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Effect of insulin on glucose transporter distribution in white fat cells from hypophysectomized rats

Susanne Keller and Juergen Zapf

Metabolic Unit, Department of Medicine, University Hospital Zurich, CH-8091 Zurich, Switzerland

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Glucose transport in white fat cells from hypophysectomized rats is increased and unresponsive to insulin. The goal of this study was to explain this observation. The number of glucose transporters, as determined by D-glucose-inhibitible cytochalasin B binding, in the plasma membranes from fat cells of hypophysectomized rats is: (1) elevated, (2) not increased by insulin, and (3) the same as in plasma membranes from insulin-stimulated fat cells of control rats. In microsomal membranes from fat cells of hypophysectomized rats the number of glucose transporters is: (1) smaller than in basal and insulin-stimulated fat cells from control rats, and (2) not changed by insulin.

Insulin; Hypophysectomy; Fat cell; Glucose transporter; Cytochalasin B

1. INTRODUCTION

Insulin enhances glucose uptake in white fat cells from normal rats at least in part through translocation of glucose transporters from an intracellular pool to the plasma membrane. Thus, the number of transporters associated with plasma membranes increases, while the number of transporters associated with intracellular organelles decreases [1–3].

In fat cells from hypophysectomized rats basal glucose transport as measured by 3-*O*-methylglucose efflux is already maximal and not further stimulated by insulin [4]. It equals insulin-stimulated 3-*O*-methylglucose efflux from fat cells of normal rats.

These observations raised the question whether the distribution of glucose transporters between the plasma membrane and the intracellular pool is changed in white fat cells of hypophysectomized rats and whether it is affected by insulin.

2. MATERIALS AND METHODS

2.1. *Animals*

Hypophysectomized male Tif-RAI rats and their normal littermates weighing between 135–195 g and 220–265 g, respectively, were generously supplied by Dr K. Mueller and R. Cortesi (Ciba Geigy AG, Basel, Switzerland). Hypophysectomy was carried out 28 days before the animals were killed for membrane preparation. Only those animals whose body weight did not increase more then 5 g/week were regarded as successfully hypophysectomized. Body weight of normal rats increased about 50 g/week. The animals were fed ad libitum with Kliba chow (Klingenthalmuehle AG, Basel, Switzerland) consisting of 69% cereals plus ingredients rich in crude fiber, 21% protein and 6% fat.

Correspondence address: S. Keller, Dartmouth Medical School, Dept. of Biochemistry, Hanover, NH 03756, USA

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2.2. *Preparation of plasma and microsomal membranes*

Forty to 60 rats were killed by decapitation. Epididymal fat pads were removed and fat cells were isolated according to the method of Rodbell [5]. They were treated for 60 min at 37°C with collagenase (Worthington; Seromed, Berlin, FRG), 1 mg/ml, in Krebs-Ringer-Hepes-buffer (131 mM NaCl, 4.8 mM KCl, 1.3 mM $CaCl_2$, 1.2 mM NaH_2PO_4, 1.2 mM $MgSO_4$, 25 mM Hepes) supplemented with 1 g/100 ml human serum albumin (Swiss Red Cross, Bern, Switzerland). The isolated fat cells were divided into two pools: one was incubated in the absence, the other in the presence of 250 μU/ml whale insulin (identical amino acid sequence to pork insulin; provided by Dr R.E. Humbel). Incubation lasted for 30 min at 37°C. Fat cells were washed twice in homogenizing buffer (10 mM Tris-HCl, 1 mM EDTA, 250 mM sucrose, pH 7.4). Plasma and microsomal membranes were prepared according to the method of McKeel and Jarett [6]. The fractions were suspended in 20 mM Tris-HCl, 1 mM EDTA, 250 mM sucrose buffer, pH 7.4, and stored in liquid nitrogen until used.

The purity of the plasma and microsomal membranes was estimated by measuring 5′ nucleotidase activity [7] and NADH-cytochrome *c* reductase activity [8]. Protein was determined by the Coomassie brilliant blue method (Protein Assay Kit, Bio Rad Laboratories, Muenchen, FRG) with human serum albumin as a standard.

2.3. *Cytochalasin B binding assay*

The number of glucose transporters in plasma and microsomal membranes was determined by measuring the D-glucose-inhibitable cytochalasin B binding to the membranes as described by Cushman and Wardzala [1] and modified by Greco et al. [9]. Membranes were suspended in 20 mM Tris-HCl, 1 mM EDTA, pH 7.4, containing either 375 mM sucrose for determination of total cytochalasin B binding or 125 mM sucrose and 500 mM D-glucose for determination of nonspecific binding. Cytochalasin E (Sigma, St. Louis, USA) was added to reduce nonspecific binding. To 100 μl aliquots of the suspen-

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Table 1

Characterization of plasma and microsomal membranes from fat cells of normal and hypophysectomized rats

		Protein (mg/2 fat pads)	5′ nucleotidase activity (nmol adenosine/ mg protein/min)	NADH-cytochrome *c* reductase activity (μmol/ mg protein/min)
Homogenate	n	6.10 ± 0.96*	2.89 ± 0.51	0.51 ± 0.33
	h	3.40 ± 0.34*	2.62 ± 0.69	0.35 ± 0.04
Plasma membranes	n	0.18 ± 0.03	9.81 ± 3.15	0.84 ± 0.46
	h	0.12 ± 0.03	9.77 ± 0.85	0.35 ± 0.04
Microsomal membranes	n	0.22 ± 0.07**	3.59 ± 0.81	2.22 ± 1.18
	h	0.13 ± 0.03**	3.05 ± 0.15	1.93 ± 0.31

Fat cells from 40–60 rats were isolated, divided into two pools and incubated in the absence or presence of insulin; each pool was subjected to fractionation as described in section 2. Fractionation was carried out twice for each group (normal (n) and hypophysectomized (h) rats). Since values for fractions from fat cells preincubated with or without insulin showed no differences, they were taken together. The values are mean values ± SD for the 4 preparations of fractions from the normal and hypophysectomized rats. Values significantly different between the two groups (as evaluated by Student's *t*-test) are marked with $*P = 0.006$, $**P = 0.05$

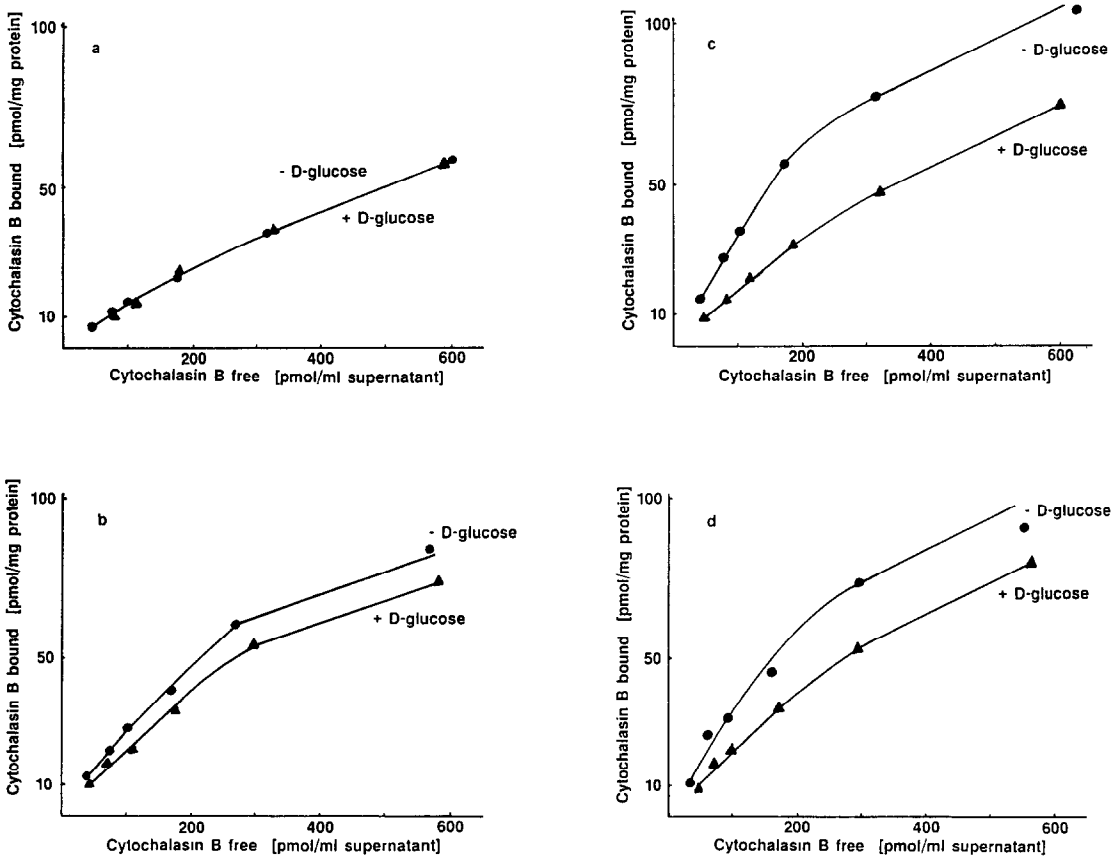

Fig.1. [^{3}H]Cytochalasin B binding to plasma and microsomal membranes of fat cells from normal rats. [^{3}H]Cytochalasin B binding to plasma membranes (a and b) and microsomal membranes (c and d) from fat cells preincubated in the absence (a and c) or presence (b and d) of insulin was measured at different cytochalasin B concentrations in the absence (●) and presence (▲) of 500 mM D-glucose and the values for bound cytochalasin B were plotted against those for free cytochalasin B [9]. Each point is the average value of duplicate determinations in a representative experiment. Ranges were within the size of the symbols and are therefore not shown.

sions unlabeled cytochalasin B (Sigma, St. Louis, USA) and tritiated cytochalasin B (Amersham International, Amersham, UK or NEN, Boston, USA) were added. Final concentrations were: cytochalasin E, 2000 nM; unlabeled cytochalasin B, 0–1280 nM; and [^{3}H]cytochalasin B, 50 or 60 nM. [^{14}C]Urea (Amersham International, Amersham, UK) was used for determination of trapped unbound [^{3}H]cytochalasin B. Incubation was carried out for 15 min at 4°C. Duplicate 50 μl aliquots of each sample were removed and protein (35–50 μg/aliquot) was precipitated with 10 μl of 0.75 M barium hydroxide and 10 μl of 0.75 M zinc sulfate and pelleted by subsequent centrifugation (Beckman microfuge, 10000 × g, 10 min at 4°C). 50 μl of the supernatant was removed for liquid scintillation counting in 10 ml Instagel (Packard, Downers Grove, USA). The lower end of the plastic tube containing the pellet was cut and 50 μl of 0.2 M sodium hydroxide was added to dissolve the pellet. The total pellet was mixed with 10 ml of Instagel. Both the pellet and supernatant were counted for 10 min in a Beckman Beta counter to determine bound and free cytochalasin B, respectively.

Results are presented as plots of bound versus free cytochalasin B [9]. The number of glucose transporters was obtained in the following way: the best line was drawn through the data points for cytochalasin B binding in the presence of D-glucose (using a curve-fitting program on a Macintosh computer), so that nonspecifically bound cytochalasin B could be calculated at any free cytochalasin B concentration. Specifically bound cytochalasin B (= D-glucose-inhibitable cytochalasin B binding) was calculated by subtraction of the calculated bound values of cytochalasin B in the presence of D-glucose from the experimental values for bound cytochalasin B in the absence of D-glucose at the respective same free cytochalasin B concentration. The reciprocal values of specifically bound cytochalasin B were plotted versus the reciprocal values of free cytochalasin B. The linear plot so obtained was subjected to simple regression analysis. The intersection of the regression line with the y-axis yielded the total number of transporter-specific D-glucose-inhibitable cytochalasin B binding sites.

3. RESULTS AND DISCUSSION

Plasma and microsomal membranes fractionated from homogenate of isolated fat cells [6] were characterized as shown in table 1. The total amount of protein in homogenate of fat cells from hypophysectomized rats was decreased by 43% compared to that from normal rats. The yields of plasma and microsomal membrane protein were reduced to about the same extent. 5′ nucleotidase activity was enriched 3.5-fold in the plasma membrane fraction, and NADH-cytochrome c reductase activity was enriched 4.5–5.5-fold in the microsomal membrane fraction. The enrichment in these marker enzyme activities was the same for the fractions from normal and hypophysectomized rats (table 1) and for the fractions prepared from basal and insulin-stimulated cells (results not shown). The crosscontamination of 5′ nucleotidase activity was the same for both groups. However, crosscontamination of NADH-cytochrome c reductase activity in plasma membranes from hypophysectomized rats was half that from normal rats. The basis for this difference has not

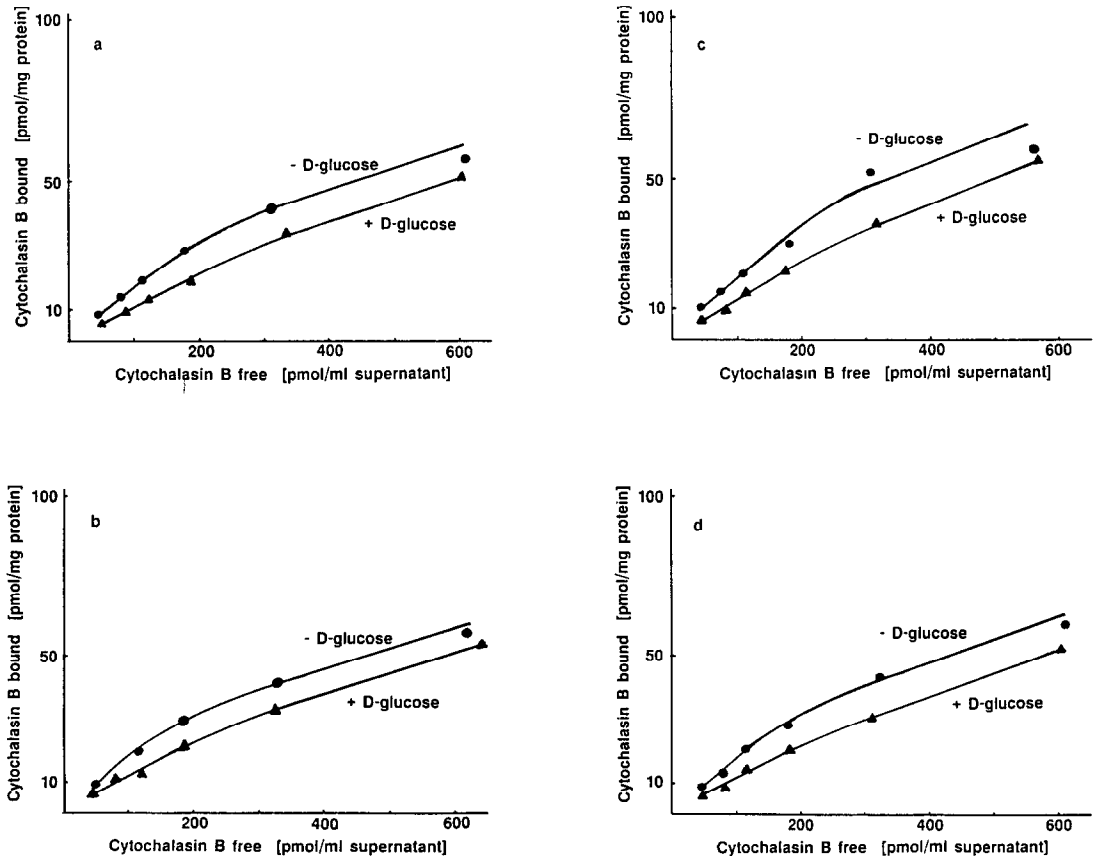

Fig.2. [^{3}H]Cytochalasin B binding to plasma and microsomal membranes of fat cells from hypophysectomized rats. For details see legend to fig.1.

been established, but its occurrence does not have an impact on the qualitative aspect of the results presented. The reason is that the number of the specific cytochalasin B binding sites is undetectably low in the plasma membranes prepared from basal fat cells of normal rats (see below) where the crosscontamination is larger.

Representative plots of cytochalasin B binding to plasma and microsomal membranes are shown in figs 1 and 2. In plasma membranes from normal rats no glucose transporters were detectable when isolated fat cells were incubated in the absence of insulin, since the cytochalasin B binding was identical with or without D-glucose (fig.1a). After insulin stimulation specific binding of cytochalasin B was apparent (fig.1b), consistent with the appearance of glucose transporters on the plasma membrane. In plasma membranes from hypophysectomized rats specific cytochalasin B binding sites were present in the basal state (fig.2a), and their number was not increased upon insulin stimulation (fig.2b). In microsomal membranes of normal rats specific binding of cytochalasin B was greater in the absence of insulin than after insulin treatment (figs 1c and d). Thus, the number of glucose transporters in the intracellular pool decreased upon insulin stimulation. In microsomal membranes from hypophysectomized rats specific cytochalasin B binding was the same with or without insulin treatment (figs 2c and d).

Table 2 summarizes the results for glucose transporter numbers, as represented by maximal specific cytochalasin B binding, of two different membrane preparations from 40–60 normal or hypophysectomized rats each.

Glucose transporter numbers determined here are lower than those reported in the literature [1,10,11]. This seems to be characteristic of the strain of rats used in our study. In another strain of rats (ZUR:SIV) we found similar numbers to those reported in [1,10,11] (results not shown).

Our results for fat cells from normal rats are in agreement with previous findings by other groups [1–3], who reported translocation of glucose transporters to the plasma membrane upon insulin stimulation. In contrast, insulin did not affect the distribution of glucose transporters in fat cells from hypophysectomized rats.

The absence of any effect of insulin on the number of glucose transporters in the plasma membranes of fat cells from hypophysectomized rats provides the explanation for lack of insulin stimulation of glucose transport in these cells [4]. Prior to this study, other explanations, such as an effect of hypophysectomy on only the intrinsic activity of the transporter, were as plausible. Moreover, our finding that the number of glucose transporters per mg protein in the plasma membrane from fat cells of hypophysectomized rats is about the same as that in the plasma membranes of insulin-treated fat cells from normal rats is consistent with the finding that the rates of glucose transport in the two cell types are the same [4,12].

Table 2

Glucose transporter numbers (= total number of D-glucose-inhibitable cytochalasin B binding sites) in plasma and microsomal membranes from fat cells of normal and hypophysectomized rats

	Without insulin	With insulin
Plasma membranes		
Normal rats	$1.1 \pm 2.4^{*+}$	$13.4 \pm 0.2^{+}$
Hypox rats	$9.8 \pm 1.9^{*}$	12.6 ± 0.9
Microsomal membranes		
Normal rats	$36.9 \pm 5.4^{*+}$	$20.8 \pm 0.6^{+}$
Hypox rats	$13.5 \pm 1.4^{*}$	15.4 ± 2.1

The numbers were obtained in the following way: the best line was drawn through the data points for cytochalasin B binding in the presence of D-glucose (using a curve-fitting program on a Macintosh computer) so that nonspecifically bound cytochalasin B could be calculated at any free cytochalasin B concentration. Specifically bound cytochalasin B (= D-glucose-inhibitable cytochalasin B binding) was calculated by subtraction of the calculated bound values of cytochalasin B in the presence of D-glucose from the experimental values for bound cytochalasin B in the absence of D-glucose at the respective same free cytochalasin B concentration. The reciprocal values of specifically bound cytochalasin B were plotted versus the reciprocal values of free cytochalasin B. The linear plot so obtained was subjected to simple regression analysis. The intersection of the regression line with the y-axis yielded the total number of transporter-specific D-glucose-inhibitable cytochalasin B binding sites. Each value given is the average of two individual values obtained in two completely separate experiments in which basal and insulin-treated fat cells from 40–60 normal and hypophysectomized rats were fractionated. The individual values are those given by adding and subtracting the ± values from the average one. The values were statistically analyzed using Student's t-test. Significantly different values ($P<0.05$) for each fraction in the comparison between normal and hypophysectomized rats are marked with *, whereas significantly different values ($P<0.05$) for each fraction in the comparison between with and without insulin are marked with $^{+}$

Hypophysectomized rats have low IGF, insulin and growth hormone levels [4]. Growth hormone, but not IGF or insulin, restore glucose transport towards normal [13]. This finding suggests that a growth hormone-dependent factor may be involved in the regulation of glucose transport [4,13,14]. This factor is hypothesized to be required for fat cells to exhibit a low basal rate of glucose transport.

In summary our results explain why the rate of glucose transport in fat cells from hypophysectomized rats is already maximal in the basal state and unresponsive to insulin. But why plasma membranes from hypophysectomized rats contain an elevated number of glucose transporters in the basal state is unknown and is a challenging question for further investigation.

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