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MEMBRANE POTENTIAL AND ION CONTENT IN THE SMOOTH MUSCLE OF THE GUINEA-PIG'S TAENIA COLI AT DIFFERENT EXTERNAL POTASSIUM CONCENTRATIONS

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SUMMARY

1. The relation between the membrane potential and the intracellular ion concentration of the taenia coli was studied in solutions with different external potassium concentrations.

2. Isotonic solution in which the sum of NaCl + KCl was constant did not produce swelling of the smooth muscle of taenia coli, even at high $[K]_o$, and did not change the intracellular chloride concentration.

3. Addition of an equivalent amount (118 mM) of NaCl or KCl to Krebs solution produced the same loss of water.

4. Solutions in which the product [K][Cl] was kept constant decreased the internal chloride concentration so that the chloride equilibrium potential became temporarily positive and returned only after 2 hr to a negative value.

5. From these results and the finding that even in normal Krebs solution E_{Cl} was about 35 mV more positive than the membrane potential, it is concluded that the chloride ions are not passively distributed. The potassium equilibrium potential is the main factor in the generation of the membrane potential. The large discrepancy between the two can be explained by the non-passive chloride distribution and by the sodium permeability of the membrane. The maintenance of the normal cell volume in solutions with constant sum of NaCl + KCl is explained by the constant intracellular chloride concentration.

INTRODUCTION

The relation between the membrane potential of taenia coli cells and the external potassium concentration has often been investigated with electrophysiological techniques (Burnstock & Straub, 1958; Holman, 1958; Kuriyama, 1963). In most of these experiments the external potassium

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concentration was increased by adding solid KCl, probably because it was assumed that the properties of smooth muscle fibres are similar to those of striated muscle. However, the water content and the intracellular ion concentration have not been investigated. A combined study of the membrane potential and the intracellular ion concentrations therefore has been made. It was confirmed that the potassium equilibrium potential is the main factor in the generation of the membrane potential but in contrast with the observations in frog striated muscle (Boyle & Conway, 1941), it was found that sodium and potassium ions were equally effective in maintaining the osmotic equilibrium of the cells. Replacement of external sodium by an equivalent amount of potassium did not affect the water content of the tissue. In addition, it was found that the intracellular chloride concentration was too high to fit a passive distribution and that it was not influenced by changes of the membrane potential.

Some of these results have been presented to a meeting of the Associations des Physiologistes (Casteels, 1966*b*).

METHODS

The technique used for the electrophysiological observations has been described previously (Bülbring, 1954; Kuriyama, 1963).

Solutions. The normal Krebs solution used in all experiments contained (mM): Na^+ 137·4, K^+ 5·9, Mg^{2+} 1·2, Ca^{2+} 2·5, Cl^- 134·1, $H_2PO_4^-$ 1·2, HCO_3^- 15·5, glucose 11·5; and was aerated with 97 % O_2 + 3 % CO_2. The pH at 35° C was 7·4.

Two different types of isotonic solution have been used to increase the external potassium concentration from 5·9 to 118 mM. The sodium, chloride and potassium content of these solutions are given in Table 1. In solution I, the increase of the potassium concentration was achieved by replacing part of the sodium chloride with potassium chloride. Therefore the sodium concentration decreased with increasing potassium concentration while the chloride concentration remained constant. In solution II the product [K] [Cl] was kept constant and an increase of potassium was also accompanied by a decrease of sodium. The reduction of the chloride concentration was achieved by replacement with ethanesulphonate (Goodford & Ing 1959).

TABLE 1. Composition of solutions (mM)

$[K]_0$		Normal	2·5×	5×	10×	20×
Solution I	K	5·9	14·8	29·5	59	118
	Na	137·4	128·6	113·8	84·3	25·3
	Cl	134·1	134·1	134·1	134·1	134·1
Solution II	K	5·9	14·8	29·5	59	118
	Na	137·4	128·6	113·8	84·3	25·3
	Cl	134·1	54	27	13·4	6·7

For some experiments hypertonic solutions were used, in which solid KCl, NaCl, or $KC_2H_5SO_3$ were added to normal Krebs solution.

Measurements of intracellular ion content. The extracellular space was measured using ^{35}S-labelled ethanesulphonate supplied by the Radiochemical Centre, Amersham.

Tissues were suspended for 10 min in Krebs solution containing ^{35}S-ethanesulphonate at 35° C. The ethanesulphonate was then extracted from the tissue at room temperature and

the activity was determined using the scintillation mixture described by Bray (1960). By following the uptake of ^{35}S-ethanesulphonate as a function of the exposure time, it was found that 10 min was sufficient to obtain a steady value for the size of the extracellular space.

The water content of the tissues was determined by comparing the wet weight and the dry weight. The latter value was obtained by drying the tissues for 20 hr at 95° C.

The ion content of the samples was determined by flame-photometry using a Zeiss spectrophotometer PMQ II with flame attachment, as described by Casteels & Kuriyama (1965).

The intracellular ion content was calculated per litre fibre water according to Boyle, Conway, Kane & O'Reilly (1941).

RESULTS

The effects of changing the external ionic composition on the water content of the taenia coli

Isotonic solutions. The changes of the water content of the tissues were studied by comparing the dry weight/wet weight ratios after 40 min exposure to the different solutions, or by following the change of the wet weight of the individual tissues as a function of time of exposure. In these experiments the tissues were blotted with filter paper before each weighing procedure. The weights expressed as percentage of the weight in Krebs solution are given in Table 2.

There was no significant change during 130 min exposure to solution I and even after 6 hr exposure no change of the wet weight could be observed. However, the wet weight decreased by about 10% in solution II at 118 mM $[K]_o$ and the largest part of this change in water content happened during the first 10 min. Replacement by ethanesulphonate of the external chloride of Krebs solution to give a chloride concentration of 6·7 mM resulted in a loss of water, similar to that in solution II with the same ethanesulphonate concentration, but with 118 mM $(K]_o$. This fall of the wet weight by about 10% corresponds to an increase of the dry wt./wet wt. ratio from 20 to 21·6% (see Table 4).

Hypertonic solutions. The addition to Krebs solution of 118 mM KCl or NaCl or $KC_2H_5SO_3$ produced an identical loss of water from the tissues, amounting to about 25% loss in wet weight. The increase of the dry weight/wet weight ratio calculated from these experiments was from 19·5 to 26% and agreed with the experimental values for the dry weight/wet weight ratios determined in other experiments, which were 19·8 % ± 0·4 (10) in Krebs solution and 26·8% ± 0·8 (10) in the hypertonic solution.

The effects of different ionic composition on the membrane potential and membrane activity

In solutions of type I with constant sum of NaCl + KCl the line relating the change of the membrane potential to the logarithm of the external potassium concentration had a maximal slope of 43 mV for a tenfold

TABLE 2. Wet weights of the tissues expressed as percentage of the wet weight in Krebs solution.
(The values are means ± S.E., number of determinations in brackets)

Time of exposure (min)	Solution I 118 mM-K	Solution II 118 mM-K	Chloride-deficient Krebs solution with 6·7 mM-Cl and 127·4 mM-$C_2H_5SO_3$	Krebs solution + 118 mM-KCl	Krebs solution + 118 mM-NaCl	Krebs solution + 118 mM-$KSO_3C_2H_5$
10	98 ± 1 (7)	92 ± 1 (7)	94 ± 0·5 (7)	75 ± 0·8 (7)	78 ± 0·9 (7)	72 ± 0·8 (7)
30	99 ± 0·9 (7)	90 ± 1·5 (7)	92 ± 0·7 (7)	71 ± 0·7 (7)	75 ± 0·8 (7)	75 ± 0·9 (7)
50	101 ± 0·5 (7)	89 ± 0·7 (7)	91 ± 0·6 (7)	72 ± 1 (7)	76 ± 1 (7)	76 ± 1 (7)
70	99 ± 0·7 (7)	90 ± 0·3 (7)	92 ± 0·8 (7)	74 ± 0·9 (7)	73 ± 1 (7)	75 ± 0·9 (7)
90	100 ± 0·6 (7)	91 ± 0·8 (7)	91 ± 1·1 (7)	73 ± 0·5 (7)	76 ± 1·5 (7)	76 ± 1·1 (7)
110	101 ± 1 (7)	93 ± 0·9 (7)	93 ± 0·7 (7)	73 ± 0·7 (7)	76 ± 0·7 (7)	78 ± 1·0 (7)
130	99 ± 1·4 (7)	92 ± 1 (7)	92 ± 0·9 (7)	73 ± 0·9 (7)	75 ± 0·6 (7)	76 ± 1·0 (7)

change of $[K]_o$ (Fig. 2). In solutions of type II with constant [K][Cl] product, the maximal slope was higher (51 mV). This was due to the fact that in solutions II the membrane potential was more reduced at high external potassium concentrations than in the corresponding $[K]_o$ in solution I. The individual values are given in Tables 3 and 4.

The change of the membrane activity and of the configuration of the spike was similar for the same potassium concentration regardless whether the sum of NaCl + KCl or the product of [K][Cl] was constant. Fig. 1 shows the effect of different potassium concentrations of solutions with constant NaCl + KCl sum (type I) on the membrane potential and activity. The decrease of membrane potential was accompanied by an increase of spike frequency and by a decrease of amplitude and rate of rise of the spike. At 29·5 mM $[K]_o$ the spike amplitude was very small and at 59 and 118 mM $[K]_o$ all membrane activity had disappeared.

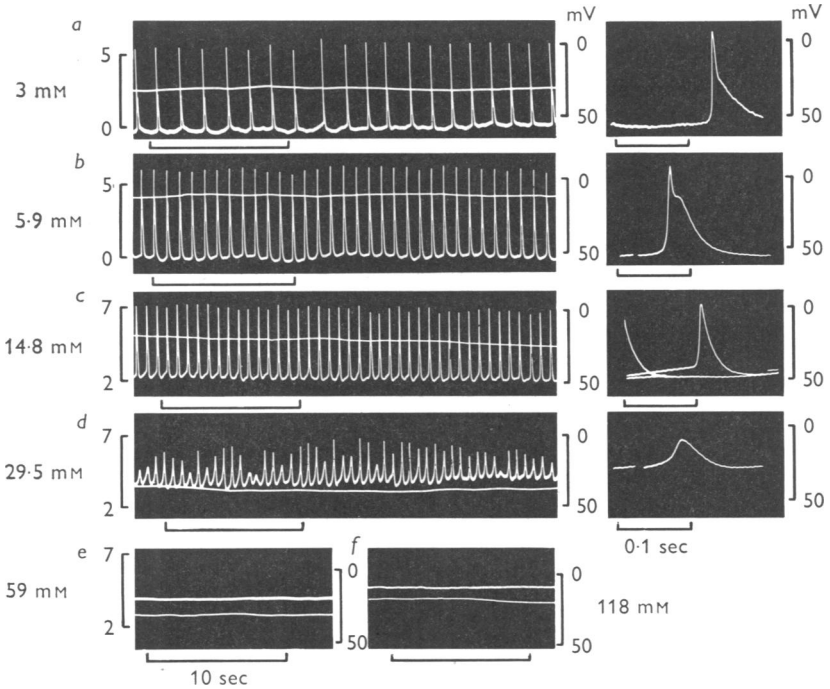

Fig. 1. The changes of the membrane potential, spike discharge and tension development by different external potassium concentrations, recorded at two different sweep speeds, *a*, 3 mM; *b*, 5·9 mM; *c*, 14·8 mM; *d*, 29·5 mM; *e*, 59 mM; and *f*, 118 mM external potassium concentration.

The effect on ion content

Solution I. When the sum of NaCl + KCl was kept constant, the water content of the tissues and the extracellular spaces remained constant at

TABLE 3. Ion content (m-moles/kg wet wt. ± S.E. of mean), extracellular spaces (ml./kg wet wt.), dry wt./wet wt. ratios, membrane potentials (mV), calculated intracellular ion concentrations (m-moles/l. cell water) and equilibrium potentials (mV) of taenia coli in solutions with different potassium concentrations of Type I, after 40 min exposure

m-moles $[K]_o$	5·9	29·5	59	118
K	86·4 ± 1·3 (13)	97·6 ± 1·1 (13)	110·4 ± 1·3 (13)	131·7 ± 0·7 (12)
Na	62·9 ± 1·1 (13)	49 ± 1·3 (13)	37·3 ± 1·9 (13)	14·5 ± 0·6 (12)
Cl	77·3 ± 1·2 (13)	78 ± 1·8 (13)	77·5 ± 1·6 (13)	88 ± 1·9 (12)
Ethanesulphonate space	351 ± 19 (6)	(340)	(340)	336 ± 11 (6)
Dry wt./wet wt.	18·5 ± 0·4 (10)	19·4 ± 0·7 (8)	19·5 ± 0·6 (12)	17·6 ± 0·5 (9)
Membrane potential	−57 ± 0·3 (30)	−31 ± 0·4 (30)	−20 ± 0·7 (20)	−9 ± 0·3 (42)
$[K]_i$	184	188	194	188
$[Na]_i$	32	22	18	12
$[Cl]_i$	66	69	69	88
E_K	−92	−49	−32	−12
E_{Na}	39	44	40	19
E_{Cl}	−19	−18	−18	−11

TABLE 4. Ion content (m-moles/kg wet wt. ± S.E. of mean), extracellular spaces (ml./kg. wet wt.), dry wt./wet wt. ratios, membrane potentials (mV), calculated intracellular ion concentrations (m-moles/l. cell water) and equilibrium potentials (mV) of taenia coli in solutions with different potassium concentrations of type II, after 40 min exposure

m-moles $[K]_o$	5·9	14·8	29·5	59	118
K	89·4 ± 0·9 (20)	93·4 ± 0·9 (16)	102·5 ± 1·2 (13)	110·1 ± 1·3 (12)	126·6 ± 1·4 (15)
Na	64·8 ± 1·3 (20)	56·9 ± 1·4 (16)	55·1 ± 1·3 (12)	39·5 ± 2·0 (13)	19·7 ± 1·8 (12)
Cl	81·2 ± 0·9 (11)	44·4 ± 0·7 (16)	28·5 ± 0·8 (11)	15·8 ± 0·7 (12)	13·9 ± 1·4 (12)
Ethanesulphonate space	336 ± 10 (9)	320 ± 11 (7)	316 ± 11 (9)	325 ± 17 (6)	321 ± 8 (8)
Dry wt./wet wt.	20 ± 0·7 (7)	—	20·3 ± 0·5 (10)	20·9 ± 0·8 (7)	21·6 ± 0·6 (5)
Membrane potential	−55 ± 0·9 (49)	−41·6 ± 0·9 (19)	−26·8 ± 0·9 (20)	−15·5 ± 0·6 (20)	−6·1 ± 0·3 (40)
$[K]_i$	187	185	194	193	192
$[Na]_i$	40	33	40	27	25
$[Cl]_i$	78	56	42	24	25
E_K	−92	−67	−50	−32	−13
E_{Na}	32	37	28	31	2
E_{Cl}	−15	+1	+12	+16	+36

the different external potassium concentrations. The analytical data, the measured membrane potential, the calculated intracellular ion concentrations and the equilibrium potentials are summarized in Table 3.

There was a slight decrease of the intracellular sodium concentration, so that the sodium equilibrium potential remained positive at concentrations of up to 25 mM of external sodium (Tables 1 and 3). The increase of the intracellular potassium concentration was relatively small, so that the slope of the calculated potassium equilibrium potential was 61 mV for a tenfold change of the external potassium concentration (Fig. 2).

The chloride equilibrium potential was in normal physiological solution about 35 mV more positive than the membrane potential. The depolarization of the membrane by high external potassium did not affect the intracellular chloride concentration except at 118 mM $[K]_o$ (Table 3 and Fig. 2). It should be mentioned that at this potassium concentration, the membrane potential became more positive than the chloride equilibrium potential.

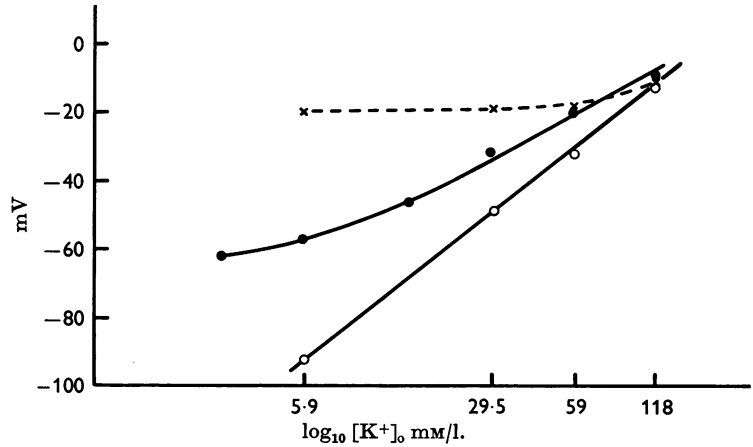

Fig. 2. The changes of the chloride equilibrium potential (×), the membrane potential (●) and the potassium equilibrium potential (○) as a function of the external potassium concentration in solutions of type I. Abscissa, the external potassium concentration on a logarithmic scale; ordinate, the potentials in mV.

Solutions II. When the product [K] [Cl] was kept constant there was a slight loss of water at 118 mM $[K]_o$ as mentioned before. The analytical data, the measured membrane potentials, and the calculated equilibrium potentials are summarized in Table 4. The intracellular potassium concentrations were similar to those observed at the corresponding external potassium concentrations in solution I, but the intracellular sodium concentrations were consistently higher. An increase in the internal sodium concentration was also observed after exposure of the taenia coli to Krebs solutions, in which 127 mM chloride was replaced either by ethanesul-

phonate or nitrate (unpublished observations). The reason for this increase of sodium by a change of the external anions is not known.

The slope of the line expressing E_K as a function of the log. of the external potassium concentration was 61 mV for a tenfold change of $[K]_o$, as in solutions I.

Solutions of type II (chloride replaced by sulphate) were used by Hodgkin & Horowicz (1959) in frog striated muscle because there should be no movement of KCl across the membrane, if potassium and chloride ions were distributed according to a Donnan equilibrium. The results given in Table 4 clearly show that in the taenia coli the chloride ions are not in equilibrium. The intracellular chloride concentration did not remain constant, but decreased progressively with decreasing external chloride concentration and increasing external potassium concentration. The chloride equilibrium potentials given in Table 4 were positive for external chloride concentrations below 54 mM (see Table 1) and for the exposure times of 40 min. However, during this period the chloride distribution had not yet reached a steady state, and after 2 hr the equilibrium potentials returned to a negative value (Table 5).

TABLE 5. Chloride content (m-moles/kg wet wt.), intracellular chloride concentration (m-moles/l. cell water) and chloride equilibrium potentials (mV) after exposure for different times to solutions of type II

Time of exposure	Total chloride content (m-moles/kg wet wt.)	Intracellular chloride concentration (m-moles/l. fibre water)	E_{Cl} (mV)
Control	73·4 ± 3 (5)	61	−21
1 hr 27 m-moles Cl	24·2 ± 1 (5)	33	+5
2 hr 27 m-moles Cl	17·5 ± 1 (5)	19	−10
3 hr 27 m-moles Cl	14·9 ± 1 (5)	13	−19
4 hr 27 m-moles Cl	15·9 ± 0·5 (4)	15	−15
1 hr 6·7 m-moles Cl	8·9 ± 0·8 (5)	15	+21
2 hr 6·7 m-moles Cl	3·0 ± 1·1 (5)	1·8	−34
3 hr 6·7 m-moles Cl	2·1 ± 0·3 (5)	2·0	−32
4 hr 6·7 m-moles Cl	3·5 ± 1·2 (4)	2·9	−22

The positive value of the chloride equilibrium potential at low external chloride concentration explains why the membrane potential was lower in solutions of type II, than at the same potassium concentrations in solutions of type I.

DISCUSSION

In normal Krebs solution, the intracellular ion concentrations calculated from the ethanesulphonate space and expressed per litre fibre water are higher than the values calculated on the basis of the inulin space and expressed per litre cell volume (Goodford & Hermansen, 1961). The intracellular potassium concentration is also higher than the concentration found in mammalian striated muscle (Creese, 1954; Giebisch, Kraupp, Pillat & Stormann, 1957) but it is in the same range as the values given by

Page (1962) for cat papillary muscle, which was calculated from the mannitol space.

When the external potassium concentration is raised, both in solutions I and II, the intracellular potassium concentration remains almost constant. In frog striated muscle, Boyle & Conway (1941) and Adrian (1956), who used a solution similar to type I, also found that the internal potassium concentration remained constant at high $[K]_o$. In this muscle, however, an appreciable amount of water is taken up and the internal potassium concentration is only maintained by a simultaneous penetration of potassium and chloride. In this way the Donnan distribution of potassium and chloride is maintained.

In the smooth muscle of taenia coli the Donnan distribution of chloride ions does not apply. Even in normal Krebs solution the intracellular chloride concentration is too high to fit a passive distribution, probably because of an active uptake of chloride against its electrochemical gradient (Casteels, 1965). Supporting evidence for this hypothesis comes from the observations that the internal chloride concentration is decreased by ouabain (Casteels, 1966*a*), by low temperature and by metabolic depletion (unpublished observations) although in these conditions the membrane is depolarized. This active transport may be able to maintain the intracellular Cl concentration even when the membrane is depolarized at high $[K]_o$, but only as long as the chloride equilibrium potential is more positive than the membrane potential. When the external potassium concentration is further increased above 59 mM, the passive distribution of chloride may also come into play and the internal chloride increases.

The large deviation between the line representing the membrane potential and the line representing the potassium equilibrium potential as a function of the log. of $[K]_o$ in solution I may be explained by the non-passive chloride distribution and by the influence of the sodium permeability of the membrane.

In solution II the internal chloride concentration decreases with the rise in external potassium concentration and the fall of external chloride concentration. However, the reduction of $[Cl]_i$ is not sufficient to maintain a negative chloride equlibrium potential. This becomes positive temporarily, although it returns to a negative value after 90–120 min. One could argue that the positive chloride equilibrium potential could be an artifact due to some binding of chloride, but measurements of the chloride activity in taenia coli homogenates gave no evidence for such binding (Casteels, 1965). It is more likely that the slow change of the chloride equilibrium potential to a negative value in the solutions in which the product of [K] [Cl] was constant, is due to the slow replacement of the intracellular chloride by other anions, as suggested by the slow penetration of ethane-

sulphonate (Goodford & Lüllmann, 1962). The alternative mechanism of a loss of intracellular cations accompanying the loss of chloride is excluded by their remaining constant (Table 4).

The striking difference between the frog striated muscle and the taenia coli is that, at increased external potassium concentrations in solutions of type I, the striated muscle takes up water with potassium chloride sufficient to maintain $[K]_i$ constant and to adjust $[Cl]_i$ to the new value of the membrane potential (Boyle & Conway, 1941). The important role of chloride ions in this swelling of the sartorius muscle is demonstrated by the fact that in solutions in which sulphate is the external anion, $[K]_o$ can be increased and $[Na]_o$ proportionally reduced without concomitant swelling of the fibre and without change of $[K]_i$ (Adrian, 1956). For the taenia coli the present experiments have shown that in solution I the smooth muscle cells maintain their normal cell volume. This can be explained by the hypothesis that the constant value of $[Cl]_i$ makes a penetration of potassium ions impossible, because electroneutrality must be maintained under all conditions. Moreover, as a consequence of the constant intracellular ion content, no water uptake can occur in solutions with high $[K]_o$ and low $[Na]_o$.

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