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

I. LACK OF EFFECT OF MEDIUM Ca^{2+}, Mg^{2+} OR PO_4^{3-}.

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SUMMARY

1. Effects of Ca^{2+}, Mg^{2+} and PO_4^{3-} on glucose metabolism and transport by fat cells have been studied in the presence and in the absence of insulin.

2. The omission of Ca^{2+} and Mg^{2+} singly or together, as well as the omission of PO_4^{3-} does not alter glucose transport by fat cells.

3. In the absence of insulin, reciprocal replacement of Na^+ and K^+ increases glucose transport.

4. In the presence of insulin, the absence of K^+ does not alter glucose transport, while it is decreased in the absence of Na^+.

INTRODUCTION

The coupling between electrolytes and the transport of noncharged molecules through biological membranes is well established in many tissues[1,2], and the general biological mechanism underlying it has been recently discussed by CRANE[3]. It has been studied most frequently in insulin-insensitive tissues performing uphill transport of amino acids and sugars[5]. However, the same coupling has been observed in diaphragm muscle for active transport of amino acids[6], whereas the reports on sugar transport in this tissue have been conflicting. While KIPNIS[6] did not find an effect of electrolytes on the transport of 2-deoxyglucose in diaphragm muscle, CLAUSEN[7,8] and CRONE[9] have reported that glucose uptake and its subsequent metabolism were affected by the ionic composition of the incubation medium.

Published studies dealing with the effects of electrolytes on glucose metabolism in intact adipose tissue and isolated fat cells are few and, again, conflicting. HAGEN *et al.*[10] have shown that the absence of either K^+ or Na^+ increases the net CO_2 production by fat tissue incubated in the presence of glucose, while omission of Na^+ depressed insulin-stimulated metabolism. On the other hand, BAKER AND RUTTER[11] reported findings suggesting that Cl^- was necessary for the maintenance of insulin responsiveness of adipose tissue, while cations were without effect. More recently, RODBELL[12]

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reported that isolated fat cells from rats exhibited decreased basal glucose metabolism in the absence of Na^+, while maximal stimulation by insulin required the presence of K^+, not of Na^+. RODBELL concluded that K^+ may be involved in the insulin-stimulated transport process for glucose, while Na^+ seemed to be necessary for normal glucose metabolism.

In 1960 ZAHND *et al.*[13] reported that replacement of K^+ by Na^+ alone, or by the combination of Na^+ and Ca^{2+} or Mg^{2+}, increased the baseline activity of glucose metabolism by adipose tissue, while somewhat decreasing insulin-stimulated metabolism. The principal result reported by these authors thus was a decreased insulin effect in the absence of K^+. They also reported a greatly diminished rate of glucose metabolism, with or without insulin, when bicarbonate in the medium was replaced by phosphate, an observation which has since been repeatedly confirmed and related to the requirement of acetyl-CoA carboxylase for CO_2 in this tissue specialized for lipogenesis[14].

Although the detailed mechanism of insulin action on glucose transport remains unclear, it is of interest that in at least five types of tissue preparations, namely muscle[15,16], toad bladder[17], skin[18] and colon[19], and finally intact adipose tissue[20] or isolated fat cells[21], the hormone has been shown to modify the electrical activity of the membrane and most probably the intracellular K^+ and Na^+ content[22–27], even in the total absence of glucose[15]. These observations clearly suggest that insulin may exert a primary and direct effect on the properties of the plasma membrane toward electrolytes[28,29] and they have prompted us to reinvestigate in some detail the nature of ionic effects on glucose transport and metabolism by the highly insulin-sensitive adipose tissue cell. In so doing, we have utilized the isolated fat cell preparation introduced by RODBELL[30], because of the advantages offered by a cell preparation in which the plasma membrane is widely exposed to the bathing medium, yet retains a high degree of sensitivity to insulin. Some of the findings to be reported have been published previously in preliminary form[31,32].

MATERIALS AND METHODS

Fed Swiss albino mice (Ivanovas, Germany) 5–7 weeks old, were used throughout. Unless otherwise stated, reagents, enzymes, insulin and incubation techniques were as described by HO AND JEANRENAUD[33]. $^{14}CO_2$ was collected according to the method of GLIEMAN[34]. The basal buffer solution, called the normal buffer, was a Krebs–Ringer bicarbonate buffer containing 3.5 % dialyzed human serum albumin (pH 7.4) equilibrated with O_2–CO_2 (95:5, by vol.), and was prepared as described by YOUNG[35]. It contained in mM: $Na^+ = 143.3$; $K^+ = 5.9$; $Ca^{2+} = 1.2$; $Cl^- = 126$; $PO_4^{3-} = 1.2$; $HCO^-_3 = 24.6$; $SO_4^{2-} = 1.2$. When a component of the medium was omitted, it was replaced by an equimolar amount of Na^+ or Cl^-.

The metabolic indices measured, namely incorporation of glucose carbon into CO_2 and total lipids, were used as the best approach to the indirect estimation of glucose uptake[36]. Together they account for 90 % or more of the total glucose uptake by adipose tissue[37,38], glucose metabolism to glycogen and lactate being almost negligible in this preparation. Since CROFFORD AND RENOLD[39] have established that in this tissue and under the conditions utilized here, glucose is transported by a mobile carrier across the fat cell wall and that this step is rate limiting for the overall glucose meta-

bolism of the fat cell, it seemed reasonable to accept the assumption that the sum of glucose carbon incorporated into CO_2 and total lipids does represent the best available approximation of glucose uptake. The disadvantage entailed by the fact that glucose uptake and fat cell water are not readily measurable in the isolated fat cell preparation were outweighed, in the opinion of the authors, by the evident advantage of dealing with cells floating free, rapidly and completely surrounded by the exact medium under scrutiny. The limitations implied by the underlying assumptions are nevertheless understood and pointed out.

RESULTS

The complete replacement of K^+, Ca^{2+} and Mg^{2+}, singly or combined, by an equimolar amount of Na^+ was studied both in the presence and in the absence of insulin (Table I). In order to free the cells from as much Ca^{2+} or Mg^{2+} as possible, they were first washed twice in 10 ml Ca^{2+}–Mg^{2+}-free medium containing 10μmoles EDTA, then 3 times in 10 ml Ca^{2+}–Mg^{2+}-free medium without the chelating agent to avoid the possible metabolic effect of the latter[40–42]. In the absence as well as in the presence of insulin, the absence of neither Ca^{2+} nor Mg^{2+} had any consistent effect on the metabolic indices measured, even though both ions have been shown to modify the hormone-induced lipolysis[43,44] in adipose tissue. However, a lack of K^+ reproducibly resulted in a stimulatory effect in the absence of insulin, an effect which was even more pronounced when K^+ depletion was combined with the omission of Ca^{2+} and/or Mg^{2+} from the buffer. In the presence of insulin (1 munit/ml), however, the complete absence of K^+, either alone or combined with lack of Ca^{2+} and/or Mg^{2+}, exerted no consistent effect on the metabolic indices measured.

TABLE I

EFFECTS OF CHANGES IN THE COMPOSITION OF KREBS–RINGER BICARBONATE BUFFER ON THE METABOLISM OF [$^{14}C_6$]GLUCOSE BY ISOLATED FAT CELLS

Incubations were carried out in 2.0 ml bicarbonate buffer containing 3.5 g/100 ml human albumin and 2.5 mM glucose. Insulin concentration, when present, was 1 munit per ml. The cations omitted were replaced by an equivalent amount of Na^+. Results are expressed as μatom glucose C metabolized per g lipids per 2 h. Each figure is the mean of 6 values ± S.E.

K^+	Ca^{2+}	Mg^{2+}	*Control*			*Insulin (1 munit/ml)*		
			CO_2	*Glyceride fatty acids*	*Glyceride glycerol*	CO_2	*Glyceride fatty acids*	*Glyceride glycerol*
+	+	+	1.73 ± 0.03	0.07 ± 0.01	4.76 ± 0.01	12.6 ± 0.48	2.79 ± 0.11	27.0 ± 0.60
−	+	+	2.41 ± 0.05	0.09 ± 0.01	6.96 ± 0.38	12.2 ± 0.36	2.50 ± 0.05	26.2 ± 0.35
+	+	−	1.90 ± 0.04	0.07 ± 0.09	5.61 ± 0.02	12.5 ± 0.36	2.70 ± 0.18	26.2 ± 0.50
+	−	+	1.60 ± 0.04	0.06 ± 0.01	4.77 ± 0.08	11.4 ± 0.40	2.63 ± 0.03	25.2 ± 0.10
−	−	−	2.98 ± 0.06	0.14 ± 0.01	8.67 ± 0.12	12.8 ± 0.38	3.24 ± 0.30	26.0 ± 0.35
+	+	+	2.49 ± 0.07	0.32 ± 0.01	5.38 ± 0.22	16.7 ± 0.34	5.94 ± 0.11	25.9 ± 0.70
−	−	+	3.45 ± 0.06	0.40 ± 0.02	7.80 ± 0.06	19.0 ± 0.37	8.64 ± 0.14	28.2 ± 0.80
+	−	−	2.49 ± 0.10	0.24 ± 0.01	5.87 ± 0.17	18.9 ± 0.24	8.02 ± 0.14	28.3 ± 0.30
−	+	−	4.22 ± 0.08	0.56 ± 0.02	7.86 ± 0.10	19.3 ± 0.64	7.94 ± 0.38	28.9 ± 0.05
−	−	−	4.26 ± 0.13	0.60 ± 0.06	10.5 ± 0.50	19.2 ± 0.86	8.30 ± 0.61	27.4 ± 0.52

The possible role of the Ca^{2+} to Mg^{2+} ratio was explored by incubating the isolated fat cells in various relative concentrations of these cations. Osmolarity was kept constant by varying the concentration of Na^+ (Tables II and III). In these experiments also, the cells were first washed with 10 ml Ca^{2+}–Mg^{2+}-free medium containing 10 μmoles EDTA, and without either Ca^{2+} (Table II) or Mg^{2+} (Table III). At 3 different glucose concentrations no consistent effect of Ca^{2+} could be elicited, either in the presence or in the absence of insulin (1 munit/ml), on CO_2 production or on the incorporation of glucose carbon into glyceride fatty acid and glyceride glycerol. Similarly, varying the Mg^{2+} concentration of the buffer was without effect on the metabolic indices studied, either in the absence or in the presence of insulin.

The only anion studied was PO_4^{3-}, which, as shown in Table IV, was without effect on the three metabolic indices measured at the concentrations studied which, however, are lower than those reported by MOSINGER AND VAUGHAN[44] to inhibit epinephrine-stimulated lipolysis.

Finally, a single experiment involving the complete reciprocal substitution of

TABLE II

EFFECTS OF INCREASING CONCENTRATIONS OF Ca^{2+} ON THE METABOLISM OF D-[$^{14}C_6$]GLUCOSE BY ISOLATED FAT CELLS

The osmolarity was kept constant by varying the Na^+ concn. Experimental conditions and expression of results as in Table I. Each figure is the mean of 6 values ± S.E.

Medium Ca^{2+} (mM)	*Control*			*Insulin (1 munit/ml)*		
	CO_2	*Glyceride fatty acids*	*Glyceride glycerol*	*CO_2*	*Glyceride fatty acids*	*Glyceride glycerol*
0	1.62 ± 0.05	0.25 ± 0.03	4.32 ± 0.38	22.8 ± 0.39	16.2 ± 0.10	26.8 ± 1.30
1.27	1.54 ± 0.07			22.8 ± 0.98		
2.54	1.80 ± 0.07	0.21 ± 0.02	4.55 ± 0.37	22.1 ± 0.75	12.2 ± 1.60	30.3 ± 1.81
5.08	1.65 ± 0.07			21.3 ± 0.54		
10.20	1.64 ± 0.05	0.21 ± 0.01	4.52 ± 0.45	20.7 ± 0.11	14.2 ± 0.50	24.7 ± 0.78

TABLE III

EFFECTS OF INCREASING CONCENTRATIONS OF Mg^{2+} ON THE METABOLISM OF D-[$^{14}C_6$]GLUCOSE BY ISOLATED FAT CELLS

Experimental conditions and expression of results as in Table I. Each figure is the mean of 6 values ± S.E.

Medium Mg^{2+} (mM)	*Control*			*Insulin (1 munit/ml)*		
	CO_2	*Glyceride fatty acids*	*Glyceride glycerol*	*CO_2*	*Glyceride fatty acids*	*Glyceride glycerol*
0	1.55 ± 0.03	0.113 ± 0.02	4.78 ± 0.10	19.1 ± 0.66	7.20 ± 0.66	32.1 ± 0.20
0.6	1.45 ± 0.04			18.9 ± 0.54		
1.2	1.38 ± 0.04	0.154 ± 0.01	5.17 ± 0.03	19.1 ± 0.61	7.50 ± 0.30	32.7 ± 0.10
2.4	1.44 ± 0.04			18.1 ± 0.43		
4.8	1.31 ± 0.03	0.132 ± 0.02	4.47 ± 0.08	18.1 ± 0.36	6.90 ± 0.10	30.3 ± 0.31

TABLE IV

EFFECTS OF INCREASING CONCENTRATIONS OF PO_4^{3-} ON THE METABOLISM OF D-[$^{14}C_6$]GLUCOSE BY ISOLATED FAT CELLS

Experimental conditions as in Table I except that the total anionic concentration of the medium was kept constant by varying the concentration of Cl^-. Expression of results as in Table I. Each figure is the mean of 6 values ± S.E.

Medium PO_4^{3-} (mM)	*Control*			*Insulin (500 μunits/ml)*		
	CO_2	*Glyceride fatty acids*	*Glyceride glycerol*	*CO_2*	*Glyceride fatty acids*	*Glyceride glycerol*
0	1.82 ± 0.02	0.29 ± 0.01	3.79 ± 0.02	25.6 ± 0.58	15.5 ± 0.12	27.8 ± 0.85
0.6	1.74 ± 0.07			26.4 ± 0.44		
1.19	1.84 ± 0.03	0.28 ± 0.01	3.86 ± 0.06	25.2 ± 0.64	16.9 ± 0.60	27.0 ± 0.55
2.38	1.74 ± 0.04			23.8 ± 0.50		
4.76	1.64 ± 0.08	0.28 ± 0.03	3.64 ± 0.06	24.0 ± 0.56	15.7 ± 0.70	25.7 ± 0.35

TABLE V

EFFECTS OF RECIPROCAL SUBSTITUTION OF K^+ AND Na^+ ON THE METABOLISM OF D-[$^{14}C_6$] GLUCOSE BY ISOLATED FAT CELLS

All components of the buffer, except for Na^+ and K^+, were as described under MATERIALS AND METHODS. Other experimental conditions and results expressed as in Table I. Each figure is the mean of 6 values ± S.E.

Medium		*Control*			*Insulin (1 munit/ml)*		
K^+ (mM)	*Na^+ (mM)*	*CO_2*	*Glyceride fatty acids*	*Glyceride glycerol*	*CO_2*	*Glyceride fatty acids*	*Glyceride glycerol*
6	144	1.57 ± 0.04	0.38 ± 0.04	3.02 ± 0.08	11.8 ± 0.10	10.02 ± 0.35	8.40 ± 0.40
—	150	4.19 ± 0.09	1.64 ± 0.05	5.13 ± 0.10	12.8 ± 0.08	12.04 ± 0.21	8.61 ± 0.26
150	—	3.69 ± 0.12	1.28 ± 0.04	5.84 ± 0.10	8.10 ± 0.20	6.25 ± 0.40	6.99 ± 0.20

Na^+ and K^+ is shown in Table V. As previously noted (Table I), when K^+ is replaced by Na^+, in the absence of insulin, the three metabolic indices measured were increased. The complete replacement of Na^+ by K^+ brought about the same metabolic response, although to a somewhat lower degree. However, a marked difference in the response to insulin was exhibited by the cells incubated in the presence of Na^+ alone or K^+ alone. In the first instance, there was only a slight effect on overall glucose metabolism when compared with control cells. These results agree well with the results shown in Table I. The complete replacement of Na^+ by K^+, on the other hand, produced a marked and highly significant diminution of all the metabolic indices measured, to values which are 69 % (CO_2), 62 % (glyceride fatty acid) and 83 % (glyceride glycerol) of the control values.

DISCUSSION

In isolated fat cells of mice aged 6–7 weeks, neither Ca^{2+} nor Mg^{2+} nor PO_4^{3-} had any consistent effect on the metabolic indices measured, namely glucose carbon incor-

poration into CO_2, glyceride fatty acid and glyceride glycerol. The authors are aware of the possibility that a sufficient amount of the divalent cations may well have been so tightly bound to structural components of the membrane that they could not be removed even by a chelating agent such as EDTA, with the Mg^{2+} of Ca^{2+} consequently remaining at sites where their presence may be essential. Nevertheless, it is reasonable to assume that divalent cations in the medium, even if they were required for the transport of glucose and related membrane functions, are at best required in minimal amounts. Furthermore, since increasing either Mg^{2+} or Ca^{2+} concentrations, singly or together, to 4 times their normal concentrations in the buffer did not alter the metabolism of isolated fat cells, it is equally reasonable to conclude that at high concentrations these extracellular cations do not inhibit or stimulate glucose transport or metabolism, at least as far as the metabolic products which were measured. The same reasoning applies to PO_4^{3-}, which most probably are firmly bound to proteins and lipids and cannot readily be washed out of the cells. Even if we are convinced that PO_4^{3-} and, for that matter, Ca^{2+} and Mg^{2+}, are essential components of the intracellular environment necessary for survival and function[43,44], it is a fact that we could not show any alteration of metabolism induced by changes in their extracellular concentration.

Of the various cations studied, only K^+ and Na^+ seem to affect the metabolism of glucose and/or its transport in the isolated fat cells. They will be studied separately and in considerable detail in the two subsequent papers.

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