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THE RELATIONSHIP BETWEEN THE TRANSPORT OF GLUCOSE AND CATIONS ACROSS CELL MEMBRANES IN ISOLATED TISSUES

VII. THE EFFECTS OF EXTRACELLULAR Na^+ AND K^+ ON THE TRANSPORT OF 3-*O*-METHYLGLUCOSE AND GLUCOSE IN RAT SOLEUS MUSCLE

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SUMMARY

1. The effect of Na^+ and K^+ on sugar transport in peripheral skeletal muscle has been assessed by measurements of 3-*O*-methylglucose uptake and efflux and glucose uptake in the isolated rat soleus muscle.

2. The transport of 3-*O*-methylglucose showed no absolute requirement for Na^+ or K^+ in the extracellular milieu, neither in the absence nor in the presence of insulin.

3. Total replacement of extracellular Na^+ by Li^+ was found to stimulate glucose uptake, glycogen deposition and 3-*O*-methylglucose transport, both in the absence and in the presence of insulin. Replacement by K^+ inhibited both basal and insulin-stimulated glucose uptake.

4. In buffers in which Na^+ had been iso-osmotically replaced by K^+ (or Rb^+) at high concentrations, the basal rates of 3-*O*-methylglucose uptake and efflux were not significantly altered. However, the replacement of extracellular Na^+ by K^+ (in the range 12–150 mM) promptly suppressed the stimulating effect of insulin, 2,4-dinitrophenol and trypsin on 3-*O*-methylglucose efflux.

5. Incubation in K^+-substituted iso-osmolar buffer leads to considerable swelling of the muscle cells. Similar degrees of swelling induced by a hypo-osmolar environment were associated with a marked suppression of the stimulating effect of insulin, trypsin and 2,4-dinitrophenol on 3-*O*-methylglucose efflux.

6. When KCl (100 mM) is added in excess of the other components of the incubation medium, there is no significant change in cell volume and the insulin-stimulated 3-*O*-methylglucose efflux is not suppressed.

7. It is concluded that the well-documented inhibitory effect of K^+ on sugar transport in skeletal muscle is, for a major part, not the result of a direct action on the sugar transport system, but related to changes in cell volume. The relative participation of different areas of the plasma membrane, *e.g.* transverse tubules, sarcoplasmic reticulum and "true" outer membrane may control the capacity for stimulation of sugar transport.

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INTRODUCTION

A number of reports have indicated that the permeability of the plasma membrane to glucose in insulin-sensitive tissues is influenced by Na^+ and K^+. In both rat hemidiaphragm[1-4] and soleus muscle[5] a high extracellular K^+ concentration was found to diminish both basal and insulin-stimulated glucose uptake. An inhibitory effect of K^+ on the uptake of xylose[1], 2-deoxyglucose[6] and 3-*O*-methylglucose[7,8] has also been described. These effects are not secondary to a lowering of extracellular Na^+ concentration, since partial or total substitution of Na^+ by Li^+, sucrose, mannitol, Tris or choline caused either no alteration, or even stimulation of sugar uptake[1,6,9-11].

In previous reports[12-15], it was shown that measurements of 3-*O*-methylglucose efflux provide a sensitive tool for the study of the function of the glucose transport system and the time-course of changes in its activity. The aim of the present study has been to characterize the effects of extracellular Na^+ and K^+ on the permeability of the plasma membrane of a peripheral skeletal muscle to 3-*O*-methylglucose and glucose. The results indicate that the extracellular Na^+ concentration has little or no direct influence on basal or insulin-stimulated transport of 3-*O*-methylglucose in soleus muscle.

However, raising the extracellular concentration of K^+ (or Rb^+) in the range 12–150 mM promptly and reversibly suppressed the stimulation of sugar transport by insulin. The effects of a number of other stimuli (2,4-dinitrophenol, trypsin and hyperosmolarity) were also considerably suppressed by raised concentrations of K^+. These effects may be related to the changes in cell volume induced by high extracellular K^+ concentrations since swelling produced by a hypo-osmolar medium is also associated with a similar suppression of 3-*O*-methylglucose transport.

METHODS

All experiments were performed using muscles from rats in the weight range 60–70 g. The procedures for the measurement of the uptake and efflux of 3-*O*-methylglucose, inulin space and K^+ content have been described in detail in previous papers[12-14]. Glucose uptake was determined by measuring the disappearance of glucose from the incubation medium [16], and the incorporation of [$^{14}C_6$]glucose into glycogen assessed as previously described[17].

Where indicated, Na^+ in the incubation medium was partially or totally replaced by equivalent amounts of K^+, Li^+, Rb^+, Cs^+ or $Tris^+$. In other experiments, varying concentrations of NaCl were replaced by a double concentration of sucrose or mannitol, to achieve approximate isotonicity. The hypo-osmolar medium was prepared by omitting 60 mM of the NaCl from the basic Krebs–Ringer bicarbonate buffer. All buffers were gassed with O_2–CO_2 (95:5, by vol.) and the pH maintained close to 7.35. Specific details are given in the appropriate legends.

Chemicals, isotopes and hormones

All chemicals were of analytical grade. 3-*O*-methylglucose was obtained from Calbiochem (Los Angeles), pancreatic trypsin (EC 3.4.4.4) from Nutritional Biochemicals Corp. (Cleveland), and the reagents for the enzymatic determination of glucose from Kabi AB (Sweden). 3-*O*-[^{14}C]Methylglucose (specific activity 50 mC/mmole), [*hydroxymethyl*-^{14}C]inulin (specific activity 9 mC/mmole), and D-[$^{14}C_6$]glucose (speci-

fic activity 335 mC/mmole) were products of the Radiochemical Centre, Amersham, England. L-Epinephrine was purchased from Rhone-Poulenc (Paris) and acetylcholine iodide from Sigma (St. Louis). Mono-component pork insulin lot No. SL-11669 MC (25 I.U./mg, purified by chromatography) was a gift from the Novo Research Laboratories (Copenhagen).

RESULTS

When soleus muscles were incubated in a Krebs–Ringer bicarbonate buffer in which all the Na^+ was replaced by K^+, the uptake of glucose and its incorporation

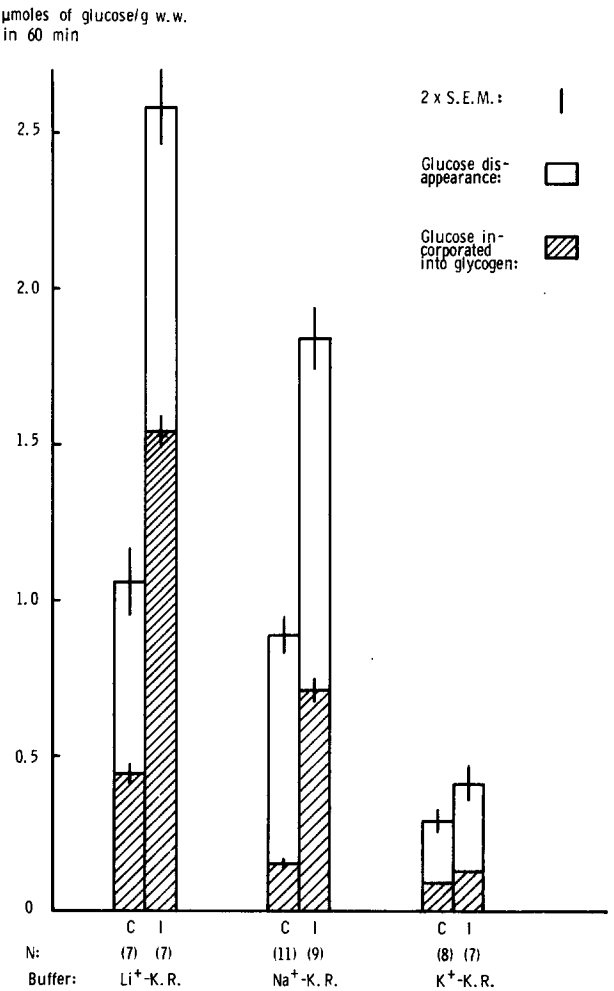

Fig. 1. Effect of Na^+ replacement and insulin on the uptake of glucose and its incorporation into glycogen by rat soleus muscle. Soleus muscles were incubated for 60 min at 30° in 1 ml of Krebs–Ringer bicarbonate buffer (K.R.) containing 1 mM pyruvate and 1 mM D-[$^{14}C_6$]glucose (0.2 μC/ml) without (C) or with (I) insulin (1 munit/ml). In Li^+-K.R. and K^+-K.R., all Na^+ was replaced by an equivalent amount of Li^+ or K^+, respectively. Uptake of glucose is expressed as μmoles/g wet wt. of tissue disappearing from the incubation medium, and the fraction hereof incorporated into glycogen is indicated by the hatched parts of the columns. The column heights represent the values from (n) observations and 2 × S.E. is denoted by the vertical bars.

into glycogen were considerably suppressed both in the absence and in the presence of insulin (Fig. 1). When Li^+ was used as the substituting ion the effects were very different. There was a slight increase in glucose uptake, and a larger fraction was incorporated into glycogen. Moreover, in the Li^+ buffer, the effects of a submaximal dose (1 munit/ml) of insulin on both parameters were enhanced.

The uptake of glucose is dependent on both its rate of transport and its subsequent metabolism and changes in the extracellular concentration of Na^+ and K^+ are known to influence glucose metabolism to a considerable degree[4]. In order to separate

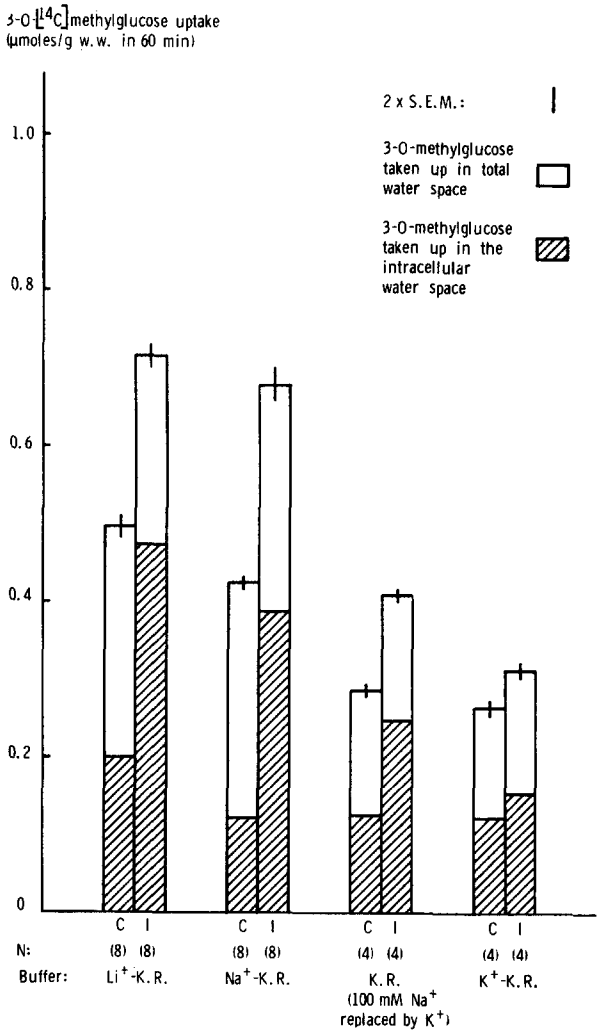

Fig. 2. Effect of Na^+ replacement and insulin on the uptake of 3-*O*-methylglucose by rat soleus muscle. Soleus muscles were incubated for 60 min at 30° in 2 ml of Krebs–Ringer bicarbonate buffer (K.R.) containing 1 mM pyruvate, 1 mM 3-*O*-[^{14}C]methylglucose (0.2 μC/ml) without (C) and with (I) insulin (1 munit/ml). Li^+- and K^+-K.R. as in Fig. 1, except for the experiment where only 100 mM Na^+ was replaced by K^+. The hatched part of the columns indicate the uptake of 3-*O*-methylglucose (as μmoles/g wet wt. of muscle) into the space not available to inulin. Other details as in Fig. 1.

out the effects on transport alone, equivalent experiments were performed using the non-metabolised glucose analogue, 3-*O*-methylglucose (Fig. 2). Again, K^+ substitution suppressed the stimulation of sugar uptake by insulin but for this sugar had no effect on the basal uptake. The apparent fall in uptake into the total tissue water can be entirely explained by the decrease in the extracellular (inulin) space which accompanies the swelling muscle cells in K^+-rich media. In the Li^+ buffer there was again a small increase in the uptake of sugar, but the insulin effect was essentially unaltered.

Thus it seems that, at least in terms of glucose transport, the absence of extracellular Na^+ has no immediate or direct effect, but a rise in extracellular K^+ leads to a loss in insulin sensitivity. In order to obtain a fuller evaluation of the role of these cations, the permeability to 3-*O*-methylglucose was examined in efflux experiments which allow a more sensitive assessment of the insulin effect and some indication of the time-course of the response to changes in the ionic milieu.

Fig. 3 shows that total replacement of Na^+ by K^+ produced no significant change in the rate of 3-*O*-methylglucose efflux during the first 20 min. The subsequent rise in the rate coefficient was not affected by phlorizin (data not presented) and may well be the result of cell damage during the transfer of muscles through the K^+-substituted efflux media. However, from some of the following graphs it can be seen that partial substitution of Na^+ by K^+ (up to 100 mM) had no effect whatever on the basal rate of 3-*O*-methylglucose efflux (dashed line in Figs. 9–11). In contrast, replacement of Na^+ by Li^+ caused a more immediate rise in the rate of efflux and this could be entirely suppressed by phlorizin (Fig. 3).

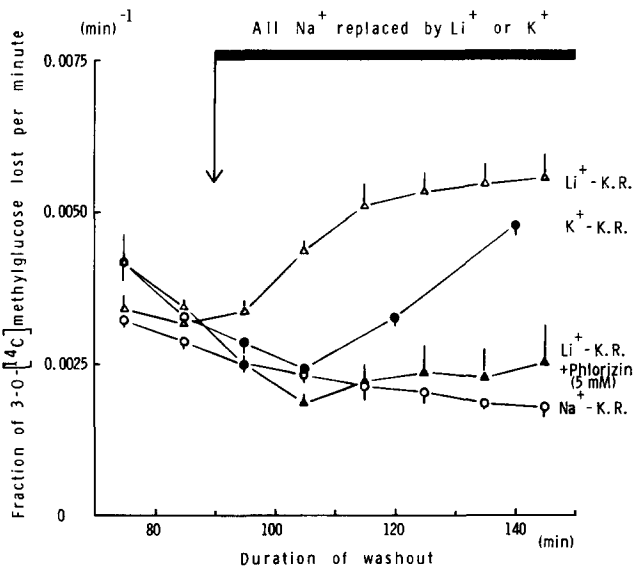

Fig. 3. Effect of Na^+ replacement and phlorizin on the rate coefficient for 3-*O*-methylglucose release from soleus. The muscles were loaded in Krebs–Ringer bicarbonate buffer (K.R.) containing 1 mM pyruvate, 1 mM 3-*O*-methylglucose and 3-*O*-[^{14}C]methylglucose (1 or 2 μC/ml) for 60 min at 30°. They were then washed out into a series of tubes containing buffer with pyruvate (1 mM), but no 3-*O*-methylglucose. The fraction of ^{14}C activity lost per minute is shown as a function of the washout time. ○—○, controls; △—△, all Na^+ replaced by Li^+; ▲—▲, all Na^+ replaced by Li^+, phlorizin (5 mM) in Li^+-K.R.; ●—●, all Na^+ replaced by K^+. Each point represents the mean of 3–11 observations and vertical bars indicate S.E. where this exceeds the size of the symbols.

Other experiments (not presented) showed that the total substitution of sucrose or mannitol for NaCl increased the rate coefficient for 3-*O*-methylglucose efflux by 93 % or 79 %, respectively, within 60 min. This increase was rather slow in onset and almost completely suppressed by phlorizin (5 mM). A recent report has shown that the uptake of 3-*O*-methylglucose in diaphragm muscle is increased to a similar extent by the substitution of mannitol for NaCl[11].

These results indicate that extracellular Na^+ is not essential for the basal level of 3-*O*-methylglucose transport, and that the omission of Na^+ from the incubation medium causes no immediate deterioration in the insulin responsiveness of the sugar transport system. Fig. 4 shows that in two different Na^+-free buffers, the stimulation of 3-*O*-methylglucose efflux following a submaximal dose of insulin (1 munit/ml) was not significantly different from that obtained in the normal buffer. These findings are in agreement with earlier studies which showed that the effect of insulin on the uptake of galactose and 2-deoxyglucose by diaphragm muscle[6], or of glucose by soleus muscle[10], is not affected by the replacement of Na^+ by choline or Tris, respectively.

In contrast, when Na^+ was replaced by K^+ the stimulating effect of the same dose of insulin was almost completely abolished (Fig. 5). Moreover, the sudden de-

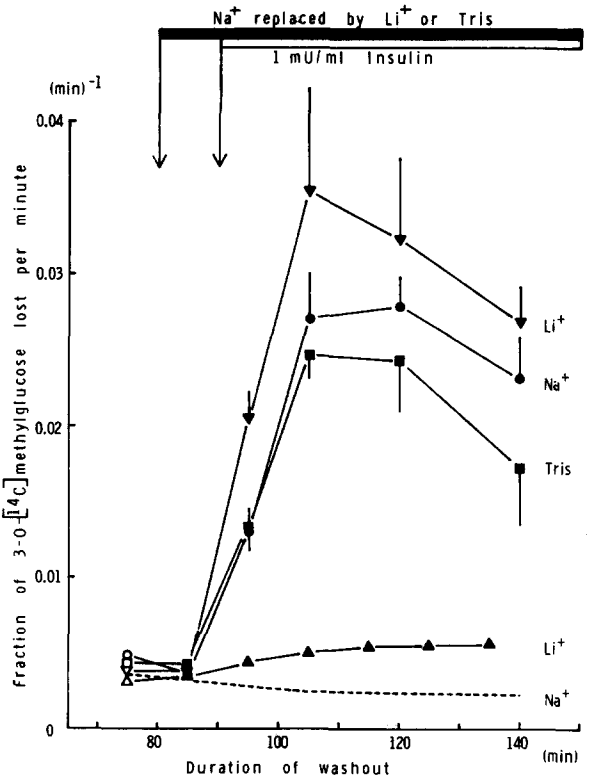

Fig. 4. Effect of Na^+ replacement on basal and insulin-stimulated 3-*O*-methylglucose release. Details as for Fig. 3. -----, controls; ●—●, normal Krebs–Ringer bicarbonate buffer with insulin (1 munit/ml); ▲—▲, all Na^+ replaced bp Li^+ (control); ▼—▼, all Na^+ replaced by Li^+, insulin (1 munit/ml); ■—■, all Na^+ replaced by Tris, insulin (1 munit/ml).

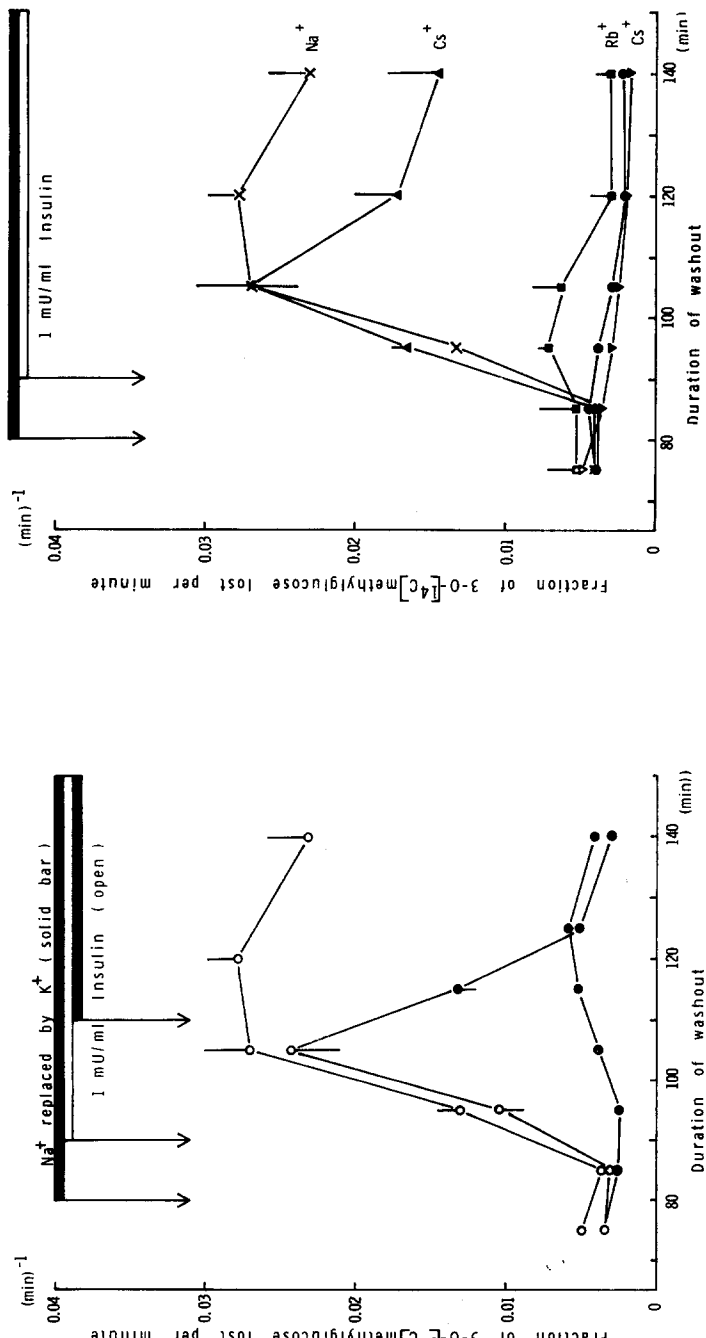

Fig. 5. Effect of K^+ and insulin on 3-*O*-methylglucose release. Experimental conditions as in Fig. 3, but 2–5 observations for each point. All muscles given insulin (1 munit/ml) from 90 min. ○—○, normal Krebs–Ringer bicarbonate buffer; ●—●, all Na^+ replaced by K^+.

Fig. 6. Effect of Cs^+ and Rb^+ on basal and insulin-stimulated 3-*O*-methylglucose release. Experimental conditions as in Fig. 3, but 3–4 observations for each point. ×—×, Krebs–Ringer bicarbonate buffer with insulin (1 munit/ml); ●—●, 100 mM Na^+ replaced by Rb^+; □—□, 100 mM Na^+ replaced by Rb^+, insulin (1 munit/ml); ▼—▼, 100 mM Na^+ replaced by Cs^+; ▲—▲, 100 mM Na^+ replaced by Cs^+, insulin (1 munit/ml).

crease in insulin-stimulated efflux suggests that K^+ has a rather rapid effect on the sugar transport system or on its activation by insulin. The change does not seem to be the outcome of an irreversible impairment of cell integrity since it could be shown in seperate experiments that a return to normal buffer after an exposure to high K^+ of up to 40 min resulted in the complete restoration of a normal insulin response (data not presented).

The specificity of the K^+ effect was assessed by performing analogous experiments using Rb^+ or Cs^+ as substituents for Na^+. From Fig. 6 it can be seen that whereas Cs^+ (100 mM) produced no significant change in the insulin-stimulated 3-*O*-methylglucose efflux, Rb^+ induces almost the same suppression as K^+. Neither of these ions themselves affect the sugar efflux in the absence of insulin. These findings again confirm that the inhibitory effects of K^+ are not due to Na^+ lack and the effect is clearly very specific among the monovalent cations.

The effects of K^+ are still evident when a supramaximal dose (100 munits/ml) of insulin is used. Increasing K^+ over the concentration range 12–100 mM resulted in a progressive suppression of the insulin effect on 3-*O*-methylglucose efflux (Fig. 7).

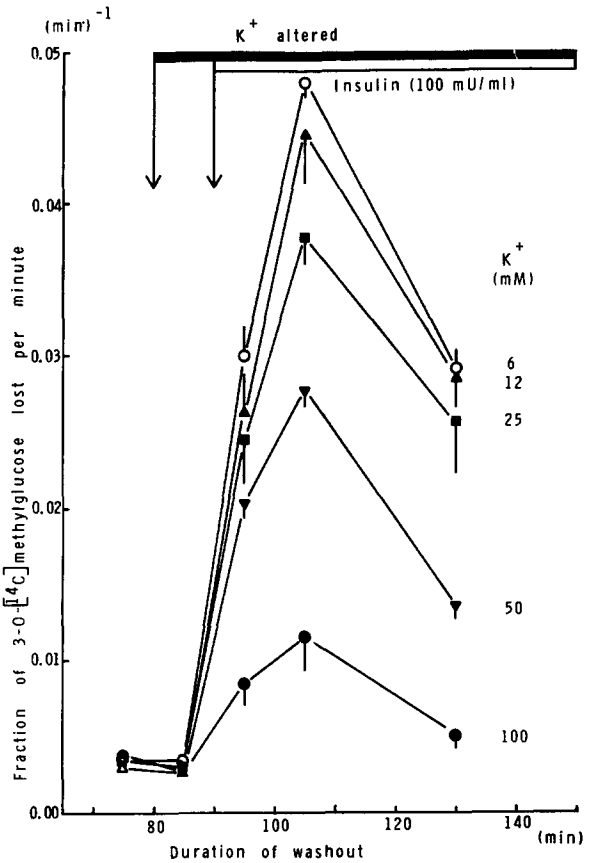

Fig. 7. Effect of varying K^+ levels on the stimulation of 3-*O*-methylglucose release by 100 munits/ml insulin. Experimental conditions as in Fig. 3. Normal Krebs–Ringer bicarbonate buffer for the first 80 min of washout, and then replacement of the indicated amount of Na^+ by K^+. Each point represents the mean of 3–10 observations.

However, using a lower level of insulin (Fig. 8 shows data for the submaximal level of 0.5 munit/ml) the sensitivity to K^+ was rather more pronounced.

The opposite situation, namely replacing all the K^+ in the bathing medium by Na^+, was examined in another group of experiments. Although the rate coefficient

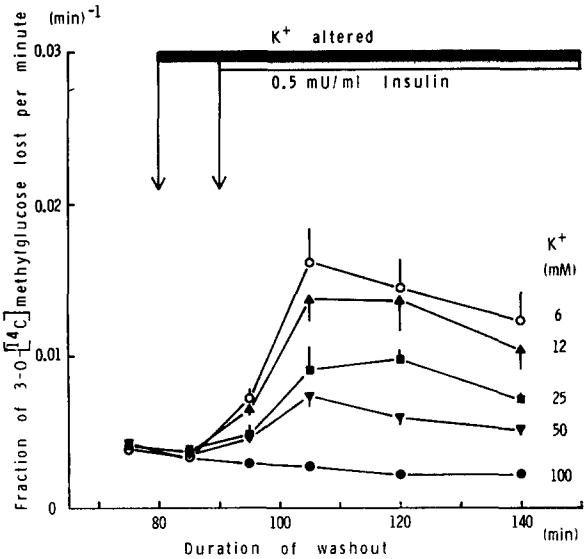

Fig. 8. Effects of varying K^+ levels on the stimulation of 3-*O*-methylglucose release by 0.5 munit/ml insulin. Details as in Fig. 7. Each point represents the mean of 3–7 observations.

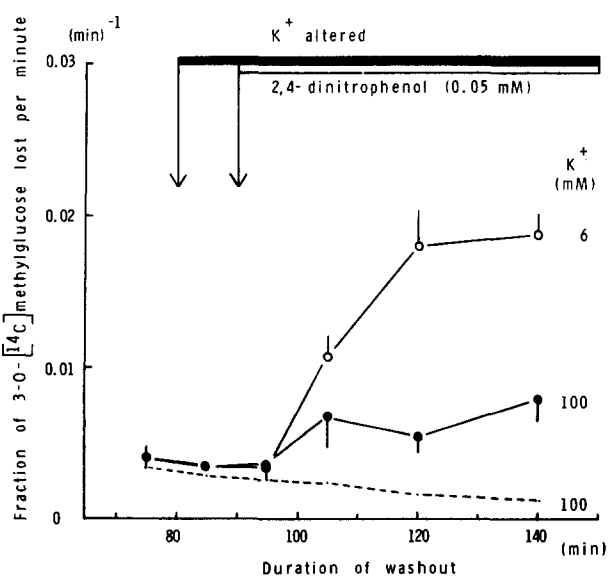

Fig. 9. Effect of K^+ on the stimulation of 3-*O*-methylglucose release by 2,4-dinitrophenol. Experimental conditions as in Fig. 3. ○—○, 2,4-dinitrophenol (0.05 mM) in Krebs–Ringer bicarbonate buffer; ●—●, 2,4-dinitrophenol (0.05 mM) in high-K^+ buffer (100 mM Na^+ replaced by K^+); – – – – –, high-K^+ (100 mM) buffer without 2,4-dinitrophenol. Each point represents the mean of 3 observations.

for 3-*O*-methylglucose efflux remained unaltered during the first 140 min, a late and somewhat variable rise of up to 20 % was observed when incubation in the K^+-free medium was prolonged to 180 min. This pattern corresponds to that previously reported for the effect of ouabain[14]. In addition, there was a variable increase in the response to submaximal doses of insulin. In three out of six experiments the size of this effect was statistically significant ($p < 0.05$).

In an attempt to determine whether K^+ had a direct effect on the sugar transport system or interfered (for example, with binding) at the site of insulin action, the sensitivity to K^+ of a number of other stimuli for sugar transport was tested. Fig. 9 shows that, in a K^+-rich buffer, the increase in 3-*O*-methylglucose efflux produced by 2,4-dinitrophenol (0.05 mM) was considerably diminished. Similarly the stimulation by trypsin (0.5 mg/ml) was also markedly suppressed (Fig. 10).

In contrast, when hyperosmolarity was used to stimulate 3-*O*-methylglucose efflux[13], the response to a raised K^+ concentration was somewhat different. As shown in Fig. 11, the early stages of the increase in sugar efflux in response to mannitol (200 mM) is unaffected by increasing the K^+ concentration to 100 mM, but a clear suppression becomes apparent in the later stages. Just as previously reported for normal buffer[13,14], the stimulating effect of hyperosmolarity in the high K^+ buffer is completely suppressed by phlorizin (5 mM). Thus the failure of K^+ to inhibit during the first 20 min does not seem to be due to tissue damage under the combined influence of a hyper-osmolar buffer and raised extracellular K^+. The delayed effects of potassium were also seen at lower concentrations (25 and 50 mM).

A possible explanation for this delayed effect of K^+ is that it might be related to the considerable and rapid swelling which occurs when muscle cells are exposed to K^+

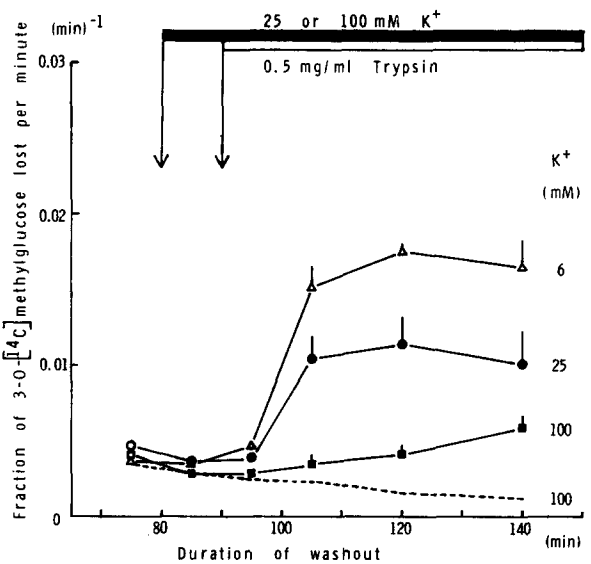

Fig. 10. Effect of K^+ on the stimulation of 3-*O*-methylglucose release by trypsin. Experimental conditions as in Fig. 3. △—△, trypsin (0.5 mg/ml) in Krebs–Ringer bicarbonate buffer; ●—●, trypsin (0.5 mg/ml) in buffer with 25 mM Na^+ replaced by K^+; ■—■, trypsin (0.5 mg/ml) in high-K^+ buffer (100 mM Na^+ replaced by K^+); -----, high-K^+ (100 mM) buffer without trypsin. Each point represents the mean of 3 observations.

rich media[6,18–21]. In the present study, exposure of soleus muscles for 60 min to a buffer in which all Na^+ was replaced by K^+ caused a decrease in the extracellular (inulin) space of about 50 % and a 26 % increase in the intracellular volume ($p < 0.01$). It was therefore interesting to examine whether swelling induced by another means (hypo-osmolarity) could mimic the effects of high extracellular K^+.

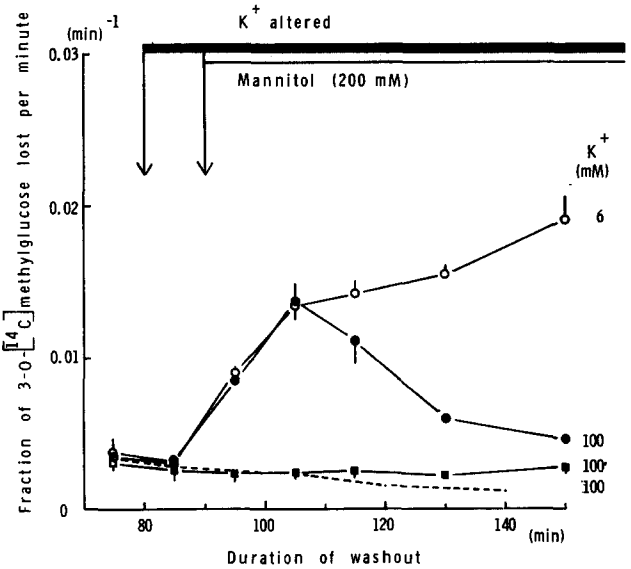

Fig. 11. Effect of K^+ on the stimulation of 3-*O*-methylglucose release by hyperosmolarity. Experimental conditions as in Fig. 3. ○—○, mannitol (200 mM) in Krebs–Ringer bicarbonate buffer; ●—●, mannitol (200 mM) in high-K^+ buffer (100 mM Na^+ replaced by K^+); ■—■, mannitol (200 mM) in high-K^+ (100 mM) buffer with phlorizin (5 mM); -----, high-K^+ (100 mM) buffer without mannitol. Each point represents the mean of 3–5 observations.

Table I shows the results from a group of uptake experiments in which the effects of hypo-osmolar media were examined. In a medium from which 60 mM of the NaCl had been omitted, a reduction in the uptake of 3-*O*-methylglucose uptake was observed both in the absence and in the presence of insulin. However, when allowance is made for the decrease in extracellular (inulin) space, the uptake into the intracellular volume in the absence of insulin was actually marginally increased. On the other hand, the insulin effect is still clearly suppressed, and to about the same level as in the high K^+ buffer (Fig. 2). When the muscles were preincubated in the hypotonic buffer for fifteen minutes prior to measuring 3-*O*-methylglucose uptake, the suppression of the insulin effect was even more marked (Table I).

The similarity between the effects of K^+ and those of hypotonicity is even more clearly illustrated in the efflux situation. Fig. 12 shows that although omission of 60 mM NaCl from the buffer has no detectable effect on unstimulated 3-*O*-methylglucose efflux, it almost abolishes the response to insulin. This inhibition was highly significant within 20 min after the onset of exposure to hypotonic environment. Separate experiments showed that incubation for 15 min in the hypotonic buffer (60 mM of the NaCl omitted) or in Na^+-free K^+-Krebs–Ringer bicarbonate buffer caused the muscle cells to swell by 11.0 ± 1.3 and 11.4 ± 1.5 %, respectively. Such rapid increases in cell

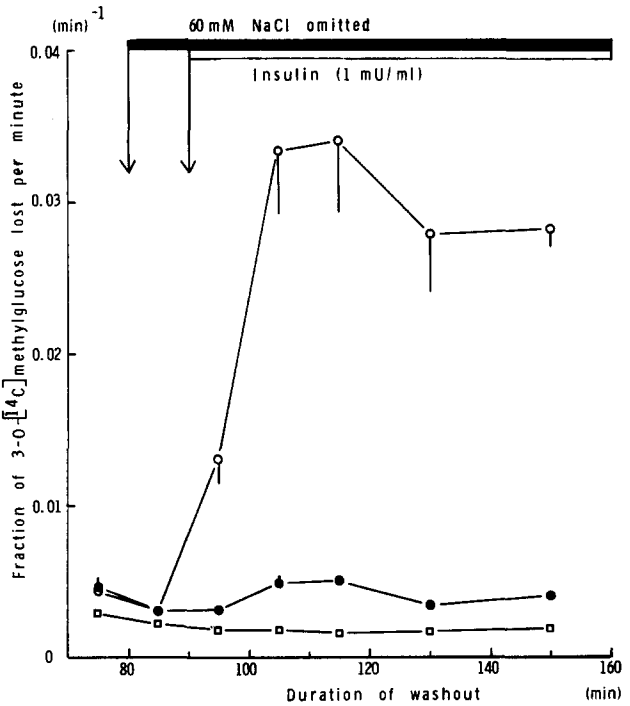

Fig. 12. Effect of hypo-osmolarity on basal and insulin-stimulated release of 3-*O*-methylglucose. Experimental conditions as in Fig. 3. ○—○, insulin (1 munit/ml) in Krebs–Ringer bicarbonate buffer; ●—●, insulin (1 munit/ml) in hypo-osmolar buffer (60 mM NaCl omitted); □—□, hypo-osmolar buffer (60 mM NaCl omitted) without insulin. Each point represents the mean of 3 observations.

TABLE I

EFFECTS OF HYPO-OSMOLARITY, K^+ AND SUCROSE ON THE UPTAKE OF 3-*O*-METHYLGLUCOSE IN SOLEUS MUSCLE

Experimental conditions as in Fig. 2. Number of observations in parentheses.

Incubation medium	*Insulin (1 munit/ml)*	*3-O-[^{14}C]Methylglucose taken up in total water space (μmoles/g wet wt. muscle)*	*3-O-[^{14}C]Methylglucose taken up in the intracellular water space (μmoles/g wet wt. muscle)*
Krebs–Ringer bicarbonate buffer	o	0.425 ± 0.008 (8)	0.123 (8)
	+	0.612 ± 0.015 (7)	0.350 (7)
Krebs–Ringer bicarbonate buffer, 60 mM NaCl omitted	o	0.315 ± 0.015 (8)	0.137 (8)
	+	0.424 ± 0.017 (4)	0.229 (4)
Krebs–Ringer bicarbonate buffer, 60 mM NaCl omitted. Muscles preincubated for 15 min in the same buffer before the measurement of sugar uptake	+	0.362 ± 0.016 (4)	0.167 (4)
Krebs–Ringer bicarbonate buffer, 60 mM NaCl replaced by 120 mM sucrose	+	0.655 ± 0.022 (4)	0.393 (4)

volume have repeatedly been demonstrated in other muscles during exposure to similar environments[19–21].

From Fig. 13 it can be seen that hypotonicity reduces the stimulating effect of trypsin (1 mg/ml) and 2,4-dinitrophenol (0.05 mM) also. Thus the effects of hypotonicity, like those of K^+ substitution, cannot readily be explained in terms of a direct effect on the binding of insulin or some other step specific to its action. They are however, confined to the mechanisms by which the sugar transport system is activated.

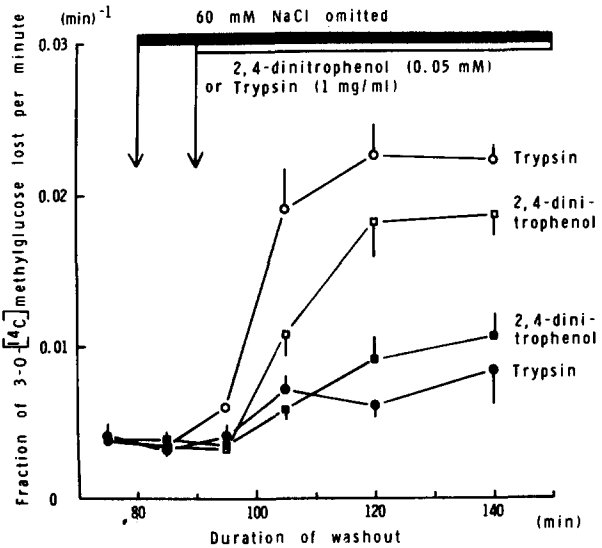

Fig. 13. Effect of hypo-osmolar buffer on the stimulation of 3-*O*-methylglucose release by trypsin or 2,4-dinitrophenol. Experimental conditions as in Fig. 3. ○—○, trypsin (1 mg/ml) in normal Krebs–Ringer bicarbonate buffer; ●—●, trypsin (1 mg/ml) in hypo-osmolar buffer (60 mM NaCl omitted); □—□, 2,4-dinitrophenol (0.05 mM) in normal Krebs–Ringer bicarbonate buffer; ■—■, 2,4-dinitrophenol (0.05 mM) in hypo-osmolar buffer (60 mM NaCl omitted). Each point represents the mean of 3 observations.

Finally, if the swelling effect of K^+ could somehow be prevented, this ion might not cause any suppression of stimulated 3-*O*-methylglucose transport. When KCl is added in excess of the other components of the buffer, the volume of muscle cell can be kept almost constant [19–20]. In soleus muscle, the addition of 100 mM of KCl (in excess of the other components of the Krebs–Ringer bicarbonate buffer) caused no significant change in wet weight or inulin space over an incubation period of 60 min. In these muscles, the intracellular K^+ concentration was 244 ± 2 mM ($n = 4$). When the muscles were incubated in a buffer containing the same concentration of K^+ (replacing an equivalent amount of Na^+), the intracellular K^+ concentration was 187 ± 14 mM ($n = 4$).

Fig. 14 shows the results of experiments in which the effect of excess KCl (100 mM) on 3-*O*-methylglucose transport was tested. It appears that the addition of KCl caused a small increase in the rate coefficient of 3-*O*-methylglucose efflux. However, in contrast to the considerable suppression of the insulin effect seen in K^+-substituted buffer (Fig. 7), the addition of hyperosmolar KCl produced no significant change. Thus, the

mere presence of K^+ does not suppress the insulin response if there is no concomitant change in cell volume.

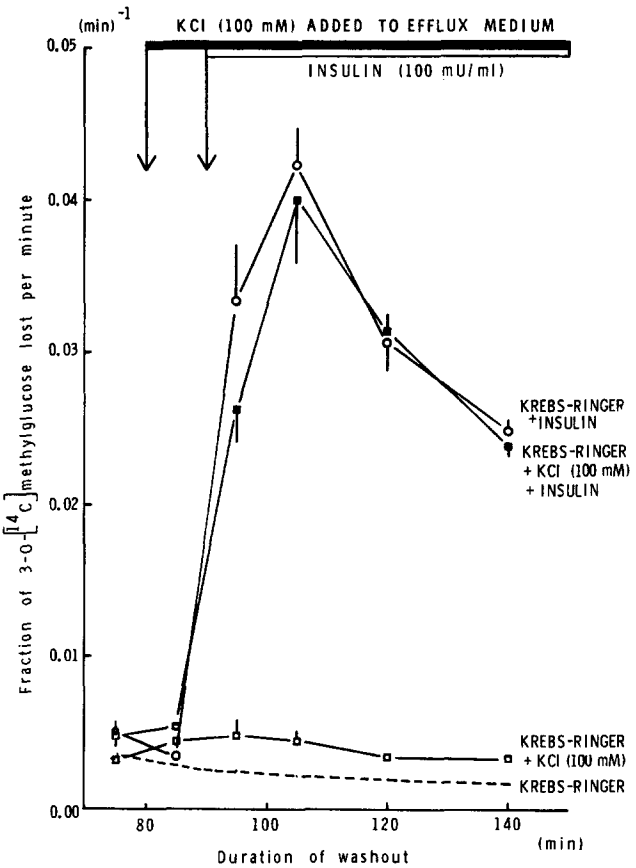

Fig. 14. Effect of KCl (hyperosmolar) on basal and insulin-stimulated 3-*O*-methylglucose release. Experimental conditions as in Fig. 3. – – – –, normal Krebs–Ringer bicarbonate buffer; ○—○, insulin (100 munits/ml) in normal Krebs–Ringer bicarbonate buffer; □—□, Krebs–Ringer bicarbonate buffer with KCl (100 mM) added in excess of the other components of the buffer; ■—■, insulin (100 munits/ml) in Krebs–Ringer bicarbonate buffer with KCl (100 mM) added in excess of the other components of the buffer.

DISCUSSION

In the evaluation of the significance of extracellular Na^+ and K^+ for sugar transport in muscle, two serious difficulties must be faced. Many of the commonly used substituents for Na^+ appear themselves to exert effects on the processes of glucose transport and metabolism. In addition, alterations in the concentration of extracellular K^+ lead to changes in cell volume.

Previous studies have shown that rather low concentrations of Li^+ (which has been widely used as a substituent for Na^+ in studies on excitable tissues) will stimulate the uptake of glucose and its incorporation into glycogen[9,22]. The present experiments show that in soleus muscle, Li^+ stimulates both glycogen deposition and a phlorizin-

sensitive sugar transport system. Replacing Na^+ by Tris stimulated sugar efflux, but Rb^+ and Cs^+ were practically without effect. When mannitol or sucrose were used to replace NaCl, a slight stimulation of 3-*O*-methylglucose transport was observed. In a medium in which 60 mM NaCl was replaced by sucrose the insulin-stimulated uptake or efflux of 3-*O*-methylglucose was not significantly different from that in the control buffer. Furthermore, when all Na^+ had been replaced by Li^+ or Tris, the stimulatory effect of insulin on 3-*O*-methylglucose efflux was unimpaired.

Collectively, the evidence shows that Na^+ is not a prerequisite for either the basal or the insulin-stimulated sugar transport. Thus the effects of high K^+ media are not simply a result of the concomitant reduction in Na^+ concentration.

The inhibitory effect of K^+ on sugar transport is well documented. The present data suggest that, when stimulated by insulin, the system mediating the transport of 3-*O*-methylglucose is promptly suppressed by a rise in extracellular K^+ concentration. Since a similar suppression of the effects of both trypsin and 2,4-dinitrophenol was also observed, the effect of K^+ does not appear to be restricted to the insulin receptor site but rather affects the mechanism responsible for augmenting the capacity of the glucose transport system. Unlike phlorizin, which completely suppresses the stimulation of sugar transport in hyperosmolar media[13], K^+ (25–100 mM) did not affect the initial rise in the rate of 3-*O*-methylglucose efflux induced by hyper-osmolarity. It is worth noting that even under basal conditions, the sugar efflux can be still further suppressed by phlorizin[14]. In contrast, raising extracellular K^+ to 100 mM appears to be completely without effect, and the full sensitivity to phlorizin is retained.

Taken together these findings suggest that the effect of K^+ is on the mechanism of activation of sugar transport, and not on the transport system itself.

K^+ has been shown to have indirect effects on a number of physiological processes. Thus, it has been shown that K^+ stimulates secretion in the exocrine pancreas, primarily by increasing the release of acetylcholine from nervous elements[23]. In muscle, K^+ has been found to stimulate adenyl cyclase[24], a property which may in part explain the considerable degradation of glycogen which occurs in soleus muscles during incubation in K^+-rich buffers[25].

However, these phenomena probably cannot explain the effect of K^+ on sugar transport since a separate series of experiments in the present study showed that neither acetylcholine (100 μg/ml), eserine (10 μg/ml) nor epinephrine (10 μg/ml) produced any significant decrease in either basal or insulin-stimulated 3-*O*-methylglucose efflux.

Another possible indirect mechanism which seemed worthy of consideration was suggested by the well-documented swelling of muscle cells produced in high K^+ media[6,18–21]. This effect has been again demonstrated for soleus muscles and seems to occur with a rapidity comparable to that induced by a lowering of the osmolarity of the bathing medium (although we have not attempted to characterise the time-course of the two processes in detail in the present study). Previous reports from this laboratory have emphasised the importance of osmolarity in determining the permeability of the plasma membrane to sugars[4,13,14], and other studies have indicated that in a hypo-osmolar buffer, insulin-stimulated glucose uptake is considerably reduced[26]. Although it cannot be excluded that an increase in extracellular K^+ concentration may influence sugar transport *via* other mechanisms, the data in Figs. 12 and 13 and in Table I suggest that swelling of the muscle cells may contribute significantly to the

inhibitory effect of K^+. This may even be true for very small increases in extracellular K^+ concentration since it has recently been reported that even at a concentration as as low as 18 mM, a rapid swelling of muscle fibres can be detected[21].

The data ilustrated in Fig. 14 show that unless the cells are caused to swell, the exposure to an extracellular K^+ concentration of 100 mM does not affect the insulin-stimulated 3-*O*-methylglucose transport. This argues that the inhibitory effect of K^+ substitution is not an effect of the ion *per se*. The addition of 100 mM of KCl in excess of the other components of the buffer caused a considerable rise in the intracellular K^+ concentration. Therefore, it seems unlikely that the rise in intracellular K^+ produced by incubation in K^+-substituted buffer can be of major significance for the inhibition of stimulated 3-*O*-methylglucose transport. It cannot yet be excluded that the changes in the intracellular concentration of Na^+ or in the K^+/Na^+ ratio might play some role for the inhibitory effect of K substitution. However, the fact that extracellular Na^+ could be entirely replaced by Li^+ or Tris (or partly be Cs^+) without any significant decrease in insulin responsiveness, suggest that intracellular Na^+ might be of minor importance – a conclusion also supported by earlier evidence[14,17].

Our present findings, taken together with those for hyperosmolarity, indicate a strong dependence of the sugar transport system on cell size. Swelling or shrinking may diminish or augment the accessability of the transverse tubule system and the sarcoplasmic reticulum[27,28]. It is now firmly established that the transverse tubule system in skeletal muscle is continuous with the extracellular space, and it is beginning to seem probable that, at least for small ions and molecules, the sarcoplasmic reticulum is also readily accessible. It has recently been suggested that the tubular system is the site of amino acid uptake into skeletal muscle[29]. These tubules consist of membranes with a considerably larger area than that of the "outer" plasma membrane (sarcolemma). If they participate in the exchange of sugars, they could therefore play a major role in determining the overall rate of uptake and efflux of 3-*O*-methylglucose. The fact that swelling markedly attenuates the capacity of the sugar transport system to be stimulated might be the result of a decrease in the area of membrane available for exchange between the cell environment and the cytoplasm.

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