Journal of Cellular Biochemistry 49:251–258 (1992)

Expression of Glucose Transporters (GLUT 1 and GLUT 4) in Primary Cultured Rat Adipocytes: Differential Evolution With Time and Chronic Insulin Effect

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Abstract We previously reported that in cultured adipose cell lines insulin increased selectively the expression of Glut 1, in contrast to in vivo regulation where variations in insulinemia have been shown to affect only GLUT 4. We have addressed here the question of the long-term regulation of GLUT 1 and GLUT 4 in fat cells by using primary cultures of rat adipocytes. Epididymal fat cells were isolated by collagenase and cultured 4 days in DMEM supplemented with BSA 1%, FCS 1%, and glucose 10 mM. GLUT 1 and GLUT 4 proteins were assessed in total cellular membranes by Western blotting, using specific antibodies against their respective C-terminal peptides. GLUT 1 steadily increased over culture time to reach at day 3, a level 3-fold higher than the initial value. In contrast, GLUT 4 decreased sharply and stabilized at day 3, at 30% of the initial value. The changes in GLUT 1 and GLUT 4 mRNAs with culture time were parallel to changes in the corresponding proteins, suggesting a pre-translational level of regulation. The expression of the lipogenic enzyme, fatty acid synthetase (FAS), highly expressed in fat cell, decreased over time following a pattern closely parallel to that of GLUT 4. Chronic exposure to insulin added at day 2 had no effect on GLUT 4 expression but increased the expression of GLUT 1 and FAS by 70% and 36%, respectively. Glucose consumption was stable over 4 days of culture, while lactate production increased from 24 to 36% of glucose utilization, in agreement with the loss in FAS. Glucose consumption increased only slightly with insulin (+160%), in good keeping with the low levels of expression of both GLUT 4 and FAS in these cultured cells. These data indicate that culture alters oppositely the expression of GLUT 1 and GLUT 4 in rat adipocytes and suggest that factor(s) other than insulin predominate in their regulation in vivo.

Key words: fatty acid synthetase, glucose consumption, lactate production, insulin resistance, GLUT 4 mRNA

Recent evidences indicate that at least two glucose transporter species are present in adipocytes: the erythroïd type (GLUT 1), an ubiquitous protein which is predominantly expressed in erythrocytes and brain (Mueckler et al., 1985; Birnbaum et al., 1986; Asano et al., 1988), and the adipose cell/muscle type (GLUT 4), which is expressed exclusively in tissues that exhibit insulin-dependent glucose transport: fat and muscles (James et al., 1988; Kaestner et al., 1989; Charron et al., 1989; Birnbaum, 1989; Fukumoto et al., 1989). Recent studies in rats in various metabolic states have pointed out a differential long-term regulation of the two isoforms in adipocytes. Decrease and increase in insulinemia by streptozotocin diabetes and insulin treatment respectively induced parallel changes in GLUT 4 expression, while GLUT 1 was relatively unaffected (Berger et al., 1989; Kahn et al., 1989; Garvey et al., 1989; Sivitz et al., 1989; Charron and Kahn, 1990). Likewise, fasting and refeeding were shown to selectively modulate the expression of GLUT 4 gene (Berger et al., 1989; Sivitz et al., 1989; Charron and Kahn, 1990). These observations in vivo have led to the hypothesis that insulin could be a regulatory factor of GLUT 4 expression in fat cells. However, observations in cultured adipose cell lines by Tordjman et al. (1989), using 3T3-L1, and ourselves, using 3T3-F442A cells (Hainque et al., 1990), did not support the role of

Received December 31, 1991; accepted February 18, 1992.

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This work was presented in part at 1991 Keystone Symposium "The adipose cell: a model for integration of hormone signalling in the regulation of cellular function," January 18–24, Park City (Utah) USA, and published in abstract form (J. Cell. Biochem., 1991, Sup 15B: 30).

insulin in the expression of GLUT 4. Unexpectedly, both studies revealed that chronic insulin treatment of these cells enhanced glucose transport activity through a specific increase in the expression of GLUT 1, whereas GLUT 4 was unchanged. These findings raise the possibility that the in vivo regulation of GLUT 4 is not directly due to insulin. Alternatively, it can be argued that adipose cell lines are not a valid model for glucose transporter regulation in adipocytes.

This prompted us to re-address the question of the long-term regulation of GLUT 1 and GLUT 4 expressions in fat cells by using primary cultures of rat adipocytes. Our data provide evidence that over the first days of culture GLUT 4 and GLUT 1 undergo reciprocal 3-fold changes, GLUT 4 being decreased and GLUT 1 increased. Chronic insulin treatment failed to modify the expression of Glut 4 but increased that of Glut 1, thus supporting further the hypothesis that in vivo other than, and/or additional factors to insulin, regulate glucose transporter expression in adipose cells.

MATERIALS AND METHODS

Fat Cell Isolation

Adipose cells were isolated by collagenase digestion of epididymal adipose tissue from 6–7-week-old lean (Fa/fa) Zucker rats, bred in our laboratory. Fat cell size (mean: 0.067 μg lipid/cell) was determined by a photomicrographic method as previously described (Lavau et al., 1977). The number of cells per culture tube was determined by dividing the lipid content of fat cell suspension by fat cell size.

Culture

Epididymal adipose cells (about 5×10^6 cells) were cultured in OPTICUL polypropylene tubes (Becton Dickinson, Clifton, NJ) in 20 ml Dulbbeco modified Eagle's medium, supplemented with BSA 1%, FCS 1%, antibiotics, and glucose 10 mM, for a maximal culture time of 4 days. The medium was not changed. Glucose and lactate concentrations in the medium were measured daily. Using a large culture medium volume ensured that glucose concentration remained higher than 5 mM over the entire culture period. Insulin (100 nM) was added at day 2, for 24 or 48 h. Insulin concentration measured in two representative experiments was found to be 30 nM and 17 nM after 24 h and 48 h, respectively.

Preparation of Membranes and Western blot Analysis

Total membranes were prepared by centrifugation of adipocyte homogenates in 10 mM Tris, 1 mM EDTA, and 255 mM Sucrose, containing 0.1 mM PMSF, 25 μg/ml Pepstatin, and 1 mM DTT, at 250,000gmax for 120 min, at 4°C. The proteins were assayed by the BioRad protein determination method. Total membrane proteins were subjected to SDS-PAGE using a 12% polyacrylamide resolving gel and transferred onto Immobilon filters (Millipore, Bedford, MA). Immunological detection of GLUT 1 and GLUT 4 isoforms was carried out using specific antibodies against their respective C-terminal peptide (gift of Dr. S.W. Cushman). For soluble proteins, a 10% polyacrylamide gel was used. Fatty acid synthetase (FAS) was detected in the supernatant by hybridization with a polyclonal rabbit antibody against rat purified FAS, prepared in our laboratory. Immunolabeled bands were vizualized by autoradiography and counted as previously described (Hainque et al., 1990).

Fatty Acid Synthetase Activity

FAS activity was measured immediately after centrifugation in the supernatants, using a spectrophotometric method as previously described (Guerre-Millo et al., 1985).

RNA Isolation and Northern Blot Analysis

Total RNA was extracted using a phenol acid procedure followed by a LiCl precipitation (A. Kimmel, personal communication). RNA was electrophoresed on 1.2% formaldehyde agarose gels, blotted, and fixed onto nylon filters (Hybond N+, Amersham France, Les Ullis, France). Hybridizations were performed with cRNA probes prepared from plasmids containing either human erythrocyte glucose transporter cDNA (Dr G. Bell) or rat muscle glucose transporter cDNA (Dr M.J. Charron). Blots were subjected to autoradiography and the relative amount of each mRNA was quantified by scanning densitometry (Cliniscan).

RESULTS

Adipocytes, cultured here in suspension, did not exhibit any detectable change in size or morphology, as assessed by photomicroscopic inspection, over the 4 days of culture. There was no appearance either of preadipocyte proliferation. We observed a decrease in the number of

floating adipocytes (20% during the first 24 h and 20% between day 3 and 4) due, at least in part, to the attachment of some cells to the culture tube walls. Protein recovery followed a pattern roughly similar to that of fat cell number, indicating that there was no major change in total protein per adipocyte over the culture time (data not shown).

The amounts of GLUT 1 and GLUT 4 proteins were determined in total cell membranes. The two types of antibody used in this study recognized proteins with apparent molecular weight in the range of 45–50 kD that did not change during culture (Fig. 1 inset). The evolution with time of GLUT 1 and GLUT 4 protein concentration is depicted in Figure 1. GLUT 1 concentration increased steadily to plateau at day 3 at a level 3-fold higher than the initial value. Reciprocally, GLUT 4 concentration decreased during the first 3 days of culture to stabilize at a level 3-fold lower than that observed in freshly isolated adipocytes.

In addition to glucose transporters, the effect of culture was assessed on a highly expressed protein in fat cells, fatty acid synthetase (FAS). FAS activity decreased gradually over culture time to stabilize at day 3 at 30% of the day 0 activity (Fig. 2). The decrease in FAS activity was fully accounted for by the decrease in FAS mass. Interestingly, the changes in FAS expression closely paralleled those in GLUT 4 expression (Figs. 2 and 1).

The molecular mechanism of the differential regulation of the two transporter sub-types by culture was investigated in two experiments, by quantification of GLUT 1 and GLUT 4 mRNA. Figure 3 shows that the evolution over culture time of GLUT 1 and GLUT 4 mRNA amounts followed a pattern very similar to that of the corresponding proteins. This is in agreement with a report by Krief et al. (1991).

We next addressed the question of whether insulin regulates the expression of glucose transporters in primary cultured rat adipocytes. The data expressed in percent variation over cells without insulin are shown in Figure 4. Under our conditions, total proteins per cell were slightly increased by insulin (+23%). A differential effect of the hormone was observed on GLUT 1 and GLUT 4 expressions. Cells maintained in the presence of insulin exhibited 76% and 61% increase in GLUT 1 after 24 and 48 h, respectively. In sharp contrast, GLUT 4 concentration was unchanged after 24 h and tended even to be decreased, although not significantly, after 48 h. An inductive effect of insulin was also observed

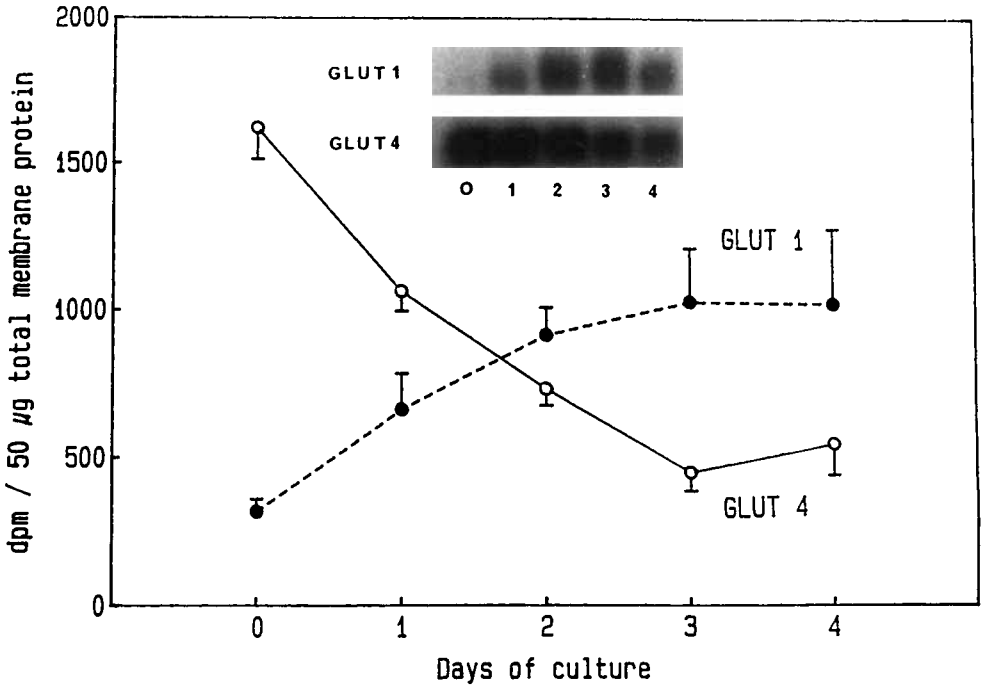

Fig. 1. Evolution with time of Glut 1 and Glut 4 proteins in cultured rat adipocytes. Total membranes were prepared as described in Methods. Means ± S.E.M. of five independent experiments. Inset: Western blot of a representative experiment.

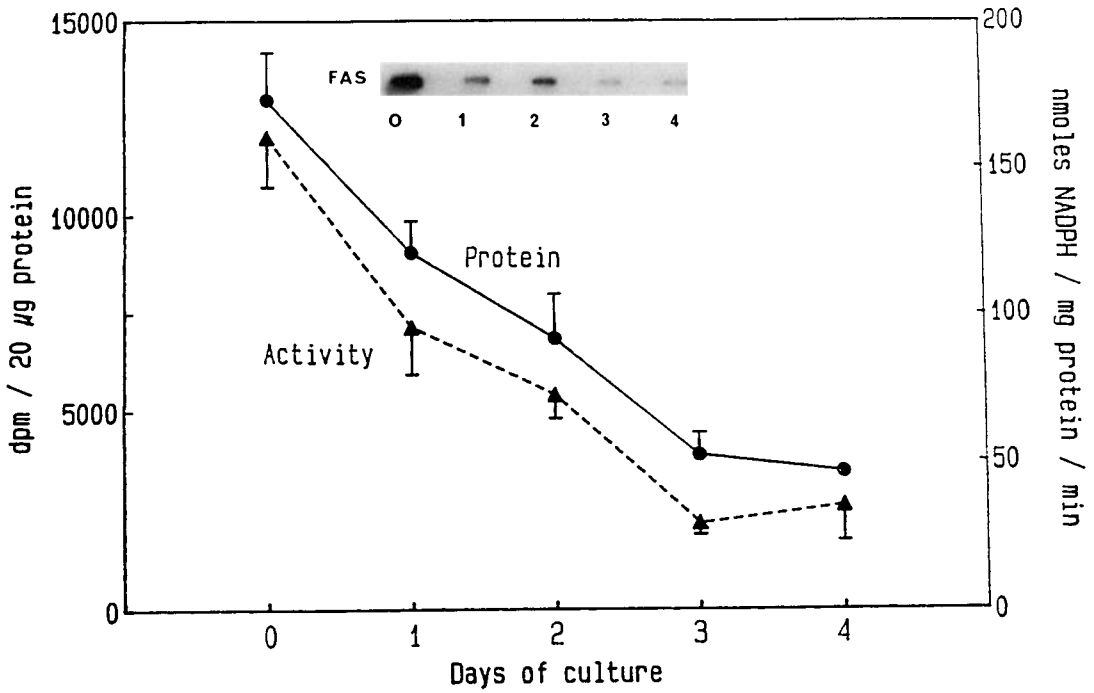

Fig. 2. Evolution with time of FAS activity and protein in cultured rat adipocytes. Means ± SEM of at least five independent experiments. Inset: Western blot of one representative experiment.

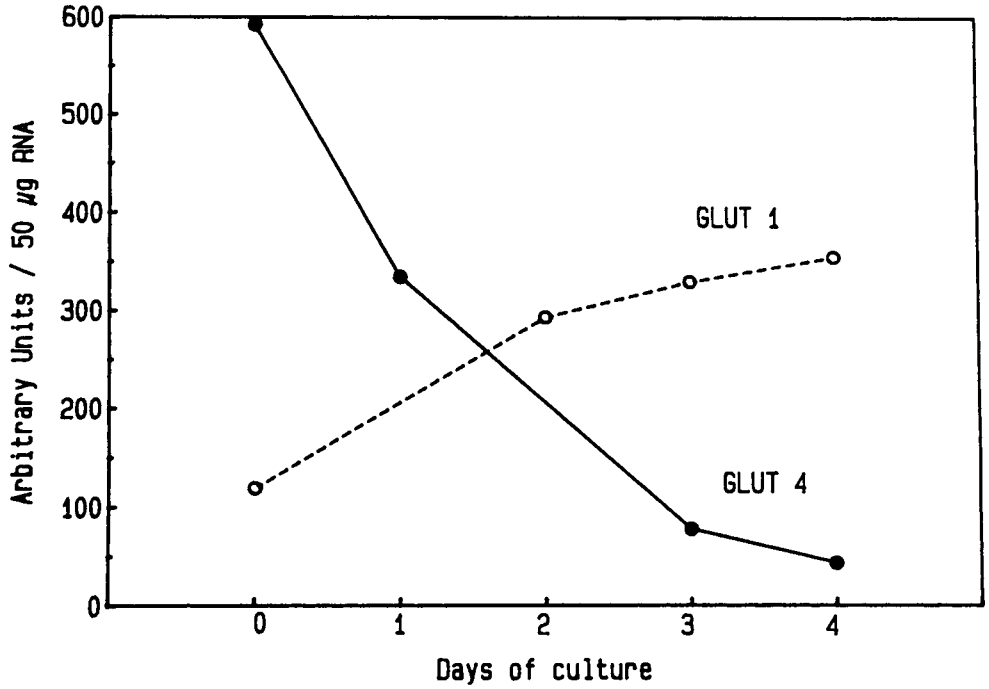

Fig. 3. Evolution with time of Glut1 and Glut 4 mRNA in cultured rat adipocytes. Total RNA were prepared as described in Methods. Means of two experiments.

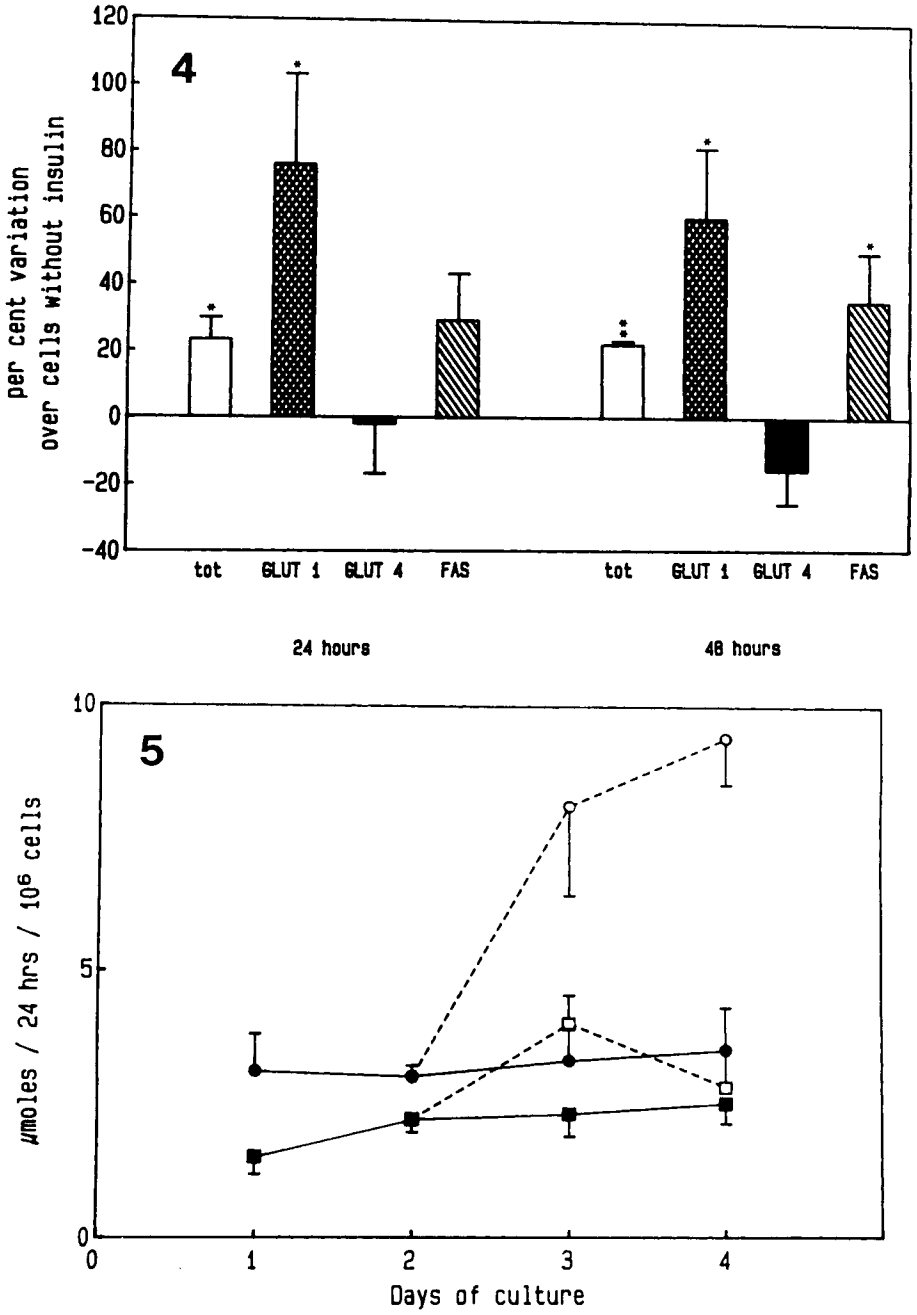

Fig. 4. Insulin effect on total protein (tot), Glut 1, Glut 4, and FAS proteins in cultured rat adipocytes after 24 and 48 h of addition. Insulin (100 nM) was added at day 2 of culture. Mean ± SEM of three to seven experiments. $*P < 0.05$, $**P < 0.01$ vs. cells without insulin.

Fig. 5. Glucose consumption (solid circles) and lactate production (solid squares) in cultured rat adipocytes without (solid lines) or with (broken lines) insulin. Means ± SEM of at least five experiments. Insulin (100 nM) was added at day 2 of culture.

on FAS expression (+36% after 2 days of treatment) the magnitude of which was higher than the hormone effect on protein content indicating a specific insulin regulation of FAS.

In order to assess the functional significance of the changes in GLUT 1, GLUT 4, and FAS expression observed in primary cultured rat adipocytes, we monitored the glucose consumption, a reflect of overall glucose metabolism, and the lactate production of these cells over the 4 days of culture. Under basal conditions, Figure 5 shows that glucose consumption was pretty sta-

ble over culture time, in spite of the substantial decrease in GLUT 4. This suggests that the effect of the loss in GLUT 4 on glucose transport, which might be the rate limiting step of glucose metabolism under basal conditions, was compensated for by the increase in GLUT 1. In adipocytes chronically exposed to insulin, glucose consumption was increased by 160%, a moderate effect as compared to the 7- to 10-fold increase in glucose consumption routinely observed in freshly isolated adipocytes. This blunted response to insulin in adipocytes expressing very low levels of GLUT 4 and FAS is consistent with the view that defects in the intracellular metabolic capacity leads to insulin resistance (Guerre-Millo et al., 1985; Lavau et al., 1979; Fried et al., 1981). Under basal conditions lactic acid production of cultured rat adipocytes increased significantly from day 1 to day 2 of culture and then remained stable. Therefore the proportion of glucose converted to lactic acid increased (from 24% to 36%) from day 1 to day 2, likely due to the loss of FAS activity and the consequent reduction of the incorporation of glucose into fatty acids. In contrast, under chronic insulin exposure, the percentages of glucose converted to lactate were reduced (to 24% and 15% after 24 h and 48 h, respectively) in good agreement with the insulin-induced increase in FAS activity.

DISCUSSION

The present study documents for the first time the regulation of glucose transporter expression in primary cultured adipocytes. We show that when rat fat cells are deprived of their in vivo environment and cultured for an extended period, the pattern of expression of the two main glucose transporters undergoes major and reciprocal changes. We observed an increase in GLUT 1 expression, concomitant with a decline in GLUT 4 expression. The coordinate changes in protein and mRNA strongly suggest a pretranslational control of the expression of both transporters.

Interestingly, studies of the effect of culture on Glut 1 expression in other cell types have shown data in line with the present findings. Wertheimer et al. (1991) have recently reported that ex vivo incubation of rat soleus muscle for 5 h induced a dramatic increase in GLUT 1 mRNA. Similarly, it has been previously demonstrated that the expression of GLUT 1 is increased in hepatocytes maintained in primary culture (Rhoads et al., 1988). It can be speculated that an over-expression of GLUT 1 is a general cellular response to culture conditions. This effect could be mediated either by the removal of repressing factor(s) that down regulate(s) the expression of GLUT 1 in vivo, or by the presence in culture medium of positive effector(s). In relevance with the latter possibility, it is interesting to note that an increase in GLUT 1 expression and/or GLUT 1 gene transcription has been repeatedly observed in cultured cell lines, either differentiated 3T3 L1 adipocytes (Clancy and Czech, 1990) or fibroblasts (NIH3T3, Hiraki et al., 1989; 3T3L1, Cornelius et al., 1991) upon the chronic exposure to cAMP. Very recently it was shown that cAMP in 3T3L1 adipocytes induced not only a 3-fold increase in GLUT 1 expression but also a reciprocal 70% decrease in GLUT 4 expression (Kaestner et al., 1991) a pattern strikingly similar to the present observations. Furthermore, it has been shown by Paulauskis and Sul (1988) that cAMP decreases FAS expression in 3T3 L1 adipocytes, an observation supporting also this candidate as a potential effector of the changes observed in our primary cultured rat adipocytes. Whether a rise in intracellular cAMP occurs in our cultured cell system remains to be documented.

The present work also documents for the first time the direct role of insulin on the expression of GLUT 1, GLUT 4, and FAS in cultured rat adipocytes. It shows that insulin has no effect on GLUT 4 expression in cultured rat adipocytes, a finding in perfect agreement with our and other's previous observations in 3T3 adipocytes (Hainque et al., 1990; Tordjman et al., 1989) or in L6 muscle cells (Koivisto et al., 1991). This negative result implies that the modulation of adipocyte GLUT4 expression observed in vivo with changes in insulinemia levels is exerted through insulin-induced variations in factors or metabolites that would be the direct effectors. At variance with GLUT 4, GLUT 1 expression was significantly increased by insulin in cultured rat adipocytes. The present observations agree with previous data in 3T3 adipose cells (Tordjman et al., 1989; Hainque et al., 1990), although the insulin effect is much smaller in cultured rat adipocytes than in adipose cell lines. This direct effect of insulin on GLUT 1 expression in rat adipocytes is difficult to reconcile with the observation that GLUT 1 expression was unchanged in hyperinsulinemic rat adipocytes (Hainault et al., 1991). It can be speculated

that Glut 1 expression is not modulated by insulin within the range of physiopathological concentrations of the hormone or that down regulating factors are present in vivo that overcome insulin action. In addition, the present work demonstrates that insulin elicited a significant increase in FAS expression in cultured rat fat cells. This is in concert with previous observation in 3T3 L1 adipocytes where insulin was shown to promote an increase in FAS rate of synthesis and mRNA content (Paulauskis and Sul, 1988). However the insulin effect reported in adipose cell lines was much larger than in the present cultured rat adipocytes. The molecular mechanism(s) underlying the insulin effect on Glut 1 and FAS expressions remain(s) to be elucidated.

In conclusion, the present study provides clear evidence that GLUT 1 and GLUT 4 expressions in rat adipocytes are dramatically altered by the culture process and/or the removal of cells from their in vivo environment, leading to a repression of GLUT 4 and an overexpression of GLUT 1. Chronic exposure to insulin was unable to reverse this pattern. These data suggest that other than, or/and, additional factor(s) to insulin are involved in the pathophysiological regulation of glucose transporter expressions in rat adipocytes.

ACKNOWLEDGMENTS

This work was supported by a grant from the Fondation pour la Recherche Medicale. We wish to thank Drs S.W. Cushman, M.J. Charron, and G.I. Bell for their kind gift of probes.

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