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THE EFFECT OF ALKALI METAL IONS ON SUGAR TRANSPORT IN MUSCLE: INTERACTION WITH THE SUGAR CARRIER OR INDIRECT: EFFECT*

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SUMMARY

1. Membrane transport of sugar in "intact" rat hemidiaphragm *in vitro* was studied by measuring fluxes of 3-*O*-methyl-D-^{14}C-glucose in and out of the intracellular water.

2. The stimulation of influx in a K^+-free medium, demonstrated earlier, was correlated with the duration of exposure to K^+-free medium and persisted for some time after transfer of tissue to a normal medium. The time course of this effect was parallel to that of concomitant changes in intracellular Na^+ and K^+ levels due to inhibition of the Na^+ pump.

3. Efflux of sugar from the tissue was stimulated under all conditions which stimulated influx. The effect of K^+-free medium also showed the same gradual onset and persistence after transfer of the tissue to normal medium.

4. These effects are not consistent with transport regulation through binding or co-transport of K^+ on the sugar carrier, nor do they support a direct link between sugar transport and the activity of the Na^+ pump as such. The data suggest that the increased Na^+ or decreased K^+ level in the cell, or both, caused by Na^+ pump inhibition, enhances sugar transport in and out of the cell to an equal extent.

INTRODUCTION

The insulin-sensitive sugar transport system is stimulated *in vitro* under conditions which inhibit sodium transport, *e.g.* the presence of ouabain or the omission of K^+ from the incubation medium. These effects were demonstrated in rat diaphragm muscle[1,2] and in adipose tissue and isolated adipocytes[3–8]. Two mechanisms for this stimulatory effect have been suggested. According to LETARTE *et al.*[6,7] the insulin-sensitive sugar carrier is thought to bind co-transport Na^+ and/or K^+, not unlike the carrier which is thought to mediate active sugar transport in the intestine (for review see ref. 9). The interpretations advanced by CLAUSEN[1,8] and from this laboratory[2,10] are less specific but favour an indirect effect of intracellular

* A preliminary report[10] of these results was made to the 4th International Congress on Pharmacology, Basel Switzerland, July 14–18th, 1969.

Na^+ or K^+, or both on the sugar carrier. These two models lead to different predictions regarding the effect of changes in ionic levels inside and outside the cell on the influx and efflux of sugar. Specifically, the first model predicts that variations in ionic levels at one side of the membrane should affect sugar transport from that side and do so without delay; this effect should be asymmetric, with flux in the opposite direction remaining unaffected. The second or indirect model does not exclude a lag period and accommodates a symmetrical and equal effect on both unidirectional fluxes, such as is also observed with insulin. We report here experiments designed to test these predictions and to provide more information on the nature of the interaction between ions and insulin-sensitive sugar transport.

MATERIALS AND METHODS

The detailed procedure for preparation and incubation of tissues and analysis of samples has been described previously[2]. Briefly, "intact" rat hemidiaphragms[11] were incubated at 37° in 5.0 ml of substrate-free KREBS–HENSELEIT bicarbonate buffer[12], pH 7.4, bubbled with 95 % O_2–5 % CO_2 and containing 0.8 % bovine serum albumin, 3-*O*-methyl-D-[^{14}C]glucose (0.0625 μC/ml, 5 mM), tracer amounts of D-[^{3}H]-mannitol, serving as extracellular marker, and other additions as indicated. In the K^+-free medium all K^+ was replaced by choline. The radioactivity of aliquots of tissue extracts and media was determined by double-label liquid scintillation spectrometry.

Sugar influx was estimated semi-quantitatively from the percent penetration (sugar in intracellular water)/(sugar in medium) × 100 reached within 10 min. As was pointed out previously[2], this value expresses net uptake and is not an accurate measure of undirectional influx because it also contains a variable contribution from sugar efflux. Thus the penetration values underestimate influx to that extent and are only semiquantititative in nature. For estimation of efflux hemidiaphragms were "loaded" by 30 min incubation in 10 mM 3-methyl-[^{14}C]glucose, followed by a 10 min "washout" period in sugar-free medium, and the amount of radioactivity left in the tissue was determined. The loss during washout was expressed as percent of the sugar content of the paired control hemidiaphragm which was loaded but not washed out. This percent loss is also not an accurate reflection of exit rates since it is uncorrected for loss from the extracellular space. It does permit, however, a comparison of effects on efflux caused by the various experimental treatments. For determinations of ionic content tissue extracts were homogenized with a teflon pestle and deproteinized with $Ba(OH)_2$–$ZnSO_4$[13]. Na^+ and K^+ levels were measured by atomic absorption spectrophotometry and the results corrected for the mannitol space determined in the same sample. Statistical evaluation of data was done by Student's t test applied to paired tissues from the same animal, unless otherwise indicated.

RESULTS

Preliminary results indicated that the sugar transport stimulating effect of ouabain or omission of K^+ from the medium is gradual in onset and related to the duration of exposure to these conditions. The effect of pretreatment on subsequent

TABLE I

EFFECT OF K^+-FREE MEDIUM ON UPTAKE OF 3-METHYLGLUCOSE

Tissues were preincubated for 30 min in sugar-free medium and then incubated with 5 mM 3-methylglucose for 10 min. Δ % penetration is the mean of differences ± S.E. between paired hemidiaphragms expressed as percentage penetration into the intracellular water space. The number of pairs for each figure is given in brackets.

Preincubation medium	*Incubation medium*	Δ % *Penetration*	% *Change*
(A) Normal	Normal K^+-free	+ 3.4 ± 0.7 (3)*	+ 143.0***
(B) K^+-free	Normal K^+-free	+ 4.1 ± 0.5 (4)**	+ 97.5***
(C) Normal K^+-free	Normal Normal	+ 2.6 ± 0.4 (6)**	+ 45.0

* $P < 0.05$ (paired comparison).
** $P < 0.0005$ (paired comparison).
*** Magnitude of effects in A and B differs significantly ($P < 0.025$, unpaired comparison).

sugar transport was, therefore, measured. Incubation or preincubation, or both, in a K^+-free medium was used, as ouabain is washed out of the tissue with difficulty and its effect on the sodium pump would not be rapidly reversed.

Table I shows that sugar transport in a K^+-free medium was significantly increased, regardless of the presence (Expt. A) or absence (Expt. B) of K^+ during preincubation. However, preincubation in the absence of K^+ increased the control sugar uptake in Expt. B, so that K^+-free incubation resulted in an effect of significantly smaller relative magnitude than in Expt. A. This difference caused by preexposure to K^+-free medium was studied in Expt. C by preincubating one half of each diaphragm in normal medium and the other half in K^+-free medium, followed

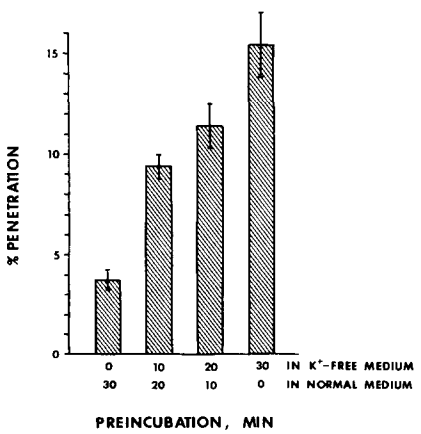

Fig. 1. Effect of duration of preincubation in K^+-free medium on the penetration of 3-methylglucose. Tissues were preincubated for a total of 30 min in K^+-free or normal medium, or both, as indicated and then incubated with 5 mM 3-methylglucose in K^+-free medium for 10 min. The bars represent percentage penetration of the intracellular water space ± S.E. indicated by brackets. $N = 6$ for each bar.

by incubation of both halves in normal medium containing 3-methylglucose. Such pre-exposure to K^+-free medium resulted in a highly significant increase in sugar transport, showing that the effect of the K^+-free medium persisted even after 10 min exposure of the tissue to normal medium.

The effect of duration of exposure to K^+-free medium on sugar transport is emphasized by the data in Fig. 1, which show that sugar transport was increasingly stimulated as duration of exposure to the K^+-free medium increased. In this series the total time of preincubation was 30 min, part in normal and part in K^+-free medium, as indicated under each bar (*e.g.* 10 min in K^+-free and 20 min in normal medium).

The results in Table I and Fig. 1 show that the absence of external K^+ *per se* is not directly responsible for the increased sugar penetration. An indirect effect *via* inhibition of the Na^+-pump is, however, consistent with the results.

Fig. 2 shows the changes in intracellular levels of Na^+ and K^+ which occur under the conditions of these experiments: When the Na^+ pump was inhibited by

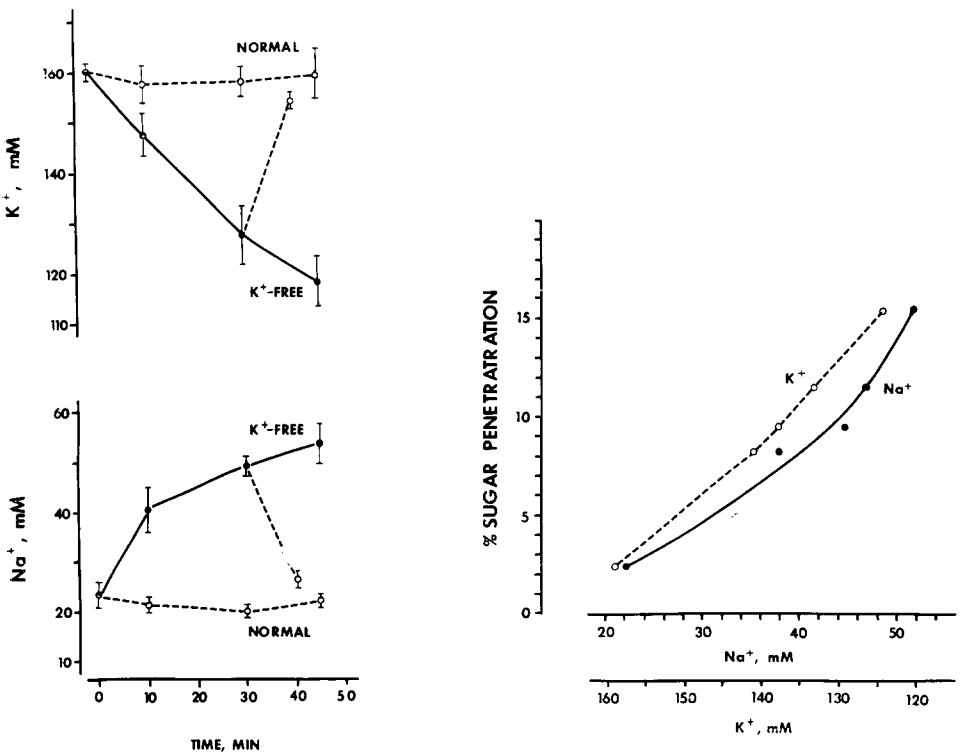

Fig. 2. Changes in intracellular Na^+- and K^+-levels during incubation. Tissues were incubated for the periods indicated on the abscissa in K^+-free medium (——) or in normal medium (– – –). The data are means ± S.E. ($N > 6$) and are corrected for the mannitol space determined in the same sample. The 30 min mannitol value was used for calculation of the zero time point (freshly excised tissue).

Fig. 3. Correlation of sugar penetration and intracellular Na^+- and K^+-levels. Ordinate, percentage penetration after 10 min incubation with 5 mM 3-methylglucose. Abscissa, "average" intracellular level of Na^+ or K^+ (arithmetic mean of values at start and end of incubation with sugar). Data taken from experiments shown in Figs. 1 and 2 and Table I.

omission of K^+ from the incubation medium there was a progressive intracellular accumulation of Na^+ and depletion of K^+. In contrast, prolonged incubation in the normal medium caused hardly any changes. When incubation in K^+-free medium for 30 min was followed by 10 min incubation in normal medium, substantial but incomplete recovery of ionic gradients occurred. The parallelism between the gradual increase in sugar penetration and the gradual changes in intracellular levels of Na^+ and K^+ upon inhibition of the Na^+ pump is obvious.

This relationship is even better illustrated in Fig. 3 which plots sugar penetration as a function of the internal levels of Na^+ and K^+. As these change continuously in some cases (see Fig. 2), the "average" ionic level during the 10 min incubation period with sugar was taken to equal the arithmetic mean of concentrations at the start and the end of incubation. The figure includes data from tissues in normal medium, in K^+-free medium for varying periods of time, and from tissues first exposed to K^+-free and then to normal medium.

TABLE II

EFFECT OF K^+-FREE MEDIUM ON EFFLUX OF 3-METHYLGLUCOSE

One hemidiaphragm of each pair was analyzed after 30 min loading with 10 mM 3-methylglucose, the other after loading followed by 10 min washout in sugar-free medium. The data refer to means of differences ± S.E. in paired experiments and give the amount of sugar lost from the tissue in percent of the amount loaded in the paired control. The number of pairs for each figure is given in brackets. P by unpaired t test (2-tailed).

	% *of sugar lost during washout*			
Preincubation (loading) medium:	*Normal*		*K⁺-free*	
Incubation (washout) medium:	*Normal*	*K⁺-free*	*Normal*	*K⁺-free*
Expt. 1	29.6 ± 2.3 (6)	49.2 ± 2.5 (6) $P < 0.005$ *	35.0 ± 2.7 (6) $P < 0.02$ *	49.6 ± 3.2 (6) $P < 0.005$ **
Expt. 2	47.0 ± 1.4 (12)		63.5 ± 1.8 (11) $P < 0.001$ *	

* As compared to loss into normal medium after preincubation in normal medium (first column).

** As compared to loss into normal medium after preincubation in K^+-free medium (third column).

If the sugar carrier were regulated by carrier-bound and co-transported Na^+ or K^+, or both, variations in ion levels should affect sugar influx and efflux in qualitatively different ways. The data in Table II show, however, that incubation and preincubation in K^+-free medium increased sugar efflux in the same manner as it did sugar influx. Moreover, as with influx, efflux into normal medium was significantly greater if the tissue was previously exposed to K^+-free medium (compare columns 1 and 3). The two experiments were performed several months apart and the difference in absolute values is probably due to seasonal factors.

Such a symmetrical activation of sugar transport is characteristic of insulin (*e.g.* see ref. 8) and is shown in Table III. The data in Table III also show that sugar efflux was activated by $1 \cdot 10^{-5}$ M ouabain, a concentration known to inhibit the Na^+ pump[14]. This complements earlier reports[1,2] of activation of sugar influx by ouabain.

TABLE III

EFFECTS OF OUABAIN AND INSULIN ON EFFLUX OF 3-METHYLGLUCOSE

Tissues were loaded and equilibrated with 3-methylglucose in normal medium as described in the text. One hemidiaphragm of each pair was then washed out for 10 min in sugar-free medium. 0.5 munit of insulin per ml and $1 \cdot 10^{-5}$ M ouabain were added as indicated. The data are expressed as in Table III. The number of pairs for each figure is indicated in brackets.

Additions	% *Sugar lost during washout*
None	35.2 ± 1.7 (10)
Insulin	55.7 ± 1.9 (12)*
Ouabain	47.1 ± 1.5 (10)*

* $P < 0.001$ as compared to loss into control (no addition).

The effect of insulin on sugar transport may show a time lag[15] and we have shown previously[2] that the effect of ouabain depends on the presence of glucose during "fixation" of ouabain to the tissue. The incubation sequence was therefore modified to include the required preincubation with insulin or ouabain. Following "loading" for 30 min with 10 mM 3-methylglucose, the tissues were exposed for 10 min to a sugar concentration equal to that reached in the tissue at the end of the first stage (determined in preliminary experiments), so that no net transport of sugar took place during this "equilibration" period. Finally, one hemidiaphragm of each pair was washed out for 10 min in a sugar-free medium as described in MATERIALS AND METHODS. Where indicated in Table III insulin was added during equilibration and washout; ouabain was present in all three stages, with 10 mM glucose added in the loading stage.

DISCUSSION

Our earlier results[2] showed clearly that sugar influx is stimulated whenever the Na^+ pump is inhibited, either by ouabain or omission of K^+ from the incubation medium or inhibition of oxidative metabolism. The results presented here provide some evidence on the nature of this link between sugar and Na^+ transport, and on the possible role of Na^+ or K^+ binding and co-transport on the sugar carrier.

Inhibition of the Na^+ pump in a K^+-free medium leads to a progressive increase in cellular Na^+ and depletion of cellular K^+, which is followed by gradual recovery of ionic gradients upon transfer of the tissue to normal medium (Fig. 2). The results in Table I and Fig. 1 show that the increase in sugar influx also develops gradually and persists for some time after return of the tissue to a normal medium, *i.e.* during the recovery period when the Na^+ pump is actually stimulated to greater than resting activity. Thus, stimulation of sugar entry is correlated neither with the activity of the Na^+ pump as such nor with the concentration of external K^+ at the time of transport. Rather, it seems to parallel the dissipation of Na^+ or K^+ gradients, *i.e.* a consequence of Na^+ pump inhibition. This relationship is best illustrated in Fig. 3 showing the correlation of sugar influx and intracellular concentrations of Na^+ and K^+. The present data not do indicate which of the two ions may be involved.

The measurements of sugar efflux in Tables II and III show that, similarly to insulin, inhibition of the Na^+ pump by ouabain or by omission of K^+ stimulates

efflux as well as influx of sugar. This stimulation of efflux also shows the same dependence upon previous exposure to K^+-free medium, as was demonstrated for influx. CLAUSEN[8] recently demonstrated a similar stimulation of sugar efflux from adipose tissue and also emphasized the apparent parallelism between depletion of cellular K^+ and stimulation of sugar transport, into and out of the cell.

Such a situation is consistent with an effect on some property of the sugar carrier, for example its mobility, making it more effective for transport in either direction. It is, however, not consistent with a modification of the carrier's properties through simultaneous binding and transport of for example Na^+, as is thought to occur in sugar transport in the small intestine[9] and in amino acid transport in many tissues (see ref. 16 for review). In such a co-transport model the activation of sugar influx and efflux depends on the concentrations of activating ions at the outside and the inside of the membrane, respectively. It is indeed this asymmetry of activation coupled to the asymmetry of ion distribution maintained by the cell, which makes these Na^+-dependent carriers capable of active transport. The similar model proposed for insulin-sensitive tissues by LETARTE AND RENOLD[7] would therefore require sugar transport in these tissues to also show differential activation of influx and efflux, depending on where alterations in ionic levels take place, and active transport (against a concentration difference) depending for its energy on the ion pump(s) responsible for maintaining ionic gradients. The collective evidence of this and other studies shows, however, that (a) any experimental manipulations affect influx and efflux of sugar equally, (b) omission of K^+ from the medium affects sugar transport *via* inhibition of the Na^+ pump, (c) stimulation of sugar transport seems to be related to the increased internal Na^+ level, or decrease K^+ level, which are the consequence of Na^+ pump inhibition, (d) neither an energy requirement nor accumulation of sugar against the gradient was demonstrated, whereas inhibition of oxidative metabolism actually stimulated transport down the gradient. These conclusions apply primarily to insulin-sensitive sugar transport in muscle but are probably also valid for the essentially identical system in white adipose tissue.

This study shows that the regulation of sugar transport in muscle does not occur *via* binding and co-transport of Na^+ and/or K^+ at the sugar carrier. Neither are the data consistent with a model assuming "activation binding" of ions at the same face of the carrier as the sugar about to be translocated, but no co-transport. Both influx and efflux of sugar appear to be regulated by the internal ionic levels. The data do not suggest by what mechanism intracellular ions may affect sugar transport nor do they provide a clue to the mode of action of insulin which is clearly unrelated to gross changes in internal ionic levels. More recent data from this laboratory suggest that the internal Na^+ level may act on sugar transport indirectly, perhaps through changes in the membrane pools of divalent ions.

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REFERENCES

1 T. CLAUSEN, *Biochim. Biophys. Acta*, 120 (1966) 361.
2 I. BIHLER, *Biochim. Biophys. Acta*, 163 (1968) 401.
3 R. J. HO AND B. JEANRENAUD, *Biochim. Biophys. Acta*, 144 (1967) 61.
4 R. J. HO, B. JEANRENAUD, TH. POSTERNAK AND A. E. RENOLD, *Biochim. Biophys. Acta*, 144 (1967) 74.
5 J. LETARTE AND A. E. RENOLD, *Biochim. Biophys. Acta*, 183 (1969) 350.
6 J. LETARTE, B. JEANRENAUD AND A. E. RENOLD, *Biochim. Biophys. Acta*, 183 (1969) 357.
7 J. LETARTE AND A. E. RENOLD, *Biochim. Biophys. Acta*, 183 (1969) 366.
8 T. CLAUSEN, *Biochim. Biophys. Acta*, 183 (1969) 625.
9 R. K. CRANE, in C. F. CODE AND W. HEIDEL, *Handbook of Physiology*, Vol. 3, Section 6, Am. Physiol. Soc., Washington, 1968, p. 1323.
10 I. BIHLER, *Abstr. 4th Intern. Congr. Pharmacol., 1969*, p. 319.
11 T. KONO AND S. P. COLOWICK, *Arch. Biochem. Biophys.*, 93 (1961) 514.
12 H. A. KREBS AND K. HENSELEIT, *Z. Physiol. Chem.*, 210 (1932) 33.
13 M. SOMOGYI, *J. Biol. Chem.*, 160 (1945) 69.
14 A. KLEINZELLER AND A. KNOTKOVA, *Physiol. Bohemoslov.*, 13 (1964) 317.
15 H T. NARAHARA, P. ÖZAND AND C. F. CORI, *J. Biol. Chem.*, 235 (1960) 3370.
16 W. D. STEIN, *The Movement of Molecules across Cell Membranes*, Academic Press, New York, 1967, p. 169.