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

V. STIMULATING EFFECT OF OUABAIN, K^+-FREE MEDIUM AND INSULIN ON EFFLUX OF 3-*O*-METHYLGLUCOSE FROM EPIDIDYMAL ADIPOSE TISSUE

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SUMMARY

1. The permeability of adipocytes to a nonmetabolizable sugar was estimated by measuring the washout of 3-*O*-[^{14}C]methylglucose from preloaded whole epididymal fat pads into a sugar-free environment.

2. After an initial rapid phase, the release of 3-*O*-[^{14}C]methylglucose was found to proceed with a rate coefficient of 0.0122 for up to 120 min.

3. The release of 3-*O*-[^{14}C]methylglucose was promptly stimulated by insulin (10–1000 μunits/ml) and inhibited by phlorizin (5 mM) and phloretin (1 mM).

4. In a K^+-free medium or in the presence of ouabain, the release of 3-*O*-[^{14}C]-methylglucose was gradually augmented, maximal effects being obtained after around 50 min.

5. Other factors (2,4-dinitrophenol, CN^-, epinephrine and ACTH), which could also be shown to reduce the K^+ content of the tissue, stimulated 3-*O*-[^{14}C]methyl-glucose release with a similar time-lag.

INTRODUCTION

It has been demonstrated that the addition of ouabain or the omission of K^+ from the incubation medium leads to a stimulation of glucose utilization in epididymal fat pad and in isolated adipocytes[1,2]. The effect of a K^+-free medium was found to be reversible upon reestablishment of the normal extracellular concentration of K^+, and kinetic evidence pointed to the fact that the augmented glucose metabolism was a result of increased v_{max} of glucose transport[3,4].

Na^+ and K^+ are fundamental for the active transport of sugars in such specialized organs as intestine or kidney[5,6], and it was already suggested by CRANE *et al.*[7] that these ions might also play a role in the transport of sugars in tissues (muscle, fat), in which no accumulation of such compounds takes place. Recently, it was reported that in the intact rat diaphragm 3-*O*-methylglucose uptake is stimulated

by ouabain or by incubation in the K^+-free buffer[8]. Hence, it seemed logical to expect analogous changes in the transport of nonmetabolizable sugars in adipose tissue. However, because of the small cytoplasmic water space, studies of the uptake of sugar in adipocytes are fraught with difficulties[9]. For the evaluation of factors affecting the permeability of the plasma membrane in adipocytes to sugars, it was therefore decided to measure the release of a nonmetabolizable sugar from preloaded whole epididymal fat pads. The observation that insulin accelerates both the influx and the efflux of arabinose and galactose in the isolated perfused rat heart suggested that this approach might be useful[10,11]. As the uptake of 3-*O*-methylglucose by epididymal adipose tissue by a carrier-mediated and insulin-responsive process had earlier been demonstrated, this sugar was selected for the present study[12–14]. Whole epididymal fat pads were loaded *in vitro* with 3-*O*-[^{14}C]methylglucose, and the efflux of this sugar was characterized by measuring the washout of radioactivity in the presence of factors known to influence glucose transport (insulin, epinephrine, ACTH, 2,4-dinitrophenol, CN^-, phlorizin and phloretin). The effect of decreased active Na^+–K^+-transport was assessed in experiments with ouabain or buffer in which all K^+ had been replaced by an equimolar amount of Na^+.

METHODS

Male Wistar albino rats weighing 90–120 g were used in this study. They had free access to Altromin laboratory chow. The animals were killed by decapitation, and the epididymal fat pads were excised and directly transferred to incubation vials containing 2 ml of buffer warmed to 37° to which ^{14}C-labeled 3-*O*-methylglucose and unlabeled 3-*O*-methylglucose (carrier) had been added. Each flask contained from 2–6 whole fat pads weighing 30–150 mg.

As it has earlier been shown that 3-*O*-methylglucose is totally equilibrated between the intracellular and extracellular water phase within 60 min (ref. 14), this time interval was chosen for the loading with labeled 3-*O*-methylglucose.

After incubation for 60 min in the presence of 3-*O*-[^{14}C]methylglucose, each fat pad was gently transferred to incubation vials containing unlabeled buffer warmed to 37° without any 3-*O*-methylglucose. The washout of radioactivity from each individual fat pad was continued in a series of vials containing 2 ml of buffer. This volume is at least 100 times larger than the total 3-*O*-methylglucose-available water space of epididymal fat pads[12]. In order to minimize damage of the tissue, a thin L-shaped polyethylene rod was used to lift the fat pad from the surface of the buffer and into the subsequent incubation vial.

Unless stated otherwise, the basic incubation medium was Krebs–Ringer bicarbonate buffer[15] (pH 7.4), containing 1 % of dialysed bovine serum albumin. Polyethylene counting vials (Packard) were used for all incubations, which took place in a Gallenkamp metabolic agitator moving 80 cycles/min. Prior to the addition of tissue, each vial was equilibrated for 10 min at 37° with a mixture of O_2–CO_2 (95:5, v/v) and gassing was repeated before closing the vial for incubation.

At the end of the washout period, each fat pad was blotted lightly, weighed and homogenized for 2 min in 2 ml of 5 % trichloroacetic acid using a Potter-Elvehjem tube. After centrifugation, aliquots of the clear interphase were withdrawn for the determination of ^{14}C activity and K^+ content. For the measurement of ^{14}C activity

released from the tissue during washout, the albumin was precipitated with trichloroacetic acid (final concn. 5 %), and after centrifugation an aliquot of the supernatant fluid was withdrawn for counting. In all experiments unlabeled 3-*O*-methylglucose (0.1 mM) was added along with the trichloroacetic acid as carrier.

Counting was performed in a Tri-Carb liquid scintillation spectrometer Model 3003 (Packard) using glass vials containing 10 ml of BRAY's solution[16]. The counting efficiency was determined using [^{14}C]toluene (Packard) for internal standardization. Buffer and tissue extracts (1-ml aliquots) were found to be counted with the same efficiency (57 %).

In each experiment the ^{14}C activity in the solution used for loading was measured, and the specific activity of the 3-*O*-[^{14}C]methylglucose calculated. On the basis of this value, the ^{14}C activity recovered during the washout and in the tissue was converted to nmoles of 3-*O*-methylglucose.

The 3-*O*-[^{14}C]methylglucose content (A_t) of the tissue at various times during the washout was calculated by adding successively in reverse order the amount of 3-*O*-[^{14}C]methylglucose released into each vial during the 10-min periods: $A_{t=10} = A_t + a_t$. A rate coefficient for the release was obtained using the following expression for the fraction of 3-*O*-[^{14}C]methylglucose lost per min within each washout period:

$$\frac{a_t}{(A_t + a_t/2) \cdot 10}$$

The K^+ content of the epididymal fat pads was determined by flame photometry using an Eppendorf flame photometer and standards containing K^+, Na^+ and trichloroacetic acid in the same concentration range as the tissue extracts. In separate experiments the K^+ content was determined after digestion of the tissue in HNO_3 (ref. 17). As seen from Table III the values obtained with this method did not differ significantly from those measured using the trichloroacetic acid homogenates.

In order to investigate the possibility that 3-*O*-[^{14}C]methylglucose was metabolized by the tissue, 4 fat pads were incubated for 60 min in 2 ml of buffer containing 0.5 μC of 3-*O*-[^{14}C]methylglucose per ml and 3-*O*-methylglucose at a concentration of 1 mM. CO_2 was collected from the buffer, and total lipids were extracted from the tissue as described elsewhere[18,19]. In two such experiments, no incorporation of ^{14}C activity into CO_2 or lipids could be detected.

In some of the washout experiments, samples of the trichloroacetic acid extracts of tissue were chromatographed[20]. More than 90 % of the total ^{14}C activity could be recovered from one spot moving with the same speed as the 3-*O*-[^{14}C]methylglucose added to the medium used for loading the tissue.

Bovine serum albumin (Fraction V) was obtained from Sigma (St. Louis) and was used after dialysis for 24 h against distilled water. Unlabeled 3-*O*-methylglucose was obtained from Calbiochem (Los Angeles) and ^{14}C-labeled 3-*O*-methylglucose (specific activity 16.4 mC/mmole) from New England Nuclear (Boston). Ouabain was purchased from Merck (Darmstadt) and phloretin from the K & K laboratories (Calif.). Phlorizin (British Drug House) was used after recrystallization from warm ethanol. The L-epinephrine and the ACTH (Synachten) were products of Rhone-Poulenc (Paris) and Ciba (Basel), respectively. Pork insulin (10 times recrystallized, Lot No. S 23267) was a gift of The Novo Laboratories (Copenhagen). All chemicals used were of analytical grade.

RESULTS

Fig. 1 describes the time-course of the washout of ^{14}C activity from epididymal fat pads loaded in buffer containing either 1 or 20 mM of 3-*O*-[^{14}C]methylglucose. After an initial rapid decrease in ^{14}C activity lasting 30–50 min, both curves approach a straight line when plotted semilogarithmically. This indicates that the release of 3-*O*-[^{14}C]methylglucose under basal conditions proceeds with approximately the same rate coefficient within a relatively wide range of tissue concentrations. Furthermore, the rate coefficient appeared to be a rather reproducible parameter. In twelve different experiments, each performed with 4–10 fat pads preloaded at a 3-*O*-methylglucose concentration of 1 mM, the rate coefficient* was found to vary between 0.0107 min^{-1} and 0.0141 min^{-1} with a mean value of 0.0122 min^{-1}. There was no significant correlation between the rate coefficient and the wet weight of the individual tissues.

Fig. 1 also shows that insulin (1 munit/ml), when added to the vials used for incubation during the washout period, produces an immediate decrease in the ^{14}C activity of the tissue.

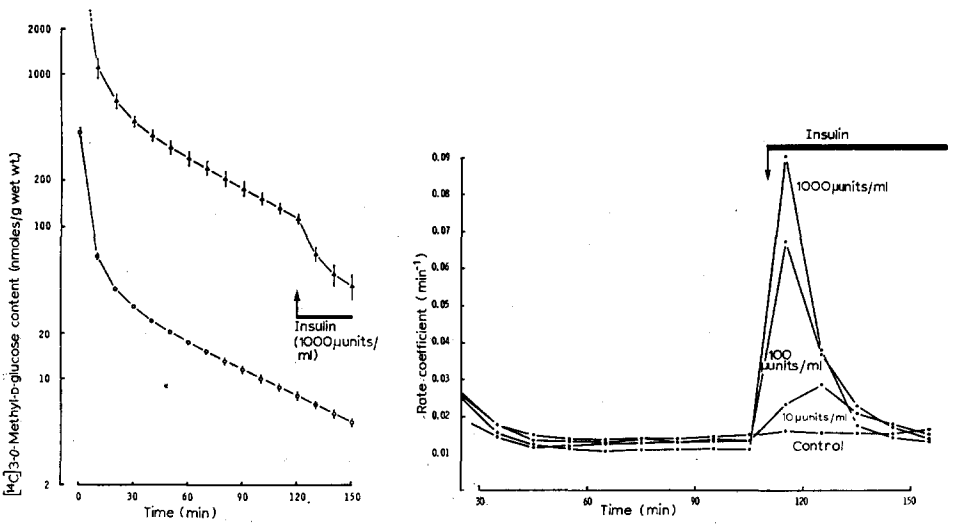

Fig. 1. 3-*O*-[^{14}C]Methylglucose content of epididymal adipose tissue during washout. Whole fat pads were loaded for 60 min in Krebs–Ringer bicarbonate buffer containing 1% albumin, 3-*O*-[^{14}C]methylglucose (1 μC/ml) and either 1 mM (○—○) or 20 mM (△—△) of 3-*O*-methylglucose. The tissues were washed in a series of vials containing the same buffer without 3-*O*-methylglucose and the ^{14}C activity released converted to nmoles of 3-*O*-methylglucose. The fat pads loaded in buffer containing 20 mM of 3-*O*-methylglucose were exposed to insulin (1000 μunits/ml) during the last 30 min of washout. Each curve represents the mean of four observations with bars indicating 2 × S.E. when this value exceeds the size of the symbols.

Fig. 2. Effect of insulin on the rate coefficient of 3-*O*-methylglucose release. Epididymal fat pads were loaded in Krebs–Ringer bicarbonate buffer containing 1% albumin, 3-*O*-[^{14}C]methylglucose (2 μC/ml) and 1 mM of 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. Insulin (10–1000 μunits/ml) was present during the last 50 min of washout. Each curve represents the mean of two observations.

* As the conditions were often changed at 90 min, the first part of the straight portion of the efflux curve from 70 to 90 min was used for the calculation of these values.

Fig. 2 shows that there is a graded response to insulin (10–1000 μunits/ml) which is detectable immediately after the first exposure to the hormone. In spite of the continued presence of insulin, the initial sharp rise in the rate coefficient was followed by a gradual decrease. As this pattern was also found in the experiments performed with tissues loaded at a 3-*O*-methylglucose concentration of 20 mM or upon addition of insulin earlier in the washout period, it seems unlikely that the decrease is only due to a lowered intracellular concentration of 3-*O*-methylglucose.

In epididymal fat pads[13,21] and in other tissues[22,23], the uptake of glucose and of nonmetabolizable sugars is inhibited by phlorizin and phloretin. As these compounds are thought to interfere with the function of a transport system for sugars in the cell membrane, their effect on 3-*O*-[^{14}C]methylglucose release was assessed. Table I shows that both phlorizin (5 mM) and phloretin (1 mM) shortly after their addition diminish the release of 3-*O*-[^{14}C]methylglucose. However, in the experiments with phloretin, ethanol (0.5 %) which was added for solubilizing the aglycone seems to be inhibitory *per se* as seen from the control. However, the effect of ethanol alone is not sufficient to explain the effect of phloretin.

A few factors which have been shown to mimick the action of insulin in augmenting the permeability of the plasma membrane to sugars were also tested for their effect on 3-*O*-[^{14}C]methylglucose release. As seen from Fig. 3, 2,4-dinitrophenol (A) and CN^- (B), which have been shown to promote the uptake of sugar in muscle and heart[24,25], accelerate the release of 3-*O*-[^{14}C]methylglucose from fat pads.

A number of lipolytic agents stimulate the utilization of glucose in adipocytes[26–28], and recently it was found that epinephrine also promotes the uptake of L-arabinose in whole epididymal fat pads[29]. Fig. 4 shows that both epinephrine

TABLE I

EFFECT OF PHLORIZIN AND PHLORETIN ON RATE COEFFICIENT OF 3-*O*-[^{14}C]METHYLGLUCOSE RELEASE

Epididymal fat pads were loaded and washed as described under Fig. 2. The rate coefficients calculated for each washout period of 10 min are shown with S.E. and the number of fat pads given in parentheses.

	Fraction of 3-O-[^{14}C]methylglucose lost per min within each washout period			
Washout periods:	*70–80 min*	*80–90 min*	*90–100 min*	*100–110 min*
Control	0.0128 ± 0.0008 (7)	0.0123 ± 0.0006 (7)	0.0123 ± 0.0008 (7)	0.0120 ± 0.0009 (7)
Phlorizin (5 mM) added at 90 min	0.0126 ± 0.0009 (4)	0.0113 ± 0.0007 (4)	0.0078 ± 0.0004 (4)	0.0063 ± 0.0003 (4)
Significance of difference between control and experimental	$P > 0.10$	$P > 0.10$	$P < 0.001$	$P < 0.001$
Control (0.5 % ethanol added at 90 min)	0.0138 ± 0.0008 (5)	0.0136 ± 0.0006 (5)	0.0122 ± 0.0012 (5)	0.0106 ± 0.0011 (4)
Phloretin (1 mM) added at 90 min	0.0136 ± 0.0008 (5)	0.0136 ± 0.0006 (5)	0.0094 ± 0.0013 (4)	0.0066 ± 0.0002 (5)
Significance of difference between control and experimental	$P > 0.10$	$P > 0.10$	$P < 0.10$	$P < 0.01$

(A) and ACTH (B) accelerate the release of 3-*O*-[^{14}C]methylglucose from fat pads.

The release of 3-*O*-[^{14}C]methylglucose was also assessed by measuring the radioactivity remaining in the tissue after 60 min of incubation in the same vial containing

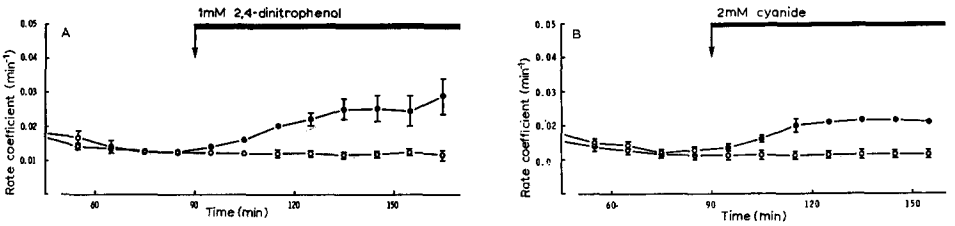

Fig. 3. Effect of 2,4-dinitrophenol (A) or CN^- (B) on the rate coefficient of 3-*O*-methylglucose release. Experimental conditions as in Fig. 2. The CN^- was added as a sodium salt. Each curve represents the mean of three to six observations with bars indicating 2 × S.E. when this value exceeds the size of the symbols. The experimental values are given by closed symbols, controls by open symbols.

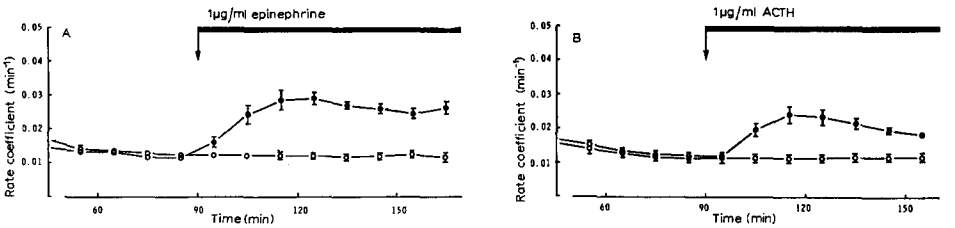

Fig. 4. Effect of epinephrine (A) or ACTH (B) on the rate coefficient of 3-*O*-methylglucose release. Experimental conditions as in Fig. 2. The hormones were added as small aliquots of freshly prepared solution immediately before the experiments. Each curve represents the mean of five to eight observations with bars indicating 2 × S.E. when this value exceeds the size of the symbols. The experimental values are given by closed symbols, controls by open symbols.

TABLE II

EFFECT OF INSULIN AND INCUBATION IN K^+-FREE BUFFER ON 3-*O*-[^{14}C]METHYLGLUCOSE CONTENT OF EPIDIDYMAL ADIPOSE TISSUE

Whole fat pads were loaded for 60 min in K^+-free or normal Krebs–Ringer bicarbonate buffer containing 1 % albumin, 3-*O*-[^{14}C]methylglucose (0.5 µC/ml) and 1 mM of 3-*O*-methylglucose. The tissues were washed twice for 10 min in 5 ml of buffer and then were incubated for 60 min in 5 ml of normal or K^+-free buffer without 3-*O*-methylglucose. Results are given as ^{14}C activity (converted to nmoles of 3-*O*-methylglucose) remaining in the tissue at the end of experiments. The values are means ± S.E. with the number of fat pads in parentheses. In the K^+-free buffer K^+ was replaced by an equimolar amount of Na^+.

Incubation medium used for loading	*Incubation medium during the washout period*	*3-O-[^{14}C]methylglucose content (nmoles/g wet wt.)*	*P*
Normal buffer	Normal buffer	9.0 ± 0.9 (4)	<0.001
	Normal buffer + insulin (10 munits/ml)	2.4 ± 0.1 (4)	
Normal buffer	Normal buffer	9.8 ± 0.9 (6)	<0.005
	K^+-free buffer	5.8 ± 1.0 (6)	
Normal buffer	Normal buffer	10.7 ± 1.7 (4)	<0.001
K^+-free buffer	K^+-free buffer	3.3 ± 0.3 (8)	

unlabeled buffer. Table II shows that under these circumstances insulin markedly stimulated the loss of 3-*O*-[^{14}C]methylglucose. It has been demonstrated that lowering of the extracellular concentration of K^+ leads to a decrease in active Na^+–K^+-trans-

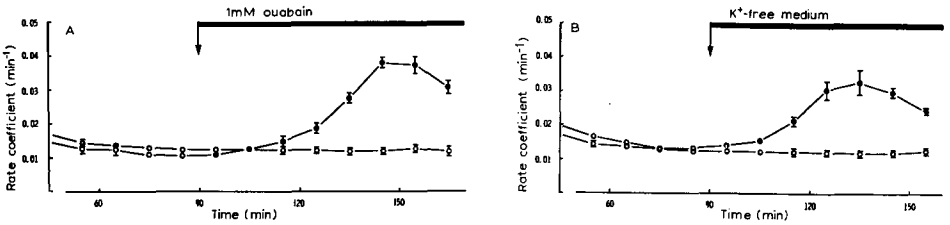

Fig. 5. Effect of ouabain (A) or K^+-free buffer (B) on the rate coefficient of 3-*O*-methylglucose release. Experimental conditions as in Fig. 2. In the K^+-free buffer all K^+ was replaced by an equimolar amount of Na^+. Each curve represents the mean of four to seven observations with bars indicating 2 × S.E. when this value exceeds the size of the symbols. The experimental values are given by closed symbols, controls by open symbols.

TABLE III

K^+ CONTENT OF EPIDIDYMAL ADIPOSE TISSUE BEFORE AND AFTER INCUBATION

All incubated tissues were loaded for 60 min as described under Fig. 2. The K^+ content was determined before and after either 110 or 170 min of washing in Krebs–Ringer bicarbonate buffer containing 1% albumin. Additions (or the omission of K^+) took place after 90 min of washing. The results are given as μmoles of K^+/g wet wt. ± S.E. with the number of fat pads in parentheses.

	Conditions	*μmoles K^+/g wet wt.*	*Significance of difference between controls and experimental*
Freshly excised "paired" fat pads from the same 8 animals		15.1 ± 0.8* (8) 15.9 ± 0.7 (8)	
After loading		16.0 ± 0.5 (12)	
After loading and washing for 110 min		14.3 ± 0.9 (5)	
After loading and washing for 170 min	Controls	12.3 ± 0.4 (12)	
	Ouabain (1 mM)	6.4 ± 0.4 (7)	$P < 0.001$
	K^+-free medium	4.5 ± 0.4 (9)	$P < 0.001$
	2,4-Dinitrophenol (1 mM)	4.9 ± 0.5 (3)	$P < 0.001$
	CN^- (2 mM)	7.1 ± 0.4 (6)	$P < 0.001$
	Epinephrine (1 μg/ml)	6.0 ± 0.3 (8)	$P < 0.001$
	ACTH (1 μg/ml)	10.7 ± 0.6 (7)	$P < 0.05$

* Results obtained using a HNO_3 digest for the flame photometry. All the other values measured in trichloroacetic acid extracts.

port[3,30,31]. From Table II it appears that fat pads upon 60 min of washing in the absence of K^+ had retained much less ^{14}C activity than the controls, an effect which became more pronounced if the loading also took place in a K^+-free medium.

A more detailed study of the time-course of the accelerating effect of inhibited active Na^+–K^+-transport is presented in Fig. 5. It appears that the effects of ouabain

(A) and of the K^+-free buffer (B) can both be demonstrated later in the efflux period and that they are gradual in onset. Maximum stimulation is only achieved after 50–60 min.

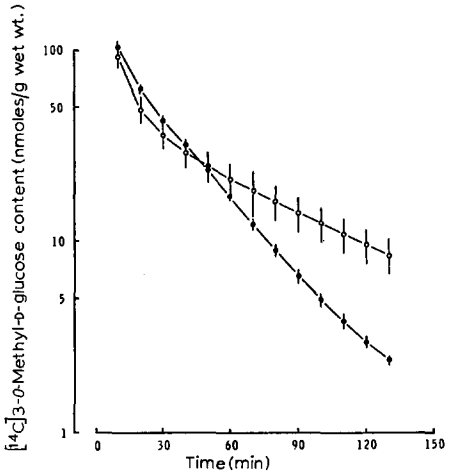

Fig. 6. Effect of K^+-free medium on 3-*O*-[^{14}C]methylglucose content. 4 epididymal fat pads were loaded in Krebs–Ringer bicarbonate buffer containing 1% albumin, 3-*O*-[^{14}C]methylglucose (1 μC/ml) and 1 mM of 3-*O*-methylglucose. The washout took place in K^+-free buffer (closed symbols). The contralateral fat pads from the same animals were loaded in a K^+-free buffer with the same additions of albumin and labeled 3-*O*-methylglucose. The washout of ^{14}C activity from these fat pads was followed in a buffer with a normal K^+ content (open symbols). The ^{14}C content was converted to nanomoles of 3-*O*-methylglucose. The bars indicate 2 × S.E.

Fig. 6 shows an experiment in which fat pads were loaded in either normal or K^+-free buffer and then were washed in the K^+-free or normal buffer, respectively. It appears that the tissues loaded in normal buffer release 3-*O*-[^{14}C]methylglucose into the K^+-free buffer considerably faster than any of the controls (*cf.* with previous figures). It should be noted that the contralateral fat pads from the same animals, when exposed to the K^+-free buffer during the loading, release 3-*O*-[^{14}C]methylglucose into normal buffer at nearly the same rate as controls. This indicates that the change in permeability to 3-*O*-methylglucose seen in the K^+-free buffer is reversible, a result analogous to the finding that stimulation of the utilization of glucose by the K^+-free medium in adipocytes is reversible upon reestablishment of the normal extracellular concentration of K^+ (refs. 3 and 4).

Table III shows the K^+ content of fat pads immediately after their preparation, after loading with 3-*O*-methylglucose and after the washout period.

Whereas there was no significant change in the K^+ content during the loading, the washing procedure caused some loss of K^+. Incubation in the K^+-free medium or in the presence of ouabain during the last 80 min of washout led to a considerably larger reduction in the K^+ content. The metabolic inhibitors and epinephrine induced a loss of K^+ of similar magnitude. ACTH had a barely significant effect, although 3-*O*-[^{14}C]methylglucose release was also stimulated by this hormone.

DISCUSSION

It has earlier been observed that insulin stimulates not only the influx but also the efflux of nonmetabolizable sugars in the isolated rat heart[10], and the effect on the efflux has been used for the quantitative evaluation of insulin responsiveness[11]. These findings indicate that factors accelerating sugar influx will influence the efflux in a similar direction. If this is the case, it seems reasonable to use efflux measurements for the detection and assessment of changes in the function of a sugar transport system in adipose tissue in which influx studies are difficult to perform.

The aim of the present study has been to assess the permeability of adipocytes to a nonmetabolizable sugar by measuring the rate of 3-*O*-[^{14}C]methylglucose release from preloaded epididymal fat pads. The finding that this process is stimulated by insulin argues that a significant portion of the 3-*O*-methylglucose is located in the cytoplasm and that the rate of release measured reflects transport across the plasma membrane. The fact that phlorizin and phloretin both inhibit the release of 3-*O*-[^{14}C]methylglucose further indicates that diffusion is not the major rate-limiting factor under basal conditions. However, upon exposure to factors accelerating the release of 3-*O*-[^{14}C]methylglucose, a biphasic change in the rate coefficient was found, which points to the fact that the washout of sugar does not occur with the same rapidity from all regions of the tissue. This might be a consequence of heterogeneity of the cell population with respect to membrane transport properties, but it seems more likely that the decrease in the rate coefficient seen after stimulation is a result of the radioactivity being retained in a core of the tissue, from which diffusion is relatively slow. With these limitations (which incidentally must be similar for studies of the uptake of sugar in adipose tissue[12,13,32]), the present approach seemed capable of yielding some information about the function of a membrane-bound sugar transport system.

It was demonstrated that a number of factors (2,4-dinitrophenol, CN^-, epinephrine and ACTH), which have already been shown to increase the uptake of sugars in muscle cells[24,25] or in adipocytes[26–29], also promote the egression of 3-*O*-methylglucose from adipose tissue.

The finding that ouabain or incubation in a K^+-free medium accelerates the release of 3-*O*-[^{14}C]methylglucose indicates that inhibition of the active Na^+–K^+-transport leads to the activation of a sugar transport system. This is possibly the basis for the increased utilization of glucose in adipocytes observed under similar conditions[1–4]. At variance with the pattern seen upon addition of insulin, the stimulatory effect of ouabain or a K^+-free medium on glucose metabolism[4] and on 3-*O*-[^{14}C]methylglucose release both show a time-lag. This suggests that some gradual change in the composition of the cytoplasm or in the structure of the plasma membrane is required for the augmentation of sugar permeability.

Ouabain, K^+-free medium, 2,4-dinitrophenol and CN^- were all found to reduce the K^+ content of the tissue. It is possible that a decrease in the concentration of K^+ or a simultaneous increase in the concentration of Na^+ of the cytoplasm leads to the activation of a sugar transport system in the plasma membrane.

Also epinephrine (and to a minor extent ACTH) was found to induce a loss of K^+. This effect has earlier been observed (B. MOSINGER, personal communication), and in isolated fat cells it has been shown that lipolytic agents inhibit the accumu-

lation of K^+ (ref. 33). As a loss of K^+ seems to be associated with an acceleration in sugar transport, it is possible that the stimulating effect of lipolytic agents on the uptake of glucose can in part be explained as secondary to alterations in the distribution of K^+ and Na^+ across the plasma membrane.

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