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RECTIFICATION IN THE SMOOTH MUSCLE CELL MEMBRANE OF RABBIT AORTA

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SUMMARY

1. The current–voltage relation of the smooth muscle cell membrane of rabbit aorta was determined by the partition method.

2. No anomalous rectification was observed in any of the following solutions: normal Krebs, Na free choline, Na sulphate, and high K–Na free sulphate.

3. Delayed rectification was seen on application of depolarizing current in both normal Krebs solution and Na free choline solution.

4. High concentration of K made the steady-state current–voltage relation almost linear in a voltage range of about 0 to −20 mV. This effect, and steady-state cathodal rectification which was seen in physiological solution, could be explained qualitatively by constant field theory without involving channels capable of anomalous rectification.

5. A slow decrease in K conductance, during application of large and long-lasting hyperpolarizing currents, which occurs in skeletal muscle and is attributed to the tubule system, was never observed in the arteries either in Krebs, Na-free choline, or Na sulphate solution.

INTRODUCTION

Current–voltage studies of skeletal muscle show rapid anomalous rectification, particularly in solutions of high K concentration, and a slow decrease in conductance during prolonged hyperpolarization (Adrian & Freygang, 1962). These properties were attributed to the T-tubule system, unlike delayed rectification which was attributed to the surface membrane. There is no T-tubule system in mammalian smooth muscle. Sarcoplasmic reticulum is found in arterial smooth muscle (Keatinge, 1972) as in other smooth muscle (Gabella, 1971; Somlyo, Devine, Somlyo & North, 1971), but does not appear to open on the surface membrane, and therefore may

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not influence the electrical properties of the cell membrane as measured by micro-electrode. No systematic analysis of the current–voltage relation of smooth muscle has yet been done, perhaps because depolarizing procedures induce electrical activity in most smooth muscle so that steady-state relations can not be obtained. The smooth muscle of large elastic arteries of mammals does not readily produce action potentials during prolonged steady depolarization (Mekata, 1971, 1974). Stable and transient current–voltage relations of the smooth muscle cell membrane of the rabbit aorta have therefore now been determined by extracellular current application and intracellular recording by micro-electrode.

METHODS

Sixty-seven strips of rabbit aorta were studied. They were always left in normal Krebs solution at 36–37° C for 2–3 hr before investigation. Depolarizing and hyperpolarizing current was applied by the partitioned chamber method (Kuriyama & Mekata, 1971). The structure of the organ bath and the method of recording of electrotonic potential were the same as those described by Kuriyama & Mekata (1971) and Mekata (1974). The composition of the bathing solutions is given in Table 1. Constant electrotonic current was generated by applying voltages of up to 45 V to the extracellular polarizing plates, with a series resistance of 10 kΩ. The input resistance of the apparatus was only 300 Ω and current was therefore effectively proportional to applied voltage. Membrane potentials were recorded by inserting a micro-electrode from the intimal surface of the aortic wall. Points of recording were within 0·45 mm from the stimulating partition, except when otherwise stated in the text. When applied current spreads as a result of cable properties to the whole tissue, the observed current–voltage curve is linearized as compared with the true curve (Noble & Stein, 1966). Therefore, in order to produce the same degree of linearizing effect when determining the current–voltage relation in different $[K]_o$, recording points roughly proportional to the space constant were selected.

RESULTS

Current–voltage relation in normal Krebs solution

When the current–voltage relation was determined over a wide voltage range, strong cathodal rectification was observed. Fig. 1*A* shows traces of electrotonic potential evoked by application of external current at different strengths in saline with 5·9 mM-$[K]_o$. When strong depolarizing current is applied, the electrotonic potentials rise to a maximum, which occurs earlier with the larger depolarizations, and then decline to a steady value. The peak potential change, indicating the clear onset of an increase in conductance, occurs at about 400 msec with the smallest depolarizing current and at about 100 msec with the largest. Fig. 1*B* shows the steady-state relation between the polarizing current and the membrane potential. Marked cathodal rectification can be seen. Thus a depolarization of 15 mV requires about 3 times larger current intensity than that producing hyperpolarization of 15 mV.

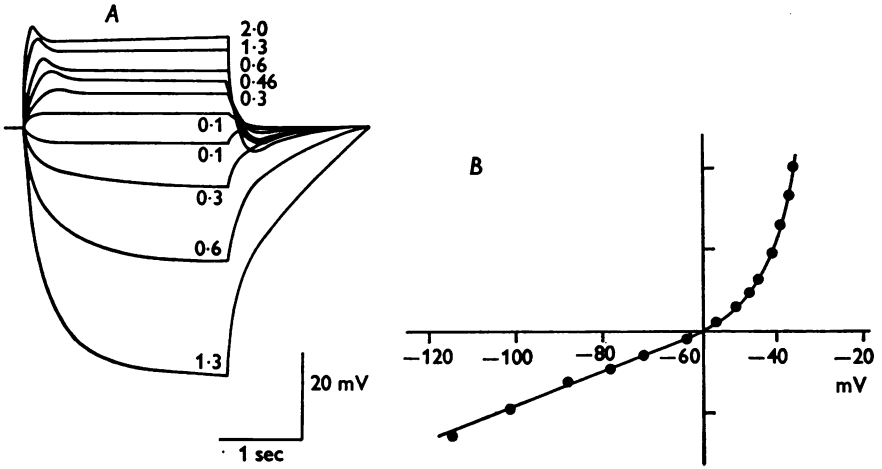

Fig. 1. *A*, superimposed traces of electrotonic potentials recorded intracellularly from aorta smooth muscle in normal Krebs solutions, which was produced by 2·5 sec square-pulses applied externally. Figures on traces indicate total polarizing current in arbitrary unit. *B*, relation between polarizing current (in arbitrary unit, ordinate) and steady-state potential (abscissa) obtained from records shown in Fig. 1*A*, upward: depolarization. Record at 0·35 mm from the stimulating partition.

Effects of changes in $[K]_0$ *in solutions containing Na and chloride ions*

Anomalous rectification might have been masked in the above experiments by cathodal rectification due to $[K]_i$ being higher than $[K]_0$ (see Discussion) and by the delayed rectification. Both of the last two can be minimized by increasing $[K]_0$. The stable current–voltage relation obtained with successive increases in $[K]_0$ is shown in Fig. 2. The current–voltage relation became nearly linear in a voltage range of about 0 to −20 mV when potassium concentration was 96 mM, indicating that cathodal rectification was reduced, but no reversal of the alinearity, which would indicate anomalous rectification, was ever observed. The time course of the electrotonic potentials in 96 mM-$[K]_0$ never showed the peak and subsequent decline seen in 5·9 mM-$[K]_0$.

Since current–voltage relations obtained in most solutions containing different external K concentrations were not linear even with hyperpolarizing current, it was impossible to calculate the space constant directly from spatial decay of amplitude of the electrotonic potential produced by applying a given current intensity. The space constants were therefore calculated from the method described by Mekata (1974). Current–voltage curves were determined at different distances from the partition, and the gradient of the current–voltage curve at zero current

was measured for each recording point. The distance at which this gradient decayed to 1/e of its value represents the space constant of the tissue. The space constants and membrane potentials are shown in Table 2. The space constants obtained in different external K concentration decrease as $[K]_0$ increases. Assuming that intracellular longitudinal resistance is not changed by $[K]_0$ as appears to be true in other smooth muscle (Tomita, 1969), membrane conductance must have increased with increase in $[K]_0$.

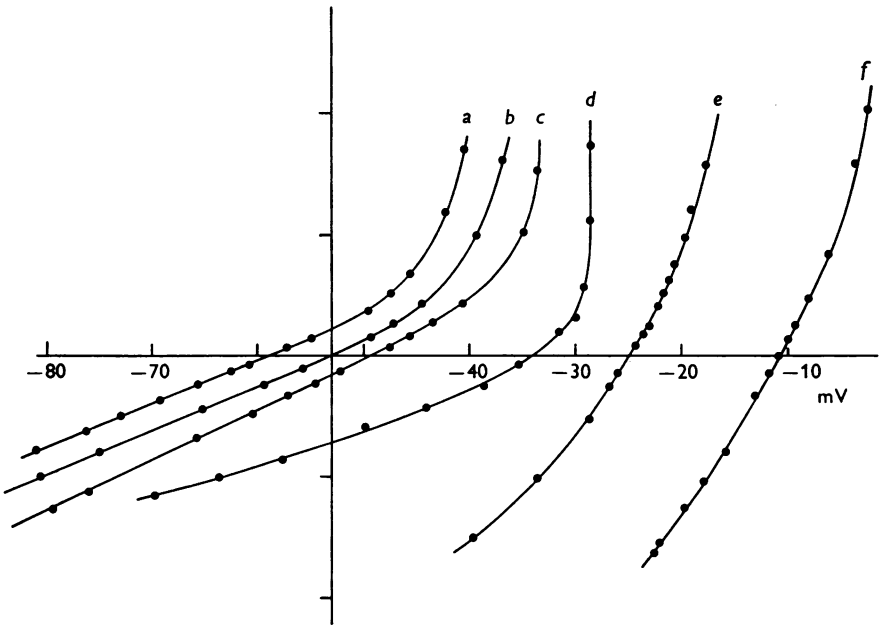

Fig. 2. Current–voltage relations recorded from the aorta in solution containing various external potassium concentrations. Abscissa: electrotonic potentials produced by 2·5 sec square-pulses. Ordinate, current intensity in arbitrary unit. Records were done at 0·36 mm for 3·0 mM-$[K]_0$ (*a*), 0·34 mm for 5·9 mM-$[K]_0$ (*b*), 0·30 mm for 12 mM-$[K]_0$ (*c*), 0·24 mm for 24 mM-$[K]_0$ (*d*), 0·12 mm for 48 mM-$[K]_0$ (*e*), and 0·09 mm for 96 mM-$[K]_0$ (*f*) from the stimulating partition.

The values for space constants obtained in this way provide a more accurate measure of the steady-state conductance change in different levels of $[K]_0$ than do the data of Fig. 2.

The effects of substitutes of Na and Cl on the electrical properties on the membrane

In choline chloride based solutions containing 5·9, 24 and 96 mM-$[K]_0$ the stable current–voltage relation were virtually identical with those measured in corresponding Na-based solutions (Fig. 3). The time course

TABLE 1. Composition of solutions (mM)

	Na^+	K^+	Ca^{2+}	Cl^-	HCO_3^-	$H_2PO_4^-$	SO_4^{2-}	Choline	Sucrose
Normal Krebs solution	137·4	5·9	2·5	134	15·5	1·2	0	0	0
3 mM-$[K]_o$ solution	140·3	3·0	2·5	134	15·5	1·2	0	0	0
12 mM-$[K]_o$ solution	131·3	12·0	2·5	134	15.5	1·2	0	0	0
24 mM-$[K]_o$ solution	119·3	24·0	2·5	134	15·5	1·2	0	0	0
48 mM-$[K]_o$ solution	95·3	48·0	2·5	134	15·5	1·2	0	0	0
96 mM-$[K]_o$ solution	47·3	96·0	2·5	134	15·5	1·2	0	0	0
5·9 mM-$[K]_o$–Na free solution	0	5·9	2·5	144·8	5·5	0·4	0	137·4	0
24 mM-$[K]_o$–Na free solution	0	24·0	2·5	134	15·5	1·2	0	119·3	0
96 mM-$[K]_o$–Na free solution	0	96·0	2·5	134	15·5	1·2	0	47·3	0
Na sulphate solution	180·3	5·9	3·8	0	15·5	1·2	89·8	0	0
High K–Na free sulphate solution	0	96·0	3·8	0	15·5	1·2	44·7	0	135·2

All solutions contain 1·2 mM-Mg^{2+} and 11·5 mM glucose.

TABLE 2. Effects of changes in $[K]_o$ on space constant and membrane potential in Na and Cl based solution

$[K]_o$ (mM)	Space constant (mm)	Membrane potential (−mV)
3	2·26 ± S.D. 0·41 (6)	56·4 ± S.D. 3·7 (30)
5·9	2·10 ± S.D. 0·35 (15)	55·2 ± S.D. 7·6 (92)
12	1·88 ± S.D. 0·48 (7)	46·1 ± S.D. 5·1 (30)
24	1·40 ± S.D. 0·42 (7)	36·1 ± S.D. 3·6 (40)
48	0·73 ± S.D. 0·26 (7)	22·7 ± S.D. 3·0 (40)
96	0·51 ± S.D. 0·08 (7)	9·2 ± S.D. 2·3 (44)

Brackets show number of observations.

of these was also similar to those in Na-based solutions. Fig. 4 shows the time course of electrotonic potentials in high K–Na free sulphate solution; it closely resembles those in 96 mM-$[K]_o$ chloride solution.

Adrian & Freygang (1962) observed a slow fall in K conductance in skeletal muscle when strong prolonged hyperpolarizing current was applied and attributed it to depletion of K ion from the tubule system. This was most clearly seen in Na sulphate based solution. Fig. 5 shows that when hyperpolarizing current was applied to aortic muscle in Na sulphate solution (5·9 mM-$[K]_o$), membrane potential increased smoothly to a steady-state value. The time course of this was rather slow. The plotted lines show the predicted time course from cable theory, assuming no time-dependent change in conductance (Hodgkin & Rushton, 1946).

$$V_x = V_{\substack{x=0 \\ t=\infty}} \cdot \tfrac{1}{2}\left\{ e^{-X}\left[1-\mathrm{erf}\left(\frac{X}{2\sqrt{T}}-\sqrt{T}\right)\right] - e^{X}\left[1-\mathrm{erf}\left(\frac{X}{2\sqrt{T}}+\sqrt{T}\right)\right]\right\}, \quad (1)$$

where V_x = amplitude of the electrotonic potential at distance (x) from the

stimulating partition at time (t) after initiation of current application, $V_{\substack{t=\infty \\ x=0}}$ = amplitude of the steady-state electrotonic potential at the stimulating partition. $T = t/\tau$ and $X = x/\lambda$; τ = time constant taken as 451 msec and λ = space constant taken as 2·48 mm. It can be seen that the actual time course followed the predicted curve closely, and so gives no indication of any time-dependent fall in K conductance of the kind seen in skeletal muscle.

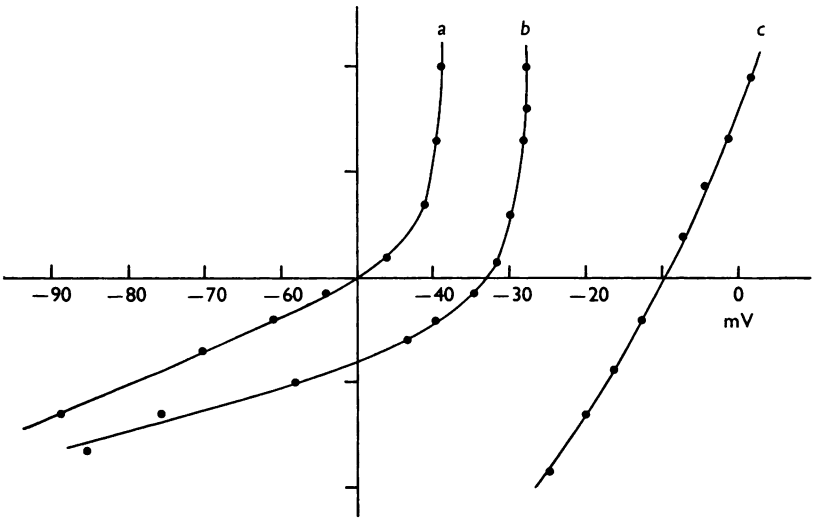

Fig. 3. Current–voltage relation obtained in Na-free choline solutions containing 5·9 (*a*), 24 (*b*) and 96 mM-$[K]_o$ (*c*). Recording at 0·31 mm for 5·9 mM-$[K]_o$, 0·22 mm for 24 mM-$[K]_o$ and 0·08 mm for 96 mM-$[K]_o$.

DISCUSSION

The fall in electrotonic potential starting 100–400 msec after application of current suggests that delayed rectification occurs in aortic muscle as in giant axon (Hodgkin, Huxley & Katz, 1952), though much more slowly.

Adrian & Freygang (1962) found anomalous as well as delayed rectification in twitch fibre of skeletal muscle. They also recorded a slow fall in membrane conductance when long-lasting hyperpolarizing current was applied. They suggested that anomalous rectification and the slow fall were due to the tubule system rather than the surface membrane, the slow fall representing decrease in K conductance in lumen of T-tubule system evoked by K depletion from the tubules. The transverse tubules of striated muscle can be disrupted from the cell membrane by glycerol (Howell, 1969; Gage & Eisenberg, 1969). After this, steady-state cathodal rectification is still seen and delayed rectification usually increased (Papir,

1973), but anomalous rectification and the slow fall in conductance on hyperpolarizing are greatly reduced or abolished. Against twitch muscle having a T-tubule system, slow fibre of skeletal muscle which has no T-tubule system shows no anomalous rectification in either normal or K sulphate solutions, in which, however, delayed and steady-state cathodal rectification were seen (Stefani & Steinbach, 1969). In Purkinje fibres of the heart, anomalous rectification is seen (Hall, Hutter & Nobel, 1963) and

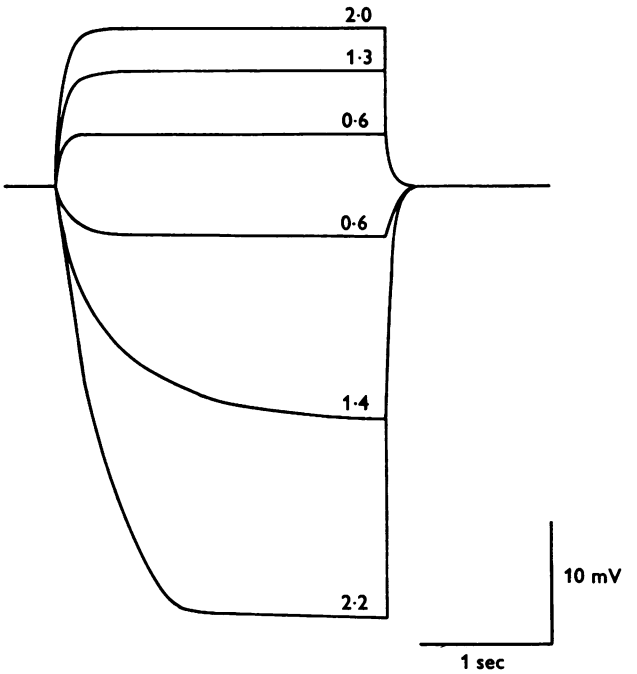

Fig. 4. Superimposed traces of the electrotonic potentials recorded in high K-Na free sulphate solution. Figures on traces indicate total applied current in arbitrary unit. Electrotonic potentials were recorded at 0·10 mm from the stimulating partition. Resting potential is 14 mV.

it is strongly suggested that a transient hyperpolarization should reduce slow potassium conductance (McAllister & Nobel, 1967; Noble & Tsien, 1968). Purkinje fibres do not have a T-tubule system but they have an extensively folded surface membrane, the area of which is 11 times that calculated by assuming that the fibre has smooth surface (Mobley & Page, 1972; Page, Power, Fozzard & Meddoff, 1969). Anomalous rectification might be due to specialized parts of this folded membrane.

No anomalous rectification was seen in the present experiments on aorta. When aorta smooth muscle was soaked in high K, steady-state

cathodal rectification was greatly weakened, as indicated by linearization of the current–voltage relationship. The theoretical conductance (G_K) is given by Goldman (1943)

$$G_K = -\frac{F^2E}{RT}.P_K.\frac{[K]_o - [K]_i \exp(FE/RT)}{\exp(EF/RT) - 1} \Big/ (E - E_K), \qquad (2)$$

where E_K = K equilibrium potential.

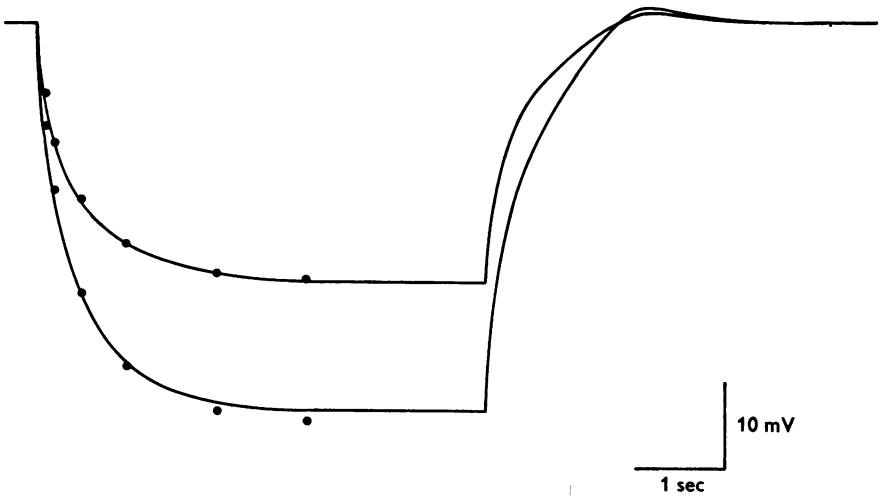

Fig. 5. Tracing of the electrotonic potentials recorded in Na sulphate solution at distances of 1·12 mm from the stimulating partition, which were produced by two different intensity of applied inward currents. The points show values calculated from the cable equation, assuming no time-dependent conductance change. Resting potential is 52 mV.

Even with constant P_K, eqn. (2) results in an alinear stable current–voltage relationship when $[K]_i$ is high and $[K]_o$ is low, as with tissue in normal Krebs solution, and it results in a more linear relationship when $[K]_o$ is increased. The linearizing effect of increased $[K]_o$ therefore does not provide any evidence of a transitional step towards anomalous rectification. Nor was any evidence obtained of a slow fall in K conductance during prolonged hyperpolarization. Both facts may be due to the absence of a communicating tubule system and of any analogous system in the smooth muscle cells.

It should be noted that because of spread of current due to cable properties of the tissue the membrane current per unit area will be proportional to total polarizing current multiplied by dI/dV, where I is the polarizing current and V is the membrane potential (Cole & Curtis, 1941). The effect of this will be to make all curved current–voltage re-

lationships, described in this paper for total polarizing current, closer to linearity than they would be if current per unit area was used. It does not affect the conclusions drawn. Stronger cathodal rectification than predicted by eqn. (2) for constant P_K with the observed space constant was recorded very close to the stimulating partition. Therefore the present experiments suggest strongly that cathodal rectification of aorta smooth muscle is due to increase in P_K with depolarization.

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