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ORIGINAL CONTRIBUTION

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Expression of glucose transporters in lactating human mammary gland epithelial cells

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Summary *Background.* Human milk contains 60–80 g/l lactose and and oligosaccharides. To synthesize this large amount of carbohydrates, the lactating mammary gland has a high demand for precursor molecules. such as glucose and galactose. *Aim of the study.* In the present study we investigated the molecular basis for the uptake of glucose and galactose into the human mammary gland. *Methods.* Using RT-PCR, Southern and Western blotting we analyzed the expression of SGLT1 (sodium glucose cotransporter 1) und GLUT1 (sodium independent glucose transporter) in epithelial cells isolated from fresh human milk. *Results.* Southern blot analysis of the amplicions revealed the expression of SGLT1 mRNA but not of GLUT1 mRNA in milk epithelial cells. Using Western blotting, SGLT1 protein was identified in human milk cells. *Conclusions.* Our findings indicate that 1) the cell fraction isolated from fresh human milk is a suitable model for investigating gene expression in the human mammary gland and 2) lactating human mammary gland epithelial cells are supplied with monosaccharides mainly via SGLT1.

Key words SGLT1 – GLUT1 – lactation – human milk

Abbreviations: SGLT, sodium dependent glucose transporter; GLUT, sodium independent glucose transporter; RT, reverse transcription; DIG, digoxigenin

Introduction

Besides 35–40 g/1 fat and 10–13 g/1 protein, human milk contains 60–80 g/l of different carbohydrates [1]. To synthesize this large amount of lactose and lactose-derived oligosaccharides the mammary gland has a high demand for precursor molecules such as glucose and galactose. Previous investigations with stable isotopes indicate that a part of orally applied D-[1-^{13}C]galactose is directly used in the human mammary gland for the biosynthesis of carbohydrates without being metabolized in other organs [2]. In the present study we investigated the first step of monosaccharide utilization, the transport of glucose and galactose into the human mammary gland. Normally, this is the rate limiting step in the biosynthesis of milk lactose as has been shown in animal studies [3].

So far, two different types of glucose transporter families are known in mammals, the sodium dependent secondary active Na^+/glucose transporters (SGLT1 and SGLT2) and the facilitative glucose transporters (GLUT 1–7). Whereas the SGLT cotransporters use the electrochemical Na^+-gradient to move glucose against its chemical gradient across the membrane, the facilitative glucose transporters move glucose only along its concentration gradient.

Within the SGLT cotransporters, only SGLT1 is capable of transporting glucose and galactose [4, 5]. SGLT1 is mainly expressed in the brush-border membrane of mammalian intestine and kidney where it transports monosaccharides from the lumen into the cell [4, 6, 7].

The family of facilitative glucose transporters is divided into seven distinct isoforms. They are encoded by different genes and their expression is tissue specific [8]. The

GLUT1 isoform transports glucose along with galactose. Its expression has been demonstrated in many tissues, such as erythrocytes, brain, placenta, and kidney [9]. Furthermore, Western and Northern blot analysis have revealed GLUT1 expression in bovine and rat lactating mammary tissue [3, 10, 11].

Up to now there are no data available an sugar transporters expressed in the human lactating mammary gland. Studies are hampered by the limited availability of breast tissue samples. Therefore, we have investigated whether this limitation can be overcome by isolating cells from fresh human milk collected at least one month post partum. At this stage of lactation mammary gland epithelial cells predominate, whereas in the early stage of lactation human milk also contains macrophages, polymorphnuclear leukocytes and lymphocytes. [12, 13, 14].

In order to analyze the expression of SGLT1 and GLUT1 mRNA, we followed two different approaches. In one, total RNA was prepared and subjected to reverse transcription; in the other, milk epithelial cells were directly used for RT-PCR without prior RNA-isolation. The second method has the advantage that the few cells isolated from approximately 3 ml milk are sufficient for the detection of expressed genes. Furthermore, the expression of SGLT1 protein was investigated in human milk cells.

Materials and Methods

Isolation of epithelial cells

Epithelial cells were isolated from fresh human milk from four lactating women (1.5–8 months post partum) following a slightly modified method described by Lindquist et al. [23] who demonstrated that these cells express the human milk typical ß-casein. In brief, 30 ml milk were centrifuged for 15 min at 4 °C and 500 *g*. The upper layer, the milk fat, was removed and the remaining supernatant discarded. The pellet, containing the epithelial cells, was resuspended in HEPES buffered saline), pH 6,8 (134 mM NaCl, 2.68 mM KCl, 1.8 mM $CaCl_2$*$2H_2O$, 1.05 mM $MgCl_2$*$6H_2O$, 2.5 mM HEPES, 0.42 mM NaH_2PO_4*$6H_2O$, 10 mM Glc*H_2O) and centrifuged at 3000 *g* (15 min, 4 °C) to remove remaining milk proteins and fat globules. After discarding the supernatant, 1 ml HEPES was added and the cells were either directly used or stored in aliquots of 100 µl at –20 °C.

Characterization of the viability of epithelial cells from fresh human milk

To test the viability, the cell fraction was isolated from 1–2 h old human milk and then directly stained with the fluorophores Calcein AM (1 µM) and Ethidium Homodimer–1 (6 µM, both Molecular Probes, Leiden, NL) for 25 min at room temperature. The excitation was at 488 nm. Due to the different emission wavelengths [515 nm for Calcein AM and 635 nm for Ethidium Homodimer–1, the fluorescence was measured in a 2-channel experiment using a dichroic mirror (580 nm)]. Both the transmission and the fluorescence images were analyzed using a confocal laser scanning microscope, CLSM Noran OZ (Middelton, USA) and a Nikon Plan 1.4 Oil DIC H objective.

RNA preparation

Total cellular RNA was isolated according to the guanidine thiocyanate method described by Chomzynski and Sacchi in 1987 [16]. In brief, approximately $7x10^6$ cells were homogenized with a Polytron in 0.5–1 ml extraction buffer (4 M guanidinium thiocyanate, 25 mM sodium citrate, 0.5 % lauroylsarcosin, 0.1 M mercaptoethanol). RNA was extracted by adding 2 M sodium acetate, pH 5.2, phenol and chloroform (0.1/1/0.2, v/v/v). After 15 min incubation an ice and 10 min centrifugation at 10 000 *g*, the RNA in the supernatant was precipitated by adding isopropanol. The pellet was resuspended in extraction buffer and again precipitated by isopropanol. After washing the RNA pellet with 70 % ethanol, it was redissolved in sterile H_2O. The concentration of RNA was determined by spectrophotometry at 260 and 280 nm.

Direct RT-PCR

Prior to RT-PCR (reverse transcription with subsequent PCR), whole cells had to be lysed to release their RNA. Cells isolated from 3 ml fresh human milk were centrifuged and the supernatant discarded. The pellet was resuspended in 10 µl lysis buffer (4 % TritonX–100/10 mM dithiothreithol). 5 µl of the lysed cells were then submitted to RT-PCR.

RT-PCR conditions

500 ng total RNA or 5 µl of the lysate were subjected to RT, which was performed with 9 U AMV-RT (avian myeloblastosis virus-reverse transcriptase, Promega, Heidelberg, Germany) in 2 µl 10x reaction buffer, with 40 U RNasin, 4 µl 25 mM $MgCl_2$, 2 µl dNTPs (each 10 mM), 250 ng Oligo-dT-primer and H_2O to a final volume of 20 µl. For each sample a negative control (without AMV-RT) was carried out. The reaction mix was incubated for 30 min at 42 °C. Immediately after terminating the reaction by heating the sample for 4 min to 92 °C, 5 µl of each sample was added to the prepared PCR reaction mix. The PCR reaction mix contained 2.5 U Taq DNA polymerase (Perkin Elmer, Weiterstadt, Germany), 4.5 µl of the corresponding 10x reaction buffer, 20 pmol of both forward and backward

primer, 2 µl dNTPs (each 2.5 mM) and H_2O to a final volume of 50 µl. After 2 min at 92 °C the PCR was performed over 5 cycles as follows: 15 s denaturation at 92 °C, 30 s annealing at the annealing temperature of the primers, minus 3 °C, and 30–60 s synthesis at 72 °C. Then 30 cycles were performed with 15 s 92 °C, 30 s annealing at the annealing temperature of the primers, and 30–60 s 72 °C. The synthesis time depended on the size of the amplified fragment. For the final synthesis the reaction was held for 5 min at 72 °C.

Nested-PCR

To specify the result obtained from the first RT-PCR, a second nested PCR was performed, in which one of the two primers reacted within the first fragment, whereas the other was adopted from the first reaction. The PCR reaction mix contained 1 µl template from the RT-PCR (diluted 1:100–1:500), 20 pmol of each primer, 2.5 U Taq DNA polymerase (Perkin Elmer, Weiterstadt, Germany), 5 µl of the corresponding 10x reaction buffer, 2 µl dNTPs (each 2.5 mM), 1 µl 25 mM $MgCl_2$ and H_2O to a final volume of 50 µl. The PCR cycles were the same as described above.

Synthetic oligonucleotides

The oligonucleotides were synthesized on a Beckmann Oligo 1000 synthesizer and were subsequently purified by NAP–10 gel filtration (Pharmacia, Freiburg, Germany). In order to discriminate between genomic and mRNA amplification, upper and lower oligonucleotides were selected in distinct exons.

The sequences and orientation of the selected oligonucleotides are listed for each transporter along with the expected fragment size and the calculated annealing temperature used for each primer pair in Table 1.

Isolation of genomic DNA

PCR amplicons from lysed cells could be derived from either mature mRNA or genomic DNA. To distinguish between the two, genomic DNA was isolated from human blood leukocytes, submitted to PCR and then compared with the PCR fragments obtained from the lysed cells. The PCR conditions and primers were the same as described above, but the synthesis time at 72 °C was prolonged to 3 min.

The isolation of genomic DNA was performed as follows: 15 ml human blood was directly added to ACD-buffer (4.8 g/l citric acid, 13.2 g/l Na-citrate*$2H_2O$, 14.7 g/l glucose) and centrifuged for 15 min at 1300 *g*. The buffy coat, led an top of the erythrocytes, was removed with a Pasteur pipette and again centrifuged for 15 min at 1300 g. The buffy coat was again removed and resuspended in 15 ml extraction buffer (10 mM TrisHCl, pH 8, 100 mM EDTA, pH 8, 20 µg/ml RNase A, 0.5 % SDS) and incubated for 1 h at 37 °C. After adding 1.5 mg of proteinase K, the suspension was incubated for 3 h at 50 °C being gently mixed throughout. The cooled solution was then extracted with the same volume of equilibrated phenol by gentle mixing for 10 min, followed by centrifugation for 15 min at 5000 g at room temperature. The supernatant was removed and again extracted with phenol twice. After the third extraction, the genomic DNA was precipitated by adding 0.4 volumes of 5 M ammonium acetate and 2 volumes of ethanol. To complete the precipitation, the genomic DNA was centrifuged for 5 min at 5000 *g*. After being washed twice with 70 % ethanol, the genomic DNA was redissolved in TE-buffer (10 mM Tris, 1 mM ETDA, pH 8) during two days. The concentration was determined photometrically. 100 ng of genomic DNA was used for each PCR reaction.

Southern blotting

Aliquots of the PCR reactions (10 µl for SGLT1 and 2 µl for GLUT1) were separated on 1 % or 1.5 % agarose gels containing ethidiumbromide. To identify specific products,

Table 1 Sequences and orientation of selected oligonucleotides for SGLT1 and GLUT1. The expected fragment size and annealing temperature used for each primer pair are given

transporter	No	orientation (5'–3')	sequence	pair	fragment size [bp]	annealing temperature [°C]
SGLT1	1	forward	ATGGTGTGGTGGCCGRTTGG			
	2	backward	CAGGTCATYGTGCAGCGCTG	1–3	1016	54
	3	backward	CTGRCCTCCATCTTCAACAG	1–2	719	59
GLUT1	1	forward	GGGCATGATTGGYTCCTTCTCT			
	2	forward	CCTGCTGCTSAGCDTCATCTT	1–3	650	60
	3	backward	TAGAAVACVGCGTTGATGCC	2–3	315	60

PCR products were transferred to a positively charged nylon membrane (Boehringer Mannheim, Mannheim, Germany). After crosslinking the filter-bound DNA with UV-light ($\lambda = 254$ nm), the filter was hybridized overnight at 42 °C with specific DIG-labelled cDNA-probes (see below). Hybridization and chemoluminescence detection were performed according to the suppliers instructions (Boehringer Mannheim, Germany).

DIG-labelling of DNA-probes

Digoxigenin (DIG)-labelled probes for hybridization were generated by PCR. The reaction mix contained 5 µl of dNTP-mix including DIG–11-dUTP:dTTP = 1:3 (Boehringer Mannheim, Mannheim, Germany), 1 µl template as described below, 20 pmol of both forward and backward primer, 2.5 U Taq DNA-polymerase (Perkin Elmer, Weiterstadt, Germany), 4.5 µl of the corresponding 10x reaction buffer, 1 µl 25 mM $MgCl_2$ and H_2O to a final volume of 50 µl. The PCR-conditions were the same as described above.

As template we chose for SGLT1 1 ng of the plasmid pAKs5c3, in which the cDNA of rabbit SGLT1 was inserted. The rabbit SGLT1 specific primers led to a 1985 bp long fragment. The GLUT1 probe was generated using total RNA from human breast cell line HS578Bst (Cancer Center Essen, Germany) as template for the RT-PCR with GLUT1 primer 1 and 3. After cleaning the amplicon with Qiaquick purification kit (Qiagen, Hilden, Germany) and alter verifying the sequencing as GLUT1 sequence, 1 µl of the diluted (1:500) RT-PCR-product was used as template to prepare the DIG-labelled probe.

Sequence analysis

The non-radioactive sequence analysis was carried out using the PRISM AmpliTaq_ FS Big Dye Terminator Cycle Sequencing Kit and following the instructions of Perkin Elmer (Weiterstadt, Germany). The annealing temperatures were matched to the used oligonucleotides. 25 cycles were carried out as follows: 10 s at 94 °C, 5 s at 45 °C and 4 min at 60 °C.

Western blotting

The cells isolated from 50 ml fresh human milk were lysed in lysis buffer [1 % Trition X–100 including the protease inhibitors Aprotinin and Leupeptin (each 20 µg/ml), Pepstatin and Chymostatin (each 5 µg/ml)]. Western blot analysis was carried out as described by Lin et al. [21]. In brief, after being denaturated (2 % SDS, 5 % mercaptoethanol, 10 mM Tris-HCl, pH 7.5), the samples were divided into 2 portions, separated in a pre-cast SDS Tris-glycine gradient polyacrylamide gel (8–16 %; Novex, San Diego, CA, USA) and transferred onto a nitrocellulose membrane. After blocking with 5 % fat-free milk solution, the SGLT1 protein was detected by using a polyclonal antipeptide-antibody Pan–3 [21], which was directed against the amino acid sequence 606 to 627 of rabbit intestinal SGLT1. This sequence is specific for SGLT 1 but is not present in SGLT 2. One sample was incubated in the presence of excess molar amounts of the corresponding antipeptide A606. The visualization was performed using the anti-sheep-IgG-peroxidase and the ECL-system of Amersham Buchler (Braunschweig, Germany).

Results

Prior to the investigation of the mRNA and protein expression of different glucose transporters, the cell fraction isolated from fresh human milk was examined for purity and viability. Besides epithelial cells, which were easily recognized by their fat droplets, 4 % non-epithelial cells were found. About 47 % of the milk epithelial cells were stained with Calcein AM indicating cell viability. The other epithelial cells were stained with Ethidium Homodimer–1, which is a marker for cell membrane damage (Fig. 1).

Since almost half of the epithelial cells were still alive, it should be possible to investigate gene expression in these cells. Therefore, SGLT1 expression was examined in epithelial cells isolated from fresh human milk. After total RNA preparation, RT-PCR and a subsequent nested-PCR

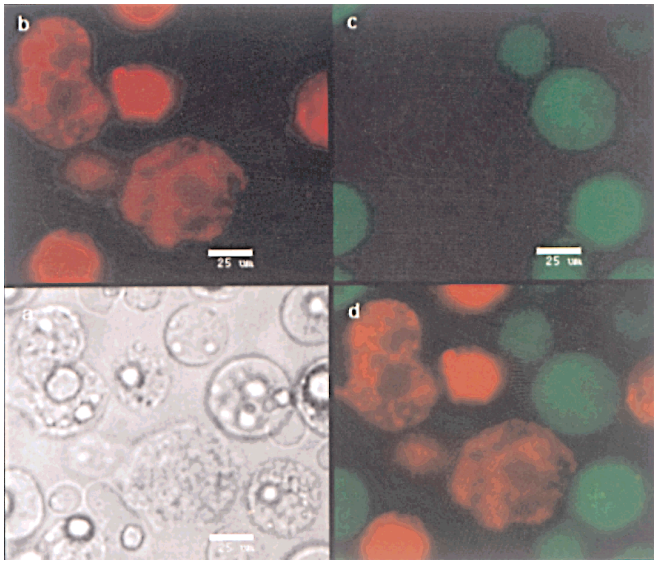

Fig. 1 Cells isolated from fresh human milk shown as a transmission (a) and as fluorescence images (b-d). The cells were stained with Calcein AM and Ethidium Homodimer–1. b: emission by Ethidium Homodimer–1, c: emission by Calcein AM, d: b and c overlapped.

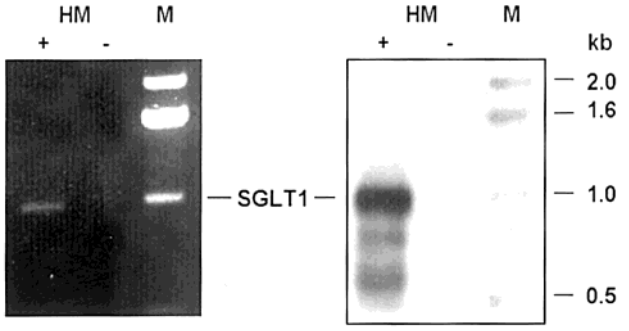

Fig. 2a Expression of SGLT1 mRNA in human mammary gland cells. Total RNA was used as template in the RT-PCR. Total RNA (500 ng each) was subjected to RT-PCR. The primers used in the PCR reaction led to the expected fragment with a size of about 1000 bp. One fifth of the PCR volume (10 µl) was separated by electrophoresis in a 1.2 % agarose gel and bands were visualized by staining with ethidiumbromide (left panel). DNA-fragments were transferred onto a nylon membrane and hybridized with a DIG-labelled SGLT1 probe (right panel). +, RT-PCR with AMV-reverse transcriptase, –, RT-PCR without AMV-reverse transcriptase, M, marker.

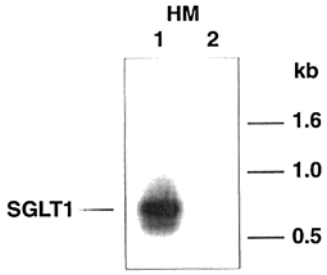

Fig. 2b Southern blot analysis of SGLT1 mRNA expression after RT-PCR and subsequent nested-PCR of epithelial cells isolated from human milk. Epithelial cells from 1.5 ml fresh human milk (1.5 month postpartum) were first lysed, then subjected to RT-PCR, followed by a second nested PCR. The amplicon was 719 bp in. size. 10 µl of the PCR products were electrophoresed in a 1.2 % agarose gel, transferred onto a nylon membrane and hybridized with a DIG-labelled SGLT1 probe. Lane 1, with AMV-reverse transcriptase; lane 2, without AMV-reverse transcriptase (negative control).

were performed using SGLT1 specific primers. The products were then separated by gel electrophoresis. Visualization by staining with ethidiumbromide resulted in several fragments, the strongest band showed the expected size of about 1000 bp (Fig. 2a). Furthermore, PCR-products were transferred onto a nylon membrane, which was hybridized with the DIG-labelled SGLT1 DNA-probe. As demonstrated in the right panel of Fig. 2a, SGLT1 mRNA expression was clearly detectable in epithelial cells isolated from the lactating mammary gland by using RT-PCR.

Since the supply of human milk is often limited, we investigated whether the expression of genes in mammary gland epithelial cells can be demonstrated without prior RNA preparation. Therefore, whole epithelial cells isolated from human milk were lysed and submitted directly to RT-PCR and subsequent nested-PCR. The Southern blot revealed a fragment with the expected size of 720 bp for SGLT1 (Fig. 2b). Sequence analysis confirmed its complete identity to human renal and intestinal SGLT1.

In addition, the expression of GLUT1 was investigated in the human mammary gland cells. However, in contrast to SGLT1 we failed to detect GLUT1 mRNA in epithelial cells from human milk, both when total RNA or whole cells were used as template in the RT-PCR and the subsequent nested-PCR (Fig. 3). The fragments seen in lane 3 and 4, which represent the amplicons from the RT-PCR and nested-PCR of lysed epithelial cells, are significantly longer than expected. Their fragment sizes are equal to the amplicons of genomic DNA isolated from human leukocytes (lane 5, 6) and fit to the expected intron length: for the RT-PCR amplicon a size of 1566 bp and for the nested-PCR product one of 404 bp. Thus, the GLUT1 amplicons seen in lysed epithelial cells seem to originate from genomic DNA.

Since only SGLT1 mRNA expression was detectable in the mammary gland epithelial cells, it was further investigated whether the expression of SGLT1 could be demonstrated at the protein level. Therefore, a Western blot analysis of cell lysats was performed. In cells isolated from fresh human milk, two bands of SGLT1 protein with sizes of about 130 kDa and about 260 kDa were detectable with the antipeptide-antibody Pan–3 (Fig. 4). The antigen reaction was suppressed by the addition of the antigen peptide A606.

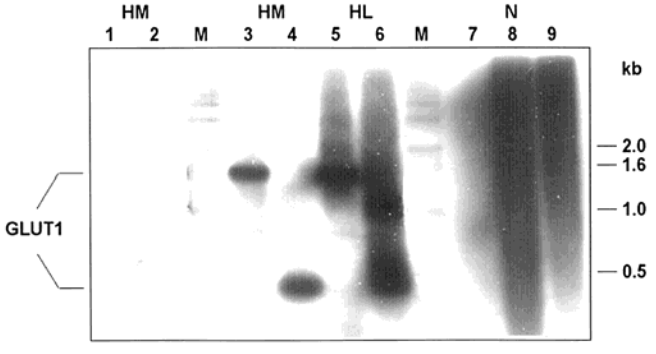

Fig. 3 Southern blot analysis of genomic GLUT1 DNA and of GLUT1 mRNA expression in human milk cells after RT-PCR and subsequent nested-PCR. 1 µl of each PCR reaction was subjected to a 1.5 % agarose gel and electrophoretically separated. The DNA was then transferred onto a nylon membrane and hybridized with a DIG-labelled GLUT1 probe. The templates were: lane 1, 2, and 7, total RNA from human milk epithelial cells (HM, 1.5 month post partum); lane 3, 4, 8 and 9, lysed epithelial cells from human milk (1.5 month post partum); lane 5 and 6, genomic DNA (HL). The first PCR led to a 650 bp fragment (lane 1, 3, 5, 7,and 8). The nested PCR was performed with the primer pair 2–3 (315 bp fragment, lane 2, 4, 6 and 9). Lane 7–9 were negative controls (N, without AMV-reverse transcriptase). M, marker.

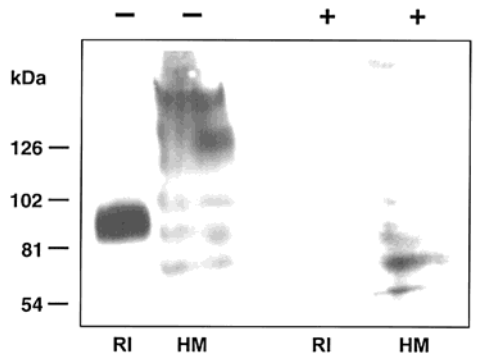

Fig. 4 Western blot analysis of SGLT1 protein expression in human milk epithelial cells. After cells from 25 ml human milk (HM) were lysed in lysis buffer (see Material and Methods for details), they were separated in a 8–16 % Tris-Glycin-Polyacylamid gel and transferred onto a nitrocellulose membrane. The blot was incubated with the antipeptide-antibody Pan–3 in the presence (+) or absence (–) of the antigene peptide A606. As control protein from rabbit intestine (RI) was used.

Discussion

In this study, we showed that the cell fraction isolated from fresh human milk contained almost exclusively epithelial cells. About 50 % of these cells were still alive 2 h after the lactating women had collected the milk. This is an important prerequisit, which had to be fulfilled regarding the examination of gene expression. Since fresh human milk is often available only in small amounts, the quantity of RNA that can be isolated is rather low. Therefore, we additionally investigated the gene expression directly in epithelial cells isolated from human milk without preceeding RNA-extraction. With both systems we were able to show that RT-PCR can be successfully performed to prove gene expression. Since SGLT1 was already found after the first PCR-reaction, it seems likely that it originated from the mammary gland epithelial cells and not from the fraction of non-epithelial cells, which was 4 % of the whole cell population. While we detected the expression of SGLT1 mRNA, we were unable to detect GLUT1 mRNA in human milk epithelial cells. These findings are in contrast to studies from other groups [2, 10], which demonstrated GLUT1 expression in the lactating bovine and rat mammary gland. This discrepancy might be explained by the use of different tissue samples. Whereas we used human milk which is not contaminated by fibroblasts and represents a source of normal epithelial cells [14, 20], other groups used either total mammary glands or tissue slices for RNA-preparation [2, 10]. Therefore, it could be possible, that in other groups GLUT1 expression originated from fibroblasts or myoepithelial cells.

In contrast to the lactating human mammary gland, GLUT1 mRNA expression was also shown in the non-lactating bovine and rat mammary gland [2, 10] and in the human breast cell line HS578Bst (data not shown). These differences in the expression of glucose transporters in different stages of breast development could be explained regarding the different tissues within the mammary gland during these periods. Before conception the mammary gland mainly consists of collagen connective tissue and some short branched duct systems embedded in adipose tissue [17]. Later in gestation and early lactation, cellular development and proliferation of the duct system occurs and therefore a change in the gene expression seems possible. During lactation, the high demand for monosaccharides, which are needed for the biosynthesis of 60–80 g lactose and oligosaccharides per litre milk [1], probably makes it necessary that a specific monosaccharide transport system with a high capacity is expressed in the lactating mammary gland.

From the brush-border membrane of the small intestine [6] and the proximal tubules of the kidney it is known that a sodium-dependent glucose transporter, SGLT1, with a molecular weight of 80–90 kDa efficiently takes up glucose as well as galactose from the lumen into the cell [4, 7]. In the human mammary gland, however, the transport of glucose and galactose, the main monosaccharides used in milk synthesis, is from the blond into the breast cells. Therefore, we suppose that in contrast to the apical localization of SGLT1 in the intestine and kidney, SGLT1 is localized in the basolateral membrane in the lactating mammary gland. A basolateral SGLT1 localization was reported from Shillingford et al. [19]. They showed in sheep parotis a SGLT1 protein with a molecular weight of 66 kDa, which is targeted to the basolateral membrane. Additionally, they also revealed in lactating sheep mammary gland a 66 kDa SGLT1 protein [19]. We, however, detected a SGLT1 protein of a molecular size of about 130 kDa and 260 kDa suggesting that SGLT1 formed multimeres. The bands were specific for SGLT1, since they disappeared after incubating the sample in the presence of excess amounts of the corresponding antipeptide. Therefore, it seems likely that in the lactating human mammary gland SGLT1 is directed rather to the basolateral than to the apical membrane. Nevertheless there is some consideration from Neville et al. [22] that SGLT1 could also be integrated in the apical membrane. While lactose is secreted into the milk via an exocytotic pathway, they suppose that glucose is most likely transported across the apical membrane into the milk where it probably regulates the milk volume. This conclusion was drawn since after i. v. infusion of [6,6-$^{2}H_{2}$]-Glc ^{2}H appeared earlier in milk Glc than incorporated in milk lactose molecules. Taken together, the functional properties and the cellular localization of the SGLT1 in the lactating human mammary gland remain to be determined.

So far, data were only available an the expression and functional properties of SGLT1 in lactating animal mammary glands [18, 19]. Shennan and Beechey [18] investigated the SGLT1 expression in lactating rat mammary gland. After total RNA preparation they showed using

Northern blot analysis that a 4 kb transcript hybridized with the cDNA for rabbit intestinal SGLT1. Western blot analysis with rabbit antibody for SGLT1 revealed that SGLT1 protein was enriched in a fraction that contained endoplasmatic, Golgi and plasma membranes. Furthermore, they showed by flux studies that 3-O-methyl-D-glucose was transported Na^+-dependent across the plasma membrane. However, the transport was not inhibited by the classical SGLT1 inhibitor phlorizin.

In conclusion, the cell fraction isolated from fresh human milk (> 1 month post partum) is a suitable model for investigating gene expression in the human mammary gland. Our findings indicate that in human lactating mammary gland only SGLT1 mRNA and protein expression occurs with no indication of GLUT1 mRNA expression. Therefore, it seems likely that in lactating mammary gland epithelial cells SGLT1 is localized in the basolateral membrane to supply the cells with glucose and galactose which are precursor molecules for the biosynthesis of lactose and oligosaccharides.

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