BBA 3906

ROLE OF PHOSPHOPROTEINS IN ION TRANSPORT: INTERACTIONS OF SODIUM WITH CALCIUM AND POTASSIUM IN LIVER SLICES

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(Received July 30th, 1962)

SUMMARY

1. Changes in ATP and phosphoprotein turnover observed when liver slices are suspended in media low in Na^+ are in part due to influx of Ca^{2+}.

2. These changes are reversed by addition of Na^+, and the amount of Na^+ required for reversal is dependent on the concentration of external Ca^{2+}.

3. Lesser amounts of Na^+ are required to stimulate ATP turnover than are required for phosphoprotein.

4. Li ions fail to stimulate phosphoprotein turnover, though they may increase the activity of ATP.

5. Na^+, Ca^{2+} and the drugs ouabain and promethazine compete for phosphoprotein groups.

6. K ions appear to be necessary for a dephosphorylating reaction of the phosphoproteins observed within 30 sec of K^+ addition. This is blocked by ouabain and promethazine.

7. The metabolism of ATP and phosphoproteins in liver cells is thus dependent on a balance between the activities of Na^+, Ca^{2+} and K^+.

INTRODUCTION

In previous work[1,2] we reported that liver slices suspended in sodium-free media showed reduced turnover of phosphoprotein phosphorus. The addition of Na^+ in small amounts increased the activity of the phosphoproteins of these systems, and drugs inhibiting Na^+ transport also inhibited phosphoprotein turnover. In the present paper we deal with the relationship between Na^+ and Ca^{2+} in the external medium. It is shown that the external Ca^{2+} level determines to a large extent the degree of response of phosphoproteins and ATP to external Na^+ concentration and that Na^+ and Ca^{2+} appear to compete for a site in the phosphoprotein. It is also shown that the turnover of ATP, which was shown to be Na^+-dependent[1], reveals the competition between Na^+ and Ca^{2+}. That phosphoprotein changes do not merely follow changes in ATP is clearly established by the use of Li^+, which stimulates ATP but not phosphoprotein turnover, and by the use of drugs such as ouabain which dissociate the one from the

other. It is also shown that Na^+ and K^+ interact at the level of the cellular phosphoproteins, and the mechanism of these interactions is discussed.

METHODS

These are identical with those used previously[1]. However, it should be emphasized that [^{32}P]P_i as received is usually impure. It should be hydrolysed in 1 N HCl for 10 min and thereafter precipitated as the magnesium ammonium salt[3]. Before dissolving this for use, it is wise to reject a small amount of the precipitate. This may be done by careful neutralization of the suspended crystals and centrifugation before all the precipitate has dissolved.

The extraction of the tissue may also present some difficulties. If tissues are extracted with organic solvents such as ethanol, ethanol–ether, etc. particularly after acid treatment as is commonly done, a large part of the phosphoprotein fraction will go into the solvent together with the lipids. In red-cells, such losses can be complete, even in the presence of carrier protein or phosphoprotein (phosvitin, casein). The losses may be minimized by extracting at room temperature, or by first washing the tissue residue with water several times to remove traces of acid. This subject has recently been touched upon[4].

RESULTS

The effect of Ca^{2+}

The effect of Ca^{2+} in the external medium has been discussed by SHANES[5] in terms of "stabilizing" activity. This means that Ca^{2+} directly influences the permeability of cells so that in higher concentration permeability is reduced, while in lower concentration, it tends to increase. The experiments shown here direct attention to another aspect of the action of Ca^{2+}, namely its metabolic effects, and the antagonism of these by Na^+. In order to investigate these phenomena, use has been made of media lower in Na^+ where choline chloride has been used as a substitute for NaCl. This makes it easier to add or withhold Na^+ at will and to vary their concentration.

In work already reported[1] we used $NaHCO_3$ buffer throughout, even in the Na^+-free systems, thus providing 12 mM Na^+ in the medium. While this was adequate for the medium then in use (containing 2.8 mM Ca^{2+}), it was found that reduction of the Ca^{2+} concentration to 0.93 mM resulted in a system which was quite different in its response to Na^+. With the lower level of Ca^{2+}, 10 mM Na^+ provides a great stimulation of phosphoprotein turnover. This is shown in Table I, where Ca^{2+} at three concentrations and the effects of zero, 10 mM and 36 mM Na^+ are compared. Two points should be noted: (a) the lower levels of Na^+ in each case stimulate ATP turnover almost to the full extent, while having relatively less effect on phosphoprotein turnover and (b) there is clearly a greater effect of Na^+ in the lower as compared to the higher level of Ca^{2+}. The conclusion from the first observation is that phosphoprotein and ATP activities do not simply follow one from the other, but that Na^+ is required for the full activation of the protein phosphorus. This conclusion is strengthened by the experiments shown in Table II, where the effect of Li^+ is compared to that of Na^+. It is clear that Li^+ has no effect upon the phosphoprotein turnover whatever, even when the radioactivity of the ATP is elevated by Li^+, as it is in the presence of 2.8 mM

TABLE I

EFFECT OF VARYING Na^+ AND Ca^{2+} ON ATP AND PHOSPHOPROTEIN

Slices (about 200 mg wet wt.) were suspended in choline Ringer with Ca^{2+} content as noted. After 10 min Na^+ or choline was added as shown, and after another 15 min, the slices were removed and homogenized at once in 5% trichloroacetic acid. ^{32}P of specific activity $2 \cdot 10^6$ counts/min/μmole P_i was present in the medium. Gas phase O_2–CO_2 (95:5). The buffer was either $NaHCO_3$ or $KHCO_3$ to give pH 7.2 Temperature 38°.

Expt.	Ca^{2+} concentration	Specific activity, counts/min/μmole P ($\times 10^{-3}$) of					
		ATP in presence of			Phosphoprotein in presence of		
		Choline	Na (10 mM)	Na (36 mM)	Choline	Na (10 mM)	Na (36 mM)
1	2.8	97	134	147	12.0	14.0	18.0
	0.93	155	216	230	17.0	28.0	39.0
2	2.8	103	130	159	5.0	5.6	8.6
	1.86	107	148	205	5.8	8.6	11.4

TABLE II

EFFECT OF Li^+ ON ATP AND PHOSPHOPROTEIN AT 2 LEVELS OF Ca^{2+}

Conditions as in Table I, except that additions of choline, Li^+ and Na^+ were all at 36 mM.

	Ca^{2+} 2.8 mM plus			Ca^{2+} 0.93 mM plus		
	Choline	Li^+	Na^+	Choline	Li^+	Na^+
ATP specific activity	134 000	198 000	190 000	98 000	103 000	132 000
Phosphoprotein, specific activity	17 000	16 800	25 000	9 000	8 500	16 000
Phosphoserine, specific activity	445	440	610	—	—	—

TABLE III

EFFECT OF Na^+ AND Ca^{2+} ON P_i AND ATP OF SLICES

Conditions exactly as in Table I, except that Na^+ was present from the start of incubation, which was of 10 min duration. ^{32}P was added to give a specific activity of 600 000 counts/min/μmole P_i.

Ca^{2+} concentration (mM)	Na^+ concentration (mM)	Specific activity (counts/min/μmole P_i) of		
		P_i	ATP	ATP/P_i
2.8	—	40 000	17 500	0.44
1.86	—	37 400	20 000	0.54
0.93	—	48 500	29 800	0.62
2.8	36	30 600	24 300	0.80
1.86	36	27 800	27 700	1.00
0.93	36	27 800	27 800	1.00

Ca^{2+}. It has been found that liver slices do not transport Li^+ to any measurable rate, and this experiment supports the view that the effect of Na^+ is specific.

Effect of Na^+ on ATP turnover

The effect of Na^+ on the turnover of ATP is shown in Table III. The experiments

were conducted at 3 levels of Ca^{2+} and one of Na^+ and show clearly that while the specific activity of the inorganic phosphate of the slices in the three different media is essentially the same, the radioactivity of the ATP is markedly higher in the presence of lower concentrations of external Ca^{2+}. The effect of Na^+ is to bring about an increase in the specific activity of the ATP relative to the specific activity of the inorganic phosphate. The experiments shown here differ from those in the earlier tables in an important respect: the effect of presence or absence of Na^+ was tested at the same time, whereas in the earlier experiments, additions of Na^+ or other ions were made after a preincubation in the appropriate Na^+-free medium. This has important effects upon the rate of response to Na^+ as will be discussed later. The significance of the results in Table III is that the increased radioactivity of the ATP in the presence of lower concentrations of Ca^{2+} is not due to greater permeability of inorganic phosphate into the cells and further, that Na^+ can be shown to have a direct effect upon the rate of transfer of P_i to ATP, as suggested previously[1].

Site of action of Ca^{2+}

The results given above show that Ca^{2+} and Na^+ certainly interact at some point or points involved in the transfer of P_i to ATP. They also suggest that Ca^{2+} and Na^+ interact with phosphoproteins.

Action of ouabain and promethazine

In order to get more direct evidence of the latter, slices were preincubated in choline Ringer containing 2.8 mM Ca^{2+} and transferred after 10 min to a medium containing 0.93 mM Ca^{2+} together with 10 mM Na^+, the balance of the monovalent cation content being made up of choline chloride and KCl (7 mM). [^{32}P]P_i was included in the latter medium. Control slices were preincubated in medium containing 0.93 mM Ca^{2+} and were transferred after 10 min to the same medium as described above, namely one containing 0.93 mM Ca^{2+}, 10 mM Na^+ and [^{32}P]P_i. After 10 min in this medium, the slices were homogenized in 5% trichloroacetic acid and the phosphoprotein and ATP assayed for level and radioactivity. It was found that slices preincubated in 2.8 mM Ca^{2+} and transferred to 0.93 mM Ca^{2+} failed to respond to 10 mM Na^+, while slices preincubated in 0.93 mM Ca^{2+} and transferred to the same, showed the normal response. The experiment was therefore repeated but this time, the preincubation in the presence of 2.8 mM Ca^{2+} was also conducted in the presence of ouabain and promethazine, drugs known to block the response of the phosphoproteins to Na^+ and to inhibit Na^+ transport[1,2,7]. The results of this experiment are shown in Table IV. It is plain that preincubation of the slices in the presence of the drugs nullified to a great extent the effect of the high calcium concentration of the surrounding medium. In order to determine any carry-over of the drugs from the preincubation to the incubation medium, controls were carried out in 0.93 mM Ca^{2+}, and as can be seen, the carry-over was very slight.

It may be concluded, therefore, that Ca^{2+} interacts with the phosphoproteins, that it is antagonized at this site by Na^+, and that drugs such as promethazine and ouabain compete with both ions for the same site.

The effect of zero external Ca^{2+}

When the external Ca^{2+} concentration is reduced to zero, liver slices lose their

intracellular K^+, gain Na^+ and water. In addition, the cell membrane becomes permeable to proteins, and a considerable loss of intracellular enzymes occurs. Net transport of K^+ against a gradient ceases when tested in suitable systems. The turnover of ATP and phosphoprotein in these systems is exceedingly rapid and relatively in-

TABLE IV

COMPETITION BETWEEN Ca^{2+}, Na^+ AND DRUGS FOR PHOSPHOPROTEIN

Slices were preincubated in choline Ringer containing 2.8 mM Ca^{2+} or 0.93 mM Ca^{2+} for 10 min in presence or absence of drugs as shown. They were then transferred to choline Ringer containing 0.93 mM Ca^{2+} + 10 mM Na^+ and ^{32}P, specific activity $1.7 \cdot 10^6$ counts/min/μmole P_i. Time of incubation was 10 min. Other conditions as in Table I.

Addition during preincubation	*Specific activity (counts/min/μmole phosphate) Preincubation in 2.8 mM Ca^{2+}*		*0.93 mM Ca^{2+}*	
	ATP	*Phospho-protein*	*ATP*	*Phospho protein*
Nil	53 000	1300	80 000	4500
Ouabain $3 \cdot 10^{-4}$ M	71 000	2500	109 000	4900
Promethazine 10^{-4} M	62 000	2000	84 500	3500

TABLE V

EFFECT OF Na^+ ON ATP LEVELS OF SLICES INCUBATED IN Ca^{2+}-FREE MEDIA

The slices were allowed to equilibrate for 10 min in choline Ringer containing the desired Ca^{2+} concentration. Additions of choline, Na^+ or Li^+ to a final concentration of 36 mM were made and incubation carried on for an additional 10 min. All other conditions as in Table I.

	Addition	*Ca^{2+} concentration (mM)*	*ATP (μmoles phosphate/g original liver)*
Expt. 1	Choline	0.93	2.10
	Na^+	0.93	2.05
	Choline	Nil	1.76
	Na^+	Nil	0.97
Expt. 2	Choline	Nil	1.30
	Na^+	Nil	0.85
	Li^+	Nil	1.32

sensitive to added Na^+. A possible reason for some of the consequences of zero external Ca^{2+} is to be seen in Table V, which shows that addition of Na^+, but not of choline or Li^+, causes a rapid loss of ATP in the slices. This loss is not prevented by ouabain or by promethazine in concentrations at which they inhibit ion transport in liver slices. The effect of zero Ca^{2+} illustrates the "stabilizer" function of this ion already referred to.

Ionic constitution of liver slices

It was shown in previous work[1] that suspension of liver slices in choline Ringer resulted in loss of intracellular K^+. Recovery was observed to begin after transfer to normal Ringer solutions, after a lag period of 20 min. It was interesting to see if this

recovery of normal constitution was related to the Ca^{2+} concentration of the choline Ringer. Table VI shows that this is indeed so. Slices were preincubated in choline Ringer of various Ca^{2+} levels and transferred to Na^+ Ringers containing either 2.8 or 0.93 mM Ca^{2+}. It is evident that the concentration of Ca^{2+} in the preincubation medium is a determining factor in the recovery. In Table VII we show the effect of ouabain

TABLE VI

RECOVERY OF K^+ LEVELS IN SLICES AFTER PREINCUBATION IN CHOLINE RINGER WITH VARYING Ca^{2+} CONCENTRATIONS

Slices (about 200 mg wet wt.) were preincubated in choline Ringer containing Ca^{2+} as shown in the first column for 10 min. They were then transferred for incubation to Na^+ Ringer as shown in the second column for 20 min. Slices were removed at the end of preincubation for K^+ analysis and again at the end of incubation. Temperature and gas phase for preincubation and for incubation were the same as in Table I.

Expt.	*Preincubated in choline Ringer containing different Ca^{2+} concentrations (mM)*	*Incubated in Na^+ Ringer containing different Ca^{2+} concentrations (mM)*	*K^+ levels, mequiv/kg dry wt.*		
			After preincubation	*After incubation*	*ΔK*
1	2.8	2.8	190	176	— 14
	1.86	2.8	183	203	+ 20
	0.93	2.8	155	205	+ 50
2	2.8	0.93	162	175	+ 13
	1.86	0.93	158	185	+ 27
	0.93	0.93	146	188	+ 42

TABLE VII

EFFECT OF OUABAIN AND PROMETHAZINE ON RECOVERY OF K^+ LEVELS AFTER PREINCUBATION IN CHOLINE RINGER

Slices were preincubated in the choline Ringer as indicated for 10 min at 38°. They were then transferred to Na^+ Ringer as indicated. The time of incubation was 15 min. Other conditions as in Table I.

Expt.	*Preincubated in choline Ringer containing 2.8 mM Ca^{2+} in presence of*	*Incubated in Na^+ Ringer containing*	*K^+ levels (mequiv/kg dry wt.)*		
			After preincubation	*After incubation*	*ΔK*
1	Nil	0.93 mM Ca^{2+}	148	147	— 1
	Promethazine 10^{-4} M	0.93 mM Ca^{2+}	144	176	+ 32
	Ouabain $5 \cdot 10^{-4}$ M	0.93 mM Ca^{2+}	112	151	+ 39
2	Nil	2.8 mM Ca^{2+}	171	156	— 15
	Promethazine 10^{-4} M	2.8 mM Ca^{2+}	170	180	+ 10
	Ouabain $5 \cdot 10^{-4}$ M	2.8 mM Ca^{2+}	139	162	+ 23

and promethazine on the recovery of K^+ levels. The drugs were present during preincubation of slices in choline Ringer containing 2.8 mM Ca^{2+}. It is clear that they offer partial protection in the sense that recovery of K^+ levels is more rapid in the slices preincubated in their presence. It may be inferred that recovery from exposure to low Na^+ media is dependent upon the Ca^{2+} concentration of such media, and that the influx of Ca^{2+} results in the paralysis of transport mechanisms until the cation is displaced by inflowing Na^+.

Demonstration of influx of Ca^{2+}

When liver slices are incubated in a medium of zero external Na^+, a net uptake of Ca^{2+} is readily demonstrable. This is shown in Table VIII, which also includes figures on slices incubated in normal Ringer solution. The movements of Ca^{2+} in the presence and absence of Na^+ and K^+ will not be discussed further here, but will be the subject of another paper. These results, however, confirm the findings in other work on muscle[10].

TABLE VIII

INFLUX OF Ca^{2+} INTO LIVER SLICES

Slices (about 200 mg wet wt.) were incubated in choline or Na^+ Ringer for 15 min at 38°. Freshly cut slices were used for the zero-time controls. All slices were soaked in Ca^{2+}-free medium to wash out the extracellular space by a predetermined technique. They were dried, weighed, and transferred to 0.1 N HCl containing 30% *n*-propanol for Ca^{2+} estimation as described previously[19].

Time	*Medium*	*Ca^{2+} Concentration (mequiv/kg dry wt.)*	*ΔCa*
0	—	3.4	—
15	Na^+	4.1	0.7
15	Choline	8.1	4.7

Interaction of Na^+ and K^+

The turnover of phosphoproteins is determined by the presence or absence of Na ions. The absence of K^+ from the external medium, while preventing Na^+ extrusion, has little effect upon the uptake of ^{32}P into ATP or phosphoproteins. In other work with red cells[6], we have found that K ions apparently take part in a dephosphorylating reaction which involves the phosphoproteins, and experiments were undertaken to see if such were also the case in liver slices. To do this, slices were first leached of most of their K^+ by cooling to 0° in 0.154 M NaCl for 30 min; after this they were transferred to a K^+-free Ringer at 38°. After 30 min their K^+ had fallen to between 30 and 50 mequiv K^+/kg dry wt. from an initial figure of about 300 mequiv K^+/kg dry wt.

TABLE IX

EFFECT OF OUABAIN AND PROMETHAZINE ON K^+ DEPLETION OF PHOSPHOPROTEIN

Slices (about 200 mg wet wt.) were cooled for 30 min in 0.154 M NaCl at 0°. They were then transferred to a K^+-free Ringer at 38°, containing ^{32}P, specific activity $2 \cdot 10^6$ counts/min/μmole P_i. After 30 min, KCl to a final concentration of 10 mM was added as shown, and 30 sec later, slices were removed and homogenized in 5% trichloroacetic acid. Drugs, when used, were added 3 min in advance of the K^+.

Drug	*Specific activity (counts/min/μmole phosphate of phosphoprotein)*		
	No K^+	*+ 10 mM K^+*	*Fall in presence of K^+ (%)*
Nil	12 300	8 200	33
Promethazine, 10^{-4} M	10 700	10 900	Nil
Ouabain $5 \cdot 10^{-4}$ M	11 600	11 000	5

Upon addition of 10 mM KCl to the flasks or upon transfer of the slices to a K^+-containing Ringer, rapid transport set in, with a rate of K^+ uptake of about 10–12 mequiv/kg dry wt./min in the initial stages. Na^+ was extruded at the same time, in a ratio of about 1:1. Fig. 1 shows the uptake of K^+ and the effect of ouabain.

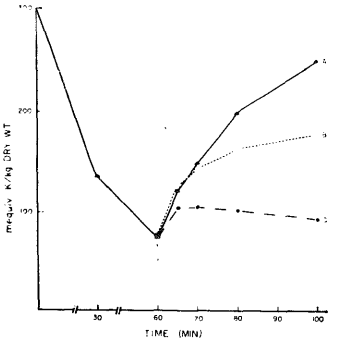

Fig. 1. Effect of ouabain on K^+ uptake by liver slices. Slices of about 200 mg wet wt. were incubated at 0° in 0.154 M NaCl (30 min) and then for 30 min at 38° in K-free Ringer. Gas phase O_2–CO_2 (95:5). $NaHCO_3$ to give a pH of 7.2 was used as buffer. At the time indicated by arrow, 10 mM K^+ was added to all flasks. Curve A, control; curve B, 10^{-4} M ouabain; curve C, $5 \cdot 10^{-4}$ M ouabain. Ouabain was added immediately before K^+.

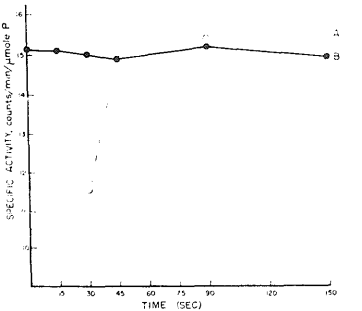

Fig. 2. Effect of K^+ on radioactivity of phosphoproteins. Slices about 200 mg wet wt. were first leached of their K^+ by incubation at 0° in 0.154 M NaCl for 35 min. They were then incubated for 30 min in a K^+-free medium at 38° containing ^{32}P, specific activity $2 \cdot 10^6$ counts per min/μmole P_i. At the end of this incubation (zero time in the figure) 10 mM KCl was added to half the flasks and samples removed as shown for phosphoprotein and ATP analysis. The specific activities of the phosphoproteins are corrected for the small variations in specific activity of ATP (150000 counts/min/μmole phosphate). Curve A, K^+ added; curve B, control.

If [^{32}P]P_i were included in the K^+-free Ringer, phosphoprotein and ATP became labelled. Addition of K^+ to 10 mM gave the result shown in Fig. 2. Within 30 sec there was a fall in radioactivity of the phosphoprotein, after which recovery to normal level took place and was complete within 45 sec from time of addition of K^+. These experiments suggest that K^+ is involved in a dephosphorylating reaction, but that in liver slices the phosphorylating reaction overshadows the breakdown so that there is but an evanescent effect. In Table IX it is shown that promethazine[7] and ouabain block this effect of K^+ at concentrations at which they inhibit ion transport in liver slices. These effects on phosphoproteins were also confirmed by assaying the activity of phosphoserine.

Effect of suspension in Li^+ Ringer

Since Li^+ has been tested in place of Na^+ in previous experiments cited in this paper, we suspended liver slices in Li^+ Ringer, containing [^{32}P]P_i and compared them

with similar slices suspended in Na^+ Ringer. The results are shown in Fig. 3, where the radioactivity of the phosphoprotein is related to that of ATP in the slices in both systems. It is evident that, in the presence of Na^+, the phosphoproteins reach isotopic equilibrium far more rapidly than in the presence of Li^+. An interesting feature of this experiment was that though the total radioactivity passing into ATP was the

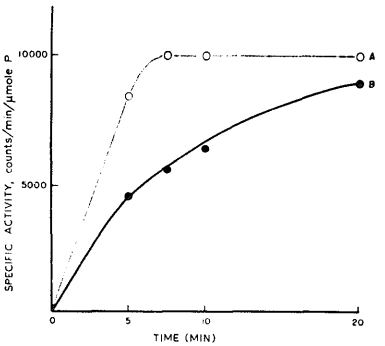

Fig. 3. Effect of suspension in Li^+ Ringer on phosphoprotein radioactivity. Slices about 200 mg wet wt. were incubated in normal Ringer solution or in a Ringer in which LiCl was substituted for NaCl. The Ca^{2+} concentration was 2.8 mM and $NaHCO_3$ was used as buffer in both systems (pH 7.2) the small effect of Na^+ addition (due to $NaHCO_3$) being disregarded. ^{32}P, specific activity $2 \cdot 10^6$ counts/min/μmole P_i was present in each case. The figures show specific activity of phosphoprotein-phosphate corrected to specific activity of ATP of 10^5 counts/min/μmole P_i. Curve A, Na^+ Ringer; curve B, Li^+ Ringer.

same both in Na^+ and Li^+ Ringers, the levels of ATP were substantially higher in the Li^+ Ringer, thus tending to reduce the specific activity, especially at 20 min.

We have also tested sucrose as a substitute for Na^+, and have obtained results identical with those already reported for choline[1] and now for Li^+.

DISCUSSION

The interaction of calcium and the transporting system of liver cells is at least two-fold.

The first type of effect, the stabilizing action of Ca^{2+} is well illustrated in work already published[7], where it is shown that slices placed in Ringer solutions containing 1.0 mM Ca^{2+} lose more K^+ than slices placed in 2.8 mM Ca^{2+}, but that recovery to normal levels is far more rapid in the former than in the latter medium. This will not be discussed further. The second type of interaction is reflected in metabolic changes induced by alteration of the Ca^{2+} content of the medium, and the relationship between Na^+ and Ca^{2+}. We have shown here that Na ions stimulate the turnover of ATP, that this effect is determined by the level of Ca^{2+} in the medium, and that it is independent of permeability of $[^{32}P]P_i$ into the cells. We have also demonstrated that Na ions are

needed for the maximum turnover of phosphoproteins and that, here again, Ca^{2+} and Na^+ compete for the active sites.

Drugs such as ouabain and promethazine which have been shown to block the effect of Na^+ on the activity of phosphoproteins also prevent to some extent the effect of Ca^{2+} on the same molecules. It is concluded, therefore, that drugs, Na^+, and Ca^{2+} all interact together at a common site.

Our results are in keeping with physiological work using heart[8,9] and skeletal muscle[10], in which good evidence is brought to show that Na^+ and Ca^{2+} share a common site of interaction. Work with smooth muscle also supports this view and will be published shortly.

The sequence of events in cells suspended in media low in Na^+ is probably as follows: first there is an influx of Ca^{2+}, together with a loss of K^+. The binding of Ca^{2+} with the active sites paralyzes the transport mechanism so that when slices are transferred to a normal Ringer solution, there is a long lag period before the normal ion distribution of the cells is restored. During this lag period, Na^+ flow in, Ca^{2+} ions are displaced and as transport becomes free to commence, the Na^+ are themselves extruded, with uptake of K^+. The effect of the drugs ouabain and promethazine, which block the action of Na^+, may be brought about by preventing the displacement of Ca^{2+} by Na^+, but our results shown here suggest that in fact they compete directly for the active sites.

The interaction of K^+ and Na^+ must be discussed briefly. In previous work[1] we postulated that Na^+ was related to a phosphorylating reaction and K^+ to a dephosphorylating one. This appears to be borne out by the results shown here, in which the addition of K^+ to Na^+-filled cells causes a rapid, but temporary decline in the radioactivity of the phosphoproteins. This effect of K^+ is also shown in other work with human red cells[6].

The rate of turnover of the phosphoprotein may be calculated from these experiments to be adequate for a role in active Na^+ transport. The level of phosphoprotein phosphate is about 4.5 mmoles/kg dry wt. liver, and assuming that 2 Na^+ are carried per mole phosphate, this means that 9 mequiv Na^+ could be accommodated/kg dry wt. The maximum rate of Na^+ extrusion observed by us is about 12 mequiv/kg dry wt./min, which means that each mole of phosphoprotein phosphate would have to turnover once in 45 sec. Our results show that this rate and more is entirely possible.

It must be emphasized, however, that despite the circumstantial evidence, there is still no proof that any phosphorylated intermediate is actually concerned with carriage of Na^+, but the evidence is now reasonable that one or more[11,12] may have a close relationship with the transport mechanism.

ACKNOWLEDGEMENTS

It is a pleasure to thank Dr. A. E. M. McLEAN of the University College of the West Indies for his advice and for numerous discussions.

Miss S. ELSEY provided valuable technical assistance throughout this work.

This work was supported (in part) by a USPHS research grant C4534 from the NCI, by grant DRG-534 from the Damon Runyon Memorial Fund for Cancer Research, and by the Burroughs-Wellcome Fund and the Charles Pfizer and Co.

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