EKSPERIMENTINIS TYRIMAS

Immunohistochemical detection of glucose transporters class I subfamily in the mouse, rat and human testis

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Summary. *A family of glucose transporters (GLUT) mediates the cellular uptake of glucose at the plasma membrane by facilitated diffusion. We investigated the presence of isoforms GLUT1-4 of class I subfamilies in different types of cells in the mouse, rat and human testis by indirect immunofluorescence technique. Immunocytochemical analyses demonstrated that GLUT1 was expressed in the rat testis, GLUT2 in the mouse and rat testis, GLUT3 in the mouse, rat and human testis and GLUT4 was not presented in the testis at all. A very intensive positive immunoreaction for GLUT3 was found in Sertoli cells, peritubular myoid cells, macrophage-like interstitial cells, testicular endothelial cells and early spermatocytes. GLUT3 positive cells were not found in the luminal part of Sertoli cells, spermatids or Leydig cells. The present results suggest that glucose uptake in different testicular cells is mediated by GLUT1, GLUT2 and GLUT3 and the GLUT3 was the prominent glucose transporter type in the testicular cells.*

Introduction

Glucose uptake and metabolism are essential for proliferation and survival of cells and is usually carried out through glucose transporters – the integral membrane proteins. Two families of glucose transporters have been identified: the facilitated-diffusion glucose transporter family (GLUT family), and the Na(+)-dependent glucose transporter one (SGLT family). Proper localization of glucose transporters and gap junctions is a prerequisite for successful transepithelial transport of sugars (1). Active transport accumulates glucose in specific, whereas facilitative transport equilibrates blood glucose and intracellular glucose inside all mammalian cells (2). Glucose, fructose and dehydroascorbic acid enter mammalian cells via facilitated diffusion, a process regulated by different glucose transporter isoforms at the plasma membrane. Thirteen members of the family of facilitative sugar transporters (GLUT1-12 and the myo-inositol transporter HMIT) are now recognized. These various transporters exhibit different substrate specificities, kinetic properties and tissue expression profiles (3). On the basis of sequence similarities and characteristic elements, the extended GLUT family can be divided into three subfamilies, namely class I (GLUT1-4), class II (GLUT5, 7, 9, 11), and class III (GLUT6, 8, 10, 12 and HMIT) (4).

It is largely known the fertility of germ cells is in direct connection with glucose metabolism of these cells and the spermatogenesis is disturbed in diabetes, causing infertility. Factors involved in the development of infertility in males with insulin-dependent diabetes mellitus are poorly characterized (5).

Malignant cells have been shown to utilize more glucose than normal cells *in vitro* and *in vivo*. GLUT3 mRNA levels has been found to be elevated in human cancers, indicating that it may play a role in glucose uptake by cancer cells. GLUT3 may be an attractive target for monoclonal therapy or imaging of testicular germ cell tumor (6). In human lung carcinomas, GLUT1 expression is seen in most cases of lung carcinoma, and a few cases of lung carcinoma show positive staining for GLUT3 and GLUT4 (7). In normal tissues GLUT3 has been detected in placenta (6). GLUT3 is also present in human adult and fetal myocardium (8) and in the rat lens (9). GLUT1, 3 and 4

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were reported in abundance in several brain regions and GLUT3 seems to uphold its suggested role in synaptic energy provision and neurotransmitters synthesis (10). The transport of glucose across the mammalian blood-brain barrier is mediated by the GLUT1, which is concentrated in the endothelial cells of the cerebral microvessels (11). To accommodate the high glucose flux, platelets express extremely high concentrations of the most active glucose transporter isoform, GLUT3 (12).

The aim of present study is to demonstrate the distribution of glucose transporters 1–4 (GLUT1-4) in the mouse, rat and human testis to see if glucose uptake mediated by these proteins is active in testicular cells.

Material and methods

Experimental animals and tissues. Adult BALB/c male mice (n=12, body weight 25–30 g) and adult male Wistar rats (n=12, body weight 180–220 g) were used as donors of normal testicular tissue. The animals had free access to food and water and they were maintained in a normal dark/light cycle. Permissions for the experiments and for use of organs from the animals after sacrifice with CO_2 were granted by the local animal authorities.

Human testis tissue was obtained from three patients undergoing orchiectomy either due to prostatic cancer (n=2) or hydrocele (n=1) in 1998–1999. The patients were being treated at the Turku University Central Hospital. Permissions for tissue donations and for use of organs for research purposes were granted by the Hospitals and Universities joint Ethical board. Neither of the patients had received any anti-gonadotropin medication prior to the castrations.

The testes were removed and frozen immediately in liquid nitrogen and kept at –70°C until use. Sections of 6 µm in thickness were cut in a cryostat, dried on slides in the air and fixed in cold (–20°C) acetone for 5 min. The acetone was allowed to evaporate before storing the sections at –20°C. The acetone fixation instead of paraformaldehyde was chosen to avoid the destruction of antigenicity of antigens by paraformaldehyde.

Immunohistochemistry. The indirect immunofluorescence method was used for the expression of GLUT1, GLUT2, GLUT3 and GLUT4 genes.

The sections were washed for 3×2 min in PBS (phosphate-buffered saline; 140 mM NaCl, 8 mM Na_2HPO_4, 2mM NaH_2PO_4, pH 7.4) at room temperature to allow stabilization of the temperature before incubation.

The non-specific binding sites were blocked by incubating the sections with 5% normal rabbit serum in PBS for 15 min. Thereafter, the sections were washed for 3×5 min in PBS and incubated for 60 min with a primary antibody at room temperature. Goat polyclonal anti-GLUT1, -GLUT2, -GLUT3, -GLUT4 IgG (Santa Cruz Biotechnology) was used as primary antibody. The primary antibody was used in a dilution of 1:100 in 1% BSA (bovine serum albumin) in PBS. In control slides the primary antibody was replaced by the 5% normal goat serum. After 3×5 min washing in PBS, the sections were incubated for 30 min with a secondary antibody. The secondary antibody was diluted 1:50 in 5% normal mouse, rat or human serum in PBS. FITC-conjugated rabbit-anti-goat Ig (Dako, cat. no. F 0234, Copenhagen, Denmark) was used as secondary antibody. The sections were washed for 3×5 min in PBS and mounted in 1,4-diazabicyclooctane (DABCO, Sigma, St. Louis, MO)-containing glycerol (50% glycerol in 2× PBS, 0.1% NaN_3 and 100 mg/ml DABCO).

The sections were examined and photographed under an ultraviolet-microscope equipped with an appropriate filters (Leitz, Wetzlar, Germany) and × 20 and × 40 objectives. There was estimated the color of fluorescence to differentiate the testicular auto-fluorescence from GLUT positive cells. The FITC-fluorescence is bright green, whereas auto-fluorescence seems yellowier. Only bright green fluorescence was seen in our samples.

Results

GLUT1

Positive immunoreaction for GLUT1 was found in the rat testis in peritubular myoid cells, macrophage-like interstitial cells, testicular endothelial cells and spermatocytes (Fig. A, Table). GLUT1 positive cells were not found in Sertoli cells or Leydig cells in the rat testis. Positive immunoreaction for GLUT1 was not detected in the mouse and human testis.

Table. **Expression of the GLUT1, GLUT2, GLUT3 and GLUT4 genes at the protein level in the mouse, rat and human testis by immunohistochemistry**

Testis	GLUT1	GLUT2	GLUT3	GLUT4
Mouse testis	0	12/12*	12/12* +++	0
Rat testis	12/12*	12/12*	12/12* +++	0
Human testis	0	0	3/3* +++	0

*$p<0.05$ vs. GLUT 4, X^2-test.
+++ intensive immunoreaction.

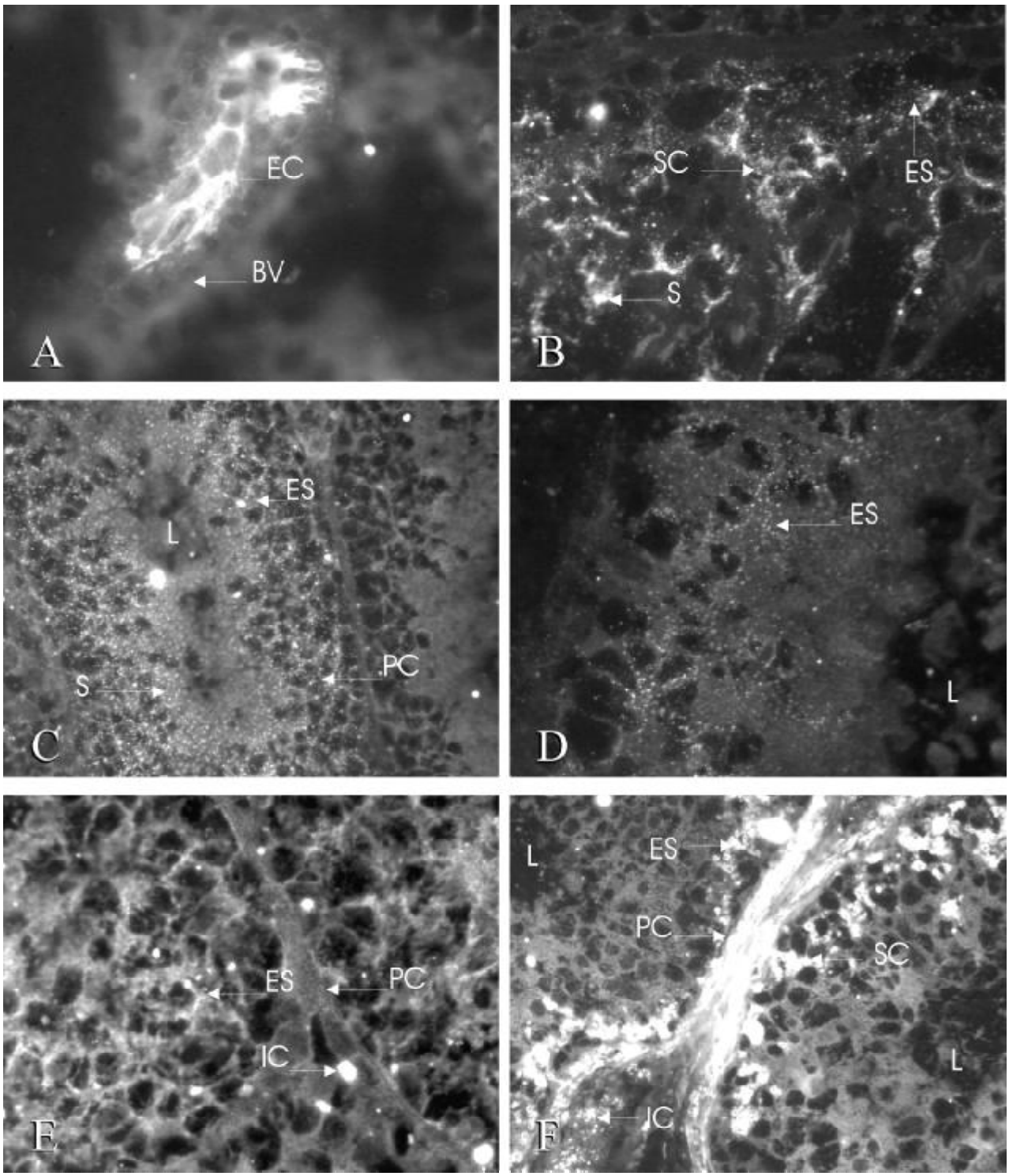

Fig. **Expression of the GLUT1, GLUT2 and GLUT3 genes at the protein level in the mouse, rat and human testis, indirect immunocytochemistry on a frozen section**

Note the presence of GLUT1 in the rat testis (A), GLUT2 in the rat testis (B), GLUT2 in the mouse testis (C), GLUT3 in the rat testis (D), GLUT3 in the mouse testis (E) and GLUT3 in the human testis (F). BV-blood vessel, EC-endothelial cell, SC-Sertoli cell, S-spermatids, ES-early spermatocytes, PC- peritubular cells, IC-interstitial cell, L-lumen of seminiferous tubule. Magnification A, B, E × 3600; D, F × 1900; C × 900.

GLUT2

Positive immunoreaction for GLUT2 was detected in the rat (Fig. B, Table) and mouse (Fig. C, Table) testis in Sertoli cells, peritubular myoid cells, spermatocytes, spermatids, testicular endothelial cells and macrophage-like interstitial cells. GLUT2 was not expressed in Leydig cells in the mouse and rat testis. Positive immunoreaction for GLUT2 was not detected in the human testis.

GLUT3

Very intensive positive immunoreaction for GLUT3 was expressed in the rat (Fig. D, Table), mouse (Fig. E, Table) and human (Fig. F, Table) testis. GLUT3 was found in Sertoli cells, peritubular myoid cells, macrophage-like interstitial cells, testicular endothelial cells and early spermatocytes. GLUT3-positive cells were not found in the luminal part of Sertoli cells, spermatids or Leydig cells.

GLUT4

Positive immunoreaction for GLUT4 was not found in the mouse, rat and human testis.

Discussion

We have demonstrated the presence of GLUT1, GLUT2, GLUT3 and GLUT4 proteins in the mouse, rat and human testis. The number of distinct gene products, together with the presence of several different transporters in certain tissues and cells indicates that glucose delivery into cells is a process of considerable complexity (3).

GLUT3 has a high affinity for glucose; 86% of testicular, 16% of ovarian, 25% of gastric and 27% of non-small cell lung carcinomas were positive for GLUT3 (6). In surgically induced unilateral abdominal cryptorchidism the GLUT3 expression was reduced by 85–95% compared with contralateral scrotal testis in rats (13). These results suggested that the degenerative changes in abdominal testis (impaired and incomplete spermatogenesis and lack of spermatozoa in the lumen of seminiferous tubules) might be associated with decreased GLUT3 mediated glucose transport in seminiferous tubules and spermatogonia. In our study, GLUT3 has a very intensive expression in the mouse, rat and human testicular cells.

There has been found that human, rat and bull spermatozoa express hexose transporter isoforms GLUT1-5 (14), but not other testicular cells are investigated yet. GLUT1, GLUT2, GLUT3, GLUT5 and low levels of GLUT4 isoform showed a typical subcellular localization in the head and the sperm tail (14, 15). The present study demonstrated that GLUT3 was the prominent glucose transporter type in the mouse, rat and human testis Sertoli cells, peritubular myoid cells, macrophage-like interstitial cells, testicular endothelial cells and early spermatocytes. GLUT1 was expressed only in the rat testis, GLUT2 in the mouse and rat testis, and GLUT4 was not present at all in the mouse, rat and human testis. This finding suggests that in case of diabetes-associated disturbances of the structure of the seminiferous epithelium (5), GLUT3 could be involved. In addition, it is very interesting that the early spermatocytes express GLUT3 at the protein level, as generally it is thought that these cell types do not use glucose in their metabolism but lactate produced by Sertoli cells. From the present results, it can be judged that possibly the early spermatocytes were ready to increase transport of glucose into their cytoplasm, although the GLUT3 proteins were present only in the cytoplasmic vesicles. The functional significance of this finding needs to be studied.

Also a novel member of GLUT family GLUT8 has been detected in the testis as well as in blastocysts, brain, muscle, and adipocytes (4). Based on homology with GLUT1-5 there has been isolated a cDNA for a novel glucose transporter GLUTX1, which mRNA was detected in testis, hypothalamus, cerebellum, brainstem, hippocampus and adrenal gland (16).

The presence of GLUT1 in the rat testis and GLUT2 in the mouse and rat testis implies that it may not be possible to use the rodent models to investigate the role of different GLUT isoforms on glucose metabolism in human testis.

The members of the extended GLUT family exhibit surprisingly diverse substrate specificity, and the definition of sequence elements determining this substrate specificity will require a full functional characterization of all members (4).

Conclusions

The present data showed that mouse, rat and human different testicular cells expressed GLUT1, GLUT2 and GLUT3 isoforms that allow for the efficient uptake of glucose, fructose, and dehydroascorbic acid by these cells. The distribution of glucose transporters isoforms class I subfamily was different in the rodent and human testis. GLUT3 was the prominent glucose transporter type in the mouse, rat and human testis.

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Pirmo tipo gliukozės nešiklių šeimos imunohistocheminis nustatymas pelių, žiurkių ir žmogaus sėklidėse

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Raktažodžiai: gliukozės nešikliai, sėklidės, imunohistochemija.

Santrauka. Gliukozės nešiklių šeima palengvina gliukozės difuziją per ląstelių membraną. Imunofluoresciniu būdu atlikti tyrimai parodė, kad pelių, žiurkių ir žmogaus sėklidžių ląstelėse egzistuoja skirtingos pirmo tipo gliukozės nešiklių izoformos. Imunohistocheminės analizės būdu nustatyta, kad pirmo tipo gliukozės nešiklių yra žiurkių sėklidėse, antro tipo gliukozės nešiklių – pelių ir žiurkių, trečio tipo gliukozės nešiklių – pelių, žiurkių ir žmogaus sėklidėse, o ketvirto tipo gliukozės nešiklių nerasta minėtose sėklidėse. Labai intensyvi trečio tipo gliukozės nešiklių teigiama imunoreakcija nustatyta Sertolio ląstelėse, apie vamzdelius esančiose mioido lastelėse, intersticinėse ląstelėse, kurios panašios į mikrofagocitus endotelinėse ląstelėse ir pirminiuose spermatocituose. Teigiamų trečio tipo gliukozės nešiklių reakcijų nenustatyta Sertolio ląstelių viršūninėje dalyje, spermatidėse bei Leidigo ląstelėse. Remiantis tyrimo duomenimis, pirmo, antro ir trečio tipo gliukozės nešikliai padeda gliukozei patekti į skirtingas sėklidžių ląsteles, o trečio tipo gliukozės nešiklis yra ryškiausias gliukozės nešiklis sėklidžių ląstelėse.

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