Effects of RHC-80267, an Inhibitor of Diacylglycerol Lipase, on Excitation of Circular Smooth Muscle of the Guinea-Pig Gastric Antrum

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Abstract

In small segments of circular smooth muscle isolated from the guinea-pig gastric antrum, the effects of RHC-80267, an inhibitor of diacylglycerol lipase, were investigated both on regenerative slow potentials (either occurring spontaneously or as the result of a depolarizing intracellular current injection) and on the actions of acetylcholine (ACh). As diacylglycerol is a known activator of protein kinase C (PKC), it would therefore be expected that RHC-80267 would activate PKC indirectly. In circular smooth muscle bundles, spontaneously generating slow potentials recorded simultaneously from two given cells were synchronized, indicating that these two cells were electrically coupled. RHC-80267 (0.3–1 μM) increased the frequency of slow potential generation, with no alteration to the amplitude of either the slow potentials or the resting membrane potential. Synchronous electrical activity in a given pair of cells was also unchanged by RHC-80267, indicating that intercellular electrical coupling was not altered. The input resistance of smooth muscle cells calculated from the amplitude of electrotonic potentials produced by injection of current was not significantly altered by RHC-80267. The refractory period for the generation of slow potentials evoked by depolarizing stimuli was about 8 s, and it was decreased to about 5 s by RHC-80267, with no significant alteration to the amplitude of spontaneous or evoked slow potentials. ACh (0.5 μM) depolarized the membrane by about 5 mV and increased the amplitude and frequency of slow potentials. The actions of ACh on the frequency of slow potentials were enhanced by RHC-80267, with no alteration to the amplitudes of both the ACh-induced depolarization and slow potentials. These results support the idea that PKC is involved in determining the frequency of slow potentials, by shortening the refractory period for excitation of gastric smooth muscle cells.

Key words: slow potential, diacylglycerol, protein kinase C, acetylcholine, refractory period

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Introduction

Slow waves generated spontaneously in gastric smooth muscle (Tomita, 1981) may originate from interstitial cells of Cajal (ICC) distributed in the gastric wall (Sanders, 1996; Huizinga *et al.*, 1997). In the gastric wall of laboratory animals such as the guinea-pig and mouse, many types of ICC are identified, such as those distributed within the myenteric plexus (ICC-MY), within the circular smooth muscle layers (ICC-IM) and within the submucosal plexus (ICC-SM) (Komuro *et al.*, 1996; Burns *et al.*, 1997). In the guinea-pig, ICC-MY generate driving potentials with initial fast transient (duration, 1–2 s) and subsequent plateau components (duration, 10–15 s), and the potentials are propagated to circular and longitudinal smooth muscle layers in an electrotonic manner through gap junctions (Dickens *et al.*, 1999; Kito *et al.*, 2002b). These slow waves consist of both voltage-sensitive and voltage-insensitive components (Ohba *et al.*, 1975; Tomita, 1981), with only the former easily inhibited by caffeine (Dickens *et al.*, 1999; Suzuki and Hirst, 1999). The driving potential generated in ICC-MY is propagated to circular muscle cells to form a voltage-insensitive component of slow waves, and this potential triggers a voltage-sensitive component (Dickens *et al.*, 1999). In gastric smooth muscle of W/W^V mutant mice lacking ICC-IM, the caffeine-sensitive component of slow waves is absent, suggesting that this component is elicited by electrotonic spread of potentials generated in ICC-IM (Dickens *et al.*, 2001).

However, regenerative slow potentials are also generated periodically in isolated circular smooth muscle cells of the guinea-pig antrum with no attached ICC-MY (Suzuki and Hirst, 1999; Nose *et al.*, 2000; Fukuta *et al.*, 2002; Suzuki *et al.*, 2002a; Kito *et al.*, 2002a). These slow potentials are sensitive to relatively low concentrations of caffeine (0.1–1 mM), and are also evoked by depolarization of the membrane, with a minimum latency of about 1 s (Suzuki and Hirst, 1999). This long latency is considered to be required for production of unidentified messengers such as inositol trisphosphate (IP_3) in response to depolarization of the membrane (Suzuki, 2000). The possible requirement of IP_3 for the generation of spontaneous activity has also been considered in mutant mice lacking the expression of IP_3 receptors (Suzuki *et al.*, 2000).

When two depolarizing stimuli are applied to a segment of circular smooth muscle from the guinea-pig gastric antrum via an intracellular microelectrode, a refractory period of about 5–8 s is required for the generation of the second slow potential, and this period may be one of the important factors for determining the maximum frequency of slow waves (Nose *et al.*, 2000; Suzuki *et al.*, 2002a; Kito *et al.*, 2002a). The effects of several types of inhibitors on the evoked slow potentials indicated that the refractory period for the slow potential generation is related to the activation of protein kinase C (PKC) whereas the amount of Ca^{2+} released from internal stores through activation of IP_3 receptors determines the amplitude of slow potentials (Kito *et al.*, 2002a). PKC is widely distributed throughout a cell and, when activated, moves towards the cell membrane (translocation) and phosphorylates functional proteins (Nishizuka, 1989). Diacylglycerol is one of the important factors involved in the activation of PKC (Nishizuka, 1989), and therefore it is speculated that inhibition of the degradation of diacylglycerol would enhance smooth muscle activity due to elevation of the activity of PKC.

RHC-80267 is a known inhibitor of diacylglycerol lipase (Balsinde *et al.*, 1991), and thus would enhance the activity of PKC indirectly by increasing the concentration of diacylglycerol.

The present experiments were aimed to investigate the effects of RHC-80267 on the refractory period for generation of slow potentials in isolated segments of circular smooth muscle of the guinea-pig gastric antrum. Experiments were carried out using small segments of smooth muscle which were of a size which was about one-tenth smaller than the length constant of the tissue (equal to 2.2 mm, Osa and Kuriyama, 1970). Two intracellular electrodes were impaled, and electrical responses were recorded simultaneously from two different smooth muscle cells, as reported previously (Suzuki and Hirst, 1999; Suzuki *et al.*, 2002a; Kito *et al.*, 2002a). Cells were stimulated by current injection through one electrode and the effects of RHC-80267 on the evoked slow potentials investigated. The effects of RHC-80267 on the actions of acetylcholine (ACh) were also observed in these preparations, since the actions involved activation of PKC (Nishizuka, 1989; Kito *et al.*, 2002a). The results indicated that RHC-80267 elevated the spontaneous activity, reduced the refractory period for generation of slow potentials and enhanced the actions of ACh. These results supported the possible importance of PKC for determining the frequency of spontaneous activity in gastric smooth muscle cells. These results were reported briefly to the 44th annual meeting of the Japanese Smooth Muscle Society at Sendai (Suzuki *et al.*, 2002b).

Materials and Methods

Male albino guinea-pigs, weighing 250–300 g, were anesthetized with fluoromethyl 2,2,2-trifluoro-1-(trifluoromethyl) ethyl ether (sevoflurane; Maruishi Pharm., Osaka, Japan) prior to decapitation. All animals were treated in accordance with the principles for the care and use of animals in the field of physiological sciences, approved by The Animal Experiments Committee of the Nagoya City University Medical School. The stomach was excised, and opened by cutting along the small curvature in Krebs solution. The mucosal layers were removed by cutting with fine scissors, and smooth muscle segments were isolated from the antrum region. The circular smooth muscle preparation (a single bundle 80–100 μm wide and 200–250 μm long) was separated from the longitudinal muscle layer by mechanical removal with fine forceps. The preparation was pinned out on a Sylgard plate (silicone elastomer, Dow Corning Corporation, Midland, Michigan, U.S.A) at the bottom of the recording chamber (volume, approximately 0.5 ml), and superfused with warmed (35°C) Krebs solution at a constant flow rate (about 2 ml/min). The recording chamber was mounted on a stage of an inverted microscope (Nikon IX-70, Tokyo, Japan). These methods were essentially those reported by Suzuki and Hirst (1999) or Kito *et al.* (2002a).

Electrical responses of smooth muscle cells were recorded using conventional microelectrode methods. Glass capillary microelectrodes filled with 0.5 M KCl had a tip resistance of between 150 and 250 MΩ. Two microelectrodes were inserted into the same tissue, and electrical responses were recorded simultaneously from two smooth muscle cells. Experiments were carried out when signals recorded from the two electrodes were synchronized. Current pulses with 0.5–10 nA intensity (duration, 1–2 s) were applied to one electrode, and electrotonic potentials produced were recorded by the second electrode. Membrane potential changes, recorded using a high input impedance amplifier (Axoclamp-2B, Axon Instruments,

Inc., Foster City California, U.S.A), were displayed on a cathode-ray oscilloscope (SS-7602, Iwatsu, Osaka, Japan) and stored on a personal computer for later analysis.

The ionic composition of the Krebs solution was as follows (in mM): Na^+ 134, K^+ 5.9, Mg^{2+} 1.2, Ca^{2+} 2.5, HCO_3^- 15.5, $H_2PO_4^-$ 1.2, Cl^- 134, glucose 15.5. The solution was aerated with O_2 containing 5% CO_2, and pH of the solution was 7.1–7.2.

Drugs used were acetylcholine chloride (ACh), atropine sulphate, caffeine and nifedipine (purchased from Sigma Chem., U.S.A.) and RHC-80267 (1,6-*bis* (Cyclohexyloximinocarbonylamino) hexane) (purchased from Calbiochem, San Diego, California, U.S.A.). Nifedipine and RHC-80267 were dissolved in dimethyl sulphoxide (DMSO) to make stock solutions (at 1 mM), and they were added to Krebs solution to make the desired concentrations. Other chemicals were dissolved in distilled water as a stock solution. These chemicals were diluted further with Krebs solution to the desired concentration (the volume ratios of the dilution were over 1:1000). The dilution procedures did not alter the pH of the Krebs solution.

Values measured were expressed as the mean ± standard error of either the mean (S.E.) or standard deviation (S.D.). Differences between values were tested using an unpaired Student's *t*-test, and probabilities of less than 5% ($P<0.05$) were considered to be significant.

Results

Effects of RHC-80267 on spontaneous electrical activities

In most segments of circular smooth muscle isolated from the guinea-pig gastric antrum (>90%), electrical responses recorded simultaneously from two smooth muscle cells were synchronized, indicating that they were electrically coupled. All cells examined demonstrated periodical generation of regenerative slow potentials of variable frequency. A burst of spike potentials was often generated on top of each slow potential. In each case, the membrane potential between slow potentials was not stable, and a random generation of transient small fluctuations (unitary potentials, Edwards *et al.*, 1999) was observed. Nifedipine (1 μM) abolished the spike potentials, with no interruption to the generation of slow potentials nor to the unitary potentials, while caffeine (1 mM) abolished all of these activities. In the presence of nifedipine, slow potentials with amplitudes ranging between 27 mV and 46 mV (mean, 35.0 ± 1.0 mV, n=25) and durations measured at the foot ranging between 5 s and 12 s (mean, 7.1 ± 0.2 s, n=25) were generated at frequencies ranging between 0.2 min^{-1} and 3.0 min^{-1} (mean, 1.11 ± 0.2 min^{-1}, n=25). These properties of the electrical responses were similar to those reported previously (Suzuki and Hirst, 1999; Edwards *et al.*, 1999; Kito *et al.*, 2002a; Suzuki *et al.*, 2002a). All the experiments were carried out in the presence of 1 μM nifedipine, to exclude possible involvement of voltage-gated Ca-channels in the evoked responses.

The effects of 0.3 μM RHC-80267 on spontaneous electrical activities recorded from a segment of circular smooth muscle isolated from the guinea-pig gastric antrum were investigated. At rest, slow potentials were generated spontaneously at a frequency of 1.12 ± 0.31 min^{-1}, and increased to 2.43 ± 0.67 min^{-1} (n=11; $P<0.05$) in the presence of 0.3 μM RHC-80267. There was no significant alteration to either the resting membrane potential (control, –67.4 ± 1.2

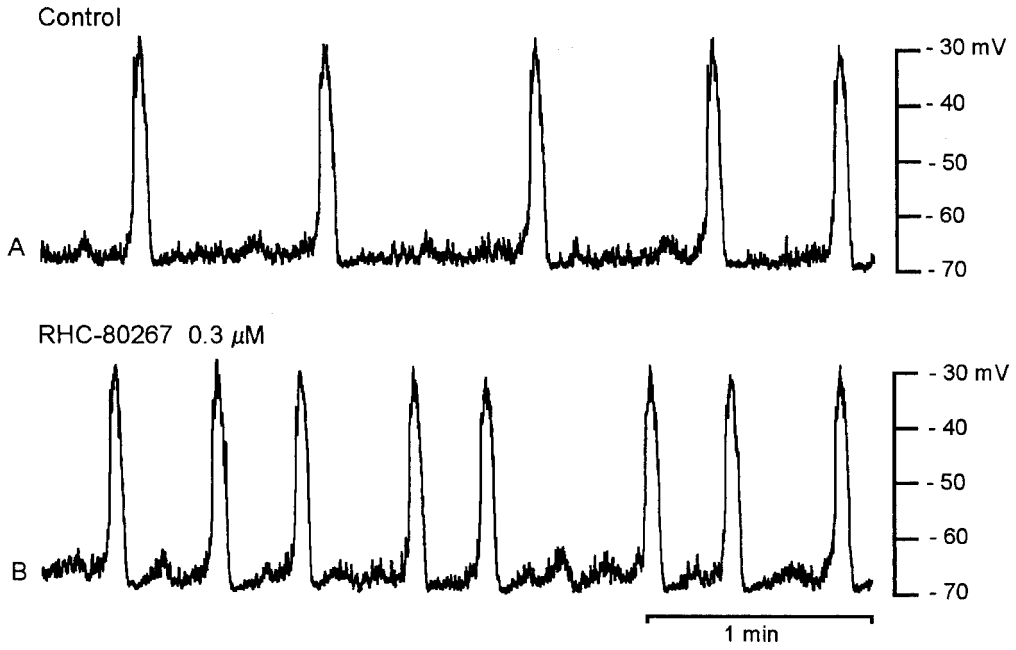

Fig. 1. Effects of RHC-80267 on spontaneous activity of antrum smooth muscle. In a segment of circular smooth muscle isolated from the guinea-pig stomach antrum, spontaneous electrical responses were recorded in the absence (A) and presence of 0.3 μM RHC-80267 (B). A and B were recorded from the same smooth muscle cell, with an interruption of 10 min.

mV; in RHC-80267, –66.9 ± 1.5 mV; n=11; P>0.05) or to the amplitude of the slow potentials (control, 37.8 ± 1.3 mV; in RHC-80267, 37.4 ± 1.4 mV; n=11; P>0.05). Increasing the concentration of RHC-80267 to 1 μM produced effects similar to those produced by 0.3 μM RHC-80267 (n=2, data not shown).

The possible alteration of the electrical coupling between a given pair of smooth muscle cells by RHC-80267 was evaluated by visualizing the activity with an expanded time scale. As shown in Fig. 2, synchronization of electrical responses recorded from two cells was not altered by RHC-80267, suggesting that intercellular electrical couplings were not altered. These results also suggested that the increased frequency of slow potentials observed in the presence of RHC-80267 was not due to changes in the biophysical properties of the membrane including intercellular electrical coupling.

Effects of RHC-80267 on the refractory period of slow potentials

In segments of gastric smooth muscle, application of current pulses (1–2 s duration) via one electrode produced electrotonic potentials in other smooth muscle cells, which could be recorded by the second electrode. The electrotonic potentials thus produced were recorded in all smooth muscle cell pairs with synchronized activity. Current injection via one electrode produced a depolarizing potential in the second electrode, and the amplitude of the electrotonic depolarization increased with the intensity of the injected current. When the amplitude of

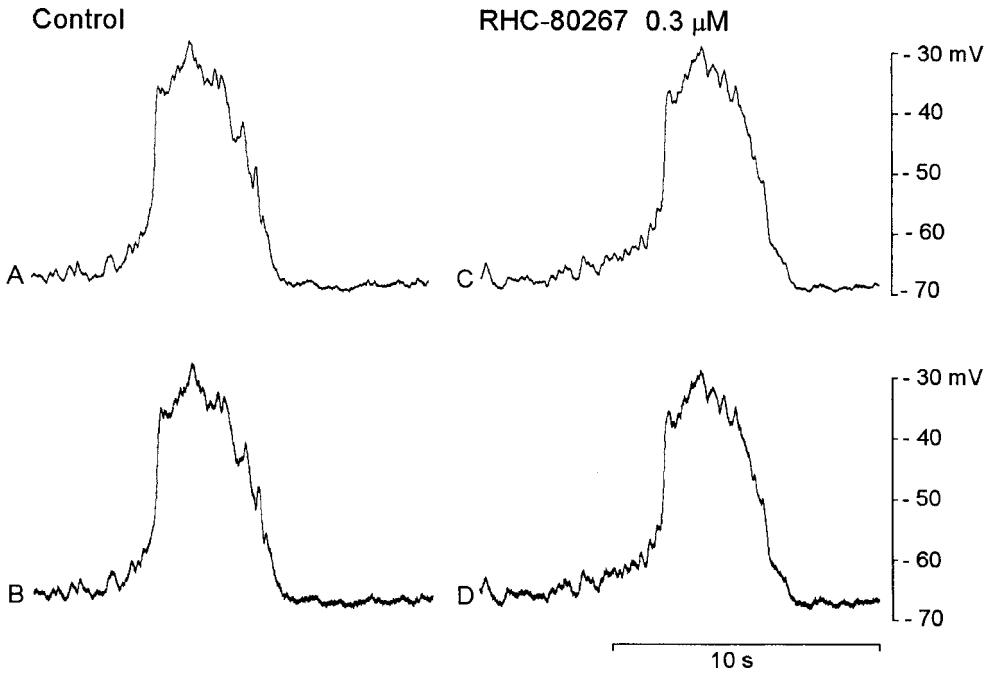

Fig. 2. Effects of RHC-80267 on the synchronized electrical responses recorded from two cells in gastric circular muscle segment. In a segment of circular smooth muscle isolated from the gastric antrum, electrical responses were recorded from two smooth muscle cells simultaneously in the absence (A and B) and presence of 0.3 μM RHC-80267 (C and D). A–C and B–D were recorded from different single cells, with an interruption of 12 min.

depolarization exceeded a certain level, smooth muscle cells produced regenerative slow potentials. The evoked slow potentials had a latency of 1–3 s after onset of the depolarization. These properties of the evoked slow potentials were similar to those reported previously (Suzuki and Hirst, 1999; Kito *et al.*, 2002a; Suzuki *et al.*, 2002a). Experiments were carried out to stimulate smooth muscle cells for 1 s with depolarizing pulses of supra-maximal intensity, at various times after cessation of a spontaneously generated slow potential. When pulses were applied within 1–2 s after the cessation of a spontaneous slow potential, only electrotonic potentials were produced (Fig. 3A). Pulses applied 3–7 s after the cessation of a spontaneous slow potential evoked electrotonic potentials and then irregular shaped potentials which looked like a cluster of unitary potentials. Increasing the time between the cessation of the slow potential and the application of depolarizing pulses to more than 8 s evoked slow potentials with amplitudes similar to those evoked spontaneously (Fig. 3B). The relationship between the time of application of pulses after cessation of spontaneous slow potentials and the peak amplitude of the evoked potentials (Fig. 3E) indicated that there was a period of about 2 s during which no response was evoked by depolarizing pulses. For the period 3–7 s after cessation of slow potentials, irregular shaped potentials evoked by stimulating pulses increased roughly linearly. Stimulation of smooth muscle cells after an interval exceeding 8 s produced complete regenerative slow potentials. As a consequence, the relationship between the interval between

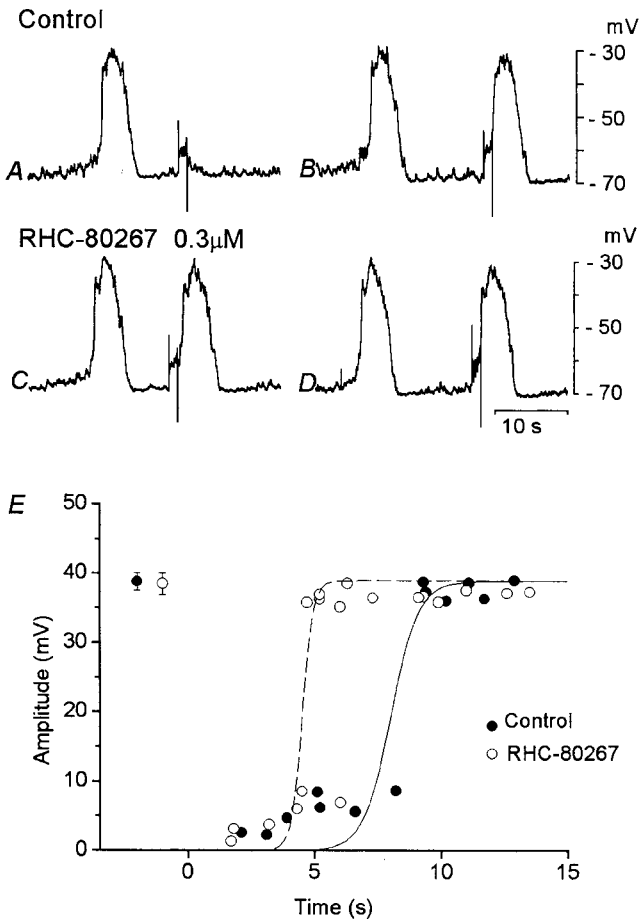

Fig. 3. Effects of RHC-80267 on the evoked slow potentials. Smooth muscle cells were stimulated by a depolarizing current (1 s duration, 2 nA) at about 5 s (A and C) and 12 s (B and D) after cessation of spontaneous slow potentials, in the absence (Control, A and B) and presence of 0.3 μM RHC-80267 (C and D). E summarizes the relationship between time of stimulation after cessation of spontaneous slow potentials and the amplitude of the evoked responses, in the absence (filled circles, continuous line) and presence of 0.3 μM RHC-80267 (open circles, dotted line). Mean amplitudes of spontaneous slow potentials generated before stimulating the smooth muscle cells are shown in the left-hand side of the graph, with symbols the same as those in the graph (n=13 for Control, n=18 for RHC-80267; P>0.05).

cessation of slow potentials, the delivery of the stimulus and the amplitude of the evoked response had a discontinuity around 3–5 s after cessation of slow potentials. Application of pulses at intervals longer than 8 s produced reproducible amplitudes of slow potentials.

These results indicate that there is a refractory period following a slow potential in gastric smooth muscle cells and during this period the generation of succeeding slow potentials is inhibited. In 6 experiments, the refractory period varied between 7 s and 12 s (mean, 7.8 ± 1.2 s). In the presence of RHC-80267 (0.3 μM), a similar relationship was observed in response to depolarizing pulse stimuli, and the refractory period for the generation of slow potentials was

reduced to 5.0 ± 0.8 s; n=6; P<0.05), with no significant alteration to the amplitude of evoked slow potentials (control, 37.7 ± 1.4 mV; in RHC-80267, 36.9 ± 0.8 mV; n=6; P>0.05).

The input resistance of the membrane calculated from the amplitude of electrotonic potentials produced by inward current pulses varied between 5.7 MΩ and 8.1 MΩ (mean, 7.2 ± 0.8 MΩ, n=6). In the presence of 0.3 μM RHC-80267, the input resistance of the membrane was not altered (7.3 ± 0.5 MΩ, n=6, P>0.05).

Modulation of the excitatory actions of acetylcholine by RHC-80267

The effects of RHC-80267 on the actions of acetylcholine (ACh) were investigated in isolated circular smooth muscle of the guinea-pig gastric antrum. The concentration of ACh tested was 0.5 μM, since this concentration of ACh depolarizes the membrane and increases spontaneous activity in the guinea-pig stomach (Komori and Suzuki, 1986; 1988). Experiments were carried out in tissues whose spontaneous activities were very low, so as to facilitate the modulation of the actions of ACh more clearly. Figure 4A shows that in quiescent tissue where only unitary potentials were generated, application of 0.5 μM ACh depolarized the membrane by about 5 mV (control, –65.9 ± 2.2 mV; in ACh, –61.1 ± 2.0 mV; n=7; P<0.05) and elicited generation of slow potentials, in a reversible manner. The frequency and amplitude of slow potentials generated in the presence of ACh was 1.56 ± 0.36 min^{-1} (n=8) and 31.1 ± 1.9 mV (n=10), respectively. Application of 0.5 μM RHC-80267 did not elicit generation of slow potentials. In the presence of RHC-80267, ACh depolarized the membrane by about 5 mV (control, –65.4 ± 1.7 mV; in RHC-80267, –60.1 ± 1.8 mV; n=7; P<0.05), which is similar to the difference seen in the absence of RHC-80267. However, the frequency (2.32 ± 0.56 min^{-1}, n=11, P<0.05), but not the amplitude (30.7 ± 1.4 mV, n=14, P>0.05), of slow potentials generated during stimulation with ACh was increased by RHC-80267 (Fig. 4B).

Discussion

The present experiments demonstrate that RHC-80267 has excitatory actions on circular smooth muscle bundles isolated from the guinea-pig gastric antrum, as evaluated from the electrical responses recorded intracellularly from smooth muscle cells. These actions were evidenced by an increase in the frequency of slow potential generation, by a reduction in the refractory period for the generation of slow potentials and by augmentation of ACh-induced responses. RHC-80267 is an inhibitor of diacylglycerol lipase (Balsinde *et al.*, 1991), and thus it is expected that this chemical may elevate the concentration of diacylglycerol. As PKC is activated by diacylglycerol (Nishizuka, 1989), it is expected that RHC-80267 will produce effects similar to the activation of PKC. Thus, the excitatory actions of RHC-80267 may be mainly related to the resultant activation of PKC.

In circular smooth muscle bundles isolated from the guinea-pig gastric antrum, slow potentials are generated by depolarization of the membrane, with a minimum latency of about 1s (Suzuki and Hirst, 1999), a time comparable to the delay required for the production of IP_3 in response to stimulation with agonists (Somlyo and Somlyo, 1994). These observations lead to the speculation that membrane depolarization produces an unidentified second messengers that

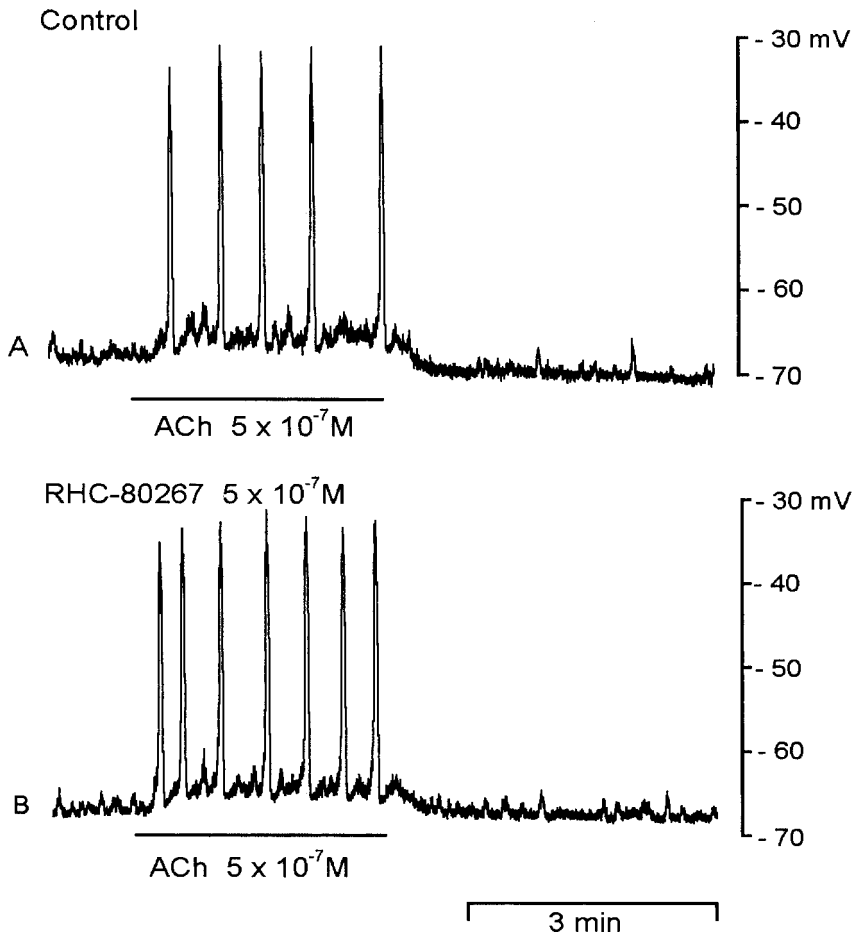

Fig. 4. Effects of RHC-80267 on the actions of ACh. In an isolated segment of gastric circular smooth muscle, electrical responses during stimulation with 5×10^{-7} M ACh were recorded in the absence (A) and presence of 0.5 μM RHC-80267 (B). A and B were recorded from the same smooth muscle cell.

accelerate the release of Ca^{2+} from internal stores (Suzuki, 2000). Slow waves are absent in the gastric smooth muscle of mutant mice lacking expression of the IP_3 receptor (Suzuki *et al.*, 2000). In gastric smooth muscle of the guinea-pig, 2-aminoethoxydiphenyl borate (2-APB), an inhibitor of the IP_3 receptor-mediated release of Ca^{2+} from internal stores (Maruyama *et al.*, 1992), abolishes slow waves (Hirst and Edwards, 2001) and also slow potentials (Fukuta *et al.*, 2002). In murine small intestine, inhibition of spontaneous activity by xestospongine C is mediated by the blockade of the release of Ca^{2+} from the IP_3-sensitive stores (Malysz *et al.*, 2001). These results suggest that the production of IP_3 may be a key requirement for the generation of slow potentials, and that the amount of Ca^{2+} released would determine the amplitude of slow potentials (Kito *et al.*, 2002a), possibly by activation of Ca^{2+}-activated Cl^--channels (Hirst *et al.*, 2002). The increased production of receptor-mediated IP_3 during depolarization of the membrane (Ganitkevich and Isenberg, 1993) supports this suggestion. In

addition to the elevated production of IP_3, PKC is also activated by stimulation with several types of agonists (Nishizuka, 1986) and also by depolarization of the membrane, possibly through an elevation of intracellular Ca^{2+} concentrations (Kong *et al.*, 1991; Maasch *et al.*, 2000). The present experiments suggest that these events also occur in antral smooth muscle, and that PKC may be one of the essential factors for the initiation of slow potentials in response to depolarization stimuli.

The refractory period of the slow potential may be one of the important factors in the determination of the frequency of slow potentials (Nose *et al.*, 2000). The refractory period for the generation of slow potentials is increased by bisindolylmaleimide I (BIM) or chelerythrine, inhibitors of PKC, indicating that the activation of PKC is related to the refractory period (Kito *et al.*, 2002a). These results could be extrapolated to indicate that PKC is a key requirement for the spontaneous initiation of slow potentials. The increase in the frequency of spontaneous slow potentials by RHC-80267 also supports this concept. In gastric smooth muscle of IP_3 receptor knock-out mice, slow waves are absent, but spike potentials are still generated spontaneously (Suzuki *et al.*, 2000). These observations further suggest that PKC is located upstream to IP_3 in the signaling pathways for the initiation of slow potentials. The spontaneous generation of slow potentials is inhibited by preventing Ca^{2+} handling in mitochondria with protonophore or mitochondrial ATP-sensitive K-channel inhibitors (Fukuta *et al.*, 2002). As PKC is activated by an elevation of $[Ca^{2+}]_i$ (Nishizuka, 1986), it is speculated that the rhythmic activation of PKC could be manipulated by Ca^{2+} released from mitochondria.

ACh, a major excitatory transmitter of gastrointestinal motor nerves, produces contraction of gastric smooth muscle cells, with and without depolarization of the membrane (Komori and Suzuki, 1986; 1988). In circular muscle of the guinea-pig stomach, ACh has a dual action on slow potentials, with an increase in both their amplitude and frequency. The former is possibly the result of an accelerated release of Ca^{2+} from the internal stores, while the latter may be the result of a shortened refractory period for generation of slow potentials through activation of PKC (Kito *et al.*, 2002a). The present experiments indicated that RHC-80267 did not increase the amplitude of spontaneous slow potentials. The amplitude of slow potentials elicited during stimulation with ACh was also not further increased by RHC-80267. These observations suggest that diacylglycerol activates PKC but not phospholipase C, an enzyme required for production of IP_3. These results also support the idea that the pathways for regulating the amplitude of slow potentials are different from those regulating the frequency (Kito *et al.*, 2002a).

Taken together, these results suggest the importance of the role of PKC in the refractory period of slow potentials. As the depolarization of the membrane activates PKC for translocation (Kong *et al.*, 1991; Maasch *et al.*, 2000), it is speculated that the refractory period for slow potential generation is related to the recovery of the amount of PKC being available for translocation, or alternatively that the refractory period is related to the time required for the supply of a sufficient amount of PKC for translocation to a certain number of pacemaker cells. IP_3 may be also involved in the generation of slow potentials, but possibly downstream to PKC, as a determinant of the amplitude of slow potentials, through regulation of the amount of Ca^{2+} released from the internal stores (Kito *et al.*, 2002a). The transient small fluctuations which appear in the interval between slow potentials may be propagated from ICC-IM through gap

junctions (Dickens *et al.*, 2001), and slow potentials may be the summed potentials of these fluctuating potentials (Edwards *et al.*, 1999). These results allow speculation that the depolarization of the smooth muscle membrane is conducted to ICC-IM and evokes transient small fluctuations, possibly through activation of PKC.

It is summarized that in circular smooth muscle of the guinea-pig gastric antrum, inhibition of diacylglycerol lipase with RHC-80267 increases the frequency of slow potentials. These actions of RHC-80267 may be mainly related to the activation of PKC. The results also support the idea that PKC is one of the important factors that determines the frequency of spontaneous activity in gastric smooth muscle.

Acknowledgments

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