FEBS Letters 347 (1994) 42–44

FEBS 14124

Parallel changes in Glut 4 and Rab4 movements in two insulin-resistant states

Jean-Marc Ricort*, Jean-François Tanti, Mireille Cormont, Emmanuel Van Obberghen, Yannick Le Marchand-Brustel

Institut National de la Santé et de la Recherche Médicale, INSERM U 145, Faculté de Médecine, Avenue de Valombrose, 06107 Nice Cedex 02, France

Received 19 April 1994

Abstract

Insulin-induced Glut 4 and Rab4 movements were studied in two insulin-resistant states. In adipocytes from streptozotocin diabetic rats, the amount of Glut 4 was decreased by 60%. The remaining Glut 4 molecules were translocated in response to insulin, and in parallel, Rab4 left the intracellular compartment. In contrast, in 3T3-L1 adipocytes rendered insulin-resistant by a prolonged insulin treatment, both Rab4 and Glut 4 remained in the intracellular compartment following an acute insulin stimulation. Those results illustrate a similar behavior of Glut 4 and Rab4 in two situations where insulin resistance results from different mechanisms, and add further support for a role of Rab4 in Glut 4 translocation.

Key words: Glut 4 translocation; Rab4; Insulin effect; Adipocyte; 3T3-L1 adipocyte; Diabetes

1. Introduction

Insulin stimulation of glucose transport in adipocytes results from the translocation of vesicles containing the Glut 4 glucose transporter from an intracellular pool to the plasma membrane [1,2], a process which presents some analogy with stimulated exocytocis. Low molecular weight GTP binding proteins have been implicated in vesicle traffic [3–5] and in a recent study [6], we have suggested that the protein Rab4 could be involved in the insulin-stimulated Glut 4 translocation. This conclusion was based on the following observations: Rab4 is associated with the Glut 4 containing vesicles and moves, under insulin stimulation, from the intracellular compartment (LDM) to the cytosol, while Glut 4 is incorporated into the plasma membrane. This movement is reversible upon insulin withdrawal. To get further insight in the possible involvement of Rab4 in the translocation of Glut 4 containing vesicles, we have studied, in the present work, whether Rab4 movement was affected in response to insulin in two situations where insulin resistance for glucose transport results from alterations at different levels [7]. In the first situation, in adipocytes from insulin-dependent diabetic rats, insulin resistant glucose transport has been attributed to a decreased Glut 4 expression while translocation remained relatively normal [8–10]. By contrast, in the second situation, in 3T3-L1 adipocytes chronically treated with insulin, insulin resistance results mainly from a defect in Glut 4 translocation [11]. We have thus compared in those two insulin-resistant states insulin effects on Rab4 subcellular distribution with those on Glut 4 translocation.

2. Materials and methods

2.1. Animals and cell culture

Rats (180–200 × *g*) were rendered diabetic by streptozotocin i.v. injection (60 mg/kg BW in citrate buffer, pH 4.5) 7 days before the experiment. All injected rats were diabetic with a glycemia higher than 20 mM. Adipocytes were isolated from epididymal fat pads by collagenase digestion as previously described [1,6]. Cells were stimulated or not for 10 min at 37°C with insulin (100 nM) before homogenization and subcellular fractionation.

Fully differentiated 3T3-L1 adipocytes were rendered insulin resistant by a prolonged insulin treatment [11]. Cells were treated for 10 h without or with 100 nM insulin. Following various washings and incubation for 1 h in Krebs–Ringer MES buffer (136 mM NaCl, 4.7 mM KCl, 1.25 mM $CaCl_2$, 1.25 mM $MgSO_4$, 10 mM MES, pH 6.0) to dissociate insulin, cells were acutely stimulated with insulin (100 nM) for 15 min and submitted to subcellular fractionation.

2.2. Glucose transport and metabolism

Lipogenesis was measured by the incorporation of ^3H from [3-^3H]glucose into lipids [12] as an index of glucose transport. Adipocyte suspensions (5%) were incubated for 1 h at 37°C without or with 100 nM insulin, before determination of [^3H]glucose incorporation into lipids. Deoxyglucose transport in 3T3-L1 adipocytes was measured as previously described [13].

2.3. Subcellular fractionation, Glut 4 and Rab4 immunodetection

Isolated adipocytes or 3T3-L1 adipocytes were homogenized in TES buffer (20 mM Tris pH 7.4, 1 mM EDTA, 250 mM sucrose, aprotinin 100 U/ml and 1 mM PMSF). Homogenates were centrifuged at 16,000 × *g* for 20 min. The supernatant was centrifuged for 90 min at 210,000 × *g* to obtain a pellet corresponding to microsomes of high and low density (HDM/LDM). Proteins (50 μg) were solubilized at room temperature in Laemmli buffer (3% SDS, 70 mM Tris, 11% glycerol) containing bromophenol blue (0.05%) and 2-mercaptoethanol (700 mM), and separated by SDS/PAGE with a 10% resolving gel. Proteins were transferred to a polyvinyliden difluoride (PVDF) sheet. The sheet was incubated with blocking buffer (PBS/5% fat skimmed dry milk) for 2 h at room temperature, and cut between the 45 and 30 k molecular weight standards. The upper part of the sheet was incubated overnight

*Corresponding author. Fax: (33) 93 81 54 32.

SSDI 0014-5793(94)00510-3

at 4°C with antibodies to the C terminus of Glut 4 [14] and the lower part with antibodies to Rab4 [15]. After washes, sheets were incubated with ^{125}I-protein A, washed and submitted to autoradiography. Quantification was performed by counting the radioactivity associated with the bands and/or by scanning (Hoefer Scientific Instrument).

3. Results and discussion

Lipogenesis from [^{3}H]glucose and [^{3}H]2-deoxyglucose uptake were measured as an index of glucose transport in adipocytes from diabetic rats (Fig. 1A) or in 3T3-L1 adipocytes treated for a prolonged period with insulin (Fig. 1B). As previously described, adipocytes from diabetic animals were unresponsive to insulin, while basal lipogenesis was similar in both groups [8]. By contrast, following prolonged treatment with insulin, basal glucose transport was higher in treated 3T3-L1 adipocytes than in control cells but did not increase further in response to an acute insulin stimulation. This increase in basal glucose uptake is due to a marked increase in Glut 1 expression in the treated cells (data not shown, [11]).

We then studied the translocation of Glut 4 and looked whether Rab4 movement was also affected in both insulin resistant states. A low and high density microsomal fraction enriched in intracellular Glut 4 containing vesicles [2] was prepared from control and insulin-treated cells and analyzed for their Glut 4 and Rab4 content. A typical autoradiogram is shown in Fig. 2 and the quantification of three fractionations performed with three different cell preparations is presented in Table 1. In adipocytes from streptozotocin diabetic animals, the intracellular Glut 4 pool was markedly depleted, as already reported by others [8–10], but Rab4 was unchanged. As shown in Fig. 2, left panel, following insulin stimulation of adipocytes from control and streptozotocin diabetic animals, about 50% of the Glut 4 and Rab4 molecules left the HDM/LDM compartment. The Glut 4 molecules, but not Rab4, appeared in a similar fashion in the plasma membranes in both situations (data not shown). These results indicate that the translocation process per se is not altered in adipocytes from streptozotocin diabetic rats, but that insulin-resistant glucose transport results mostly from the depletion of the Glut 4 pool. A different result was observed in 3T3-L1 adipocytes following a prolonged treatment with insulin (Fig. 2, right panel). The intracellular Glut 4 amount was also diminished in insulin resistant cells, although to a lower extent (by about 30%) than in diabetic adipocytes. However, those Glut 4 molecules were no longer able to be recruited in response to an acute insulin stimulation (and did not appear in the plasma membranes) and in parallel, Rab4 molecules remained in the intracellular compartment of treated 3T3-L1 adipocytes.

In summary, insulin-resistant glucose transport was observed in the two situations studied but the alterations underlying this resistance are clearly different. In both cases, the Glut 4 intracellular pool was depleted, although to a different degree. However, while the translocation of the remaining Glut 4 molecules happened normally in streptozotocin diabetic adipocytes, a total blockade in the Glut 4 translocation was observed in 3T3-L1 chronically treated with insulin. The insulin induced movement of the low molecular weight GTP binding protein Rab4 was in both states parallel to the Glut 4 translocation. Although the cascade of phosphorylations following the insulin receptor tyrosine kinase activation is better understood (for reviews see [16–19]), the pathways implicated in the Glut 4 translocation process are still largely unknown. The close correlation between the movements of Glut 4 and Rab4 is a further indication that Rab4 could be involved in the process of the Glut 4 containing vesicles under hormonal stimulation. Further studies will be required to understand the precise role of Rab4 in this process.

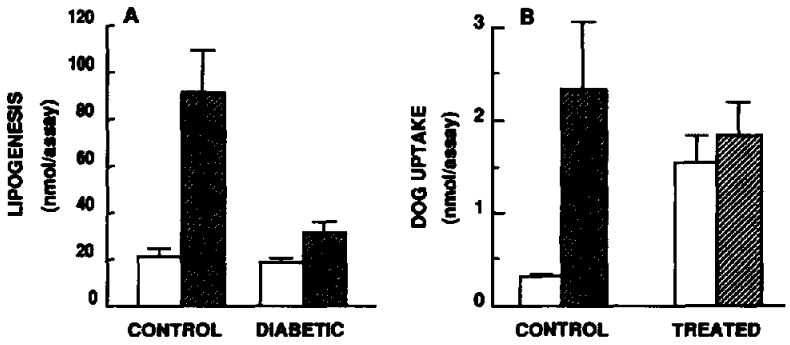

Fig. 1. Glucose transport and metabolism in two insulin resistant states. Left panel: adipocytes were isolated from control or streptozotocin diabetic rats and incubated without (open bars) or with (hatched bars) 100 nM insulin for 1 h in the presence of [^{3}H]glucose. At the end of the incubation, glucose incorporation into lipids was measured as described in section 2. Right panel: 3T3-L1 adipocytes were treated without (control) or with (treated) 100 nM insulin. Following washings and incubation for 1 h in Krebs–Ringer MES buffer, cells were acutely incubated without (open bars) or with (hatched bars) 100 nM insulin for 15 min before measuring deoxyglucose (DOG) uptake. Values are presented as the means ± S.E.M. of 3–5 experiments.

Table 1
Effect of insulin on Glut 4 and Rab4 movements in insulin-resistant adipocytes and 3T3-L1 adipocytes

	Adipocytes from		3T3-L1 adipocytes	
	Control rats	Diabetic rats	Control	Treated
Glut 4 in HDM/LDM fraction				
Basal	1	0.42 ± 0.06	1	0.67 ± 0.06
Insulin	0.45 ± 0.04*	0.23 ± 0.06*	0.60 ± 0.01*	0.54 ± 0.05
Rab4 in HDM/LDM fraction				
Basal	1	0.94 ± 0.24	1	0.75 ± 0.03
Insulin	0.51 ± 0.04*	0.34 ± 0.03*	0.39 ± 0.04*	0.66 ± 0.07

Adipocytes were prepared from control or streptozotocin diabetic rats, and incubated for 10 min without (basal) or with insulin (100 nM). In a similar fashion control or chronically treated 3T3-L1 adipocytes were incubated without insulin (basal) or stimulated for 15 min with 100 nM insulin. Microsomes of high and low density (HDM/LDM) were prepared as described in section 2, proteins were analyzed by SDS/PAGE and Glut 4 and Rab4 were quantified by scanning and/or counting following immunoblotting. To allow comparison amongst experiments, the amounts of Glut 4 and Rab4 were expressed relative to the amount of proteins present in control basal conditions. Results are the means S.E.M. of 3 different experiments (or the range of 2 experiments for Rab4 in streptozotocin diabetic animals).
*Insulin effect was significant with $P < 0.05$.

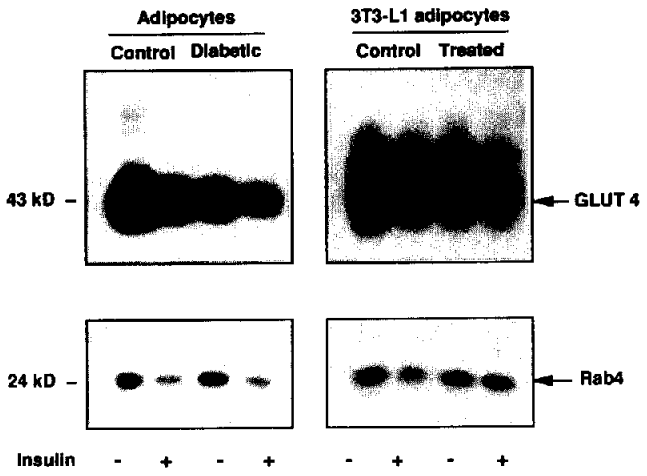

Fig. 2. Glut 4 and Rab4 translocation process in two insulin resistant states. Adipocytes from control and diabetic rats (left panel) and control or treated 3T3-L1 adipocytes were incubated as described in Fig. 1 before subcellular fractionation. Microsomes of low and high density were prepared, proteins (50 μg) were separated by SDS/ polyacrylamide gel electrophoresis, transferred on membranes, and immunoblotted with antibodies to Glut 4 (upper panels) of Rab4 (lower panels).

Acknowledgements: This work was supported in part by the Institut National de la Santé et de la Recherche Médicale (France), the University of Nice and Grant 6979 from the Association pour la Recherche contre le Cancer (ARC). We thank Dr. A. Zahroui (INSERM U 248, Paris) for his generous gift of antibody to Rab4, and G. Visciano for illustration work.

References

[1] Simpson, I.A. and Cushman, S.W. (1986) Annu. Rev. Biochem. 55, 1059–1089.
[2] Gould, G.W. and Holman, G.D. (1993) Biochem. J. 295, 329–341.
[3] Bucci, C., Parton, R.G., Mather, I.H., Stunneberg, H., Simons, K., Hoflack, B. and Zerial, M. (1992) Cell 70, 715–728.
[4] van der Sluijs, P., Hull, M., Webster, P., Mâle, P., Goud, B. and Mellman, I. (1992) Cell 70, 729–740.
[5] Novick, P. and Brennwald, P. (1993) Cell 75, 597–601.
[6] Cormont, M., Tanti, J.-F., Zahraoui, A., Van Obberghen, E., Tavitian, A. and Le Marchand-Brustel, Y. (1993) J. Biol. Chem. 268, 19491–19497.
[7] Kahn, B.B. and Pedersen, O. (1993) Endocrinology 132, 13–22.
[8] Kahn, B.B., Charron, M.J., Lodish, H.F., Cushman, S.W. and Flier, J.S. (1989) J. Clin. Invest. 84, 404–411.
[9] Sivitz, W.I., DeSautel, S.L., Kayano, T., Bell, G.I. and Pessin, J.E. (1989) Nature 340, 72–74.
[10] Berger, J., Biswas, C., Vicario, P.P., Strout, H.V., Saperstein, R. and Pilch, P.F. (1989) Nature 340, 70–72.
[11] Kozka, I.J., Clark, A.E. and Holman, G.D. (1991) J. Biol. Chem 266, 11726–11731.
[12] Moody, A.J., Stan, M.A. and M.S. (1974) Horm. Metabol. Res. 6, 12–16.
[13] Tanti, J.-F., Grémeaux, T., Van Obberghen, E. and Le Marchand-Brustel, Y. (1994) J. Biol. Chem. 269, 6054–6057.
[14] Le Marchand-Brustel, Y., Olichon-Berthe, C., Grémeaux, T., Tanti, J.F., Rochet, N. and Van Obberghen, E. (1990) Endocrinology 127, 2687–2695.
[15] Cormont, M., Tanti, J.-F., Zahraoui, A., Van Obberghen, E. and Le Marchand-Brustel, Y. (1994) Eur. J. Biochem. 219, 1081–1085.
[16] Lawrence Jr., J.C. (1992) Annu. Rev. Physiol. 54, 177–193.
[17] White, M.F. and Kahn, C.R. (1994) J. Biol. Chem. 269, 1–4.
[18] Van Obberghen, E. (1994) Diabetologia, in press.
[19] Lee, J. and Pilch, P.F. (1994) Am. J. Physiol. 266, C319–C334.