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# EFFECTS OF SODIUM, POTASSIUM AND CALCIUM IONS ON THE SLOW WAVE IN THE CIRCULAR MUSCLE OF THE GUINEA-PIG STOMACH

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SUMMARY

1. The contribution of Na, K and Ca ions to the generation of slow waves in the circular muscle of the guinea-pig stomach was studied.

2. The slow waves had a lower, potential-independent (first) and an upper, potential-dependent (second) component. Reduction of the external Na prolonged the first component, but complete removal of Na depolarized the membrane and caused deterioration of the slow wave.

3. Readmission of Na (5–10 mM) restored the slow wave; this action was not abolished by ouabain.

4. Removal of K depolarized the membrane and slightly reduced the amplitude and duration of the slow waves. Readmission of K hyperpolarized the membrane and increased the amplitude and duration of the slow waves, particularly of the first component. Ouabain blocked the effects on the membrane potential, but not the effects on the slow waves.

5. An increase in extracellular Ca prolonged the first component and reduced the frequency. Removal of extracellular Ca abolished the slow wave activity. Excess Ca enhanced, and low Ca reduced the effects of altering the concentrations of external K.

6. It is concluded that the ouabain-sensitive Na-K pump may not be directly involved in generating slow waves, but that some other metabolic process is involved, which is regulated to a large extent by Ca, and possibly also by Na and K.

## INTRODUCTION

In previous papers (Ohba, Sakamoto & Tomita, 1975, 1976) the spontaneous electrical activity of the circular muscle of the guinea-pig stomach was described. The activity consisted of rhythmic slow waves of depolarization, which could be resolved into two components (see Fig. 1): a lower first component, and an upper second component. There was often a small spike superimposed upon the top of the second component. It

was shown that changes in the membrane potential had marked effects upon the spike and second components, but had almost no effect upon the first component, which seemed to determine the rhythm of the spontaneous activity.

Under voltage-clamp conditions, when the membrane potential is held close to the resting potential, rhythmic waves of inward current can be recorded; these probably correspond to the first component of the slow

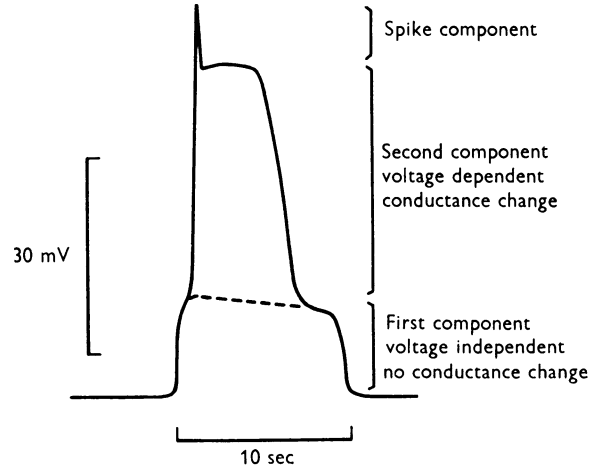


Fig. 1. Diagram of typical slow wave intracellularly recorded from circular muscle of guinea-pig stomach. The component parts of the complex wave have been indicated (based on Ohba *et al.* 1975).

wave. If the membrane is hyperpolarized or depolarized, it makes very little difference to the slow inward current, suggesting that a change in membrane conductance is not involved. This observation, together with the high temperature coefficient for the frequency of slow waves ($Q_{10}$ = 2·7) suggests that an active transport system is involved in the generation of the first component of the slow wave. This paper is largely concerned with factors modifying this first component.

In the longitudinal muscle of the cat's duodenum, there are slow waves which, at least superficially, resemble the slow waves in stomach. These duodenal slow waves may be generated by the oscillatory action of an electrogenic Na pump, as they are blocked by ouabain or the removal of external K (Connor, Prosser & Weems, 1974). According to these authors, the repolarization phase of the slow wave is due to activation of the pump, and depolarization corresponds to a cessation of pump activity.

In the present experiments we set out to test the hypothesis that the

slow waves in stomach are due to cyclic activity of the sodium pump. If this were the case, they should be abolished by ouabain or by removal of K, and should be stimulated by readmission of K. We present evidence that the electrogenic Na pump is not implicated in the genesis of the slow waves, but that Ca ions have an important, if not fully understood, role in the regulation of spontaneous activity. We also show that Na ions are not an important charge carrier during the slow waves.

## METHODS

The double sucrose-gap method was mainly employed for recording the electrical activity. A piece of the smooth muscle was dissected in a circular direction from the stomach after guinea-pigs were stunned and bled. In some experiments, voltage-clamp analysis was performed using the same double sucrose-gap set-up. These methods were the same as previously described (Ohba *et al.* 1975). The centre node was 700 $\mu$m in width, and experiments in which micro-electrodes were inserted into single cells in the node during voltage-clamp pulses showed that the membrane potential was reasonably stable during the clamping (Ohba *et al.* 1975).

The slow wave recorded with the double sucrose-gap method usually showed a hump at the falling phase. It may be argued that this hump is a kind of artifact, related to the fact that both sides of an active region of the preparation are exposed to sucrose solution. In order to clarify this point, the conventional method for intracellular recording with micro-electrodes was also employed in some experiments, mounting a piece of preparation in a small organ bath. The hump was still observed with this method.

Krebs solution was used as the control 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. When the Na concentration was reduced, NaCl was replaced on an equimolar basis with choline chloride, dimethyl diethanol ammonium chloride (DDA), Tris-hydroxymethyl aminomethane chloride (THAM) or lithium chloride (Li). In solutions containing less than 15·5 mM-Na, $NaHCO_3$ was also replaced with the substitute and KCl was substituted with $KHCO_3$. Atropine ($2 \times 10^{-6}$ M) was added when choline or DDA substitute was used. In K-free solution, KCl was replaced with NaCl or its substitute.

The experiments were usually performed at a constant temperature of 35–36° C.

## RESULTS

### *Effects on the Na-K pump*

*Removal of Na.* If Na ions were important as charge carriers during any phase of the slow wave, then reduction or removal of external Na should markedly decrease the amplitude of the slow waves. On the other hand, if the Na pump were important in the genesis of the slow waves, then removal of external Na, by depleting the cells of Na, should eventually cause cessation of pump activity and abolish the slow waves. Readmission of Na to the bathing medium would allow Na to enter the cells, stimulating

the pump and re-starting the slow waves. The effect of Na readmission should be blocked by ouabain or by lack of external K.

When the external Na concentration was reduced from 137 to 14 mM by substituting with choline or DDA, the slow wave was prolonged, particularly at the lower part as seen by the appearance of the 'hump' in the repolarizing phase, and its frequency was decreased. Fig. 2 (*A–D*) shows such an example. When the membrane potential was clamped at

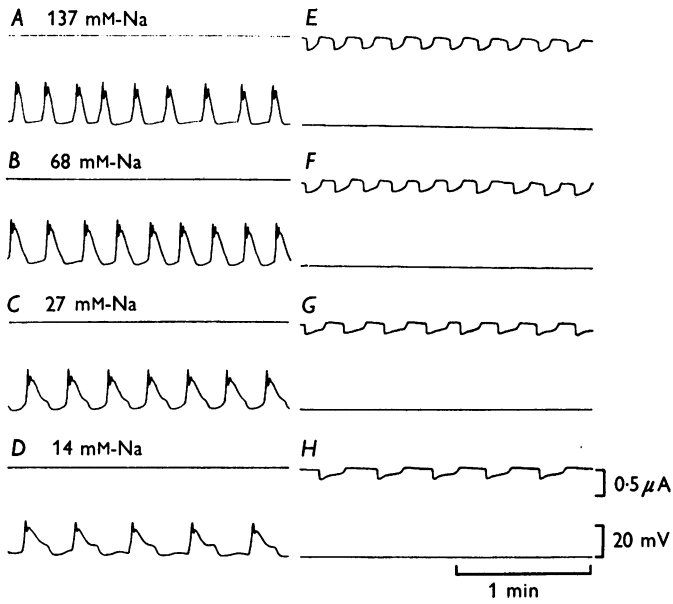


Fig. 2. Effects of reduction (from 137 mM, *A* and *E* to 14 mM, *D* and *H*) of Na concentration on electrical activity. Choline substitution. After 10 min exposure to each solution, records were taken before (*A–D*) and during voltage clamp (*E–H*). Upper trace: current (inward, downward); lower trace: membrane potential. Note prolongation of lower part of slow wave (*C*, *D*) and of inward current (*G*, *H*) in Na-deficient solution.

the level between the slow waves, inward currents were recorded with more or less the same rhythm as that of the slow wave, as previously reported (Ohba *et al.* 1975). The inward currents showed similar changes to the first component of slow wave (*E–H*). The duration of the inward current was prolonged and the interval was increased with the decrease in external Na concentration. However, the magnitude of the inward current was not much changed.

When Na ions were completely replaced with other substitutes, such as choline, DDA, THAM or Li ions, the membrane was depolarized by 5–20 mV, the amplitude of the slow wave was decreased, and its frequency

was increased. Since the pattern of responses was similar irrespective of the different substitutes for Na, the depolarization does not seem to be due to stimulation of a cholinergic receptor. The slow wave sometimes disappeared for 2–10 min at an early phase of Na removal, but it re-appeared with some repolarization of the membrane (Fig. 3*A*). The slow oscillatory activity disappeared after 20–60 min exposure to Na-free

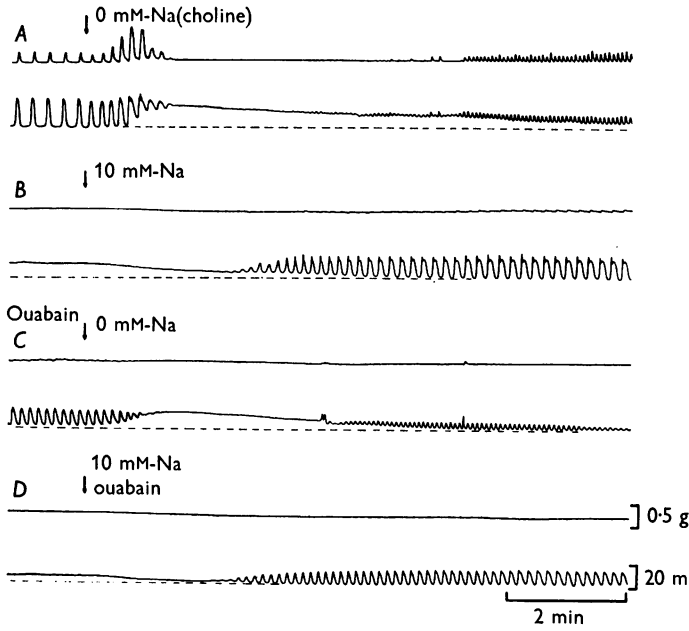


Fig. 3. Effects of Na-free (choline) solution (*A*, *C*) and the recovery of slow wave activity by addition of 10 mM-Na (*B*, *D*). 30 min elapsed between *A* and *B*, and between *C* and *D*. Records *C* and *D* were taken in the presence of ouabain ($2 \times 10^{-6}$ M) which had been present for 20 min before record *C*. Upper trace: mechanical activity; lower trace: electrical activity. Interrupted lines indicate the control level of membrane potential.

solutions. The change in pattern of electrical activity in Na-free solutions was not simply due to depolarization, because repolarization by applying prolonged inward current did not significantly improve the activity.

The effects of Na removal were confirmed using intracellular recordings. Configuration of the slow wave was essentially the same as recorded with the sucrose-gap method, and it was independent of the size of preparations, the smallest being less than 1 mm × 1 mm.

The sensitivity to Na removal varied from preparation to preparation.

In general, preparations taken from the upper corpus part of the stomach wall showed a small first component, and depolarization of the membrane and suppression of the slow wave on Na-removal were more pronounced than in preparations from the lower antrum part, which had a large first component in the slow wave.

After complete abolition of the activity following a prolonged exposure to Na-free solution, the slow wave recovered on addition of 10 mM-Na, this was accompanied by repolarization of the membrane (Fig. 3*B*). The slow wave gradually reappeared after about 2 min in 10 mM-Na solution and the amplitude reached 80% of the control and the frequency 140%. In some preparations, a similar recovery was observed with 5 mM-Na and the hump in the repolarizing phase of the slow waves due to the first component was clearly observed.

Ouabain ($5 \times 10^{-6}$–$10^{-5}$ M) depolarized the membrane by about 5 mV, reduced the amplitude of slow waves and slightly increased the frequency. The rhythmic activity was never completely suppressed by ouabain. When the concentration of ouabain was low ($5 \times 10^{-7}$ M), the slow wave, particularly at the lower part, was often prolonged.

Fig. 3*C* and *D* show effects of Na removal and readmission of 10 mM-Na in the presence of ouabain ($5 \times 10^{-6}$ M). The preparation was pre-treated with ouabain for 20 min before Na was removed. The results were similar to those observed in the absence of ouabain (*A*, *B*), i.e. the recovery of the slow wave in 10 mM-Na was also observed in the presence of ouabain.

*K removal and its readmission.* If Na-K pumping made an important contribution to the genesis of the slow waves, then removal of K from the external solution should abolish pump activity and reduce the slow waves. Readmission of K should stimulate the pump and enhance or initiate the slow waves. The effect of K admission should be abolished by ouabain.

The membrane was depolarized by about 5 mV in K-free solution, and hyperpolarized beyond the control level following readmission of normal K concentration (6 mM), as observed in the taenia coli (Casteels, Droogmans & Hendrickx, 1971*a*, *b*; Tomita & Yamamoto, 1971). The slow wave was decreased in amplitude by removal of K ion and the first component became difficult to distinguish from the second component (Fig. 4*A*). Its frequency was transiently increased and then often decreased. On readmission of K, the first component of the slow wave was prolonged in duration, increased in amplitude, and its interval also prolonged, before recovering towards the control (Fig. 4*B*). In the presence of ouabain (Fig. 4*C*) there was almost no depolarization on removal of K ion and almost no hyperpolarization when K ion was readmitted (Fig. 4*D*). However, the effects of K removal and K readmission on the slow wave

were essentially the same as the control. The augmentation of the first component by K readmission was even greater than the control.

At 36° C it was not so clear whether the effect of K readmission appeared first as a hyperpolarization of the membrane between the slow waves or first as a prolongation of the first component (Fig. 5*A*, *B*). Therefore the sequence of slow waves was slowed down by lowering the temperature to

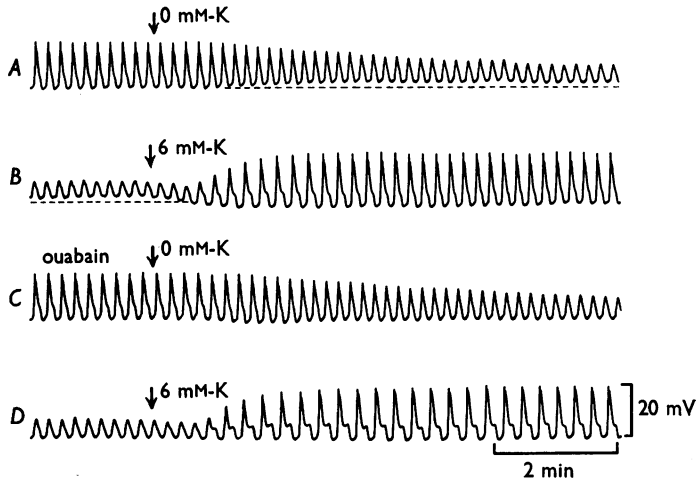


Fig. 4. Effect of K-free solution and its recovery in the absence (*A*, *B*) and in the presence (*C*, *D*) of ouabain ($5 \times 10^{-7}$ M). Note that, in the presence of ouabain, changes in the membrane potential are very small in response to K removal and K readmission, but that potentiation of the slow wave is clear, particularly at the lower part, during recovery from K-free solution.

25° C (*C* and *D*). At this temperature, removal and readmission of K ions produced more or less the same effects as at 36° C, although hyperpolarization on readmission was less at a low temperature. From Fig. 3*D*, it is clear that a prolongation of the first component could appear before the membrane potential between slow waves was hyperpolarized following K readmission.

The effects of K removal and K readmission in Na-deficient solution (15·5 mM-Na) were similar to those in the presence of ouabain (Fig. 6). After observing the effects in normal Krebs solution (*A*, *B*), NaCl was reduced to 15·5 mM (*C*). When K was removed after 10 min exposure to the Na-deficient solution, the membrane was hyperpolarized by 5–7 mV and the slow wave was reduced following a transient increase (*D*). Although the membrane was depolarized on K readmission a marked prolongation and augmentation of the first component was observed (*E*).

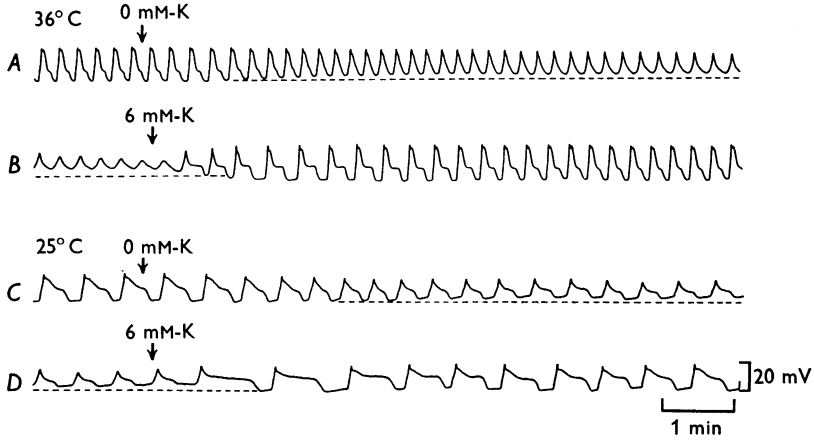


Fig. 5. Removal (0 mM-K) and readmission (6 mM) of K ion at 36° C (*A*, *B*) and at 25° C (*C*, *D*). Exposure to K-free solution was for 10 min. Note prolongation of lower part of slow wave on readmission of K ion.

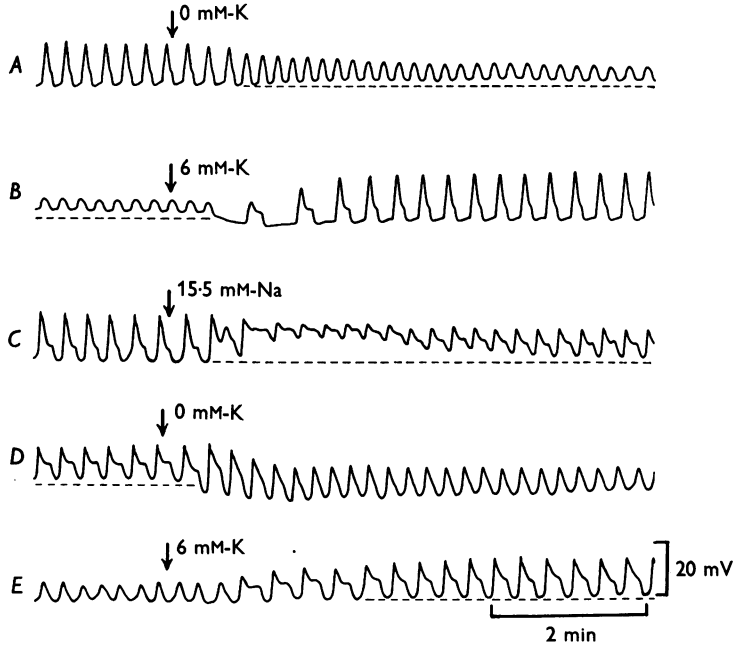


Fig. 6. Effects of K-removal and K-readmission in 137 mM-Na (*A*, *B*) and 15·5 mM-Na (*D*, *E*) solution. Record *C* shows the effect of Na-deficient solution. Exposure to K-free solution was for 10 min and exposure to Na-deficient solution was also for 10 min before K was removed in (*D*). Note that in Na-deficient solution changes in membrane potential to alteration of K concentration are opposite to those in the control solution, but that there is clear recovery of the slow wave on readmission of K ion.

*Role of Ca in control of slow waves*

Ca ions have an important role in the regulation of membrane permeabilities (Tomita & Watanabe, 1973), and may carry the depolarizing current during the spike in smooth muscle (Brading, Bülbring & Tomita,

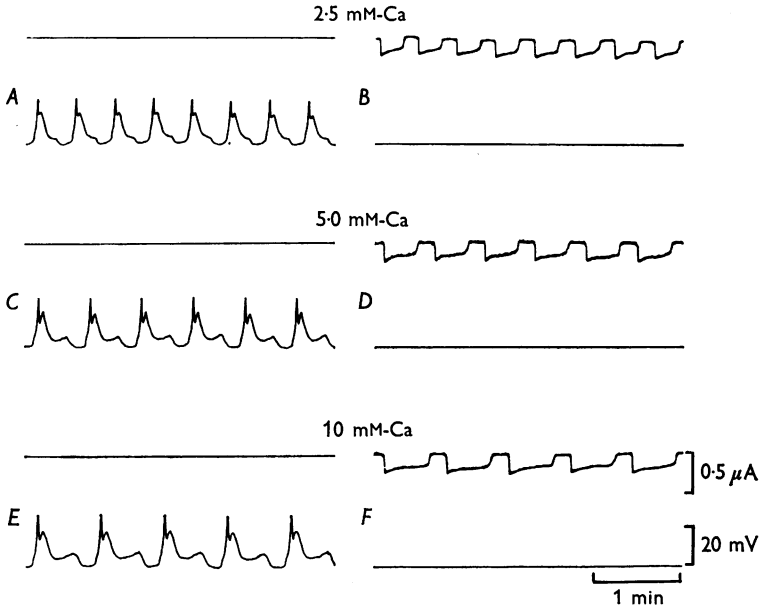


Fig. 7. Effects of excess Ca on the slow wave (*A*, *C*, *E*) and the inward current observed during voltage clamp (*B*, *D*, *F*). *A* and *B*, control (2·5 mM-Ca); *C* and *D*, after 5 min in 5 mM-Ca; *E* and *F*, after 5 min in 10 mM-Ca.

1969). It was therefore of interest to see whether Ca ions had a role in the control of slow wave activity, and the effects of both increased and decreased levels of extracellular Ca concentration were observed, first on the slow waves themselves, and then on the potassium effect previously described.

*Excess Ca.* Fig. 7 shows the effects of excess Ca (5 and 10 mM) on the slow wave. The main effects were prolongation of the first component and reduction of the frequency (*C*, *E*). In excess Ca solution, the second component occasionally appeared twice on a prolonged first component. In such a case, the apparent frequency was increased by increasing Ca concentration.

The right-hand side of Fig. 7 shows the inward current recorded under voltage-clamp condition in three different Ca concentrations. The inward current was prolonged without much change in the magnitude by increasing

the external Ca concentration (*D* and *F*), as observed for the first component of the slow wave (*C* and *E*).

A similar change in the slow waves was also confirmed using intracellular micro-electrodes in the preparations exposed to Krebs solution containing different concentrations of Ca. In excess Ca solution, the membrane was not hyperpolarized but sometimes slightly (2–3 mV) depolarized and the amplitude of the first component was not much changed.

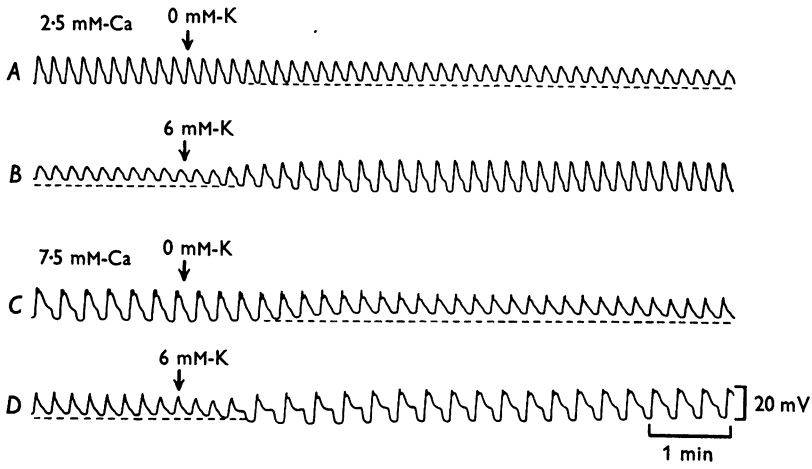


Fig. 8. Effects of K removal (*A*, *C*) and K readmission (*B* and *D*) in normal Ca (*A*, *B*) and 7·5 mM-Ca Krebs solution (*C*, *D*). Exposure to K-free solution was for 10 min, and in *C* the preparation was treated with 7·5 mM-Ca Krebs solution for 10 min before K was removed.

*Effect of K in different Ca concentrations.* After observing the effect of removal of external K (Fig. 8*A*), and also the effect of readmission of K (*B*), the external Ca concentration was increased from 2·5 to 7·5 mM (*C*). Excess Ca increased the duration of slow waves and reduced the frequency. Although the effects of K removal and readmission were essentially the same, the increases in duration and amplitude of the first component during the recovery from K-free solution were more pronounced in the presence of excess Ca (*D*).

When Ca ion was also omitted from K-free solution, deterioration of the slow wave was facilitated (Fig. 9*C*), compared with simple removal of K ion only (*A*). In the absence of K, readmission of 2·5 mM-Ca caused only a partial recovery in amplitude (*D*), but further addition of K to this solution produced a typical recovery (*E*) very similar to that from the simple K removal (*B*). In Fig. 9*F* and *G*, after 10 min exposure to 0 mM-Ca, 0 mM-K solution, K was first readmitted, then Ca ion. Re-

admission of K, in the absence of Ca, increased the amplitude, but not the frequency (*F*), while readmission of Ca in the presence of K caused a rapid recovery of the slow wave without a transient potentiation of the first component and decrease in frequency (*G*). When Ca and K were

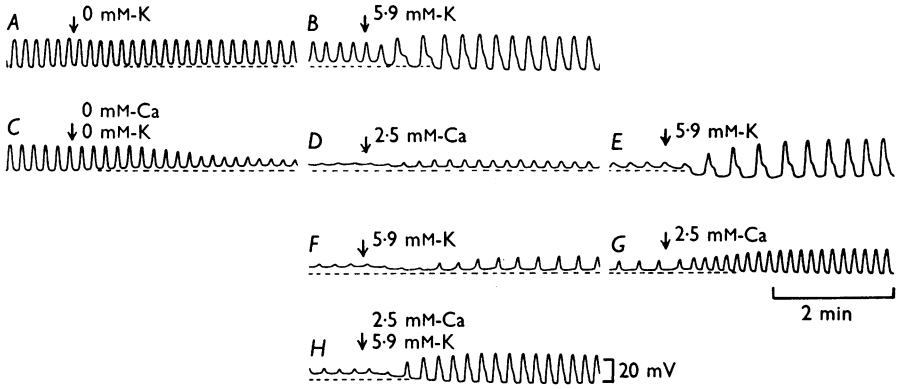


Fig. 9. Effects of K removal with and without simultaneous omission of Ca, and the recovery process. K was removed (*A*) and readmitted after 10 min (*B*) in the presence of 2·5 mM-Ca. After simultaneous removal of K and Ca (*C*). Ca was first readmitted 10 min later (*D*), then after further 10 min K was reintroduced (*E*). After 10 min in K- and Ca-free solution, K was first readmitted (*F*), and then Ca after further 10 min (*G*). After exposing to K- and Ca-free solution for 10 min, K and Ca were simultaneously readmitted (*H*). 0 mM-Ca solution was made without adding Ca and no chelating agent was used. All records are from the same preparation.

simultaneously readmitted, there was only a weak potentiation of the first component (*H*). Therefore, it may be concluded that the presence of Ca in K-free solution is a prerequisite for demonstrating a marked potentiation of the first component during the recovery process.

## DISCUSSION

The slow wave is rather resistant to a reduction of the external Na concentration. The duration, particularly of the first component, is prolonged in Na-deficient solution, but the amplitude is less dependent on the Na concentration until it is reduced to less than 10 mM.

A rapid depolarization is observed on exposure to Na-free solutions. The observations that depolarization is small in Na-deficient solution containing more than 10 mM-Na and that repolarization is produced by an addition of 5–10 mM-Na suggest that Na lack is responsible for the depolarization rather than the presence of a substitute. Similar

depolarization has been reported for the uterine smooth muscle of the mouse (Osa, 1971, 1973).

Depolarization by Na-removal in the mouse myometrium has been explained by an increase in Ca permeability, because the depolarization is prevented by Mn ions (Osa, 1973). The mechanism of the depolarization in the guinea-pig stomach muscle has not been investigated in the present experiments. However, an increase in Ca permeability may partly be involved, since a reduction of the external Ca concentration reduces the depolarization in Na-free solution (unpublished observation).

In the canine cardiac Purkinje fibre exposed to Na-free solution, an addition of 4–24 mM-Na produces hyperpolarization (Wiggins & Cranefield, 1974). This hyperpolarization is interpreted as due to electrogenic Na extrusion, because it is inhibited by ouabain, K removal and cooling. However, in the guinea-pig stomach muscle, ouabain does not essentially modify the recovery process from Na-free solutions caused by addition of 5–10 mM-Na.

Removal of the external K ions from normal Krebs solution depolarizes, and readmission of K hyperpolarizes the membrane. After treatment with ouabain or in Na-deficient solutions these responses are abolished or reversed, suggesting that in this preparation, as in the taenia coli (Casteels *et al.* 1971*a*, *b*; Tomita & Yamamoto, 1971), the Na-K pump is responsible for the changes in membrane potential during alterations in external K. However, in the presence of ouabain or the absence of K ions, the slow wave activity persists, so it is unlikely that Na-K pumping activity is responsible for these slow waves. The slow waves also persist in Na-deficient solutions, except where the Na concentration falls below 10 mM; it is possible that the disappearance of the slow waves in completely Na-free solutions is related to the mechanism, already discussed, for depolarization in Na-free solution.

The readmission of K to previously K-free solutions produces not only changes in membrane potential (blocked by ouabain) but also a marked increase in the first component of the slow wave. This latter effect is not blocked by ouabain, nor by Na deficiency. It seems that some active transport system is responsible for the first component, for which function the presence of external K ion is necessary. However, this system is probably not the ouabain-sensitive Na pump. In this respect, the slow wave in the circular muscle of guinea-pig stomach differs from that in the longitudinal muscle of cat small intestine, in which the Na pump seems to play an important role for the generation of the slow wave (Connor *et al.* 1974; Daniel, 1965; Job, 1969; Liu, Prosser & Job, 1969).

Reduction of the external Na to less than 10 mM, removal of the external K and addition of a high concentration of ouabain all cause

deterioration in the slow wave. It is possible that these effects are not due to suppression of the Na pump, but mediated through a modification of Na distribution, and secondarily of Ca distribution, across the membrane.

The duration of the first component is found to be dependent on the external Ca concentration. The potentiation of the first component produced by K readmission is suppressed in Ca-free solution and augmented in excess Ca solution. If an active ionic transport system is responsible for electrogenesis of the slow wave, Ca and K ions seem to be involved in this process.

Na may have controlling actions on the Ca distribution. One possibility is that Na competes with Ca at a site on the outside of membrane, as assumed for the cardiac muscle (Lüttgau & Niedergerke, 1958). Another is that Ca transport across the membrane may be controlled by a Na–Ca exchange mechanism, as demonstrated in the squid giant axon (Baker, Blaustein, Hodgkin & Steinhardt, 1969; Baker, 1972) and in the cardiac muscle (Reuter & Seitz, 1968; Reuter, 1973). In the circular muscle of the guinea-pig stomach, the effect of a reduction of the external Na to about 14 mM is similar to an increase in the external Ca concentration. This may be explained by the competitive action between Na and Ca. When the external Na is reduced to less than 10 mM, the membrane is depolarized and the slow wave becomes very small or is abolished. This is probably caused by a disturbance in Ca distribution as a result of suppression of the Na–Ca exchange mechanism. The presence of about 10 mM-Na seems enough for this mechanism and also for the generation of the first component of the slow wave.

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