Regional differences in the frequency of slow waves in smooth muscle of the guinea-pig stomach

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Abstract

The frequency of slow waves recorded from circular muscle bundles with attached longitudinal muscle (intact muscle) was compared with that of slow potentials recorded from isolated circular muscle bundles (isolated muscle) from the guinea-pig stomach. In intact muscle preparations, slow waves were generated in the corpus, antrum and pylorus with a higher frequency in the corpus (about 5 min⁻¹) than the other regions (about 2 min⁻¹ in antrum, about 1.5 min⁻¹ in pylorus). The resting potential amplitude was graded across the stomach, at about −50 mV in the fundus, −60 mV in the corpus, −65 mV in the antrum and −70 mV in the pylorus. A similar distribution of resting membrane potential and slow potential frequency was also observed in isolated muscle bundles from the different regions. Caffeine (1 mM) abolished slow waves in some corpus preparations and inhibited the 2nd component of slow waves in the antrum and pylorus, and also abolished slow potentials in isolated muscle preparations from any region of the stomach. This suggests that myenteric interstitial cells of Cajal (ICC-MY) are heterogeneously distributed in the stomach (pylorus, antrum and part of the corpus regions), with a homogeneous distribution of muscular interstitial cells of Cajal (ICC-IM) within the circular muscle bundles. The frequency of slow potentials in smooth muscle isolated from any region of the stomach changed linearly in response to membrane potential changes produced by either current injection or high potassium solutions. The frequency of slow potentials after setting the membrane potential at −60 mV was larger in the corpus than the antrum, suggesting that the high frequency discharge of corpus muscle is produced by the low membrane potential and additional unidentified factors. We suggest that the regional difference in slow wave discharge is produced mainly by ICC-IM, and the role of ICC-MY may be little, if any.

Key words: gastric smooth muscles, slow waves, slow potentials, membrane potential, interstitial cells of Cajal

Introduction

Gastric smooth muscle spontaneously generates slow waves (Tomita, 1981), which may
originate from interstitial cells of Cajal (ICC) distributed in the gastric wall (Sanders, 1996; Huizinga et al., 1997; Sanders et al., 1999). There are two types of ICC in the guinea-pig stomach, ICC-MY distributed in the myenteric layers and ICC-IM distributed within smooth muscle bundles (Burns et al., 1995). In smooth muscle cells of the guinea-pig gastric antrum, recording electrical responses of the membrane using intracellular microelectrodes revealed that ICC-MY generate driving potentials (or pacemaker potentials) spontaneously in the presence of nifedipine (Dickens et al., 1999). ICC-MY have gap junctional connections with surrounding ICC-MY, ICC-IM and smooth muscle cells (Komuro et al., 1999; Komuro, 2006), and the driving potentials generated in ICC-MY propagate to ICC-IM and smooth muscle cells in an electrotonic manner and elicit follower potentials in the longitudinal muscle and slow waves in the circular muscle (Dickens et al., 1999; Hirst and Edwards, 2001; Cousins et al., 2003; Hirst and Ward, 2003; Hirst and Edwards, 2006; Hirst et al., 2006). However in circular muscle bundles isolated from the gastric antrum of the guinea-pig, spontaneous electrical activity, unitary potentials and slow regenerative potentials, were also produced spontaneously, even in the absence of ICC-MY (Suzuki and Hirst, 1999; Suzuki, 2000). Frequency analysis of the slow regenerative potentials suggested that this potential was formed by a summation of unitary potentials (Edwards et al., 1999; Hirst and Ward, 2003; Hirst et al., 2006). The properties of slow regenerative potentials recorded from isolated circular muscle bundles differed from slow waves recorded from circular muscle of the intact tissue, in that the slow regenerative potentials but not the slow waves could be abolished by low concentrations (0.5–2 mM) of caffeine (Suzuki and Hirst, 1999), and therefore the potentials produced in isolated circular muscle bundles were identified as slow potentials (Kito et al., 2002). Thus, these data indicate that two types of pace-producible ICC are functioning in the stomach wall, ICC-MY distributed in myenteric layers and ICC-IM distributed in circular muscle bundles.

Slow waves have two components referred to as the 1st and 2nd components (Tomita, 1981). Examination of the properties of slow waves generated in gastric muscle of W/Wv mice lacking the c-kit gene, indicates that the 1st component is produced by an electrotonic spread of driving potentials generated in ICC-MY and that the 2nd component which is superimposed on the 1st component is produced by summed unitary potentials generated in ICC-IM (Dickens et al., 2001; Hirst and Ward, 2003; Hirst et al., 2006). In considering the triggering of the discharges of unitary potentials and slow potentials in response to membrane depolarization (Suzuki and Hirst, 1999; Kito et al., 2002), the 2nd component of slow waves may be produced by the depolarization caused by the driving potentials. In this case, it is reasonable to consider that ICC-MY are taking a pacemaker role in the stomach (Hirst and Edwards, 2001). However, it has been shown recently that in the guinea-pig stomach, smooth muscle cells showing slow waves with the highest frequency are distributed in the corpus region along the greater curvature (Hashitani et al., 2005; Hirst et al., 2008). The smooth muscle of this region has a distribution of only ICC-IM, with no ICC-MY, and produces 4–6 slow waves every min (Hashitani et al., 2005), the frequency being much higher than that in the antrum region in which it ranges between 1 and 2 times per min (Nose et al., 2000; Fukuta et al., 2002).

The question arises as to why the frequency of slow wave discharge differs between regions of the stomach. Why is the frequency of slow waves high in the corpus and low in the antrum
or pylorus? Attempts were made to investigate the properties of slow potentials generated in smooth muscle bundles isolated from different regions of the guinea-pig stomach. There is a graded distribution of resting membrane potential in the guinea-pig stomach, with the least negative potential in the fundus region and the most negative potential in the pylorus region (Komori and Suzuki, 1986). The frequency of slow waves is sensitive to the membrane potential, and is increased by depolarization and decreased by hyperpolarization of the membrane (Nose et al., 2000). Therefore, the relationship between membrane potential and the frequency of spontaneous activity (slow waves and slow potentials) was examined in muscle bundles isolated from different regions of the stomach. The results indicate that differences in the resting membrane potential may make some, but not all, contribution to the production of the regional difference in frequency of slow waves. It was also found that the regional difference in the frequency of slow waves may be causally related to the difference in the properties of ICC-IM distributed in the circular muscle.

Materials and methods

Preparations

Male albino guinea-pigs, weighing 200–300 g, were anesthetized with fluoromethyl 2,2,2-trifluoro-1-(trifluoromethyl) ethyl ether (sevoflurane, Maruishi Pharm., Osaka, Japan), and exsanguinated by decapitation. All animals were treated ethically according to the guiding principles for the care and use of experimental animals in the field of physiological sciences, approved by The Experimental Animal Committee of the Nagoya City University Medical School. The stomach was excised, and a segment of tissue (5 × 5 mm) was dissected from the stomach wall, along the greater curvature, and kept in Krebs solution. After removing the mucosal layers by cutting with fine scissors, single circular muscle bundle preparations either with the longitudinal muscle layer attached together with the myenteric plexus (intact muscle) or without attached longitudinal muscle and myenteric layers (isolated muscle). Circular muscle bundles (250–300 µm long) were then pinned on the silicone rubber plate, with the mucosal side uppermost. The bundles were superfused with oxygenated Krebs solution (warmed to 36.5°C), at a constant flow rate of about 2 ml/min.

Conventional microelectrode techniques were used to record the electrical activity of smooth muscle cells, using glass capillary microelectrodes (capillary outer diameter, 1.2 mm, inner diameter 0.6 mm; Hilgenberg, Germany) filled with 0.5 M KCl. The tip resistance of the electrodes ranged between 150 and 300 MΩ. In some experiments, electrical responses were recorded simultaneously from two different cells in the same segment, using two electrodes. When the electrical responses of two cells were synchronized (which occurred in over 99% of cell pairs examined), stimulating current pulses were applied to one electrode and changes in membrane potential were recorded from the second electrode, as reported previously (Suzuki and Hirst, 1999). Electrical responses recorded through 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 were also stored on a personal computer for later analysis. As no significant change in the general properties of slow waves
had been reported in the presence of several types of Ca-antagonists (Tomita, 1981), all experiments were carried out in the presence of $10^{-6} \text{M}$ nifedipine throughout, which also minimized muscular movement.

The ionic composition of the Krebs solution was as follows (mM): $\text{Na}^+ 137.4$, $\text{K}^+ 5.9$, $\text{Ca}^{2+} 2.5$, $\text{Mg}^{2+} 1.2$, $\text{HCO}_3^− 15.5$, $\text{H}_2\text{PO}_4^− 1.2$, $\text{Cl}^− 134$, and glucose 11.5. Solutions with a high concentration of potassium ions (high-K solutions) were prepared by elevating the volume of KCl solution and reducing the volume of NaCl solution, thus keeping the concentration of chloride ions constant. These solutions were aerated with $\text{O}_2$ containing 5% $\text{CO}_2$, and the pH of the solutions was maintained at 7.2–7.3. Chemicals used were nifedipine and caffeine (both purchased from Sigma-Aldrich Chem., St. Louis, MI, USA). Nifedipine was dissolved first in dimethyl sulphoxide (DMSO) at a concentration of 10 mM, and it was further diluted to $1 \mu\text{M}$ with Krebs solution, just before use.

The voltage-dependency of slow potentials was expressed by fitting the distribution of values with a linear line $Y = aX + b$ ($Y$, parameters of slow potentials; $X$, membrane potential; $a$, slope; $b$, value $Y$ at $X = 0$) using the least square method, and the fit of $Y$ values to the line was evaluated by calculating the correlation coefficient ($r$).

Experimental values were expressed as the mean value ± standard deviation (S.D.). Statistical significance was tested using Student’s $t$-test (two-tailed), and probabilities of less than 5% ($P<0.05$) were considered to be significant.

### Results

**Distribution of slow waves and slow potentials in intact muscle of the stomach**

In intact muscle preparations, recording membrane activity from circular smooth muscle cells with microelectrodes revealed that slow waves could be recorded from corpus, antrum and pylorus muscle bundles (Fig. 1). Most of the muscle bundles isolated from the fundus region showed a random generation of unitary potentials with an amplitude ranging between 0.1 and up to 2 mV, and in only 2 out of 11 bundles examined, slow waves with low amplitude (5–10 mV) were recorded (data not shown). In corpus, antrum and pylorus preparations, the shape of slow waves differed between regions, as reported previously (Komori and Suzuki, 1986). Briefly, slow waves generated in the corpus muscle appeared successively with no stable potential in the interval, and the rising and falling phases of each wave were formed by a similar time course in each opposite direction (Fig. 1B). In the antrum and pylorus, slow waves were formed with initial rapidly rising and following slowly falling phases, with a stable potential, lasting from 3–5 s to up to 2 min, in the interval between the slow waves (Fig. 1, C–E). In some preparations isolated from the antrum, slow waves were observed to have 2 components, with the 1st component being the initial rapidly rising phase and slowly falling phases, while the 2nd component appeared when the falling phase had reached about 80% of the peak amplitude (Fig. 1D).

When the most negative level of the membrane potential in the interval between slow waves was defined as the resting membrane potential, it differed between regions, with the least negative values in smooth muscle isolated from the fundus region (about −50 mV), and
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becoming more negative in successively lower regions of the stomach (about –60 mV in corpus, about –65 mV in antrum, and about –70 mV in pylorus, see Fig. 5A).

Figure 2 shows the distribution of both the amplitude and frequency of slow waves in the stomach, plotted against the resting membrane potential. The amplitude of slow waves was negatively correlated with the membrane potential, being least in the corpus and largest in the pyloric region (Fig. 2A). The frequency of slow waves tended to increase as the resting membrane potential became less negative, being lowest in the pyloric region and highest in the corpus region (Fig. 2B). These results agreed with previous observations which had examined the distribution of slow waves in gastric smooth muscle of the guinea-pig using a large segment of tissue (Komori and Suzuki, 1986).

Isolated circular smooth muscle generated slow potentials in corpus, antrum and pylorus preparations, but not in those of the fundus. The distribution of slow potentials in the stomach was similar to that of slow waves (Fig. 3). Isolated single circular smooth muscle bundles of the fundus showed a random generation of unitary potentials with an amplitude ranging from 0.5 mV to up to 15 mV (Fig. 3A). The amplitude of slow potentials was least in corpus muscle compared to antrum and pylorus muscle, while the frequency was higher in corpus muscle and
lower in antrum and pyloric muscle (Fig. 3, B–E). The resting membrane potential was least negative in fundus muscle, and became more negative from the corpus to the pyloric muscle. Thus, the results again showed regional differences in the distribution of the resting membrane potential as well as both the amplitude and frequency of slow potentials. The difference between intact muscle and isolated muscle appeared in the random generation of unitary potentials, which were more marked in the latter than in the former, although their generation could be recognized in both types of preparation.

The plot of the amplitude and frequency of slow potentials against membrane potential (Fig. 4) indicated that the relationship was similar to that of slow waves (see Fig. 2). The amplitude of slow potentials increased with increase in negativity of the resting membrane potential, being larger in antrum and pyloric muscle compared to that of corpus muscle (Fig. 4A). The distribution of the frequency of slow potentials was also dependant on the membrane potential, and the frequency was higher in cells with less negative membrane potentials (Fig. 4B). The frequency of slow potentials was higher in corpus muscle and lower in antrum and pyloric muscle (Fig. 3, B–E). The resting membrane potential was least negative in fundus muscle, and became more negative from the corpus to the pyloric muscle. Thus, the results again showed regional differences in the distribution of the resting membrane potential as well as both the amplitude and frequency of slow potentials. The difference between intact muscle and isolated muscle appeared in the random generation of unitary potentials, which were more marked in the latter than in the former, although their generation could be recognized in both types of preparation.

The plot of the amplitude and frequency of slow potentials against membrane potential (Fig. 4) indicated that the relationship was similar to that of slow waves (see Fig. 2). The amplitude of slow potentials increased with increase in negativity of the resting membrane potential, being larger in antrum and pyloric muscle compared to that of corpus muscle (Fig. 4A). The distribution of the frequency of slow potentials was also dependant on the membrane potential, and the frequency was higher in cells with less negative membrane potentials (Fig. 4B). The frequency of slow potentials was higher in corpus muscle and lower in antrum and pyloric

Fig. 2. Distribution of slow waves (A, amplitude; B, frequency) recorded from circular muscle of the intact muscle preparations isolated from corpus (●), antrum (○) and pylorus regions (▲) of the guinea-pig stomach, plotted against the resting membrane potential. Mean ± S.D. (n=8–15). Nifedipine 10⁻⁶ M was present throughout.
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Thus, the results indicate that the distribution of the resting membrane potential, and both the amplitude and frequency of spontaneous activity of circular muscle did not show a significant difference between intact and isolated preparations.

The difference between intact and isolated circular muscle bundles had been considered to be due to the heterogeneous distribution of the type of ICC in the tissue, with both ICC-MY and ICC-IM in the intact muscle, while there is only ICC-IM in isolated circular muscle bundles. The possible involvement of ICC-IM in a given segment of circular muscle bundle could be examined by using caffeine. Low concentrations of caffeine (0.5–2 mM) selectively abolish unitary potentials and slow potentials, but partially inhibit slow waves, possibly due to selective inhibition of the activity of ICC-IM (Suzuki and Hirst, 1999). Experiments were carried out to observe the effects of 1 mM caffeine on slow waves or slow potentials recorded from circular smooth muscle bundles isolated from different regions of the guinea-pig stomach.

When 1 mM caffeine was applied to intact muscle preparations, the membrane was transiently hyperpolarized by 1–5 mV and abolished slow waves in corpus muscle preparations (Fig. 5A) or inhibited the amplitude and frequency of slow waves in antrum and pyloric muscle and also in some of corpus muscle preparations (Fig. 5, B–D). Caffeine abolished slow waves in 15 of the 24 corpus muscle preparations examined. The amplitude of hyperpolarization

**Fig. 3.** Slow potentials recorded from isolated circular muscle bundles taken from different regions of the stomach. A, fundus; B and C, corpus; D, Antrum; E, pylorus. All responses were recorded from different tissues. Nifedipine 10⁻⁶ M was present throughout.

![Slow potentials recorded from isolated circular muscle bundles taken from different regions of the stomach.](image-url)
produced by caffeine was 2.4 ± 1.0 mV (n=15) in preparations which showed disappearance of slow waves with caffeine, and 3.0 ± 1.5 mV (n=9) in preparations which showed generation of slow waves in the presence of caffeine. As the amplitude of caffeine-induced hyperpolarization was not significantly different between the two (P>0.05), it was suggested that the presence or absence of ICC-MY was not causally related to the frequency of slow waves in corpus muscle. The relationship between the amplitude of caffeine-induced hyperpolarization (peak amplitude) and the frequency (Fig. 6A) or the amplitude of slow waves (Fig. 6B) generated in the absence of caffeine, indicated that there was no causal relationship between these parameters. These results suggest that the responses to caffeine were not causally related to the type of ICC distributed in the given preparation. Examination of electrical responses with an expanded time scale revealed that the reduction in amplitude of slow waves generated in antrum muscle was due to the inhibition of the 2nd component (data not shown).

In circular muscle bundles isolated from any region of the stomach, caffeine abolished both
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Slow potentials and unitary potentials, with an associated transient hyperpolarization of the membrane (Fig. 5, E–G). Thus, although the effects of caffeine varied between preparations of intact muscle isolated from the corpus region, there was no significant regional difference in the
inhibitory actions of caffeine on both slow potentials and unitary potentials. The inhibitory actions of caffeine were reversible, and required 5–7 min for recovery (data not shown).

Comparison was made of the resting membrane potential, the amplitude and the frequency of both slow waves and slow potentials recorded in muscle isolated from different regions of the stomach. In intact muscle preparations, the resting membrane potential of circular smooth muscle cells was least negative in the fundus muscle, and more negative in lower regions of the stomach (Fig. 7A). The distribution of resting membrane potentials in isolated circular muscle bundles was again similar to that of intact muscle bundles, suggesting that the effects of both longitudinal muscle and ICC-MY on the resting membrane potential of the circular muscle were negligibly small, if any.

Both the amplitude and frequency of slow waves were compared with those of slow waves.
potentials in different regions of the stomach, and the results summarized in Fig. 7, B and C, respectively. The amplitude of slow potentials was significantly larger for slow potentials than for slow waves, at any given region (Fig. 7B). The frequency of slow waves was higher in corpus muscle and lower in pyloric muscle, with an intermediate value for the antrum, while the distribution of slow potential frequency was similar to that of slow waves (Fig. 7C). In each region, the frequency of slow waves was not significantly different from that of slow potentials. These results strongly suggest that in the guinea-pig stomach, ICC-MY do not have a major role in the determination of the slow wave frequency, while ICC-IM are taking an important role in the production of the regional differences in slow wave frequency.

Voltage-dependency of slow potentials

The results indicated that there was a graded distribution of the resting membrane potential in the circular smooth muscle of the stomach, being the least negative in the fundus and most negative in the pylorus, confirming previous observations (Komori and Suzuki, 1986). The present experiments further indicate that this was also the case in isolated circular muscle bundles (Fig. 7). The frequency of slow waves or slow potentials is sensitive to the membrane potential, and is increased by depolarization and decreased by hyperpolarization (Nose et al., 2000; Suzuki et al., 2006). Attempts were therefore made to examine the possible effects of the membrane potential in producing the regional differences in the discharge of slow potentials. Experiments were carried out to observe the effects of changes in the membrane potential on the frequency of slow potentials in circular muscle bundles isolated from different regions of the stomach. Membrane potentials were changed using either high-K solutions or current injections via intracellular electrodes.

Figure 8 shows the effects of membrane hyperpolarization by current injection on slow potentials generated in isolated circular muscle from the corpus region. At rest (i.e., the membrane potential was –59 mV), slow potentials with amplitude of about 35 mV were generated rhythmically at a frequency of about 4.5 times min⁻¹. Application of three different intensities (0.5, 1.5 and 2.5 nA) of inward current pulse for 1 min to the muscle produced a sustained hyperpolarization with an intensity-dependent amplitude. During this time, the amplitude of slow potentials was increased and their frequency was decreased in an intensity-dependent manner (Fig. 8A). The changes in both the amplitude and frequency of slow potentials were plotted against the membrane potentials produced by current injection (Fig. 8, B and C, respectively). The results showed that for both amplitude and frequency, there was a linear response against membrane potential, with amplitude being negatively related and frequency positively related to the membrane potential. The regression lines calculated by using the least square methods were Y = –19.2 – 1.0X (correlation coefficient r=0.97) for the amplitude change and Y = 8.9 + 0.1X (r=0.91) for the frequency change (Y, amplitude or frequency; X, membrane potential).

In isolated circular muscle bundles from the antrum region, the effects of changes in membrane potential on the frequency of slow potentials were also examined by applying current stimulation, using two-electrode experiments. Depolarization of the membrane increased the frequency and decreased the amplitude of slow potentials, while hyperpolarization increased the
amplitude and decreased the frequency, and these changes were again linear against the membrane potential (Fig. 9). Hyperpolarization of the membrane below −80 mV stopped the generation of slow potentials, and clustered unitary potentials alone were generated periodically (data not shown). The changes in amplitude and frequency of slow potentials against membrane potential were again linear, and the regression lines for the amplitude and frequency were shown by $Y = -50.8 - 1.28X$ ($r=0.96$) and $Y = 12.9 + 0.14X$ ($r=0.79$), respectively ($Y$, amplitude or frequency; $X$, membrane potential; $r$=regression coefficient).

In circular muscle isolated from the antrum region, the effects on slow potentials of depolarization of the membrane with high-K solutions were examined. Figure 10 shows a typical example of the effects of high-K solution on slow potentials, with application of a solution containing 10.6 mM [K+]o (10.6 mM-K) producing a sustained depolarization of the membrane by about 5 mV and increasing the frequency of slow potentials from $0.59 \pm 0.12$ times min$^{-1}$ in control to $1.10 \pm 0.36$ times min$^{-1}$ ($P<0.05$) in 10.6 mM-K solution, with a decrease in amplitude
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of about 8% (control, 27.8 ± 1.4 mV, n=10; in 10.6 mM-K, 23.3 ± 1.6 mV, n=10; P<0.05). Similar experiments were repeated in this tissue by applying different concentrations of [K⁺]o (8.7–20 mM), and the amplitude and frequency of slow potentials generated at each high-K solution were plotted against the membrane potential (Fig. 9, B and C, respectively). The results indicated that both amplitude and frequency of slow potentials changed linearly in response to depolarization of the membrane. The amplitude decreased and frequency increased in response to high-K solutions, and their regression lines were as follows; amplitude, Y = −114.4 − 2.1X (r=0.97, Fig. 10B) and frequency, Y = 30.9 + 0.5X (r=0.96, Fig. 10C).

These results indicated that both amplitude and frequency of slow potentials were sensitive to the membrane potential in both corpus and antrum muscle, and that they changed linearly in response to changes in the membrane potential. Attempts were made to calculate the frequency of slow potentials generated at −60 mV in both the corpus and antrum muscle, by using the regression lines obtained in each experiment. The “theoretical” frequency of slow potentials measured at −60 mV was 4.37 ± 0.67 min⁻¹ (n=18) in the corpus muscle and it was significantly
lower in the antrum muscle (3.08 ± 0.68 min⁻¹, n=11, P<0.05). Thus, the results have indicated that the difference in frequency of slow potentials between the corpus and antrum muscle preparations was not simply due to the difference in membrane potential, although the frequency of slow potentials was a function of membrane potential in muscle obtained from any region of the stomach.

**Discussion**

In the guinea-pig stomach, electrophysiological examination of circular smooth muscle using a large segment of tissue revealed a regional difference in electrical properties with the resting membrane potential being graded, at its least negative value in the fundus muscle and its most negative value in the pyloric muscle, with a correlated difference in both the shape and frequency of slow waves (Komori and Suzuki, 1986). The present experiments showed that this was also the case in isolated single circular muscle bundles of the stomach. Moreover, the
graded changes in both the membrane potential and frequency of slow potentials were also found in isolated circular muscle bundles without attached longitudinal muscle and myenteric layers. Slow potentials may be produced by a summation of unitary potentials generated in ICC-IM (Edwards et al., 1999). Slow waves have two components, and the 1st component is produced by an electrotonic propagation of driving potentials from ICC-MY, while the 2nd component is formed by slow potentials (Hirst and Ward, 2003; Suzuki et al., 2006). These regional differences in frequency were found in both slow waves and slow potentials, suggesting that ICC-IM, but not ICC-MY, are taking the main role in the production of spontaneous activity in each region of the stomach. However, this does not necessarily mean that ICC-IM are the only pacemaker cells in the stomach. Periodic generation of follower potentials is observed in isolated longitudinal smooth muscle without attached circular muscle (Nakamura et al., 2006), indicating that ICC-MY could generate rhythmic activities by themselves. These results indicate that there are two types of rhythm-producing cells, ICC-IM and ICC-MY, in the guinea-pig stomach. In considering the homogeneous distribution of ICC-IM throughout the circular muscle (Burns et al., 1995) and the partial distribution of ICC-MY in the stomach (Hashitani et al., 2006), the regional difference in the frequency of slow waves may be dependant on the distribution of ICC-IM in different regions.

Caffeine is useful as a tool to identify the type of ICC distributed in a segment of smooth muscle bundles, since its chemical selectively inhibits the activity of ICC-IM but not ICC-MY in the stomach (Suzuki and Hirst, 1999). However, the mechanism by which caffeine causes this selective inhibition of the activity of ICC-IM remains unclear. In many types of tissues, caffeine releases Ca\(^{2+}\) from internal stores and also increases the content of cyclic AMP by inhibiting the activity of phosphodiesterases (Arnoud, 1987). A causal relationship between cyclic AMP and the inhibitory actions of caffeine has also been suggested in guinea-pig gastric muscle (Tsugeno et al., 1995). However, this does not seem to be the case for gastric smooth muscle of the guinea-pig, as inhibition of the rhythmic activity of the antral smooth muscle by caffeine is not causally related to an elevated concentration of cyclic AMP in the smooth muscle (Nakamura et al., 2004). The present experiments indicate that both slow potentials and unitary potentials generated in any region of the stomach can be abolished by caffeine, suggesting that the cellular mechanism for generating ICC-IM activity is homogenous throughout the stomach.

The present experiments also indicate that in intact muscle preparations from the antrum and pyloric regions of the guinea-pig stomach, application of caffeine inhibited but could not abolish slow waves, indicating that these regions had a distribution of both ICC-MY and ICC-IM. In intact muscle preparations isolated from the corpus, however, the actions of caffeine on slow waves were not the same in all tissues examined, causing them to be abolished in some and to be partially inhibited in others, suggesting that the distribution of ICC-MY is not homogenous in the corpus region. The results further indicated that in the corpus muscle, the frequency of slow wave discharge was not causally related to the distribution of ICC-MY (H. Suzuki, unpublished observation). These results again support the idea that ICC-IM distributed in circular muscle of the corpus region have the main role in the production of rhythmic gastric peristaltic movement. This is in agreement with the findings that in the dog stomach the myoelectrical activity related to gastric peristaltic activity originates in the corpus region along
the large curvature (Cannon, 1898; Kelly and Code, 1971).

The present experiments were aimed to examine the mechanism for the production of the regional difference in the frequency of slow waves in the stomach and to explain why the frequency of slow waves is higher in the corpus region and lower in antrum and pylorus regions. In circular smooth muscle isolated from any region of the stomach, the frequency of slow waves or slow potentials is a function of the membrane potential, and depolarization increases and hyperpolarization decreases the frequency (Nose et al., 2000; Fukuta et al., 2002; Suzuki et al., 2006). However, examination of the change in frequency of slow potentials observed in individual cells indicated that the value of the resting membrane potential of the corpus muscle did not fit the frequency of slow potentials in the corpus region. Thus, the regional difference in the frequency of slow potentials may only be partly related to differences in resting membrane potential. However, in antral muscle preparations for example, change of membrane potential to the resting membrane potential level of the corpus muscle did not alter the frequency to be close to that in isolated corpus muscle. In muscles isolated from the corpus region, hyperpolarization of the membrane to levels close to the resting membrane potential of the antrum muscle again did not reduce the frequency of slow potential discharges to values comparable to that in antrum muscle preparations. These results suggest that differences in the frequency of slow waves between regions is not due to the difference in resting membrane potential alone, and the possible involvement of additional unidentified factors must be considered.

It is concluded that in the guinea-pig stomach, the difference in the frequency of slow waves between regions is produced by the difference in membrane potential and additional unidentified factors. These regional differences in the frequency of slow waves are produced mainly by the difference in the properties of ICC-IM, with little if any contribution by ICC-MY.

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