Characteristics of Electrogenic Sodium Pumping in Rat Myometrium

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ABSTRACT Sodium-rich myometrium, obtained from the uteri of pregnant rats, rapidly hyperpolarized when 4.6–120 mM potassium was added to the bathing medium at 37°C. Hyperpolarization was due to sodium pumping since the process was markedly temperature dependent, was abolished by ouabain, and required both intracellular sodium and extracellular potassium. The observed membrane potential exceeded the calculated potassium equilibrium potential during hyperpolarization providing evidence that sodium pumping was electrogenic. Hyperpolarization was reduced in the presence of chloride. The rate of sodium pumping may influence potassium permeability since potassium apparently did not short-circuit the pump during hyperpolarization.

The sodium pump in skeletal muscle (Kernan, 1962; Cross, Keynes, and Rybová, 1965; Adrian and Slayman, 1966), in cardiac muscle (Page and Storm, 1965; Tamai and Kagiyama, 1968), in snail, leech, and *Aplysia* neurons (Kerkut and Thomas, 1965; Nicholls and Baylor, 1968; Carpenter and Alving, 1968; Thomas, 1969), in crustacean stretch receptors (Nakajima and Takahashi, 1966), and in mammalian nonmyelinated nerve fibers (Rang and Ritchie, 1968) can be electrogenic. In these tissues the sodium pump is capable of generating a potential difference across the cell membrane by the extrusion of sodium. The presence and properties of such a pump have been most readily demonstrated and studied during the net extrusion of sodium from tissues whose intracellular sodium content has been artificially elevated. Electrogenic sodium pumping in such tissues occurs only in the presence of potassium or a suitable cation substitute and is abolished by procedures (e.g. removal of external potassium or lowering the temperature) and by drugs (e.g. ouabain) which inhibit the sodium pump. The characteristic feature of such pumps is that during sodium extrusion, the observed membrane potential (E_m) exceeds the calculated potassium equilibrium potential (E_K) (Straub, 1967).

Until recently it was uncertain whether the sodium pump in rat myometrium was neutral or electrogenic. It appeared, however, that in this tissue sodium efflux was not tightly linked to potassium influx and therefore the existence of an electrogenic sodium pump had to be considered (Daniel, 1963 *a*, *b*).

When uteri, obtained from pregnant rats, were exposed to potassium-free Krebs solution at 4°C, they gained sodium and lost potassium (i.e. the tissues became sodium-rich). The myometrial cells of these tissues had a membrane potential of about −15 mv, inside negative (Taylor, Paton, and Daniel, 1969). Upon the addition of 4.6 mM potassium at 37°C to such tissues E_m increased within 2 min to about −70 mv. At this time no significant changes in ion content had occurred and E_m exceeded E_K by 5–14 mv, the value of E_K depending on the volume of the extracellular space used in the calculation of E_K. 30–40 min later spontaneous mechanical and electrical activity commenced and E_m had decreased to −46 mv (Taylor et al., 1969). These findings provided evidence that electrogenic sodium pumping was occurring.

The studies presented here were designed to investigate further the changes in membrane potential produced by exposure of sodium-rich myometrium from pregnant rats to potassium and to determine whether the changes were due to the operation of an electrogenic sodium pump. Preliminary accounts of this work have been presented elsewhere (Taylor and Paton, 1969; Daniel, Paton, Taylor, and Hodgson, 1970; Daniel, Robinson, Kidwai, Wolowyk, Taylor, and Paton, in press).

METHODS

1. *Tissue Preparation*

Pregnant rats (Wistar strain) were killed near term (about 20 days pregnant). A segment of uterus (about 70 mm × 30 mm) was mounted serosal surface uppermost on a neoprene block in an organ bath by means of two pins at one end and two silk ties at the other connected to a force displacement transducer (Grass [Medical Instruments, Quincy, Mass.] Model FT 03C) via a plastic fulcrum. A resting tension of 0.5–1.0 g was applied to all tissues and tension changes were monitored on a polygraph (Grass: Model 5A).

Uteri from pregnant rats were used in this study because the E_m of myometrial cells could be recorded more easily and for longer periods in such tissues as compared to uteri from nonpregnant rats. However, preliminary results we have obtained indicate that sodium pumping by myometrium from nonpregnant rats is also electrogenic and has similar properties to those found in the present study. The endometrium was not removed from the myometrium because this procedure was found to be difficult and traumatic when performed on uteri from pregnant animals. The recovery of ionic gradients in sodium-rich myometrium was less complete in such cases than in intact undissected tissues.

2. *Electrical Recording*

Membrane potentials of myometrial cells were measured with glass capillary microelectrodes filled with 3 M potassium chloride and 48–80 MΩ resistance. Glass tubing (Corning [Glass Works, Corning, N. Y.]: 7740 tubing, about 1.18 mm O.D. × 0.44 mm I.D.) was drawn into fine pipettes with a microelectrode puller (Industrial Science Associates, Inc., Ridgewood, N. Y.). The electrodes were filled with water: methanol mixture (50:50 v/v) under reduced pressure and left to stand overnight in 3 M potassium chloride to fill by diffusion. The microelectrode was mounted on a platinum wire of 0.002 inch diameter formed into a helix of 1 cm diameter and approximately 1½ revolutions. The microelectrode was connected via a high input impedance probe head to a negative capacitance electrometer (Model A-35, Medistor Instrument Co., Seattle, Wash.) and the membrane potential was displayed on a polygraph (Grass: Model 5A). An Ag:AgCl reference electrode completed the circuit.

Tip potentials were measured by breaking the microelectrode after a series of penetrations; data from electrodes with tip potentials greater than 5 mv were rejected. In some experiments 1 μM thorium chloride was added to the organ bath to minimize tip potentials (Agin and Holtzman, 1966). No correction was made for differences in junction potentials between the microelectrode and intracellular fluid. Any small changes in the junction potential that occurred when the bathing medium was changed were balanced out before penetrations were made.

Impalements were accepted if they fulfilled the following criteria: (*a*) a sharp negative deflection was produced upon penetration, (*b*) the potential remained stable for at least 4 sec, and (*c*) the potential returned to the original base line upon withdrawal from the cell (Taylor, Paton, and Daniel, 1969).

3. *Determinations of Ion Content of Tissues*

50–100 mg samples of uterine tissues were used for ion content analysis and were maintained under tension similar to tension maintained for the tissues used for membrane potential measurements. Samples were removed from the organ bath, blotted carefully, weighed, dried in an oven at 105°C for 48 hr, and reweighed. The samples were then dissolved in 0.2 ml concentrated nitric acid and 0.1 ml hydrogen peroxide at 200°C and dried to a white powder. The residue was dissolved in 25 ml distilled water and the ion content determined by flame photometry. Ion contents are expressed in terms of the wet weight of the tissue.

4. *Determination of Extracellular Space*

Measurements of the extracellular space were made by following the uptake of inulin-^{14}C (0.05 μCi/ml:33.6 μg/ml) over a 3–4 hr period, by uterine segments obtained from pregnant rats (*a*) after 30 min immersion in Krebs solution and (*b*) after overnight immersion at 4°C in Krebs solution. After the appropriate uptake period the tissues were rapidly removed, rinsed for 1 or 2 sec in the corresponding solution free of inulin-^{14}C, and blotted gently. The tissues were then weighed and each tissue was dissolved in a scintillation vial using NCS solubilizer (Amersham-Searle Corp., Don Mills, Ontario, Canada). To each vial Bray's phosphor (Bray, 1960) was then

added and total ^{14}C counted in a scintillation spectrometer (Picker X-Ray Corp., White Plains, N.Y.). Duplicate 1.0 ml aliquots of the uptake media were counted with each set of samples. Each sample was counted for at least 10 min and corrected for quenching, using the channels ratio method. Inulin-^{14}C uptake was expressed as ml per 100 g tissue (wet weight). The space occupied by inulin-^{14}C did not increase significantly between 2 and 4 hr incubation. The inulin space was 50.3 ± 1.7 and 52.1 ± 2.4 ml per 100 g in fresh and sodium-rich tissues, respectively. In all determinations of E_K, the extracellular space was assumed to be 52.1 ml per 100 g.

5. *Solution*

The composition of the solutions used is given in Table I. All solutions were made using double-distilled water and were equilibrated with 95% oxygen and 5% carbon

TABLE I

COMPOSITION OF SOLUTIONS

Solution	NaCl	$NaHCO_3$	NaH_2PO_4	$NaCH_3SO_4$	LiCl	KCl	$KHCO_3$	$CaCl_2$	$MgSO_4$	Glucose	Sucrose
	mM per liter	*mM per liter*	*mM per liter*	*mM per liter*	*mM per liter*	*mM per liter*	*mM per liter*	*mM per liter*	*mM per liter*	*mM per liter*	*mM per liter*
Krebs	115.5	21.9	1.2	—	—	4.6	—	2.5	1.2	49.2	—
Potassium-free Krebs	120.1	21.9	1.2	—	—	—	—	2.5	1.2	49.2	—
Sodium-free, sucrose substituted	—	—	—	—	—	4.6	—	2.5	1.2	49.2	250.8
Sodium-free, lithium-substituted	—	—	—	—	138.6	—	5.7	2.5	1.2	49.2	—
Low chloride	—	21.9	1.2	115.5	—	4.6	—	2.5	1.2	49.2	—

dioxide at 37°C, unless otherwise stated. The pH of solutions was initially 7.3–7.5, any required adjustment in pH having been made with tris (hydroxymethyl)aminomethane.

Krebs solutions containing varying concentrations of potassium were prepared by the substitution of KCl for NaCl.

6. *Statistical Analysis*

The variability of samples is expressed as mean ± standard error. Significant differences between samples were determined using Student's *t* test.

RESULTS

As reported previously (Taylor et al., 1969) uteri immersed for approximately 18 hr in potassium-free Krebs solution at 4°C gained sodium (about 70 mEq/kg) and lost potassium (about 67 mEq/kg). After being rewarmed to 37°C in the continued absence of potassium they had a membrane potential of about

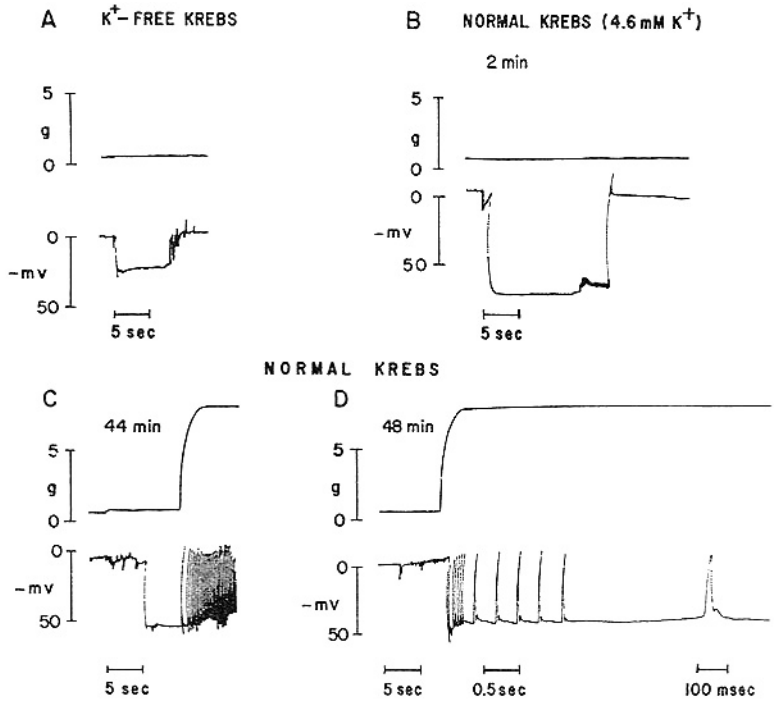

FIGURE 1. Recovery of the membrane potential and spontaneous contractility of a sodium-rich uterus when exposed to Krebs solution. Panel A shows the E_m recorded in sodium-rich myometrium in potassium-free Krebs solution at 37°C. Panel B shows the large increase in E_m measured 2 min after the addition of Krebs solution, containing 4.6 mM potassium; no spontaneous activity was present at this stage. Panel C shows the onset of spontaneous contractility, at an E_m of about −50 mv, 44 min after the addition of Krebs solution to a sodium-rich tissue. Panel D shows spontaneous electrical and mechanical activity after 48 min.

−15 mv. Upon the addition of 4.6 mM potassium to the bathing medium at 37°C the membrane potential of myometrial cells increased rapidly to about −70 mv within 1–2 min. 30–40 min later E_m had decreased to −46 mv and spontaneous electrical and mechanical activity commenced. One such experiment is shown in Fig. 1.

Evidence that the Hyperpolarization is Produced by the Sodium Pump

A possible explanation for the hyperpolarization which occurred on the addition of potassium to sodium-rich tissues was that the potassium was activating the sodium pump, the subsequent extrusion of sodium by the pump producing the increase in E_m. In order to examine this possibility we determined the effects of various procedures known to inhibit the sodium pump on the increase in E_m produced by potassium.

In other tissues electrogenic sodium extrusion has been shown to be inhibited by removal of external potassium (Carpenter and Alving, 1968;

Moreton, 1969), by ouabain (Rang and Ritchie, 1968; Tamai and Kagiyama, 1968), or by lowering the temperature to 0°C (Cross et al., 1965). In addition such pumps are unable to extrude lithium from tissues loaded with lithium instead of sodium (Tamai and Kagiyama, 1968; Thomas, 1969).

a. THE EFFECTS OF TEMPERATURE

The effect of adding 4.6 mM potassium to the solution bathing sodium-rich muscles was tested at three different temperatures (4°, 25°, and 37°C).

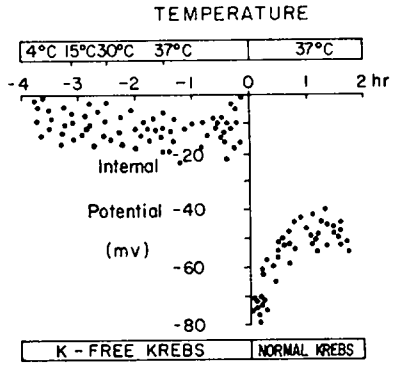

FIGURE 2. Effect of temperature increase and potassium addition on the membrane potential of sodium-rich myometrium of pregnant rats. Sodium-rich uteri were incubated in potassium-free Krebs solution and E_m recorded as the temperature of the bathing medium was increased from 4° to 37°C (shown to the left of the ordinate). At zero time, Krebs solution at 37°C was added and the subsequent E_m determinations are shown to the right of the ordinate. Each point represents a single penetration. Ordinate is E_m (in millivolts, inside negative).

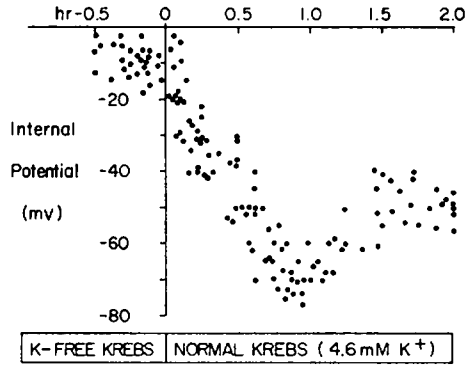

FIGURE 3. Recovery of the membrane potential of sodium-rich myometrium of pregnant rats at 25°C. The E_m of sodium-rich myometria in potassium-free Krebs solution at 25°C were determined and are shown to the left of the ordinate. At zero time, Krebs solution, containing 4.6 mM potassium, was added to the tissues at 25°C and the changes in E_m monitored. Each point represents a single penetration, the data being plotted from three experiments. Ordinate is E_m (in millivolts, inside negative).

At 4°C addition of potassium had no effect on the membrane potential and no mechanical activity occurred. When the temperature was increased from 4° to 37°C in potassium-free solution there was no change in membrane potential (Fig. 2) but addition of potassium at 37°C caused a rapid hyperpolarization, the membrane potential reaching −70 to − 80 mv within 1 or 2 min (Fig. 2). The membrane potential then decreased to about −50 mv in 40 min.

When potassium was added at 25°C the hyperpolarization developed very slowly over a period of 45–70 min (Fig. 3). Bursts of action potentials, accompanied by contraction, occurred when the membrane potential approached −50 mv.

b. THE EFFECTS OF OUABAIN

A large concentration of ouabain (10^{-3} M) was used throughout these experiments as this concentration had previously been found necessary for the induction of downhill ion movements in freshly dissected uterine horns and for the prevention of recovery following sodium enrichment (Daniel, 1964).

The effect of ouabain on the recovery of the membrane potential of sodium-rich myometria at 37°C is shown in Fig. 4. Ouabain was added 10 min before changing the bathing medium from potassium-free Krebs solution to Krebs solution containing 4.6 mM potassium. The presence of ouabain completely inhibited recovery of the membrane potential. When the ouabain was washed out, a slow increase in membrane potential was observed indicating the re-

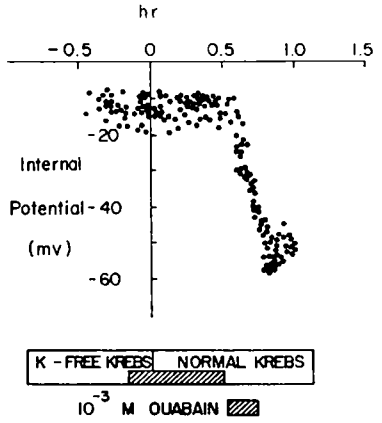

FIGURE 4. Effect of ouabain on the recovery of the membrane potential of sodium-rich myometrium of pregnant rats. The E_m of a sodium-rich myometrium in potassium-free Krebs solution at 37°C was determined and is shown to the left of the ordinate; 10^{-3} M ouabain was present for the final 10 min in potassium-free Krebs solution. At zero time, Krebs solution, containing 4.6 mM potassium and 10^{-3} M ouabain, was added to the tissues and E_m measured. After a further 30 min, ouabain was removed. Each point represents a single penetration. Ordinate is E_m (in millivolts, inside negative).

versible nature of the effect of ouabain. 15–20 min after washing out the ouabain the membrane potential in 3 experiments was −54 ± 1.0 mv (12 penetrations). Approximately 30 min later spontaneous mechanical and electrical activity appeared.

C. THE EFFECTS OF REMOVAL OF EXTERNAL POTASSIUM

As discussed earlier, potassium was essential for the development of hyperpolarization. When sodium-rich myometria to which 4.6–18.4 mM potassium

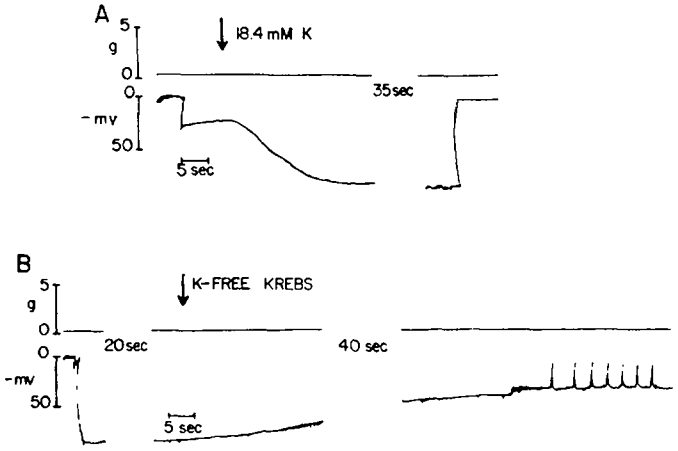

FIGURE 5. Effect of potassium on the membrane potential of sodium-rich myometrium from a pregnant rat. Panel A shows the effect of the addition (at the arrow) of 18.4 mM potassium at 37°C to a sodium-rich uterus; after 35 sec, the microelectrode was withdrawn. Panel B shows the effects of the addition (at the arrow) of potassium-free Krebs solution to a sodium-rich tissue recovering in 18.4 mM potassium.

had been added shortly before, were subjected to the removal of potassium from the bathing medium a rapid depolarization resulted (Fig. 5).

d. THE EFFECTS OF LITHIUM

Segments of uteri were caused to accumulate lithium by exposure at 4°C for 18 hr to a solution containing no sodium or potassium, but to which lithium had been added. The membrane potential of the myometria of such tissues was −7.1 ± 1.8 mv (52 penetrations in 3 tissues). After 40 min exposure to a solution containing 4.6 mM potassium to which lithium had been added, the membrane potential had not altered and was −7.0 ± 0.4 mv (30 penetrations). Likewise the addition of neither a solution containing 46 mM potassium to which lithium had been added, nor of Krebs solution containing 4.6 mM potassium for 60 min, produced any increase in membrane potential in such tissues.

Effect of Potassium on Hyperpolarization

The above studies established that the hyperpolarization resulting from the addition of potassium to sodium-rich uteri was dependent upon sodium pumping which appeared to be electrogenic. We have shown previously that when sodium-rich myometrium was exposed to 4.6 mM potassium E_m exceeded E_K by 5 mv during the early phase of sodium extrusion, providing further evidence that sodium pumping in this tissue was at least partially electrogenic (Taylor et al., 1969). The nature of sodium pumping in rat myometrium has been studied further using concentrations of potassium from 0.6 to 120 mM.

In general, the rate of recovery of membrane potential and of spontaneous contractility following the addition of potassium was inversely related to the potassium concentration, e.g. at a concentration of 120 mM, contractility commenced within 5–10 min whereas at a concentration of 0.6 mM, contractility only returned after about 300 min. Four tissues exposed to potassium, 2.3 mM, did not develop a membrane potential greater than −60 mv.

The exposure of sodium-rich myometria to 46 mM potassium produced marked hyperpolarization within 1–2 min of its addition, the membrane potential being -77.4 ± 0.4 mv (56 penetrations in 7 tissues). The ion content changes occurring during recovery in 46 mM potassium were also determined. For this purpose, samples of sodium-rich tissues were analyzed for their sodium, potassium, and water contents (*a*) in the sodium-rich state (immediately before the addition of potassium), (*b*) 2 min after the addition of 46 mM potassium (at the time when hyperpolarization was present), and (*c*) when the first spontaneous contraction occurred (at about 12 min after the addition of potassium). The ion and water contents at these times as well as the calculated potassium equilibrium potentials (E_K) are shown in Table II. After 2 min exposure to 46 mM potassium, the tissues had gained about 19 mEq potassium and lost 18 mEq sodium/kg wet weight. Potassium-free Krebs solution contained 138 mM sodium and zero potassium whereas the modified Krebs solution contained 92 mM sodium and 46 mM potassium. Thus on the basis of an inulin space of 52.1 ml/100 g the tissues would be expected to have gained about 23 mEq potassium and lost about 23 mEq sodium/kg wet weight as a consequence of the change in the ionic composition of the extracellular fluid. It appears likely therefore that the changes in ions found at 2 min mainly reflect changes in the composition of the bathing medium. At this time the membrane potential had hyperpolarized from -16.4 ± 0.8 mv to -77.0 ± 0.9 mv while E_K had fallen from a very high value to between −9.0 and +2.5 mv. After 12 min exposure, about 41 mM sodium had been lost and 43 mM potassium gained. Clearly at this time intracellular sodium had been extruded and potassium had been reaccumulated intracellularly since the observed changes cannot be accounted for by the alterations in the ionic

TABLE II

RECOVERY OF ION CONTENT AND MEMBRANE POTENTIAL OF SODIUM-RICH UTERI OF PREGNANT RATS IN KREBS SOLUTION CONTAINING 46 mM POTASSIUM

	In potassium-free Krebs solution (Na-rich)	In Krebs solution, containing 46 mM potassium	
		After 2 min	After 12 min
Na_t	138 ± 3.9 (35)	120 ± 2.0 (39)	97 ± 1.5 (39)
K_t	13 ± 1.3 (35)	32 ± 1.1 (39)	56 ± 1.4 (39)
H_2O_t	829 ± 3.9 (38)	831 ± 3.4 (39)	824 ± 3.5 (39)
E_m	−16.4 ± 0.8 (42)	−77.0 ± 0.9 (31)	−47.9 ± 0.9 (30)
K_i	41.9	41.9*	106.5
		64.5‡	
E_K	−161.1§	+2.5*	−21.7
		−9.0‡	

Na_t, K_t, total tissue sodium or potassium content (in mEq/kg wet weight).
H_2O_t, total tissue water content (in g/kg wet weight).
E_m, observed membrane potential (in mv, inside negative).
K_i, calculated intracellular potassium content (in mEq/liter).
E_K, calculated potassium equilibrium potential (in mv).
Numbers in parentheses indicate numbers of tissues used except in the case of E_m where the numbers of penetrations are indicated.
* Calculated assuming that all potassium gained was extracellular.
‡ Calculated assuming that 50% of extracellular space had equilibrated with Krebs solution, containing potassium, 46 mM.
§ Calculated assuming an extracellular potassium concentration of 0.1 mEq/liter.

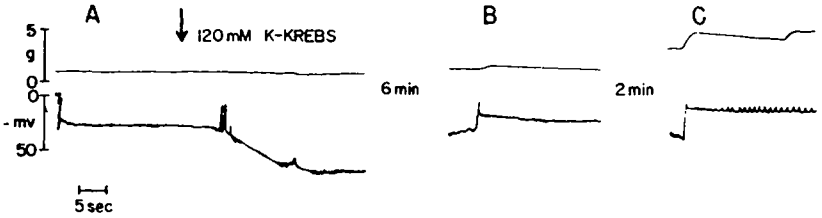

FIGURE 6. The effect of Krebs solution containing 120 mM potassium on the recovery of sodium-rich myometrium. Panel A shows the changes produced by the addition (at the arrow) of Krebs solution containing 120 mM potassium to a sodium-rich tissue. Panel B shows the first spontaneous contraction in the same solution 6 min later. Panel C shows further spontaneous contractions 2 min later.

content of the extracellular fluid. At this time the observed membrane potential was −47.9 ± 0.9 mv and still exceeded E_K which was about −22 mv.

The addition of 120 mM potassium to sodium-rich tissues also produced rapid hyperpolarization, the membrane potential being −79 ± 0.9 mv (16 penetrations in 3 tissues). The membrane potential had fallen to about −40 mv 5 min later (Fig. 6).

Thus the addition of potassium to sodium-rich tissues in concentrations of

4.6, 9.2, 46, and 120 mM resulted, within 2 min, in observed membrane potentials of -69.4 ± 1.7, -76.2 ± 0.8, -77.0 ± 0.9, and -79.0 ± 0.9 mv (inside negative), respectively.

Effects of Other Ions on Hyperpolarization

a. SODIUM

The addition of a solution in which sucrose was substituted for sodium and which contained 5.7 mM potassium to sodium-rich uteri resulted in an immediate hyperpolarization to -70.6 ± 0.5 mv (30 penetrations in 3 tissues)

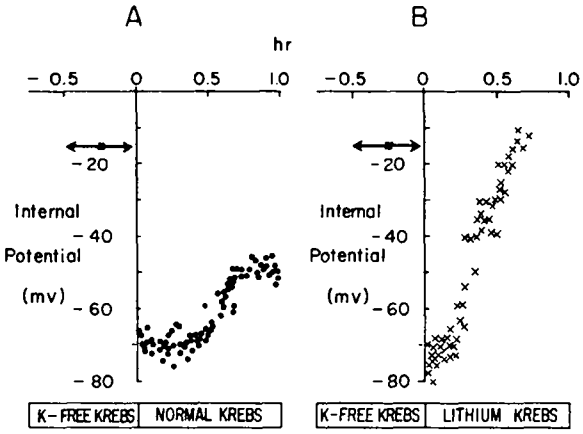

FIGURE 7. Effect of a solution to which lithium had been added, which was sodium-free, on the membrane potential of sodium-rich myometrium. The two graphs show the change in E_m produced by the addition of either Krebs solution (A) or sodium-free solution to which lithium had been added (B). The horizontal lines to the left of each ordinate represent the main $E_m \pm$ SE in potassium-free Krebs solution. Each point represents a single penetration. Ordinate is E_m (in millivolts, inside negative).

followed by a subsequent decline in E_m to about -30 mv in 30 min. Similarly the addition of a solution in which lithium was substituted for sodium and which contained 4.6 mM potassium to sodium-rich uteri produced an increase in membrane potential to -74.4 ± 1.2 mv (18 penetrations in 2 tissues), the membrane potential declining over the next 30 min to about -20 to -30 mv (see Fig. 7).

b. CHLORIDE

If the hyperpolarization reflected the functioning of an electrogenic sodium pump, the E_m should increase when the short-circuiting effect of outwardly moving chloride ions was removed. In order to examine this possibility, uteri were rendered sodium-rich by exposure to a low chloride solution, which was potassium-free, at 4°C for 18 hr. The addition of a low chloride solution con-

taining 4.6 mM potassium to such uteri resulted in rapid hyperpolarization to -79.9 ± 0.6 mv (19 penetrations in 2 tissues) as compared to a control value of -70.2 ± 0.7 mv (25 penetrations in 2 tissues) when Krebs solution was added to other portions of the same uteri, rendered sodium-rich in the usual manner.

Effect of Inhibition of the Sodium Pump on the Membrane Potential of Fresh Tissues

Because the hyperpolarization of sodium-rich uteri caused by potassium appeared to depend upon the activity of the sodium pump, the effect of inhibition of the sodium pump on the membrane potential of myometrial cells was examined immediately after the removal of the uteri from the animals.

The withdrawal of external potassium did not alter the membrane potential of such myometria; after 3 hr in potassium-free Krebs solution the membrane potential of three tissues was -49.3 ± 0.7 mv (27 penetrations) and did not differ significantly from the control value of -50.2 ± 0.2 mv (39 penetrations). By contrast, as described earlier, the removal of external potassium from sodium-rich myometria recovering in the presence of 4.6–18 mM potassium, resulted in an immediate depolarization (Fig. 5).

The effects of 10^{-3} M ouabain on four fresh uteri were also determined. These myometria had control membrane potentials of -48.3 ± 0.5 mv. The addition of ouabain produced within 5–10 min a small but significant decrease in membrane potential to -44.1 ± 0.8 mv accompanied by a sustained contraction; after 60 min exposure, a larger decrease in membrane potential to -33.2 ± 0.6 mv had occurred.

DISCUSSION

The main finding in this study was that a marked hyperpolarization occurred in response to the addition of 4.6–120 mM potassium to sodium-rich tissues. The observed hyperpolarization was produced by the operation of the sodium pump since it was abolished by ouabain, low temperature, absence of intracellular sodium or of extracellular potassium, procedures known to inhibit the sodium pump (Baker, 1967). Further, the properties of this pump are clearly similar to those of the Na^+-K^+–activated adenosinetriphosphatase enzyme (Skou, 1965).

Considerable evidence was obtained that sodium pumping in this tissue is electrogenic. The first evidence arises from a consideration of the changes with time of E_m resulting from the addition of potassium to sodium-rich uteri; E_m was largest soon after the addition of potassium when very little potassium had been reaccumulated intracellularly and E_m fell towards the level found in fresh tissues as potassium was reaccumulated. Second during recovery E_m was dependent upon sodium pumping; rapid depolarization occurred on

removal of extracellular potassium. By contrast the E_m of fresh tissues was not altered by removal of extracellular potassium presumably because in such tissues E_m is maintained mainly by passive ion fluxes dependent on the electrochemical potentials and permeabilities to various ions. Furthermore during recovery E_m exceeded E_K. These findings could be produced by electrogenic but not by neutral sodium pumping.

The calculated value of E_K is, however, open to question for several reasons: (*a*) The exact size of the extracellular space is uncertain, thus making calculation of intracellular ion concentrations difficult (Kao, 1967). (*b*) If the concentration of potassium immediately outside the cell membrane is less than in the bathing medium, the value for E_K calculated by using the concentration of potassium in the external medium would underestimate the actual value of E_K (Page and Storm, 1965; Adrian and Slayman, 1966). (*c*) Compartmentation of potassium may occur intracellularly so that the concentration of potassium immediately inside the cell membrane is higher than within the rest of the intracellular water while some of the intracellular water may be bound or sequestered. Either of these possibilities would produce an increase in the intracellular concentration of potassium and hence E_K would increase. However, none of these possibilities appears to be a very likely explanation for the discrepancy between E_m and E_K noted when 46 or 120 mM potassium was added.

The pieces of uteri of whole wall thickness used for ion determinations in this study included both myometrium and endometrium. Attempts to separate the myometrium from the endometrium using uteri from pregnant rats showed that the process was very traumatic to the tissue as shown by a reduced ability to maintain a normal ionic composition. As pointed out by Kao (1967) the ionic compositions of epithelial and muscular tissues may differ considerably thus making it difficult to interpret the ionic contents of mixed tissues. In view of this the observations summarized in Table II are particularly important. 2 min after the addition of 46 mM potassium E_m had hyperpolarized to -77.0 mv. At this time the tissue had gained a small amount of potassium at least some of which can be accounted for by that present in the bathing medium. It seems very unlikely that under these circumstances E_K could have equaled E_m since a large amount of potassium would have had to be gained by the myometrium very rapidly and the intracellular concentration of potassium in the myometrium would have had to be considerably higher than any previously reported (see review of Kao, 1967, for values for rat myometrium). Thus in spite of the problems associated with the accurate determination of E_K, the marked hyperpolarization observed cannot be accounted for in terms of E_K and thus reflects the activity of an electrogenic sodium pump.

Mammalian nonmyelinated nerves have been shown to possess an electrogenic sodium pump; in this tissue, posttetanic hyperpolarization was increased

gradually over 10–15 min from about −3 mv to about −20 mv when external chloride was replaced by an impermeant anion (Rang and Ritchie, 1968). As these authors have pointed out, if an electrogenic sodium pump is responsible for the hyperpolarization observed, then "removal of the short-circuiting effect of chloride ions moving outwards would be expected to increase the potential developed." Similarly in our studies we found that the hyperpolarization produced by the addition of potassium was increased when the internal chloride concentration was markedly reduced by prolonged exposure to a low chloride solution. The small increase in E_m produced by this alteration may be due to the fact that the external chloride concentration was 9.6 mM. There was a nonlinear relationship between the external chloride concentration and the amplitude of the posttetanic hyperpolarization developed in nonmyelinated nerves; 10 mM chloride reduced the hyperpolarization to 56% of the amplitude developed in chloride-free medium (Rang and Ritchie, 1968).

In myometrium exposed to normal concentrations of external chloride, ECl has been calculated to be −20 to −30 mv (Casteels and Kuriyama, 1965). Furthermore this value decreases in sodium-rich tissues since total tissue chloride increases (Daniel, 1963 *b*). Therefore the hyperpolarization produced by the addition of external potassium could not have resulted from the movement of chloride ions. Furthermore the finding that exposure of uteri to low chloride solutions did not reduce the hyperpolarization developed, rules out a significant contribution to the hyperpolarization of an inward chloride pump. Such a pump would have been inhibited by reduced external chloride.

In contrast to the findings at 37°C, hyperpolarization developed slowly at 25°C. Since the tissue appears to be undergoing electrogenic sodium pumping at this time the hyperpolarization would be expected to reflect the rate of sodium pumping. Since pumping is apparently due to the activity of the Na^+-K^+–activated ATPase enzyme (Skou, 1965; Baker, 1967) it would be anticipated that pumping would be considerably reduced by a 12°C fall in temperature. Whether this can account entirely for the delay in hyperpolarization, however, is not certain.

In mammalian nonmyelinated nerve fibers the amplitude of posttetanic hyperpolarization produced by electrogenic sodium pumping declined at external potassium concentrations greater than 2 mM (Rang and Ritchie, 1968). These workers suggested that this might result from potassium short-circuiting the sodium pump. By contrast in the present study potassium did not apparently short-circuit the pump, the E_m developed at potassium concentrations of 4.6–120 mM ranging from −69 to −79 mv. Why short-circuiting did not occur if the sodium pump was working maximally at these concentrations as seems likely is not clear. It is possible that the rate of sodium pumping regulated potassium permeability in some way so that the greater the rate of

sodium pumping, the smaller was the potassium permeability. Most procedures which inhibit sodium pumping in rat uteri have been found to increase potassium permeability (Daniel et al., in press).

The resting membrane potential of fresh tissues was not altered by removal of external potassium and was slightly depolarized by ouabain. These findings suggest that the rate of electrogenic sodium pumping in fresh tissues was considerably lower than in tissues recovering from sodium enrichment. Withdrawal of external potassium would, however, be expected to increase E_m whereas in fact E_m did not alter. Ouabain increased potassium permeability in such tissues (Daniel et al., in press). Both these factors would oppose the effects of E_m thus making the interpretation of the results difficult and resulting possibly in an underestimate of the degree of electrogenic sodium pumping in fresh tissues.

Kao and Nishiyama (1969) have recently reported the absence of any period in which E_m exceeds E_K during recovery of sodium-rich rabbit myometrium. This may represent a species difference between rabbit and rat myometrium. Other possible explanations are the apparent failure in their study to measure membrane potentials immediately after restoring external potassium and the use of tissues which had not been thoroughly depleted of their potassium content.

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