quinidine).

Various depolarizing or hyperpolarizing step pulses were applied in the presence and absence of CCh. CCh-activated currents (I_{CCh}) were obtained by subtracting the control currents from the currents recorded in the presence of CCh. Time-dependent relaxation of inward currents was seen at negative membrane potentials lower than −40 mV (Figure 1b). The steady-state current-voltage (I-V) relationship of I_{CCh} showed inward rectification at around +15 mV and a negative slope conductance at negative potential range (< −40 mV, Figure 1c). All these properties are similar to the reports about non-selective cation channels activated by muscarinic stimulation in other gastrointestinal smooth muscle cells (Benham *et al.*, 1985; Inoue & Isenberg, 1990b; Sims, 1992).

Effects of quinidine on I_{CCh}

As shown in Figure 2a, I_{CCh} was dose-dependently suppressed by externally-applied quinidine at concentration ranges of 0.1–2 μM. This effect was fully reversible. The rather slow development of suppression can be attributed to a superfusion delay. The dose-dependency of the inhibitory effects of quinidine on I_{CCh} is shown in Figure 2b. The degree of inhibition was estimated as the ratio of remaining current to the control current at −20 mV. The curve was drawn by a non-linear regression equation ($1/\{1+([\text{quinidine}]/IC_{50})^n\}$) with a coefficient ($n$) of 1.8 and a half-inhibiting concentration of quinidine (IC_{50}) of 0.25 μM from 20 different cells. It was tested whether quinidine can also block the nonselective cation channel from the internal side of the membrane. Quinidine was added at various concentrations in the pipette solution (10, 30, 80 μM). Five to ten minutes after making a whole-cell configuration, CCh was applied to the bath solution. Values of I_{CCh} in the control and in the presence of quinidine applied internally were normalized to the membrane capacitance and are compared in Figure 2c. Quinidine applied to the cytoplasmic side apparently had only a minor effect until its concentration reached 10 μM, and much higher concentrations of quinidine were needed to induce a significant suppression of I_{CCh}.

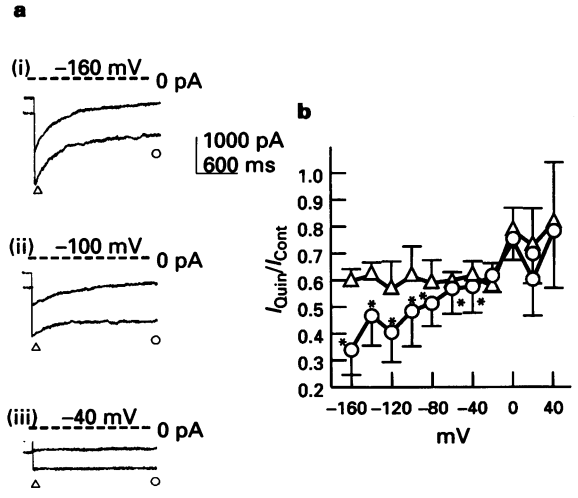

Figure 3 Voltage-dependent effect of quinidine on carbachol (CCh)-induced current (I_{CCh}). (a) The step pulses to −160 (i), −100 (ii) and −40 mV (iii) were applied for 1.8 s from a holding potential of −20 mV. I_{CCh} was partially suppressed by 0.3 μM of quinidine. (b) The ratios of peak (△) and sustained (○) currents (measured at the end of each step pulse) to those of control currents were plotted against membrane potential (mean ± s.e., $n=4$). The degrees of inhibition for sustained current at a given membrane potential were compared with that at −20 mV. Statistical significance was determined using the paired t test. The asterisks indicate $P<0.05$.

To inspect the effect of quinidine more precisely, the membrane potential was stepped to depolarized or hyperpolarized levels during treatment with 0.3 μM quinidine. Representative traces are shown in Figure 3a. The amplitude of the currents gradually decreased in the presence of 0.3 μM quinidine at −160 mV or −100 mV, while it remained constant at −40 mV. The ratio of peak and sustained currents after treatment with quinidine to those of control currents are plotted in Figure 3b. It is evident that the degree of inhibition for the sustained values at a given membrane potential is reduced as the membrane is depolarized and the ratio of the degree of inhibition at −160 mV over that at −20 mV is 1.9±0.2 (mean±s.e., $n=4$) for 0.3 μM quinidine.

Effects of quinidine on GTPγS-induced current

It was reported that muscarinic stimulation activates non-selective cation channels through the activation of GTP-binding protein (G-protein) mediated signal transduction (Inoue & Isenberg, 1990c; Komori *et al.*, 1992). Analogues of GTP resistant to hydrolysis (guanosine 5-O-(γ-thio) triphosphate (GTPγS) or guanosine 5'-(β,γ-imido) triphosphate (GPPNHP)) combine with the α-subunit of G-protein, converting it to a permanently activated state. Another analogue (guanosine 5-O-(β-thio) diphosphate (GDPβS)) is thought to compete with GTP for binding on the G-protein, resulting in irreversible inhibition.

We found that intracellular application of 200 μM GTPγS from the patch electrode slowly induced an inward current. The current increased with time and saturated within 15–20 min. In this state, the inward current could no longer be enhanced by a simultaneous application of 50 μM CCh (Figure 4a). Moreover, with GDPβS (0.3 mM) in the pipette, no or only a minimal inward current could be activated by externally applied CCh ($n=5$, data not shown). This GTPγS-induced current showed time-, and voltage-dependent characteristics that are very similar to I_{CCh} (Figure 4b,c). GTPγS-induced inward currents were also sensitivity blocked by quinidine as shown in Figure 4a (54±4.4% suppression by 0.3 μM of quinidine at −20 mV, $n=5$).

Effects of quinidine on depolarization induced by muscarinic stimulation

From results shown above, it was suggested that quinidine can be an effective blocker of I_{CCh} in a concentration-range be-

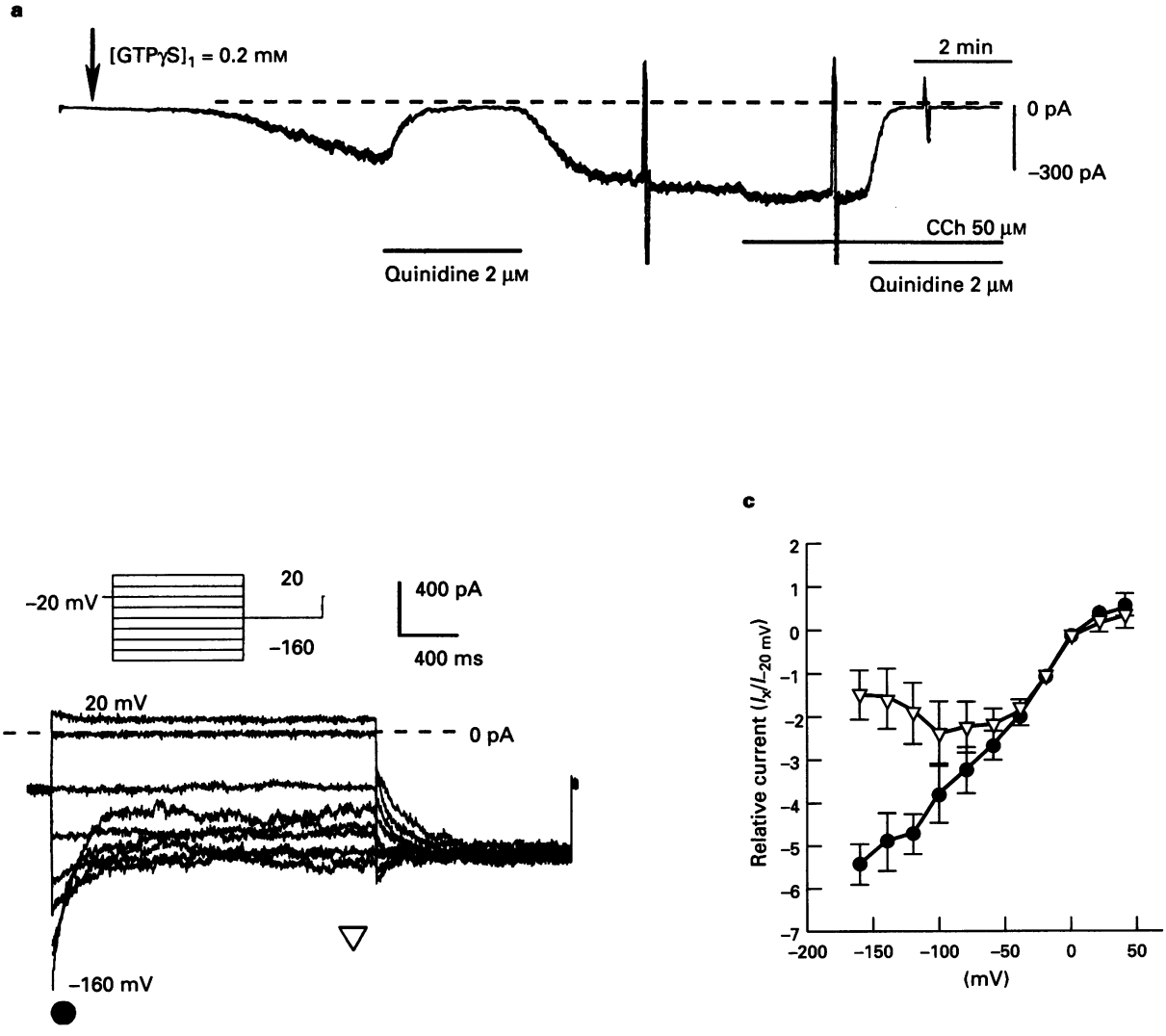

Figure 4 Effects of quinidine on GTPγS-induced current. (a) GTPγS (0.2 mM) was added in the pipette solution. Slowly increasing inward current was induced 2 min after making whole-cell configuration ($V_h=-20$ mV). The application of carbachol (CCh, 50 μM) had minimal effect when this inward current had reached a steady-state. Responses of currents to step voltage pulses (b) or their *I-V* relationships (c) are very similar to those induced by CCh (see Figure 1). Symbols in (b) and (c) (● and ▽) represent initial peak current and steady state current, respectively.

tween 1 and 2 μM. We therefore tested whether quinidine was able to block the depolarization induced by ACh without itself affecting membrane potential or slow waves. Membrane potential was measured by conventional intracellular microelectrode techniques and isometric contraction was also recorded by a force transducer connected to one end of the muscle strip. A brief application of ACh (2 μM) resulted in transient depolarization (Figure 5a). The amplitude of depolarization was measured from the dotted line (5.1 ± 1.4 mV, $n=6$). In Figure 5b, the same muscle strip was pretreated with quinidine (2 μM) and the shape of slow waves as well as resting membrane potential were not affected by quinidine only. In this condition, addition of ACh could not depolarize the membrane while the contractile response still occurred. Similar results were obtained in another 3 preparations.

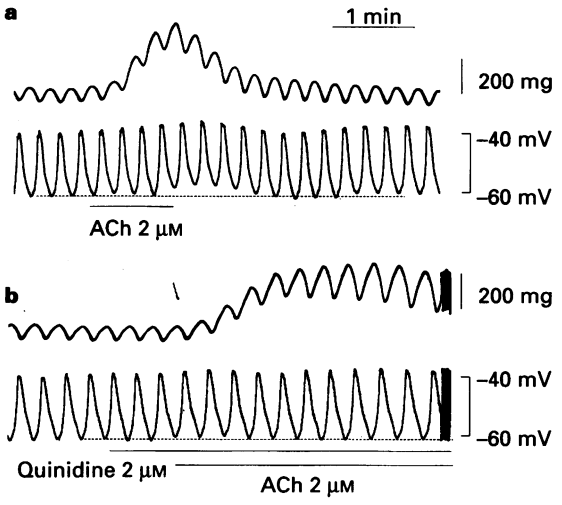

Figure 5 Effects of quinidine on depolarization induced by muscarinic stimulation. (a) The isometric contraction (upper trace) and membrane potential (lower trace) were recorded simultaneously by the intracellular microelectrode technique. Brief application of acetylcholine (ACh 2 μM) induced transient depolarization (5 mV from resting membrane potential (dotted line)) and potentiated contraction. (b) In the same strip of muscle, the membrane potential and contraction were not affected by quinidine (2 μM) itself but the ACh-induced depolarization was selectively blocked by quinidine.

Effects of other ion channel blockers on I_{CCh}

I_{CCh} was also suppressed by other K channel blockers, 4-aminopyridine (4-AP) or tetraethylammonium (TEA). However, the inhibitory effects of these drugs were much less potent than that of quinidine (Figure 6, $IC_{50}=3.3$ mM and 4.1 mM for 4-AP and TEA, respectively). We also tested a number of ion channel blockers (apamin (1 μM), glibenclamide (20 μM), tetrodotoxin (1 μM), and amiloride (100 μM)) that were ineffective ($n=2-3$ respectively, data not shown). Niflumic acid showed a partial inhibitory effect at 10 μM (24 ± 5% decrease at −20 mV, $n=3$). Blocking effects of diphenylamine-2-carboxylate (DPC) derivatives (including niflumic acid) on cation channels have been reported in other cells (Gögelein *et al.*, 1990; Chen *et al.*, 1993).

Effects of quinidine on voltage-activated Ca and K currents

To evaluate the selectivity of quinidine as a blocker of I_{CCh}, effects of quinidine on voltage-activated Ca or K currents were investigated. To record voltage-activated Ca currents, 20 mM of internal Cs was replaced with TEA and the external solution was changed to normal Tyrode solution.

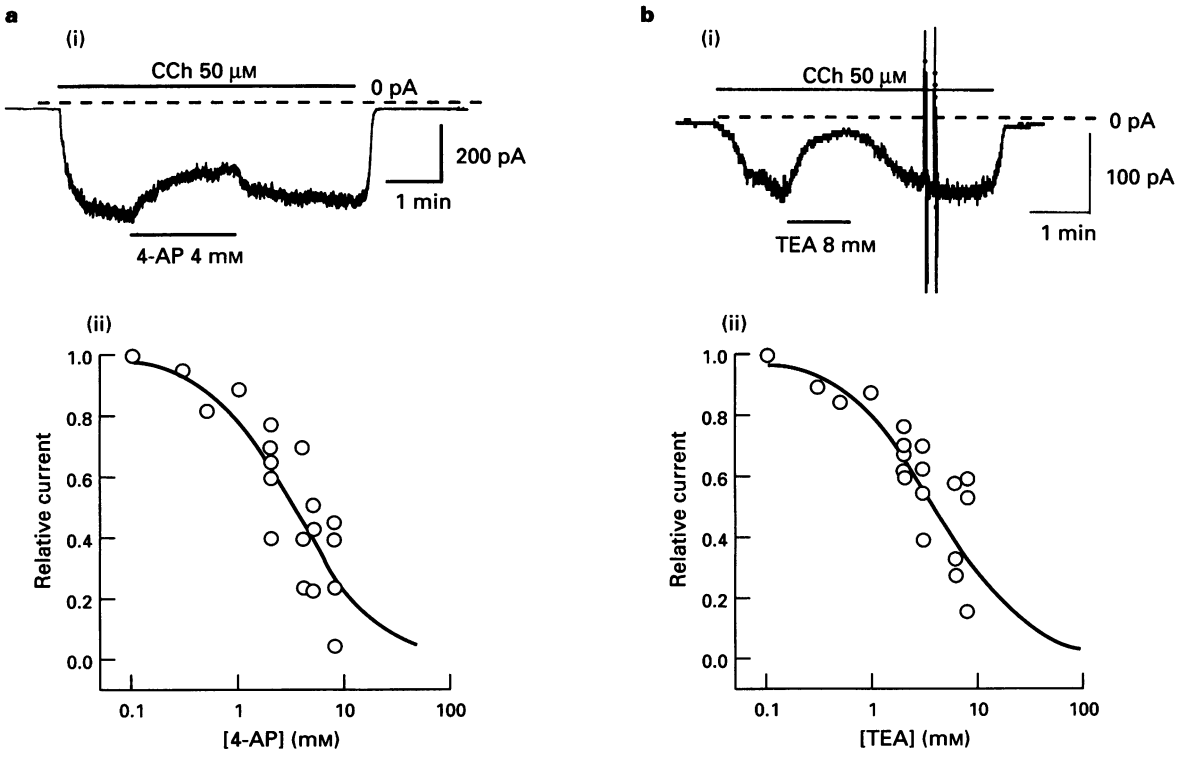

Figure 6 Effects of 4-aminopyridine (4-AP) and tetraethylammonium (TEA) on I_{CCh}. I_{CCh} (at −20 mV) was inhibited by millimolar concentrations of 4-AP (a) or TEA (b) applied extracellularly in a dose-dependent manner. The continuous line in (aii) and (bii) were fitted by the non-linear regression equation shown in Figure 2. IC_{50}s were 3.3 mM and 4.1 mM, respectively and coefficients (n) were 1.1 and 1.0, respectively.

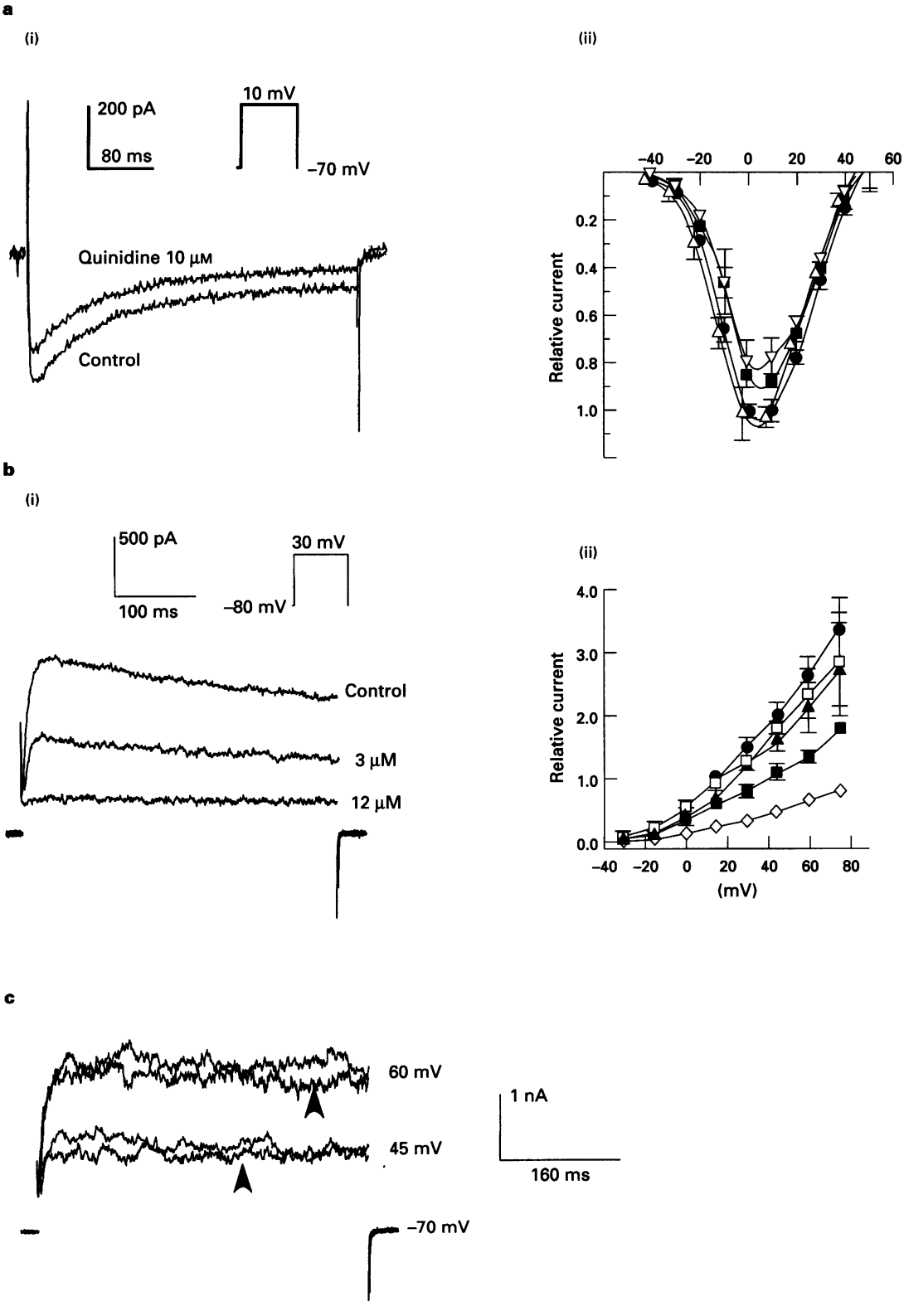

Figure 7 Effects of quinidine on voltage-activated Ca and K currents in gastric myocytes. (a) Calcium currents were elicited by depolarizing pulses from −70 mV and Cs, TEA-Cl solution in the pipette. The effects of quinidine in bath solution on Ca currents at various concentrations (1, 5, 10 μM, $n=3$) are compared in (ii). In (aii) symbols indicate (▽) 10 μM; (■) 5 μM and (●) 1 μM quinidine; (△) control. (b) Voltage-activated K currents were evoked by depolarizing pulses using high EGTA (10 mM), KCl solution in the pipette. Quinidine suppressed these outward currents in a dose-dependent manner and the half-inhibitory concentration was about 3 μM ($n=4$ for 1, 2 and 3 μM, $n=3$ for 12 μM). In (bii) symbols indicate (□) 1 μM; (▲) 2 μM; (■) 3 μM and (◇) 12 μM quinidine; (●) control. (c) Using KCl solution in the pipette where pCa was clamped at 6.5, large outward currents were evoked by depolarizing pulses. Quinidine (2 μM, arrow head) had a weak inhibitory effect on these currents (11 ± 1.3% decrease at +45 mV, $n=5$).

Membrane potential was held at −70 mV and stepped to various depolarizing levels. As can be seen in Figure 7a, 10 μM quinidine was able to decrease the amplitude of the Ca current by only a small proportion. Figure 7b shows the effects of quinidine on voltage-activated K currents. KCl-rich, Ca^{2+}-free solution with 10 mM EGTA was used as the pipette solution. In this condition, quinidine showed quite prominent and dose-dependent suppression of outward currents ($n=4$). The IC_{50} was about 3 μM. In Figure 7c, the intracellular Ca^{2+} concentration was raised and clamped (pCa = 6.5) by adding an appropriate concentration of Ca^{2+} to the pipette solution according to Fabiato & Fabiato (1979). In this condition, depolarizing pulses induced much larger currents that presumably included Ca-activated K currents in a large proportion. Quinidine, 2 μM, could suppress only a minute proportion of the outward current (11.1 ± 1.3% decrease at 45 mV, $n=5$) and these results suggest that Ca-activated K channels are not much affected by quinidine at this concentration. A much higher concentration of quinidine was needed to block the Ca-activated K channel with large conductance (BK_{Ca}) in other reports (>200 μM in guinea-pig gastric myocyte, Wong, 1989).

Discussion

The actions of agents tested on I_{CCh} in gastric myocytes can be summarized as follows: (1) Quinidine, 4-AP and TEA suppress I_{CCh} in a dose-dependent manner, with quinidine as the most potent agent. (2) Quinidine seems to act directly on the cation channel mainly from the external side of membrane, and in a voltage-dependent manner.

Quinidine is frequently used as a class I antiarrhythmic agent in the management of cardiac rhythm disturbances. Previously, the major effect of quinidine was thought to be a voltage- and frequency-dependent inhibition of the sodium current (70% inhibition by 20 μM, Lee *et al.*, 1981). In addition, the delayed rectifier type K current (80% decrease by 6.7 μM in vetricular myocyte, Hiraoka *et al.*, 1986), the transient outward current ($IC_{50} \cong 7$ μM, Imaizumi & Giles, 1987) and the Ca inward current (70% decrease by 6.7 μM in ventricular myocyte, Hiraoka *et al.*, 1986; 30% decrease by 6.7 μM in A-V nodal cell, Nishimura *et al.*, 1987) in cardiac myocytes have been shown to be blocked by quinidine. In gastrointestinal smooth muscle, quinidine inhibited nonselectivity the inward Ca current and voltage-dependent K current (about 20 and 25% decrease at 3 μM respectively, Nakao *et al.*, 1986), and the activity of the BK_{Ca} channel (dissociation constant >200 μM in guinea-pig gastric myocyte, Wong, 1989). Quinine, the stereoisomer of quinidine, was reported to be a blocker for the Ca-activated K channel in red blood cells (200–1000 μM, Burgess *et al.*, 1981).

In this study, the inhibitory effect of quinidine on I_{CCh} in guinea-pig gastric myocyte ($IC_{50} = 0.25$ μM) was much more potent than any of the above reports. In guinea-pig gastric myocytes, quinidine suppressed the voltage-activated K current with a potency ten times lower than that on I_{CCh}. As can be seen in Figure 2, 1.5–2 μM quinidine suppressed I_{CCh} almost completely whereas only 15–20% of K currents were suppressed (Figure 7b). Quinidine also suppressed the voltage-activated Ca current but much less potently (15–20% block at 10 μM, Figure 7a). Relatively selective effect of quinidine on I_{CCh} could be seen more clearly in the experiment recording membrane potential, where 2 μM quinidine almost completely blocked ACh-induced depolarization whereas quinidine itself had no or only a minute effect on membrane potential and contractility (Figure 5).

The signal transduction pathway between muscarcinic receptor activation and ion channels has been studied on myocytes in guinea-pig ileum (Inoue & Isenberg, 1990a; Komori *et al.*, 1992). It has been found that when ACh binds to the muscarinic receptor the information is transferred to the channel protein through a pertussis toxin-sensitive GTP-binding protein. Substances affecting neurotransmitter-operated channels like this CCh-activated channel may act at any of the steps between ligand-receptor binding and channel gating. However, indirect interference of quinidine with muscarinic receptor activation or interference with activation of the G-protein by the receptor is not likely because: (1) quinidine potently suppressed the GTPγS-activated current (Figure 4) and (2) quinidine acts on the channel mainly from the external side of membrane (Figure 2). Although the time course of the development of the inhibitory effect was slower than those of K channel blockers in ileal myocytes (Chen *et al.*, 1993; 100 ms for full effect), this delay seemed to be due to the superfusion system.

Chen *et al.* (1993) have also reported effects of well known ion channel blockers on muscarinic receptor-activated cationic current in guinea-pig ileal myocytes from the longitudinal layer. They tested a number of K^+ channel blockers (TEA, 4-aminopyridine, procaine and quinine) and DPC derivatives (DPC, 3',5'-dichloro DPC, flufenamic acid and niflumic acid). According to their results, quinine showed the most potent effect ($IC_{50} \cong 1$ μM) on the cationic current and seemed to interact directly with the cation channel. In our experiments, quinidine was also found to be highly potent ($IC_{50} = 0.25$ μM), and rather selectively acted on I_{CCh} in these gastric myocytes. Inhibitory effects of 4-AP and TEA were also observed in our experiments, although their effective concentrations overlap with those typically used for K channel blockade. It has also been reported that 4-AP applied to the cytoplasmic side of membrane had blocking effects on a nonselective cation channel from rat insulinoma cell line (Sturgass *et al.*, 1987).

The voltage-dependence of the degree of inhibition by quinidine (almost two fold more inhibited at −160 mV than at −20 mV, Figure 3b) was similar to those reported for TEA, 4-AP, caffeine and quinine in ileal myocytes (Chen *et al.*, 1993; see Table 1 in that paper). These authors suggested a blocking mechanism of these organic agents for the muscarinic receptor-activated cation channel. According to their suggestion, positively charged large organic molecules can be driven into the orifice or attracted into the middle of the cation channel along the transmembrane potential gradient to interfere with cation permeability. Such blockade tends to be voltage-dependent because the interference would be relieved by depolarization due to the reduction of transmembrane electrochemical gradient. Further studies of the blocking mechanism and properties of these agents, considering their chemical structures and molecular sizes, may give some clues about the nature of nonselective cation channels activated by CCh.

In conclusion, our results suggest a novel and important effect of quinidine: a quite selective and potent blockade of a nonselective cation current induced by muscarinic stimulation in the guinea-pig gastric myocyte. Quinidine can be a useful pharmacological tool in the study of gastrointestinal physiology.

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