Properties of Unitary Potentials Recorded from Myenteric Interstitial Cells of Cajal Distributed in the Guinea-Pig Gastric Antrum

Yoshihiko Kito¹, Hikaru Suzuki¹ and Frank R. Edwards²

¹Department of Physiology, Nagoya City University Medical School, Mizuho-ku, Nagoya 467-8601, Japan, and
²Department of Zoology, University of Melbourne, Parkville, Victoria 3052, Australia

Abstract

Intracellular recordings were made from myenteric interstitial cells of Cajal (ICC-MY) distributed in the guinea-pig gastric antrum to investigate the properties of unitary potentials. In most cells studied, pacemaker potentials with initial fast transient and following plateau components were generated periodically, and intervals between the potentials were quiescent. However, there were few cells (less than 5% of cells examined) which showed discharge of unitary potentials spontaneously in the intervals between pacemaker potentials. The amplitude and frequency of unitary potentials appeared to be random variables, as observed in isolated circular smooth muscle bundles of the guinea-pig gastric antrum. BAPTA-AM (an intracellular Ca²⁺ chelator) or papaverine (a non-selective phosphodiesterase inhibitor) reduced the discharge frequency of unitary potentials, with associated decrease in the frequency of pacemaker potentials. These agents finally abolished both unitary potentials and pacemaker potentials. In preparations showing no detectable generation of unitary potentials, depolarization of the membrane with high-K solution ([K⁺]o = 10.6 mM) elicited generation of unitary potentials during intervals between pacemaker potentials. Pinacidil (an opener of KATP-channels) hyperpolarized the membrane and increased the frequency and amplitude of unitary potentials with no alteration to the relationship between the amplitudes of unitary potentials and their half-widths. These results suggest that the elevation of intracellular Ca²⁺ concentration is causally related to the generation of unitary potentials in pacemaker cells. They are consistent with the proposition that the depolarization produced by a burst of unitary potentials triggers the primary component of pacemaker potentials in ICC-MY, which induces a release of Ca²⁺ from inositol 1,4,5-trisphosphate (IP₃)-sensitive internal stores and then activates Ca²⁺-sensitive Cl⁻-channels to form the plateau component. Similarities and differences in unitary potentials between circular muscle and pacemaker cells are discussed.

Key words: unitary potential, pacemaker potential, calcium, ICC-MY, stomach
Introduction

Spontaneous contractions of gastric smooth muscles are elicited by rhythmic electrical activities of the membrane such as slow waves or action potentials or both (Tomita, 1981). It was thought that the spontaneous activity is myogenic and that pacemaker cells are distributed nearly homogeneously in smooth muscle tissues (Kuriyama, 1970). However, the distribution and properties of pacemaker cells have long remained uncertain (Tomita, 1981). Thuneberg (1982) considered that the rhythmic activity might originate in the interstitial cells of Cajal (ICC) distributed in the myenteric region of gastrointestinal tracts, since these cells are rich in mitochondria and have close contact with surrounding ICC and smooth muscle cells. ICC are of mesenchymal origin and are triangularly shaped or stellate-shaped cells with long processes (Thuneberg, 1982). ICC express c-Kit immunoreactivity and form gap junctional connections with each other and with nearby smooth muscle cells (Komuro et al., 1996; Sanders, 1996; Huizingar et al., 1997; Sanders et al., 1999). ICC are heterogeneous, and many types of cell with different immunohistochemical and electrical properties, such as myenteric ICC (ICC-MY), intramuscular ICC (ICC-IM), deep muscular plexus ICC (ICC-DMP) and submucosal ICC (ICC-SM), are distributed in the gastrointestinal tract (Sanders et al., 1999).

In the gastric antrum of the guinea-pig, ICC-MY generate pacemaker potentials with initial transient and following plateau components, called driving potentials (Dickens et al., 1999). In animal models which lack development of ICC-MY due to mutation of the c-kit gene (Maeda et al., 1992), rhythmic activity of smooth muscles in the small intestine is strongly attenuated, with associated absence of slow waves, indicating that these cells are indeed essential for pacing intestinal activity (Ward et al., 1994; Huizinga et al., 1995). In smooth muscle tissues of the guinea pig gastric antrum, simultaneous recordings of electrical responses from ICC-MY and smooth muscle cells indicate that the activity appears first in the former and then propagates passively to the latter (Dickens et al., 1999). In circular muscle bundles with no attached ICC-MY isolated from the guinea-pig stomach antrum, spontaneous generation of membrane noise (unitary potentials, Edwards et al., 1999) and regenerative slow potentials are also recorded (Suzuki and Hirst, 1999). Analysis of the frequency spectrum of unitary potentials suggests that the slow potentials are formed by summation of unitary potentials (Edwards et al., 1999). In W/Wv mice, which are c-kit gene mutated mice (Huizinga et al., 1997), antral smooth muscles fail to generate unitary potentials and there is associated impairment of the development of ICC-IM, suggesting a causal relationship between these cells and unitary potentials (Dickens et al., 2001). Electrical properties of pacemaker potentials recorded from ICC-MY are similar to those of regenerative slow potentials recorded from circular muscle bundles (Edwards et al., 1999; Suzuki and Hirst, 1999; Hirst and Edwards, 2001; Kito et al., 2002a), suggesting that both of these potentials are formed by similar mechanisms.

In the present study, properties of unitary potentials recorded from ICC-MY were investigated in antral smooth muscle tissues of the guinea-pig stomach. The results suggest that unitary potentials recorded from ICC-MY have characteristics similar to those found in isolated circular smooth muscle bundles. That is, chelating intracellular Ca²⁺ with BAPTA-AM or applying papaverine, a chemical which elevates cyclic AMP levels, abolished unitary...
potentials, while depolarization of the membrane with high [K+]o solution or hyperpolarization of the membrane with pinacidil, an activator of K_{ATP} channels, elevated the generation of unitary potentials. These results suggest that an increase in intracellular Ca^{2+} concentration is required for the generation of unitary potentials in ICC-MY of the guinea-pig gastric antrum.

**Methods**

Albino guinea-pigs of either sex, weighting 200–300 g, were anesthetized with fluoromethyl 2,2,2-trifluoro-1-(trifluoromethyl) ethyl ether (sevoflurane, Maruishi Pharm., Osaka, Japan), and exsanguinated from the femoral artery. All animals were treated ethically according to the guiding principles for the care and use of animals in the field of physiological sciences, approved by The Physiological Society of Japan. The stomach was excised, and opened by cutting along the small curvature in Krebs solution (see below). The mucosal layers were removed by cutting with fine scissors, and smooth muscle tissues were isolated from the antral region. The serosal layer was carefully removed under a dissecting microscope. A tissue segment (about 1.5 mm width and 3 mm long), with longitudinal and circular muscles attached, was pinned out with the serosal side uppermost on a silicone rubber plate fixed at the bottom of an organ bath (8 mm wide, 8 mm deep, 20 mm long). The tissue was superfused with warmed (35°C) and oxygenated Krebs solution, at a constant flow rate of about 2 ml min^{-1}. Experiments were carried out in the presence of 1 µM nifedipine throughout, and this minimized muscle movement and allowed sustained recordings.

Conventional microelectrode techniques were used to record intracellular electrical responses of smooth muscle tissues, and the glass capillary microelectrodes (outer diameter, 1.2 mm, inner diameter 0.6 mm; Hilgenberg, Germany) filled with 3 M KCl had tip resistances ranging between 50 and 80 MΩ. Electrical responses recorded via 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 also stored on a personal computer for later analysis. Spectral analysis of unitary potentials was carried out by the method described by Edwards et al. (1999).

The ionic composition of the Krebs solution was as follows (mM): Na+, 137.4; K+, 5.9; Ca^{2+}, 2.5; Mg^{2+}, 1.2; HCO_{3}−, 15.5; H_{2}PO_{4}−, 1.2; Cl−, 134; and glucose, 11.5. Solutions containing high-potassium ion concentrations (high-K solutions) were prepared by replacing NaCl with KCl. The solutions were aerated with O_{2} containing 5% CO_{2}, and the pH of the solutions was maintained at 7.2–7.3.

Drugs used were diazoxide, nifedipine, pinacidil (all from Sigma, St. Louis, Missouri, U.S.A), and 1,2-bis (2-aminophenoxy) ethane-N,N,N’,N’-tetraacetic acid (BAPTA-AM) (from Dojindo, Osaka, Japan). Nicorandil was a gift from Chugai Pharmaceutical Co. Ltd. BAPTA-AM, diazoxide, nicorandil, nifedipine and pinacidil were dissolved in dimethyl sulphoxide (DMSO) to make stock solutions, and were added to Krebs solution to make the desired concentrations immediately prior to use. Other drugs tested were dissolved in distilled water. The final concentration of the solvent in Krebs solution did not exceed 1/1000. Addition of these chemicals to Krebs solution did not alter the pH of the solution.
Experimental values were expressed by the mean value ± standard deviation (S.D.). Statistical significance was tested using Student’s t-test, and probabilities of less than 5% (P<0.05) were considered significant.

Results

General observations

In the presence of 1 µM nifedipine, three distinct patterns of ongoing discharge of rhythmical electrical activities were recorded from antral smooth muscle tissues of the guinea-pig stomach: triangular potentials with slow (<0.2 Vs⁻¹) rates of rise and with 20–30 mV amplitudes (slow waves), square-shaped potentials with fast (>0.2 Vs⁻¹) rates of rise and subsequent plateau components having 40–50 mV amplitudes (pacemaker potentials), and square-shaped potentials with slow (<0.2 Vs⁻¹) rates of rise and subsequent plateau components having 20–30 mV amplitudes (follower potentials). Comparison of these properties of the potentials with those reported previously (Dickens et al., 1999) suggested that the slow waves, follower potentials and pacemaker potentials may be recorded from circular muscle, longitudinal muscle and ICC-MY, respectively.

As reported previously (Kito et al., 2002a), pacemaker potentials consist of a primary component and subsequent plateau component. The former had a mean peak amplitude of 48.9 ± 6.3 mV (n=11; each n value represents the number of animals examined), while the latter had a mean amplitude of 50.4 ± 5.9 mV (n=11). Pacemaker potentials were generated at a frequency of 3.42 ± 0.37 min⁻¹ (n=11), and their mean half-width and the maximum rate of rise (dV/dtmax) were 7.25 ± 0.29 s and 0.56 ± 0.14 Vs⁻¹ (n=11), respectively. The resting membrane potential of these cells was −64.1 ± 3.7 mV (n=11). In some ICC-MY (less than 5% of over 100 impalements), unitary potentials were detected during the interval between pacemaker potentials, as has been reported by Hirst and Edwards (2001). These unitary potentials were generated at apparently random times (Fig. 1A), but usually the rate of generation dropped to near zero following a pacemaker potential and progressively increased with time, and the highest rate of generation was observed just before the occurrence of a pacemaker potential. The amplitude of unitary potentials ranged between 0.7 and 10.1 mV, with the mean value being 2.9 ± 1.5 mV (n=117, where n refers to the number of unitary potentials counted in three different cells; Fig. 2A). The expanded traces show that the potentials were formed by a fast component alone with no following plateau component (Fig. 1, B–D). The half-width of unitary potentials (the period for which the amplitude exceeded 50% of peak amplitude) ranged between 40 and 280 ms (mean value, 117 ± 51 ms, n=117). When the half-width of unitary potentials was plotted as a function of unitary potential amplitude, there was a directly proportional relationship between these two factors (Fig. 2B). These properties of unitary potentials recorded from ICC-MY were similar to those of unitary potentials recorded in isolated circular smooth muscle bundles (Edwards et al., 1999) and in ICC-MY (Hirst and Edwards, 2001).

Effects of BAPTA-AM and papaverine on unitary potentials

In isolated circular smooth muscle bundles, unitary potentials are due to increased
Properties of unitary potentials in ICC-MY 169

conductance of Ca\(^{2+}\)-activated Cl\(^{-}\)-channels (Hirst et al., 2002). Therefore, experiments were carried out to investigate the effects of BAPTA-AM (an internal Ca\(^{2+}\) chelator) and papaverine (a non selective phosphodiesterase inhibitor) on unitary potentials, since both of these chemicals are considered to reduce intracellular Ca\(^{2+}\) concentration (Kuriyama et al., 1998), but through different mechanisms, in gastric smooth muscles.

In preparations which showed spontaneous generation of unitary potentials (mean amplitude, 3.2 ± 2.4 mV; half-width, 181 ± 84 ms; n=42), BAPTA-AM (50 µM) reduced the amplitude and frequency of unitary potentials, with associated decrease in the duration of pacemaker potentials (Fig. 3A). In the initial 3–6 min of BAPTA-AM application, unitary potentials disappeared and dV/dt\(_{\text{max}}\) and half-width of pacemaker potentials both decreased (Fig. 3C). In 10 min all spontaneous activities were abolished with associated depolarization of the membrane. These results were similar to those reported previously (Kito et al., 2002a). The unitary potentials analysed by the method reported previously (Edwards et al., 1999) indicated that the decrease in the discharge of unitary potentials by BAPTA-AM was associated with a fall in the power spectral density of unitary potentials, yielding spectral density curves similar to those obtained from records with quiescent baselines (Fig. 4). The increased density at 10 Hz was considered artifacts elicited by unidentified sources in the recording system, since it remained unaltered.

**Fig. 1.** Unitary potentials generated in ICC-MY. A. Typical recording of unitary potentials generated in the interval between pacemaker potentials recorded from ICC-MY. Three examples of unitary potentials (B, small size, <2 mV; C, middle size, about 5 mV; D, large size, about 10 mV) are shown with expanded time scale. The resting membrane potential, −65 mV.
when the tip of recording electrode was located outside of the cell (data not shown).

In preparations with spontaneously discharged unitary potentials (mean amplitude, 1.3 ± 0.5 mV; half-width, 90 ± 26 ms; n=38 for both), application of papaverine (30 μM) reduced the discharge of unitary potentials and subsequently reduced the frequency and half-width of pacemaker potentials, finally abolishing both unitary potentials and pacemaker potentials (Fig. 5A). The inhibitory actions on unitary potentials and pacemaker potentials of papaverine were
Properties of unitary potentials in ICC-MY

Reversible, and in the recovery process, the discharge rate of unitary potentials accelerated beyond control level before returning to baseline (mean amplitude, $2.1 \pm 2.1$ mV; half-width, $220 \pm 250$ ms; n=51 for both) (Fig. 5, B and C). Spectral analysis of unitary potentials was made in the absence (Fig. 6A) and presence (Fig. 6B) of papaverine, and during recovery from the inhibition by papaverine. High-speed traces of responses recorded in the absence (B) and after wash out of $30 \mu$M papaverine for 5 min (C).

![Fig. 4. Frequency analysis of the generation of unitary potentials in ICC-MY. Spectral density relationships for unitary potentials were measured before (A) and during application of 50 $\mu$M BAPTA-AM (B).](image)

![Fig. 5. Effects of papaverine on pacemaker and unitary potentials generated in ICC-MY. In a spontaneously active preparation, papaverine (30 $\mu$M) was applied for about 4 min (shown by bar under the record). Note that unitary potentials appeared with high frequencies during the recovery process from the inhibition by papaverine. High-speed traces of responses recorded in the absence (B) and after wash out of $30 \mu$M papaverine for 5 min (C).](image)
Fig. 6. Effects of papaverine on the spectral analysis of the discharge of unitary potentials recorded from ICC-MY. Spectral density relationships for unitary potentials recorded before (A), during (B) and after (C) application of 30 µM papaverine. The increased density at 10 Hz was considered artifacts elicited by unidentified sources in the recording system, since it remained unaltered when the tip of recording electrode was located outside of the cell (data not shown).
actions of papaverine (Fig. 6C). The results indicated that in the presence of 30 \( \mu \)M papaverine, the power spectral density curves resembled those determined when the recording electrode was outside the tissue (Fig. 6B). The power of the spectral density curves exceeded control values during the recovery process from the inhibition by 30 \( \mu \)M papaverine (Fig. 6C). The increased density at 10 Hz was again considered artifacts elicited by unidentified sources in the recording system, as described above (data not shown).

Taken together, these results suggest that intracellular Ca\(^{2+}\) concentration is an important factor in maintaining the discharge of unitary potentials in ICC-MY, confirming previous observations (Edwards et al., 1999).

**Effect of high-K solution on the generation of unitary potentials**

Since high-K solution depolarizes the membrane and increases the frequency of slow waves (Nose et al., 2000; Fukuta et al., 2002) and pacemaker potentials (Kito et al., 2002a) recorded from the guinea pig gastric antrum, the effects of membrane depolarization with high-K solution on the generation of unitary potentials during intervals between pacemaker potentials were investigated. Application of 10.6 mM \([K^{+}]_o\) solution depolarized the membrane by about 7 mV and increased the frequency of pacemaker potentials, as has been reported previously (Kito et al., 2002a). Following high-K depolarization, discharge of unitary potentials appeared in 2 out of 10 preparations. Unitary potentials evoked during depolarization had amplitudes of 3.0 ± 1.9 mV and half-widths of 96 ± 39 ms (n=13 for both; Fig. 7B). In these two preparations, the discharge of unitary potentials was further augmented by increasing the concentration of \([K^{+}]_o\) to 24.7 mM; the evoked unitary potentials had an amplitude of 2.6 ± 1.5 mV and a half-width of 106 ± 43 ms (n=50 for both; Fig. 7C). Thus, the membrane depolarization accelerated the generation of unitary potentials in a potential-dependent manner.

**Effect of pinacidil on the generation of unitary potentials**

It has been reported that in circular muscle strips of the antrum of guinea-pig stomach, cromakalim, an opener of K\(_{ATP}\) channels, inhibits contractile activity, with associated hyperpolarization of the membrane (Katayama et al., 1993; Huang et al., 1999). Experiments were carried out to investigate the effects of membrane hyperpolarization with an opener of K\(_{ATP}\) channels on the generation of unitary potentials in pacemaker potential-generating cells. Pinacidil (10 \( \mu \)M) hyperpolarized the membrane (8.4 ± 5.9 mV, n=10), and increased the amplitude (control, 48.8 ± 6.9 mV; in pinacidil, 55.9 ± 6.3 mV; n=10; P<0.05) and decreased the frequency (control, 3.23 ± 0.72 min\(^{-1}\); in pinacidil, 2.45 ± 0.72 min\(^{-1}\); n=10; P<0.05) of pacemaker potentials, with no alteration to the dV/dt\(_{max}\) of the primary component (control, 0.62 ± 0.10 Vs\(^{-1}\); in pinacidil, 0.53 ± 0.11 Vs\(^{-1}\); n=10; P>0.05). In the presence of pinacidil, generation of unitary potentials was increased (not quantified), and the unitary potentials were larger in amplitude than in the absence of pinacidil (control, 0.9 ± 0.5 mV, n=39; in pinacidil, 1.2 ± 0.8 mV, n=126; P<0.01; Figs. 8 and 9A). However, the half-width of unitary potentials was not significantly altered by pinacidil (control, 89 ± 42 ms, n=39; in pinacidil, 97 ± 35 ms; n=126; P>0.05). The relationship between the amplitudes of unitary potentials and their half-widths was not altered by 10 \( \mu \)M pinacidil (Fig. 9B). Similar results were obtained by application of 300 \( \mu \)M nicorandil.
or 200 µM diazoxide, which are alternative K<sub>ATP</sub>-channel openers (data not shown). In 2 preparations with no detectable unitary potentials, pinacidil elicited the generation of unitary potentials during intervals between pacemaker potentials.

Thus, hyperpolarization of the membrane with K<sub>ATP</sub>-channel openers increases the amplitude and frequency of unitary potentials, with no alteration to the relationship between the amplitudes and half-widths of unitary potentials.

**Discussion**

In isolated circular smooth muscle bundles of the guinea pig gastric antrum, slow potentials are thought to be produced by a high frequency burst of unitary potentials (Edwards et al., 1999). Unitary potentials are generated by the opening of Ca<sup>2+</sup>-activated Cl<sup>-</sup>-channels (Hirst et al., 2002). In the gastric muscle of W/W<sup>v</sup> mutant mice lacking ICC-IM, the second component of slow waves is absent (Dickens et al., 2001). The second component of slow waves is thought

---

**Fig. 7.** Effects of high-K solution on pacemaker and unitary potentials generated in ICC-MY. Responses were recorded before (A) and during application of high-K solution (B, [K<sub>i</sub>]<sub>o</sub> = 10.6 mM; C, [K<sub>i</sub>]<sub>o</sub> = 24.7 mM). All responses were recorded from the same cell. The resting membrane potential, –64 mV (shown by dotted line in B and C).
to operate through the same mechanism as slow potentials generated in circular smooth muscle bundles with no attached ICC-MY (Suzuki and Hirst, 1999). Therefore, slow waves are likely to include a component due to electrotonic spread of unitary potentials generated in the ICC-IM. Similar unitary potentials are detected in the plateau components of pacemaker potentials and during intervals between pacemaker potentials recorded from ICC-MY of the guinea pig gastric
antrum (Hirst and Edwards, 2001). The present study confirms that the electrophysiological properties of unitary potentials recorded from ICC-MY are similar to those of unitary potentials generated in ICC-IM of the guinea pig gastric antrum.

In the present study, both spontaneously generated unitary potentials and pacemaker potentials are abolished by application of BAPTA-AM, an internal Ca\(^{2+}\) chelator, or papaverine, a nonselective inhibitor of phosphodiesterase. Although both chemicals inhibited the generation of pacemaker potentials, their effects on the configuration of pacemaker potentials differed (Kito et al., 2002a). The inhibitory action of BAPTA-AM acted first on the primary component, and subsequently on the plateau component. These effects may be consequent upon a decrease in the level of [Ca\(^{2+}\)] (Fukuta et al., 2002). On the other hand, papaverine abolished the plateau component first, and then inhibited the generation of the primary component. Papaverine is known to increase intracellular cAMP levels due to nonselective inhibition of the activity of phosphodiesterases (Berndt et al., 1976). In several types of smooth muscle tissues, cAMP decreases [Ca\(^{2+}\)], through activation of protein kinase A (PKA; Kuriyama et al., 1998). These results suggest that in ICC-MY, the generation of unitary potentials requires an elevation of [Ca\(^{2+}\)], as in the case of unitary potentials generated in ICC-IM (Edwards et al., 1999).

Interestingly, during recovery from inhibition by papaverine, the generation of unitary potentials recommences before the reappearance of pacemaker potentials, and pacemaker potentials having only a primary component are often observed before recovery of the plateau component. These results support the hypothesis presented previously (Hirst and Edwards, 2001; Kito and Suzuki, 2003a) that depolarization of the membrane due to summation of unitary potentials can exceed threshold for activation of ion channels (possibly voltage-dependent Ca\(^{2+}\)-permeable channels). The consequent influx of Ca\(^{2+}\) generates the primary component of the pacemaker potential, and this activates the release of Ca\(^{2+}\) from IP\(_3\)-sensitive internal stores to open Ca\(^{2+}\)-sensitive Cl\(^{-}\)-channels in the cell membrane which generates the plateau component (Hirst et al., 2002).

Depolarization of the membrane with high-K solution also increased the frequency of unitary potential generation in ICC-MY of the guinea pig gastric antrum. Furthermore, this occurred in preparations in which spontaneous generation of unitary potentials was not obvious in control solution. Depolarization of the membrane with high-K solution has been shown to elevate the level of resting [Ca\(^{2+}\)], even in the presence of nifedipine (Fukuta et al., 2002). Isolated segments of antral smooth muscle tissue contain fragments of cholinergic nerves, and high-K solution may depolarize the membranes of nerve terminals and accelerate the release of acetylcholine (ACh) from the nerve endings. In gastric muscles, ACh elevates [Ca\(^{2+}\)] through IP\(_3\) pathways, and diacylglycerol produced via this pathway could activate PKC (Kito et al., 2002b). Thus, an alternative possibility may be that cholinergic mechanisms enhance generation of unitary potentials. It is reported that in isolated circular smooth muscle bundles of the guinea-pig gastric antrum, atropine does not alter the frequency or amplitude of slow potentials generated in high-K solution (Nose et al., 2000). Taken together, these observations suggest that the elevated generation of unitary potentials during depolarization with high-K solution is causally related to the elevated level of resting [Ca\(^{2+}\)], in ICC-MY.

The effects of pinacidil on unitary potentials generated in ICC-MY seem rather complicated.
In ICC-MY, pinacidil hyperpolarizes the membrane and increases the amplitude and frequency of unitary potentials. Preliminary experiments indicated that in the guinea-pig gastric antrum, application of K\textsubscript{ATP}-channel openers inhibited the second component of slow waves with associated reduction in amplitude of the first component, however stimulation of muscles with pinacidil for a long period of time (20–40 min) resulted in the reappearance of the second component of slow waves. The restored second component in the sustained presence of pinacidil was again abolished by 5-hydroxydecanoic acid (5-HDA), an inhibitor of mitochondrial K\textsubscript{ATP}-channels (Kito and Suzuki, 2003b). These observations suggest that the reappearance of the second component of slow waves in the presence of K\textsubscript{ATP}-channel openers is related to the activation of mitochondrial K\textsubscript{ATP}-channels. Mitochondrial K\textsubscript{ATP}-channels distributed in the mitochondrial inner membrane are an isoform different from sarcolemmal K\textsubscript{ATP}-channels (Inoue et al., 1991), and may contribute to mitochondrial volume control, mitochondrial Ca\textsuperscript{2+} handling or production of reactive oxygen species (O’Rourke 2000; Grover et al., 2000). It has been reported that both diazoxide and pinacidil activate Ca\textsuperscript{2+} release from isolated rat cardiac mitochondria, possibly by depolarizing the mitochondrial membrane and decreasing the driving force for the entry of Ca\textsuperscript{2+} (Holmuhamedov et al., 1999). In intact cardiomyocytes, diazoxide decreases mitochondrial [Ca\textsuperscript{2+}]\textsubscript{i}, and this action of diazoxide is antagonized by 5-HDA (Holmuhamedov et al., 1999). It is therefore possible that in gastric muscles, K\textsubscript{ATP}-channel openers release Ca\textsuperscript{2+} from mitochondria in ICC-IM and this accelerates the generation of unitary potentials, possibly through activation of Ca\textsuperscript{2+}-activated Cl–-channels. This may also be the case for ICC-MY during exposure to K\textsubscript{ATP}-channel openers. Further experiments are required to elucidate the precise mechanisms for the actions of K\textsubscript{ATP}-channel openers on the generation of unitary potentials in ICC-MY.

Previous studies have suggested that the following processes are involved in the generation of pacemaker potentials in ICC-MY (Kito and Suzuki, 2003a); (1) Ca\textsuperscript{2+} released from mitochondria activates Ca\textsuperscript{2+}-activated Cl–-channels to generate unitary potentials; (2) When the membrane potential exceeds the threshold level for excitation, voltage-gated Ca\textsuperscript{2+} permeable channels are opened and generate the primary component of a pacemaker potential; (3) This depolarization elicits an influx of Ca\textsuperscript{2+} and elevates production of IP\textsubscript{3} through the activation of a Ca\textsuperscript{2+}-sensitive enzyme such as phospholipase C\textgreek{d} (Rebecchi and Pentyala, 2000); (4) Elevated IP\textsubscript{3} causes the release of Ca\textsuperscript{2+} from internal stores, which induces the generation of the plateau component by opening Ca\textsuperscript{2+}-activated Cl–-channels. K\textsubscript{ATP}-channel openers may enhance the generation of unitary potentials by elevating the release of Ca\textsuperscript{2+} from mitochondria, while BAPTA-AM and papaverine may decrease the generation of unitary potentials by reducing the available Ca\textsuperscript{2+} in mitochondria.

In conclusion, these results suggest that in ICC-MY, the generation of unitary potentials are causally related to an increase in [Ca\textsuperscript{2+}]\textsubscript{i}, and intracellular Ca\textsuperscript{2+} handling mechanisms may be maintained by mitochondrial functions. Electrophysiological properties of ICC-MY were similar to those of unitary potentials generated in ICC-IM.
Acknowledgements

Nicorandil was a gift from Chugai Pharmaceutical Co. Ltd (Japan). The present experiments were supported partly by the Grant-in-Aid for the Scientific Research (C) from the Ministry of Education, Culture, Sports, Science and Technology (MEXT) of Japan to H. S. (No. 14570044).

References

Berndt, S.F., Schulz, H.U. and Stock, K. (1976). Influence of papaverine derivatives on phosphodiesterase activity, cyclic 3',5'-AMP levels and relaxing effect on rabbit ileum. Naunyn-Schmiedeberg's Arch. Pharmacol. 294: 271–275.

Dickens, E.J., Hirst, G.D.S. and Tomita, T. (1999). Identification of rhythmically active cells in guinea-pig stomach. J. Physiol. (Lond.) 514: 515–531.

Dickens, E.J., Edwards, F.R. and Hirst, G.D.S. (2001). Selective knockout of intramuscular interstitial cells reveals their role in the generation of slow waves in mouse stomach. J. Physiol. (Lond.) 531: 827–833.

Edwards, F.R., Hirst, G.D.S. and Suzuki, H. (1999). Unitary nature of regenerative potentials recorded from circular smooth muscle of guinea-pig antrum. J. Physiol. (Lond.) 519: 235–250.

Fukuta, H., Kito, Y. and Suzuki, H. (2002). Spontaneous electrical activity and associated changes in calcium concentration in guinea-pig gastric smooth muscle. J. Physiol. (Lond.) 540: 249–260.

Grover, G.J. and Garlid, K.D. (2000). ATP-sensitive potassium channels: A review of their cardioprotective pharmacology. J. Mol. Cell. Cardiol. 32: 677–695.

Hirst, G.D.S. and Edwards, F.R. (2001). Generation of slow waves in the antral region of guinea-pig stomach—a stochastic process. J. Physiol. (Lond.) 535: 165–180.

Hirst, G.D.S., Bramich, N.J., Teramoto, N., Suzuki, H. and Edwards, F.R. (2002). Regenerative component of slow waves in the guinea pig gastric antrum involves a delayed increase in [Ca$^{2+}$] and Cl$^{-}$ channels. J. Physiol. (Lond.) 540: 907–919.

Holmuhamedov, E.L., Wang, L. and Terzic, A. (1999). ATP-sensitive K$^{+}$ openers prevent Ca$^{2+}$ overload in rat cardiac mitochondria. J. Physiol. (Lond.) 519: 347–360.

Huang, S.-M., Nakayama, S., Iino, S. and Tomita, T. (1999). Voltage sensitivity of slow wave frequency in isolated circular muscle strips from guinea pig gastric antrum. Am. J. Physiol. 276: G518–G528.

Huizinga, J.D., Thuneberg, L., Vanderwinden, J.-M. and Rumessen, J. (1997). Interstitial cells of Cajal as targets for pharmacological intervention in gastrointestinal motor disorders. Trend. Pharmacol. Sci. 18: 395–403.

Inoue, I., Nagase, H., Kishi, K. and Higuti, T. (1991). ATP-sensitive K$^{+}$ channel in the mitochondrial inner membrane. Nature 352: 244–247.

Katayama, N., Huang, S.-M., Tomita, T. and Brading, A.F. (1993). Effects of cromakalim on the electrical slow waves in the circular muscle of guinea-pig gastric antrum. Br. J. Pharmacol. 109: 1097–1100.

Kito, Y., Fukuta, H. and Suzuki, H. (2002a). Components of pacemaker potentials recorded from the guinea-pig stomach antrum. Pflüg. Arch. 445: 202–217.

Kito, Y., Fukuta, H., Yamamoto, Y. and Suzuki, H. (2002b). Excitation of smooth muscle isolated from the guinea-pig gastric antrum in response to depolarization. J. Physiol. (Lond.) 543: 155–167.

Kito, Y. and Suzuki, H. (2003a). Pacemaker frequency is increased by sodium nitroprusside in the guinea pig gastric antrum. J. Physiol. (Lond.) 546: 191–205.

Kito, Y. and Suzuki, H. (2003b). Modulation of slow waves by hyperpolarization with potassium-channel openers in antral smooth muscle of the guinea-pig stomach. J. Physiol. (Lond.) (in press).
Properties of unitary potentials in ICC-MY

Komuro, T., Tokui, K. and Zhou, D.S. (1996). Identification of the interstitial cells of Cajal. Histol. Histopathol. 11: 769–786.

Kuriyama, H. (1970). Effects of ions and drugs on the electrical activity of smooth muscle. In: Smooth Muscle, ed. by Bülbüring, E., Brading, A.F., Jones, A.W. and Tomita, T., Edward Arnold, London, pp. 366–395.

Kuriyama, H., Kitamura, K., Itoh, T. and Inoue, R. (1998). Physiological features of visceral smooth muscle cells, with special reference to receptors and ion channels. Physiol. Rev. 78: 811–920.

Nose, K., Suzuki, H. and Kannan, H. (2000). Voltage-dependency of the frequency of slow waves in antrum smooth muscle of the guinea-pig stomach. Jpn. J. Physiol. 50: 625–633.

O’Rourke, B. (2000). Pathophysiological and protective roles of mitochondrial ion channels. J. Physiol. 529: 23–36.

Rebecchi, M.J. and Pentyala, S.N. (2000). Structure, function, and control of phosphoinositide-specific phospholipase C. Physiol. Rev. 80: 1291–1335.

Sanders, K.M. (1996). A case for interstitial cells of Cajal as pacemakers and mediators of neurotransmission in the gastrointestinal tract. Gastroenterol. 111: 492–515.

Sanders, K.M., Ördög, T., Koh, S.D., Torihashi, S. and Ward, S.M. (1999). Development and plasticity of interstitial cells of Cajal. Neurogastroenterol. Motil. 11: 311–338.

Suzuki, H. and Hirst, G.D.S. (1999). Regenerative potentials evoked in circular smooth muscle of the antral region of guinea-pig stomach. J. Physiol. (Lond.) 517: 563–573.

Thuneberg, L. (1982). Interstitial cells of Cajal: intestinal pacemaker cells? Adv. Anat. Embryol. Cell Biol. 71: 1–130.

Tomita, T. (1981). Electrical activity (spikes and slow wave) in gastrointestinal smooth muscles. In: Smooth Muscle, ed. by Bülbüring, E., Brading, A.F., Jones, A.W. and Tomita, T., Edward Arnold, London, pp. 127–156.

(Received December 5, 2002: Accepted December 27, 2002)