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REGULATION OF SUGAR TRANSPORT IN MUSCLE: EFFECT OF INCREASED EXTERNAL POTASSIUM *IN VITRO**

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SUMMARY

(1) Membrane transport of 3-*O*-methyl-D-[^{14}C]glucose and intracellular levels of Na^+ and K^+ were measured in "intact" rat hemidiaphragms, *in vitro*.

(2) Incubation in media with high K^+ content led to decreased Na^+ and increased K^+ levels in the muscle cells, corresponding to stimulation of the Na^+ pump.

(3) In parallel with the decrease in internal Na^+ and increase in internal K^+ levels, sugar transport both into and out of the cells was decreased. This effect was greater in the presence of a submaximal concentration of insulin but was also observed in its absence.

(4) "High-potassium" media were prepared by isosmotic substitution of K^+ for Na^+ or by addition of KCl to normal medium. The resulting decrease in external Na^+ or increase in osmolarity, respectively, both stimulated sugar transport and partially counteracted the inhibitory effect of high K^+.

(5) The inhibition of sugar transport by high K^+ is the converse to the previously described stimulation of sugar transport following inhibition of the Na^+ pump. The results thus support our earlier suggestion that the internal concentrations of Na^+ and/or K^+ may regulate sugar transport in muscle.

INTRODUCTION

Recent work in this and other laboratories has shown that the insulin-sensitive specific sugar transport mechanism in muscle[1–3] and in adipose tissue[4–9] is stimulated when the Na^+ pump is inhibited by a variety of means including anoxia, decreased external K^+ and inhibitory concentrations of cardioactive steroids. The data suggest that the effect on sugar transport may be linked to an increase in internal Na^+ or decrease in internal K^+ levels, or both, caused by inhibition of the Na^+ pump. We have also shown recently[10] that in these conditions sugar influx and efflux are both stimulated. These observations are consistent with a regulatory effect of intracellular ions on the sugar carrier, perhaps its "mobility", but not with the formation of a ternary ion–sugar–carrier complex. Consequently, we have proposed, as a working

* These results were presented[1] in part to the 24th International Congress of Physiological Sciences, Washington, D.C., 1968.

hypothesis, that the Na^+ pump through its effect on internal ion levels exerts a modulating effect on sugar transport in muscle and adipose tissue.

If there exists such a regulatory relationship between increased sugar transport and increased internal Na^+ (or decreased internal K^+), one would expect that opposite changes in internal ion levels should lead to inhibition of sugar transport. It has been shown that high external K^+ stimulates the Na^+–K^+-dependent ATPase of mammalian tissues and may cause shifts in the ionic balance which are consistent with a stimulation of the Na^+ pump. For example, incubation in a K^+-rich solution has led to an increase in the rate of Na^+ efflux and in the level of internal K^+ in frog muscle[11–14] and to a decrease in the internal Na^+ level in intact rat diaphragms[15,16]. We have, therefore, used incubation in a high-K^+ medium to test the above prediction, namely that sugar transport in muscle should be inhibited following stimulation of the Na^+ pump and the consequent changes in internal levels of K^+ and Na^+.

METHODS

Sugar transport was estimated by measuring the distribution of the nonmetabolized glucose analog, 3-*O*-methyl-D-glucose in the rat diaphragm by methods described previously[3,10]. Briefly, "intact" rat hemidiaphragms[17] were incubated at 37° in 5.0 ml substrate-free KREBS–HENSELEIT[18] bicarbonate buffer (pH 7.4) bubbled with 95 % O_2–5 % CO_2 and containing 0.8 % of 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. Two types of high-K^+ media were prepared, (a) by adding appropriate amounts of solid KCl to the basal medium and (b) by isosmotic substitution of K^+ for Na^+. The radioactivity of tissue extracts and media was determined by double label liquid scintillation spectrometry and ionic content was measured by atomic absorption spectrophotometry. Where, applicable, the data were corrected for the extracellular space determined in the same tissue sample.

Sugar influx was estimated from the percentage penetration (sugar in intracellular H_2O/sugar in medium) × 100, reached at the end of an incubation period with sugar. This expression underestimates unidirectional influx to the extent of simultaneously occuring efflux and is, therefore, only semiquantitative in nature[3,10]. For estimation of efflux the tissues were first "loaded" by incubation for 30 min in 10 mM 3-methylglucose, then "washed out" in sugar-free medium and the radioactivity left in the tissue determined. Loss during washout was expressed as percentage of the sugar content of the paired hemidiaphragm which was loaded but not washed out. Statistical evaluation of results was done by Student's *t*-test.

RESULTS

Most of the experiments described below were done by adding KCl to the basal medium, resulting in a slightly or moderately hyperosmolar solution. The effect of high external K^+ on internal ion levels under these conditions is shown in Fig. 1. Incubation with 16 mM K^+ (10 mM KCl added) resulted in a highly significant ($P < 0.0005$, unpaired comparison) increase in intracellular K^+ and decrease in intracellular Na^+, when compared to ion levels after incubation in normal medium.

Incubation in the 36 mM K^+ medium (30 mM KCl added) had an even more pronounced effect on ion levels. In such a hypertonic medium the osmotic shift of water out of the cell would tend to increase the intracellular concentrations of both K^+ and Na^+. Part of the observed increase in the level of K^+ could be due to this effect but the decrease in the level of Na^+ is clearly the opposite from that expected as a result of osmotic change. The effect of high K^+ on ion levels was not significantly influenced by insulin. It has been shown that insulin may increase K^+ and decrease Na^+ concentrations in rat diaphragm muscle[19]. We have also noted this effect (unpublished observations) but found it negligible under the present conditions of incubation in the absence of metabolic substrates.

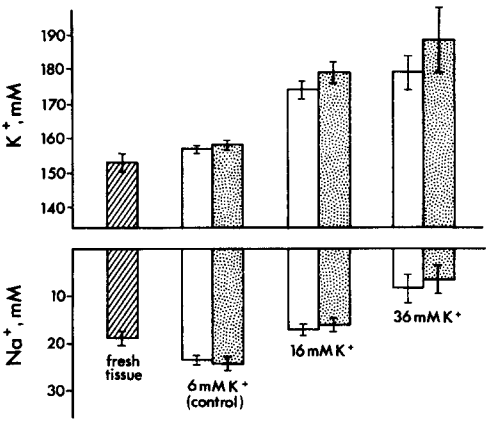

Fig. 1. Dependence of intracellular Na^+ and K^+ on the level of extracellular K^+. K^+ concentrations were adjusted by adding KCl to the basal medium. Incubation time, 50 min. Shaded bars indicate the addition of 0.25 munit/ml insulin. The height of the bars refers to the mean (± S.E.) intracellular concentration of 13–26 samples.

The effect of high K^+ on the uptake of 3-methylglucose is shown in Table I. With 16 mM K^+ sugar penetration was significantly decreased in comparison to the control in normal K^+. This decrease was greater in the presence of a submaximal dose of insulin, 0.25 munit/ml, but was highly significant even in its absence. If the decrease is expressed as per cent change from control uptake, it appears that insulin increases the inhibitory effect of high K^+. With 36 mM K^+ (30 mM KCl added) the inhibition of sugar penetration is also highly significant. However, hyperosmolarity as such increases sugar transport, as shown by CLAUSEN[20] and confirmed by the experiments in Table I where mannitol was added to produce media approximately isosmolar with the 16 and 36 mM K^+ medium, respectively. 20 mM mannitol did not affect sugar transport significantly but 60 mM did. The last two columns in Table I show the calculated effects of high K^+ media on sugar penetration when the mean values with the appropriate hyperosmolar (mannitol) controls were taken as reference. With this correction, the inhibition of sugar penetration by 36 mM K^+ appears to be much greater than with 16 mM K^+.

Table II shows that the efflux of 3-methylglucose is also significantly decreased by 16 mM K^+ in the presence of insulin. The decrease in the absence of insulin was not significant at the 5 % level. This decrease in efflux under conditions inhibiting influx

TABLE I

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

Incubation for 30 min with 5 mM 3-methylglucose. KCl added to normal medium to obtain total concentrations of K^+ shown. 0.25 munit/ml insulin added as indicated. 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 parentheses. The "corrected" values were calculated with reference to the mean of the appropriate value with mannitol (see text). P by paired comparison.

Incubation medium	*Observed*		*"Corrected"*	
	Δ% penetration	*% change*	*Δ% penetration*	*% change*
Normal (6 mM K^+), 16 mM K^+	− 3.7 ± 0.6 (11) $P < 0.0005$	−31	− 5.3	−39
Normal + insulin, 16 mM K^+ + insulin	−15.7 ± 2.0 (11) $P < 0.0005$	−45	−17.2	−47
Normal, 36 mM K^+	− 2.9 ± 0.9 (5) $P < 0.025$	−24	−13.5	−60
Normal + insulin, 36 mM K^+ + insulin	−21.4 ± 1.9 (5) $P < 0.0005$	−61	−34.1	−71
Normal, 20 mM mannitol	+ 1.6 ± 0.7 (3) $P > 0.05$	+13		
Normal + insulin, 20 mM mannitol + insulin	+ 1.0 ± 1.3 (3) $P > 0.25$	+ 3		
Normal, 60 mM mannitol	+10.6 ± 0.5 (5) $P < 0.0005$	+88		
Normal + insulin, 60 mM mannitol + insulin	+12.7 ± 1.4 (5) $P < 0.0005$	+35		

TABLE II

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

Tissues were loaded with sugar and then washed out in sugar-free solution as described in the text. The composition of media was as described in Table I. The data give the amount of sugar lost from the tissue (mean ± S.E.) in percent of the amount loaded in the paired control. The number of pairs for each figure is given in parentheses. P by unpaired comparison.

Incubation medium	*% of sugar lost during washout*	
Normal (6 mM K^+)	50.8 ± 1.3 (10)	$P > 0.05$
16 mM K^+	47.5 ± 2.7 (10)	
Normal + insulin	61.7 ± 1.8 (8)	$P > 0.05$
16 mM K^+ + insulin	57.1 ± 1.3 (8)	

agrees with earlier data that efflux and influx of sugar in muscle are stimulated in parallel by the same factors.

Fig. 2 summarizes the results obtained when K^+ was isosmotically substituted for part of Na^+ in the medium. This procedure avoids the complications from hyperosmolarity but introduces new ones due to the variations in external Na^+. When exposed to the high K^+–low Na^+ media, tissues showed an increase in internal K^+ and a decrease in internal Na^+ but these changes were not as clearly correlated with variations in external K^+ as those shown in Fig. 1. Moreover, there was an appreciable discrepancy between the increase in K^+ and the decrease in Na^+ in the tissues exposed

to the medium with 78 mM K^+. The effect of insulin at each level of K^+ on the changes in internal ion levels was not statistically significant. The influence of the high K^+–low Na^+ media on sugar penetration differed depending on the addition of insulin. In its presence sugar transport was progressively more inhibited as external K^+ was increased, although the inhibition was less than under the conditions of Table I. In the absence of insulin sugar transport was decreased to a small but significant extent ($P < 0.05$, paired comparison) with 16 mM K^+, yet was significantly increased ($P < 0.01$) with 36 mM K^+. The small increase with 78 mM K^+ was not significant.

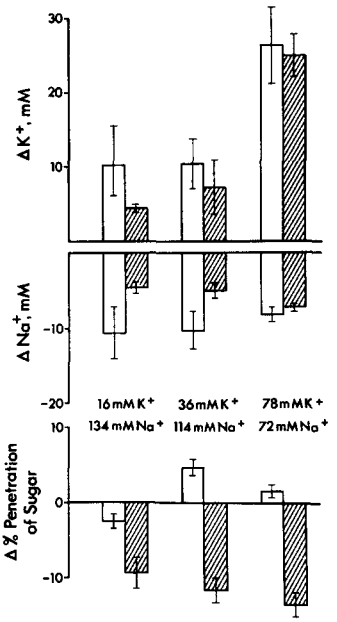

Fig. 2. Changes in tissue ion levels and in 3-methylglucose penetration following partial isosmotic substitution of K^+ for Na^+ in the medium. Incubation time, 30 min. Shaded bars indicate the addition of 0.25 munit/ml of insulin. The height of the bars in the upper part indicates mean (± S.E.) changes in intracellular ion concentrations when compared to paired controls in basal medium (6 mM K^+, 144 mM Na^+). The lower part shows the corresponding changes in sugar penetration (mean ± S.E.). $N = 5$ pairs for each bar.

These aberrant effects are due to the variations in external Na^+ which have complicated effects of their own on sugar transport in muscle[21] which are both time and concentration dependent (unpublished observations). With 30 min of incubation, sugar transport increases to a maximum at 100–110 mM Na^+ and gradually decreases at still lower Na^+ levels. Thus, in the concentration range of Fig. 2, the combined effects of high K^+ and low Na^+ oppose each other, accounting for the pattern of sugar penetration in the absence of insulin and probably also for the lesser decrease in insulin stimulated transport than seen in Table I.

DISCUSSION

A dependence of the Na^+ pump in muscle and other tissues on the concentration of external K^+ has been known since the work of STEINBACH[22,23]. The stimulatory

effect of high external K^+ has been demonstrated, among others, in skeletal muscle[11–16,22,23], intact erythrocytes[24] and their reconstituted ghosts[25], in crab nerve[26] and squid giant axons[27]. The mechanism of this dependence appears to be 2-fold, at least in muscle. Firstly, the activity of the Na^+–K^+-dependent ATPase, thought to be a functional part of the Na^+ pump, is increased by K^+ in a saturable manner[28,29]. Secondly, high K^+ has a depolarizing effect on muscle, and it has been suggested[12,16] that the Na^+ pump is activated by a decrease in membrane potential. Regardless of the mechanism(s) responsible, it is well established that in skeletal muscle high K^+ stimulates the Na^+ pump, resulting in increased internal K^+ and decreased internal Na^+ levels. Our data confirm that significant electrolyte shifts of this type also occur in the present experiments. We have shown earlier[10] that sugar transport in muscle is affected by the intracellular concentrations of Na^+ or K^+, or both, and not by the activity of the Na^+ pump at the time of transport; HOLLOSZY AND NARAHARA[30] have found that the stimulation of sugar transport by muscular activity is not linked to electrical events. Hence, one may reasonably exclude the possibility that sugar transport is directly affected by changes in Na^+ pump activity as such or by K^+-induced membrane depolarization. Incubation in high K^+ should, therefore, be a useful and valid tool for the study of interactions between the internal levels of Na^+ and K^+ and the activity of the sugar transport system.

The results presented here show that the transport of 3-methylglucose, a non-metabolized glucose analog, both into and out of rat diaphragm muscle was significantly decreased when internal K^+ was raised and internal Na^+ lowered through incubation in a high-K^+ medium. These results are the exact converse of earlier findings[1–10] that sugar transport was stimulated when internal Na^+ was increased and internal K^+ decreased through inhibition of the Na^+ pump. We have proposed[3,10] that the internal levels of Na^+ and/or K^+ exert a regulatory control over sugar transport in muscle and that this control mechanism may serve *in vivo* to increase the supply of glucose for glycolysis when the "aerobic" Na^+ pump is depressed through a decrease in the supply of oxidative ATP. In other words, the well known increase in sugar transport during anoxia is thought to be mediated by a negative feedback from the aerobic Na^+ pump. Clearly, if such a regulatory arrangement exists, it should function in either direction, *i.e.* to inhibit as well as to stimulate sugar transport. The present experiments are evidence for such an effect, thereby providing support for our hypothesis.

The data indicate that increasing concentrations of external K^+, whether replacing Na^+ or added to the complete medium, have a graded inhibitory effect on sugar transport, consistent with the effect on ion distribution. This relationship is partly obscured by the effects on sugar transport of variations in external Na^+ or osmolarity, one or the other being inevitable, depending on the method used to increase the K^+ content of the medium. Both these factors tend to stimulate sugar transport under certain conditions[20,21]. In Table I an attempt was made to correct for the stimulating effect of hyperosmolarity by comparing sugar transport in high-K^+ media to that in isosmotic mannitol-containing media. The calculation assumes that the effects of high external K^+ and of hyperosmolarity are algebraically additive which may not be strictly true, so that the correction represents an outside limit. In Fig. 2 the data are complicated by the stimulatory effect of variations in external Na^+ which is biphasic under the conditions of the experiments (unpublished data).

The observed changes in sugar transport may be explained, therefore, by a summation of the opposing effects of internal ion levels and of low external Na^+. Thus, low external Na^+ or hyperosmolarity may partially antagonize or even reverse the inhibitory effect of high external K^+ but cannot be responsible for the clearcut inhibition of sugar transport demonstrated here. Furthermore, significant inhibition is seen in 16 mM K^+ media of both types in which interference by both factors is minimized. As shown in Table I, the effect on sugar transport of an equally hypertonic medium with 20 mM mannitol was not significant. Also, the tissue water content remained unchanged, and the possible error due to the effect of high K^+ on extracellular space[15] was obviated in all experiments by correcting the sugar and ion content data for the extracellular space determined in the same tissue sample. The effect of reducing the external Na^+ from 144 to 134 mM during 30 min (as in Fig. 2) is also negligible.

Only a qualitative correlation between electrolyte shifts and changes in sugar transport was sought at this stage for the following reasons: (1) As pointed out above, the sugar transport data do not strictly reflect unidirectional fluxes. (2) The studies of LEV[31] on frog muscle, using Na^+-sensitive microelectrodes, suggest than an appreciable fraction of Na^+ usually considered intracellular is located in the transverse tubular system which may be in contact with the extracellular space; the "true" sarcoplasmic concentration of Na^+ is therefore uncertain. (3) It has been reported[32] that in rat diaphragm Na^+ (together with Cl^- and H_2O) may be extruded without exchange for K^+ by a mechanism different from the classical ouabain-sensitive pump, and the relative importance of this process is at present not clear. Finally, (4) there are strong indications[33] that the regulatory effect of internal Na^+ (or K^+) on sugar transport is not direct but may involve the participation of Ca^{2+}. This ion appears also to play a role in mediating the increase in sugar transport by muscular contraction[30], prompting speculations that a specific membrane bound pool of Ca^{2+} may be the ultimate and common mediator of a variety of regulatory influences in sugar transport.

Earlier reports on the effect of high external K^+ on sugar transport in muscle are mostly in agreement with the results reported here. BHATTACHARYA[34] was the first to describe a decrease in the uptake of glucose and xylose by rat diaphragm upon replacement of Na^+ by K^+ in an isotonic sucrose medium. RYBOVÁ[35] found that xylose uptake by intact rat hemidiaphragms was significantly inhibited when 30–100 mM K^+ was substituted for Na^+ in a saline medium. KIPNIS AND PARRISH[15] reported that the penetration of 2-deoxyglucose into insulin-treated intact rat diaphragms was depressed as increasing concentrations of K^+ from 40 to 150 mM replaced Na^+ in the medium. In contradiction to all these and our own results are the data of CRONE[36] on the isolated perfused cat hind limb, showing that perfusion with K^+-rich blood increased glucose uptake. His finding that 10^{-5} M ouabain strongly depressed glucose uptake is also opposed to other observations[1–10]. We have no ready explanation for this discrepancy. While this paper was in preparation there appeared a preliminary report by CLAUSEN AND KOHN[37] that high K^+ had a graded inhibitory effect on 3-methylglucose efflux from isolated rat soleus muscle. The effect was greater with low than with high insulin concentrations but was not seen in the absence of insulin. These results agree with our data, except that we could demonstrate an effect of high K^+ on sugar uptake also in the absence of insulin. It would appear from their and from our own results that the tissue is most responsive to inhibitory regulatory

interventions when transport is moderately increased *e.g.* with low doses of insulin, whereas the response is less when transport is repressed (in the basal state) or very strongly stimulated (with supramaximal doses of insulin).

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