J. Physiol. (1975), **253**, *pp.* 505–516
With 9 text-figures
Printed in Great Britain

THE SLOW WAVE IN THE CIRCULAR MUSCLE OF THE GUINEA-PIG STOMACH

By M. OHBA, Y. SAKAMOTO AND T. TOMITA

From the Department of Physiology, School of Medicine, Fukuoka University, Fukuoka 814, Japan

(*Received 5 May 1975*)

SUMMARY

1. The slow wave in the circular muscle of guinea-pig stomach was investigated with the double sucrose-gap method.

2. The amplitude of the slow wave was reduced by depolarization, and it was increased by a small hyperpolarization (5–10 mV). With hyperpolarization greater than 15 mV the amplitude decreased, and the slow wave became reduced, and less dependent on polarization. This residual was not abolished by strong hyperpolarizing current pulses.

3. The frequency of the slow waves was not much affected by membrane polarization. The change was only 15–20% by depolarization or hyperpolarization of 12 mV.

4. Rhythmic inward currents could be recorded under voltage-clamp conditions. The frequency of the inward currents was the same as that of the slow wave. The intensity of inward current was little affected by membrane polarization.

5. Lowering the temperature reduced the frequency of the slow wave. The rates of rise and fall of the component which remained during strong hyperpolarization were similarly decreased by lowering the temperature. The Q_{10} of the frequency was about 2·7.

6. It is suggested that the slow wave consists of two different components. One is generated by a potential independent process, and triggers the second component which is potential dependent. The first component may be controlled by some metabolic process.

INTRODUCTION

The electrical activity in most smooth muscles is composed of at least two different components: the action potential and the slow wave. Verapamil, which competitively blocks an increase in the membrane conductance for Ca ions, selectively suppresses the action potential without affecting the slow wave (Golenhofen & Lammel, 1972). The smooth

muscles of guinea-pig stomach and of rabbit or cat small intestine produce large slow waves, providing suitable isolated preparations for studies of the slow wave.

There is some evidence that the membrane conductance is increased during the slow wave (rabbit intestine: Mills & Taylor, 1971; guinea-pig stomach; Magaribuchi, Ohbu, Sakamoto & Yamamoto, 1972; Tomita & Watanabe, 1973). However, the mechanism of generation of slow waves is still a matter of speculation (Bortoff, 1972; Prosser, 1974), although a contribution from the active transport of Na ion has been suggested (Job, 1969; Conner, Prosser & Weems, 1974).

In the present experiments, the dependency of the slow wave on the membrane potential was investigated in the smooth muscle of guinea-pig stomach. The results suggest the possible involvement of two different mechanisms in the slow wave.

METHODS

Guinea-pigs of either sex were stunned and bled. Small strips of the circular muscle (0·5 mm in diameter and 2 cm in length) were dissected from the antrum region of the stomach. The mucous membrane was carefully removed from the strip and in some preparations the longitudinal muscle layer was also stripped off. There was no fundamental difference whether the preparations contained the longitudinal muscle or not. The strip was mounted in a double sucrose-gap apparatus, as previously described (Bülbring & Tomita, 1969). The centre pool where the test solution flowed was 700 μm in width.

The slow waves could be recorded for more than 2 hr without significant deterioration when the width of the centre pool was about 700 μm. However, when the centre pool was less than 500 μm, the amplitude and the frequency of slow waves gradually decreased during the course of experiments. The circuit used for the voltage-clamp experiments was essentially similar to that described by Anderson (1969). A high-voltage operational amplifier, Teledyne-Philbrick 1022, was used as the output amplifier of the feed-back circuit.

Krebs solution was used as the normal medium and had the following composition (mM): NaCl 122, KCl 6, $CaCl_2$ 2·5, $MgCl_2$ 1·2, $NaHCO_3$ 15·5, glucose 11·5. It was aerated with a gas mixture of 3% CO_2 and 97% O_2. The temperature of the test solution was kept at 35° C, except in the experiments in which temperature effects were studied.

RESULTS

After mounting the preparation in the double sucrose-gap apparatus, the spontaneous slow wave gradually increased in amplitude reaching a steady state within 15–20 min. Action potentials appeared on the top of the slow wave in many preparations, but in some preparations the action potential was very small or absent. The slow wave started abruptly from a steady membrane potential level, and the membrane potential between slow waves was more or less stable. The amplitude of the slow wave was

usually between 15 and 30 mV (21·3 ± 2·4 mV, average and S.D., $n = 34$) and the duration at half amplitude was between 3 and 4 sec (3·31 ± 0·7 sec). The frequency of slow waves was 5·2 ± 0·7/min ($n = 23$) at 35° C. The slow wave usually had humps at a level between 6 and 12 mV depolarization (8·3 ± 1·2 mV, $n = 34$), often on the repolarizing phase, but sometimes on the depolarizing phase or on both phases, suggesting the presence of two components. There was no correlation between 'humps' and the action potential.

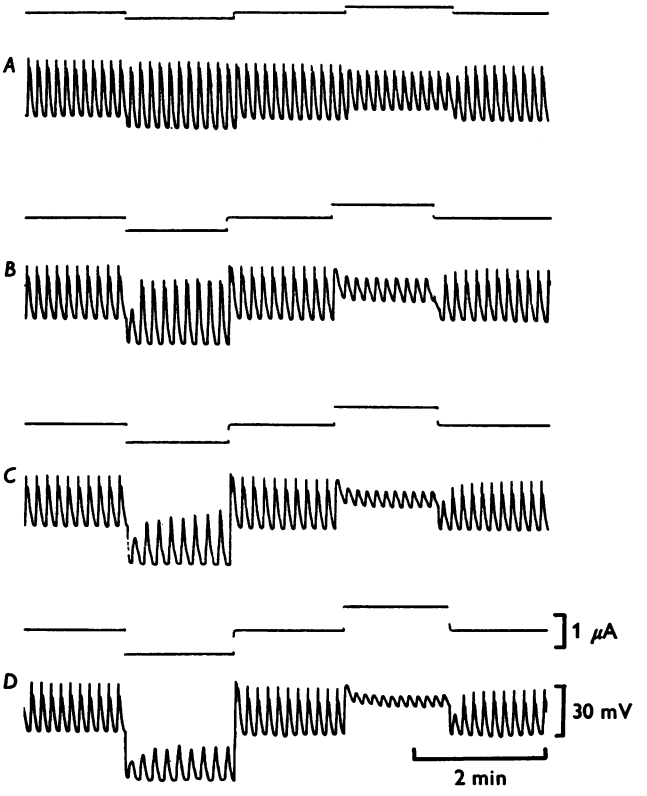

Fig. 1. Effects of membrane polarization on slow wave. Upper trace: current intensity; lower trace: membrane potential. Note reduction of amplitude by both depolarization and hyperpolarization, except by small hyperpolarization, and also note weak effect on frequency.

Fig. 1 shows the effect of membrane polarization on the slow wave. The amplitude was reduced by depolarization of the membrane. When the membrane was hyperpolarized by 5–15 mV the amplitude increased (*A* and *B*). However, during application of strong hyperpolarizing current, the amplitude decreased (*C* and *D*). The suppression of amplitude was

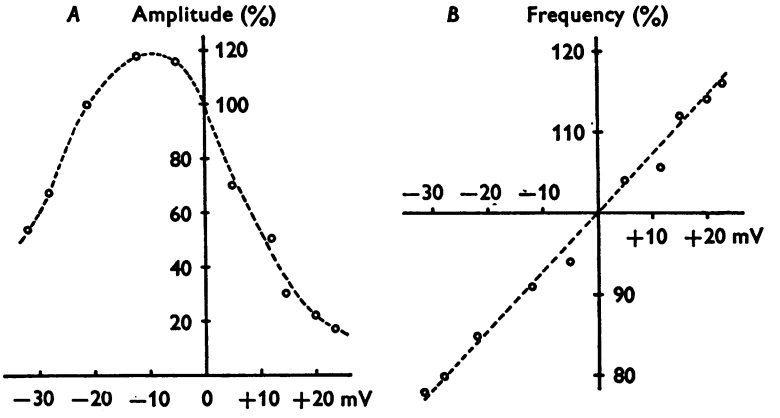

Fig. 2. Relationship between amplitude of slow waves and membrane polarization (*A*), and between frequency and membrane polarization (*B*), expressed as percentages of control. +, depolarization, −, hyperpolarization.

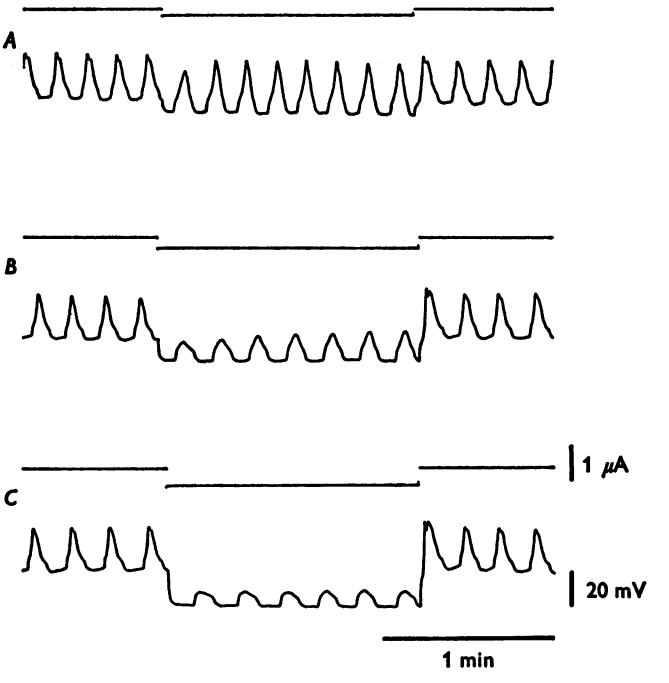

Fig. 3. Effects of membrane polarization on the configuration of slow wave. Note that the upper part of slow wave was reduced by hyperpolarization, and that the remaining lower part responsible for the 'hump' in the slow waves, was not greatly affected.

greatest on the first slow wave after the current application. A typical relationship between the amplitude of slow waves and membrane polarization is shown in Fig. 2*A*. When the membrane was depolarized or hyperpolarized more than 20 mV, the amplitude became slightly less dependent on the membrane potential.

The effect of polarization on the frequency of slow waves was less marked compared with the effect on the amplitude. Usually, there was a roughly linear relationship between the frequency and the membrane polarization (Fig. 2*B*). The degree of frequency modulation by membrane polarization varied with different preparations, but the average change was only 15–20 % of the control in response to a polarization of 12 mV. The effects of membrane polarization were unaffected by atropine (10^{-6} g/ml.) or tetrodotoxin (10^{-6} g/ml.), suggesting no contribution by nervous elements.

The 'humps' in slow wave were very clear in some preparations though not in others. However, hyperpolarization of the membrane always revealed the component responsible for the 'humps'. Fig. 3 shows an example of the effect of membrane polarization on the slow wave. In this preparation, the humps appeared both on the depolarizing and repolarizing phases when it had reached about 10 mV depolarization. Hyperpolarization of the membrane reduced the amplitude of the slow waves but caused no change in the duration of the lower part of slow wave whose amplitude was scarcely affected even when hyperpolarization was increased to more than 20 mV. It is suggested from this result that hyperpolarization reduced mainly the second component of the slow wave, leaving the underlying first component unmasked. When the membrane was hyperpolarized by more than 20 mV, the membrane resistance became smaller.

A potential deflexion produced by a small current pulse (500 msec in duration) was smaller when applied near the peak of slow waves than between the slow waves (Fig. 4*A*). However, the amplitude of electrotonic potentials was not much changed when the second component was removed by strong membrane hyperpolarization (Fig. 4*B*). The configuration of slow waves was modified by a strong relatively brief current pulse (200 msec duration) applied during the slow wave (Fig. 4*C*, *D*). The upper part of the slow wave could be abolished with a strong hyperpolarizing current pulse when it was applied near the peak of the wave. However, there always remained some underlying potential wave which could not be abolished (6 in Fig. 4*D*). This remaining potential probably corresponds to the first component which was observed during strong hyperpolarization and which was responsible for the 'humps' in the slow waves.

When the membrane potential is clamped at a constant level in an excitable membrane, in which the membrane conductance is determined

only by a voltage-dependent mechanism, the membrane current will reach a steady intensity after a transient response which depends on the difference between the holding potential and the test voltage pulse. However, in the smooth muscle of guinea-pig stomach, rhythmic inward currents could be observed when the membrane potential was clamped at the resting potential level (Fig. 5*F*), similar to those observed in the

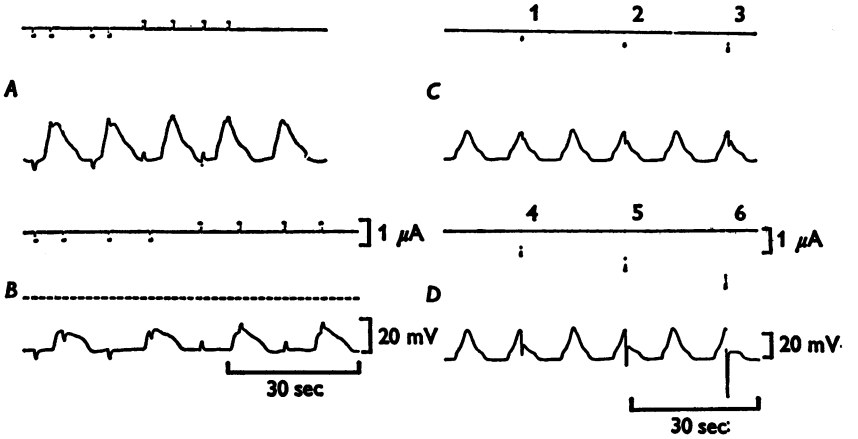

Fig. 4. *A*, electrotonic potentials produced by the same current pulse (500 msec duration) between slow waves and near the peak. *B*, the same as *A*, but during sustained membrane hyperpolarization which suppressed the upper part of slow wave. Dotted line indicates original membrane potential level. *C* and *D*, effects of short current pulses (200 msec duration) with increasing intensities on configuration of slow wave. See text for further detail.

longitudinal smooth muscle of cat duodenum (Conner *et al.* 1974). The frequency of the inward currents was the same as that of the slow waves observed in the same preparation without clamping the membrane potential (Fig. 5*A*). This suggests that the inward current is generated by some mechanisms other than a voltage-dependent mechanism.

It may be argued that the inward currents are due to imperfect clamping of the membrane potential. However, the results shown in Fig. 5 seem to exclude this possibility. Fig. 5 shows simultaneous recordings of the membrane potential and the membrane current with various feed-back gains of the voltage-clamp circuit. After recording the slow waves (*A*), the feed-back circuit was closed (*B*). From *B* to *F* the gain of feed-back amplifier was increased from 15× to 1000×. When the feed-back circuit with a low gain was closed, large inward currents were observed corresponding to the generation of slow waves (*B* and *C*). The configuration of this inward current was similar to a mirror image of the slow wave.

The intensity of inward current decreased on increasing the feed-back gain, accompanied by a reduction of the amplitude of slow waves. The intensity of inward current became nearly constant beyond a certain degree of the gain of the feed-back amplifier (*D–F*), but fluctuation of the membrane potential disappeared with increasing the gain. The frequency of the inward currents was independent of perfectness of the clamp.

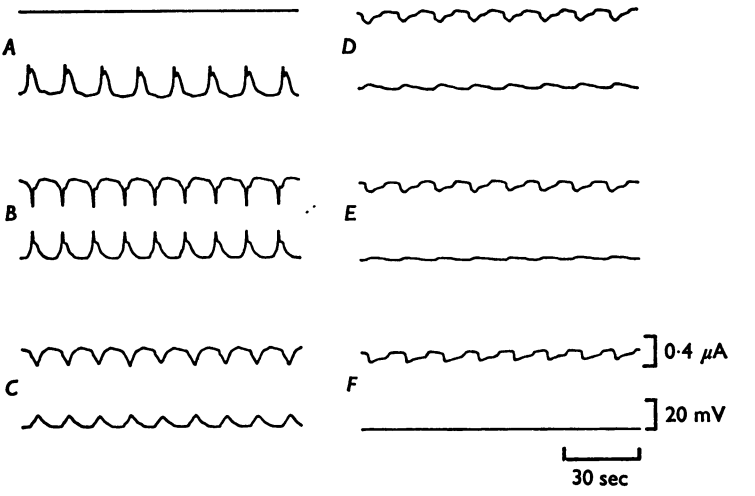

Fig. 5. Simultaneous recording of membrane current (upper trace) and membrane potential (lower trace) with the feed-back amplifier set at various gains. *A*, slow waves recorded without feed-back. From *B* to *F*, the gain of the feed-back circuit was increased (*B*, 15; *C*, 30; *D*, 50; *E*, 100, and *F*, 1000 ×).

In order to check the actual transmembrane potential, an intracellular micro-electrode was inserted into a muscle fibre in the middle part of the centre pool before and during the voltage clamp. The slow waves recorded intracellularly were 50–70 % larger than those recorded by the sucrose-gap method (Fig. 6*A*), as previously observed for the electrotonic potential in the taenia coli and ureter (Kuriyama & Tomita, 1970). The intracellular recording indicated that the membrane potential was reasonably stable during the clamping (Fig. 6*B*). In some preparations a small hyperpolarization was observed during the inward current, but the amplitude was less than 3 mV.

Spontaneous inward currents observed under voltage-clamp conditions are also shown in Fig. 7. The frequency of the rhythmic inward currents was modified by membrane polarization, increasing with depolarization (*A*), and decreasing with hyperpolarization (*C*) as observed for the slow

waves without clamping the membrane potential. However, the change in the frequency was slightly larger under voltage-clamp conditions. In most preparations there was no very marked effect of membrane polarization on the intensity of inward current, in contrast to the effect on the amplitude of the slow wave.

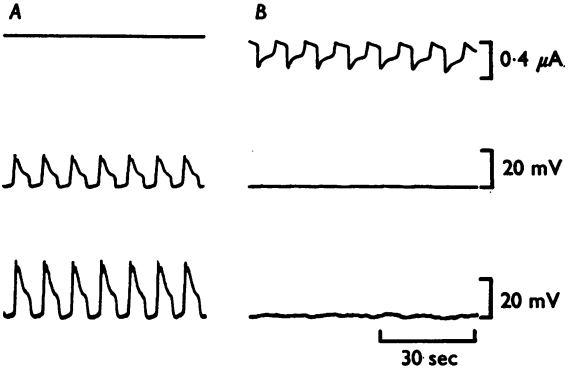

Fig. 6. Simultaneous recordings across the sucrose-gap (middle trace) and with an intracellular micro-electrode (bottom trace), before (*A*) and during the voltage-clamp (*B*). Top trace shows current recording (downward deflexion indicates inward current).

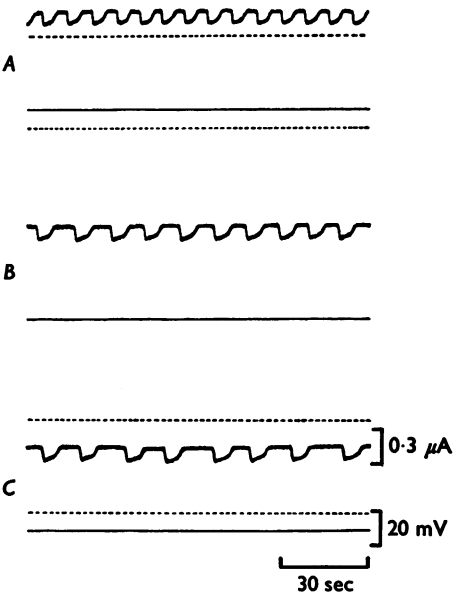

Fig. 7. Rhythmic inward currents recorded under voltage-clamp condition, and effects of membrane polarization on the inward current. *A*, depolarization; *B*, control; and *C*, hyperpolarization. Dotted lines indicate control levels. Upper traces: membrane current; lower traces: membrane potential.

The frequency of slow waves was highly sensitive to changes in temperature. When the logarithm of frequency, i.e. the reciprocal of the interval between slow waves, was plotted against the reciprocal of the absolute temperatures, a linear relationship was observed. An example is shown in Fig. 8. The Q_{10} of the frequency change measured between 25 and 35° C

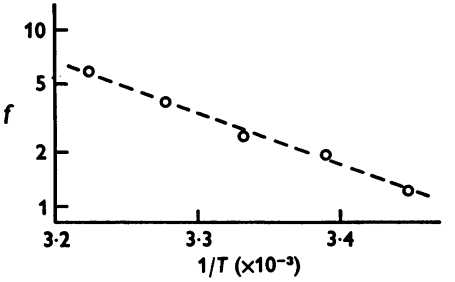

Fig. 8. An example of relationship between the frequency of slow wave per min (f) and the reciprocal of absolute temperature (T).

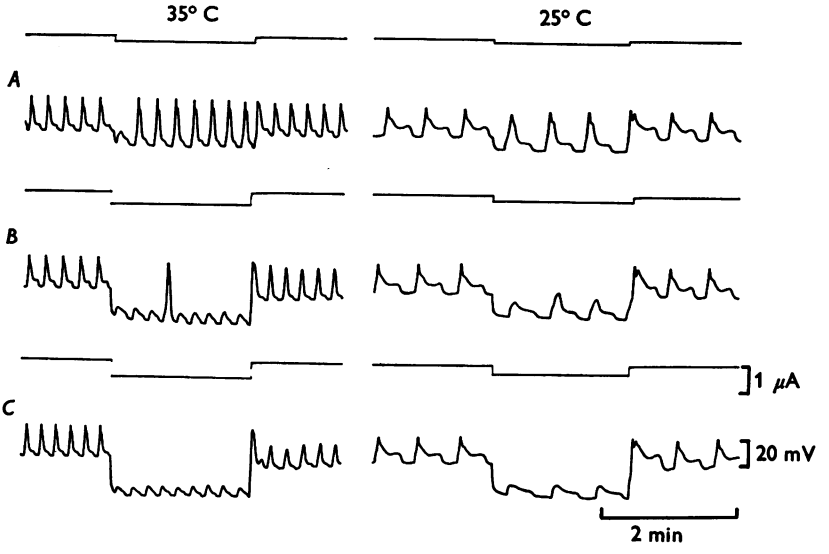

Fig. 9. Slow waves with and without hyperpolarization observed at 35 and 25° C. See text for further details.

was $2{\cdot}7 \pm 0{\cdot}3$ (average of four experiments and S.D.) which was similar to the values previously reported for peristaltic waves of the guinea-pig stomach fundus (2·4: Golenhofen, Loh & Milenov, 1970) and for the slow wave in the cat small intestine (2·73: Job, 1969). The high Q_{10} value is consistent with the hypothesis that the generation of the slow wave is controlled by some enzymic processes.

When the temperature was lowered, not only the interval between slow waves but also the duration of the slow wave were prolonged. The increased duration appeared mainly on the first component, which was unmasked by strong hyperpolarization (Fig. 9). Low temperature slowed both the rising and the falling phase of the first component of slow waves to a similar degree.

DISCUSSION

The frequency of slow waves in the circular muscle of the guinea-pig stomach seems to be much less dependent on the membrane polarization than that of the longitudinal muscle of the cat duodenum (Conner *et al.* 1974). In the cat duodenum, the frequency increases by 50% with 5 mV depolarization, while the frequency decreases to 20% with 25 mV hyperpolarization of the membrane. On the other hand, in the guinea-pig stomach, the frequency change is only 15% of the control with depolarization or hyperpolarization of 12 mV. Furthermore, the reduction in amplitude of the slow wave observed in the guinea-pig stomach when the membrane is hyperpolarized by more than 15 mV has not been noted for the cat duodenum, although the slow wave of cat duodenum is reported to be abolished when the membrane is hyperpolarized by more than 30 mV.

When the membrane potential is clamped at the resting potential, spontaneous inward currents can be recorded, which appear at the same rhythm as the slow waves. The same results have been obtained in the smooth muscle of the cat duodenum (Conner *et al.* 1974). The space constant of the circular muscle of guinea-pig is 1·4 mm (Kuriyama, Osa & Tasaki, 1970) and the width of the centre gap is 700 μm in the present experiments. Therefore, there is a potential reduction of about 10% along the preparation exposed to Krebs solution, if it is assumed that the preparation in the centre pool behaves like a short cable. It is nevertheless difficult to prove decisively that the inward current is generated by a voltage independent process. However, this inward current is probably not due to imperfect clamping of the membrane potential, because the inward current is not changed when the small fluctuation of the membrane potential is completely suppressed by increasing the gain of feed-back amplifier for the voltage-clamp. Thus, it is more likely that the inward current is generated by a process which is independent of the membrane potential. However, this inward current is probably responsible only for part of the slow wave, i.e. for the underlying process (the first component). The depolarization produced by this inward current, i.e. the first component of the slow wave triggers the second component by increasing the voltage-dependent membrane conductance for some as yet unidentified ions. The failure to abolish the whole slow wave by a strong inward current also

suggests the presence of some component which is not dependent on the membrane potential.

It may also be argued that the slow wave is generated in the longitudinal muscle and electrotonically spreads to the circular muscle, as is proposed for the cat jejunum (Bortoff & Sachs, 1970). Therefore, if the first component of slow waves corresponds to an electrotonic spread from the longitudinal muscle, the effect of polarization of the circular muscle may be very small. However, this is unlikely because preparations which contain only circular muscle produce an identical electrical response to that observed in the circular preparation to which the longitudinal muscle is attached. Also, the slow wave has been recorded intracellularly from the dissected pyloric circular muscle of guinea-pig (Kuriyama *et al.* 1970).

In the cat duodenum, the slow wave is assumed to be generated by electrogenic Na-pump activity (Conner *et al.* 1974). According to this hypothesis, the depolarizing phase of the slow wave corresponds to a reduction of the electrogenic transport rate, which in turn leads to an increase in net Na influx. The present experiments do not provide decisive evidence about the underlying mechanism responsible for the slow wave of guinea-pig stomach. The observations of an inward current which is voltage-independent and the high-temperature coefficient of the frequency of slow waves may suggest that the slow wave in the guinea-pig stomach is also related to an active transport process. However, the configuration of the inward current and the similarity of temperature effect on the rising and falling phases of the slow wave cast some doubts on the above hypothesis. The small but real dependence of frequency of the slow wave on the membrane potential may indicate that the process responsible for the generation of slow waves is somehow modified by the membrane potential or the membrane current.

The authors are grateful for Professor E. Bülbring and Dr A. F. Brading, Oxford University, for their suggestion on the intracellular recordings and for improving the manuscript.

REFERENCES

ANDERSON, N. C. (1969). Voltage-clamp studies on uterine smooth muscle. *J. gen. Physiol.* **54**, 145–165.

BORTOFF, A. (1972). Digestion: motility. *A. Rev. Physiol.* **34**, 261–290.

BORTOFF, A. & SACHS, F. (1970). Electrotonic spread of slow waves in circular muscle of small intestine. *Am. J. Physiol.* **218**, 576–581.

BÜLBRING, E. & TOMITA, T. (1969). Increase of membrane conductance by adrenaline in the smooth muscle of guinea-pig taenia coli. *Proc. R. Soc.* B **172**, 89–102.

CONNER, J. A., PROSSER, C. L. & WEEMS, W. A. (1974). A study of pace-maker activity in intestinal smooth muscle. *J. Physiol.* **240**, 671–701.

GOLENHOFEN, K. & LAMMEL, E. (1972). Selective suppression of some components of spontaneous activity in various types of smooth muscle by iproveratril (verapamil). *Pflügers Arch. ges. Physiol.* **331**, 233–243.

GOLENHOFEN, K., v. LOH, D. & MILENOV, K. (1970). Elektrophysiologische Untersuchungen zur Spontanaktivität isolierter Muskelpräparate aus verschiedenen Abschnitten des Meerschweinchen-Magens. *Pflügers Arch. ges. Physiol.* **315**, 336–356.

JOB, D. D. (1969). Ionic basis of intestinal electrical activity. *Am. J. Physiol.* **217**, 1534–1541.

KURIYAMA, H., OSA, T. & TASAKI, H. (1970). Electrophysiological studies of the antrum muscle fibers of the guinea-pig stomach. *J. gen. Physiol.* **55**, 48–62.

KURIYAMA, H. & TOMITA, T. (1970). The action potential in the smooth muscle of the guinea-pig taenia coli and ureter studied by the double sucrose-gap method. *J. gen. Physiol.* **55**, 147–162.

MAGARIBUCHI, T., OHBU, T., SAKAMOTO, Y. & YAMAMOTO, Y. (1972). Some electrical properties of the slow potential changes recorded from the guinea pig stomach in relation to drug action. *Jap. J. Physiol.* **22**, 333–352.

MILLS, R. G. & TAYLOR, G. S. (1971). Studies of intestinal slow wave activity with a double sucrose gap apparatus. *Life Sci., Oxford* **10**, 347–353.

PROSSER, C. L. (1974). Smooth muscle. *A. Rev. Physiol.* **36**, 503–535.

TOMITA, T. & WATANABE, H. (1973). Factors controlling myogenic activity in smooth muscle. *Phil. Trans. R. Soc.* B **265**, 73–85.