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IONIC EFFECTS ON GLUCOSE TRANSPORT AND METABOLISM BY ISOLATED MOUSE FAT CELLS INCUBATED WITH OR WITHOUT INSULIN

II. EFFECT OF REPLACEMENT OF K^+ AND OF OUABAIN

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SUMMARY

1. This report deals with the effects of K^+ and of ouabain on the metabolism of glucose by isolated mouse fat cells.

2. In the absence of insulin, both K^+ lack and the presence of ouabain increased glucose uptake and metabolism. These effects were present at various concentrations of glucose and were reversible.

3. In the presence of amounts of insulin sufficient for near-maximal stimulation of glucose metabolism, neither K^+ lack nor ouabain had any significant effect.

4. Intracellular K^+ content was decreased by incubation in medium with low K^+ concentrations or containing ouabain. These results were the same, whether insulin was present in the incubation medium or not.

5. A carrier system forming a ternary complex with glucose and K^+ and/or Na^+ is postulated.

INTRODUCTION

The effects of K^+ and of ouabain on glucose metabolism by diaphragm muscle *in vitro* have recently been reviewed by CLAUSEN[1,2], who has shown that either replacement of K^+ or the presence of ouabain in the medium enhances glycogen synthesis and decreases lactate output, without altering glucose uptake. Under the same experimental conditions, yet using adipose tissue or isolated fat cells, MOSINGER and co-workers[3,4] as well as HO *et al.*[5] have reported that the release of free fatty acids resulting from lipolytic stimulation was diminished, while the latter authors also demonstrated that ouabain or the absence of K^+ increases glucose oxidation and its utilization for lipogenesis[6]. It was suggested that all of these effects might be mediated by inhibition of adenyl cyclase[4,7], an activity which ouabain and absence of K^+ might thus share with insulin[8,9]. The present studies of the effects of K^+ and of ouabain on glucose metabolism and K^+ content of isolated mouse fat cells are part of a detailed analysis of the relationship of insulin action to that of modifications in the ionic

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composition of the incubation medium[10]. Some of these findings have been reported previously in preliminary form[11,12].

MATERIALS AND METHODS

Unless otherwise stated, the animals, reagents, enzymes, insulin, buffer and incubation techniques were as described in the first paper of this series[10] and by HO AND JEANRENAUD[6]. When K^+ was omitted from the incubation medium, it was replaced by an equimolar amount of Na^+, the other ionic components being unchanged. $^{42}K^+$ was purchased from the Radiochemical Centre, Amersham, Great Britain; ouabain (strophantin G) was purchased from Fluka (Buchs, Switzerland).

The intracellular K^+ measurements were performed as follows. Isolated fat cells were prepared as previously described[13]. They were then washed with normal buffer containing $^{42}K^+$ and preincubated for 30–60 min in a normal buffer containing the same concentration of $^{42}K^+$. Most of the medium was then removed, and the thick suspension of cells was distributed into different tubes containing the agent being studied. The specific activity of K^+ was kept constant throughout. After incubation for 1 h, the medium was removed with a very thin needle, and its radioactivity and K^+ content were measured for estimation of K^+ specific activity. The thick fat cell suspension was immediately weighed in a preweighed cup, dried under vacuum to constant weight and reweighed. The water space (wet wt. *minus* dry wt.) was about equal to the cell's dry weight. Since the proportion of intracellular water under these conditions is very small[14], all of the water was considered to represent extracellular water. The dried fat cells (containing dried extracellular electrolytes), as well as a corresponding amount of medium mixed with nonlabeled cells, were separately counted in a Packard Auto gamma spectrometer, Model 402. The counts corresponding to the contaminating medium (water space in μl multiplied by counts/min per μl medium) were then subtracted from the total counts of the dried radioactive cells. The difference in counts was divided by the specific activity of K^+ so as to convert it into μmoles, and then into μmoles of K^+ per g dried fat cells. The values so obtained were reproducible within any one experiment (Table I) but varied considerably from day to day. Many explanations can be invoked for this variability[15] such as variations of body weight, age, state of nutrition, *etc.* However, they remain speculative for the time being, since we did not further investigate the phenomenon.

RESULTS

The stimulatory action of a lack of K^+ on the baseline metabolic activity of mouse fat cells toward glucose was easily seen at all concentrations of glucose tested (Table II). When plotted according to LINEWEAVER AND BURK[16] two straight lines were obtained, merging near the abscissa (Fig. 1) and suggesting that the presence of K^+ decreases the maximal velocity of overall glucose metabolism, without altering the apparent K_m of the reactions involved. The excellent linearity of the points obtained also suggests a single controlling step in each instance, most probably at the level of transport[14,17].

The effect of the replacement of K^+ by Na^+ was also examined in the presence of varying concentrations of insulin, as shown in Fig. 2. The cells incubated in normal

TABLE I

REPRODUCIBILITY OF THE MEASUREMENT OF K^+ CONTENT OF ISOLATED FAT CELLS OF MICE

The two experiments reported here were carried out at an interval of 1 week. The incubation medium contained 6 mM K^+ and 2.5 mM D-glucose. Each figure is the mean of 12 values ±S.E.

Expt. No.	*Incubation time (min)*	*Age (weeks)*	*K^+ (μM/g dry wt.)*
1	30	5–6	5.25 ± 0.13
2	30	6–7	3.94 ± 0.07

TABLE II

EFFECT OF K^+ ON D-[$^{14}C_6$]GLUCOSE METABOLISM BY ISOLATED FAT CELLS OF MICE IN THE PRESENCE OF INCREASING CONCENTRATIONS OF GLUCOSE

The cells were incubated in Krebs–Ringer bicarbonate buffer, with or without 6 mM K^+. Results are expressed as μatoms C incorporated into CO_2 or total lipids per g lipid over 2 h. Each figure is the mean of 6 values ±S.E.

Medium glucose (mM)	*Medium K^+, 6 mM*		*Medium K^+, 0 mM*	
	CO_2	*Total lipids*	*CO_2*	*Total lipids*
1.25	1.48 ± 0.04	1.96 ± 0.04	2.25 ± 0.05	4.22 ± 0.11
1.67	1.71 ± 0.04	2.36 ± 0.03	2.67 ± 0.06	4.84 ± 0.09
2.5	2.19 ± 0.06	3.02 ± 0.08	3.33 ± 0.05	5.27 ± 0.12
5.0	3.48 ± 0.09	4.24 ± 0.09	4.24 ± 0.08	6.92 ± 0.16
10.0	5.23 ± 0.11	5.95 ± 0.13	5.93 ± 0.36	7.23 ± 0.16

medium exhibited a good insulin response, with maximal metabolic activity attained 100–500 μunits/ml for all three metabolic indices measured. In the absence of K^+, basal metabolism was again increased, and this was also true at submaximal levels of stimulation by insulin, although the increased glucose metabolism induced by the absence of K^+ never went beyond the maximal insulin-stimulated level already observed in the presence of K^+. With fully stimulating concentrations of insulin, glucose metabolism in the absence of K^+ was either the same (glyceride fatty acids) or slightly less (CO_2 and glyceride glycerol) than in normal medium.

The reversibility of the stimulating effects of a lack of K^+ is shown in Fig. 3. The isolated fat cells were washed and suspended in a medium free of K^+. They were then distributed into vials containing 0 or 6 mM K^+ and preincubated for 1 h, in the presence of unlabeled glucose. At the end of this period, most of the incubation medium was removed, and the cells were resuspended in fresh medium containing radioactive glucose and incubated for the next 2 h in the presence or absence of 6 mM K^+. It is evident from the results shown in this figure that the effect of preincubation in the absence of K^+ was readily reversed by the subsequent addition of K^+. A nonspecific and irreversible disruption of the cell membrane, leading to glucose "leakage" into the cell, therefore appears to be an unlikely explanation for the mechanism underlying the effects of K^+ lack.

A possible relationship between K^+ and ouabain[6,18,19] was also investigated. Isolated fat cells were washed and suspended in a K^+-free medium and distributed

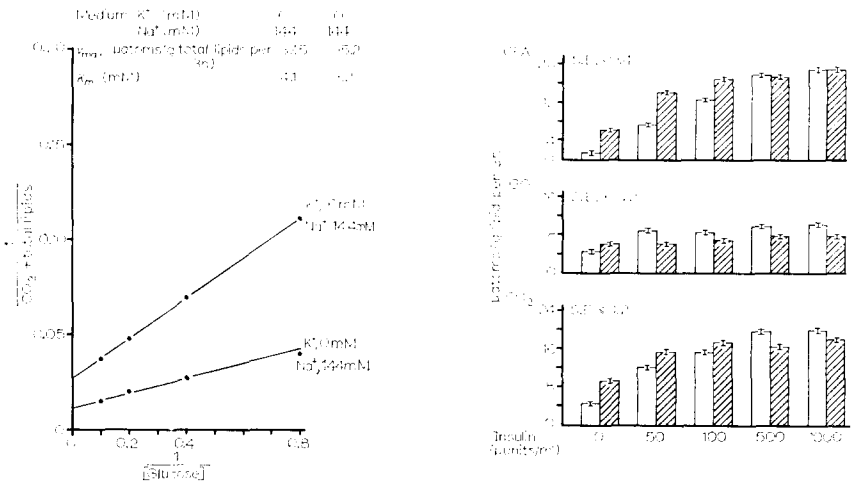

Fig. 1. Effect of K^+ on D-[$^{14}C_6$]glucose metabolism by isolated mouse fat cells in the presence of increasing concns. of glucose. Experimental conditions are the same as in Table II. Oxidation of glucose carbon to CO_2 and glucose carbon incorporation into total lipids were included and used as an expression of overall velocity of glucose uptake. Values were plotted according to LINEWEAVER–BURK. Each point is the mean of 6 values.

Fig. 2. Effect of K^+ on D-[$^{14}C_6$]glucose metabolism by isolated mouse fat cells in the presence of increasing concns. of insulin. Cells were incubated for 2 h in 2.0 ml Krebs–Ringer bicarbonate buffer containing 2.5 mM glucose and 3.5 g/100 ml albumin. Blank columns represent results obtained in the presence of 6 mM K^+, hatched columns those obtained in the absence of added K^+. Each column is the mean of 6 values ±S.E. GFA = glyceride fatty acids; GG = glyceride glycerol.

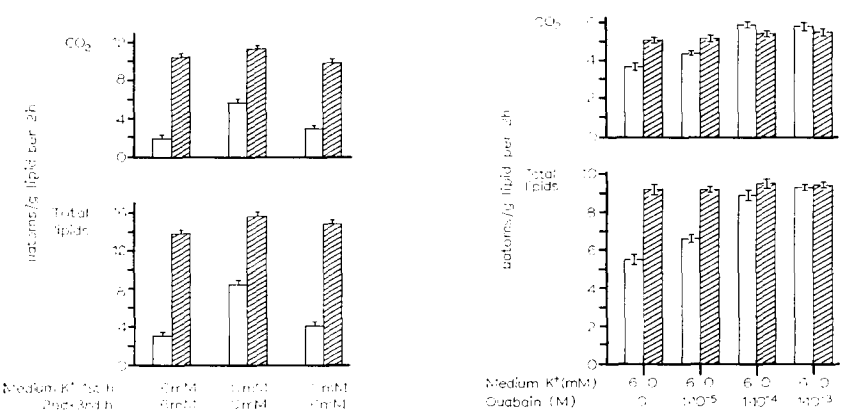

Fig. 3. Reversibility of the effect of lack of K^+ on D-[$^{14}C_6$]glucose metabolism by isolated mouse fat cells in the absence (blank columns) and presence (hatched columns) of insulin (1 munit/ml). Cells preincubated for 1 h with or without K^+, as indicated, then transferred to new medium with or without K^+. Glucose (2.5 mM) and insulin (when added) were present throughout. Labeled glucose was added only during the 2nd and 3rd h. Each figure is the mean of 6 values ±S.E.

Fig. 4. Effect of increasing concns. of ouabain on D-[$^{14}C_6$]-glucose metabolism by isolated mouse fat cells in the presence of 6 mM K^+ (blank columns) or in the absence of added K^+ (hatched columns), K^+ being replaced by Na^+. Glucose concn., 2.5 mM. Each figure is the mean of 6 values ±S.E.

into vials containing either no K^+ or a sufficient amount to give a final K^+ concentration of 6 mM. They were then incubated in the presence of increasing concentrations of ouabain. After 2 h, the incorporation of glucose into CO_2 and total lipids was measured (Fig. 4). The addition of ouabain to the cells incubated in the absence of

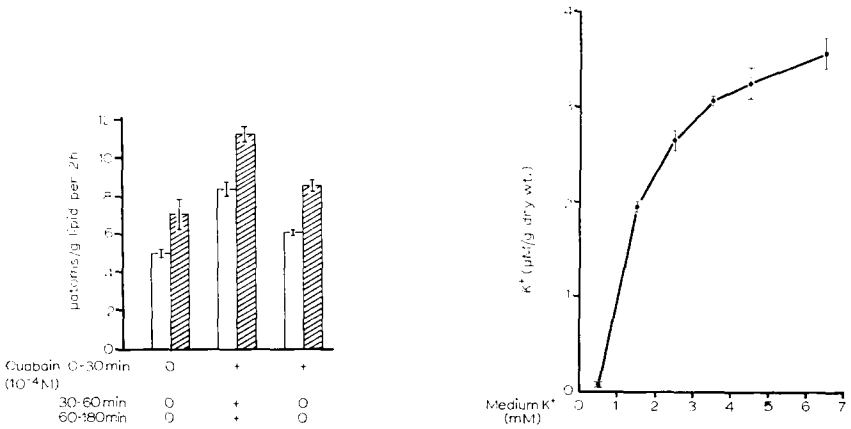

Fig. 5. Reversibility of ouabain effect on D-[$^{14}C_6$]glucose metabolism by isolated mouse fat cells. The presence or absence of ouabain during two preincubation periods of 30 min each, and during the 2-h incubation is indicated. 2.5 mM glucose is present throughout. Labeled glucose was added only during the 2 h of incubation (60–180 min). Each figure, mean of 6 values ± S.E. Open columns, oxidation of labeled glucose to CO_2. Hatched columns, incorporation of glucose carbon into total lipids.

Fig. 6. Effect of medium K^+ concn. on the intracellular K^+ content of isolated mouse fat cells. For experimental conditions see MATERIALS AND METHODS. Glucose concn. 2.5 mM. Mice were 6–7 weeks of age. Each point is the mean of 5 values ± S.E.

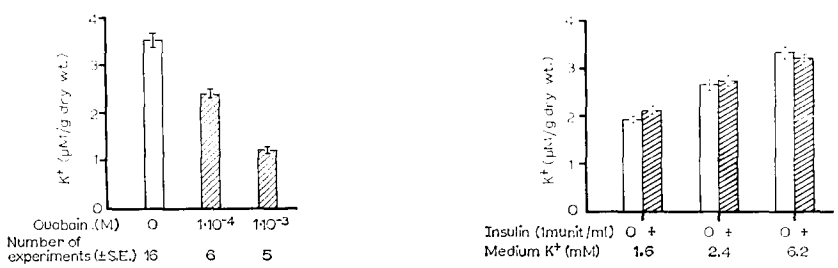

Fig. 7. Effect of ouabain (hatched columns) on the intracellular K^+ content of isolated mouse fat cells. Other experimental conditions were as Fig. 6.

Fig. 8. Intracellular K^+ content of isolated mouse fat cells in the presence (hatched columns) or absence (blank columns) of insulin, and in the presence of varying concns. of extracellular K^+. Other experimental conditions were as in Fig. 6. Each figure is the mean of 6 values ± S.E.

K^+ did not alter their glucose metabolism. However, when the glycoside was added to cells incubated in medium containing K^+, a progressive increase in metabolic activity was seen. At an ouabain concentration of 0.1 mM the ouabain-induced stimulation of glucose metabolism attained the level induced by K^+-free medium alone. These results are in agreement with observations in other systems which have shown that the effects of K^+ can be blocked by ouabain[20]. In the present instance, the depressive effect of K^+ upon glucose metabolism by isolated fat cells was neutralized by the presence of ouabain.

We have also investigated whether the ouabain effect is reversible, utilizing a protocol similar to that used for the effects of K^+ lack (Fig. 5). The cells were washed, suspended and incubated in normal medium or in medium containing 0.1 mM ouabain. After 30 min, they were resuspended in normal medium or in medium containing ouabain for another 30 min, radioactive glucose was added and incubation continued for another 2 h. The stimulatory effect of ouabain was seen in all cells exposed to ouabain, but partial reversibility was evident from the reduced metabolic activity toward glucose of the cells stimulated by ouabain during the first 30 min only.

Although the stimulatory effect of ouabain is evident only in the presence of K^+ (Fig. 4) and is reversible (Fig. 5), implying that the process interfered with by ouabain is a K^+-dependent one, increasing K^+ in the presence of even low concentrations of ouabain did not overcome the ouabain-induced stimulation of glucose metabolism (Table III).

TABLE III

LACK OF EFFECT OF K^+ ON OUABAIN-INDUCED STIMULATION OF D-[$^{14}C_6$]GLUCOSE METABOLISM BY ISOLATED FAT CELLS OF MICE

Glucose concn. was 2.5 mM. Results as in Table II. Each figure is the mean of 6 values ±S.E.

Ouabain (mM)	*Medium K^+ (mM)*	*CO_2*	*Total lipids*
0	6	5.83 ± 0.06	7.50 ± 0.02
0.01	6	6.28 ± 0.16	7.61 ± 0.08
	12	6.24 ± 0.14	7.72 ± 0.14
	24	6.74 ± 0.09	8.28 ± 0.22
	32	6.59 ± 0.08	8.21 ± 0.26
0.1	6	7.44 ± 0.17	9.02 ± 0.22
	12	7.23 ± 0.13	9.14 ± 0.03
	24	7.33 ± 0.12	8.75 ± 0.20
	32	7.26 ± 0.08	8.82 ± 0.18

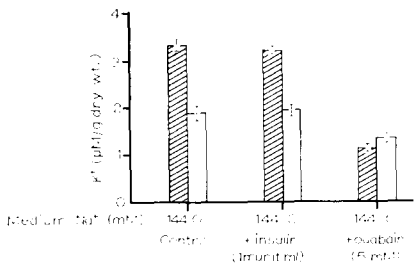

Fig. 9. Intracellular K^+ content of isolated fat cells of mice in the presence (hatched columns) or absence (blank columns) of extracellular Na^+, and in the presence or absence of insulin or ouabain. Na^+ was replaced by choline$^+$; the concn. of K^+ in the medium was 6 mM throughout. Other experimental conditions were as in Fig. 6. Each figure is the mean of 5 values ±S.E.

Because the results described so far, at least in the absence of insulin, suggested the possible existence of a link between glucose transport or metabolism and the electrolyte pump[21], we have also measured the intracellular K^+ content of isolated fat

cells after 1 h of incubation in various media. A clear relationship between the cellular K^+ content and the K^+ concentration in the medium (Fig. 6) was demonstrated. Furthermore, and as expected, ouabain decreased the cellular K^+ content (Fig. 7) over a range of concentrations similar to that effective in stimulating the metabolic activity (Fig. 4). Lowering the extracellular concentration of Na^+ similarly decreased the K^+ content of fat cells (Fig. 9). However, no effect of insulin on the K^+ content of cells incubated under these different experimental conditions was seen (Figs. 8 and 9).

We have succeeded in preparing an ATP-splitting system from fat cell membranes. As reported recently by others[22], we confirm that this enzymatic activity was partially blocked in the presence of ouabain after activation by K^+ and Na^+. An insulin effect could not be detected, whether the intact cells were preincubated with insulin (1 munit/ml) or whether the hormone was added directly to the cell homogenate used for the assay.

DISCUSSION

We have investigated the possible effects of K^+ and ouabain on glucose transport in isolated mouse fat cells. In general, our results are in agreement with other studies of intact adipose tissue[23–25] and isolated cells[6], although they differ from the data reported by RODBELL[26] in isolated rat fat cells, since this author observed a K^+ dependency of the insulin effect. In experiments not reported here, we have shown that this discrepancy cannot be accounted for on the basis of the difference in the species used, and we are forced to assume that the difference may be the result of as yet unrecognized differences in the technique used for the isolation of the fat cells.

The concentration of K^+ in the incubation medium affects the intracellular K^+ content of the cells, in accordance with the known dependence on K^+ of the Na^+ pump activity in various tissues[27] and in fat cells in particular[22]. How then can we link this modified K^+ content to the concomitant alterations of glucose uptake? In other words, are K^+ content of fat cells, activity of the Na^+ pump, and glucose transport somehow interrelated?

When fat cells are stimulated by insulin, neither K^+ depletion nor ouabain, even at concentrations effective in altering K^+ content and ATPase activity, modifies glucose transport and/or metabolism. In this instance at least, we must assume that neither intracellular K^+ nor the state of activity of the Na^+ pump directly influences glucose transport and its subsequent metabolism. Yet, in the absence of the hormone, a definite relationship exists: whenever the activity of the Na^+ pump is decreased, as in the absence of either K^+ or Na^+ (ref. 27) or in the presence of ouabain[20], glucose uptake is markedly increased. A relationship of inverse proportionality, not necessarily linear, is therefore clearly suggested. Since decreased activity of the Na^+ pump would result in changes in both intracellular content of K^+ and the intracellular ratio of Na^+/K^+, glucose uptake could equally well be related to either of these quantities, although again only in the absence of insulin.

Ouabain and low amounts of K^+ also decrease adenyl-cyclase activity of fat cells[7], presumably lowering the 3′,5′-cyclic AMP concentration within the cells. Accordingly, the stimulation of glucose uptake might also be related to the concentration of 3′,5′-cyclic AMP. However, recent reports indicating a lack of correlation between

3′,5′-cyclic AMP levels and glucose transport in fat cells[9] or ghosts[28] suggest that this explanation is an unlikely one.

Again, even these explanations, which of necessity are still hypothetical, would be valid only in the absence of insulin. In its presence, K^+ lack and addition of ouabain did not significantly alter the metabolic indices measured, while, as will be emphasized further on[29], the lack of Na^+ markedly decreased insulin-stimulated metabolism of glucose. It therefore becomes necessary to postulate different mechanisms for glucose transport in fat cells in the presence and in the absence of insulin.

Glucose transport in isolated fat cells, as well as in intact adipose tissue, is now known to exhibit the characteristics of facilitated diffusion[17,30] presumably involving a specific carrier[31]. As in the gut, the active carrier might result from the formation of a complex with monovalent ions[32]. Since both Na^+ and K^+ may, under certain conditions, alter glucose uptake and metabolism, a hypothesis deriving these effects from ionic effects on the glucose carrier system would have to postulate the existence of a ternary complex, involving glucose and both cations, singly and/or together. For example, if the number of binding sites available for glucose on the carrier were directly or inversely related to the presence of one or both cations, the observations made could be explained. Thus, when cells are incubated in the absence of either K^+ or Na^+, the carrier is exposed to only one active cation, and all cationic binding sites are occupied by only one type of cation. This might make more binding sites for the hexose available, or result in a more mobile and hence more effective carrier; in either case the overall result would be increased maximal velocity without significant change in the affinity of the carrier for glucose, as was found experimentally (Fig. 1). Similarly, ouabain might prevent binding of K^+, not of Na^+, to the carrier: the carrier molecule would again be exposed to only one cationic species, Na^+, again allowing for more glucose molecules to be transported into the cells. As in the case of cation-linked glucose transport in the intestinal mucosa, glucose and cations would be liberated inside the cytoplasmic membrane and recycling would occur. The number of available carrier molecules per unit of time would depend on the relative concentrations of monovalent cations at both the inner and outer surfaces of the membrane.

In the presence of insulin, additional carrier units would be made available or else more binding sites for glucose would be uncovered on each carrier molecule. This would perhaps occur through a change in the binding of carrier to Na^+ and/or K^+, since the transport process can no longer be altered by the omission of K^+ or the presence of ouabain, while it does appear to require Na^+ for optimal efficacy, as will be described further in the next paper of this series[29].

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