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IONIC BASIS OF THE RESTING POTENTIAL OF SUBMUCOSAL ARTERIOLES IN THE ILEUM OF THE GUINEA-PIG

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SUMMARY

1. The changes in the resting membrane potential of arterioles produced by rapid and brief changes in external ionic concentrations were measured.

2. The resting membrane potential was insensitive to changes in the external concentrations of both sodium and chloride ions but sensitive to changes in the external concentration of potassium ions.

3. Increasing the external concentrations of potassium ions produced depolarizations that were well described by the Nernst equation.

4. Decreased external concentrations of potassium ions produced membrane depolarizations which appeared to result not from inhibition of an electrogenic sodium pump but rather from a change in the resting conductance of the arteriolar membrane to potassium ions.

5. Ouabain caused both membrane depolarization and an increase in membrane resistance.

6. It is suggested that at rest, arteriolar smooth muscle is permeant predominantly to potassium ions, with only small contributions from chloride and sodium ions. No evidence was obtained which would support the idea that an appreciable proportion of the resting membrane potential depended upon current flow from an electrogenic sodium pump.

INTRODUCTION

Smooth muscle cells, like other cells, have a negative resting potential with respect to the extracellular fluid (see Casteels, 1970; Bennett, 1972). With intestinal smooth muscle, the membrane potential is decreased as the external potassium is raised (Holman, 1958; Kuriyama, 1963; Bennett, 1966). When the extracellular chloride is reduced by substituting chloride by anions to which the membrane is less permeable, membrane depolarization occurs; generally the depolarization is transient (Kuriyama, 1963). This is qualitatively similar to the effects of potassium and chloride ions on skeletal muscle fibres (Hodgkin & Horowicz, 1959).

Studies on the rates of efflux of the isotopes of potassium, sodium and chloride have indicated that intestinal muscle is permeant to each of these ions with permeability

ratios of potassium 1 : sodium 0·05 : chloride 0·6 (Brading, 1979, 1981). When attempts have been made to relate these ratios of permeability, and the measured distributions of these ions to the observed values of resting membrane potential using the Goldman (1943) and Hodgkin & Katz (1949) equations there is, however, a difference between the prediction and experimental observations (Casteels, 1970; Casteels, Droogmans & Hendrick, 1971*a*, *b*; see however Brading 1971; Bennett, 1972). This has led to the suggestion that the resting membrane potential arises in part from current flow across the membrane generated by an electrogenic sodium pump (Casteels, 1981; Thomas, 1972). Support for this view came from experiments in which either the extracellular potassium concentration was reduced to low levels or the tissue was exposed to ouabain. Both of these procedures which inhibit the activity of electrogenic sodium pumps (Thomas, 1972) caused membrane depolarization (Tomita & Yamamoto, 1971).

Taken together, these observations suggest that the resting membrane potential of intestinal muscle results from a balance between potassium, sodium and chloride permeabilities, along with a contribution from the activity of an electrogenic sodium–potassium pump.

The resting membrane potential of *arterial* and *arteriolar* smooth muscle is some 10–20 mV more negative than that of intestinal smooth muscle. Recent reports suggest it lies in the range −65 to −75 mV (Hirst, 1977; Kajwara, Kitamura & Kuriyama, 1981; Kuriyama & Suzuki, 1981). These values can only be accepted with some caution, imposed by the need to use high-resistance micro-electrodes for intracellular recording. In common with intestinal muscle, the membrane potential is sensitive to changes in extracellular potassium concentration (Hermsmeyer, 1976; Casteels, Kitamura, Kuriyama & Suzuki, 1977*a*, *b*). However, a contribution by chloride ions has been questioned (Harder & Sperelakis, 1978). Again, like intestinal muscle, there are data which suggest that current flow produced by the activity of an electrogenic sodium pump contributes to the resting membrane potential (Hendrickx & Casteels, 1974; Hermsmeyer, 1976).

The experiments described in this report were carried out in an attempt to determine the ionic basis of the resting potential in arteriolar smooth muscle. The method chosen was essentially the same as that used by Hodgkin & Horowicz (1959). Tissues were exposed for brief periods to rapid changes in external ion concentration and the resultant changes in membrane potential determined. Arterioles, whose muscle wall is only one cell thick, were used to minimize diffusional delays. Use of this tissue also avoided some of the complications of interpretation of data obtained from muscular arteries, where the smooth muscle cells form three dimensional syncitia and which are subjected to dissimilar concentration gradients before equilibrium is reached.

METHODS

Experiments were carried out using arterioles isolated from the submucosa of guinea-pig small intestine (for details of the dissection and recording techniques see Hirst, 1977). The preparations were pinned onto a microscope coverslip which had previously been coated with a fine layer of silicone rubber; the coverslip formed the base of a Perspex recording chamber of volume 0·1 ml. Tissue fluid, composition (mM): NaCl, 120; KCl, 5; $CaCl_2$, 2·5; $MgCl_2$, 2; $NaHCO_3$, 25; NaH_2PO_4, 1; glucose 11, which had been gassed with 95 % O_2 : 5 % CO_2, flowed continuously through the chamber at 6 ml./min at a temperature of 37 °C.

Solution changes were effected by means of a hydraulic valve positioned immediately prior to a heating block which formed one wall of the recording chamber. The valve allowed the test solutions to run to waste, again at 6 ml./min before switching; after switching the control solution ran to waste. In this way the gaseous concentrations of both solutions were the same. Using this system the bath composition was completely changed within 6 sec (see fig. 2, Hirst, Neild & Silverberg, 1982). The membrane potential of the arterioles was measured continuously before, during and after the test solution. Records obtained where the micro-electrode was dislodged during this procedure were discarded. Except in the cases where the effects of ouabain and low-temperature solutions were examined, the exposure time was limited to 1 min.

In the experiments where membrane resistance changes were determined, short segments of arteriole were prepared by cutting the arteriole with a fragment of razor blade (Hirst & Nield, 1978, 1980). Segments were cut to have lengths of 180 to 300 μm, as these have electrical lengths of 0·1–0·2 length constants and with little error could be considered to be isopotential during current injection (Hirst & Neild, 1978, 1980). The input resistance of a segment was determined using a bridge circuit, the bridge being balanced after impalement of a segment using the method suggested by Martin & Pilar (1963).

Increases in extracellular potassium concentration were produced by substituting potassium chloride for sodium chloride. Reductions in potassium were obtained by simple removal of potassium chloride; control experiments suggested that the small changes in tonicity associated with this procedure produced no detectable errors. The extracellular chloride concentration was changed by replacing sodium chloride with sodium isothionate. The extracellular sodium concentration was changed by substituting sucrose (240 mM) for sodium chloride. In these experiments the membrane potential was measured differentially, a second extracellular micro-electrode being positioned close to the intracellular recording electrode. In some experiments, ouabain (Sigma or Fluka) was used.

RESULTS

Effects of changing the extracellular concentrations of chloride and sodium ions on the resting membrane potential of arterioles

Arteriolar smooth muscle cells had resting membrane potentials of -60 to -70 mV immediately after impalement; brief transmural stimuli (pulse width 50–100 μsec) initiated excitatory junction potentials (e.j.p.s). Over the next few minutes the resting potential increased to a stable value of -65 to -80 mV; during this time evoked e.j.p.s increased in amplitude. Thereafter the resting membrane potential and the amplitude of e.j.p.s remained constant, often for up to 3 hr. The mean value of this stabilized resting potential, determined by sudden withdrawal of the micro-electrode, was $-72{\cdot}1$ mV (S.E. of mean 1·1 mV; $n = 16$ separate preparations), a value very similar to that reported by Kuriyama & Suzuki (1981) for guinea-pig mesenteric artery ($-69{\cdot}6$ mV).

When the extracellular chloride concentration was reduced by substituting the appropriate proportion of chloride ions with isothionate ions (see Methods) no change in resting membrane potential could be detected during the 1 min exposure to this test solution (seven experiments). In comparable experiments using frog skeletal muscle fibres a similar change in chloride concentration, again using isothionate ions as the substitute, produced a rapid membrane depolarization which was sufficiently large (> 30 mV) to initiate action potentials.

If chloride ions were passively distributed across the membrane of arteriolar smooth muscle cells as they are in skeletal muscle fibres (Hodgkin & Horowicz, 1959), it could be argued that their large surface to volume ratio allowed a new equilibrium to be established during the time taken to change the extracellular chloride concentration. If this were the case, since both the extracellular and intracellular

chloride concentrations would fall, the membrane resistance would increase (Hodgkin & Katz, 1949). An increased membrane resistance can be detected with intestinal muscle, which has an appreciable permeability to chloride ions when the extracellular concentration of chloride ions is reduced (Ohashi, 1970; Bolton, 1973). However, when recordings were made from short segments of arteriole, and current pulses were passed through the recording electrode to determine arteriolar membrane resistance, no change in resistance could be detected following the reduction of extracellular chloride to one tenth of that of the control solution. These observations suggest that with arteriolar smooth muscle, the contribution of chloride permeability to the observed value of resting membrane potential must be much smaller than it is in either skeletal or intestinal smooth muscles.

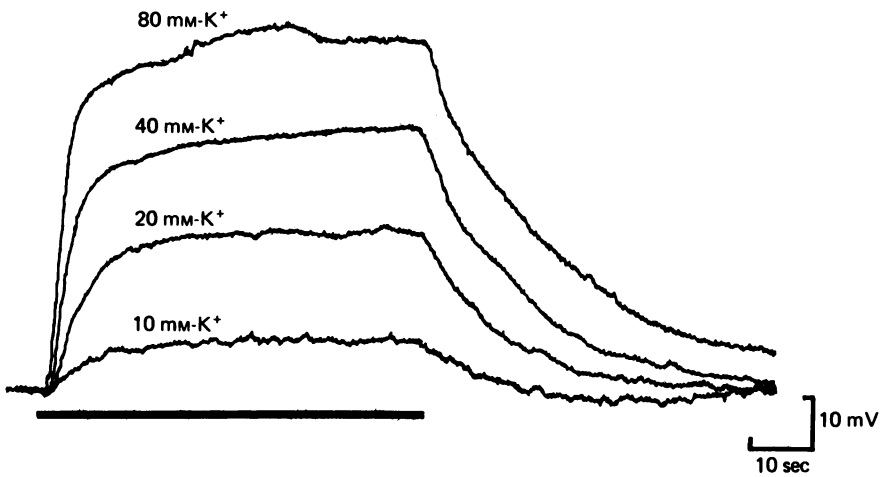

Fig. 1. Changes in membrane potential of arteriolar smooth muscle cells produced by bathing the arteriole in solutions containing increased concentrations of potassium ions. In this and subsequent records the period during which the test solution flowed through the recording chamber is indicated by the horizontal bar. It can be seen that the membrane potential increased by approximately 20 mV for each doubling of external potassium concentration. Calibration bars apply to each record.

Similarly, a concurrent reduction in both extracellular sodium and chloride ions, by substituting the sodium chloride in the control solution with sucrose, failed to produce a detectable change in resting membrane potential. Evidently, like other smooth muscles, the resting sodium permeability of arteriolar muscle cells is low when compared with the permeability of another ion (or ions) (Brading, 1981; Casteels, 1981).

Effects of changing the extracellular concentration of potassium on the resting membrane potential of arterioles

The resting membrane potential of arteriolar smooth muscle was changed by either increasing or decreasing the external concentration of potassium ions. When the potassium concentration was increased (see Fig. 1), the resting membrane potential decreased. Whereas a doubling of potassium concentration from control (5 mM) to

10 mM produced a depolarization of 10–15 mV; subsequent 2-fold increases in potassium produced increments of about 20 mV depolarization. As shown in Fig. 1, the membrane potential change resulting from an increase in potassium concentration was essentially complete after 30 sec. This change was reversible and repeatable. Since arterioles have a high specific membrane resistance (Hirst & Neild, 1978), it is likely that little change in internal potassium concentration could occur during these short exposure times and this assumption has been made for the treatment of all data. The

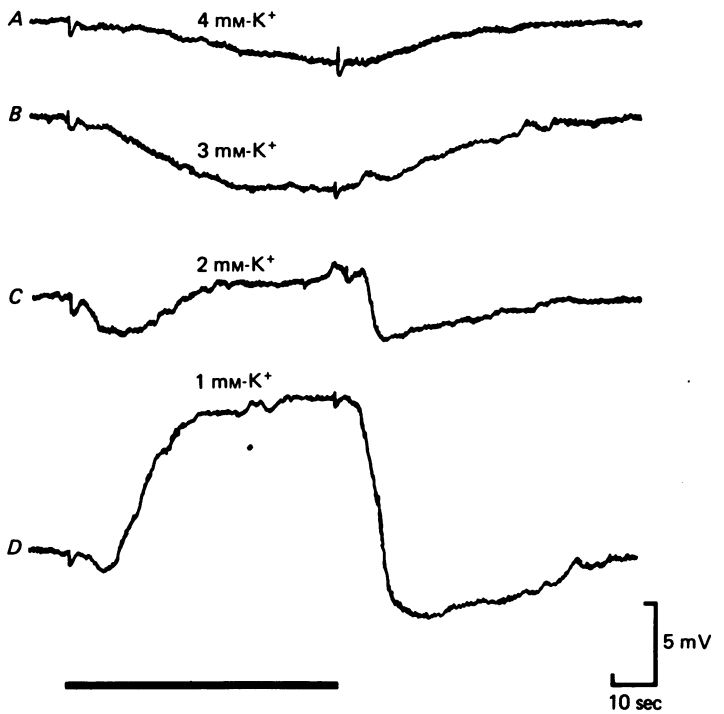

Fig. 2. Changes in membrane potential of arteriolar smooth muscle cells produced by bathing the arteriole in solutions containing decreased concentrations of potassium ions. Small reductions of the external concentration of potassium ions cause membrane hyperpolarization, but more dramatic reductions result in a more complex series of membrane-potential changes. The membrane first hyperpolarizes and then depolarizes. Restoration of normal potassium solutions then produces a further transient hyperpolarization. Calibration bars apply to each record.

mean data from the experimental series are shown in Fig. 3. At concentrations of potassium of 10–80 mM the slope of the relationship between the logarithmic concentrations of potassium ions and depolarization is approximately linear, with the slope of 57 mV per log unit of potassium concentration. The concentration of potassium, obtained by extrapolation, which gave a resting membrane potential of zero millivolts was 120 mM. This agrees well with the value of 130 mM estimated in arterial smooth muscle (Casteels *et al.* 1977*a*).

When the potassium concentration was reduced from 5 mM to either 3 or 4 mM, the resting membrane potential increased (Fig. 2*A* and *B*). With greater reductions

than this, however, the responses were complex (see Fig. 2*C* and *D*). The membrane potential transiently hyperpolarized and subsequently depolarized; on returning to 5 mM-potassium solution the membrane potential then transiently hyperpolarized. Again the mean data for changes in membrane potential produced by reductions in extracellular potassium concentration after 60 sec exposure are shown in Fig. 3.

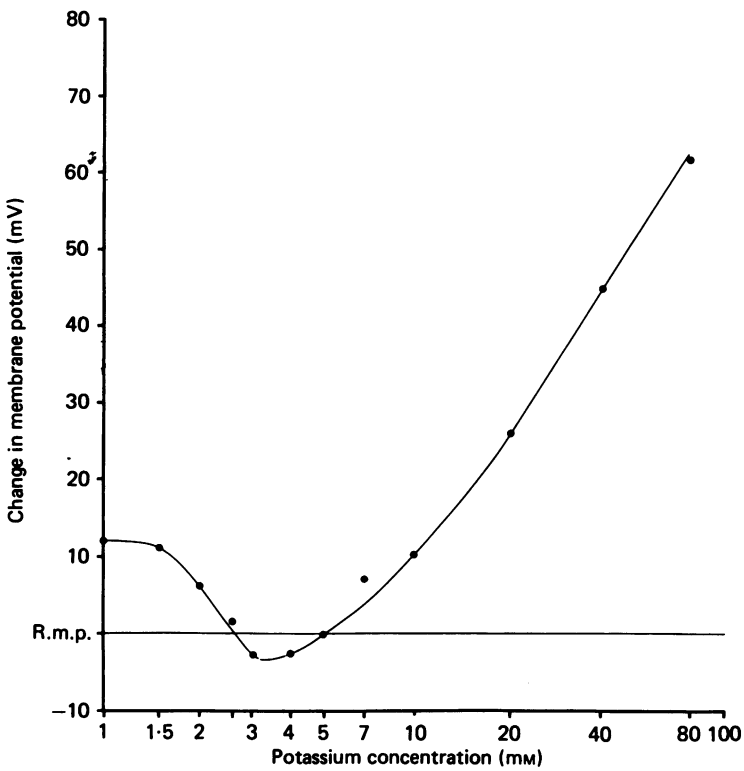

Fig. 3. Relationship between membrane potential of arteriolar smooth muscle cells and external concentration of potassium ions. Each point is the mean of four to eight determinations of the change in membrane potential observed at the end of the 1 min exposure to each test solution. The largest standard error of the mean was that for the responses produced by exposure to 80 mM-potassium and had a value of 1·4 mV, $n = 5$. The line was drawn by eye through the points. R.m.p. = resting-membrane potential.

When the potassium concentration was reduced to less than 2 mM, a mean depolarization in excess of 10 mV was observed. When the extracellular potassium concentration was reduced to very low levels, by superfusing the preparation with potassium-free solution, a similar depolarization was detected (mean depolarization in potassium-free solution 12·6 mV; S.E. of mean, 1·0 mV; $n = 9$).

These observations show that at high extracellular concentrations of potassium ions, the membrane potential of arterioles behaves much as would be predicted by the Nernst equation for a potassium electrode. The deviation from the Nernst potential at concentrations near the physiological range can perhaps be accounted for by minor contributions from both sodium and chloride ions (Fig. 6), as is the case

in other tissues (see Hille, 1977). In this arteriolar preparation these contributions must be small as they were not detected by ion substitution experiments. However, the depolarization seen at very low concentrations of potassium ions is at variance with predictions by the Goldman (1943) and Hodgkin & Katz (1949) equations.

Effects of extracellular potassium concentration on the membrane resistance of arterioles

From the experiments just described, it appears that the resting membrane potential of arterioles is primarily determined by the distribution of potassium ions, the membrane being predominantly permeable to this ion. Thus, provided the internal concentration of potassium does not change, the membrane resistance should be a function of external potassium concentration and membrane potential (Hodgkin & Katz, 1949).

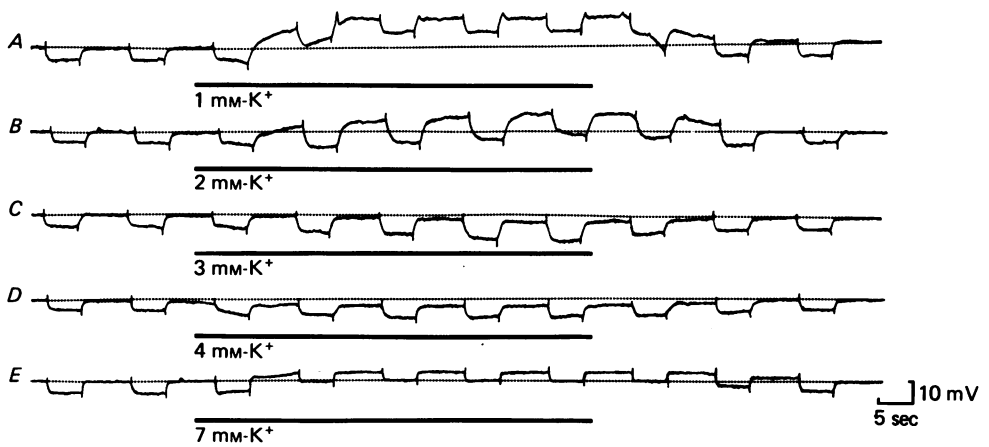

Fig. 4. Effect of changing the external concentration of potassium ions on the input resistance of an isolated segment of arteriole. Each record was obtained from the same segment of arteriole which was exposed at approximately 4 min intervals to the test solutions. In each record a 5 sec constant-current pulse of 0·1 nA, was passed through the recording electrode at 11 sec intervals. As the external concentration of potassium was decreased, the amplitude of the electrotonic potentials increased (*A*, *B*, *C* and *D*). Conversely, when the external concentration of potassium was increased, the amplitude of the electrotonic potentials decreased (*E*). Calibration bars apply to each record.

The relationship between membrane resistance and external potassium concentration was examined in a series of experiments using short isolated segments of arteriole. Each segment of arteriole was less than 300 μm in length (range 150–280 μm). The input resistance of such segments gives a direct measure of membrane resistance (see Hirst & Neild, 1978, 1980 for details and justification). It can be seen from Fig. 4 that the membrane resistance of such a segment of arteriole was changed by changing the extracellular concentration of potassium ions. When the concentration of potassium was increased, the amplitude of the membrane-potential change produced by constant current pulses passed through the recording electrode fell. With this change in input resistance there was a parallel change in membrane time constant, indicating that input-resistance change reflected a true change in membrane

resistance rather than changed coupling between smooth muscle cells. Conversely, as the potassium concentration was decreased the amplitude of these potential changes increased. The entire range of changed input resistances was not simply a consequence of the changes in membrane potential. The membrane resistance of arterioles and arteries is constant over a membrane potential range ± 10 mV resting value; however, when the membrane potential changes exceed ± 10 mV, voltage-dependent

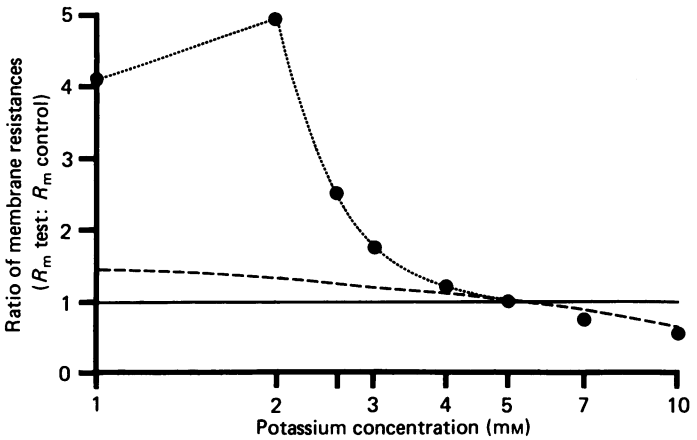

Fig. 5. Relationship between the membrane resistance of arterioles and external potassium concentration. The results (dotted line) are expressed as a ratio of the membrane resistance in changed potassium to that determined in control solution (potassium 5 mM). The ratio for each determination was obtained by measuring the membrane resistance immediately before the solution change and during the period 30 sec to 60 sec after exposure to test solution. Each point is the mean of a minimum of four determinations from four different preparations. The theoretical relationship between membrane resistance and potassium concentration is shown as a dashed line crossing a ratio of unity (continuous line) at a potassium concentration of 5 mM (see text for details of calculation).

rectification occurs (Hirst & Neild, 1978; Casteels *et al.* 1977*a*). The pooled results from this series of experiments is shown in Fig. 5. In this Figure the ratios of input resistance in given concentrations of potassium to input resistance in 5 mM-potassium are plotted (dotted line) against the concentrations of potassium. Also plotted is the theoretical curve (dashed line) for the ratio of membrane resistance as a function of potassium concentration (Hodgkin & Katz, 1949).

It is apparent from Fig. 5 that as the extracellular potassium concentration was reduced, the membrane resistance of the arteriolar segment increased more than would be expected on theoretical grounds. The maximum increase in resistance occurred at a concentration of about 2 mM-external potassium. At a lower potassium concentration the increase in resistance was not as great. This was at least in part a consequence of the depolarization produced by the low potassium solution (Fig. 3.); returning the membrane potential towards its original resting value by passing steady currents through the recording electrode caused a further increase in the amplitude of the electrotonic potential produced by the resistance monitoring pulses. A comparison of Figs. 3 and 5 indicates that where the membrane potential

changes in response to reduced potassium concentration behave anomalously, there is a corresponding disparity between predicted membrane resistance and observed values.

These observations indicate that the membrane depolarization produced by exposure to low potassium solutions does not result simply from inactivation of a sodium/potassium pump, but they suggest that it results from a decrease in the potassium permeability of the arteriolar membrane. That is the relative selectivities of the membrane to ions were changed (see also Hodgkin & Horowicz, 1959). Support for this idea was obtained in experiments where the effects of changing chloride concentration on membrane potential were examined. As indicated above, a reduction of extracellular chloride concentration in the presence of 5 mM-potassium ions produced no detectable change in membrane potential. However, when the arteriole was bathed in 2 mM-potassium solution, a solution which increases the resting resistance by a factor of five (Fig. 5), the same reduction in chloride concentration caused a depolarization of 8–10 mV. Evidently, the relative contribution of potassium was decreased and that of chloride consequently increased.

Relative permeabilities of arterioles to potassium, chloride and sodium ions

In order to determine the relative permeabilities of these three ions an attempt was made to describe our data using the Goldman/Hodgkin & Katz equation. For these calculations, the free internal ion concentration should be known. As the values for sodium and chloride are not known for arterioles, figures of 36 mM for chloride and 20 mM for sodium (Bennett, 1972) were assumed. The free internal potassium concentration was taken to be 120 mM (see preceding sections). A fit between the experimental observations was obtained with the ratios of $P_K : P_{Cl} : P_{Na}$ having the values 1 : 0·09 : 0·005 (Fig. 6, continuous line) over the potassium concentration range 4–80 mM. Clearly, the permeability ratios would be slightly changed if the assumed internal concentrations of sodium and chloride ions were inappropriate. Using these calculated permeability ratios it was then possible to predict the expected changes in membrane potential resulting from experimentally used changes in external chloride and sodium chloride concentrations. The predicted changes were +1·8 mV and −2·1 mV respectively. Changes of these magnitude were at the limits of our resolution. Thus over a limited range of concentrations, the present findings were adequately described by the Goldman/Hodgkin & Katz equation; this analysis indicates that the dominant permeability at rest is that of potassium and the sodium permeability is very low.

However, the depolarizations observed in low potassium solutions were clearly not in accordance with the Goldman/Hodgkin & Katz equation (Fig. 6). The measurements of membrane resistance (Fig. 5) indicated that in these concentrations there was a deviation from that predicted by the Hodgkin & Katz (1949) conductance equation. Since the dominant conductance at rest is to potassium ions, we attempted to test by calculation whether the decrease in membrane potential seen at low potassium concentrations could result from a simple decrease in potassium conductance. This was done by assuming that the increase in cell resistance resulted solely from a decrease in potassium conductance. Values of membrane potential were therefore calculated where the ratios of permeabilities to the three ions, potassium, chloride

and sodium, changed by an amount designated by the increased observed resistance. The agreement between prediction and observed data over the range of potassium concentration 2–3 mM was good (Fig. 6, dotted line). At lower concentrations of external potassium than this, the fit was poor and this may well reflect the activation of other conductances since these lower concentrations often gave rise to regenerative membrane responses and arteriolar constrictions (see also Casteels *et al.* 1977*b*).

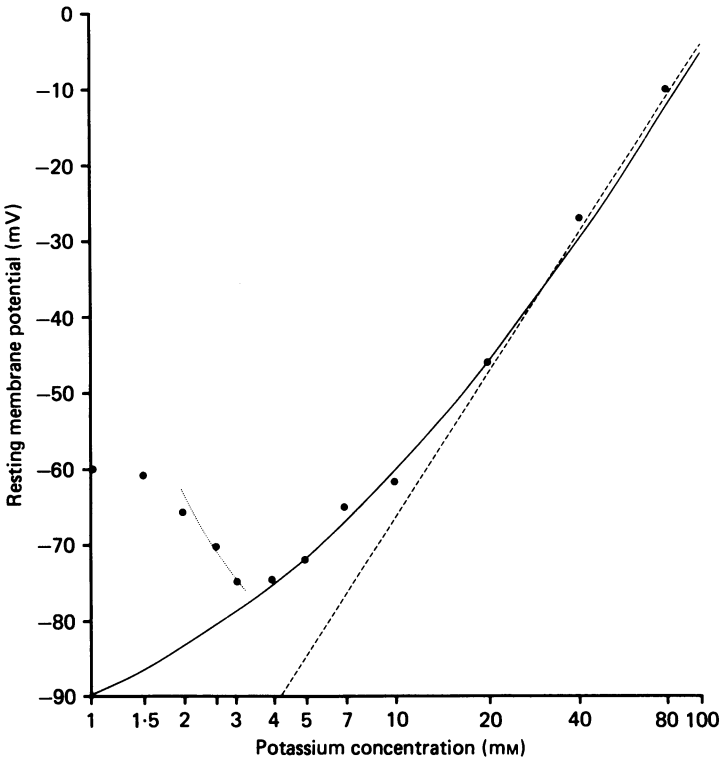

Fig. 6. Predicted relationships between membrane potential and potassium concentration. The experimental data shown in Fig. 3 are plotted (●) but normalized to a resting membrane potential of −72 mV. The continuous line shows the relationship predicted by the Goldman/Hodgkin & Katz equation for the permeability ratios $P_K:P_{Cl}:P_{Na}$ 1:0·09:0·005. The dotted line shows the expected relationship between potassium concentration and membrane potential predicted by the Goldman/Hodgkin & Katz equation where the ratios $P_K:P_{Cl}:P_{Na}$ vary according to the assumption that the membrane resistance changes shown in Fig. 5 reflect changing values of P_K. The dashed line shows the Nernst potential calculated for an intracellular potassium concentration of 120 mM.

These calculations support the idea that the membrane potential of arterioles is determined largely by its permeability to potassium ions, but suggest that when the external potassium concentration is reduced ratios of permeabilities to potassium, sodium and chloride change.

Effect of ouabain on the resting membrane potential and membrane resistance of arterioles

The experiments in which arterioles were bathed in solution of low potassium concentration gave little indication of the magnitude of the electrogenic component

of the resting membrane potential. Sodium–potassium transport systems in a variety of tissues, including smooth muscle, may also be inhibited by ouabain (Thomas, 1972; Bolton, 1973). As has been reported for arterial and other smooth muscles (Hendrickx & Casteels, 1974; Tomita & Yamamoto, 1971), ouabain in a concentration range of 10^{-6} to 10^{-4} M caused a decrease in the resting membrane potential of arterioles. As shown in Fig. 7*A*, the depolarization, unlike that produced by a reduction in extracellular potassium concentration, was slow in onset and, as might be expected, was not preceded by membrane hyperpolarization. The depolarization amounted to

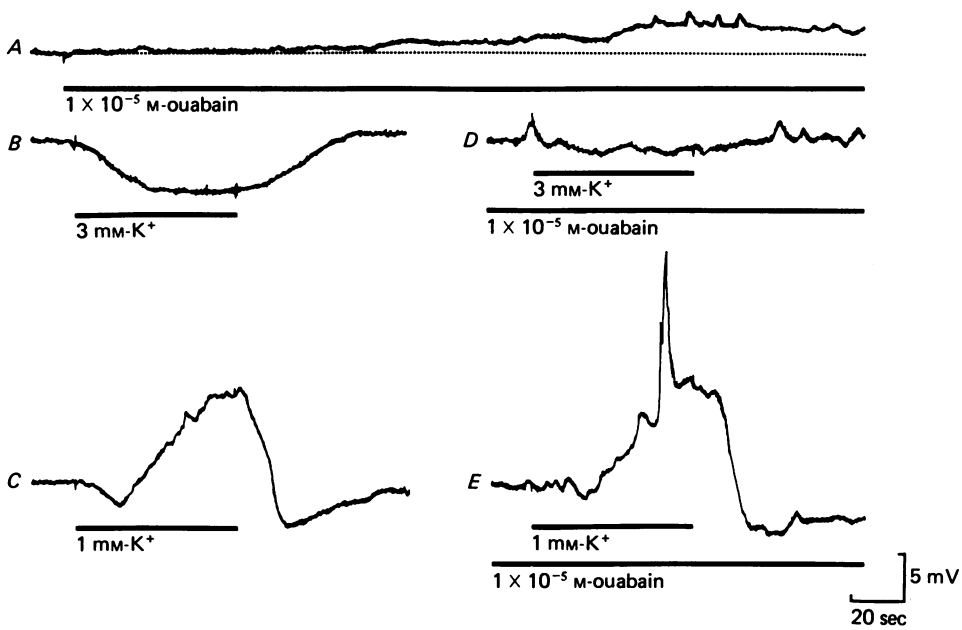

Fig. 7. Comparison between the membrane-potential changes produced by ouabain and reduced potassium concentration. *A*, shows the slow depolarization produced by ouabain (10^{-5} M). Records *B* and *C* show the membrane potential changes produced by reducing the external potassium to 3 and 1 mM respectively. The records *D* and *E* show the membrane-potential changes produced by the same changes in potassium concentration 15–18 min after exposure to ouabain (10^{-5} M). Here the ouabain has caused a depolarization of 7 and 8 mV respectively. The hyperpolarization produced by exposure to 3 mM-potassium is much reduced in the presence of ouabain (records *B* and *D*). The net depolarization caused by 1 mM-potassium is little altered by ouabain. However, the initial hyperpolarization is suppressed (records *C* and *E*). All records obtained from the same cell voltage and time calibrations apply to all records.

3–8 mV (six preparations) after 2 min exposure to ouabain (10^{-5} M). During more prolonged exposure (10–15 min) the membrane potential progressively decreased, presumably reflecting a redistribution of ions across the membrane (Casteels, 1981). During these times the membrane potential records became 'noisy', and occasionally spontaneous action potentials were recorded which were associated with spasmodic constriction of regions of the arteriolar tree. When ouabain was washed from the recording chamber, the membrane potential progressively repolarized but, again

unlike the low potassium responses, was not followed by a hyperpolarization (see also Hendrickx & Casteels, 1974).

In order to determine the degree of pump inhibition of a concentration of 10^{-5} M-ouabain in arterioles, the rates of onset of membrane depolarization in ouabain at concentrations of 10^{-5} and 10^{-4} M were compared. No differences between the two solutions were apparent. This would suggest that the lower concentration had inhibited the sodium pump. Despite this a reduction in external potassium concentration, sufficient to cause membrane depolarization in ouabain-free solutions (Fig. 7*C*), continued to cause a membrane depolarization (Fig. 7*E*) in ouabain-containing solutions. Moreover, a reduction in the external concentration of potassium which in the absence of ouabain produced only a hyperpolarization (Fig. 7*B*), no longer gave such a response in ouabain-treated tissues (Fig. 7*D*).

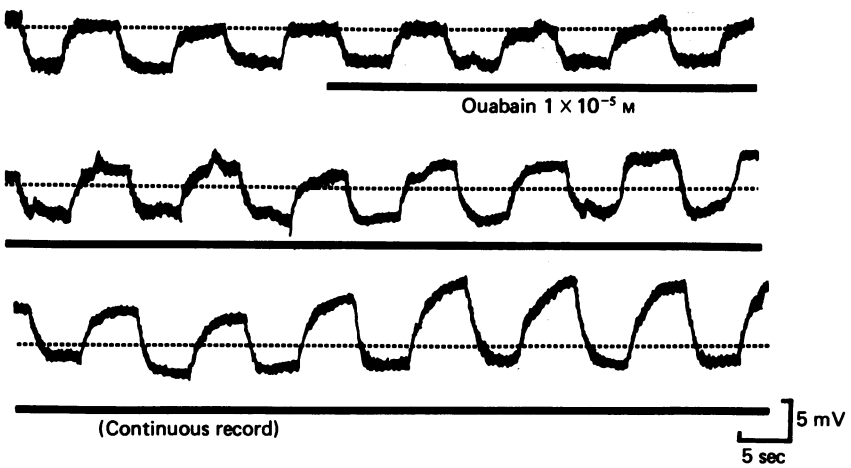

Fig. 8. Effect of ouabain (10^{-5} M) on the membrane potential and membrane resistance of arteriolar smooth muscle. Ouabain caused membrane depolarization and this was associated with an increase in membrane resistance. Amplitude of constant pulses 0·15 nA. The record is a continuous recording. The dashed line indicates the value of membrane potential before exposure to ouabain. After washing ouabain from the recording chamber, the membrane potential and the membrane resistance returned to their control values (not shown).

These latter observations suggested that the action of ouabain may be more complex than one of simply inhibiting the sodium pump. Consequently, the effect of ouabain on the membrane resistance was examined. The depolarization produced by ouabain was associated with an increase in arteriolar resistance (Fig. 8).

An alternative way in which ion transport systems may be slowed is to lower the temperature (Thomas, 1972). As had been described for other smooth muscles (Brading, Bulbring & Tomita, 1969), cooling the tissue from 37 to 27 °C, caused the membrane potential of the arteriole to decrease. This again was associated with an increased membrane resistance. Thus three procedures, reduction in external potassium concentration, ouabain and cooling, all of which reduced the activity of sodium pump transport systems, each resulted in membrane depolarization and each was associated with an increase in membrane resistance.

DISCUSSION

The changes in resting membrane potential resulting from increases and small decreases in the extracellular concentration of potassium ions indicate that the membrane potential of arterioles is predominantly set by this ion. However, unlike both skeletal muscle (Hodgkin & Horowicz, 1959) and intestinal muscle (Kuriyama, 1963; Casteels, 1981) our experiments where the extracellular concentration of chloride was changed imply that the permeability of arterioles to this ion is quite low. Neither the membrane potential nor the membrane resistance were altered by reducing the external concentration of chloride. Similarly, no change in membrane potential was detected when the concentration of sodium and chloride ions was reduced simultaneously (see also Bennett, 1972). Evidently, like other smooth muscles (Bennett, 1972; Brading, 1981; Casteels, 1981) and other excitable cells (Hille, 1977), arterioles are relatively impermeant to sodium ions at rest.

A large portion of the relationship between the potassium ion concentration and observed membrane potentials could be accounted for using the Goldman/Hodgkin & Katz equation with the relative values of $P_K:P_{Cl}:P_{Na}$ being 1:0·09:0·005 (see Fig. 6). The specific permeabilities of these three ions may be calculated (Hodgkin & Katz, 1949). Taking an average value of membrane resistance of 66 $k\Omega/cm^2$ (table 1, Hirst, & Neild, 1978) the specific permeabilities of potassium chloride and sodium are 22·5, 2·0 and $0{\cdot}1 \times 10^{-8}$ cm/sec respectively. Since the R_m values given by Hirst & Neild (1978) are lower limits for specific membrane resistance, the above values are upper limits for specific permeabilities. A more realistic value may perhaps be obtained by assuming a specific membrane capacitance (C_m) of 1 $\mu F/cm^2$ (Cole, 1968) and scaling the membrane resistances appropriately using the mean C_m value from Table 1 of Hirst & Neild (1978). This yields permeabilities of potassium, chloride and sodium of 3·3, 0·3 and $0{\cdot}02 \times 10^{-8}$ cm/sec. These values for potassium and sodium ions are in good agreement with those determined for other smooth muscles using efflux measurements (Brading, 1981; Casteels, 1981). The value for chloride permeability calculated above is clearly less than that determined for intestinal muscle (Brading, 1971; Casteels, 1981). It could be that the high value of membrane time constants of arterioles (Hirst & Neild, 1978, 1980) compared to that of intestinal muscle (Abe & Tomita, 1968) simply reflects the lack of an appreciable chloride conductance in arterioles.

The depolarization seen in low potassium concentrations can be accounted for, not by a reduction in the magnitude of an electrogenic sodium–potassium-pump current, but rather by a decreased permeability to potassium ions. A similar suggestion has been made for the depolarization observed when skeletal muscle fibres were exposed to low potassium solutions after chloride removal (Hodgkin & Horowicz, 1959). Support for the idea that under these conditions arterioles become less selective to potassium ions is provided by the experiments where the chloride concentration was changed in the presence of low potassium concentrations. The observed depolarization was 8–10 mV whereas that predicted by the Goldman/Hodgkin & Katz equation under these conditions is a depolarization of 7 mV. A similar change in the selectivity of resting membranes in the presence of low external potassium concentration is implicit in the observation that in axons this procedure leads to a fall in the rate of potassium efflux (Keynes & Lewis, 1951). It is however, difficult to understand how

a reduction in external potassium concentration could lead directly to such a marked decrease in resting potassium conductance since the movement of these ions through the resting channels is outwardly directed.

The observation that ouabain causes membrane depolarization has been made on many tissues other than smooth muscle (Thomas, 1972) and is often taken to indicate that current flow from an electrogenic sodium pump contributes directly to the observed value of resting membrane potential. This clearly is not the case for the resting membrane potential of arterioles. The depolarization produced by ouabain was associated with an increase in membrane resistance (see also Hope, Simpson & Walker, 1966). If this increased resistance also results from a suppression of potassium conductance, the depolarization can be entirely accounted for by the predicted changes in permeability ratios to potassium, chloride and sodium ions. Evidently, the direct contribution of current flow across the cell membrane resistance from an electrogenic sodium pump is likely to be small. A theoretical prediction for the magnitude of the steady-state polarization produced by the sodium/potassium pump can also be made. If a sodium/potassium exchange ratio of 3/2 is assumed, then for P_{Na} in the range of $0{\cdot}02$–$0{\cdot}11 \times 10^{-8}$ cm/sec, R_m 66 kΩ/cm², $[Na]_o$ 146 mM, $[Na]_i$ 20 mM, E_m −72 mV, the calculated hyperpolarization produced by the sodium/potassium pump is 0·2–0·9 mV.

It is not known how the three procedures used in these experiments, namely reduced extracellular potassium concentration, ouabain and cooling, each of which undoubtedly slow the activity of sodium/potassium pumps, cause a decrease in resting potassium conductance. It is possible that each procedure has a different mechanism, alternatively it could be that changed permeability is a consequence of sodium-pump inhibition. For example, an increased internal sodium concentration could directly depress potassium permeability or conceivably indirectly depress potassium permeability by displacing calcium ions from critical membrane sites.

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REFERENCES

ABE, Y. & TOMITA, T. (1968). Cable properties of smooth muscle *J. Physiol.* **214**, 173–190.

BENNETT, M. R. (1966). Model for the membrane of smooth muscle cells of the guinea-pig taenia coli muscle during transmission from inhibitory and excitatory nerves. *Nature, Lond.* **211**, 1149–1152.

BENNETT, M. R. (1972). *Autonomic Neuromuscular Transmission. Monographs of the Physiological Society* **30**, Cambridge: University Press.

BOLTON, T. B. (1973). Effects of electrogenic sodium pumping on the membrane potential of longitudinal smooth muscle from terminal ileum of guinea-pig. *J. Physiol.* **228**, 693–712.

BRADING, A. F. (1971). Analysis of the effluxes of sodium, potassium and chloride ions from smooth muscle in normal and hypertonic solutions. *J. Physiol.* **214**, 393–416.

BRADING, A. F. (1979). Maintenance of ionic composition smooth muscle. *Br. med. Bull.* **35**, 227–234.

BRADING, A. F. (1981). Ionic distribution and mechanisms of transmembrane ion movements in smooth muscle. In *Smooth Muscle, an Assessment of Current Knowledge*, ed. BÜLBRING, E., BRADING, A. F., JONES, A. E. & TOMITA, A. pp. 65–92. London: Edward Arnold.

BRADING, A., BÜLBRING, E. & TOMITA, T. (1969). The effect of temperature on the membrane conductance of the smooth muscle of the guinea-pig taenia coli. *J. Physiol.* **200**, 621–635.

CASTEELS, R. (1970). The relation between the membrane potential and the ion distribution in smooth muscle cells. In *Smooth Muscle*, ed. BÜLBRING, E., BRADING, A. F., JONES, A. W. & TOMITA, T., pp. 70–99. London: Edward Arnold.

CASTEELS, R. (1981). Membrane potential in smooth muscle cells. In *Smooth Muscle, an Assessment of Current Knowledge*, ed. BÜLBRING, E., BRADING, A. F., JONES, A. W. & TOMITA, T., pp. 105–126. London: Edward Arnold.

CASTEELS, R., DROOGMANS, G. & HENDRICKX, H. (1971*a*). Membrane potential of smooth muscle cells in K-free solution. *J. Physiol.* **217**, 281–295.

CASTEELS, R., DROOGMANS, G. & HENDRICKX, H. (1971*b*). Electrogenic sodium pump in smooth muscle cells of the guinea-pig taemia coli. *J. Physiol.* **217**, 297–313.

CASTEELS, R., KITAMURA, K., KURIYAMA, H. & SUZUKI, H. (1977*a*). The membrane properties of the smooth muscle cells of the rabbit main pulmonary artery. *J. Physiol.* **271**, 41–61.

CASTEELS, R., KITAMURA, K., KURIYAMA, H. & SUZUKI, H. (1977*b*). Excitation–contraction coupling in the smooth muscle cells of the rabbit main pulmonary artery. *J. Physiol.* **271**, 63–79.

COLE, K. S. (1968). *Membranes, Ions and Impulses.* Los Angeles: University of California Press.

GOLDMAN, D. E. (1943). Potential, impedance and rectification in membranes. *J. gen. Physiol.* **27**, 37–60.

HARDER, D. R. & SPERELAKIS, N. (1978). Membrane electrical properties of vascular smooth muscle from the guinea-pig superior mesenteric artery. *Pflügers Arch.* **378**, 111–119.

HENDRICKX, H. & CASTEELS, R. (1974). Electrogenic sodium pump in arterial smooth muscle cells. *Pflügers Arch.* **346**, 299–306.

HERMSMEYER, K. (1976). Electrogenesis of increased norepinephrine sensitivity of arterial vascular muscle in hypertension. *Circulation Res.* **38**, 362–367.

HILLE, B. (1977). Ionic basis of resting and action potentials. *The Nervous System*, sect. 1., vol. 1. *Cellular Biology of Neurones*, ed. KANDEL, E. R., pp. 99–136. Handbook of Physiology, American Physiological Society, Baltimore.

HIRST, G. D. S. (1977). Neuromuscular transmission in arterioles of guinea-pig submucosa. *J. Physiol.* **273**, 263–275.

HIRST, G. D. S. & NEILD, T. O. (1978). An analysis of excitatory junction potentials recorded from arterioles. *J. Physiol.* **280**, 87–104.

HIRST, G. D. S. & NEILD, T. O. (1980). Some properties of spontaneous excitatory junction potentials recorded from arterioles of guinea-pigs. *J. Physiol.* **313**, 43–60.

HIRST, G. D. S., NEILD, T. O. & SILVERBERG, G. D. (1982). Noradrenaline receptors on the rat basilar artery. *J. Physiol.* (in the Press).

HODGKIN, A. L. & HOROWICZ, P. (1959). The influence of potassium and chloride ions on the membrane potential of single muscle fibres. *J. Physiol.* **148**, 127–160.

HODGKIN, A. L. & KATZ, B. (1949). The effect of sodium ions on the electrical activity of the giant axon of the squid. *J. Physiol.* **108**, 37–77.

HOLMAN, M. E. (1958). Membrane potentials recorded with high-resistance micro-electrodes; and the effects of changes in ionic environment on the electrical and mechanical activity of the smooth muscle of the taenia coli of the guinea-pig. *J. Physiol.* **141**, 464–488.

HOPE, A. B., SIMPSON, A. & WALKER, N. A. (1966). The efflux of chloride from cells of *Nitella* and *Chara*. *Aust. J. biol. Sci.* **19**, 355–362.

KAJIWARA, M., KITAMURA, K. & KURIYAMA, H. (1981). Neuromuscular transmission and smooth muscle membrane properties in the guinea-pig ear artery. *J. Physiol.* **315**, 283–302.

KEYNES, R. D. & LEWIS, P. R. (1951). The resting exchange of radio-active potassium in crab nerve. *J. Physiol.* **113**, 73–98.

KURIYAMA, H. (1963). The influence of potassium, sodium and chloride on the membrane potential of the smooth muscle of taemia coli. *J. Physiol.* **166**, 15–28.

KURIYAMA, H. & SUZUKI, H. (1981). Adrenergic transmission in the guinea-pig mesenteric artery and their cholinergic modulations. *J. Physiol.* **317**, 383–396.

MARTIN, A. R. & PILAR, G. (1963). Dual mode of synaptic transmission in the avian ciliary ganglion. *J. Physiol.* **168**, 443–463.

OHASHI, H. (1970). An estimate of the proportion of the resting membrane conductance of the smooth muscle of guinea-pig taenia coli attributable to chloride. *J. Physiol.* **210**, 405–419.

THOMAS, R. C. (1972). Electrogenic sodium pump in nerve and muscle cells. *Physiol. Rev.* **52**, 563–594.

TOMITA, T. & YAMAMOTO, T. (1971). Effects of removing the external potassium on the smooth muscle of the guinea-pig taenia coli. *J. Physiol.* **212**, 851–868.