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REGULATION OF CELLULAR VOLUME IN RAT MYOMETRIUM

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SUMMARY

1. Pieces of rat myometrium that had been made Na^+-rich by incubation in K^+-free medium at 4 °C, subsequently lost weight and extruded Na^+ and water on being rewarmed at 37 °C. In the present study the mechanism of these changes was examined.

2. The process was not dependent on the coupled Na^+–K^+ pump since it was not abolished by omission of K^+ or by absence of external Na^+. It was however abolished by metabolic inhibition, produced by anaerobiosis and omission of D-glucose from the medium.

3. Shrinkage on rewarming was not Na^+ dependent, since Na^+ could be replaced by K^+ or Li^+. Shrinkage was prevented when Cl^- was replaced by an impermeant anion, *e.g.* propionate or methylsulphate.

4. In the absence of Ca^{2+}, shrinkage did not occur. The process was also inhibited by iodoacetamide, isopropylnoradrenaline and papaverine.

5. The results are compatible with a mechanochemical mechanism for the ouabain-insensitive control of cell volume. It is possible that water and Na^+ extrusion result from an alteration in ion binding in, and subsequent contracture of, micropinocytotic vesicles. It is also possible that with cooling the plasma membrane undergoes a phase transition with resultant loss of volume control.

INTRODUCTION

Until recently it was generally believed that cellular volume was determined by the balance between passive ion fluxes (*i.e.* a leak component) and active Na^+ extrusion (*i.e.* a pump component), with water following net ionic movements passively[1]. Considerable evidence has, however, been obtained for an additional mechanism contributing to the control of cellular volume in several tissues, including renal cortical cells[2–13], skeletal muscle[14], frog bladder[15] and vascular smooth muscle[16].

Recent studies have also provided evidence for the operation of such a mechanism in rat myometrium: (1) inhibition of coupled Na^+-K^+ pumping was not invariably accompanied by swelling[17] as would be predicted from the pump and leak hypothesis[1]; (2) a ouabain-insensitive and K^+-independent net extrusion of water

Abbreviation: EGTA, ethyleneglycol-bis(β-aminoethyl ether)-*N,N*-tetraacetic acid.

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and Na^+ occurred when Na^+-rich tissues were rewarmed[17–19]; and, (3) exposure of tissues to iodoacetamide did not inhibit coupled Na^+–K^+ pumping but caused tissue swelling[20]. Since several metabolic inhibitors (*e.g.* anoxia, iodoacetic acid, 2,4-dinitrophenol) caused isotonic tissue swelling and inhibited or abolished contractility of rat myometrium, it was suggested[20] that a link might exist in rat myometrium between inhibition of a contractile mechanism and tissue swelling. Kleinzeller and his associates[1,2,11–14] had earlier proposed that a mechanochemical system is involved in volume control in renal cortical cells and in diaphragm.

The purpose of the present investigation was to examine further the mechanisms regulating cellular volume in rat myometrium. For this purpose we have examined the effect of various factors on the loss of weight and extrusion of Na^+ and water that occurs when Na^+-rich segments of rat myometrium are rewarmed.

METHODS AND MATERIALS

Preparation of tissues

Female rats (Wistar strain), weighing 150–170 g, were pretreated with 50 μg estradiol 17-β, injected subcutaneously for 2–3 days. After the animals had been killed by a blow on the head, their uterine horns were rapidly removed and dissected free of surrounding tissues. The endometrium was then removed to obtain myometrial pieces consisting predominantly of longitudinal muscle.

Measurement of weight changes

After dissection, tissues were incubated at 37 °C for 45–60 min in Krebs solution, equilibrated with 95% O_2–5% CO_2. The tissues were then removed and weighed to obtain their "fresh weights". These tissues were then loaded with Na^+, or other cations, by incubation at 4 °C for 18–24 h in appropriate solutions described in detail below. Tissues were then removed, reweighed and incubated in the corresponding solutions at 37 °C for 120 min. Their weights were recorded after 20, 60 and 120 min incubation. All weights are expressed as percentages of the "fresh weight". At the end of this period, tissues were removed for ion analyses.

Solutions

The Krebs solution had the following composition (mM): NaCl, 116; $NaHCO_3$, 22; NaH_2PO_4, 1.2; KCl, 4.6; $CaCl_2$, 1.5; $MgSO_4$, 1.2; and, D-glucose, 20. K^+-free Krebs solution had the same composition except for the omission of K^+. NaCl, $NaHCO_3$ and NaH_2PO_4 were omitted from the following Na^+-free solutions and replaced as indicated: (a) in lithium solution, by LiCl (116 mM) and $LiCO_3$ (22 mM), the pH being adjusted to 7.3 with HCl; (b) in KCl solution, by KCl (116 mM) and $KHCO_3$ (22 mM); and, (c) in sucrose solution, isoosmotically by sucrose (200 mM), histidine (25 mM) being used as the buffer, the pH being adjusted by HCl. All solutions were equilibrated with 95% O_2–5% CO_2. The pH of all solutions was 7.3–7.5.

Determination of ion content of tissues

The Na^+, K^+ and Li^+ contents of tissues were determined by flame photo-

TABLE I

EFFECT OF COOLING AND REWARMING ON RAT MYOMETRIUM

Group I: Tissues incubated in Krebs solution for 45 min after dissection; Group II: Tissues made Na^+ rich and removed for ion analysis after incubation for 18–24 h at 4 °C, and Groups III and IV: Na^+-rich tissues rewarmed in K^+-free Krebs solution at 37 °C for 15 or 120 min. The number of tissues in each experiment was 4–6.

Group and condition		*Tissue weight (% fresh weight)*	*Tissue contents of*							
			Water (g/kg wet wt)		*Solids (g/kg wet wt)*		*Na^+ (mmoles/kg dry wt)*		*K^+ (mmoles/kg dry wt)*	
			final weight	*relative to fresh weight*	*final weight*	*relative to fresh weight*	*final weight*	*relative to fresh weight*	*final weight*	*relative to fresh weight*
I	Fresh	100	823.2 ± 2.5	823.2	176.8	176.8	533.5 ± 15.5	533.5	266.5 ± 15.7	266.5
II	Na^+-rich	111.2 ± 2.4	852.1 ± 5.8 *	947.5	147.9	164.5	876.9 ± 34.5 *	975.1	5.1 ± 2.4 *	5.7
III	Na^+-rich, rewarmed (15 min)	100.4 ± 2.5 **	837.6 ± 5.0 *, **	841.0	162.4	163.0	766.0 ± 23.5 *, **	769.1	6.6 ± 2.8 *	6.6
IV	Na^+-rich, rewarmed (120 min)	90.5 ± 3.7 *, **	831.9 ± 5.9 *, **	752.9	168.1	152.1	800.5 ± 44.7 *, **	724.5	8.7 ± 1.9 *	7.9

* Differences found significant ($P < 0.05$) by Scheffe's test, using fresh tissue as the control.

** Na^+ and water contents in Groups III and IV were significantly difference from those in Group II ($P < 0.05$, Scheffe's test).

metry as described previously[20]. When Li^+ was measured, appropriate corrections were made for interference from K^+.

Chemicals and drugs

The following drugs and chemicals were used in this study and obtained from the sources indicated: ethyleneglycol-bis(β-aminoethylether)-*N*,*N*-tetraacetic acid (EGTA) (Sigma); α-iodoacetamide (Calbiochem); isopropylnoradrenaline hydrochloride (Sigma); and, papaverine hydrochloride (Lilly). Chemicals and drugs were weighed out daily and added directly to the media.

Statistical analysis

The variability of samples is expressed as mean ± standard error of the mean. The significance of differences between paired samples was determined using the paired "*t*" test. Where paired experiments were not done, Scheffe's test for multiple comparisons was used[21]. The differences were regarded as significant when $P<0.05$.

RESULTS

Effects on Na^+-rich tissues of re-warming

Incubation of tissues in K^+- and glucose-free Krebs solution for 18–24 h at 4 °C resulted in a loss of K^+ and a gain of Na^+, *i.e.* the tissues became Na^+ rich (Table I). The net Na^+ gain significantly exceeded the net K^+ loss and was accompanied by net gains in tissue weight and tissue water content. It should be noted however that Na^+ enrichment did not invariably result in tissue swelling; this point is illustrated in subsequent tables.

On re-warming such Na^+-rich tissues at 37 °C in the continued absence of external K^+, tissues rapidly lost weight; the process was essentially complete by

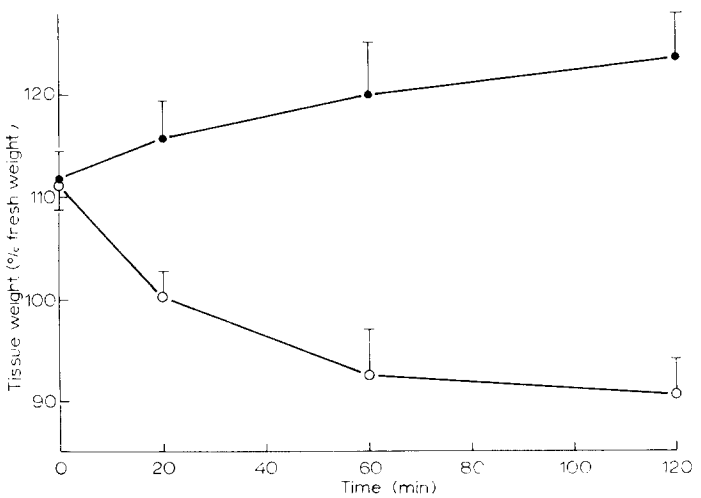

Fig. 1. Effect of temperature on the weight of rat myometrium. Pieces of rat myometrium were incubated at 4 °C for 18–24 h in K^+-free Krebs solution then weighed (weight at zero time). Thereafter they were either re-warmed at 37 °C (○—○) or kept at 4 °C (●—●), and weighed at the time shown. The values of tissue weight (% fresh weight) are the mean ± S.E. ($n=6$).

60 min (Fig. 1). The loss of weight was almost entirely due to loss of tissue water, alterations in the content of tissue solids being very much smaller. The loss of tissue water was also accompanied by a marked loss of Na^+ from the tissues (Table I).

The residual K^+ content of Na^+-rich tissues was extremely small and was not altered by re-warming. Previous studies[17,20,22] have shown that coupled Na^+–K^+ pumping does not occur in Na^+-rich rat myometrium when such tissues are re-warmed in the absence of external K^+. In all subsequent studies changes in tissue weight, and in tissue contents of water and Na^+ were determined in Na^+-rich tissues during re-warming at 37 °C in K^+-free media.

The loss of weight observed on re-warming was not due to excessive handling of the tissues. Na^+-rich tissues kept at 2 °C but otherwise handled identically, did not lose weight but rather gained weight over 120 min, the gain in weight amounting to 12% of fresh weight (Fig. 1).

Factors affecting the response of Na^+-rich tissues to re-warming

(1) Sodium. Previous studies have shown that the control of cell volume in rabbit renal cortex was not dependent upon external Na^+ but that external Na^+ could be replaced by Li^+, Tris or choline[11]. In order to determine whether Na^+ was required for the process responsible for shrinkage on re-warming of Na^+-rich rat myometrium, tissues were loaded with either K^+ or Li^+ by incubation at 4 °C for 18–24 h in the KCl or LiCl solutions. Tissues were then rewarmed at 37 °C in the same solutions. The resultant changes in weight are shown in Table II.

TABLE II

EFFECT OF CATIONS ON WEIGHT CHANGES OF RAT MYOMETRIUM

Pieces of rat myometrium were incubated at 4 °C for 18–24 h in the various media indicated below and then re-warmed at 37 °C. The values of tissue weight (% fresh weight) are the mean ± S.E. (n=4–7).

Media	*Tissue weight (% fresh weight)*				
	After incubation at 4 °C for 18–24 h *A*	*After rewarming at 37 °C for* *20 min* *B*	*60 min* *C*	*120 min* *D*	*Net weight change (D − A)*
K^+-free Krebs	96.5 ± 2.1	92.8 ± 1.9	89.6 ± 1.6	88.8 ± 1.9	− 7.7 ± 1.4
LiCl solution	98.3 ± 1.2	92.5 ± 2.0	88.4 ± 2.6	89.1 ± 3.1	− 9.2 ± 1.6
K^+-free Krebs	102.2 ± 1.4	95.5 ± 1.6	91.1 ± 2.1	90.1 ± 2.7	−12.1 ± 1.7
KCl solution	115.8 ± 1.9*	105.9 ± 1.3*	104.6 ± 1.9*	103.2 ± 2.1*	−12.6 ± 1.9

* $P<0.05$ by paired "t" test.

The weights of tissues loaded with Li^+ did not differ significantly from those of tissues made Na^+ rich. Further, on rewarming at 37 °C, they lost as much weight as did the control Na^+-rich tissues. After re-warming for 120 min at 37 °C, the water contents of tissues exposed to Li^+ did not differ from the Na^+-rich tissues (Table III). Li^+ had also essentially completely replaced both Na^+ and K^+, the contents of

TABLE III

EFFECT OF PROCEDURES ON H_2O AND Na^+ CONTENTS OF RAT MYOMETRIUM

Full details of the conditions to which tissue were subjected may be found in the tables indicated.

Condition	*Details in Table*	*Tissue contents of*			
		Water (g/kg wet wt)	*Na^+ (mmoles/kg dry wt)*	*Li^+ (mmoles/kg dry wt)*	*K^+ (mmoles/kg dry wt)*
K^+-free Krebs	II	817.3 ± 6.5	< 20	747.3 ± 41.9	
LiCl solution		823.2 ± 6.7	734.8 ± 28.3		
K^+-free Krebs	II	811.7 ± 4.3	736.5 ± 16.3		12.3 ± 2.7
KCl solution		830.3 ± 2.9*	19.9 ± 2.4*		836.1 ± 33.5*
Ca^{2+}-free + EGTA	V	866.1 ± 3.6	1218.9 ± 7.5		
Ca^{2+}-free + EGTA + 0.3 mM Ca^{2+}		846.6 ± 4.1*	1046.5 ± 24.0*		
Not depleted	VI	822.6 ± 8.6	760.0 ± 59.0		
Depleted		861.4 ± 6.4*	966.7 ± 65.4*		
No inhibitor	VI	820.9 ± 4.3	799.4 ± 28.4		
Iodoacetamide		845.3 ± 1.8*	934.1 ± 15.2*		
No drug	VII	803.8 ± 3.2	674.6 ± 19.7		
Papaverine		835.9 ± 5.9*	806.5 ± 22.3*		

* $P < 0.05$ by paired "t" test.

Na^+ and K^+ being less than 20 and 10 mmoles/kg dry wt, respectively. This is in keeping with previous studies of Li^+ movements in myometrial tissue[22,23] that demonstrated that Li^+ replaced both Na^+ and K^+ and that K^+ accumulation did not occur in such tissues.

Tissues loaded with K^+ gained considerably more weight than control tissues after incubation at 4 °C for 18–24 h. Under certain conditions other smooth muscles have also been found to swell when exposed to KCl solutions[24]. Rewarming such tissues in the KCl solution led to a rapid loss of weight; the total weight loss at the end of 120 min was not significantly different from that of control tissues, loaded with Na^+. At any given time, the K^+-loaded tissues were, however, more swollen than control, Na^+-rich tissues (Table II).

Tissues that had been re-warmed for 120 min in the KCl solution also had significantly greater water contents than control Na^+-rich tissues (Table III). It should be noted that K^+ had essentially replaced Na^+ on a mole for mole basis in such tissues, *i.e.* they had indeed become K^+ rich, the residual Na^+ content being less than 20 mmoles/kg dry wt.

(*2*) *Anions.* The effect of Cl^- on shrinkage was studied by loading tissues in K^+-free media in which NaCl was replaced by sodium nitrate, sodium propionate or sodium methylsulphate. Tissues were incubated at 4 °C for 18–24 h in such solutions, then rewarmed at 37 °C and the resulting weight changes followed.

Tissues gained weight on overnight incubation in the presence of NO_3^- but rapidly lost as much weight on being rewarmed as did corresponding pairs loaded in a Cl^- medium (Table IV). Tissues placed in sodium propionate overnight did not lose weight on being re-warmed; in fact, a significant increase in weight, amounting

TABLE IV

EFFECT OF ANIONS ON WEIGHT CHANGES OF RAT MYOMETRIUM

Pieces of rat myometrium were incubated at 4 °C for 18–24 h. in various K^+-free media either containing NaCl or a substitute Na^+ salt as indicated below. They were then re-warmed in the same media at 37 °C. The values of tissue weight (% fresh weight) are the mean ±S.E. ($n=4$–6).

Predominant anion in medium	*Tissue weight (% fresh weight)*				*Net weight changes (D − A)*
	After incubation at 4 °C for 18–24 h A	*After rewarming at 37 °C for*			
		20 min B	*60 min C*	*120 min D*	
Chloride	102.7±1.3	99.0±1.0	92.3±1.5	88.8±1.6	−15.0±1.8
Nitrate	114.7±1.6*	108.6±1.9*	98.6±1.3*	96.2±2.3*	−12.6±2.7
Chloride	100.1±4.7	92.9±3.6	91.1±3.1	88.9±2.8	−11.2
Propionate	96.0±2.0	100.0±2.0*	100.7±2.7*	103.5±2.4*	+ 7.5*
Chloride	96.8±3.2	88.6±2.3	86.2±1.9	85.7±2.2	−10.9±2.9
Methylsulphate	89.9±2.6*	87.5±2.3	86.9±2.1	86.9±1.8	− 3.1±2.1*

* $P<0.05$ by paired "t" test.

TABLE V

EFFECT OF CALCIUM ON WEIGHT CHANGES OF RAT MYOMETRIUM

Pieces of rat myometrium were incubated at 4 °C for 18–24 h in K^+-free Krebs or depleted of Ca^{2+} by exposure to Ca^{2+} and K^+-free medium, containing 0.1 mM EGTA. They were then re-warmed at 37 °C under the conditions shown. The values of tissue weight (% fresh weight) are the mean ±S.E. ($n=4$–6)

Conditions	*Tissue weight (% fresh weight)*				*Net weight changes (D − A)*
	After incubation at 4 °C for 18–24 h A	*After rewarming at 37 °C for*			
		20 min B	*60 min C*	*120 min D*	
1.5 mM Ca^{2+}	99.1±2.1	94.7±1.5	92.2±2.0	92.1±2.3	− 7.8±1.2
Ca^{2+} depleted	120.3±1.8*	123.9±4.9*	122.9±6.9*	124.3±2.0*	+ 1.2±2.6*
Ca^{2+} depleted	112.3±1.7	108.5±1.5	111.7±0.9	110.5±1.2	− 1.8±2.6
Ca^{2+} depleted, 0.3 mM Ca^{2+} added	114.1±1.2	109.5±2.5	105.4±2.3*	98.8±1.3*	−15.3±0.4*
Ca^{2+} depleted	116.1±4.1	113.9±2.9	116.3±2.8	118.3±1.7	+ 2.2±3.3
Ca^{2+} depleted, 0.5 mM Ca^{2+} added	116.8±0.9	110.0±1.3	105.8±3.1*	101.6±0.4*	−15.3±0.4*
Ca^{2+} depleted	116.8±2.0	114.3±1.9	115.6±1.1	114.1±0.7	− 1.9±2.2
Ca^{2+} depleted, 0.3 mM Sr^{2+} added	117.2±0.9	110.6±1.9	104.6±1.1*	101.7±1.9*	−15.5±1.4*

* $P<0.05$ by paired "t" test.

to approximately 8%, occurred. Tissues exposed to sodium methylsulphate lost weight on overnight incubation in the cold. Subsequent incubation at 37 °C led to a slight reduction in weight; this was, however, significantly less than that observed with control tissues loaded in the NaCl-containing medium.

(*3*) *Calcium.* Ca^{2+} depletion was produced by incubating tissues for 18–24 h in a K^+- and Ca^{2+}-free Krebs solution, containing 0.1 mM EGTA. This procedure inhibits the contractile response of rat myometrium to all agonists[23]. Tissues thus depleted of Ca^{2+} gained a considerable amount of weight, about 20% (see Table V). On rewarming in the same medium, there was a further slight increase in weight. Paired controls incubated in K^+-free Krebs containing 1.5 mM Ca^{2+}, lost weight on rewarming.

In another set of experiments, Ca^{2+} was restored to the incubation medium before rewarming to determine whether the impairment of weight loss in Ca^{2+}-depleted tissues was reversible. The addition of 0.3 or 0.5 mM Ca^{2+} restored the ability of tissues to lose weight on rewarming in K^+-free Krebs (Table V) and resulted in a net extrusion of Na^+ and H_2O (Table III). Since the incubation media contained 0.1 mM EGTA, the amount of free Ca^{2+} present would be about 0.2–0.4 mM Ca^{2+}. Rewarming Ca^{2+}-depleted tissues that had been subsequently exposed to 0.5 mM Sr^{2+}, resulted in weight loss whereas the control Ca^{2+}-depleted tissues did not do so (Table III). Sr^{2+} could thus adequately substitute for Ca^{2+}.

(*4*) *Metabolic inhibition.* If cellular volume is regulated by a process dependent on metabolic energy, then prior depletion of endogenous metabolic stores should prevent or reduce the extrusion of water produced by rewarming Na^+-rich tissues. Fresh tissues were therefore incubated at 37 °C for 4 h in glucose-free Krebs solution, equilibrated with 95% N_2–5% CO_2. Corresponding pairs were incubated for the

TABLE VI

EFFECT OF ENERGY METABOLISM ON WEIGHT CHANGES OF RAT MYOMETRIUM

In series A, pieces of rat myometrium were incubated at 37 °C for 4 h in Krebs medium in the presence of 20 mM D-glucose ("not depleted group") or in the absence of D-glucose and equilibrated with 95% N_2–5% CO_2 ("depleted group"). All tissues were then incubated in K^+ and glucose-free Krebs medium at 4 °C for 18–24 h then re-warmed at 37 °C. In series B, tissues were exposed to 10^{-4} M iodoacetamide for 30 min prior to rewarming. The values of tissue weight (% fresh weight) are the mean ± S.E. ($n=6$).

	Energy state	*Tissue weight (% fresh weight)*				*Net weight changes (D − A)*
		After incubation at 4 °C for 18–24 h A	*After rewarming at 37 °C for*			
			20 min B	*60 min* C	*120 min* D	
Series A	Not depleted	97.1 ± 2.1	89.4 ± 1.6	87.3 ± 1.7	85.8 ± 1.9	−11.3 ± 1.6
	Depleted	110.8 ± 1.1*	107.5 ± 1.2*	106.7 ± 2.3*	107.8 ± 2.4*	− 3.0 ± 1.0*
Series B	No inhibitor	101.9 ± 2.2	94.0 ± 2.5	91.2 ± 3.9	89.7 ± 5.3	−12.2 ± 3.1
	Iodoacetamide	97.7 ± 1.1	96.1 ± 1.8	101.4 ± 2.2*	102.4 ± 0.6*	+ 4.7 ± 1.2*

* $P<0.05$ by paired "t" test.

same duration in Krebs solution containing 20 mM D-glucose. All tissues were then made Na^+ rich in glucose-free, K-free Krebs solution, rewarmed at 37 °C and weight changes followed. The results (Table VI) show that the tissues that had undergone the pre-incubation under anoxic conditions, gained considerable weight on Na^+ enrichment. The slight loss in weight observed on rewarming such tissues was significantly less than that observed in control tissues (−11%) and, in addition, net extrusion of Na^+ and water was inhibited (Table III). Thus metabolic depletion significantly impaired the extrusion of fluid that occurs on rewarming.

Previous studies have shown that exposure of rat myometrium to 0.1 mM iodoacetamide for 30 min inhibited contractions without causing any reduction of tissue ATP content or significant impairment of glycolysis[20]. Na^+-rich rissues in glucose-free, K^+-free solution at 4 °C were exposed to 0.1 mM iodoacetamide for 30 min. Tissues were then transferred to a glucose-free, K^+-free Krebs solution, containing no inhibitor at 37 °C. Tissues treated in this way did not lose weight but instead a slight but significant increase in weight occurred. Control tissues that had not been exposed to iodoacetamide, lost weight (Table VI). Iodoacetamide also inhibited net extrusion of Na^+ and water (Table III).

(5) Drugs. If the loss in weight observed on rewarming were related to a contractile mechanism, then drugs known to inhibit contractions of the rat uterus might reduce or abolish the loss in weight. Accordingly the effects of several drugs known to inhibit contractions of rat myometrium were studied. Na^+-rich tissues were therefore exposed to K^+-free Krebs solution at 37 °C containing 10^{-4} M papaverine or 10^{-6} M isopropylnoradrenaline. Such tissues did not lose weight to any significant extent whereas control tissues, not treated with these drugs, did lose weight (Table VII). Papaverine also significantly impaired Na^+ and water extrusion (Table III).

TABLE VII

EFFECT OF DRUGS ON WEIGHT CHANGES OF RAT MYOMETRIUM

Pieces of rat myometrium were incubated at 4 °C for 18–24 h in K^+-free Krebs medium and then rewarmed at 37 °C in the presence or absence of the drugs shown. The values of tissue weight (% fresh weight) are the mean ± S.E. (n = 4–6).

Drug	*Tissue weight (% fresh weight)*				*Net weight changes (D − A)*
	After incubation at 4° C for 18–24 h A	*After rewarming at 37 °C for*			
		20 min B	*60 min C*	*120 min D*	
	102.7 ± 4.0	91.4 ± 3.3	86.6 ± 3.3	86.6 ± 3.6	− 16.1 ± 2.4
10^{-4} M papaverine	98.4 ± 2.9	96.9 ± 2.5	95.3 ± 3.5	101.5 ± 4.4*	+ 3.2 ± 2.6*
	106.6 ± 0.9	100.7 ± 1.1	99.3 ± 1.2	97.0 ± 1.4	− 9.5 ± 2.2
10^{-6} M isopropyl-noradrenaline	103.4 ± 3.0	103.5 ± 2.1	102.0 ± 1.5	102.2 ± 2.9	− 0.9 ± 2.6*

* $P < 0.05$ by paired "t" test.

DISCUSSION

The following characteristics of the loss of weight that occurs when Na^+-rich segments of rat myometrium are rewarmed, have been established in the present and earlier studies[17–19]. (1) The loss of weight was due to a net loss of water and NaCl, and cannot be accounted for by a loss of tissue cells or solids. (2) The process was not inhibited by ouabain or by omission of K^+, procedures that inhibit coupled Na^+–K^+ pumping in this tissue. (3) The ouabain-insensitive extrusion of water was dependent on metabolic energy, being completely blocked by anoxia and omission of D-glucose, and by ethacrynic acid. (4) The process displayed a lack of cation specificity since tissue shrinkage still occurred in tissues rendered Li^+ or K^+ rich. Under these conditions, the Na^+ content remaining in the tissues was less than 20 mmoles/kg dry wt. (5) The mechanism for volume control was also Ca^{2+} dependent since Ca^{2+} depletion resulted in abolition of shrinkage on re-warming. In addition, Ca^{2+} depletion induced considerable swelling presumably as a consequence of increased membrane permeability. Sr^{2+} could substitute for Ca^{2+}. (6) The process was inhibited by the sulphydryl inhibitor, iodoacetamide, in concentrations shown previously[20] not to reduce the ATP content of such tissues or to inhibit Na^+–K^+ pumping or glycolysis. (7) Shrinkage was dependent upon the presence of a permeant anion since shrinkage occurred when Cl^- was replaced by the permeant NO_3^- but not when replaced by the impermeant methylsulphate anion.

The characteristics of the process are thus very similar to those of the ouabain-insensitive mechanism of volume control described by Kleinzeller and his associates[1,11–14] in renal cortical cells and in rat diaphragm. Several hypotheses have been advanced to account for ouabain-insensitive volume control in cells; these have been recently extensively reviewed by Kleinzeller[1].

According to the "cryptic pump" hypothesis[25], the Na^+–K^+ pump could continue working in the absence of K^+ in the medium since K^+ would leak out of the cells and maintain such pumping. However, the present and previous results[22] have shown that this hypothesis cannot account for the ouabain-insensitive process observed: (i) The addition of K^+ to Na^+-rich segments of rat myometrium at 37 °C resulted in the rapid onset of electrogenic Na^+–K^+ pumping; this was absolutely dependent, however, upon the addition of K^+ to the medium: and (ii) Tissue shrinkage that occurred on re-warming, was not Na^+ dependent whereas Na^+–K^+ pumping in this tissue was absolutely dependent upon Na^+ (ref. 22).

Kleinzeller has recently extensively reviewed and discussed[1] the evidence in favour of a mechanochemical process being responsible for the ouabain-insensitive control of cellular volume. Some indirect evidence has been obtained that regulation of cellular volume in rat myometrium may also be dependent upon the operation of a mechanochemical system (Rangachari, P. K., Paton, D. M. and Daniel, E. E., unpublished observation). Firstly, when Na^+-rich tissues are re-warmed, they undergo a contracture. Secondly, the following procedures, shown in the present study to prevent tissue shrinkage on re-warming, also inhibited contractility of rat myometrium: omission of Ca^{2+}, metabolic inhibition, iodoacetamide, and relaxant drugs[20,26].

In an earlier study, Daniel and Robinson[27] postulated a mechanism to explain the complex movements of Na^+ in the rat uterus. They based their model on the

hypothesis of Goodford *et al.*[28] that the so-called micro-pinocytocic vesicles in the plasma membrane could serve as binding sites for Na^+. The model of Daniel and Robinson suggested that the formation of the vesicles was an ATP-dependent but ouabain-insensitive process; it was proposed that after their formation, the vesticles accumulated Na^+ from the cytoplasm through the agency of an ouabain-sensitive, Na^+–K^+ pump; the accumulated Na^+ was bound within the vesicles and subsequently extruded by reverse pinocytosis.

The results of the present study have suggested that the above model requires modification. It is possible that the vesicular membrane contains an actomysin-like contractile protein that binds Na^+ (or K^+, Li^+ *etc.*) at 4 °C. It is suggested that an increase in temperature leads to a rapid reduction in ion binding and an increase in osmotic pressure within the vesicle. This in turn causes a net movement of water into the vesicles leading to a transient vesicular swelling and activates the vesicular contractile protein which then squeezes out the vesicular contents. ATP and Ca^{2+} depletion would alter both the binding of the ions on the protein and the contractile mechanism squeezing out the vesicular contents.

Another possible mechanism is that with cooling the plasma membrane undergoes a phase transition with resultant loss of cellular volume control and swelling, these changes being reversed on rewarming. Certainly a number of membrane lipids undergo phase transitions when cooled[29,30] while the activation energies of a number of membrane-bound enzymes[31], notably the $Na^+ - K^+$ activated adenosine triphosphatase[32,33], alther significantly. Similar changes may occur in the rat myometrium since the Q_{10} for Na^+ efflux between 15 °C and 5 °C was about 15 (ref. 27).

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