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TERMINATION OF INSULIN-INDUCED HEXOSE TRANSPORT IN ADIPOCYTES

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Summary

The hexose transport of insulin-pretreated (80 pM) adipocytes remained elevated for at least 45 min when the cells were depleated of ATP by treatment with dinitrophenol. On the other hand, the half-time of deactivation of hexose transport in insulin-pretreated cells was of the same magnitude as that of dissociation of receptor-bound insulin both in the absence and presence of glucose (about 8 min). Thus, a high ATP-level, but not ongoing glucose metabolism, appears to be important for termination of the insulin effect shortly after dissociation of insulin from its receptor.

Introduction

Ciaraldi and Olefsky [1] recently published data indicating a marked difference in the time course of dissociation of receptor-bound insulin (concentration in preincubation step 0.5—1 ng/ml, i.e., 80—160 pM), and deactivation of hexose transport in rat adipocytes. In the presence of glucose, $t_{1/2}$ for deactivation of transport of the non-metabolizable sugar analogue, 3-*O*-methylglucose, was 3-times that of receptor dissociation. This appeared to disagree with previous studies by Crofford [2] on deactivation of insulin-induced glucose oxidation and by Gliemann et al. [3] on deactivation of lipogenesis from glucose. Furthermore, hexose transport remained activated for 2 h in the absence of glucose and Ciaraldi and Olefsky [1] concluded that deactivation was dependent upon some function of glucose metabolism. Kono et al. [4] and Siegel and Olefsky [5] have observed that cells depleted of ATP were unresponsive to insulin. We have recently observed that some properties of the hexose transport

Abbreviation: Hepes, *N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid.

system are modified when glucose is metabolized at high rates [6]. In the light of these results we have investigated the possible effects of ATP depletion and glucose metabolism on the deactivation of transport of glucose and methylglucose.

Materials and Methods

Highly purified pig insulin was obtained from Nordic Insulin, Gentofte, Denmark. A14 [^{125}I]monoidoinsulin with a specific activity of 1.9 Ci/μmol was prepared by Dr. S. Linde, Hagedorn Research Laboratory, Gentofte, as described [7]. 3-*O*-[*methyl*-^{14}C]Glucose, [U-^{14}C]glucose and [3-^{3}H]glucose were supplied by The Radiochemical Centre, Amersham. Crude collagenase (type 1) and bovine serum albumin were from Sigma, St. Louis. Phloretin was from K and K Laboratories, Plain View, NY. 2,4 dinitrophenol was from B.D.H. Laboratory Chemicals Division, U.K.

Isolated fat cells were prepared from epididymal fat pads of 120—170 g ad lib fed male Wistar rats. [3-^{3}H]Glucose (0.3 μCi/ml, spec. act. 0.5 Ci/mol) was present in the cell preparation step. The triglycerides were, thereby, prelabelled and the ^{3}H-radioactivity used as a measure of sample size.

The rate of synthesis of ^{14}C-labeled lipids from tracer [U-^{14}C]glucose (0.05 μCi/ml, 250 Ci/mol) and the initial velocity of methylglucose (2.4 μCi/ml, 60 Ci/mol) influx were measured at 37°C, as described previously [3,6,8]. (For details, see legend to Fig. 1.)

Results

Fig. 1A shows that the rate of lipogenesis from glucose in cells preincubated with 80 pM insulin (i.e., the slope of the upper curve) decreased as a function of time and became indistinguishable from that of basal cells after 15—20 min. This result with 0.2 μM [U-^{14}C]glucose is basically in agreement with our previous results using 0.55 mM glucose [3], and those of Crofford [2] using 1 mM glucose. We have previously found that the rate-limiting step for conversion of tracer [U-^{14}C]glucose to lipids is glucose transport [9], and the findings therefore argue against the hypothesis [1] that glucose metabolism is important for deactivation of the transport system. The point is demonstrated more directly in Fig. 1B, which shows the initial velocity of 3-*O*-methylglucose influx in the absence of glucose (ordinate) as a function of time after removal of the 80 pM insulin. The transport rate declined rapidly and was indistinguishable from that of basal cells by 40 min. The inset shows the dissociation of 80 pM A14 [^{125}I]-monoiodoinsulin [7] from similar cells and it appears that the $t_{1/2}$ for deactivation of hexose transport was of the same magnitude as that for dissociation of receptor-bound insulin. Fig. 1C shows that 1 mM glucose had no demonstrable effect on the time course of deactivation of methylglucose transport. On the other hand, it is also seen that cells incubated with 0.5 mM dinitrophenol for 15 min, after association with 80 pM insulin for 45 min, followed by removal of the insulin showed no significant deactivation of hexose transport for at least 40 min. This treatment with dinitrophenol depletes the cells for ATP [4]. It appears therefore that energy, but not metabolism of extracellular glucose, is necessary for deactivation of transport.

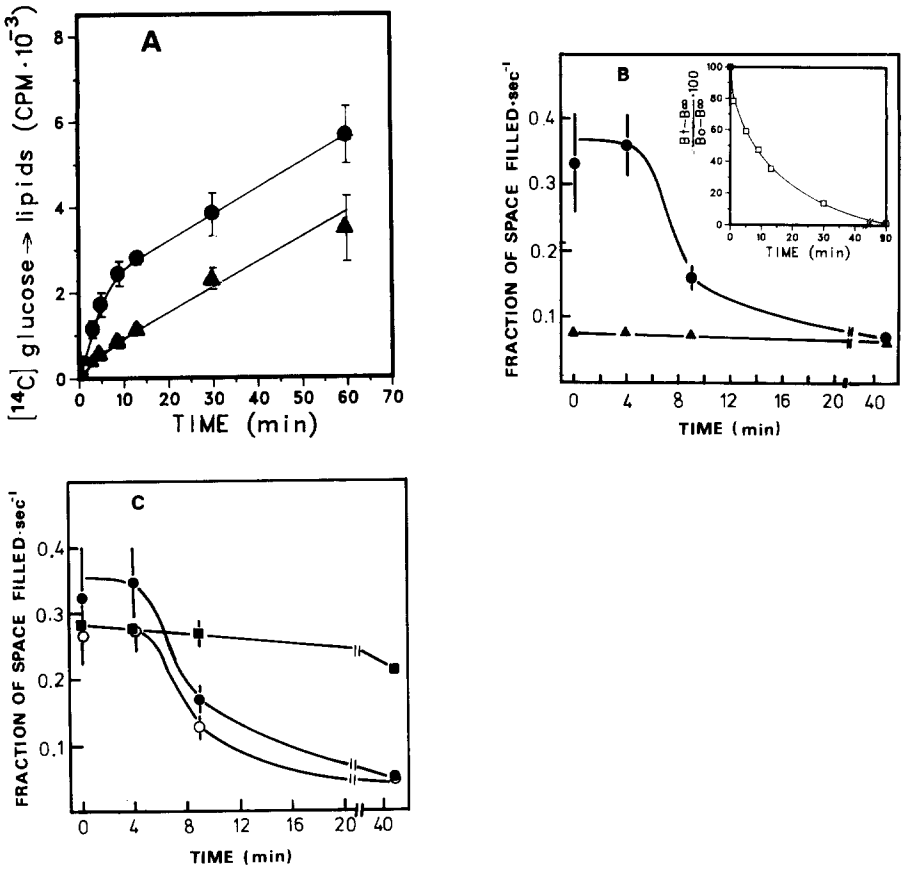

Fig. 1. Isolated fat cells (8% v/v, i.e., about $8 \cdot 10^5$ cells/ml) were incubated at 37°C in Krebs-Ringer Hepes buffer (pH 7.4) containing 5% bovine serum albumin and 0.35 mM bacitracin, in order to block protease-mediated degradation of insulin [6]. By 45 min (60 min in dinitrophenol studies), the cells were allowed to float and infranatant medium was removed to give a packed cell volume of 50%. A. Lipogenesis from glucose. At time zero the cells were diluted to a packed cell volume of 0.8% (i.e., dilution factor for extracellular-medium was 67) at 37°C, in buffer containing 0.05 μCi [U-^{14}C]glucose/ml and the accumulated ^{14}C-radioactivity was measured as a function of time. The results are from cells preincubated in the absence (▲——▲) or in the presence (●——●) of 80 pM insulin. Mean of four experiments ±1 S.D. B. Initial velocity of methylglucose influx. The same cell preparations were used for measuring transport both immediately before dilution (time zero) and as a function of time after dilution (in buffer not containing [U-^{14}C]glucose) and reconcentration to about 30% (v/v). Transport was measured using incubations for 1—4 s and the ordinate shows the fraction of the equilibrium distribution for methylglucose space filled/s. The points are mean values of four replicates from a representative experiment ±1 S.D., for basal (▲——▲) or insulin-pretreated cells (●——●). The inset shows the dissociation of 80 pM A14-[^{125}I-monoiodoinsulin as determined in four parallel experiments. The ordinate indicates the amount bound at time t as a percentage of that at time zero. The amount bound at 120 min (B_∞) was about 10% and has been subtracted from all points. C. Effect of dinitrophenol and lack of effect of glucose on the initial velocity of methylglucose influx. The results are mean values from a representative experiment (±1 S.D., $n = 4$) with insulin-pretreated cells incubated in buffer without additions (●——●), in the presence of 1 mM glucose (○——○) or in the presence of 0.5 mM dinitrophenol from time 45—60 min in the preincubation step (■——■).

Discussion

The present results of experiments in the absence of glucose are markedly different from those of Ciaraldi and Olefsky [1], who found that insulin-

induced 3-*O*-methylglucose transport remained constant for 2 h. In the presence of 1 mM glucose Ciaraldi and Olefsky found a 3-times larger $t_{1/2}$ for transport deactivation (i.e., 43 min) than for insulin dissociation from receptors, whereas $t_{1/2}$ for the two processes was similar in the present experiments both in the absence and presence of glucose. In other words, the possible lag time between dissociation of the insulin molecule and termination of its effect on the hexose transport system does not exceed a few minutes and is independent of extracellular glucose. This conclusion is also supported by the results of Vega and Kono [10] who found a $t_{1/2}$ of deactivation of insulin-induced methylglucose transport of less than 15 min in the presence of 1 mM glucose. However, the conclusion is valid only when cells are preexposed to low concentrations of insulin. With increasing concentrations there is an increasing discrepancy between the $t_{1/2}$ of insulin dissociation and that of deactivation [9,3]. This phenomenon is a consequence of the presence of 'spare receptors', as previously discussed in some detail [3].

Ciaraldi and Olefsky [1] reported a $t_{1/2}$ for dissociation of 160 pM [^{125}I]-insulin at 37°C of 14 min, in agreement with the $t_{1/2}$ reported by Crofford [2] for deactivation of glucose oxidation, when the insulin concentration is abruptly changed from 125 pM to 25 pM. These authors [1] claimed that one-half of the transition between the two steady states of insulin binding would occur within 7 min. This is surprising since 25 pM is much below the dissociation constant for insulin binding (3 nM) even when some receptor heterogeneity and negative homotropic cooperativity is taken into account [9,11]. Thus, $t_{1/2}$ for dissociation would not be changed appreciably by the presence of 25 pM insulin in the efflux medium and there is no reason to imply, on the basis of Crofford's data [2], that there is a lag between dissociation and deactivation of glucose oxidation.

The present results with dinitrophenol suggest that energy (ATP) is required for deactivation of hexose transport following removal of insulin. The energy requirement for activation has previously been demonstrated by Kono et al. [4] and Siegel and Olefsky [5]. The combined results are consistent with the recent studies by Cushman and Wardzala [12] and Suzuki and Kono [13], showing a transfer of transport sites from an intracellular pool to the cell membrane when cells are stimulated by insulin and with those of Karnieli et al. [14] showing a reversal of this process when insulin is removed.

Our results indicate that the receptor occupancy at any moment, and independent of ongoing glucose metabolism, is the major determinant for the activation state of the transport system of normal cells. The difference between our results and those of Ciaraldi and Olefsky [1] may reflect a difference in the ability of the cells to maintain energy levels, especially in the absence of glucose.

Note added in proof (Received January 8th, 1981)

It was recently reported, in agreement with the present results, that the hexose transport system of insulin treated adipocytes remained activated for at least 1 h when cells were depleated of ATP [15].

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