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THE RELATIVE CONTRIBUTION OF K AND Cl TO THE TOTAL INCREASE OF MEMBRANE CONDUCTANCE PRODUCED BY ADRENALINE ON THE SMOOTH MUSCLE OF GUINEA-PIG TAENIA COLI

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SUMMARY

1. The relative contribution of K and Cl to the total increase of membrane conductance produced by adrenaline on the smooth muscle of guinea-pig taenia coli has been investigated. Constant current pulses were applied with a pair of large external electrodes, the voltage change was recorded with an intracellular micro-electrode, and the change in the size of the electrotonic potential by adrenaline was measured.

2. Adrenaline hyperpolarized the membrane by about 8 mV with a range from 5 to 15 mV and it reduced both the size and the time course of the electrotonic potential. On the average the size was reduced to $65 \pm 1{\cdot}8\,\%$ ($n = 75$) of the normal size, from which a decrease of the membrane resistance to $45 \pm 2{\cdot}2\,\%$ was calculated, or 48 % allowing for the spatial decay.

3. The magnitude of the reduction of the electrotonic potential caused by adrenaline was decreased as the concentration of K or Cl ions in the medium was reduced by substituting NaCl or sucrose for KCl or an impermeant anion, benzene sulphonate, for Cl ions. The adrenaline effect remained unaltered when the NaCl was replaced with Tris-Cl.

4. It is therefore concluded that adrenaline opens the ion pathways mainly for K and Cl.

5. On the basis of the changes in potential and in conductance caused by adrenaline, the equilibrium potential for its action was calculated as -75 mV.

6. The ratio of the Cl component to the K component of the additional conductance increase was obtained as 0·36 using the calculated equilibrium potential and the Nernst potentials for K (-91 mV) and Cl (-31 mV).

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INTRODUCTION

There is at present little doubt that adrenaline and noradrenaline increase membrane permeability of intestinal smooth muscle cells to inorganic ions. This conclusion is based on evidence obtained both from electrophysiological studies (Kuriyama, 1963; Bülbring & Kuriyama, 1963; Bülbring & Tomita, 1969) and studies of transmembrane ion movements (Bülbring, Goodford & Setekleiv, 1966; Jenkinson & Morton, 1965, 1967). However, the results obtained by the two different approaches are not always in agreement. Measurements of changes in the membrane resistance of the guinea-pig taenia coli, using the double sucrose-gap method, indicated that adrenaline increased the Cl conductance as well as the K conductance of the membrane (Bülbring & Tomita, 1969). On the other hand, measurements of ion fluxes, though showing that adrenaline and noradrenaline increased the transmembrane movement of K (Bülbring *et al.* 1966; Jenkinson & Morton, 1965, 1967), did not show an increase in Cl movement (Jenkinson & Morton, 1967). It was therefore desirable to reinvestigate the problem.

In the present experiments, further electrophysiological evidence was obtained, with the micro-electrode technique, that both an increase in K conductance and an increase in Cl conductance are involved in the action of adrenaline. Since the Nernst potential for Cl ions is less negative than the resting potential (Casteels, 1964, 1965, 1969; Casteels & Kuriyama, 1966), an increase in Cl conductance would displace the membrane potential in a more positive direction. In fact, however, adrenaline normally hyperpolarized the membrane. If a possible action of adrenaline on an electrogenic Na pump (Burnstock, 1958) is neglected the question that remains to be answered is, how much does the change in Cl conductance contribute to the total effect on the membrane conductance.

METHODS

The isolated smooth muscle of the taenia coli of the guinea-pig was used throughout. The bathing solution was a modified Krebs solution of the following composition (mM): Na 136·9, K 5·9, Ca 2·5, Mg 1·2, Cl 133·5, HCO_3 15·5, H_2PO_4 1·2 and glucose 11·5, equilibrated with a gas mixture of 3 % CO_2 and 97 % O_2. To this solution sucrose (292 mM) was added to make it hypertonic (about twice the normal tonicity). The hypertonic solution suppresses spontaneous activity which interferes with the shape of electrotonic potentials. Changes of the ionic composition are described in detail in the appropriate sections. The experiments were carried out at 35° C.

Changes in the membrane resistance were measured with the method used by Tomita (1966) and Abe & Tomita (1968). A pair of large external electrodes was used for passing current (one of which also served to partition the stimulating from the recording compartment) and an intracellular electrode inserted at a given distance

(0·5 mm) from the stimulating partition for voltage recording. The equation from which changes in membrane resistance or membrane conductance were calculated from changes in the size of steady-state electrotonic potentials was the same as that used in the previous paper (Ohashi, 1970).

Adrenaline was applied by adding it to the solution flowing continuously through the bath to obtain a concentration of 10^{-6} g/ml. This high concentration was chosen to obtain maximal effects.

RESULTS

Effect of adrenaline in the normal Krebs solution

Exposure of the tissue to the solution containing adrenaline caused a hyperpolarization of the membrane by 5–15 mV (mean, 8 mV) and reduced the electrotonic potentials both in amplitude and in time course as shown in Figs. 1 and 2. The strength of the depolarizing current required to evoke a spike was increased and occasionally a spike failed to fire with its full size even if the stimulating current was increased. As soon as hyperpolarization was observed, electrotonic potentials were recorded and those obtained at the peak of the effect were chosen for measurement and calculation. The adrenaline effect was completely reversible and reproducible if at least a 10 min interval between applications was allowed to prevent development of tachyphylaxis. The graph in Fig. 2 shows that the relationship between the size of electrotonic potentials and the strength of the potential field in the absence and presence of adrenaline is linear. In every experiment, the slope of the line obtained in the presence of adrenaline was less steep than that in the normal solution. This indicates that adrenaline decreased the membrane resistance. The amount of reduction in the size of the electrotonic potential varied from preparation to preparation. On the average the amplitude was reduced to $65\% \pm 1{\cdot}8\%$ ($n = 75$) of the size in the normal solution, from which it was calculated that adrenaline decreased the membrane resistance to $45 \pm 2{\cdot}2\%$ ($n = 75$). However, the spatial decay of the electrotonic potential at the usual distance (0·5 mm) from the stimulating electrode may not be negligible since, when the membrane resistance is reduced the space constant would become small. This would lead to a slight overestimation of the effect of adrenaline on the membrane resistance. According to Tomita (1966), the value for the space constant in normal solution is 1·6 mm. When the membrane resistance is reduced to 45% of normal, the space constant is calculated to decrease to about 1·1 mm, assuming that the internal resistance remains constant. In this case, the error arising from the spatial decay is about 12%. This means that the value of the membrane resistance is reduced not to 45% but only to 51%. Thus it can be argued that the real value would be somewhere between these two values. The mean, 48%, will be taken for the following calculations.

The effect of adrenaline in solutions with modified ionic compositions

K deficiency. In order to study the effect of K deficiency KCl was replaced either with NaCl or with sucrose. At least 10 min were allowed to ensure that the tissue had reached a steady state in the modified solution. The effect of adrenaline in normal solution (5·9 mM-K) was compared with

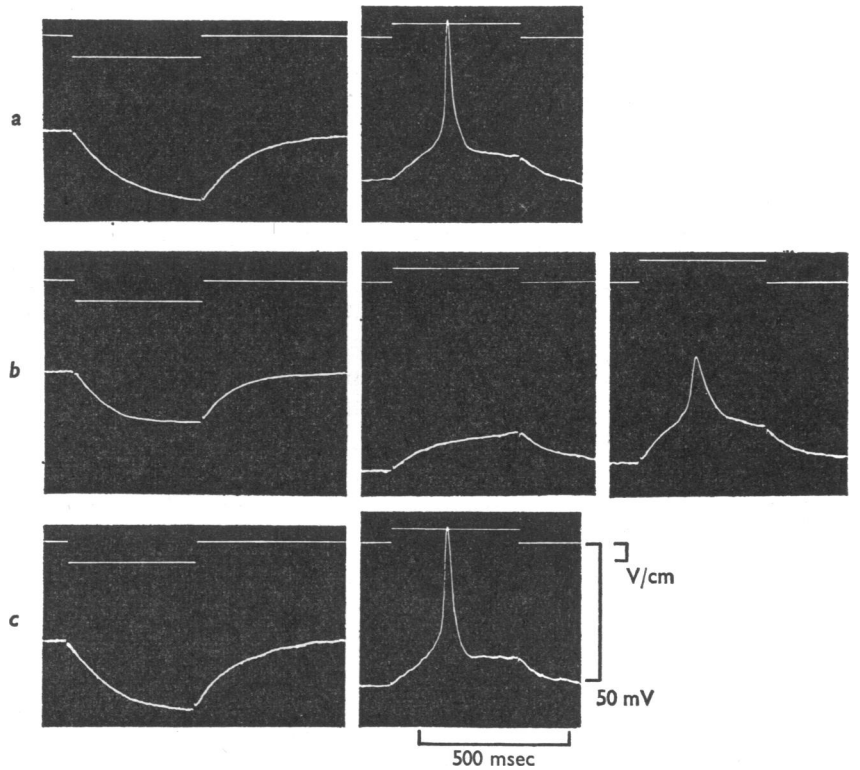

Fig. 1. The effect of adrenaline on the electrotonic potential and on the evoked action potential. Upper trace: potential field (V/cm). Lower trace: intracellular record (mV). *a*, Response to anodal and cathodal current pulses of 400 msec duration, recorded in normal solution; *b*, After 4 min exposure to a solution containing adrenaline (10^{-6} g/ml.); *c*, after 15 min readmission of normal solution. Note, in the presence of adrenaline, a decrease in the size and the time course of the electrotonic potential. A graded response is evoked by applying a cathodal current pulse of higher intensity.

that in 3 mM-K, 1·5 mM-K and 0·7 mM-K. Though K deficiency itself hyperpolarized the membrane, adrenaline caused still further hyperpolarization, but its magnitude was not significantly greater than in normal solution. A greater hyperpolarization would be expected if adrenaline increased solely the membrane permeability to K ions, since deviation of

E_m from E_K becomes larger as the $[K]_o$ is reduced from the normal value (Kuriyama, 1963). Therefore this result may be taken as evidence that some depolarizing mechanism is also involved.

In K deficient solution the size and the time course of the electrotonic potential were increased. Adrenaline decreased both the size and the time course, as in normal solution, but to a lesser extent when $[K]_o$ was reduced below 3 mM. The procedure used to express the magnitude of the decrease in the size of electrotonic potentials caused by adrenaline was as

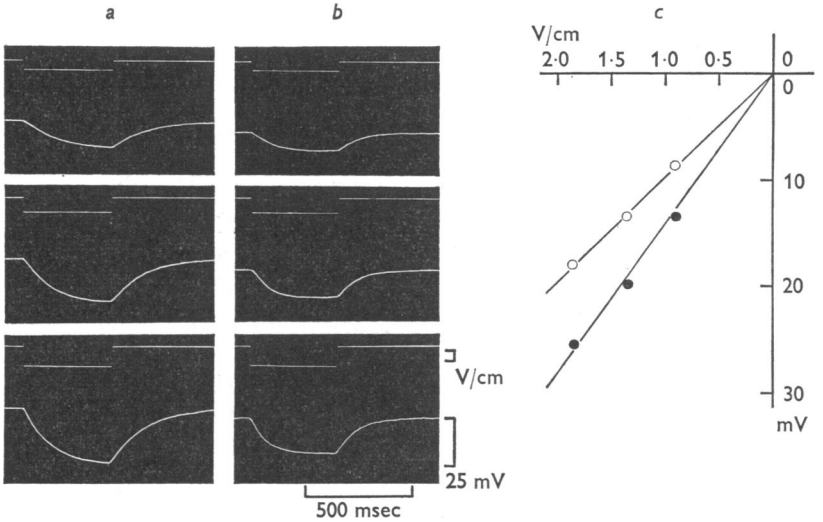

Fig. 2. The effect of adrenaline on the electrotonic potentials produced by anodal current pulses of 400 msec duration at three different intensities. Records as in Fig. 1. *a*, In normal solution; *b*, after 3 min exposure to a solution containing adrenaline (10^{-6} g/ml.); *c*, the graph shows the relationship between the strength of the potential field (abscissa) and the size of the electrotonic potential (ordinate) in the presence (open circles) and absence (filled circles) of adrenaline.

follows. The amplitude of electrotonic potential during exposure to the adrenaline in K-deficient solution (V^*) was divided by the corresponding value during the immediately preceding period in normal solution (V). The square of this ratio $(V^*/V)^2$ is an estimate of the ratio in the membrane resistance (Ohashi, 1970). The results are summarized in Table 1.

Cl deficiency. To obtain information of the relation between adrenaline induced changes in membrane resistance and the Cl concentration in the medium, the effect of reducing the Cl ions was investigated. Benzene sulphonate was selected for Cl substitution, because the membrane is effectively impermeable to this anion (Brading & Tomita, 1968). The method for calculating the effect of Cl deficiency on the action of adrenaline was the same as that described for K deficiency. When the tissue was

TABLE 1. Effects of adrenaline on membrane potential and electrotonic potential in K deficient solutions

$[K]_o$ (mM)	5·9	3·0	5·9	1·5	5·9	0·7
E_m (mV) before	55·5 ± 0·5	55·8 ± 0·6	58·9 ± 0·6	59·3 ± 1·1	57·7 ± 0·7	63·0 ± 1·5
and	↓	↓	↓	↓	↓	↓
during adrenaline	68·0 ± 1·3	67·5 ± 0·9	67·5 ± 0·9	65·4 ± 1·1	64·7 ± 0·4	67·7 ± 0·7
	(12)	(12)	(8)	(8)	(6)	(6)
V^*/V	0·70 ± 0·02	0·69 ± 0·03	0·75 ± 0·02	0·79 ± 0·01	0·74 ± 0·02	0·82 ± 0·02
	(12)	(12)	(8)	(8)	(6)	(6)
$(V^*/V)^2$	0·50 ± 0·03	0·48 ± 0·04	0·57 ± 0·02	0·63 ± 0·01	0·55 ± 0·03	0·68 ± 0·03

TABLE 2. Effects of adrenaline on membrane potential and electrotonic potential in Cl deficient solutions

$[Cl]_o$ (mM)	134	67	134	34	134	17	134	7
E_m (mV) before	60·8 ± 1·2	62·0 ± 0·8	61·4 ± 1·0	63·4 ± 0·2	61·0 ± 0·9	67·0 ± 0·4	60·3 ± 0·5	63·8 ± 1·1
and	↓	↓	↓	↓	↓	↓	↓	↓
during adrenaline	67·5 ± 1·2	66·5 ± 1·3	71·4 ± 1·0	62·8 ± 0·5	70·5 ± 1·7	70·0 ± 3·1	69·8 ± 0·6	66·5 ± 2·1
	(12)	(12)	(8)	(8)	(8)	(8)	(6)	(6)
V^*/V	0·77 ± 0·02	0·78 ± 0·02	0·73 ± 0·03	0·76 ± 0·02	0·65 ± 0·04	0·75 ± 0·03	0·55 ± 0·02	0·69 ± 0·01
	(12)	(12)	(8)	(8)	(8)	(8)	(6)	(6)
$(V^*/V)^2$	0·60 ± 0·03	0·61 ± 0·03	0·53 ± 0·04	0·58 ± 0·04	0·43 ± 0·05	0·57 ± 0·05	0·32 ± 0·02	0·48 ± 0·01

exposed to the Cl-deficient solutions, the membrane was transiently depolarized and, after about 10 min, the membrane potential returned to its initial level. The size and time course of electrotonic potential increased progressively with time. An almost steady level was usually reached after 20 min. The effect of adrenaline was therefore tested after 20 min or longer exposure to the solution. The results are summarized in Table 2. In the presence of 67 mM-Cl solutions, the membrane was always hyperpolarized by adrenaline as in normal solution, but when the Cl was reduced below 67 mM no change or either a slight depolarization or a slight hyperpolarization was produced by adrenaline. The effect on the membrane resistance was invariably diminished as $[Cl]_o$ was reduced.

Na deficiency. For the experiments described here the NaCl in the Krebs solution was replaced with Tris-(hydroxymethylaminomethane) Cl. Exposure to the Na deficient solution in which only 16 mM-Na was present caused a hyperpolarization of the membrane by 3–10 mV, but no change of the electrotonic potentials or a slight reduction. Adrenaline produced about the same effect on the membrane resistance in the Na deficient solution as in normal solution, as observed by Bülbring & Tomita (1969).

Relationship between the adrenaline effect and $[K]_o$ or $[Cl]_o$

Since the action of adrenaline on the membrane resistance is decreased both when one reduced $[K]_o$ or $[Cl]_o$, adrenaline must increase the membrane conductance for both K and Cl ions. One way of showing the dependence of the adrenaline effect on $[K]_o$ or $[Cl]_o$ is to compare the reduction of membrane resistance in modified solutions with that in normal solution (Bülbring & Tomita, 1969). For example, for the present results, in K-deficient solutions, the mean percentage changes were 103 % at 3·0 mM-K, 88 % at 1·5 mM-K and 74 % at 0·7 mM-K. In Cl-deficient solutions, they were 98 % at 67 mM-Cl, 90 % at 34 mM-Cl, 76 % at 17 mM-Cl and 74 % at 7 mM-Cl. However, this approach does not take into account any effects of the changed ionic environment on the normal membrane resistance, and a more accurate assessment of the dependence of the adrenaline effect on the external K or Cl concentration is described below. The amount by which adrenaline reduced the membrane resistance can be expressed by $1-(V^*/V)^2$. Since $(V'/V)^2$ is the ratio of the membrane resistance in modified solution to that in normal solution, i.e. $= r'_m/r_m$, the ratio of membrane conductance is g_m/g'_m (Ohashi, 1970). Therefore, the relative adrenaline effect

$$A = \frac{1-\left(\frac{V^*}{V}\right)^2 \text{ in modified solution}}{1-\left(\frac{V^*}{V}\right)^2 \text{ in normal solution}} = \frac{1-\frac{g'_m}{g'_m+\Delta g'_m}}{1-\frac{g_m}{g_m+\Delta g_m}} \qquad (1)$$

where r_m = membrane resistance, g_m = membrane conductance, Δg_m = additional membrane conductance produced by adrenaline, and r'_m, g'_m and $\Delta g'_m$ are the same quantities in K- or Cl-deficient solution. The eqn. (1) can be rearranged as follows:

$$A = \frac{1 + \frac{\Delta g_m}{g_m}}{\frac{g'_m}{g_m} \cdot \frac{\Delta g_m}{\Delta g'_m} + \frac{\Delta g_m}{g_m}}. \quad (2)$$

This equation predicts that the magnitude of A varies not only with $\Delta g'_m/\Delta g_m$ (the ratio of the additional membrane conductance produced by adrenaline in the two solutions), but also with g'_m/g_m (the ratio of the membrane conductance in the two solutions). The ratios for different K and Cl concentrations g'_m/g_m have been calculated in a previous paper (Ohashi, 1970) and are used in Table 3. From the present result

$$(g_m/g_m + \Delta g_m = 0{\cdot}48)$$

the ratio, $\Delta g_m/g_m$, is 1·08. Now, $\Delta g'_m/\Delta g_m$ at different K or Cl concentrations can be obtained by inserting the experimentally obtained values of A (Table 3) into eqn. (2). The calculated values for K deficiency were 0·74 at 3·0 mM-K 0·47 at 1·5 mM-K and 0·34 at 0·7 mM-K. For Cl deficiency, they were 0·65 at 67 mM-Cl, 0·43 at 34 mM-Cl, 0·31 at 17 mM-Cl and 0·28 at 7 mM-Cl. The graph of $\Delta g'_m/\Delta g_m$ against the K or Cl concentrations (Fig. 3) shows that membrane conductance produced by adrenaline is decreased in proportion to the reduction of $[K]_o$ or $[Cl]_o$.

TABLE 3. Relationship between the external K and Cl concentrations and the effect of adrenaline on conductance

External K concentration (mM)	3·0	1·5	0·7	
g'_m/g_m (Ohashi, 1970)	0·69	0·61	0·58	
Relative adrenaline effect (A)	103	88	75	
$\Delta g'_m/\Delta g_m$	0·74	0·47	0·34	
External Cl concentration (mM)	67	34	17	7
g'_m/g_m (Ohashi, 1970)	0·68	0·53	0·51	0·48
Relative adrenaline effect (A)	98	90	76	74
$\Delta g'_m/\Delta g_m$	0·65	0·43	0·31	0·28

Effect of K deficiency on the action of adrenaline in a Cl-deficient solution

If the K component and Cl component of the conductance change produced by adrenaline are independent of one another, the K component would become relatively larger in a Cl-deficient solution. Thus one can expect that reduction of $[K]_o$ reduces the effect to a larger extent.

Reduction of the effect on the membrane resistance by lowering $[K]_o$ was observed in a Cl-deficient solution in which the Cl, except $CaCl_2$ and $MgCl_2$, in the Krebs solution was replaced with benzene sulphonate. The results from the experiments in two preparations are summarized in Fig. 4*a*. The increase of conductance produced by adrenaline, Δg_m, was plotted against $[K]_o$ in Cl-deficient solution (7 mM-Cl), and the same plot in normal Cl concentration (134 mM-Cl), derived from the data in Fig. 3, has been inserted for comparison. Every value in either solution was

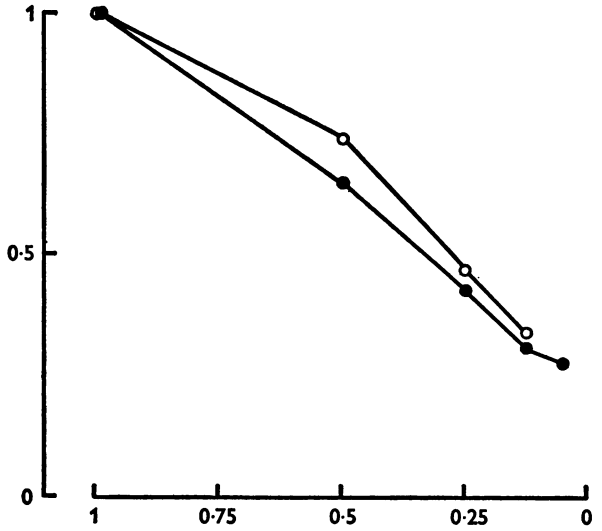

Fig. 3. Relationships between the increase of membrane conductance by adrenaline and the external K concentration (open circles), and external Cl concentration (filled circles). Abscissa: external K concentration and Cl concentration (normal K and Cl concentrations taken as 1). Ordinate: the relative values of the increased membrane conductance, $\Delta g'_m/\Delta g_m$ (the value in normal solution, Δg_m, taken as 1). For further description see text.

expressed as a ratio to the value at the normal K concentration. It can be seen that the magnitude of the ratio, i.e. the conductance change produced by adrenaline, depends on the K content of both solutions. However, the reduction of the adrenaline effect by reducing the K content is less in the Cl-deficient solution. This is contrary to expectation. Moreover, in the Cl-deficient solution with different K concentrations (5·9, 3·0 and 1·5 mM-K) adrenaline produced not only much less hyperpolarization than in the normal Cl concentration but frequently a depolarization.

Effect of Cl deficiency on the action of adrenaline in a K-deficient solution

On the basis of the hypothesis outlined in the preceding paragraph, the effect of lowering Cl ions on the action of adrenaline was examined in a K-deficient solution in which the K was reduced to 0·7 mM by replacement with Na. The results from the experiments in two preparations are summarized in Fig. 4*b*. Reduction of Cl in K-deficient solution diminished the

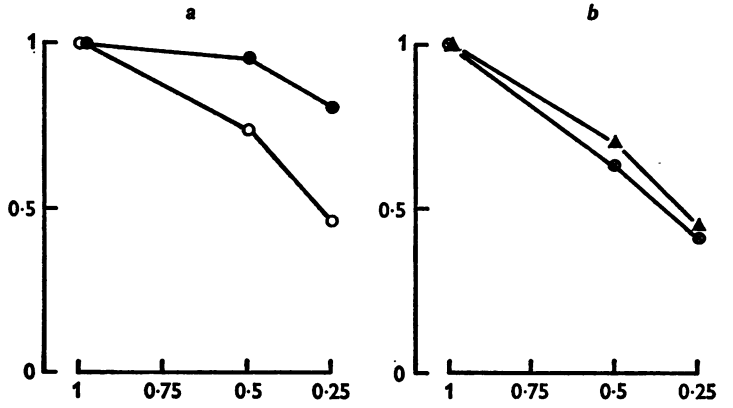

Fig. 4. Comparison of (*a*) the effect of K deficiency in two different Cl concentrations, and (*b*) of the effect of Cl deficiency in two different K concentrations, on the action adrenaline. *a*, Relationships between the external K concentration and the increased conductance by adrenaline in the presence of 134 mM-$[Cl]_o$ (○) and 7 mM-$[Cl]_o$ (●); *b*, the relationship between the external Cl concentration and the increased conductance by adrenaline in 5·9 mM-$[K]_o$ (⊗) and in 0·7 mM-$[K]_o$ (▲). Abscissa: (*a*) external K concentration and (*b*) Cl concentration (normal K and Cl concentrations taken as 1). Ordinate: the relative values of the increased conductance (the value in 5·9 mM-K for *a* and the value in 134 mM-Cl for *b* taken as 1). For further description see text.

effect of adrenaline on membrane resistance, but this effect was only slightly less than in the normal K concentration. The hyperpolarization was always increased. In one preparation, the Cl-deficient and K-deficient solution (0·7 mM-K and 34 mM-Cl) itself increased the membrane potential from 59 to 69 mV and adrenaline increased it by about 15 mV further.

Calculation of the equilibrium potential for the action of adrenaline

The changes in potential and in conductance caused by adrenaline, in normal ionic environment, makes it possible to calculate the equilibrium potential, if the same model, as has been used by Fatt & Katz (1951) to explain the action of acetylcholine on the end-plate membrane, can be

used to account for the action of adrenaline on smooth muscle. The essential features are illustrated in Fig. 5*a*, where E_m is the resting potential, g_m the conductance in the absence of adrenaline, e the equilibrium potential for the action of adrenaline, Δg_m additional conductance produced by adrenaline. Its effect is thus mimicked by closing the switch, S. The increment in potential difference, ΔE, is given by $\Delta E = (e-E)(\Delta g_m/g_m + \Delta g_m)$. From the present experiments, E: -60mV, ΔE: -8 mV, the ratio $\Delta g_m/g_m$: 1·08, and e is calculated as -75 mV.

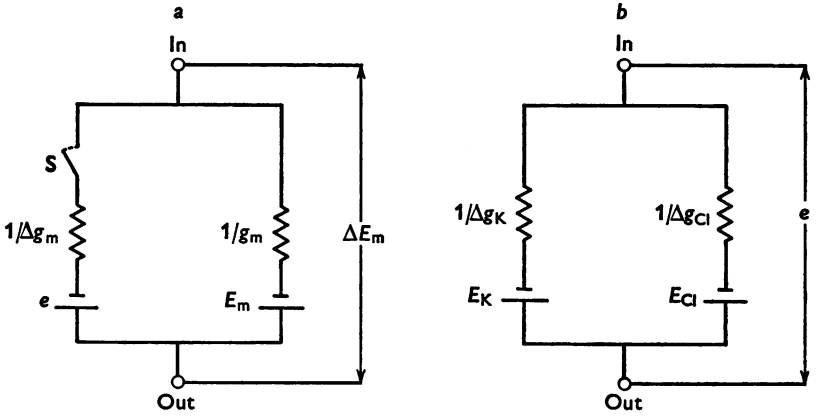

Fig. 5. *a*, Equivalent circuit for the action of adrenaline; *b*, equivalent circuit for adrenaline equilibrium potential, *e*, in terms of Nernst potentials and conductances, Δg, produced by adrenaline. For further description see text.

Contribution of the increment in chloride conductance to the total effect of adrenaline on the membrane conductance

The results described in the previous sections throw some light on the relative K and Cl conductance changes produced by adrenaline. The equilibrium potential for the action of adrenaline, e, would be given by the equivalent circuit as shown in Fig. 5*b*, where E_K and E_{Cl} are the Nernst potentials for K and Cl ions, Δg_K and Δg_{Cl} the K and Cl conductances produced by adrenaline. The equilibrium potential, e, is given by

$$e = \{E_K + (\Delta g_{Cl}/\Delta g_K)E_{Cl}\}(1 + \Delta g_{Cl}/\Delta g_K)^{-1}.$$

The ratio, $\Delta g_{Cl}/\Delta g_K$, can be calculated from $e = -75$ mV, $E_K = -91$ mV and $E_{Cl} = -31$ mV, derived from the data reported by Brading & Setekleiv (1968). The ratio $\Delta g_{Cl}/\Delta g_K$ was calculated as 0·36.

DISCUSSION

The present results, that the effect of adrenaline on membrane conductance was sensitive to changes in the external concentration of K ions as well as Cl ions, but that it was insensitive to a change in Na ions confirmed the observations of Bülbring & Tomita (1969). It is therefore concluded that adrenaline opens the ion pathways for K and Cl. The changes of membrane potential caused by adrenaline are also in favour of this conclusion. It is, however, very difficult to evaluate the potential changes precisely, because the conductances of the ion species involved and their concentrations on each side of the membrane are not known. The similarity of the degree of hyperpolarization caused by adrenaline in K-deficient solution to that in normal solution indicates that the ion pathways opened by adrenaline must allow the passage not only of K but also of at least one other ion species for which the Nernst potential is less negative than the membrane potential (E_m). This has been shown for E_{Cl} (Casteels, 1964, 1965, 1969; Casteels & Kuriyama, 1966). The depolarization by adrenaline observed in Cl-deficient solution can be interpreted by a larger deviation of E_{Cl} from E_m so that an increase in Cl conductance becomes more effective in displacing E_m towards E_{Cl} (depolarization). However, the deviation between E_{Cl} and E_m has been shown to decline with time by continual loss of Cl ions from the tissue (Casteels, 1964). In this case, the depolarizing effect of adrenaline would be expected to diminish also with time. In fact, it is time-dependent and its magnitude declines gradually in the course of 1 hr (E. Bülbring, unpublished observations).

From the fact that adrenaline increases the membrane potential and membrane conductance it is evident that the level of the equilibrium potential for the action of adrenaline is more negative than the resting potential (E_m). From the present results the equilibrium potential is -75 mV. Bülbring & Tomita (1969), using the sucrose-gap method, observed that conditioning hyperpolarization of the membrane of 10–20 mV by passage of anodal current converted the hyperpolarization produced by adrenaline to depolarization. This also means that the equilibrium potential should be about 10–20 mV more negative than the resting potential, which is about 54 mV (Holman, 1958; Bülbring & Kuriyama, 1963; Casteels & Kuriyama, 1966; Kuriyama, Osa & Toida, 1967). Since the potential difference between E_m and E_{Cl} is about the same as that between E_m and E_K (Casteels & Kuriyama, 1966; Casteels, 1969; Brading & Setekleiv, 1968), the additional conductance produced by adrenaline must be greater for K than for Cl ions. In fact, from the present experiments, the ratio of the Cl component to the K component, $\Delta g_{Cl}/\Delta g_K$, has been calculated as

0·36. This value is based on the ion distribution given by Brading & Setekleiv (1968) which has been used for the calculation.

If Δg_m consists of a K component (Δg_K) and Cl component (Δg_{Cl}), which are independent of one another and not greatly affected by small changes in membrane potential, $\Delta g'_m$, may be expressed by the following equations: In K-deficient solution, $\Delta g_m = \Delta g'_K + \Delta g_{Cl}$ or in Cl-deficient solution, $\Delta g_m = \Delta g_K + \Delta g'_{Cl}$, where Δg_K and $\Delta g'_{Cl}$, are K component in K-deficient solution and Cl component in Cl-deficient solution, respectively. Then

$$\frac{\Delta g'_m}{\Delta g_m} = \frac{\Delta g'_K + \Delta g_{Cl}}{\Delta g_K + \Delta g_{Cl}}, \quad \text{or} \quad \frac{\Delta g_K + \Delta g'_{Cl}}{\Delta g_K + \Delta g_{Cl}}$$

which can be rearranged as follows:

$$\frac{\Delta g'_m}{\Delta g_m} = \frac{\Delta g'_K/\Delta g_K + \Delta g_{Cl}/\Delta g_K}{1 + \Delta g_{Cl}/\Delta g_K}, \quad \text{or} \quad \frac{\Delta g_K/\Delta g_{Cl} + \Delta g'_{Cl}/\Delta g_{Cl}}{\Delta g_K/\Delta g_{Cl} + 1}. \qquad (3)$$

From these equations the expected ratio, $\Delta g'_m/\Delta g_m$, can be calculated for K-deficient and Cl-deficient solutions assuming that Δg_K and Δg_{Cl} are decreased in a simple proportional manner by reducing $[K]_o$ and $[Cl]_o$, respectively, if the ratio, $\Delta g_{Cl}/\Delta g_K$ is known. In K-deficient solutions, the calculated and experimentally obtained values fit fairly well. In Cl-deficient solution, however, the calculated ratios do not fit at all. The expected value with a ratio of $\Delta g_{Cl}/\Delta g_K = 0{\cdot}36$ should be more than 0·73 even when $\Delta g'_{Cl}/\Delta g_{Cl}$ becomes zero (see eqn. (3)). The experimentally obtained values were, however, less than 0·73 at any of the Cl concentrations used (see Fig. 3). This may mean that the increase in the K component and the increase in the Cl component of membrane conductance caused by adrenaline are not independent of one another, but that both are affected when either ion concentration in the medium is changed. If the two components were independent, the K component of conductance increased by adrenaline would become relatively larger in Cl-deficient solution, so that the reduction of $[K]_o$ should reduce the effect to a larger extent. Actually, it was observed that the adrenaline effect on the membrane resistance was less diminished by a given reduction of one species of ions, e.g. K, in the Cl-deficient solution (7 mM-Cl) than in the normal Cl concentration (134 mM-Cl) (Fig. 4*a*).

An alternative explanation for this might be that the Nernst potential, E_{Cl}, is closer to the membrane potential than calculated, because of the considerable uncertainty of the exact distribution of ions in smooth muscle. In this case, it would be expected that the ratio, $\Delta g_{Cl}/\Delta g_K$, could be larger than 0·36. If the ratio is 0·5, the similar relationship of $\Delta g'_m/\Delta g_m$ to $[K]_o$ and to $[Cl]_o$ (Fig. 3) would be explained. However, the possibility is made

rather unlikely by the fact that the observed decrease in $\Delta g'_m/\Delta g_m$ is much larger than that predicted from eqn. (3).

The present experiments indicate that the ratio of the Cl component to the K component of the increase in membrane conductance produced by adrenaline is about 0·36, without allowing for some uncertainty of the distribution of ions, but that these two components are not affected in an independent manner when either K or Cl concentration in the medium is reduced by substituting Na or sucrose for K and benzene sulphonate for Cl.

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