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Effects of diltiazem on electrical responses evoked spontaneously or by electrical stimulation in the antrum smooth muscle cells of the guinea-pig stomach

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1 In circular smooth muscle cells of the guinea-pig stomach (antrum), diltiazem (10^{-6}–10^{-5} M) blocked the overshooting spike potential generated either spontaneously or by electrical stimulation in the presence of 2 mM tetraethylammonium chloride, but did not block the slow wave and the abortive spike potential.

2 The membrane was depolarized by high concentrations of diltiazem (more than 3×10^{-6} M), and this depolarization was associated with an increase in the membrane resistance.

3 The interval between slow waves was shortened to about 0.90 times the control (14.7 s) by 10^{-5} M diltiazem.

4 Transmural nerve stimulation evoked an inhibitory junction potential (i.j.p.) and enhanced the subsequently generated slow wave. Tetrodotoxin (3×10^{-7} M) blocked both responses but atropine (10^{-6} M) blocked only the latter.

5 Diltiazem (more than 10^{-6} M) increased the amplitude of the i.j.p. and depressed the enhancement of the slow wave produced by transmural nerve stimulation, presumably due to depolarization of the membrane. The latency for the i.j.p. remained the same in the presence of diltiazem (10^{-5} M).

6 It is concluded that in the guinea-pig stomach, diltiazem blocks Ca-influx during the generation of the overshooting spike potential, but not the Ca-influx related to generation of the abortive spike potential or the slow wave. The cholinergic excitatory and the non-adrenergic, non-cholinergic inhibitory transmission may not be much affected by diltiazem.

Introduction

Diltiazem dilates vascular smooth muscle tissues and reduces cardiac force development due to suppression of Ca-influxes during excitation of muscles, and these actions of diltiazem are much the same as those of so-called Ca antagonists or Ca-channel blockers such as nifedipine, nisoldipine and verapamil (Fleckenstein, 1983; Stone & Antman, 1983). The dilator actions of diltiazem have been studied mainly on the cardiovascular system due to the clinically important uses of this drug, i.e., hypertension, cardiac ischaemia or coronary spasm is protected or prevented by diltiazem (Fleckenstein, 1983; Stone & Antman, 1983). Blockade of Ca currents by diltiazem has also been found in visceral smooth muscles, e.g., in the guinea-pig taenia-coli, diltiazem blocks the generation of spontaneous or evoked spike potentials without a marked change in the membrane potential (Magaribuchi *et al.*, 1977).

The antrum smooth muscles of the guinea-pig stomach generate two types of electrical response, a slow wave and a spike potential (Tomita, 1981). These electrical responses may be generated primarily by an increase in Ca permeability of the membrane (Ohba *et al.*, 1977; Tomita, 1981). We studied the effects of diltiazem on electrical responses evoked spontaneously or by electrical stimulation in this tissue. The experiments were carried out to observe whether diltiazem has any selectivity in blocking the Ca influxes associated with the electrical responses as is the case with verapamil which blocks the spike potential but not the slow wave (Golenhofen & Lammel, 1972).

Differential effects of Ca antagonists on autonomic nerves and smooth muscle have been reported in visceral smooth muscle tissues including vascular tissues, i.e., verapamil blocks electrical or mechanical responses of smooth muscle cells at concentrations below those affecting transmitter release from nerve terminals (Haeusler, 1972). However, diltiazem blocks

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transmitter release from adrenergic nerves in the guinea-pig vas deferens (Tajima *et al.*, 1980) and the mesenteric artery (Suzuki *et al.*, 1982), when the amount of released transmitter is estimated from the amplitude of the excitatory junction potential. In the guinea-pig stomach antrum, stimulation of intramural nerves generates a non-adrenergic, non-cholinergic inhibitory potential (Holman, 1970; Kuriyama, 1981) followed by an enhancement of the subsequent slow wave, and an effect on the cholinergic transmission can be estimated from changes in the latter component (Komori & Suzuki, 1985). Experiments were also carried out to observe the effect of diltiazem on the inhibitory potential and the increased amplitude of the slow waves due to transmural nerve stimulation in the antrum smooth muscle of the guinea-pig stomach.

Methods

Albino guinea-pigs of either sex, weighing 200–250 g, were stunned and bled. The stomach was excised and cut in the longitudinal direction along the greater curvature. The contents of the stomach and mucosal layer were removed in Krebs solution at room temperature. The circular muscle strips, 1–1.5 mm wide and 10–15 mm long, were dissected from the antrum region. The tissue was mounted with fine needles on a rubber plate fixed at the bottom of an organ bath which was made from Lucite plate and had a capacity of about 2 ml. The tissue was superfused with warmed (35.5°C) Krebs solution at a flow rate of 2–3 ml min^{-1}.

Electrical responses of smooth muscle cells were recorded by glass capillary microelectrode filled with 3 M KCl. The tip resistance of the electrode ranged between 40–80 MΩ. The electrode was inserted from the mucosal side of the tissue, and the electrical responses were displayed on a pen-writing recorder (Nihon Kohden Recticorder RJG4024). Electrotonic potentials were produced by the partition stimulating method (Abe & Tomita, 1968). Field stimulation was applied to the muscle to stimulate intramural nerves using the point stimulation method, i.e., a silver wire (0.3 mm in diameter) was gently attached to the surface of the muscle and brief anodal current pulses (0.05–0.3 ms in duration, 10–50 V in intensity) were supplied by an electric stimulator (Nihon Kohden SEN7103). Electrical responses produced by nerve stimulation were recorded from the surrounding areas of the silver wire (usually within 300 μm).

The ionic composition of the Krebs solution was as follows (mM): Na^+ 137.4, K^+ 5.9, Mg^{2+} 1.2, Ca^{2+} 2.5, HCO_3^- 15.5, $H_2PO_3^-$ 1.2, Cl^- 134, glucose 11.5. The pH of the solution was kept at 7.2–7.4 by gassing with 97% O_2 plus 3% CO_2.

Drugs used were: diltiazem (Tanabe), tetrodotoxin and atropine sulphate (Sigma), tetraethylammonium chloride and guanethidine sulphate (Tokyo Kasei), phentolamine mesylate (Ciba-Geigy) and propranolol-HCl (Sumitomo).

Experimental values were expressed as means ± s.d. Statistical significances were evaluated by Student's *t* test, and probabilities of less than 5% ($P < 0.05$) were considered significant.

Results

Effects of diltiazem on spontaneous electrical activity

Smooth muscle tissues isolated from the antrum region of the guinea-pig stomach showed spontaneous oscillatory potentials (slow waves) and, in many tissues isolated from regions closer to the pylorus, spike potentials were superimposed on the slow wave (Tomita, 1981). The spontaneous electrical activities were classified into three types: (1) slow waves which

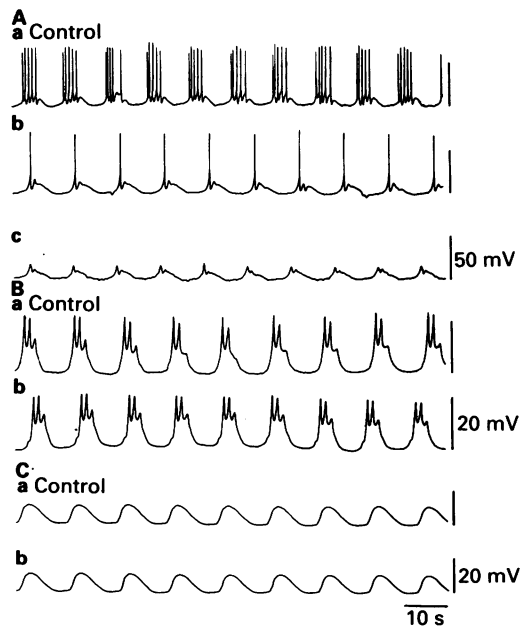

Figure 1 Effects of diltiazem on spontaneous electrical responses recorded from circular smooth muscle cells of the guinea-pig antrum. (A) Spike potentials with overshoot potentials were generated on the slow wave. Responses were recorded before (a, control) and after application of 10^{-6} M diltiazem (b, 7 min; c, 20 min). (B) Slow waves accompanied by abortive spike potentials; (a) control (b) the effect of diltiazem 10^{-5} M, 9 min. (C) Slow waves with smooth potential change; (a) control (b) effect of diltiazem 10^{-5} M, 14 min. Traces (A) (B) and (C) were recorded from different tissues.

were accompanied by the generation of spikes with an overshoot potential at the top (Figure 1Aa), (2) slow waves which were accompanied by abortive spike potentials with a long duration (0.5–1.0 s) and with no overshoot potential (10–30 mV in amplitude) (Figure 1Ba), and (3) slow waves with a smooth potential change (Figure 1Ca).

Figure 1 shows the effects of diltiazem on these types of spontaneous electrical activity recorded from circular muscles of the antrum region of the guinea-pig stomach. Diltiazem (10^{-6}–10^{-5} M) blocked the overshooting spike potential; this inhibitory effect of diltiazem was slow in onset. The first response to diltiazem was a reduction in the number of spike potentials superimposed on each slow wave (Figure 1Ab) and then the spike potential ceased within 15–20 min after the application of diltiazem (Figure 1Ac). The generation of the slow wave with abortive spike potential was still apparent, for up to 60 min after the complete inhibition of the overshooting spike potential by diltiazem (10^{-6}–10^{-5} M; Figure 1Bb). The slow waves with a smooth potential change were not altered by diltiazem (10^{-6}–10^{-5} M) even after 60 min (Figure 1Cb). Increasing the concentration of diltiazem to 10^{-4} M reduced the amplitude of any type of slow wave, within 10 min, together with the associated depolarization of the membrane.

The interval between the generation of each slow wave varied between 11 s and 30 s, depending on the tissue. The mean interval between the slow waves recorded from different tissues was 14.7 ± 4.1 s ($n = 29$). However, when slow waves were recorded from individual cells, the interval was moderately constant and showed variations of less than 5% from the mean value. Slow waves were recorded from single cells for up to 20 min after the application of diltiazem (10^{-6}–10^{-5} M), and the effects of diltiazem on the interval between slow waves were expressed relative to that before application of the drug. The interval between slow waves was decreased to 0.90 ± 0.07 times ($n = 12$, $P < 0.05$) the control by 10^{-5} M diltiazem but was not significantly changed by 10^{-6} M diltiazem (0.97 ± 0.05 times the control, $n = 15$, $P > 0.2$).

Effects of diltiazem on membrane potential and membrane resistance

Figure 2 shows the effects of diltiazem on the membrane potential of circular muscle cells of the antrum, measured at the most negative part of the slow wave. Figure 2a shows the time course of the changes in membrane potential before, during and after application of 5×10^{-5} M diltiazem for 40 min. Diltiazem depolarized the membrane and the amplitude of the depolarization increased slowly and reached about 6 mV within 30 min. The depolarized membrane did not repolarize completely, after wash out of diltiazem for up to 60 min.

Such experiments were repeated in different tissues with various concentrations of diltiazem. The dose-response relationship of the effects of diltiazem on the steady amplitude of the depolarization in the circular muscle of the antrum is shown in Figure 2b. Diltiazem at concentrations above 3×10^{-6} M depolarized the membrane.

Changes in the membrane resistance of the stomach smooth muscle during the diltiazem-induced depolarization were estimated from the amplitude of electrotonic potentials produced by the partition stimulating method (Abe & Tomita, 1968). The electrotonic potential was recorded from cells located in close proximity to the stimulating electrode (usually within 0.2 mm). The length constant of the circular muscle of the antrum of guinea-pig stomach is about 1.4 mm (Kuriyama *et al.*, 1970), and therefore, changes in membrane resistance could be estimated from changes in the amplitude of electrotonic potentials, assuming that changes in the internal resistance in-

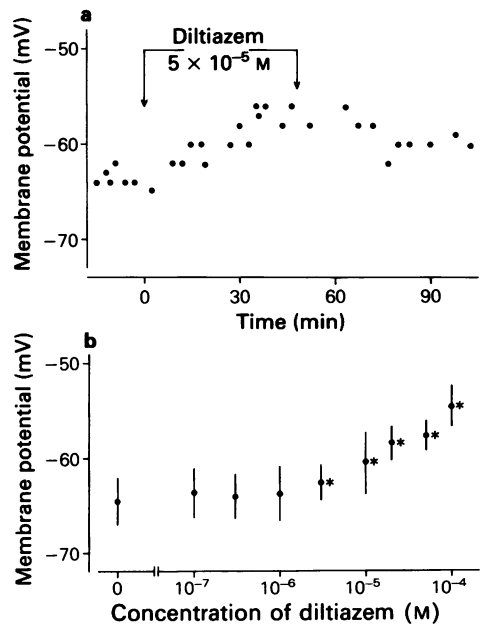

Figure 2 Effects of diltiazem on the membrane potential of circular smooth muscle cells of the antrum. (a) Time course of the change in the membrane potential after application of 5×10^{-5} M diltiazem for 40 min. Each point shows the membrane potential recorded by successive penetration of different cells by the electrode. (b) Dose-response relationship for the effects of diltiazem on the membrane potential. Each point shows mean, and vertical lines s.d. ($n = 20$–30), recorded from 2–3 tissues. * Significantly different from the control ($P < 0.05$).

cluding intercellular resistance are negligibly small due to the short distance between stimulating and recording electrodes (Hodgkin & Rushton, 1946). In 3 experiments, the amplitude of electrotonic potentials produced by inward current pulses (1 s duration) was increased during the diltiazem-induced depolarization. Figure 3 shows an example of such experiments, in which application of 10^{-4} M diltiazem for 10 min depolarized the membrane by about 4 mV and increased the amplitude of the electrotonic potentials produced by 4 different intensities of the current pulse. The current-voltage relationship showed a slope which was steeper after the membrane was depolarized by diltiazem (Figure 3c). In different experiments, the slope was still steeper than that of the control, even after the depolarized membrane was repolarized close to the resting membrane potential by applying a constant inward current.

These results suggest that in smooth muscle of the antrum, the diltiazem-induced depolarization is accompanied by an increase in the membrane resistance.

Effects of diltiazem on spike potentials evoked by a current pulse

Tetraethylammonium chloride (TEA) increases membrane resistance, enhances the amplitude and rate of rise of spike potentials in electrically active tissues, or induces spike generation in electrically quiescent tissues (Kuriyama, 1970). In smooth muscles of the guinea-pig stomach, TEA enhanced spike potentials or induced a generation of spike potentials in tissues which showed only slow waves (Ito *et al.*, 1970).

Figure 4 shows the effects of diltiazem on electrical responses of circular muscles of the guinea-pig antrum in the presence of 2 mM TEA. Application of 2 mM TEA generated spikes with an overshoot potential which was not seen before the application of TEA. Additional application of 10^{-6} M diltiazem reduced the amplitude of the spike potentials to 10–15 mV; however, occasionally spike potentials of amplitudes between 30–50 mV were observed, for up to 20 min. Increasing the concentration of diltiazem to 10^{-5} M completely blocked the generation of spike potentials within 5 min and only slow waves with abortive spike potentials were observed, similar to those seen before the application of TEA (Figure 4A). The inhibitory effects of diltiazem continued after wash out of diltiazem for up to 60 min.

Effects of diltiazem on the spike potential evoked by an outward current pulse (1 s in duration) in the presence of 2 mM TEA are shown in Figure 4B. In the control condition, this particular cell did not generate spike potentials accompanying depolarization of the membrane by up to 20 mV with a current pulse. TEA 2 mM increased the amplitude of electrotonic potentials produced by an inward current pulse (1 s duration) and generated spike potentials accompanying an outward current pulse. Application of 10^{-5} M dil-

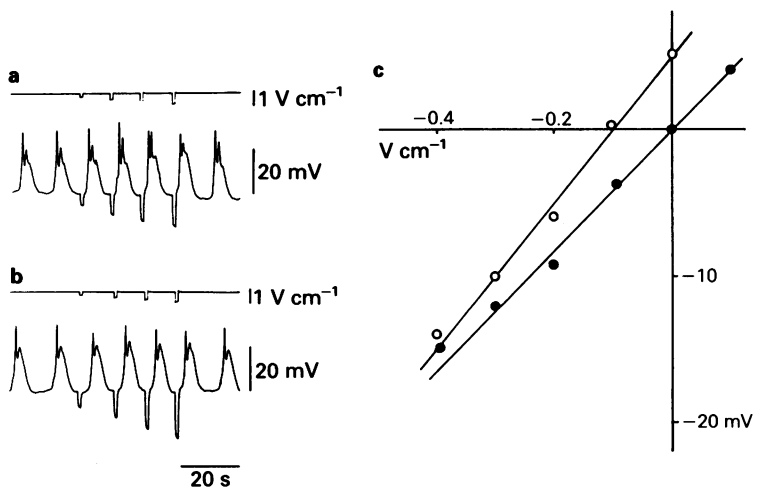

Figure 3 Electrotonic potentials produced by 4 different intensities of inward current pulse (1 s in duration) recorded from a smooth muscle cell of the stomach antrum, before (a, control) and after application of 10^{-4} M diltiazem for 10 min (b). (c) Current-voltage relationship. The amplitude of the electrotonic potential was plotted against the intensity of the current (shown by V cm^{-1}). Vertical axis: membrane potential change measured from the resting membrane potential (negative value indicates hyperpolarization). All the responses were recorded from the same cell which was located 0.15 mm from the stimulating electrode. (●) Control; (○) in the presence of 10^{-4} M diltiazem.

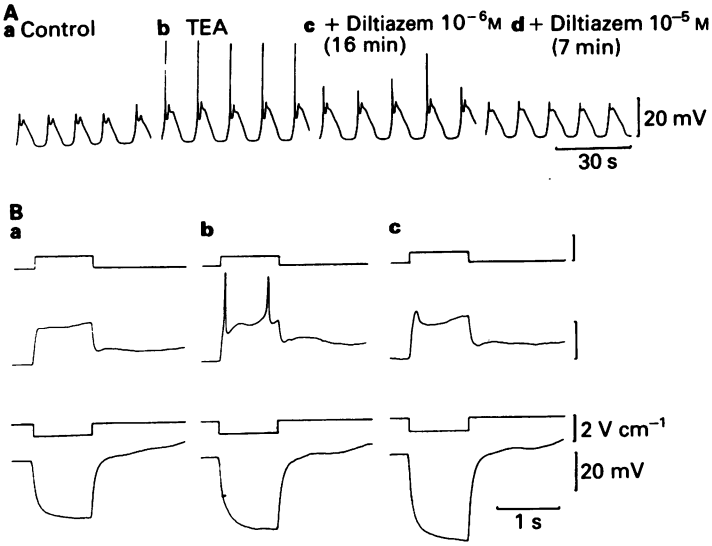

Figure 4 Effects of diltiazem on electrical responses of circular smooth muscle cells of stomach antrum in the presence of 2 mM tetraethylammonium (TEA). Spontaneous (A) and current-evoked electrical responses (B) were recorded from different cells. (B) Electrotonic potentials produced by the same intensity and duration (0.9 V cm^{-1}, 1 s) of outward (upper records) and inward current pulse (lower records) were recorded before (a, control), after application of 2 mM TEA (b) and after additional application of 10^{-5} M diltiazem for 12 min (c). In each record, upper and lower traces indicate current monitor and membrane potential change, respectively. Guanethidine (5×10^{-6} M), tetrodotoxin (3×10^{-7} M) and atropine (10^{-6} M) were present throughout.

tiazem, in addition to 2 mM TEA, blocked the generation of spike potentials induced by an outward current pulse and increased the amplitude of electrotonic potentials produced by an inward current pulse.

Effects of diltiazem on neuromuscular transmission

In smooth muscle cells from the antrum circular muscle of the guinea-pig stomach, transmural stimulation with a brief current pulse (0.05 ms in duration, 30 V in intensity), applied at the most negative part of a slow wave, evoked an inhibitory junction potential (i.j.p.) and then enhanced the amplitude of the subsequent slow wave (Figure 5a). Both electrical responses induced by transmural stimulation were blocked by tetrodotoxin (3×10^{-7} M), therefore these responses were considered to be the result of intramural nerve stimulation (Figure 5b). Atropine (10^{-6} M) blocked the enhancement of the slow wave after nerve stimulation, but not the i.j.p. (Komori & Suzuki, 1985). The i.j.p. was not blocked by phentolamine (10^{-6} M), propranolol (10^{-6} M) or guanethidine (5×10^{-6} M), therefore this potential had properties similar to that seen in smooth muscle cells of the mammalian gastrointestinal tract (Holman, 1970).

Diltiazem 10^{-5} M given for more than 10 min increased the amplitude of the i.j.p. and decreased that of the slow wave generated after nerve stimulation (Figure 5c), and these changes went with depolarization of the membrane. Increasing the number of stimuli (frequency 10 Hz) increased the amplitudes of the i.j.ps and subsequent slow waves. Diltiazem (10^{-5} M) consistently increased the amplitude of the i.j.ps and reduced the enhancement of the subsequently generated slow wave produced by transmural nerve stimulation, at any given number of stimuli (Figure 6). Figure 7 shows the dose-response relationship of the effects of diltiazem on the amplitude of the i.j.p. and the subsequently generated slow wave evoked by 3 stimuli (frequency 10 Hz) in the circular muscle of the antrum. The amplitudes of the i.j.ps, expressed relative to those before the application of diltiazem, were increased by concentrations of diltiazem above 3×10^{-6} M, while diltiazem inhibited the enhanced amplitude of the slow wave provoked by nerve stimulation.

The amplitude of the i.j.p. was dependent on the membrane potential (Holman, 1970). The membrane potential was displaced to various levels by applying inward or outward current pulses (2 s in duration), and the i.j.p. was generated during the steady level of the electrotonic potential. When the i.j.p. amplitudes were plotted against the membrane potential, the relationship was linear (Figure 8). From this relationship, the

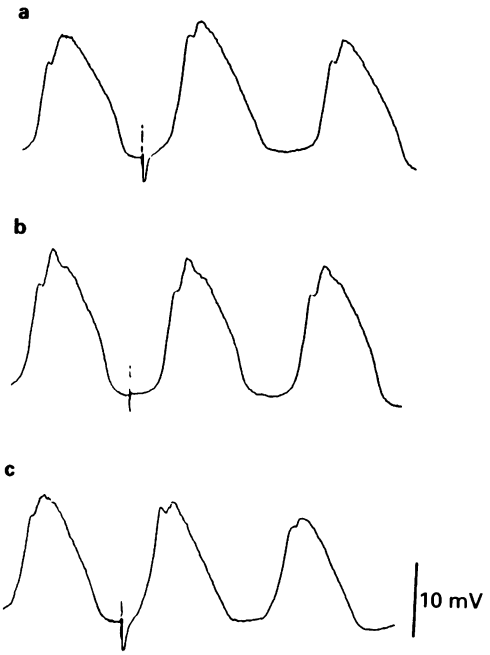

Figure 5 Electrical responses of a smooth muscle cell in the circular muscle of the antrum produced by transmural nerve stimulation (0.05 ms in duration, 30 V in intensity). (a) Control; (b) in the presence of tetrodotoxin 3×10^{-7} M; (c) in the presence of 10^{-5} M diltiazem (26 min). All the responses were recorded from the same cell.

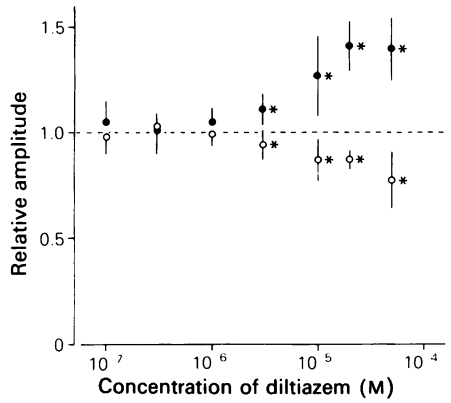

Figure 7 Effects of diltiazem on the amplitude of the i.j.p. (●) and the slow wave (○) produced by transmural nerve stimulation. Transmural nerve stimulation (0.1–0.05 s in duration; 30 V in intensity) was applied (3 pulses at 10 Hz) at the most negative part of the slow waves. The relative amplitude of i.j.p. and the subsequent slow wave produced by nerve stimulation, compared to the control (= 1.0), were plotted. Each point shows the mean, and vertical lines s.d. ($n = 8-10$) of results obtained from different tissues. *Statistically significant change ($P < 0.05$). Mean amplitude of the i.j.p. and the enhancement of the subsequent slow wave in the controls was 4.4 ± 0.8 mV ($n = 54$) and 1.43 ± 0.14 times ($n = 54$), respectively.

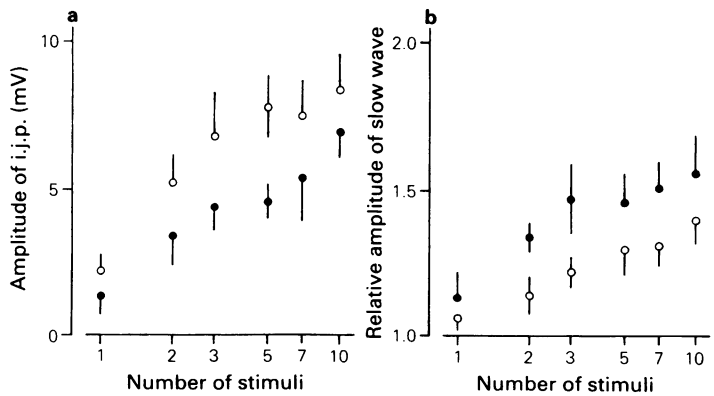

Figure 6 Effect of diltiazem (2×10^{-5} M) on the amplitude of i.j.ps (a) and the subsequent slow waves (b) produced by transmural nerve stimulation in the circular muscle of the guinea-pig stomach antrum. Transmural nerve stimulation (0.1 ms in duration, 30 V in intensity) was applied (1–10 pulses in a train at 10 Hz) before (control, ●) and during a 20–60 min application of diltiazem 2×10^{-5} M (○). The amplitude of the slow wave is expressed relative to that before nerve stimulation. Each point is the mean and vertical lines show s.d. ($n = 6-15$). All the data were obtained from the same tissue.

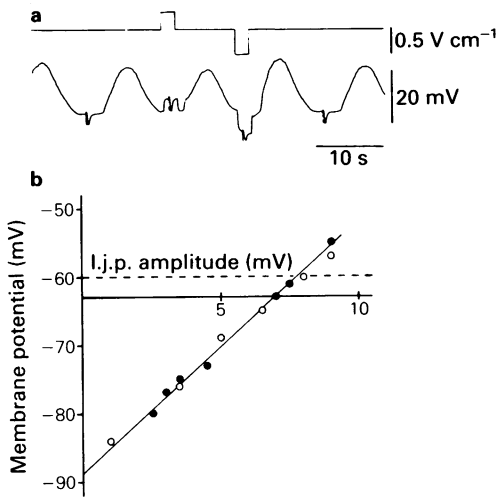

Figure 8 Effects of displacement of the membrane potential on the i.j.p. amplitude. (a) I.j.ps were evoked at different membrane potentials displaced by a current pulse (2 s in duration). (b) Relationship between the amplitude of the i.j.p. and the membrane potential. Vertical axis: membrane potential (resting membrane potential = -63 mV and in the presence of 5×10^{-5} M diltiazem = -60 mV). Amplitude of i.j.p. (●, control; ○, in the presence of 5×10^{-5} M diltiazem) was plotted against the membrane potential. The straight line in the figure shows the relationship between the membrane potential and the i.j.p. amplitude in the control condition, determined by the Least Squares Method ($Y = 3.7X - 88.8$, where Y = membrane potential and X = amplitude of i.j.p.).

reversal potential for the i.j.p. was calculated by extrapolating the line to a potential which shows no potential change on nerve stimulation. The calculated reversal potential for the i.j.p. was -88.9 ± 4.7 mV ($n = 16$) in the control condition, and this value was not significantly changed by application of diltiazem (5×10^{-5} M) for 20–40 min (-85.0 ± 2.1 mV, $n = 6$, $0.05 < P < 0.1$).

The latency for the generation of the i.j.p. after transmural nerve stimulation was measured in the circular muscles of the antrum region. The mean value of the latency was 156.5 ± 20.0 ms ($n = 93$) in the control and 152.3 ± 17.3 ms ($n = 98$) in the presence of 10^{-5} M diltiazem (20–40 min). These two values were not significantly different ($P > 0.1$).

Discussion

Smooth muscle cells of the guinea-pig stomach generated two types of spontaneous electrical responses, slow waves and spike potentials, the spike potentials appearing with or without overshoot potentials. All of these electrical responses are postulated to be generated by influxes of Ca ions (Tomita, 1981). Diltiazem blocked only the spike potential with overshoot potential, but not the slow wave and the abortive spike potential, as was the case with verapamil (Golenhofen & Lammel, 1972). These observations suggest that diltiazem inhibits Ca channels, but the inhibition is limited only to Ca channels which are employed in the generation of the overshoot spike potential. Ca channels involved in the generation of the abortive spike potential or the slow wave are not inhibited by diltiazem. Selectivity of the inhibition of Ca-influxes by diltiazem is observed in the guinea-pig stomach (Itoh *et al.*, 1982), basilar (Fujiwara *et al.*, 1982) or mesenteric artery (Suzuki *et al.*, 1982), i.e., in these tissues, contractions produced by high-potassium or sodium-free solution are abolished in Ca-free solution but only the potassium-induced contraction is inhibited by diltiazem. However, nifedipine and nisoldipine do inhibit contractions produced by both the potassium and the sodium-free solution in the rabbit mesenteric or coronary arteries (Kanmura *et al.*, 1983; Itoh *et al.*, 1984). Therefore, each Ca antagonist may exhibit selectivity in inhibiting Ca-channels.

The slow wave (slow potential change) is observed in stomach smooth muscle from many species including man, cat, dog and guinea-pig (Kuriyama, 1970; Tomita, 1981). In the dog stomach, D-600 blocks the slow potential change but not the spike potential (El-Sharkawy *et al.*, 1978); this observation is in contrast to that seen in the guinea-pig stomach. In both cases, spike potentials and slow potential changes are abolished in Ca-free solution (Ohba *et al.*, 1977; El-Sharkawy *et al.*, 1978), suggesting that the mechanism responsible for the generation of these electrical responses are different in the various species.

The frequency of the slow waves observed in the guinea-pig stomach changes with polarization of the membrane, i.e., depolarization increases and hyperpolarization decreases the frequency, although the change is very little in comparison with that in the amplitude of the slow wave (Ohba *et al.*, 1975). Diltiazem reduced the interval between slow waves (and thus increased their frequency) at a concentration of 10^{-5} M, but not at 10^{-6} M. The former concentration of diltiazem, but not the latter, depolarized the smooth muscle membrane. Presumably, the increase in the frequency of the slow wave is a secondary effect occurring as a result of depolarization of the membrane.

In the guinea-pig stomach, high concentrations of diltiazem (above 3×10^{-6} M) depolarized the membrane and increased the membrane resistance, as has been observed in the guinea-pig mesenteric (Suzuki *et al.*, 1982) or basilar artery (Fujiwara *et al.*, 1982). Dihydropyridine derivatives such as nifedipine, nisol-

dipine, nimodipine and nitrendipine have Ca antagonistic actions on cardiac and smooth muscle (Fleckenstein, 1983), but do not depolarize smooth muscle membrane in the rabbit mesenteric artery (Kanmura *et al.*, 1983; Makita *et al.*, 1983). All of these Ca antagonists inhibit spike potentials generated either spontaneously or by current pulses. Once again it has been suggested that each Ca antagonist has a different action on Ca-related phenomena of smooth muscle tissues. The depolarizing effects of diltiazem may be due to inhibition of potassium permeability of the membrane, as a secondary consequence of a decrease in the supply of Ca ions which contribute to the maintenance of the ionic permeability of the membrane (Suzuki *et al.*, 1982).

The amplitude of the i.j.p. was increased while that of the slow wave following nerve stimulation decreased by diltiazem at concentrations ($> 3 \times 10^{-6}$ M) which depolarized the smooth muscle membrane of the guinea-pig stomach. The i.j.p. is generated by an increase in the potassium permeability of the membrane, and the amplitude is voltage-dependent (Holman, 1970). The enhancement of the amplitude of the slow wave produced by nerve stimulation is attributed to the release of acetylcholine from vagal nerves, and the amount of acetylcholine released can be estimated from the change in amplitude of the subsequent slow wave (Komori & Suzuki, 1985). Diltiazem (above 10^{-6} M) suppressed the enhancement of the slow wave following nerve stimulation in the guinea-pig antrum, and this was associated with depolarization of the membrane. The amplitude of the slow wave decreases with depolarization of the membrane (Ohba *et al.*, 1975). Therefore, the increase in i.j.p. amplitude and also the decrease in the enhancement of the subsequent slow wave by diltiazem may be due to depolarization of the membrane, rather than to changes in the amount of transmitter substances released from nerve terminals. The latency of the i.j.p. remained unchanged by diltiazem, and this suggests that diltiazem does not affect intramural nerve excitation in the guinea-pig stomach. These observations contrast with those on adrenergic transmission, i.e., in the guinea-pig vas deferens (Tajima *et al.*, 1980) or mesenteric artery (Suzuki *et al.*, 1982), or in the dog basilar artery (Fujiwara *et al.*, 1982), diltiazem decreases the amount of transmitter released. Verapamil does not change the amount of transmitter released from sympathetic nerves in the cat heart (Haeusler, 1972); however, it does reduce the amount released in the rabbit heart (Gothert *et al.*, 1979) and in the dog saphenous vein (Takata & Kato, 1984). A decrease in transmitter release by verapamil has also been found in the guinea-pig mesenteric artery when the amount of transmitter released was estimated from the change in amplitude of the excitatory junction potential (Zelcer & Sperelakis, 1981). These observations suggest that the effects of Ca antagonists on nerve terminals vary between tissues.

It is concluded that in the antrum circular muscle of the guinea-pig stomach, diltiazem blocks the Ca-channels through which the Ca ions are carried for the generation of overshooting spike potentials. However, this drug does not block the Ca-channels involved in the generation of abortive spike potentials or slow waves. The depolarization and the increase in membrane resistance induced by diltiazem may be secondary effects resulting from inhibition of Ca-dependent potassium channels. The increase in i.j.p. amplitude and the decrease in the enhancement of slow waves induced by transmural nerve stimulation in the presence of diltiazem may be due to depolarization of the membrane, and the release of transmitters from intramural nerves may not be affected by diltiazem.

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