J. Physiol. (1971), **217**, *pp.* 297–313
With 4 text-figures
Printed in Great Britain

ELECTROGENIC SODIUM PUMP IN SMOOTH MUSCLE CELLS OF THE GUINEA-PIG'S TAENIA COLI

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(*Received 15 February 1971*)

SUMMARY

1. The changes of the membrane potential, of the K equilibrium potential, and of the membrane conductance during K accumulation by K-depleted tissues have been studied. Three subsequent characteristic periods can be described.

2. Readmission of 5·9 mM-K after complete depletion results in a rapid extrusion of Na and uptake of K, and in a rapid hyperpolarization of the cells. Initially the time course of the K equilibrium potential and the membrane potential are similar except in propionate solution. This initial period is characterized by a high membrane conductance. No change of membrane potential occurs if 10^{-5} M ouabain is present.

3. After 5–7 min the membrane potential becomes more negative than the K equilibrium potential. The difference between both values is larger in solutions containing propionate or in hypertonic solutions. This second phase of the recovery period is characterized by a progressive decrease of the membrane conductance.

4. In a third phase both the membrane potential and the membrane resistance return to their steady-state value.

5. If the external K concentration in the recovery solution is increased, the maximal hyperpolarization is less and has a shorter duration. A decrease of the temperature of the recovery solution results in a slower initial rate of repolarization and in a decrease of the maximal value of the hyperpolarization.

6. These observations demonstrate the existence of an electrogenic sodium pump in smooth muscle cells during stimulation of the Na pump. An analysis of the experimental data obtained under steady-state conditions in normal Krebs solution suggests that also under these conditions an electrogenic Na pump might take part in the maintenance of the resting potential.

INTRODUCTION

The hypothesis that the resting potential of smooth muscle cells might be influenced by an electrogenic Na pump is not new. Burnstock (1958) and Bülbring (1962) suggested that the hyperpolarization produced by adrenaline might be due to a transitory activation of an electrogenic Na pump. The hypothesis that the steady-state resting potential is partially determined by a continuously operating electrogenic Na pump could explain the observation that the measured resting potential in taenia coli cells is about 20 mV more negative than the potential calculated by the Goldman equation (Casteels, 1969). Such a mechanism, which has been described in molluscan neurones (Gorman & Marmor, 1970) and in *Aplysia* neurones (Carpenter, 1970), could also affect the membrane potential in taenia coli cells considerably because their membrane resistance is rather high, as measured by electrophysiological methods (Tomita, 1966) and by tracer experiments (Casteels, 1969). It has, however, to be proved that the Na pump can be electrogenic in taenia coli, and one of the accepted criteria for such a mechanism is the finding that the resting potential becomes more negative than the K equilibrium potential during pronounced stimulation of the Na pump. Therefore K-depleted, Na-rich preparations of taenia coli (Casteels, Droogmans & Hendrickx, 1971) have been used in this study and the changes of the membrane potential, the K equilibrium potential and of the membrane conductance have been investigated during reaccumulation of K. These data strongly support the existence of an electrogenic Na pump during K reaccumulation. A further analysis of the experimental data, obtained in normal Krebs solution, suggests that also in these steady-state conditions, the electrogenic pump may take part in maintaining the resting potential. Preliminary reports of some of these observations have been given (Casteels & Hendrickx, 1969; Casteels, Hendrickx & Droogmans, 1969*a*, *b*).

METHODS

The methods, used in these experiments, have been described in the preceding paper (Casteels, Droogmans & Hendrickx, 1971). Because the loss of K in K-free solutions containing large anions, is rather slow, complete K-depletion can only be obtained by exposing the tissues for 3 hr to K-free solution containing chloride and then transferring them for 1 hr to K-free solution containing propionate. A similar procedure was used to obtain complete K-depletion in hypertonic K-free solution. For solutions at lowered temperature, the CO_2 content in the gas mixture was reduced in order to maintain the same pH. In solutions with high K concentration, NaCl has been replaced by equivalent amounts of KCl so as to maintain the same tonicity. The extracellular space of taenia coli cells in solutions at different temperatures or containing different concentrations of K has only been measured in some

conditions. Because extreme conditions did not affect this value significantly, it has been assumed that the extracellular space was not affected by the intermediate conditions.

RESULTS

Changes of E_K and of the membrane potential during K reaccumulation in solutions containing different anions. Exposure of K-depleted and depolarized smooth muscle cells to a Krebs solution containing 5·9 mM-K results in a rapid reaccumulation of K and in a fast decrease of the membrane potential. Table 1 summarizes the changes of total ion content, the extracellular space and the dry wt./wet wt. ratio during reaccumulation of K in Krebs solutions containing different anions and in Krebs solution made hypertonic by adding sucrose. The amounts of K, taken up in the solutions containing different anions during the same period of time, are rather similar. From these values the intracellular K concentrations and the corresponding K equilibrium potentials (E_K) have been calculated. Fig. 1 represents the change of E_K, of the membrane potential and of the measured electrotonic potential during the uptake of K. The change of E_K in function of the time of exposure is similar for all solutions containing 5·9 mM-K, except for hypertonic solution where the decrease of E_K proceeds faster. The membrane potential follows initially nearly the changes of E_K. Later on the changes of the membrane potential are markedly affected by the type of anion present in the recovery solution. After the first 5–7 min this potential becomes more negative than E_K in all solutions. For solutions containing large anions as propionate or benzenesulphonate, the membrane potential becomes already more negative at an earlier stage of the recovery and the maximal difference between both potentials is appreciably higher. Moreover, the duration of the hyperpolarization is prolonged.

If the external solution contains chloride or nitrate, the membrane hyperpolarizes over E_K by a maximal value of 15 mV. The changes occurring in hypertonic solution are intermediate between the changes in solutions containing permeant and large anions.

Effect of ouabain on the K uptake and the concomitant potential change. Readmission of 5·9 mM-K in the external solution does not produce a hyperpolarization in K-depleted cells if 10^{-5} M ouabain is present. Moreover, the uptake of K by the tissue is very small. In some experiments it was observed that readmission of K caused a short-lasting reversal of the membrane potential to positive values. The high value of P_K, the large surface/volume ratio and the inward directed gradient for K allow a rapid increase of the intracellular K concentration so that the membrane potential returns rapidly to its original value.

TABLE 1. Total ion content (m-mole/kg wet wt.), extracellular space (ml./kg wet wt.) and dry wt./wet wt. ratio (percentage) of taenia coli during reaccumulation of K in solutions containing different anions and in hypertonic solution. From these values the intracellular ion concentrations (m-mole/l. fibre water) and the K equilibrium potentials (mV) have been calculated

Recovery solution	Time of recovery	Na^+	K^+	Cl^-	ECS	Dry wt. / Wet wt.	$[Na]_i$	$[K]_i$	$[Cl]_i$	E_K
Chloride solution	5	132 ± 1 (20)	15·1 ± 0·8 (20)	92 ± 2 (15)	360 ± 15 (7)	16·9 ± 0·2 (7)	174	27	92	−40
	10	118 ± 1 (13)	27·5 ± 0·9 (14)	93 ± 2 (9)	—	—	145	53	94	−58
	20	97 ± 1 (25)	50·4 ± 1·0 (28)	93 ± 2 (15)	365 ± 17 (7)	16·0 ± 0·3 (7)	100	102	94	−76
	30	76 ± 1 (12)	63 ± 1·0 (12)	90 ± 1 (12)	340 ± 18 (5)	—	56	128	88	−82
	60	55 ± 3 (5)	94 ± 1·3 (4)	90 ± 3 (5)	—	—	12	193	88	−92
Nitrate solution	5	127 ± 2 (6)	15·4 ± 0·4 (6)	—	—	—	166	29	—	−42
	10	114 ± 2 (6)	27·0 ± 0·7 (6)	—	370 ± 15 (7)	17·3 ± 0·2 (7)	138	54	—	−59
	20	100 ± 2 (6)	44 ± 2·0 (6)	—	—	—	107	91	—	−73
	30	83 ± 2 (6)	60 ± 2·1 (6)	—	—	—	70	126	—	−81
	60	61 ± 1 (6)	84 ± 2·4 (6)	—	—	—	22	178	—	−90
Propionate solution	5	120 ± 1 (14)	15·0 ± 0·5 (14)	—	318 ± 17 (5)	16·0 ± 0·3 (5)	146	25	—	−38
	10	108 ± 2 (14)	25 ± 1·0 (14)	—	—	—	123	44	—	−53
	20	86 ± 2 (13)	40 ± 1·5 (13)	—	320 ± 10 (16)	16·0 ± 0·1 (16)	81	73	—	−67
	30	74 ± 2 (7)	60 ± 1·2 (7)	—	—	—	58	112	—	−78
	60	58 ± 2 (7)	82 ± 1·3 (7)	—	—	—	27	154	—	−87
Hypertonic solution	5	159 ± 2 (14)	14·5 ± 0·9 (14)	105 ± 1 (10)	486 ± 11 (10)	27·1 ± 1·6 (10)	378	48	163	−56
	10	142 ± 1 (13)	23·5 ± 1·0 (12)	98 ± 1 (9)	—	—	308	84	135	−70
	20	132 ± 1 (13)	32·1 ± 2·1 (12)	94 ± 2 (10)	—	—	268	119	118	−78
	30	121 ± 2 (14)	45·7 ± 2·5 (13)	88 ± 1 (10)	484 ± 15 (8)	26·8 ± 1·5 (10)	223	175	94	−90
	60	97 ± 2 (4)	78 ± 2·8 (8)	—	—	—	125	307	—	−104

Reaccumulation of K at different external concentrations. Fig. 2 summarizes the changes of E_K and of the membrane potential during K reaccumulation in solutions with one of the following K concentrations: 1·2; 2·95; 11·8 or 23·6 mM. The E_K values have been calculated from the analytical data given in Table 2. All values of $[K]_o$ higher than 1·2 mM cause a similar

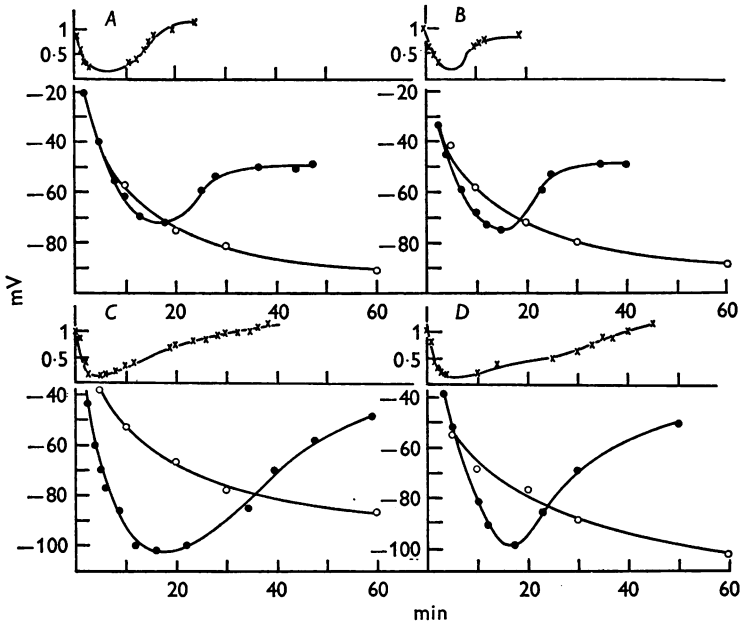

Fig. 1. The resting potential (filled circles), the K equilibrium potential (open circles) and the electrotonic potential (crosses) of smooth muscle cells during reaccumulation of K in solutions containing chloride (*A*), nitrate (*B*), propionate (*C*), and in hypertonic solution (*D*). Upper scale of the ordinate: relative values for the electrotonic potential; lower scale: potential in mV. The time of exposure is indicated on the abscissa in min.

initial rate of repolarization. For increasing values of $[K]_o$ the maximal hyperpolarization appears earlier and attains a less negative value than at 5·9 mM-$[K]_o$. This finding is in clear contrast with the observations of Taylor, Paton & Daniel (1970) who described in K-depleted myometrium cells an increase of the maximal hyperpolarization for increasing $[K]_o$. In our experiments also the total duration of this hyperpolarization is reduced for increasing values of $[K]_o$ and the maximal difference between E_K and the membrane potential becomes larger. If the external solution contains 2·95 mM-K the initial rate of hyperpolarization is similar to the one observed for the higher K concentrations but E_K and the membrane potential have the same course for up to 25 min. The beginning of the

depolarization occurs appreciably later than at 5·9 mM. Exposure to a solution containing 1·2 mM-K produces a rather slow decrease of the membrane potential, so that the most negative value is only reached after 60 min and this low value is still maintained after a recovery for more than 90 min. Moreover, E_K remains always more negative than the membrane potential.

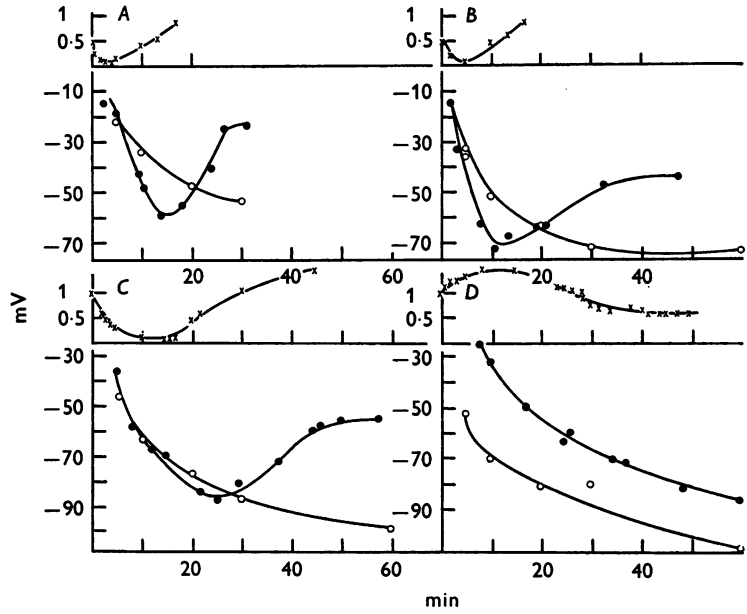

Fig. 2. The resting potential (filled circles), the K equilibrium potential (open circles) and the electrotonic potential (crosses) of smooth muscle cells during reaccumulation of K in solutions containing 23·6 (*A*), 11·8 (*B*), 2·95 (*C*), and 1·2 mM-K (*D*). Upper scale of the ordinate: relative values for the electrotonic potential; lower scale: potential in mV. The time of exposure is indicated on the abscissa in min.

At all $[K]_o$ there exists a striking similarity between the rate of Na extrusion and K uptake (Fig. 3). If the initial decrease of $[Na]_i$ after readmission of K is entirely active, we may consider the initial slope of the curves representing $[Na]_i$ as a function of time, as a measure of the initial pumping rate. The increase of this slope for increasing K concentrations indicates that $[K]_o$ exerts an important effect on the pumping rate.

Changes of the membrane conductance during K reaccumulation. The influence of an electrogenic Na pump on the membrane potential is determined not only by its pumping intensity but also by the membrane resistance. We have therefore determined the relative values of the total membrane resistance during K reaccumulation by measuring the electrotonic

TABLE 2. Total ion content (m-mole/kg wet wt.), sorbitol space (ml./kg wet wt.) and dry wt./wet wt. ratio (percentage) of taenia coli during reaccumulation of K in solutions of different K concentrations. From these values the intracellular ion concentrations (m-mole/l. cell water) and the K-equilibrium potentials have been calculated

K concentration in the recovery solutions	Time of recovery	Na^+	K^+	ECS	Dry wt. / Wet wt.	$[Na]_i$	$[K]_i$	E_K
23·6 mM-K^+	5	115 ± 1 (20)	32·1 ± 1·2 (20)	—	—	152	50	−21
	10	98 ± 1 (19)	49·4 ± 1·4 (19)	—	—	116	86	−34
	20	74 ± 3 (17)	75 ± 4·0 (16)	362 ± 8(7)	15·8 ± 0·3(7)	65	140	−47
	30	64 ± 2 (10)	89 ± 3·1 (10)	—	—	44	169	−53
11·8 mM-K^+	5	124 ± 1 (22)	23·0 ± 0·8 (21)	—	—	162	39	−32
	10	104 ± 2 (13)	43·3 ± 1·7 (13)	—	—	119	82	−51
	20	88 ± 2 (17)	64 ± 2·0 (17)	—	—	86	126	−63
	30	62 ± 3 (9)	89 ± 1·2 (9)	—	—	31	178	−72
	60	56 ± 1 (8)	93 ± 2·1 (8)	—	—	18	187	−73
5·9 mM-K^+	5	132 ± 1 (20)	15·1 ± 0·8 (20)	360 ± 15 (7)	16·9 ± 0·2 (7)	174	27	−40
	10	118 ± 1 (13)	27·5 ± 0·9 (14)	—	—	145	53	−58
	20	97 ± 1 (25)	50·4 ± 1·0 (28)	365 ± 17 (7)	16·0 ± 0·3 (7)	100	102	−76
	30	76 ± 1 (12)	63 ± 1·0 (12)	—	—	56	128	−82
	60	55 ± 3 (5)	94 ± 1·1 (4)	—	—	12	193	−92
2·95 mM-K^+	5	136 ± 1 (9)	9·0 ± 0·2 (9)	—	—	180	17	−46
	10	130 ± 2 (8)	16·4 ± 0·6 (8)	—	—	168	32	−63
	20	125 ± 2 (9)	26·5 ± 1·6 (8)	—	—	157	54	−77
	30	110 ± 2 (8)	39·1 ± 1·7 (8)	—	—	125	80	−87
	60	91 ± 3 (6)	59 ± 2·0 (6)	—	—	85	122	−99
1·2 mM-K^+	5	138 ± 3 (8)	4·7 ± 0·2 (8)	—	—	183	9	−53
	10	142 ± 1 (4)	10·3 ± 1·7 (4)	—	—	192	21	−76
	20	135 ± 2 (8)	12·6 ± 1·3 (8)	359 ± 12 (6)	15·9 ± 0·4 (6)	177	26	−82
	30	142 ± 1 (3)	12·3 ± 0·3 (3)	—	—	192	25	−81
	60	113 ± 2 (4)	31·1 ± 2·6 (4)	—	—	131	65	−106
	100	99 ± 3 (2)	45 ± 4·1 (8)	—	—	101	94	−115
	140	84 ± 4 (4)	57 ± 2·0 (4)	—	—	69	119	−121
	180	83 ± 4 (4)	68 ± 2·2 (4)	—	—	67	142	−126

potential. The effect of readmitting K in the external solution on the electrotonic potential is shown for different experimental conditions in Figs. 1 and 2 together with the values of E_K and of the membrane potential. Adding 5·9 mM-K to the external solution produces in all conditions at 35° C a pronounced decrease of the membrane resistance. The lowest membrane resistance is reached after 5–7 min. The period during which the membrane resistance has its lowest value coincides with the period

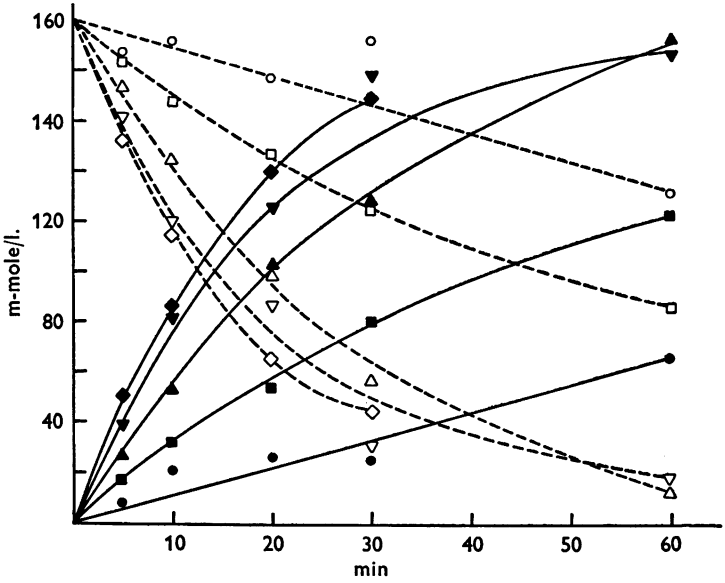

Fig. 3. The changes of the intracellular Na and K concentration during reaccumulation of K from solutions containing different K concentrations. The curves were fitted by eye to the experimental points. The sets of open symbols represent the Na concentrations, the filled symbols the K concentrations. The following external K concentrations have been used (mM): 1·20 ○; 2·95 □; 5·9 △; 11·8 ▽; 23·6 ◇.

during which the difference between E_K and the membrane potential is not significant. As soon as the membrane resistance increases, the membrane potential becomes more negative than E_K. The membrane resistance increases further to its steady-state value, while the membrane depolarizes to the normal resting potential. Higher concentrations of $[K]_o$ (11·8 and 23·6 mM) produce a fall of the membrane resistance which is similar to the one observed in 5·9 mM $[K]_o$, but the onset of the return to the steady-state value of the membrane resistance appears earlier. At 2·95 mM $[K]_o$ the decrease of the resistance develops more slowly and the return to the steady-state value occurs later. Unexpectedly, readmission of 1·2 mM-K results in an increase of the membrane resistance, which is followed after

about 30 min by a return to a steady value. It must be pointed out that the measurement of the electrotonic potential becomes impossible as soon as the mechanical activity reappears.

Adding 10^{-5} M ouabain to the K-free solution after K depletion does not affect the membrane resistance. The readmission of K in the external solution in the presence of 10^{-5} M ouabain causes a decrease of the membrane resistance, which is however much smaller than the decrease observed in the presence of 5·9 mM-K without ouabain. The question therefore arises whether an active pumping mechanism has a direct effect on the membrane conductance. This problem has not been further investigated.

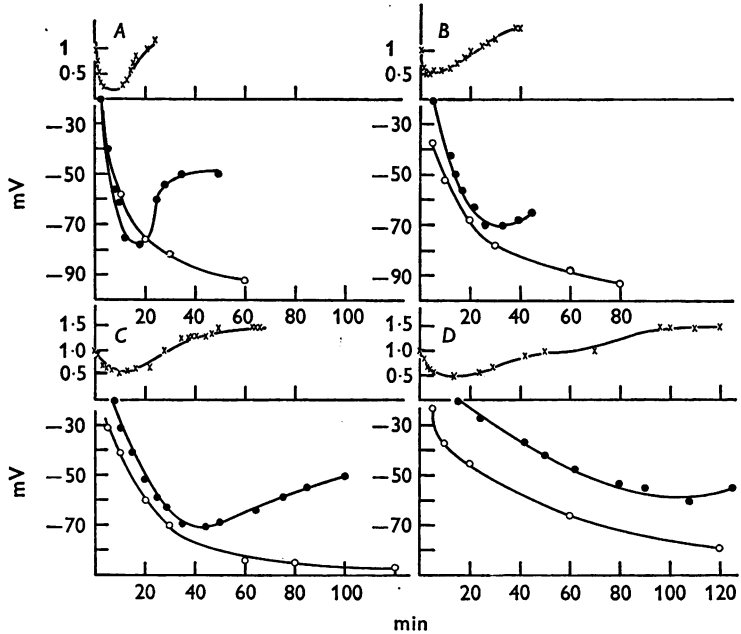

Fig. 4. The resting potential (filled circles), the K equilibrium potential (open circles) and the electrotonic potential (crosses) of smooth muscle cells during reaccumulation of K in solutions containing 5·9 mM-K at 35° C (*A*), 30° C (*B*), 25° C (*C*), and 20° C (*D*). Ordinate: upper scale: relative values for the electrotonic potential; lower scale: potential in mV. Abscissa: time of exposure in min.

K accumulation and changes of membrane potential in K-depleted tissues at different temperatures. Fig. 4 represents the change of E_K, of the membrane potential and of the electrotonic potential at 35, 30, 25 and 20° C. The E_K values have been calculated from the analytical data, summarized in Table 3. It is shown that the temperature decreases the rate of hyperpolarization and of the change of E_K. The minimal value of the membrane

TABLE 3. Total ion content (m-mole/kg wet wt.), ECS space (ml./kg wet wt.) and dry wt./wet wt. ratio (percentage) of taenia coli cells during reaccumulation of K from Krebs solution at different temperatures. From these data the intracellular Na and K concentrations (m-mole/l. cell water) and the E_K have been calculated

Temperature of recovery solution	Time of recovery	Na^+	K^+	ECS	Dry wt. / Wet wt.	$[Na]_i$	$[K]_i$	E_K
35° C	5	132 ± 1 (20)	15·1 ± 0·8 (20)	360 ± 15 (7)	16·9 ± 0·2 (7)	174	27	−40
	10	118 ± 1 (13)	27·5 ± 0·9 (14)	—	—	145	53	−58
	20	97 ± 1 (25)	50·4 ± 1·0 (28)	365 ± 17 (7)	16·0 ± 0·3 (7)	100	102	−76
	30	76 ± 1 (12)	63 ± 1·1 (12)	—	—	56	128	−82
	60	55 ± 3 (5)	94 ± 1·2 (4)	—	—	12	193	−92
30° C	5	128 ± 2 (10)	13·6 ± 0·4 (16)	—	—	164	24	−37
	10	121 ± 2 (11)	23·1 ± 0·7 (11)	—	—	150	44	−52
	20	101 ± 3 (11)	40·6 ± 1·5 (11)	—	—	108	80	−68
	30	90 ± 2 (10)	57 ± 2·0 (11)	—	—	85	114	−78
	60	61 ± 2 (10)	85 ± 4·0 (10)	—	—	24	173	−88
	80	58 ± 3 (9)	92 ± 2·4 (9)	—	—	18	187	−93
25° C	5	136 ± 2 (7)	11·0 ± 0·1 (4)	—	—	181	19	−31
	10	132 ± 3 (9)	15·5 ± 0·7 (5)	—	—	172	28	−41
	20	125 ± 5 (9)	29·9 ± 0·9 (5)	—	—	158	58	−60
	30	131 ± 2 (5)	44·5 ± 2·7 (5)	—	—	170	88	−70
	60	112 ± 4 (5)	73 ± 5·1 (5)	—	—	131	148	−84
	80	94 ± 5 (9)	80 ± 4·0 (5)	—	—	93	156	−85
	120	77 ± 5 (7)	82 ± 5·0 (4)	—	—	58	166	−87
20° C	5	132 ± 2 (5)	9·2 ± 0·6 (5)	—	—	172	15	−23
	10	131 ± 1 (5)	14·0 ± 1·0 (5)	—	—	170	25	−37
	20	124 ± 2 (5)	18·7 ± 0·9 (5)	363 ± 11 (10)	16·1 ± 0·2 (10)	156	35	−45
	60	111 ± 3 (4)	40 ± 2·0 (4)	—	—	129	79	−66
	120	81 ± 4 (5)	67 ± 3·0 (5)	359 ± 10 (4)	15·9 ± 0·1 (4)	66	135	−79

potential during K reaccumulation is less negative for decreasing temperature, and the onset of the return of the membrane potential to its steady-state value occurs later. Only at 35° C does the membrane potential become significantly more negative than E_K. At all lower temperatures it is found that E_K remains more negative than the membrane potential.

The values of the electrotonic potentials, shown in Fig. 4, indicate that the membrane resistance decreases transiently during K accumulation at different temperatures. The onset of the increase of the membrane resistance to steady-state values occurs later for decreasing temperature.

DISCUSSION

Readmitting K in the external solution causes a marked hyperpolarization and a rapid uptake of K by K-depleted smooth muscle cells. It is observed that the increase of the intracellular K proceeds rather fast, so that during the first 5 min the time courses of the membrane potential and of E_K are not significantly different in solutions at 35° C containing permeant anions and in which the K concentration is higher than 1·2 mM. These findings are in contrast with the description given by Taylor *et al.* (1970) of the relation between K reaccumulation and hyperpolarization occurring in K-depleted myometrium cells. These authors conclude that the initial hyperpolarization is only accompanied by a very small uptake of K even at very high values of $[K]_o$. Our results also show that the hyperpolarization is less and disappears sooner if the external K concentration is high. This finding again is in contrast with the observations of Taylor *et al.* (1970) who describe an increasing hyperpolarization for increasing values of $[K]_o$. These differences might be due to differences in experimental procedure, as i.e. the degree of K depletion and to differences in tissue and species.

The hyperpolarization and the uptake of K by the cells do not occur if the Na-K pump is inhibited by a cardiac glycoside as ouabain (Schatzmann, 1953). At lowered temperature the K uptake and the hyperpolarization proceed more slowly. It may therefore be concluded that the hyperpolarization and the K accumulation are produced by a stimulation of the Na-K exchange pump. The essential factor to obtain this large transient stimulation of the sodium pump seems to be an increased value of $[Na]_i$. The same procedure to stimulate the Na pump by increasing $[Na]_i$ has also been used in skeletal muscle fibres (Kernan, 1962; Cross, Keynes & Rybová, 1965; Adrian & Slayman, 1966), heart muscle (Page & Storm, 1965), snail neurones (Kerkut & Thomas, 1965) and non-myelinated nerve fibres (Rang & Ritchie, 1968). Our experiments differ from all the previous ones by the fact that the tissues are completely depleted of K and

contain the maximal concentration of Na. Consequently the experimental conditions before starting the *K* reaccumulation are well defined and always the same.

The generally accepted evidence in favour of an electrogenic activity of the Na pump is that the membrane potential becomes more negative than E_K. Under these circumstances the K uptake is partially electrically coupled to the Na extrusion, as demonstrated for frog skeletal muscle by Kernan (1962), Cross *et al.* (1965), and by Adrian & Slayman (1966). A different explanation for these large hyperpolarizations is that by the intense pumping rate of an electroneutral pump the fluid in contact with the cell membrane becomes depleted of K, so that the real E_K would be appreciably more negative than the value calculated from the given K concentration in the extracellular solution (Ritchie & Straub, 1957). In the case of striated muscle this possibility has been excluded by Adrian & Slayman (1966). These authors showed that substitution of K by Rb in the extracellular solution produced a larger hyperpolarization in K-depleted cells, which could not be explained by a neutral pump, because the diffusion constants for K and Rb in free solutions are similar. We have observed that in smooth muscle the hyperpolarization during Rb accumulation is larger than during K accumulation. This finding can more easily be reconciled with an electrogenic pump than with a neutral one, because in smooth muscle as in striated muscle the membrane conductance is lower in solutions containing Rb than in solutions containing K (unpublished observations). Our observations that the hyperpolarization of K-depleted smooth muscle tissues is larger in recovery solutions containing large anions than in chloride solutions support the hypothesis of an electrogenic Na pump. An increase of the $[K]_i/[K]_o$ ratio due to the action of an electroneutral pump would produce a smaller hyperpolarization in propionate solution than in chloride solution, because large anions not only decrease the membrane conductance by cancelling the chloride conductance but also by reducing the K conductance (Casteels, 1971; Casteels *et al.* 1971).

The potential changes, occurring during K reaccumulation can be explained by an equation given by Finkelstein & Mauro (1963) and Kornacker (1969).

$$E = (\Sigma g_\gamma)^{-1}.i_p + \frac{\Sigma g_\gamma . E_\gamma}{\Sigma g_\gamma}, \qquad (1)$$

where E is the membrane potential, g_γ the membrane conductance for the γth ion, defined by $i_\gamma/(E-E_\gamma)$ where i_γ represents the net passive current of the γth ion, i_p is the pump current and E_γ the equilibrium potential for the γth ion. This equation can be deduced from Ohm's law and makes a clear distinction between the electrogenic component $(\Sigma g_\gamma)^{-1}.i_p$ and the ionic component $(\Sigma g_\gamma . E_\gamma)/\Sigma g_\gamma$ of the membrane potential. From this

equation we can deduce that a necessary and a sufficient condition for an electrogenic Na pump is that the membrane potential is more negative than the ionic component $(\Sigma g_\gamma . E_\gamma)/\Sigma g_\gamma$. E_K is the most negative equilibrium potential, and is consequently more negative than the ionic component $(\Sigma g_\gamma . E_\gamma)/\Sigma g_\gamma$, which is a weighed mean of the three equilibrium potentials (E_{Na}, E_K and E_{Cl}). It can therefore be concluded that if the membrane potential is more negative than E_K, the existence of an electrogenic pump is proved above all doubt. If, however, E is less negative than E_K, the pump is still electrogenic on condition that E is more negative than $(\Sigma g_\gamma . E_\gamma)/\Sigma g_\gamma$.

Because the membrane resistance in smooth muscle cells is much higher than in striated muscle (Tomita, 1966; Casteels, 1969), one would expect that during Na extrusion the hyperpolarization beyond E_K in smooth muscle would be much larger than in striated muscle. The finding that the difference between E_K and the membrane potential is initially rather small can be explained by assuming that the important decrease of the membrane resistance during K accumulation is mainly due to an increase of the K conductance (g_K). As a consequence the second term in eqn. (1) becomes approximately equal to E_K. The first term or the electrogenic component of this equation remains small in spite of a high value of i_p, because $(\Sigma g_\gamma)^{-1}$ is now very low. The high value of g_K causes the initial short-circuit current to be largely a potassium current. This is also suggested by the finding that the initial rates of Na extrusion and K uptake are about equal.

After the first 10 min the K conductance starts to decrease, while the pumping rate remains high because $[Na]_i$ is still large. As a consequence the membrane potential becomes more negative than E_K. The hyperpolarization becomes still larger if the membrane resistance is increased by replacing chloride by large anions, or by using hypertonic solutions.

The return of the membrane potential to its steady value is partially due to the decrease of the pumping rate, which is determined by $[Na]_i$ and $[K]_o$, and this progressive decrease of the pumping rate limits the contribution of the electrogenic term in spite of an increase of the membrane resistance. At the same time the further decrease of g_K shifts the ionic component of the membrane potential to a more positive value between E_K and E_{Na}.

The fact that at 1·2 mM-$[K]_o$ the membrane potential remains always less negative than E_K does not exclude that also under these conditions the Na pump is electrogenic. Applying eqn. (1), we may conclude firstly that the ionic component has a value which is different from E_K because increasing $[K]_o$ to 1·2 mM does not cause the characteristic increase in g_K, and secondly that the contribution of the electrogenic term to the

TABLE 4. Experimental data, as given by Casteels (1969) from which the net passive fluxes and the corresponding ion currents have been calculated by the equation of Ussing (1949) for a membrane potential of −55 mV

Ion species	Mean value of the unidirectional flux (p-mole.cm^{-2}.sec^{-1})	Extracellular ion concentration (m-mole/l.)	Intracellular ion concentration (m-mole/l.)	Equilibrium potential (mV)	Net passive flux (p-mole.cm^{-2}.sec^{-1})	Ionic current (μA.cm^{-2})
Na^+	6	137	13	+62	+7	+0·7
K^+	5·4	5·9	164	−89	−4	−0·4
Cl^-	9·4	134	58	−22	−6	+0·6

membrane potential remains low in spite of a high membrane resistance because the pumping current i_p is small at this low $[K]_o$.

The effect of lowering the temperature on the relation between E_K and the membrane potential can be explained similarly. One must take into account that changing the temperature not only affects the pumping rate, but also changes the membrane conductances.

The above experimental results prove that the Na pump is electrogenic in smooth muscle cells under some experimental conditions. The hypothesis that this pump plays a direct role in the maintenance of the normal resting potential of taenia coli cells was somehow suggested by the immediate depolarizing action of ouabain on these cells (Casteels, 1966) and was further supported by the recent study of the Goldman potential in smooth muscle cells (Casteels, 1969). The potential of -37 mV calculated by the Goldman equation can be considered as the value of the diffusion potential, i.e. the potential at which the sum of the ionic currents is zero for the measured values of ion concentrations and membrane permeabilities. By applying the equation

$$\ln \frac{m_i}{m_o} = -\frac{zF}{RT}(E - E_\gamma) \quad \text{(Ussing, 1949)}$$

for calculating the ratio of the passive unidirectional fluxes, on the flux values given by Casteels (1969), we have calculated the net passive fluxes and the corresponding ion currents as summarized in Table 4. Considering only the currents carried by Na, K and Cl, a net inward current of $+0{\cdot}9\ \mu A.cm^{-2}$ is calculated. In steady-state conditions the net passive fluxes must equal the active fluxes. Therefore the electrogenic pump generates a current of $-0{\cdot}9\ \mu A.cm^{-2}$. In order to calculate the potential produced by this current we have to introduce the value of the chord conductance. Because the current–voltage relation for taenia coli cells is almost linear in the potential range between -130 mV and $+10$ mV (Kumamoto & Horn, 1970) we may assume that the slope and the chord conductance are almost equal. Introducing the slope resistance of $25\ k\Omega.cm^2$ given by Tomita (1966) as the value of the chord conductance, a value of -22 mV is found for the electrogenic contribution to the membrane potential. The membrane potential under steady-state conditions would then amount to -59 mV, which is in good agreement with the measured value.

The authors are indebted to Mr Y. Parijs and Mr J. Cuypers for their skilled technical assistance. The work was supported in part by research grant 1182 from the F.G.W.O. (Belgium).

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