Metabolic component of the temperature-sensitivity of slow waves recorded from gastric muscle of the guinea-pig

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Abstract

The effects of changes in temperature on slow waves were investigated in smooth muscle tissues isolated from the guinea-pig gastric antrum. Within the range 24°C to 42°C, elevation of temperature increased the frequency and maximum rate of rise of the upstroke phase \((dV/dt)\) of slow waves and decreased their duration, with no alteration to amplitude or resting membrane potential. These observations also applied to follower potentials and pacemaker potentials recorded from longitudinal muscle and myenteric interstitial cells, respectively. Slow waves were comprised of 1st and 2nd components, and the latency for generating the 2nd component was decreased exponentially by elevating temperature, reaching a stable value of about 1 s above 32°C. The temperature coefficient was \(>2\) for the frequency, \(dV/dt\) and latency of the 2nd component, about 1.7 for the duration and about 1 for amplitude. Potassium cyanide (KCN), an inhibitor of mitochondrial metabolic activity, reduced the frequency and duration of slow waves, with no alteration to other parameters (amplitude, \(dV/dt\), latency). In the presence of 30 \(\mu\)M KCN, the temperature-dependency of the frequency of slow waves was diminished or abolished, while other parameters of slow waves remained unaltered. These results indicate that in slow waves the frequency may be related to metabolic activities, while the temperature-dependent changes in the \(dV/dt\), latency for the 2nd component and duration of slow waves are produced largely by mechanisms other than metabolic activity.

Key words: gastric muscle, temperature, slow wave, cyanide, ICC

Introduction

Gastrointestinal smooth muscles are spontaneously active with periodic generation of slow waves and/or spike potentials (Tomita, 1981). Thuneberg (1982) considered that a group of cells called interstitial cells of Cajal (ICC) might be the pacemakers of spontaneous activity in gastrointestinal smooth muscles, since they were rich in mitochondria and had close contact with other ICC and smooth muscle cells. Investigation of the pathophysiological alteration of c-
kit mutant mice revealed that a lack of ICC distributed in the myenteric region (ICC-MY) was causally related to the absence of slow waves in intestinal smooth muscle, thereby suggesting that slow waves might originate in ICC-MY (Ward et al., 1994; Huizinga et al., 1995; Sanders, 1996; Huizinga et al., 1997; Sanders et al., 1999).

In isolated smooth muscle tissues of the guinea-pig gastric antrum, three types of electrical signals are recorded; slow waves recorded from circular smooth muscle cells, pacemaker potentials recorded from ICC-MY and follower potentials recorded from longitudinal smooth muscle cells (Dickens et al., 1999). Pacemaker potentials generated in ICC-MY propagate to both circular and longitudinal smooth muscle cells in an electrotonic manner (Dickens et al., 1999; Cousins et al., 2003). However, the shapes of slow waves differ from follower potentials, despite both potentials being produced by electrotonic spread of pacemaker potentials. Isolated circular smooth muscle bundles of the guinea-pig stomach antrum are also spontaneously active with periodic generation of regenerative potentials (slow potentials) (Suzuki and Hirst, 1999). The addition of a slow potential to a pacemaker-derived electrotonic potential forms a slow wave (Tomita, 1999). Thus, slow waves comprise two components, the 1st component formed by a propagated pacemaker potential and the 2nd component formed by a slow potential generated in the circular muscle bundle (Dickens et al., 2001; Hirst and Edwards, 2001).

Slow waves are absent in mice which lack expression of the inositol 1,4,5-trisphosphate (IP₃) receptor, even in the presence of c-Kit positive interstitial cells (Suzuki et al., 2000), suggesting that the Ca²⁺ releasing processes from internal stores through IP₃ receptors take a key role in the generation of pacemaker potentials. The importance of mitochondria in the generation of pacemaker activity is also suggested by the inhibitory effects of chemicals (CCCP or FCCP) which interfere with Ca²⁺ handling in mitochondria indirectly by impairing proton transport (Ward et al., 2000; Fukuta et al., 2002; Kito et al., 2002a). Slow waves are inhibited by metabolic inhibitors in the guinea-pig stomach (Nakayama et al., 1997). Slow waves are sensitive to temperature, and the temperature coefficient (Q₁₀) of their frequency is high (Q₁₀ = 2–3) (Job, 1969; Golenhofen et al., 1970; Ohba et al., 1975). These observations again suggest that mitochondrial activity may be causally related to the generation of spontaneous activity.

It is expected that any of the components or characteristics of slow waves related directly to mitochondrial activity may be sensitive to temperature. Attempts were made to investigate the changes in configuration and parameters of slow waves recorded from gastric smooth muscle tissues of the guinea-pig, in response to changes in temperature. The effects of KCN, a metabolic inhibitor, on the temperature-sensitive parameters were further examined. The results indicate that the frequency, duration and rate of rise of slow waves were temperature-sensitive, while the amplitude was insensitive to temperature. Of the temperature-sensitive parameters, only the frequency of slow waves was attenuated by KCN, suggesting that the frequency of slow waves (i.e. corresponding to that of pacemaker activities) is coupled to metabolic activity.

**Methods**

Male albino guinea-pigs, weighting 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 animals in the field of physiological sciences, approved by The Physiological Society of Japan. The stomach was excised, and opened by cutting along the lesser curvature in Krebs solution. The mucosal layers were removed by cutting with fine scissors, and smooth muscle tissues were isolated from the antral region.

Two types of tissue were prepared from the gastric smooth muscle. The intact tissue had a single circular muscle bundle with attached longitudinal muscle layers, the dimensions being about 100 μm wide and 200–300 μm long. The tissue segment was pinned out on a silicone rubber plate fixed at the bottom of an organ bath (8 mm wide, 8 mm deep, 20 mm long), with the mucosal side uppermost, and slow waves were recorded from circular smooth muscle cells. The longitudinal muscle tissue (2 mm × 5 mm) was prepared by removing the circular muscle layers, and it was pinned out on the silicone rubber plate with the mucosal side uppermost. Follower potentials and pacemaker potentials were recorded from the longitudinal muscle cells and ICC, respectively. These tissues were superfused with oxygenated Krebs solution, at a constant flow rate of about 2 ml/min. The temperature of superfusate was changed indirectly by controlling the temperature of the warming bath, and the temperature of the bath was measured with a thermometer (MGA III-219, Nihon-Kohden, Tokyo, Japan).

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. Electrical responses recorded with 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.

The parameters of the slow wave measured were amplitude, duration, frequency, rate of rise of the upstroke phase, and the latency for the generation of the 2nd component. Details for the measurement of each parameter are described in the Results section. Each of these values was plotted against temperature, and the temperature coefficient (Q10 value) of each parameter was calculated from the slope of the relationship.

The ionic composition of the Krebs solution was as follows (mM): Na⁺ 137.4, K⁺ 5.9, Ca²⁺ 2.5, Mg²⁺ 1.2, HCO₃⁻ 15.5, H₂PO₄⁻ 1.2, Cl⁻ 134, and glucose 11.5. The solution contained 1 μM nifedipine (from Calbiochem, San Diego, California, U.S.A.) throughout, so as to minimize muscle movements. Nifedipine was dissolved in dimethyl sulphoxide (DMSO) to make a stock solution (10 mM), and it was added to Krebs solution, just prior to use. In some experiments, potassium cyanide (KCN), dissolved in distilled water, was added to the solution. The solutions were aerated with O₂ containing 5% CO₂, and the pH of the solutions was maintained at 7.2–7.3. The pH of the solution was not changed by adding nifedipine or KCN.

Experimental values were expressed by the mean value ± standard deviation (SD). Statistical significance was tested using Student's t-test, and probabilities of less than 5% (P<0.05) were considered significant.
Fig. 1. Modulation of slow waves in response to changes in temperature. Slow waves were recorded from a single circular smooth muscle cell in intact tissue, during change in temperature from 36.2 to 38.3°C. A, parameters measured from each slow wave were the peak amplitude (a), amplitude of the 1st component (b), duration of the 1st component (c) and 2nd component (d), and interval between the foot of the upstroke phases of successive slow waves (e). Frequency of slow waves was calculated by dividing 60 by e (interval between slow waves) and was expressed as cycles per min (cpm). Time-courses of the change in amplitude (B), duration (C) and frequency (D) of slow waves generated during a change in temperature (E) are plotted. In B and C, the amplitude and duration of the 1st and 2nd components of slow waves are shown by open and filled squares, respectively.

Results

Effects of change in temperature on slow waves

Slow waves were recorded from circular smooth muscle cells of the intact tissue preparation. As reported by Tomita (1981), each slow wave was formed by two components, the 1st component being followed by a superimposing 2nd component. Experiments were carried out to observe the changes in form of slow waves during elevation of temperature by several degrees, in the range between 24°C and 42°C. The parameters of slow waves measured were the amplitude, duration, frequency, maximum rate of rise of upstroke phase (dV/dt) and the time required for the generation of the 2nd component following the 1st component (latency). The methods for measurement of the former 3 parameters are shown in Fig. 1A. The amplitude of slow waves was measured from the resting level to the peak of the 1st and 2nd components, and as a consequence the peak of the 2nd component was the sum of 1st and 2nd components. These values varied between tissues but were relatively constant for cells within the same tissue. The time of onset and offset for a slow wave was measured at each foot, and the elapsed
time between these two points was defined as the duration of the slow wave, including both the 1st and 2nd components. The time until the generation of the next slow wave was measured, and the frequency of slow waves was calculated as cycles per min (cpm) \( \text{i.e.,} 60 \) was divided by the time between slow waves.

Figure 1 shows the changes in amplitude, duration and frequency of slow waves during elevation of temperature from \( 36.2^\circ \text{C} \) to \( 38.3^\circ \text{C} \), recorded from a circular muscle cell in intact muscle tissue. Changes in slow waves in response to the elevation of temperature were a decrease in duration and an increase in frequency, with no significant alteration to the amplitude. These changes appeared equally for both 1st and 2nd components of slow waves. The resting membrane potential, defined as the stable membrane potential observed at the interval between slow waves, was not changed during the elevation of temperature (in \( 36.2^\circ \text{C} \), \( -67.6 \pm 2.5 \text{ mV, n=15}; \) in \( 38.3^\circ \text{C} \), \( -68.1 \pm 2.2 \text{ mV, n = 16}; \text{P}>0.05 \)). Lowering temperature by several degrees produced responses opposite to those produced by elevating temperature, \( \text{i.e.,} \), an increase in duration and a decrease in frequency, but again with no alteration to the resting membrane potential or the amplitudes of slow waves (data not shown). These changes were observed following a modulation from any given temperature, in the range between \( 24^\circ \text{C} \) and \( 42^\circ \text{C} \). In some tissues, the frequency was increased transiently for 2–3 min during the change in temperature. However in any case, the overshoot in frequency was a transient phenomenon and a new stable level was reached within at most 3 min after the change in temperature. Therefore, further experiments were carried out to observe slow waves after the tissues had been exposed to a new level of temperature for at least 5 min.

In intact tissues, the resting membrane potential varied between preparations, from \(-60 \text{ mV} \) to \(-75 \text{ mV} \) (mean, \( -66.8 \pm 2.5 \text{ mV, n=16} \)), and it was a similar value in cells from the same preparation. In all preparations, no significant change was elicited in the resting membrane potential, in response to change in temperature between \( 24 \) and \( 42^\circ \text{C} \) (Figs. 2 and 3A). The amplitude of the 1st component and the peak of the slow wave were measured, and in 10 preparations these values were similar at any given temperature between \( 24 \) and \( 41^\circ \text{C} \) (Figs. 2 and 3B). The \( Q_{10} \) values calculated for the amplitude of slow waves generated at different temperatures were nearly 1 (Table 1), indicating that the amplitude was insensitive to temperature. The duration of slow waves was increased by lowering temperature and was decreased by elevating temperature, and the change was linear between \( 24^\circ \text{C} \) and \( 42^\circ \text{C} \), for both 1st and 2nd components. The slope of the change in duration was larger for the 1st component than for the 2nd component (Fig. 3C). The frequency of slow waves was a temperature-dependent characteristic, and it increased linearly with elevation of temperature (Fig. 3D). The calculated \( Q_{10} \) values were much higher for the frequency than for the duration (Table 1).

The maximum value of the rate of change of voltage during the upstroke phase \( (dV/dt) \) was measured for the 1st and 2nd components (Fig. 4A). The \( dV/dt \) was much higher for the 2nd component than for the 1st component, and each was increased by elevating temperature and decreased by lowering temperature (Fig. 4B). The relationships were linear between \( 24 \) and \( 42^\circ \text{C} \) for both components (Fig. 4B), and their \( Q_{10} \) values were similar (Table 1).

The latency for the generation of the 2nd component plotted against temperature indicated that it decreased exponentially with elevating temperature between \( 24 \) and \( 32^\circ \text{C} \), and it
Fig. 2. Slow waves recorded in different temperature. Slow waves were recorded from circular muscle in different temperature (A, 24.3°C; B, 29.2°C; C, 35°C; D, 38.7°C). All responses were recorded from the same tissue.

Fig. 3. Modulation of slow waves recorded from circular muscle of intact tissue in response to change in temperature. The resting membrane potential (A) and amplitude (B), duration (C) and frequency (D) of slow waves recorded at different temperatures (mean ± S.D.) are plotted against temperature (1st component, ○; 2nd component, ●). All values were obtained from the same tissue.
Table 1. Effects of KCN on Q10 values of the parameter of slow waves

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Control</th>
<th>in KCN</th>
</tr>
</thead>
<tbody>
<tr>
<td>Amplitude (peak amplitude)</td>
<td>1.03 ± 0.09 (n=10)</td>
<td>1.08 ± 0.05 (n=6)</td>
</tr>
<tr>
<td>Duration (1st component)</td>
<td>1.73 ± 0.19 (n=10)</td>
<td>1.68 ± 0.36 (n=6)</td>
</tr>
<tr>
<td>Duration (2nd component)</td>
<td>1.70 ± 0.12 (n=10)</td>
<td>1.86 ± 0.43 (n=6)</td>
</tr>
<tr>
<td>dV/dt (1st component)</td>
<td>2.14 ± 1.17 (n=10)</td>
<td>2.02 ± 0.40 (n=6)</td>
</tr>
<tr>
<td>dV/dt (2nd component)</td>
<td>2.49 ± 0.65 (n=10)</td>
<td>2.18 ± 0.49 (n=6)</td>
</tr>
<tr>
<td>Frequency</td>
<td>2.46 ± 0.95 (n=10)</td>
<td>1.20 ± 0.28 (n=6)*</td>
</tr>
<tr>
<td>Latency of 2nd component</td>
<td>2.42 ± 1.25 (n=8)</td>
<td>2.24 ± 0.89 (n=6)</td>
</tr>
</tbody>
</table>

The temperature coefficient (Q10) of each parameter of slow waves was calculated in the absence and presence of 30 μM KCN. Mean ± S.D. (n = number of observations). *, significantly different from control (P<0.05).

Fig. 4. Effects of changes in temperature on the rate of rise and the latency of the 2nd component of the slow wave. A, Slow waves were recorded at different temperatures, and the maximum value of the rates of rise of the 1st and 2nd components were measured, as was the time required for the initiation of 2nd component after the start of the 1st component (latency). B, Rate of rise of the 1st and 2nd components (● and ○, respectively) of slow waves. C and D, Latency of the 2nd component of the slow wave plotted against temperature, on a linear scale (C) and semi-logarithmic scale (D). All values, shown by mean ± S.D., were obtained from slow waves recorded from circular muscle in the same intact tissue.

remained unchanged above 34°C (Fig. 4C). This was more clearly shown when the relationship was plotted on a semi-logarithmic scale (Fig. 4D). The latency measured above 34°C remained unchanged, and the value varied between tissues from 0.6 to 1.5 s, with a mean value of 0.92 ± 0.17 s (n=8). The Q10 value for the temperature-sensitive phase of the latency was 2.42 ± 1.25 (n=8), while that of the temperature-insensitive phase was 1.14 ± 0.39 (n=8).
Modulation of follower and pacemaker potentials by changing temperature

The effects of changes in temperature on pacemaker potentials recorded from ICC-MY were investigated, since these potentials formed the 1st component of slow waves (Hirst and Ward, 2003). The changes in follower potentials in response to temperature were also investigated, as these potentials were also formed by the electrotonic propagation of pacemaker potentials (Hirst and Ward, 2003). When follower potentials or pacemaker potentials were recorded with the temperature elevated by several degrees, the changes in amplitude, duration, frequency and $dV/dt$ of these potentials varied directly with change in temperature (data not shown), as was the case for slow waves (see Figs. 1 and 2). Therefore, these parameters were measured after stabilizing the tissue at the new temperature for at least 5 min. Actual traces of follower and pacemaker potentials recorded at different temperatures are shown in Fig. 5. The changes in follower potentials (Fig. 5, A–D) in response to the elevation of temperature from 24.4°C to 37.9°C were a reduction in duration and an increase in frequency. The amplitude of follower potentials was similar at all temperatures. This was also the case for pacemaker potentials recorded in solutions at different temperatures (26.8°C and 37.3°C) (Fig. 5, E–H), the durations of which were decreased and the frequencies of which were increased (data for the latter were not shown), with no marked change in amplitude.

Typical examples of the quantified changes in amplitude, duration, frequency and $dV/dt$ of the upstroke phase of follower and pacemaker potentials are shown in Fig. 6. The resting membrane potential was more negative for ICC-MY than for longitudinal muscles, and was not
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Fig. 6. Effects of change in temperature on parameters of follower potentials and pacemaker potentials. Follower potentials (●) and pacemaker potentials (○) were recorded from longitudinal muscles and ICC-MY, respectively, and the membrane potential (B), amplitude (C), duration (D), frequency (E) and rate of rise (F) measured at different temperatures were shown by the mean ± S.D. (n=10-15). A, methods for the measurement of parameters of follower potentials are shown.

significantly changed by elevating temperature (Fig. 6B). Pacemaker potentials were larger in amplitude than follower potentials by about 15 mV at any given temperature, and were insensitive to change in temperature (Fig. 6C). The Q_{10} values were 1.00 ± 0.04 (n=7) for follower potentials and 1.01 ± 0.06 (n=6) for pacemaker potentials. The duration and frequency of pacemaker potentials were similar to those of follower potentials, and in both waveforms, the decrease in duration and the increase in frequency varied linearly with elevation of temperature (Fig. 6, D and E). The Q_{10} values for the duration (follower potentials, 1.69 ± 0.18, n=10; pacemaker potentials, 1.86 ± 0.28, n=7, P<0.05) and frequency (follower potentials, 2.72 ± 1.01, n=10; pacemaker potentials, 2.56 ± 0.7, n=7, P>0.05) were again similar for both waveforms. The dV/dt values were much higher for pacemaker potentials than for follower potentials at any given temperature, and varied linearly with elevation of temperature (Fig. 6F). The Q_{10} values for the change in dV/dt of pacemaker potentials (2.68 ± 0.58, n=7) were similar to those for follower potentials (2.28 ± 0.59, n=10; 0.1<P<0.2). The comparison of these Q_{10} values with those of slow waves (Table 1) indicated that the temperature sensitivities of the parameters of follower and pacemaker potentials were similar to those of slow waves. Parallel changes in the temperature-sensitive parameters between follower potentials and pacemaker potentials also suggest that the electrical coupling between these two types of cells is not markedly modulated by temperature.
Fig. 7. Effects of KCN on slow waves. Slow waves were recorded from circular muscle in intact preparations, in the presence of different concentrations of KCN (1–100 μM), and the frequency of slow waves was plotted against the concentration (A). Mean ± S.D. (n=6 for each point). Typical slow waves recorded from the same cell, in the absence (B) and presence of 30 μM KCN (C). The effects of 30 μM KCN on the amplitude (D), duration (E) and frequency (F) of slow waves recorded from 6 preparations were summarized. * significantly different from control (P<0.05). All data were collected at 36.5°C.

Effects of KCN on slow waves

As KCN (>100 μM) has been reported to abolish slow wave generation in gastric muscle of the guinea-pig (Nakayama et al. 1997), attempts were made to test the effects of different concentrations (1–100 μM) of KCN on slow waves at 36.5°C. Low concentrations of KCN (<3 μM) did not produce any detectable change in the measured parameters of slow waves (amplitude, duration, rate of rise, frequency). KCN in concentrations ranging between 10 and 30 μM reduced the frequency of slow waves by 20–80%, while a high concentration (100 μM) reduced the frequency of slow waves below 0.2 cpm (Fig. 7A). A series of experiments indicated that the concentration of KCN which reduced the frequency of slow waves to about 50% of the control value ranged between 20 μM and 50 μM (mean 31.5 ± 5 μM, n=5). Therefore, the effects of 30 μM KCN on slow waves were examined.

Figures 7 and 8 show typical examples of the effects of 30 μM KCN on slow waves. Administration of KCN reduced the frequency of slow waves, with no significant alteration to their configuration (Fig. 7, B and C). The time courses of changes in some parameters of slow waves (amplitude, duration, frequency) upon administration of KCN indicated that the reduction in frequency appeared rapidly, and required about 60 min to reach a stable value of about 50% of control (Fig. 8C). The amplitudes of slow waves remained unchanged during application of 30 μM KCN for up to 60 min (Fig. 8A), while their duration was reduced by about 10% of control in
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Fig. 8. Changes in parameters of slow waves in the presence of KCN. Slow waves were recorded from a single smooth muscle cell in an intact tissue, and amplitude (A), duration (B) and frequency (C) of successive slow waves were measured, while 30 μM KCN was applied at time 0 (shown by dotted line). Temperature, 36.5°C.

the presence of KCN (Fig. 8B). These responses were observed in 6 different tissues examined, and summarized data indicated that under stabilized conditions, KCN significantly reduced the frequency and duration of slow waves, with no alteration to amplitude (Fig. 7, C, D and E respectively). The latency of the 2nd component of slow waves and dV/dt were also not significantly changed by KCN (data not shown).

After the actions of 30 μM KCN had stabilized, the effects of changing temperature on slow waves were examined. First the temperature was reduced to 24–26°C, and then it was elevated beyond control level by 3–5 degrees, to reach 38–40°C. The changes in parameters of slow waves (amplitude, duration, frequency, dV/dt, latency for the 2nd component) were measured at different temperature, and a typical result is illustrated in Fig. 9. In the presence of KCN, elevation of temperature resulted in a reduction in duration (for both 1st and 2nd components, Fig. 9B) and latency and an increase in dV/dt (for both 1st and 2nd components, Fig. 9C), with no alteration to the amplitude (for both 1st and 2nd components, Fig. 9A). In the presence of KCN, the frequency of slow waves increased in 4 tissues (Fig. 9E), with low Q_{10} values ranging between 1.07 and 1.80, and remained unchanged in 2 tissues (data not shown). The latency for generating the 2nd component was temperature-dependent at temperatures below 32°C, and it remained unaltered in the presence of KCN (Fig. 9D). The Q_{10} values calculated from these relationships were summarized in Table 1. KCN markedly reduced the Q_{10} value for frequency, with no significant alteration to that for amplitude, duration, dV/dt or latency of the 2nd component of slow waves.
Fig. 9. Effects of changes in temperature on parameters of slow waves in the presence of KCN. In the presence of 30 µM KCN, slow waves were recorded from intact tissue at different temperatures, and amplitude (A), duration (B), rate of rise of the upstroke phase (C), latency of the 2nd component (D) and frequency of slow waves (E) were measured. The 1st and 2nd components of the amplitude, duration and rate of rise of upstroke phase were measured (●, 1st component; O, 2nd component). In D, the latency is plotted on a log scale. Mean ± S.D. (n=10-15).

Discussion

The present experiments were designed to investigate which parameters of slow waves were sensitive to changes in temperature, since those might be closely related to metabolic activity. The results indicate that the parameters sensitive to change in temperature were the duration, frequency, dV/dt of the upstroke phase and the latency preceding the 2nd component. For pacemaker and follower potentials, the first three parameters showed similar temperature sensitivity. Follower potentials and the 1st component of slow waves are formed by an electrotonic spread of pacemaker potentials to longitudinal and circular smooth muscles, respectively (Dickens et al., 1999; Cousins et al., 2003). The 2nd component of slow waves is formed by slow potentials generated in circular muscles (Dickens et al., 1999; Cousins et al., 2003), and the results indicate that the temperature sensitivities of parameters for slow waves are similar to those for pacemaker potentials. These results indicate that, although the conduction of electrical signals from ICC-MY to smooth muscle cells is a decremental phenomenon (Edwards and Hirst, 2005), temperature-induced changes in pacemaker potentials are conducted to smooth muscles, possibly through gap junctions, at all temperatures examined. This suggests that the conductance between pacemaker cells and smooth muscle cells is a temperature-insensitive structure within the range of temperatures examined in the present
experiments (between 24°C and 42°C).

It is generally considered that parameters with high Q₁₀ values are related to metabolic activity whereas those with low Q₁₀ values are related to physical processes. Activation of voltage-sensitive Ca²⁺-channels has a Q₁₀ value of about 1.63 (Klöckner et al., 1990). In slow waves, temperature-insensitive parameters (amplitude, resting membrane potential) showed low Q₁₀ values around 1, while temperature-sensitive parameters (frequency, duration, dV/dt, latency) showed high Q₁₀ values. Amongst the latter parameters, the Q₁₀ values for the duration ranged between 1.6 and 2, while those for the dV/dt and latency were over 2. The rising phase of pacemaker potentials may depend upon the activation of Ca²⁺-permeable channels which are not sensitive to nifedipine but are sensitive to Ni²⁺ or to voltage change (Hirst and Edwards, 2001; Hirst et al., 2002b; Kito et al., 2002a; Kito and Suzuki, 2003a). If the Ca²⁺-permeable channel has a Q₁₀ value similar to that for other ion channels, then the high Q₁₀ value for dV/dt suggests the contribution of unidentified additional temperature-dependent factors in determining the speed of the upstroke phase. On the other hand, the Q₁₀ value for duration was comparable to those for ion channels. The plateau component (Kito et al., 2002a) and slow potentials (Hirst et al., 2002a) may depend upon activation of Ca²⁺-sensitive Cl⁻-channels, and therefore the duration may be causally related to the level of [Ca²⁺]ᵢ. High temperatures would facilitate the reduction of intracellular Ca²⁺ concentration due to an increased rate of Ca²⁺ uptake into the internal stores, and as a consequence the activity of Ca²⁺-sensitive Cl⁻-channels could be terminated more rapidly. In bladder smooth muscles, low temperatures result in a decrease in the rate of rise and an increase in duration of action potentials, with associated elevation in amplitudes and duration of contractions, possibly due to a reduction in the speed of [Ca²⁺]ᵢ decay (Hashitani and Brading, 2003). Thus, it is possible that the factors responsible for the temperature-dependent change in the duration of slow waves are the properties of ion channels and intracellular [Ca²⁺].

The frequency of pacemaker potentials is the parameter most sensitive to changes in temperature, suggesting that this parameter is tightly related to the metabolic activities carried out in mitochondria. It is proposed in murine small intestine that Ca²⁺ extrusion from mitochondria activates ion channels at the plasma membrane to form pacemaker potentials (Ward et al., 2000), while in the guinea-pig stomach, IP₃ formation is stimulated by elevation of cytosolic Ca²⁺ due to mitochondria-derived Ca²⁺ release, possibly through activation of protein kinase C (Kito et al., 2002b; Suzuki et al., 2002). In both hypotheses, mitochondrial Ca²⁺ handling may have a pivotal role in the production of rhythmic activity (Kito and Suzuki, 2003b). In addition, the properties of ion channels contributing to the formation of the primary phase of the pacemaker potential might be another factor influencing frequency, since an increase in duration will reduce the frequency due to the refractory period following each slow wave (Kito et al., 2002b). Membrane potential is also one of the factors determining the frequency of slow waves (Nose et al., 2000; Fukuta et al., 2002; Kito and Suzuki, 2003c), and the insensitivity of membrane potential to temperature change suggests that the temperature-dependent change in frequency is not causally related to the changes in membrane potential.

The present experiments revealed that the inhibition of mitochondrial metabolism by KCN significantly reduced the frequency of slow waves, with a slight reduction in the duration and no
marked alteration to the amplitude, $dV/dt$ and latency of slow waves. These results suggest that the frequency and a part of the duration of slow waves are causally related while other parameters are mostly independent from mitochondrial activity. Furthermore, the effects of KCN on temperature-dependent changes in parameters of slow waves applied only to the frequency. These results suggest that only the frequency, but not other parameters, of slow waves is causally related to mitochondrial metabolism. The duration of slow waves was reduced by KCN at 36.5°C, but the $Q_{10}$ values for the duration were not altered by KCN, and these results suggest that most factors determining the duration of slow waves have no causal relation to metabolic activity.

The temperature-dependence of the latency of the 2nd component of the slow wave differed depending on the temperature range, being temperature-sensitive below 32°C and temperature-insensitive above 34°C. The 2nd component of the slow wave may be generated by a summation of unitary potentials (Edwards et al., 1999) in response to depolarization of the membrane (Suzuki and Hirst, 1999), and possible involvement of depolarization-activated production of IP$_3$ has been considered (Suzuki et al., 1999; Suzuki, 2000; Hirst et al., 2002a; Hirst and Ward, 2003). The generation of slow potentials is accelerated by depolarization of the membrane, and increasing the amplitude of depolarization reduces the latency to a minimum of about 1 s (Suzuki and Hirst, 1999; Hirst et al., 2002b; Kito et al., 2002b). IP$_3$ is the major intracellular messenger for receptor activation in smooth muscle, and its production is also delayed by about 1 s after stimulation with agonists (Somlyo and Somlyo, 1994). The release of Ca$^{2+}$ from internal stores by IP$_3$ is not sensitive to temperature (Hirata et al., 1985), suggesting that the high-sensitivity to temperature of the latency involves mechanisms other than IP$_3$-mediated activation of Ca$^{2+}$ release from the internal stores, such as a voltage-sensitive activation of phosphoinositide phosphatase coupled with ion channels appearing in the sperm tail of the sea squirt (Murata et al., 2005). The absence of the effects of KCN on the $Q_{10}$ value for the latency also suggests that the factors determining this parameter are not coupled with mitochondrial metabolism. It may be that only the voltage-activated production of IP$_3$ is sensitive to temperature. On the other hand, the latency remains unaltered during elevation of temperature above 32–34°C, possibly because IP$_3$-mediated processes are saturated at a maximum level in this range of temperature.

In summary, there are temperature-sensitive and insensitive parameters describing slow waves generated in the guinea-pig stomach antrum; the former include duration, frequency and $dV/dt$ of the upstroke phase, while the latter includes amplitude. Parallel changes are also observed for follower and pacemaker potentials. KCN, a metabolic inhibitor, markedly reduced the frequency and slightly reduced the duration of slow waves, with no marked alteration to other parameters. The $Q_{10}$ for the frequency, but not other parameters, was also reduced by KCN. These results suggest that the frequency of slow waves (equal to that of pacemaker potentials) may be related to the level of metabolic activity, while the duration and $dV/dt$ of slow waves are determined mainly by the mechanisms other than metabolic activity. The temperature-sensitivity is biphasic for the latency of the generation of the 2nd component of slow waves, and KCN did not significantly alter the relationship, suggesting a possible involvement of factors other than IP$_3$. 
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