Glucose Transport by Acinar Cells in Rat Parotid Glands

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Key Words

Glucose transporters • Salivary glucose • SGLT1 • GLUT1 • GLUT2 • GLUT4 • Acinar cells

Abstract

Background/Aims: Salivary glucose is often considered as being from glandular origin. Little information is available, however, on the possible role of glucose transporters in the secretion of the hexose by salivary glands. The major aim of the present study was to investigate the expression and localization of several distinct glucose transporters in acinar cells of rat parotid glands. **Methods:** Quantitative real-time PCR analysis, immunohistochemistry and western blotting techniques were used to assess the presence of SGLT1, GLUT1, GLUT2 and GLUT4 in acinar cells of rat parotid glands. **Results:** Quantitative real-time PCR documented the expression of SGLT1 and GLUT1 in parotid tissues, with a much lower level of GLUT4 mRNA and no expression of GLUT2 mRNA. Western blot analysis revealed the presence of SGLT1, GLUT1 and GLUT4 proteins, but not GLUT2 proteins in the parotid extract. Immunohistochemistry confirmed these findings. SGLT1 was specifically located at the baso-lateral membrane, co-localizing

with Na⁺/K⁺ ATPase. GLUT1 was found both at the baso-lateral and apical level. GLUT4 appeared to be also located at the baso-lateral level. However, too little GLUT4 was present to allow co-localization labeling. **Conclusion:** Based on these findings, a model is proposed for the transport of glucose into the acinar cells and thereafter into the acinar lumen.

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Introduction

Saliva is a fluid with a specific composition and specific roles [1]. Like every biological fluid, some salivary components could be used to measure general parameters. In the view of a simple and rapid diagnosis of diabetes mellitus, it could be interesting to establish a correlation between salivary glucose and plasmatic glucose concentrations, and many authors found higher glucose salivary levels in diabetic patients versus control patients [2-4]. Unfortunately, this correlation is not so evident [5-7]. To explain this supposed correlation, a study of the biochemical secretion of glucose by glandular cells is required. The glandular origin of salivary glucose was showed [5, 6, 8]. The variations of glucose concentration between diabetic and control patients supported this

hypothesis [2-4, 7]. In 1983, a paracellular pathway for glucose secretion in rat submandibular and sublingual glands was described [9]. Recently, Sabino-Silva and col. described the presence of SGLT1 transporters in basolateral membranes of acinar parotid cells [10, 11]. This last hypothesis seems to corroborate with the glucose concentration measured during an oral glucose tolerance test: a similar time course pattern was observed for salivary glucose levels and glycemia [7]. A preferential diffusion mediated by specific glucose transporters has to be implicated. The most popular glucose transporters are part of the GLUT family. To date, 14 members of the GLUT family have been identified [12]. The aim of this study is to determinate the glucose transporters present in the acinar cells of parotid glands of rats and their localization, in order to understand the mechanism of glucose secretion. By analogy with the kidney or enterocyte models, if SGLT1 is present at the baso-lateral membrane of the acinar cells, another transporter has to be implicated at the apical membrane. GLUT1, GLUT2 GLUT4 and SGLT1 are considered in this article as potential transporter. It is well known that GLUT1 is a ubiquitous transporter; GLUT2 is specific for liver tissue; GLUT4 for muscle tissue. SGLT1 is a Na-Glucose co-transporter [13].

Materials and Methods

Animals and experimental design

Six Wistar female rats (weight: 250g) were purchased from Charles River Company, France, and housed in the animal facilities of our laboratory. They had access to food and water ad libitum and were maintained in a 12:12 h day:night cycle. They received care in agreement with the national legal requirements, the local ethic committee rules and the European Communities Council Directive (86/609/EEC). Euthanasia by exsanguination and decapitation was further achieved under deep anesthesia using ketamine (60 mg/kg) and xylazine (7.5 mg/kg).

Parotid glands, liver, kidney, muscle and pancreas were removed and further processed for quantitative real-time PCR analysis, western blot and immunohistochemistry studies.

Quantitative real-time PCR analysis

The quantitative real-time PCR analyses were realized using a previously described method [14]. Prior to extraction, the organs were kept at -80°C. Total RNA was extracted using a Tripure lysis solution and a High Pure RNA Tissue Kit (Roche Diagnostics, Vilvoorde, Belgium) according to the manufacturer's protocol, which included DNase treatment. cDNA was obtained from total RNA after a reverse-transcription step using SuperScript II RT enzyme (Invitrogen, Belgium). The mRNA quantification was performed next using a real-time

Gene name Accession n°	Sense primer (5'-3')	Antisense primer (5'-3')	Amplicon size (bp)
SGLT-1 NM_013033.2	CATGATTGCAGGAAGGTTGTT	GGTAAGTGGTGATGGACTGGA	122
GLUT-1 NM_138827.1	ACTCCATGCTGATGATGAACC	CAGGCCACAGTACACTCCAAT	125
GLUT-2 NM_012879.2	CTTGTTTCATGTTGCTGAAT	GAAGTCCCAATGTACTGGAA	125
GLUT-4 NM_012751.1	CTCTCAGGCATCAATGCTGTT	GAGACCAACGTGAAGACGGTA	122
GAPDH NM_017008	AATGTAATCCGTTGTGGATCT	CAAGAAGGTGGTGAAGCAGG	79

Table 1. This table specifies the primer sequences of the antibodies used for quantitative real-time PCR analysis.

quantitative polymerase chain reaction on a LightCycler 480. Primer sequences (Table 1) were designed with Primer3 software (Whitehead Institute for Biomedical Research, Cambridge, MA). Quantitative PCR reaction was achieved using a commercial kit containing SYBR Green (Eurogentec, Seraing, Belgium) and the cycling program was: 5 minutes at 95°C, 15 seconds at 95°C for 40 cycles, and 30 seconds at 60°C. According to the delta Ct method, the gene expression level of each mRNA was calculated, further normalized to GAPDH (glyceraldehyde 3-phosphate dehydrogenase enzyme) mRNA, and related to control samples. Specific amplification was confirmed by melting curve analysis and migration on agarose gel.

Immunohistochemistry

The immunohistochemistry analyses were realized using a previously described method [15]. After fixation of the organs in 10% formalin followed by a paraffin-embedding procedure, 7µm-sections were rehydrated, endogenous peroxidases were blocked (0.3% H2O2 in methanol) and non-specific binding antibodies inhibited for 1 hour with 10% goat serum.

Polyclonal GLUT1 (Millipore, ref. #07-1401, Overijse, Belgium), GLUT2 (Millipore, ref.#07-1402, Overijse, Belgium), GLUT4 (Millipore, ref.#07-1404, Overijse, Belgium) or SGLT1 (Millipore, ref.#07-1417, Overijse, Belgium) antibodies were incubated overnight at room temperature. Manipulations were conducted at 1/500 dilution. After washing, the bound antibodies were revealed by staining with a secondary biotinylated goat anti-polyvalent IgG (Immunologic, Duiven, The Netherlands) followed by avidin-biotin-peroxidase and diaminobenzidine as a substrate. Used as control, sections were incubated with secondary antibody alone and organs expressing different glucose transporters were also included. To confirm localization of the GLUT transporters on the membrane, co-localizations studies of Na⁺/K⁺ ATPase and GLUT transporter were realized. Immunohistochemical labeling was performed using a fluorescein-labeled secondary antibody.

Western blotting

The western blotting analyses were derived from a previously described method [16]. Prior to extraction, the organs were kept at -80°C. Tissue extracts were loaded on 6% polyacrylamide gels. Proteins were then transferred to polyvinylidene-difluoride membranes and immuno-labeled using specific polyclonal GLUT1, GLUT2, GLUT4 or SGLT1 antibodies at a dilution of 1/500. Bound antibodies were then detected using an enhanced chemiluminescence method.

Fig. 1. Comparison of SGLT1 mRNA expression level between parotid, kidney (positive control) and muscle tissues (negative control). Mean values ($\pm$ SEM) refers to 4 individual determinations. The asterisks indicate significant differences ($p < 0.05$).

Fig. 2. Comparison of GLUT1 mRNA expression level between parotid, lung (positive control) and muscular tissues (negative control). Mean values ($\pm$ SEM) refers to 4 individual determinations. The asterisks indicate significant differences ($p < 0.05$).

Statistical analysis

All results are presented as mean values ($\pm$ SEM). The statistical significance of differences between mean values was assessed by the use of Student's *t*-test.

Results

Quantitative real-time PCR analysis

SGLT1. As shown in Fig. 1, SGLT1 mRNA was well expressed in parotid cells. Relative to GAPDH mRNA, SGLT1 mRNA expression levels was twice higher ($p < 0.05$) in comparison with the kidney tissue used as a positive control. The negative control was a muscle tissue.

GLUT1. As shown in Fig. 2, the parotid cells expressed mainly GLUT1 mRNA. Lung tissue was chosen as a positive control and muscle tissue as a negative control. Relative to GAPDH mRNA, the expression level of GLUT-1 mRNA was about twice

Fig. 3. Comparison of GLUT2 mRNA expression level between parotid, liver tissues (positive control) and muscular tissues (negative control). Mean values ($\pm$ SEM) refers to 4 individual determinations. The asterisks indicate significant differences ($p < 0.05$).

Fig. 4. Comparison of GLUT4 mRNA expression level between parotid, muscle (positive control) and pancreatic tissues (negative control). Mean values ($\pm$ SEM) refers to 4 individual determinations. The asterisks indicate significant differences ($p < 0.05$).

Fig. 5. SGLT1 (A), GLUT1 (B) and GLUT4 (C) glucose transporters proteins detected in rat parotid glands by western blotting.

higher ($p < 0.05$) in the parotid gland versus the positive control tissue.

GLUT2. As shown in Fig. 3, there was no expression of GLUT2 mRNA in the parotid tissue. Liver tissue was used as positive control. The muscle tissue was chosen as negative control.

GLUT4. As shown in Fig. 4, GLUT4 mRNA was unexpectedly present in parotid cells. The expression level

Fig. 6. Immunodetection of SGLT1 in kidney (A, scale bar = 50 μ m) and parotid tissues (B, scale bar = 10 μ m and C, scale bar = 50 μ m). Immunohistochemical labeling was performed using secondary biotinylated goat anti-polyvalent IgG.

Fig. 7. Double immunolabeling on parotid tissue with specific Na^+/K^+ ATPase antibody (A) and SGLT 1 antibody (B). Double labeling combined (C) specifies the presence of SGLT 1 on the baso-lateral membrane of acinar cells (scale bar = 50 μ m).

Fig. 8. Immunodetection of GLUT1 in exocrine pancreas (A, scale bar = 50 μ m) and parotid tissues (B, scale bar = 10 μ m and C, scale bar = 50 μ m). Immunohistochemical labeling was performed using secondary biotinylated goat anti-polyvalent IgG.

was just a little lower ($p < 0.05$) than the expression level in the skeletal muscle. The pancreatic tissue was chosen as a negative control.

Western blotting

Western blot analysis revealed the presence in parotid tissue of GLUT1, GLUT4 and SGLT1 transporters (Fig. 5). No GLUT2 protein was detected by western blotting.

Immunohistochemical studies

SGLT1. The immunohistochemical method showed the presence of SGLT1 transporter at the level of the acinar cells (Fig. 6). Kidney tissue was used as a positive control. Transporter SGLT1 was specifically located at

the baso-lateral membrane of acinar cells, as judged by fluorescein co-localization with a Na^+/K^+ ATPase antibody (Fig. 7).

GLUT1. Immunohistochemistry showed the presence of GLUT1 transporter in the acinar cells (Fig. 8). Exocrine pancreas was used as a positive control. This transporter was present both at the baso-lateral membrane and at the apical membrane. A double fluorescein-labeling with the baso-lateral Na^+/K^+ ATPase confirmed this observation (Fig. 9).

GLUT2. As expected, there was no GLUT2 transporter in rat parotid glands. Endocrine pancreatic tissue was used as a positive control (Fig. 10).

GLUT4. Immunohistochemical labeling showed the presence of GLUT4 transporter on acinar cell at baso-

Fig. 9. Double immunolabeling on parotid tissue with specific Na^+/K^+ ATPase antibody (A) and SGLT 1 antibody (B). Double labeling combined (C) specifies the presence of GLUT 1 on the baso-lateral and apical membrane of acinar cells (scale bar = 50 μm).

Fig. 10. Immunodetection of GLUT2 in pancreatic islet (A, scale bar = 50 μm) in comparison with parotid tissue (B, scale bar = 10 μm and C, scale bar = 50 μm). Immunohistochemical labeling was performed using secondary biotinylated goat anti-polyvalent IgG

Fig. 11. Immunodetection of GLUT4 in muscle (A, scale bar = 50 μm) and parotid tissues (B, scale bar = 10 μm and C, scale bar = 50 μm). Immunohistochemical labeling was performed using secondary biotinylated goat anti-polyvalent IgG.

lateral membrane (Fig. 11). Muscle tissue was used as a positive control. No double labeling technique was used in this case because the sensitivity of the fluorescent method did not allow a labeling of GLUT4 transporter on acinar cells.

Discussion

The present results contribute to determine the mechanisms of glucose transport by acinar cells inside rat parotid glands [7, 9]. It could correspond to a

transporter-mediated process rather than a paracellular one as suggested by some authors [9].

The main glucose transporters are from the GLUT1 and SGLT1 families. Relative to GAPDH, quantitative Real-time PCR analysis pointed out a double mRNA quantity for SGLT1 and GLUT1 when compared to the respective positive control tissues. In the parotid gland, the expression of GLUT1 exceeded that of SGLT1 ($p < 0.05$).

Unexpectedly, a GLUT4 transporter was found in the acinar cells, the latter transporter being otherwise typically associated to muscular tissue. The analysis of

immunohistochemical figures showed a localization on the baso-lateral membrane. Hence, it does not seem to be attributable to the labeling of myoepithelial cells because it is also present in isolated acinar parotid cells (data not shown). However, the GLUT4 mRNA was found in smaller amounts when compared to the mRNA amounts of the other transporters ($p < 0.05$). This low expression level was responsible for the low fluorescence level measured with a double labeling.

A western blot analysis was performed in order to confirm the presence of these glucose transporting proteins. Immunohistochemical techniques allowed localizing all of these transporters on cellular membranes of acinar cells.

GLUT1 transporter was located as well on the apical as on the baso-lateral level of acinar cells, whereas SGLT1 transporter was only located on the baso-lateral level of acinar cells. Last, the insulin-dependent GLUT4 trans-

porter was located on the baso-lateral level of acinar cells.

Based on these results and on the presence of various transporters in other cellular types such as enterocytes [17] and kidney [18, 19], it is actually possible to suggest a cellular model for glucose secretion by acinar cells.

Thus, glucose is supposed to enter in acinar cells by ways of SGLT1 transporter and GLUT1 transporter. Glucose is supposed to use the GLUT1 transporter to reach the luminal domain of acini. The role of the insulin-dependent GLUT4 transporter requires further investigation.

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