BBA 75472

STIMULATING EFFECT OF HYPEROSMOLARITY ON GLUCOSE TRANSPORT IN ADIPOCYTES AND MUSCLE CELLS

T. CLAUSEN, J. GLIEMANN, J. VINTEN AND P. G. KOHN

Institute of Physiology, University of Aarhus, Aarhus and Institute of Medical Physiology C, University of Copenhagen, Copenhagen (Denmark)

(Received March 16th, 1970)

SUMMARY

1. The effect of hyperosmolarity on sugar permeability was assessed in isolated fat cells, epididymal fat pads and soleus muscles of the rat.

2. Hyperosmolarity, whether induced by the addition of sucrose, mannitol or sorbitol, gave an up to 15-fold increase in glucose metabolism of isolated fat cells. The stimulating effect of mannitol (400 mM) and a submaximal concentration of insulin (5 μunits/ml) were similar with respect to the rates of onset and reversal, kinetics and sensitivity towards 3-*O*-methylglucose or phlorizin.

3. In whole epididymal fat pads and soleus muscles hyperosmolarity produced a marked increase in the release of 3-*O*-methylglucose without causing any decrease in K^+ content. This effect could be abolished by phlorizin (5 mM) and showed nearly the same time-course as that induced by a submaximal concentration of insulin.

4. It is concluded that hyperosmolarity stimulates the carrier system mediating glucose transport without impairing the overall integrity of the plasma membrane.

INTRODUCTION

Hyperosmolarity has been found to stimulate the uptake and metabolism of glucose in adipocytes and muscle[1–3]. In the experiments reported here, an attempt was made to determine whether hyperosmolarity resembles insulin in activating the system which mediates the transfer of glucose and other sugars across the plasma membrane or, alternatively, produces a nonspecific impairment of membrane integrity.

Furthermore, since it is known that changes in extracellular osmolarity induce alterations in the ultrastructural configuration of cell membranes[4–6], the problem is of more general interest with respect to an understanding of carrier-mediated transport processes.

The present report describes experiments in which the transport of glucose and a nonmetabolized sugar was evaluated using epididymal fat pads, isolated fat cells and soleus muscle from the rat. The evidence obtained indicates that hyperosmolarity mimics the effect of insulin in causing a rapid and reversible acceleration of carrier-mediated sugar transport across the plasma membrane. In the fat cells, both hyperosmolarity and insulin were found to cause a marked increase in the overall apparent v_{max} and a decrease in the apparent K_m of glucose utilization.

METHODS

Experiments with isolated fat cells

The animals, chemicals and the methods used for the preparation and incubation of isolated fat cells were similar to those described previously[7] with one exception: the epididymal fat pads were disintegrated in bicarbonate buffer containing 0.4 mg/ml of crude collagenase (Worthington Biochemical Corp. Lot No. CLS 7 IC). It should be stressed that, except when stated otherwise, the incubation medium (referred to as basal buffer) contained 0.55 μmole of glucose per ml. The recovery of $^{14}CO_2$ and extraction of cell glycerides followed published methods[7,8].

Experiments with epididymal fat pads

Whole epididymal fat pads from fed Wistar rats (90–120 g) were loaded for 60 min in Krebs–Ringer bicarbonate buffer[9] containing 1 % bovine serum albumin, 3-*O*-[^{14}C]methylglucose (2 μC/ml) and unlabeled 3-*O*-methylglucose (1 mM). The washout of ^{14}C activity into unlabeled buffer was measured and a rate coefficient for this process calculated as earlier described[10]. At stated intervals after the onset of the washout period, mannitol, sucrose, urea, NaCl or phlorizin was added to the efflux medium to give the concentrations indicated in the figures.

Experiments with soleus muscle

Fed female Wistar rats weighing 60–70 g were used for these studies. The soleus muscles were gently dissected out, rinsed in 154 mM NaCl and equilibrated for 15 min in the incubation medium. All incubations took place at 30° in centrifuge tubes containing 3 ml of Krebs–Ringer bicarbonate buffer modified to contain 1.27 mM of Ca^{2+} and with the addition of 1 mM sodium pyruvate. The solution was agitated by continuous bubbling with a mixture of O_2–CO_2 (95:5, v/v). For the measurement of 3-*O*-methylglucose uptake, the muscles were incubated for 60 min in buffer containing ^{14}C-labeled 3-*O*-methylglucose. ^{14}C-labeled inulin was used for the determination of extracellular space. For the measurement of 3-*O*-methylglucose release, the muscles were loaded with the ^{14}C-labeled sugar for 60 min. During the washout, each individual muscle was attached by its lower tendon to the polyethylene tube used for gas distribution. This arrangement minimized the handling of the tissue and facilitated its transfer through the series of efflux tubes. At the end of the washout period each muscle was blotted, weighed and homogenized in 4 ml of 5 % trichloroacetic acid containing 0.1 mM of carrier 3-*O*-methylglucose. After centrifugation, 1.5 ml of the clear supernatant was withdrawn for counting and 2 ml for the determination of Na^+ and K^+ by flame photometry[10]. In order to obtain the same counting efficiency, trichloroacetic acid and carrier 3-*O*-methylglucose were added to the washing medium to give the same final concentrations as in the tissue extracts. The rate coefficient for the release of 3-*O*-[^{14}C]methylglucose was calculated as previously described[10].

Counting

The ^{14}C activity in CO_2 and extracts of lipids obtained in the experiments with fat cells was determined using plastic counting vials containing 10 ml of toluene with 0.5 % 2,5-diphenyloxazole, and 0.03 % 1,4-bis-2-(4-methyl-5-phenyloxazolyl)-

benzene. For the counting of ^{14}C activity recovered from tissue extracts and buffers in the experiments with 3-*O*-methylglucose, 1–1.5-ml aliquots were mixed with 10 ml of the following scintillator: 2 l toluene, 1.25 l Triton X-100, 0.5 l ethanol, 5 g 2,5-diphenyloxazole, and 0.25 g 1,4-bis-2-(4-methyl-5-phenyloxazolyl)benzene. All counting was performed in a Packard liquid scintillation spectrometer Model 3320, and for the quench correction either external standardization or internal standardization with [^{14}C]toluene was used.

Chemicals, isotopes and hormones

Bovine serum albumin (Fraction V) was obtained from Sigma (St. Louis) and human serum albumin from the Swiss Red Cross (Berne). Both were used after dialysis against distilled water for 24 h. D-[$^{14}C_6$]Glucose (specific activity 288 mC per mmole) was purchased from the Radiochemical Centre, Amersham; ^{14}C-labeled inulin (specific activity 7.7 mC/mmole) and 3-*O*-[^{14}C]methylglucose (specific activity 10 mC/mmole) from New England Nuclear (Boston). Unlabeled 3-*O*-methylglucose was obtained from Calbiochem (Los Angeles). Pork insulin (10 times recrystallized, 24.4 units/mg, Lot No. 018864) was a gift of the Novo Research Institute, Copenhagen. All chemicals used were of analytical grade.

RESULTS

Effect of hyperosmolarity on glucose metabolism of isolated fat cells

Hyperosmolarity induced by the addition of sucrose, mannitol, NaCl, LiCl or choline chloride was previously found to stimulate glucose uptake in epididymal fat pads and diaphragm muscle[1,3]. In the same tissues mannitol and sucrose have been shown to be valid markers for the extracellular space[11,12], and therefore, these compounds were used for increasing the osmolarity in most of the present experiments.

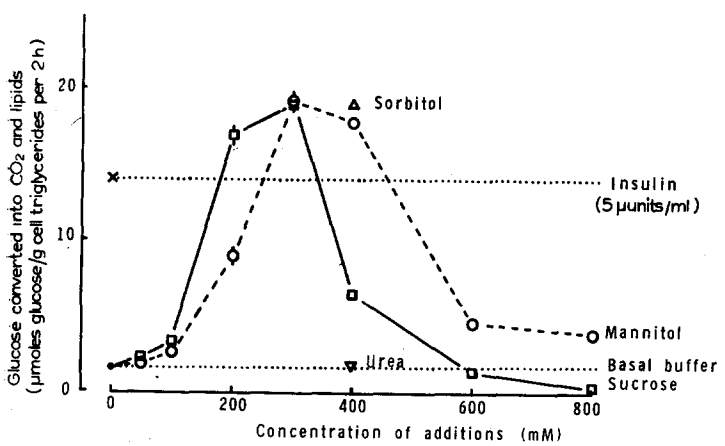

Fig. 1. Dose response relationship for the effect of mannitol and sucrose on glucose metabolism. Isolated fat cells were incubated for 120 min in Krebs–Ringer bicarbonate buffer containing 1% dialyzed human serum albumin, 0.55 mM D-glucose, 0.05 μC/ml D-[$^{14}C_6$]glucose and the indicated additions. The horizontal dotted lines show the glucose conversion by cells incubated in basal buffer with or without insulin (5 μunits/ml). The effects of sorbitol and urea (400 mM) are also shown. Each point represents the mean of 3 observations with bars indicating $\pm$ 1 S.E. when this value exceeds the size of the symbols.

From Fig. 1 it appears that in isolated fat cells the conversion of D-[$^{14}C_6$]glucose into CO_2 and glycerides was increased by the addition of mannitol in the concentration range 100–800 mM with a maximal effect around 300–400 mM. In the presence of a maximal concentration of insulin (10^3 μunits/ml) the addition of mannitol (0–400 mM) produced no marked change, whereas higher concentrations of mannitol clearly diminished insulin-stimulated glucose metabolism (data not presented). The action of sorbitol was similar to that of mannitol, whereas the cells seemed more sensitive to sucrose. Urea, which is known to be rapidly equilibrated across the plasma membrane of adipocytes[13], did not produce any stimulation of the glucose utilization even at a concentration of 400 mM.

As it is difficult to exclude the possibility of trace contamination of the sucrose by glucose, mannitol was chosen for further studies. With 400 mM mannitol glucose metabolism was increased to about the same level as that obtained in the presence of insulin (5 μunits/ml) (Fig. 1), and therefore, the effects of these factors were compared with respect to time-course and the kinetics of glucose utilization.

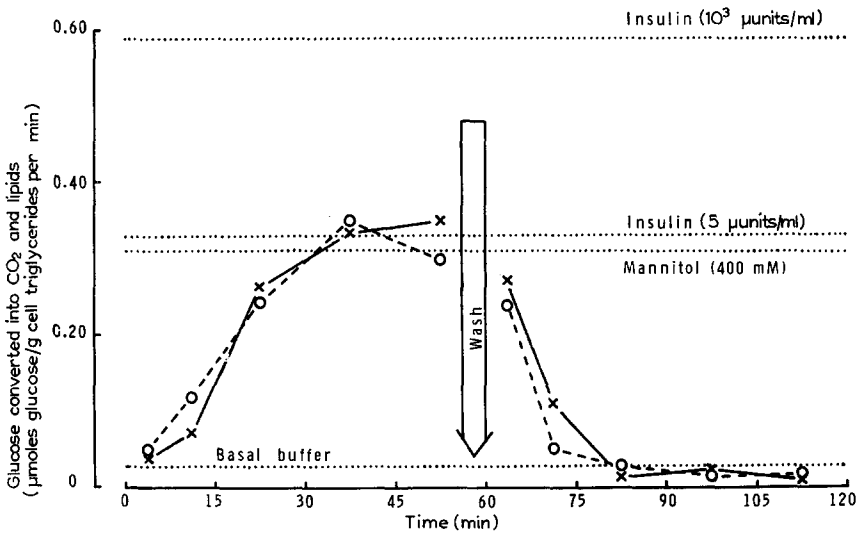

Fig. 2. Time-course and reversal of the action of mannitol and insulin on glucose metabolism. Experimental conditions as in Fig. 1. Isolated fat cells were suspended in buffer containing either 5 μunits/ml insulin (×—×) or 400 mM mannitol (○- - - -○). The conversion of glucose per min measured in time intervals of 7.5 or 15 min duration is illustrated by the points in the middle of these periods. The horizontal dotted lines show the mean glucose conversion per min as measured throughout the entire 2-h period in basal buffer, with mannitol or insulin. The arrow indicates replacement of hyperosmolar and insulin-containing buffer with basal buffer. Each point represents the mean of 4 observations.

Fig. 2 shows that insulin (5 μunits/ml) and mannitol (400 mM) stimulated glucose metabolism with almost the same time-lag, the plateau being obtained only after about 30 min of exposure. In a separate series of experiments[25], the delay in the onset of insulin action was found to be similar over a range of glucose concentrations from 0.55 to 5.5 mM. When the stimulated cells were washed and reincubated in the same medium as the controls, glucose metabolism returned to basal levels within about 20 min.

Both in the absence and in the presence of insulin the metabolism of glucose in fat cells shows saturation phenomena[14]. The results shown in Fig. 3 confirm this finding and illustrate that in a hyperosmolar buffer, the metabolism of glucose also takes place by a saturable process. It appears that insulin (5 μunits/ml) caused an increase in apparent v_{max} from 0.02 to 0.06 mmole/g cell triglyceride per 2 h, and a decrease in K_m from 3.4 to 0.74 mM. Mannitol (400 mM) induced very similar changes in these parameters.

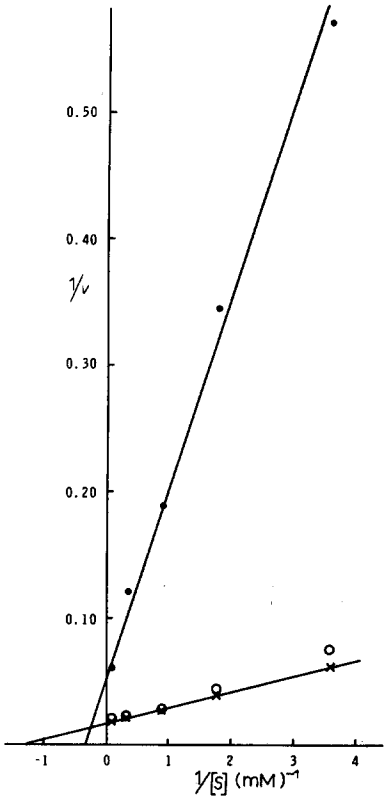

Fig. 3. Effect of insulin and hyperosmolarity on the kinetics of glucose metabolism. Experimental conditions as in Fig. 1. The reciprocal value of D-[$^{14}C_6$]glucose converted into CO_2 and triglycerides (v in μmoles/g triglycerides per 2 h) is plotted against the reciprocal value of the glucose concentration (mM). ●—●, basal buffer; ×—×, in the presence of insulin (5 μunits/ml); ○—○, in the presence of mannitol (400 mM). Each point represents the mean of 4 observations. The lines were drawn by inspection.

Several studies indicate that 3-*O*-methylglucose and phlorizin can interact with the carrier system for glucose transport so as to decrease its capacity for translocating glucose[15,16]. From Fig. 4 it appears that, whether stimulated by insulin or by mannitol, the metabolism of glucose displays very much the same sensitivity towards these two compounds. It should be noted that in the presence of mannitol (400 mM) the glucose metabolism was further increased by insulin at a submaximal concentration (5 μunits/ml).

Effect of hyperosmolarity on the transport of 3-O-methylglucose

Previous experiments have demonstrated that hyperosmolarity causes a pronounced stimulation of glucose metabolism and glycogenolysis[3]. In fat cells, the sum of the conversion of glucose into CO_2 and glycerides accounts for about 80% of the glucose disappearance both in the absence and presence of insulin[8]. Nevertheless, it seemed desirable to assess sugar transport in a more direct fashion. From Fig. 5 it can be seen that the release of 3-*O*-methylglucose, which has earlier

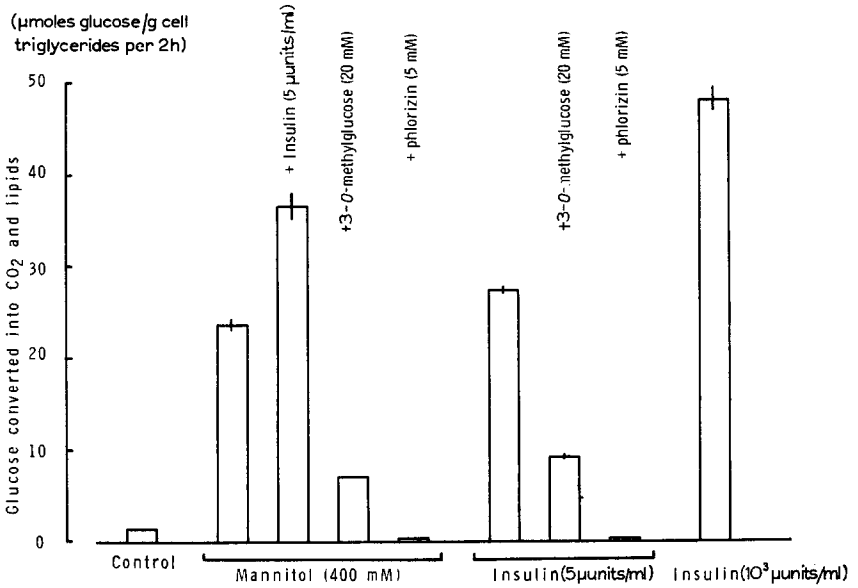

Fig. 4. Effect of 3-*O*-methylglucose and phlorizin on the stimulation of glucose metabolism induced by mannitol or insulin. Experimental conditions as in Fig. 1. Note that the molar ratio between 3-*O*-methylglucose and glucose is 36. Each value is the mean of 4 observations with bars indicating ± 1 S. E.

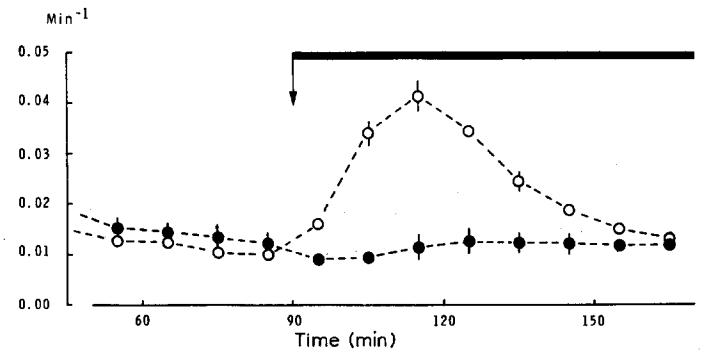

Fig. 5. Effect of mannitol without or with phlorizin on the rate coefficient of 3-*O*-methylglucose release. Whole epididymal fat pads were loaded in Krebs–Ringer bicarbonate buffer containing 1% dialyzed bovine serum albumin, 3-*O*-[^{14}C]methylglucose (2 μC/ml) and 1 mM 3-*O*-methylglucose. The fraction of ^{14}C activity lost per min from the tissue during each interval of the washout period is shown as a function of time. Mannitol (200 mM) was present from 90 min onwards – either alone (○- - - -○) or with (5 mM) phlorizin (●- - - -●). Each curve represents the mean of 3 observations with bars indicating ± 1 S. E. when this value exceeds the size of the symbols.

been shown to be accelerated by insulin and a number of other stimuli of glucose transport[10], was also augmented by hyperosmolarity. The fact that this effect could be completely abolished by the simultaneous addition of phlorizin (5 mM) argues that the increase in 3-*O*-methylglucose release was due to a stimulation of carrier-mediated sugar transport and was not the consequence of an indiscriminate leak of intracellular solutes.

Fig. 6 shows that also when the osmolarity of the efflux medium was increased

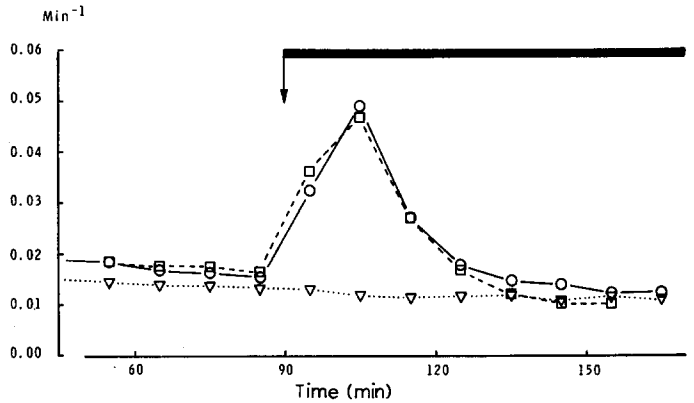

Fig. 6. Effect of sucrose, NaCl and urea on the rate coefficient of 3-*O*-methylglucose release from epididymal fat pads. Experimental conditions as described in Fig. 5. 200 mM sucrose (□- - - -□), 100 mM NaCl (○- - - -○) or 200 mM urea (▽. . . .▽) were present from 90 min onwards. Each curve represents the mean of 2–3 observations.

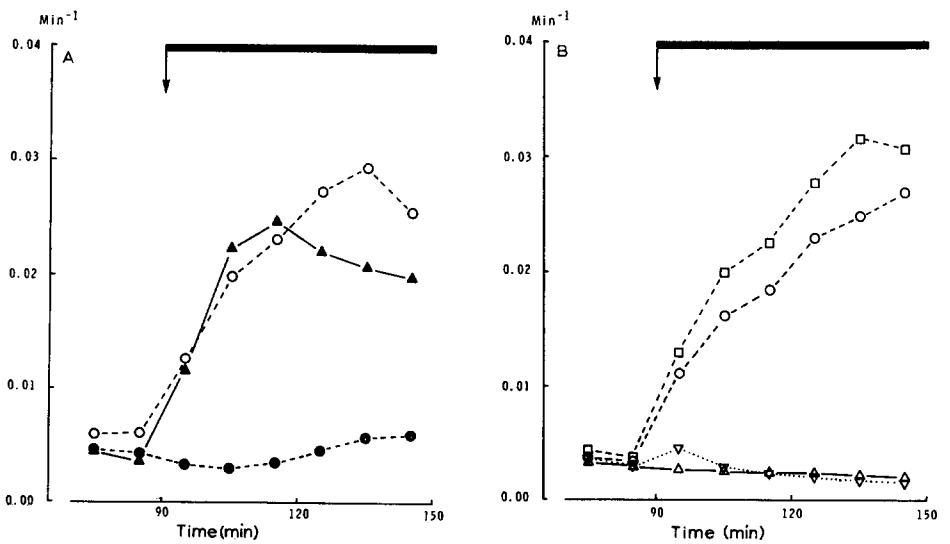

Fig. 7. Effect of hyperosmolarity and insulin on the rate coefficient of 3-*O*-methylglucose release from soleus muscle. The muscles were loaded in Krebs–Ringer bicarbonate buffer containing 3-*O*-[^{14}C]methylglucose (2 μC/ml), 1 mM 3-*O*-methyl glucose and 1 mM sodium pyruvate. 90 min after the onset of washout the following additions were made: (A) ▲—▲, insulin (10^3 μunits/ml); ○- - - -○, mannitol (200 mM); ●- - - -●, mannitol (200 mM) + phlorizin (5 mM). (B) □- - - -□, sucrose (200 mM); ▽. . . .▽, urea (200 mM); ○- - - -○, mannitol (200 mM); △—△, none. Each curve represents the mean of 2–3 observations.

by the addition of sucrose or NaCl, the release of 3-*O*-methylglucose was considerably increased, whereas an equiosmolar concentration of urea failed to produce any stimulation.

The release of 3-*O*-methylglucose from fat pads seemed to be somewhat more sensitive to hyperosmolarity than the glucose metabolism of fat cells. Thus, 50 mM of mannitol was found to be sufficient to produce a clearly detectable (2-fold) increase in the rate coefficient (data not presented).

Since the transport of glucose in adipocytes is very sensitive to a number of factors which have little or no effect on this process in muscle[17,18], the above evidence was supplemented by experiments performed with soleus muscle.

Fig. 7 shows that in this preparation both mannitol and sucrose (200 mM) stimulated the release of 3-*O*-[^{14}C]methylglucose to about the same level (and with approximately the same time-course) as a submaximal concentration of insulin (10^3 μunits/ml). As with the epididymal fat pads, the effect of mannitol was nearly entirely suppressed by phlorizin (Fig. 7A). Again, urea (200 mM) gave a barely detectable change in the rate coefficient (Fig. 7B).

Recent reports indicate that conditions which lead to a loss of K^+ from the tissue cause an increase in the transport of glucose and 3-*O*-methylglucose in adipocytes and muscle[19–21,10]. From the data presented in Table I it seems unlikely that the stimulating effect of hyperosmolarity on 3-*O*-methylglucose transport can be related to K^+ loss. Both in the fat pads and in the soleus muscles exposed to hyper-

TABLE I

EFFECT OF HYPEROSMOLARITY ON TISSUE K^+

For one group of measurements, muscles were loaded for 60 min in Krebs–Ringer bicarbonate buffer with or without the listed additions. Alternatively, muscles or fat pads were loaded for 60 min and washed as described in Figs. 7 and 5, respectively. The additions were made after 90 min of washing and the K^+ content determined after the total washing periods indicated. The results are given as μmoles of K^+ per g wet wt. $\pm$ S.E. with the number of observations in parentheses.

Tissue	*Conditions*	*μmoles K^+ per g wet wt.*	*Significance of difference between control and experiment*
Soleus muscles	After loading for 60 min		
	Controls	83 ± 2 (10)	
	Mannitol (200 mM)	93 ± 2 (10)	$P<0.0025$
	Sucrose (200 mM)	87 ± 3 (9)	$P>0.10$
	After loading and washing for 150 min		
	Controls	73 ± 3 (3)	
	Mannitol (200 mM)	84 ± 3 (2)	
	Sucrose (200 mM)	82 ± 2 (2)	
	Urea (200 mM)	78 ± 3 (2)	
Whole epididymal fat pads	After loading and washing for 170 min		
	Controls	13.2 ± 0.4 (9)	
	Mannitol (100 mM)	14.8 ± 0.7 (7)	$0.05<P<0.10$
	Mannitol (200 mM)	15.9 ± 1.0 (7)	$P<0.02$
	Sucrose (200 mM)	15.1 ± 2.1 (3)	

TABLE II

EFFECT OF HYPEROSMOLARITY ON 3-*O*-METHYLGLUCOSE UPTAKE IN SOLEUS MUSCLE

The muscles were incubated for 60 min in Krebs–Ringer bicarbonate buffer containing 3-*O*-[^{14}C]-methylglucose (0.1 μC/ml) and 0.3 mM 3-*O*-methylglucose. The extracellular water space was measured using the contralateral muscles and the same incubation media containing [^{14}C]inulin (0.1 μC/ml). The total water content of each muscle was determined and the intracellular concentration of 3-*O*-[^{14}C]methylglucose calculated as per cent of the extracellular.

Additions	*% Filling of intracellular water (mean ± S.E.)*	*Significance of difference between controls and experimental*
Control	42.7 ± 4.1 (5)	
Mannitol (200 mM)	81.9 ± 4.9 (5)	$P<0.001$
Sucrose (200 mM)	76.0 ± 5.3 (5)	$P<0.001$

osmolar buffer, the K^+ content was similar to, and in some experiments even higher than, that of the controls.

Finally, it could be argued that solvent drag along the osmotic gradient across the cell membrane might account for the stimulation of 3-*O*-methylglucose transport. However, measurements of 3-*O*-methylglucose uptake showed that when the tonicity of the incubation medium was increased by the addition of sucrose or mannitol (200 mM), considerably more 3-*O*-methylglucose gained access to the cytoplasm (Table II). As found for glucose, hyperosmolarity stimulated the transport of 3-*O*-methylglucose against the possible forces of solvent drag.

The addition of 200 mM sucrose or mannitol was found to reduce the intracellular water space to 63 ± 4 % of that of the controls. If the muscle cells behaved as perfect osmometers one would expect a reduction to 61 %. The close agreement between these values is a further indication of the cell membrane integrity.

DISCUSSION

The aim of the present study has been to characterize the hyperosmolarity-induced increase in sugar permeability of adipocytes and muscle cells. In agreement with earlier work[2], hyperosmolarity was found to augment the metabolism of glucose in isolated fat cells. The considerable suppression of this effect by phlorizin and 3-*O*-methylglucose, in addition to the fact that the utilization of glucose displayed saturation kinetics, argues that in a hyperosmolar medium fat cells take up glucose mainly by a carrier-mediated process. Both in adipose tissue and in muscle, hyperosmolarity also accelerated the uptake and the release of a nonmetabolized sugar, which is probably transported by the system mediating the transfer of glucose across the plasma membrane[12]. The stimulation of 3-*O*-methylglucose transport was not associated with any decrease in the K^+ content of the tissue, and it could be abolished by the addition of phlorizin. This, together with the reversibility of the stimulating effect on glucose metabolism (Fig. 2 and refs. 1 and 3), adds further support to the contention that hyperosmolarity augments the functional capacity of the system involved in glucose transport without impairing the overall integrity of the plasma membrane.

Both in muscle and adipocytes, hyperosmolarity seems to increase the energy metabolism[1,3], and as this could lead to a lowering of the ATP availability, the stimulation of sugar transport might have the same origin as that seen during anoxia or exposure to metabolic inhibitors[22]. However, in epididymal fat pads, cyanide and dinitrophenol were found to cause a considerably smaller and more delayed rise in the release of 3-*O*-methylglucose, which, at variance with hyperosmolarity, was associated with a marked loss of K^+ (ref. 10). The early effect of hyperosmolarity on both glucose utilization in fat cells and 3-*O*-methylglucose transport in epididymal fat pads and soleus muscles suggests a more direct effect on the glucose transport system.

In fat cells hyperosmolarity was found to cause the same change in the apparent v_{max} and K_m of glucose utilization as produced by a submaximal concentration of insulin (5 μunits/ml). Also, with respect to the rates of onset and return to basal level hyperosmolarity produced very much the same pattern as insulin. Again, in soleus muscle, hyperosmolarity stimulated the release of 3-*O*-methylglucose with nearly the same time-lag as insulin (10^3 μunits/ml). These observations suggest that hyperosmolarity can mimic insulin in causing the structural transformation of the plasma membrane required for the activation of the glucose transport system.

In frog muscle, ultrastructural studies have demonstrated that the transverse tubules are accessible to extracellular markers[23], and that the surface of their walls is 5–7 times larger than that of the entire outer plasma membrane of the muscle cell[23,24]. In addition, hyperosmolarity has been found to produce a rapid and reversible (up to 4-fold) increase in the diameter of the T-tubules of both rat heart and frog muscle[4–6]. This change might render their lumen more accessible to extracellular solutes and at the same time augment the area of their walls. If the T-tubules contain a glucose transport system, both changes could increase the maximal capacity for the exchange of glucose between the cytoplasm and the surroundings. At present, attempts are being made to determine whether hyperosmolarity can induce similar alterations in the T-tubules of soleus muscle.

ACKNOWLEDGMENTS

The skilled technical assistance of Miss Kirsten Skaarup and Mrs Grethe Schou is gratefully acknowledged.

REFERENCES

1 T. KUZUYA, E. SAMOLS AND R. H. WILLIAMS, *J. Biol. Chem.*, 240 (1965) 2277.
2 J. GLIEMANN, *Diabetes*, 14 (1965) 643.
3 T. CLAUSEN, *Biochim. Biophys. Acta*, 150 (1968) 56.
4 H. E. HUXLEY, S. PAGE AND D. R. WILKIE, *J. Physiol. London*, 169 (1963) 325.
5 L. GIRARDIER, J. P. REUBEN, P. W. BRANDT AND H. GRUNDFEST, *J. Gen. Physiol.*, 47 (1963) 189.
6 W. H. FREYGANG JR., D. A. GOLSDTEIN, D. C. HELLAM AND L. D. PEACHEY, *J. Gen. Physiol.*, 48 (1964) 235.
7 J. GLIEMANN, *Diabetologia*, 3 (1967) 382.
8 J. GLIEMANN, *Acta Physiol. Scand.*, 72 (1968) 481.
9 P. P. COHEN, in W. W. UMBREIT, R. H. BURRIS AND J. F. STAUFFER, *Manometric Techniques and Tissue Metabolism*, Burgess Publishing Co., Minneapolis, 2nd Ed., 1951, p. 119.
10 T. CLAUSEN, *Biochim. Biophys. Acta*, 183 (1969) 625.
11 D. M. KIPNIS AND J. E. PARRISH, *Federation Proc.*, 24 (1965) 1051.

12 O. B. CROFFORD AND A. E. RENOLD, *J. Biol. Chem.*, 240 (1965) 14.
13 O. B. CROFFORD, W. STAUFFACHER, B. JEANRENAUD AND A. E. RENOLD, *Helv. Physiol. Pharmacol. Acta*, 24 (1966) 45.
14 M. RODBELL, *J. Biol. Chem.*, 241 (1966) 130.
15 W. WILBRANDT AND T. ROSENBERG, *Pharmacol. Rev.*, 13 (1961) 109.
16 O. B. CROFFORD AND A. E. RENOLD, *J. Biol. Chem.*, 240 (1965) 3237.
17 J. H. HAGEN AND E. G. BALL, *J. Biol. Chem.*, 235 (1960) 1545.
18 A. MIRSKY, in A. E. RENOLD AND G. F. CAHILL, JR., *Handbook of Physiology*, Section 5, American Physiological Society, Washington, 1965, p. 407.
19 R. J. HO AND B. JEANRENAUD, *Biochim. Biophys. Acta*, 144 (1967) 61.
20 J. LETARTE, B. JEANRENAUD AND A. E. RENOLD, *Biochim. Biophys. Acta*, 183 (1969) 357.
21 I. BIHLER, *Biochim. Biophys. Acta*, 163 (1968) 401.
22 P. J. RANDLE AND G. H. SMITH, *Biochem. J.*, 70 (1958) 490.
23 L. D. PEACHEY, *J. Cell Biol.*, 25, No. 3, Part 2 (1965) 209.
24 G. FALK AND P. FATT, *Proc. Roy. Soc. London, Ser. B*, 160 (1964) 69.
25 J. GLIEMANN, in preparation.