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Glucose transporter expression in rat mammary gland

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The expression of different glucose transporter isoforms was measured during the development and differentiation of the rat mammary gland. Before conception, when the mammary gland is mainly composed of adipocytes, Glut 4 and Glut 1 mRNAs and proteins were present. During pregnancy, the expression of Glut 4 decreased progressively, whereas that of Glut 1 increased. In the lactating mammary gland only Glut 1 was present, and was expressed at a high level. The absence of Glut 4 suggests that glucose transport is not regulated by insulin in the lactating rat mammary gland.

INTRODUCTION

The rat mammary gland utilizes large amounts of glucose for the synthesis of milk. The first step of glucose utilization, glucose transport, has been measured in isolated acini. It is specific, saturable and inhibitable by cytochalasin B and phloretin, as for other mammalian cell glucose transporters (Threadgold *et al.*, 1982). The apparent K_m for glucose transport in rat mammary acini (Threadgold *et al.*, 1982) and in mouse mammary cells (Prosser & Topper, 1986) is about 15 mM. Although insulin has been shown to acutely stimulate several enzyme activities involved in glucose metabolism, such as phosphofructokinase I, pyruvate dehydrogenase and acetyl-CoA carboxylase (Baxter & Coore, 1978; McNeillie & Zammit, 1982; Kilgour & Vernon, 1987; Burnol *et al.*, 1988; A.-F. Burnol, M. Loizeau & J. Girard, unpublished work), the presence of a stimulatory effect of insulin on glucose transport in mammary gland remains controversial. It has been reported that glucose transport is under the control of insulin *in vivo* in the rat mammary gland (Threadgold & Kuhn, 1984). However, insulin had no effect on glucose transport in isolated acini or mammary cells (Prosser & Topper, 1986; Munday & Williamson, 1987).

The cellular uptake of glucose is mediated by a family of glucose transporters. At the present time, five distinct facilitative glucose transporters have been described, the expression of which is tissue-specific (Bell *et al.*, 1990). One isoform, Glut 1, is expressed in many tissues, and is most abundant in placenta, brain and erythrocytes (Bell *et al.*, 1990; Mueckler, 1990). Another isoform, Glut 4, is present only in white adipose tissue and muscle, the two tissues in which glucose transport is sensitive to insulin (Bell *et al.*, 1990). Thus the presence of Glut 4 in the mammary gland would suggest that glucose transport could be regulated by insulin.

This study was aimed at identifying the glucose transporter isoforms present in the rat mammary gland during lactation, and also investigated the possible regulation of glucose transport by insulin, by measuring the Glut 4 isoform in mammary gland.

MATERIALS AND METHODS

Animals

Female Wistar rats were obtained from Iffa-Credo (L'Arbresle, France) at 8 weeks of age, and were mated in the laboratory. Rats were studied at 16 and 21 days of gestation, and at 3 and 12 days of lactation. For the lactating groups, the number of pups was adjusted to 10 in each litter on the day of delivery. Animals were killed by cervical dislocation and the tissues were rapidly removed, and either frozen in liquid N_2 and stored at −80 °C for subsequent RNA extraction, or treated extemporaneously for protein analysis.

Western blotting

Crude membrane fractions were prepared from the mammary glands of virgin rats, 21-day-pregnant and 12-day-lactating rats. The tissues were homogenized in ice-cold 0.3 M-sucrose/25 mM-Tris/HCl (pH 7.4), containing 2 mM-EGTA, 5 mM-EDTA, 1 mM-phenylmethanesulphonyl fluoride, 2 mg of bacitracin/ml and 1000 trypsin-inhibitory units/ml. The homogenate was centrifuged at 3000 *g* for 15 min at 4 °C. The supernatant from the mammary glands of virgin rats was then centrifuged for 45 min at 50 000 *g* (Klein & Freidenberg, 1985), and the supernatants from the mammary glands of pregnant and lactating rats were centrifuged for 90 min at 105000 *g* (Djiane *et al.*, 1977). The membrane pellet was dissolved in the same buffer and proteins were quantified using the Bio-Rad protein assay (Bio-Rad, Munich, Germany). Equal amounts of membrane proteins were solubilized in Laemmli buffer (Laemmli, 1970) and submitted to SDS/PAGE (12% gels). The proteins were electrophoretically transferred to nitrocellulose membranes (BA 85; Schleicher & Schuell, Dassel, Germany). Protein markers (Rainbow; Amersham) were used as molecular mass standards and also to assess the efficiency of the transfer. The blots were blocked with non-fat dry milk (Carnation Company, Los Angeles, CA, U.S.A.), and incubated either with an antibody directed against Glut 4 (R 820, kindly donated by Dr. D. James, Washington University, St. Louis, MO, U.S.A.), or with an antibody directed against Glut 1 (kindly donated by Dr. C. Carter-Su, University of Michigan, Ann Arbor, MI, U.S.A.). The immune complex was detected using ^{125}I-Protein A. After several washes the blots were autoradiographed (Hyperfilm MP; Amersham) and quantified by scanning densitometry (GS 300; Hoeffer, San Francisco, CA, U.S.A.) coupled to a Macintosh microcomputer.

Northern blot analysis

Total cellular RNA was isolated by a guanidine thiocyanate method (Chomczynski & Sacchi, 1987). Briefly, tissue samples (100 mg–1 g) were homogenized in 4 M-guanidium thiocyanate, 25 mM-sodium citrate, pH 7, 0.5% sarcosyl, and 0.1 M-2-mercaptoethanol. The extraction was continued by adding 0.2 M-sodium acetate (pH 4.0)/phenol/chloroform (0.1:1.0:0.2, by vol.). RNA was precipitated with propan-2-ol and washed with ethanol. The RNA pellet was dissolved in diethyl pyrocarbonate-treated double-distilled sterile water, and the RNA concentration

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was determined by spectrophotometry (absorbance at 260 nm). Aliquots (20 μg) of total RNA were submitted to electrophoresis in a denaturing 1% agarose/0.66 M-formaldehyde gel, and transferred to a nylon membrane (Hybond N; Amersham). The filter-bound RNA was then hybridized overnight at 42 °C with ^{32}P-labelled cDNA probes. The hybridization medium contained 42% formamide, 8 × Denhardt's solution, 1% SDS, 7.5% dextran sulphate, salmon sperm DNA (80 μg/ml) and 40 mM-Tris/HCl (pH 7.5). The membranes were washed (0.1% SSC/0.1% SDS at 55 °C) and autoradiographed. Quantification was performed by scanning densitometry. The cDNA probes used were Glut 4 (James *et al.*, 1989) (a gift from Dr. D. James), Glut 1 and Glut 2 (Shows *et al.*, 1987; Fukumoto *et al.*, 1988; Kayano *et al.*, 1989) (gifts from Dr. G. Bell, Howard Hughes Medical Institute, University of Chicago, Chicago, IL, U.S.A.).

Statistical analysis

Results are expressed as means ± S.E.M. Statistical analysis was performed by Student's *t* test for unpaired data.

RESULTS AND DISCUSSION

Before conception, the rat mammary gland consists only of a short branching duct system lying at the site of the inguinal fat depot, with no true lobulo-alveolar secretory tissue (Knight, 1984). Cellular proliferation and development of the tissue occur during gestation and early lactation. The presence of two isoforms of the glucose transporter, Glut 1 and Glut 4, was tested in the mammary gland at different stages of gestation and lactation (Fig. 1). During the development and differentiation of the mammary gland, from the virgin rat to parturition (at 21 days), the amount of Glut 4 mRNA was decreased by 90%, whereas the amount of Glut 1 mRNA was increased by 8-fold. During lactation Glut 1 mRNA was present at a level similar to that observed in the mammary gland of the virgin rat. The concentration of mRNA encoding for Glut 1 was 5-fold lower in mammary glands from 12-day-lactating rats than in rat brain (results not shown), but Glut 4 mRNA was undetectable, even after a long exposure (10 days) of the autoradiograms.

The variations in the amounts of Glut 4 and Glut 1 mRNAs during the development of the mammary gland and during lactation were accompanied by similar modifications in the expression of the encoded proteins, as shown in Fig. 2. The concentration of Glut 4 protein was 7-fold lower in the mammary gland at parturition than in the mammary gland from a virgin rat, whereas Glut 1 protein was 3-fold higher. From parturition

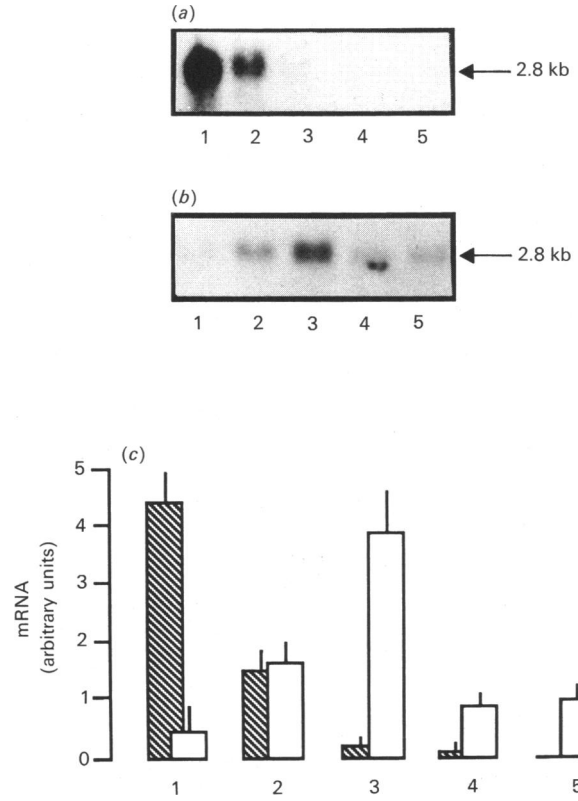

Fig. 1. Northern blot analysis of Glut 4 and Glut 1 mRNA expression during differentiation and development of the mammary gland

Total RNA (20 μg) was electrophoresed after denaturation on a 1% agarose gel, transferred to a nylon membrane and hybridized with the Glut 4 (*a*) or Glut 1 (*b*) cDNA inserts labelled by random priming (see the Materials and methods section). Both mRNAs detected were ~ 2.8 kb in size. Mammary glands were from: lane 1, virgin rat; lane 2, 16-day-pregnant rat; lane 3, 21-day-pregnant rat; lane 4, 3-day-lactating rat; lane 5, 12-day-lactating rat. (*c*) Quantification by scanning densitometry of autoradiograms from Northern blot analysis of Glut 4 (▨) and Glut 1 (□) mRNAs. Results are the means ± S.E.M. of three different blots.

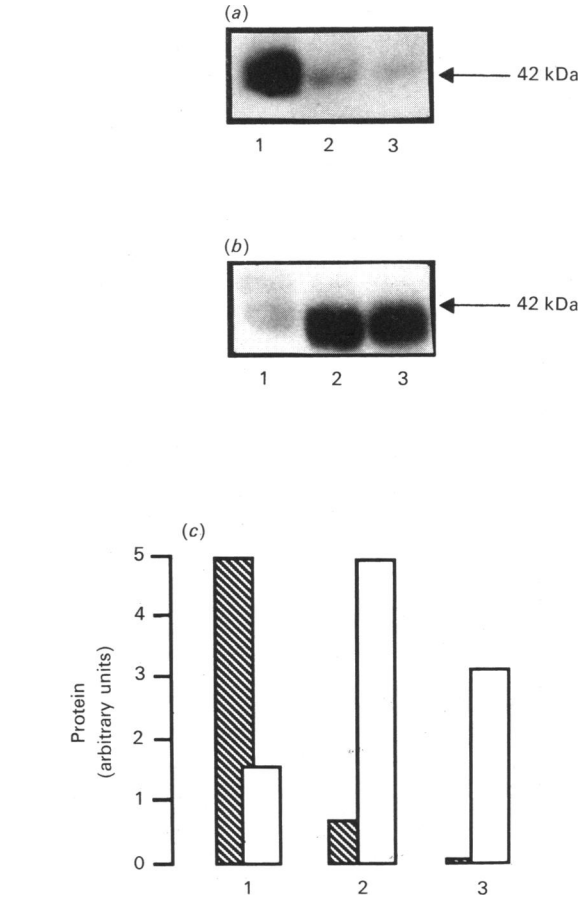

Fig. 2. Western blot analysis of glucose transporter proteins during differentiation and development of the mammary gland

Membrane proteins were subjected to SDS/PAGE and electrophoretically transferred to nitrocellulose. Glut 4 (*a*) and Glut 1 (*b*) were detected using antibodies kindly provided by Dr. D. James and Dr. C. Carter-Su respectively, as described in the Materials and methods section. Mammary glands were from: lane 1, virgin rat; lane 2, 21-day-pregnant rat; lane 3, 12-day-lactating rat. (*c*) Quantification by scanning densitometry of autoradiograms (▨, Glut 4; □, Glut 1). Results are the means of two experiments.

to peak lactation, Glut 1 protein was decreased by about 40%, whereas the Glut 4 protein level was very low in lactating mammary gland.

These results indicate that Glut 4, which is known to be specific for muscle and adipose tissue (James *et al.*, 1989; Charron *et al.*, 1989; Birnbaum, 1989; Kaestner *et al.*, 1989) is not expressed in the differentiated mammary gland. The high levels of Glut 4 measured in mammary glands from virgin rats are due to the predominance of the adipocytes at this state of mammary gland development (Knight, 1984). The observed decrease in the expression of Glut 4 mRNA and protein throughout pregnancy can be attributed to the decrease in the percentage of fat cells, whereas the secretory cells progressively constitute the major part of the tissue as development proceeds.

The increase in the expression of Glut 1 occurs simultaneously with the development and differentiation of the mammary gland. Several studies have reported that the expression of Glut 1 mRNA and protein is induced by factors that stimulate cellular growth and division, such as oncogenes, tumour promoters (Flier *et al.*, 1987), and polypeptide growth factors (Hiraki *et al.*, 1988; Rollins *et al.*, 1988; De Herreros & Birnbaum, 1989). Therefore it is likely that the high expression of Glut 1 in the mammary gland during pregnancy is linked with the increased cellular division during the development of the mammary gland.

The results presented in Figs. 1 and 2 suggested that, when the data were analysed per equal amount of total RNA, the expression of Glut 1 mRNA was similar in mammary glands of lactating and virgin rats. However, the concentration of total RNA varied with the tissue considered, and was respectively 0.36 ± 0.06 ($n = 3$), 2.4 ± 0.4 ($n = 3$) and 5.5 ± 0.8 ($n = 6$) μg of RNA per mg of tissue in the mammary glands from virgin rat, at parturition and at peak lactation. When the relative abundance of transporter mRNA was normalized per mg of tissue, it appeared that the expression of Glut 1 was respectively 60-fold and 40-fold higher in mammary glands at parturition and at peak lactation than in the mammary glands of virgin rats. The concentration of total extracted RNA was 0.6 ± 0.1 μg of RNA per mg of tissue for the rat brain. Normalizing the expression of Glut 1 per mg of tissue indicated that Glut 1 was 2-fold more abundant in lactating rat mammary gland than in rat brain. Thus Glut 1, the glucose transporter isoform expressed in most tissues, mainly in brain and placenta, is also expressed in the mammary gland at a high level during gestation and lactation.

To assess if the concentration of Glut 1 expressed in the mammary gland during lactation was sufficient to explain the high glucose utilization, the presence of other glucose transporter isoforms was tested. Glut 2 is the liver-type glucose transporter, and is also expressed in kidney, intestine and β-cells (Fukumoto *et al.*, 1988; Thorens *et al.*, 1988). This glucose transporter exhibits a high K_m for glucose (23–48 mM), indicating that transport never becomes rate-limiting when the glucose concentration rises (Mueckler, 1990). This characteristic should be useful for an organ like the lactating mammary gland, with a high glucose utilization rate tightly linked to the increased food intake (Page & Kuhn, 1986). However, Glut 2 mRNA was detected in rat liver but not in rat mammary gland (results not shown).

In conclusion, this study has shown that Glut 1 is present in the rat mammary gland at a higher concentration than in rat brain, and that the high glucose utilization in the former tissue may be accounted for by the expression of Glut 1. This work gave also clear evidence that the glucose transporter specific for the insulin-sensitive tissues, Glut 4, is not expressed. Since Glut 4 is the major isoform of glucose transporter present in tissues where glucose transport is stimulated by insulin (Bell *et al.*, 1990; Mueckler, 1990), this would suggest that glucose transport is not regulated by insulin in the rat mammary gland.

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