Journal of Cellular Biochemistry 71:189–203 (1998)

Hexose Transporter Expression and Function in Mammalian Spermatozoa: Cellular Localization and Transport of Hexoses and Vitamin C

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Abstract We analyzed the expression of hexose transporters in human testis and in human, rat, and bull spermatozoa and studied the uptake of hexoses and vitamin C in bull spermatozoa. Immunocytochemical and reverse transcription-polymerase chain reaction analyses demonstrated that adult human testis expressed the hexose transporters GLUT1, GLUT2, GLUT3, GLUT4, and GLUT5. Immunoblotting experiments demonstrated the presence of proteins of about 50–70 kD reactive with anti-GLUT1, GLUT2, GLUT3, and GLUT5 in membranes prepared from human spermatozoa, but no proteins reactive with GLUT4 antibodies were detected. Immunolocalization experiments confirmed the presence of GLUT1, GLUT2, GLUT3, GLUT5, and low levels of GLUT4 in human, rat, and bull spermatozoa. Each transporter isoform showed a typical subcellular localization in the head and the sperm tail. In the tail, GLUT3 and GLUT5 were present at the level of the middle piece in the three species examined, GLUT1 was present in the principal piece, and the localization of GLUT2 differed according of the species examined. Bull spermatozoa transported deoxyglucose, fructose, and the oxidized form of vitamin C, dehydroascorbic acid. Transport of deoxyglucose and dehydroascorbic acid was inhibited by cytochalasin B, indicating the direct participation of facilitative hexose transporters in the transport of both substrates by bull spermatozoa. Transport of fructose was not affected by cytochalasin B, which is consistent for an important role for GLUT5 in the transport of fructose in these cells. The data show that human, rat, and bull spermatozoa express several hexose transporter isoforms that allow for the efficient uptake of glucose, fructose, and dehydroascorbic acid by these cells. J. Cell. Biochem. 71:189–203, 1998.

Key words: glucose transporters; sperm; dehydroascorbic acid; fructose; 2-deoxy-D-glucose

Mammalian cells posses transport systems of the facilitative type that mediate the movement of glucose across the plasma membrane down a chemical gradient [Carruthers, 1990]. Six different facilitative glucose transporter isoforms, GLUT1, GLUT2, GLUT3, GLUT4, GLUT5, and GLUT7, have been molecularly cloned [Mueckler, 1994]. GLUT1 is responsible for the basal uptake of glucose in all cells and is especially abundant in erythrocytes and brain [Birnbaum et al., 1986; Mueckler et al., 1985]. GLUT2 is a low-affinity transporter of glucose that also transports fructose with even lower affinity [Thorens et al., 1988]. GLUT2 is involved in the transport of glucose in the liver, small intestine, and kidney and in rodents is part of the glucose-dependent sensory mechanism related to the secretion of insulin by the β cells of the pancreas [Thorens et al., 1990]. GLUT3 is a high-affinity transporter of glucose that is abundant in brain but appears to be expressed in other tissues as well [Haber et al., 1993; Kayano et al., 1988; Maher and Simpson, 1994]. GLUT4 is restricted mainly to the adipose and skeletal tissues and is responsible for

Contract grant sponsor: Universidad Austral de Chile; Contract grant numbers: S9524 and S9541; Contract grant sponsor: Fondecyt, Chile; Contract grant number: 1960485; Contract grant sponsor: Memorial Sloan-Kettering Institutional Funds.

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Received 22 April 1998; Accepted 20 May 1998

the acute increase of glucose transport and utilization in response to insulin [Birnbaum, 1989]. GLUT5 is abundantly expressed in the epithelial cells that line the small intestine, in human spermatozoa, and in red cells [Burant et al., 1992; Concha et al., 1997; Kayano et al., 1990; Rand et al., 1993]. GLUT5 is not a glucose transporter but transports fructose with high affinity [Burant et al., 1992; Rand et al., 1993]. GLUT7 has been identified in microsomes of liver cells and is similar to GLUT2 in terms of primary sequence [Waddell et al., 1992].

Mature spermatozoa can use fructose and glucose as an external source of energy, indicating that they possess efficient transport systems for the uptake of extracellular sugars [Grootegoed and den Boer, 1990; Hyne and Edwards, 1985; Peterson and Freund, 1975]. Although evidence indicating that human and rat spermatozoa express the glucose transporter GLUT3 [Haber et al., 1993] and the fructose transporter GLUT5 [Burant et al., 1992] are consistent with the participation of facilitative hexose transporters in the transport of sugars by the spermatozoa, there is limited information on the functional properties of the hexose transporters expressed in mammalian sperm and on the subcellular distribution of the transporters [Hiipakka and Hammerstedt, 1978b; Nakamura et al., 1987; Peterson et al., 1977]. For instance, there are no data indicating that a fructose transporter with the expected functional properties of GLUT5 is functionally active in mammalian spermatozoa. Similarly, there are no definitive functional data indicating that GLUT3 is the transporter involved in the uptake of glucose by mammalian spermatozoa [Glander and Dettmer, 1978a,b]. These are important considerations because each hexose transporter isoform presents distinct functional properties, including the ability to transport different hexoses and sensitivity to inhibitors, with the result that cells that express different transporters show unique functional properties in terms of their ability to transport different hexoses. The distinct functional properties of the transporters can also be used to assess the functional status of different transporters in cells expressing more than one isoform [Carruthers, 1990; Mueckler, 1994].

In addition to their role in transporting the energy-producing sugars glucose and fructose, the presence of hexose transporters in mammalian spermatozoa may be particularly relevant to a proposed functional role for vitamin C in normal sperm physiology. Vitamin C is fundamental to human physiology and appears to be essential for the normal development of the male germinal cells [Chinoy et al., 1986]. Low concentrations of vitamin C are associated with the production of poor quality sperm and decreased fecundating capacity [Dawson et al., 1987, 1992]. Humans cannot synthesize vitamin C, and therefore it must be provided in the diet and transported intracellularly [Rose, 1988]. The glucose transporters are efficient transporters of the oxidized form of vitamin C, dehydroascorbic acid [Vera et al., 1993]. Normal and neoplastic human cells accumulate high concentrations of ascorbic acid by a complex mechanism involving the facilitated transport of dehydroascorbic acid through glucose transporters, followed by the intracellular reduction of dehydroascorbic acid and the trapping of ascorbic acid [Vera et al., 1994, 1995]. No information is available, however, on the capacity of mammalian spermatozoa to take up vitamin C, including the chemical form of the vitamin that is transported (reduced or oxidized) or the functional properties of the transporters involved in the transport of vitamin C by the spermatozoa.

We performed a detailed immunochemical analysis of the expression of facilitative hexose transporters in human, rat, and bull spermatozoa and in adult human testis and characterized the transport of deoxyglucose, fructose, and vitamin C by bull spermatozoa. Our data indicate that adult human testis express five different hexose transporters, GLUT1, GLUT2, GLUT3, GLUT4, and GLUT5. We identified GLUT1, GLUT2, GLUT3, and GLUT5 as the main hexose transporters expressed in human, rat, and bull spermatozoa and determined their cellular localization. We also provide evidence indicating that bull spermatozoa transport deoxyglucose, fructose, and the oxidized form of vitamin C, dehydroascorbic acid, in a manner compatible with the direct participation of several facilitative hexose transporters in the transport of these substrates.

MATERIALS AND METHODS

Sample Collection

Human semen was collected in sterile plastic containers from healthy young men, and the spermatozoa were obtained by the swim-up procedure [Aitken and Clarkson, 1988]. Sperm cell

vitality was greater than 98% as assessed by eosin-nigrosin exclusion. Bull spermatozoa ejaculates were obtained from the Center for Artificial Insemination, Universidad Austral de Chile. Rat spermatozoa were isolated from the epididymis of adult male Holtzman rats [Vera et al., 1984].

Immunoblotting

For immunoblot analysis, spermatozoa membrane proteins were obtained by homogenizing the spermatozoa in buffered Tris-HCl (pH 7.4) containing 0.5% deoxycholic acid, 0.5% Triton X-100, 1 mM EDTA, 1 mM EGTA, 5 µg/ml each of aprotinin, leupeptin, and soybean trypsin inhibitor, and 1 mM phenylmethylsulfonyl fluoride. Total membranes were collected by high-speed centrifugation. For immunoblotting, 30 µg of membrane protein were loaded on each lane and fractionated by polyacrylamide gel electrophoresis in the presence of sodium dodecylsulfate, transferred to nitrocellulose membranes, and probed with glucose transporters antibodies (East Acres Biologicals, Southbridge, MA) or preimmune sera (1:500–1:1,500). The secondary antibody was a goat anti-rabbit IgG coupled to alkaline phosphatase (1:500). The reaction was developed with 4-nitroblue tetrazolium chloride and 5-bromo-4-chloro-3-indolyl phosphate. In experiments in which the secondary antibody was an anti-rabbit IgG coupled to horseradish peroxidase, the reaction was developed by enhanced chemiluminescence according to the ECL Western blotting analysis system (Amersham Corporation, Arlington Heights, IL).

Immunocytochemistry

For immunocytochemistry, spermatozoa were spread onto superfrosted slides, fixed in buffered formaldehyde-acetone for 30 sec, and washed with phosphate buffered saline (PBS; pH 7.4). The fixed cells were incubated in PBS containing 5% bovine serum albumin (BSA) for 1 h, followed by incubation for 1 h with the different GLUT antibodies or with preimmune rabbit serum, diluted 1:100 in PBS containing 1% BSA and 0.3% Triton X-100. The spermatozoa were washed in PBS and incubated with anti-rabbit IgG-colloidal gold (1:40; Auroprobe, Amersham Corporation) for 1 h, washed with distilled water, and subjected to silver enhancement according to the manufacturer's instructions. The cells were counterstained with azure C and mounted. Parallel samples were stained by immunofluorescence by using a goat anti-rabbit IgG coupled to fluorescein isothiocyanate or with the immunoperoxidase procedure by using a goat anti-rabbit IgG coupled to horseradish peroxidase.

The expression of hexose transporters in adult human testis was determined by histochemical analysis of a set of thin sections prepared from archived paraffin-embedded tissue blocks. Paraffin was removed by incubating the sections in xylene followed by absolute alcohol and then rehydrated by immersion in graded alcohol solutions. Endogenous peroxidases were inactivated by treating the tissue sections with 3% hydrogen peroxide for 15 min at room temperature. Sections were then incubated in PBS containing 5% skim milk followed by incubation with the GLUT antibodies (1:100) for 2 h. After extensive washing, sections were incubated with a goat anti-rabbit IgG coupled to horseradish peroxidase. Immunostaining was developed with 0.05% 3,3′-diaminobenzidine and 0.03% hydrogen peroxide. Cells were counterstained with hematoxylin.

Reverse Transcription–Polymerase Chain Reaction (RT-PCR)

For reverse transcription, 1 µg of total RNA obtained from normal human adult testis (Clontech Laboratories, Inc., Palo Alto, CA) was incubated in a 20-µl reaction volume containing 10 mM Tris pH 8.3, 50 mM KCl, 5 mM MgCl2, 1 mM dNTPs, 200 ng of random dN6, and 50 units of avian myeloblastosis virus reverse transcriptase (Life Sciences, FL) for 10 min at 23°C followed by 30 min at 42°C and 5 min at 94°C. Parallel reactions were performed in the absence of reverse transcriptase to control for the presence of contaminant DNA. For amplification, a cDNA aliquot in a volume of 25 µl containing 10 mM Tris, pH 8.3, 50 mM KCl, 5 mM $MgCl_2$, 1 mM dNTPs, 0.5 units of Taq DNA polymerase (Boehringer Mannheim Corporation, Indianapolis, IN), and appropriate primers (Fig. 2) [Takagi et al., 1994] was incubated at 94°C for 50 sec, 55°C for 50 sec, and 72°C for 90 sec for 32 cycles. PCR products were separated by 1.5% agarose gel electrophoresis and visualized by staining with ethidium bromide. Beta-actin primers were used as controls for the efficiency of the RT-PCR.

Uptake Assays

For uptake assays, the cells were suspended in incubation buffer (15 mM Hepes, pH 7.6, 135 mM NaCl, 5 mM KCl, 1.8 mM $CaCl_2$, 0.8 mM $MgCl_2$) [Vera et al., 1995]. Deoxyglucose uptake assays were performed in a final volume of 0.2 ml containing 1×10^8 cells and 0.5–3 µCi of 2-[1,2-^{3}H]deoxy-D-glucose (26.2 Ci/mmol, NEN Life Science Products, Boston, MA) and 0.5–30 mM deoxyglucose. Fructose uptake assays contained 0.1–0.2 µCi of D-[U-^{14}C]fructose (285 mCi/mmol; Amersham Corporation). For ascorbate uptake, the incubation buffer contained 0.2 µCi of L-[1–14C]ascorbic acid (6.4 mCi mmol; NEN Life Science Products) and 50 µM to 5 mM ascorbic acid. For dehydroascorbic acid uptake, 0.5–5 units of ascorbate oxidase (Sigma, St. Louis, MO) were added to the incubation medium containing appropriate concentrations of ascorbic acid and incubated for 2 min at room temperature before adding the cells. The oxidation of ascorbic acid was monitored by the decrease in the absorbance at 266 nm [Vera et al., 1994]. Uptake was performed at room temperature for the times indicated in the figure captions and was stopped by adding 10 volumes of cold PBS. The cells were collected by centrifugation and washed twice with cold PBS. Cells were dissolved in lysis buffer (10 mM Tris-HCl, pH 8.0, containing 0.2% sodium dodecylsulfate), and the incorporated radioactivity was assayed by liquid scintillation spectrometry. When appropriate, competitors and inhibitors were added to the uptake assays simultaneously with the assayed substrates.

RESULTS

We used a panel of antibodies specific for each of five members of the family of facilitative hexose transporters (GLUT1–GLUT5) to identify the isoforms expressed in tissue sections obtained from adult human testis by using horseradish peroxidase-labeled secondary antibodies (Fig. 1). The immunochemical analysis demonstrated the expression of five different hexose transporters in adult human testis, GLUT1, GLUT2, GLUT3, GLUT4, and GLUT5, with the degree of immunoreactivity decreasing in the order GLUT3 > GLUT5 > GLUT4 ≥ GLUT2 ≥ GLUT1. The data also showed that the different transporter isoforms were not homogeneously expressed in all testicular cells (Fig. 1).

Spermatids at different stages of maturation, located near the lumen of the seminiferous tubules, were strongly immunoreactive with anti-GLUT3 (Fig. 1). The immunoreactivity was associated with the plasma membrane and was also observed intracellularly. Strong GLUT3 immunostaining was also observed in the sperm

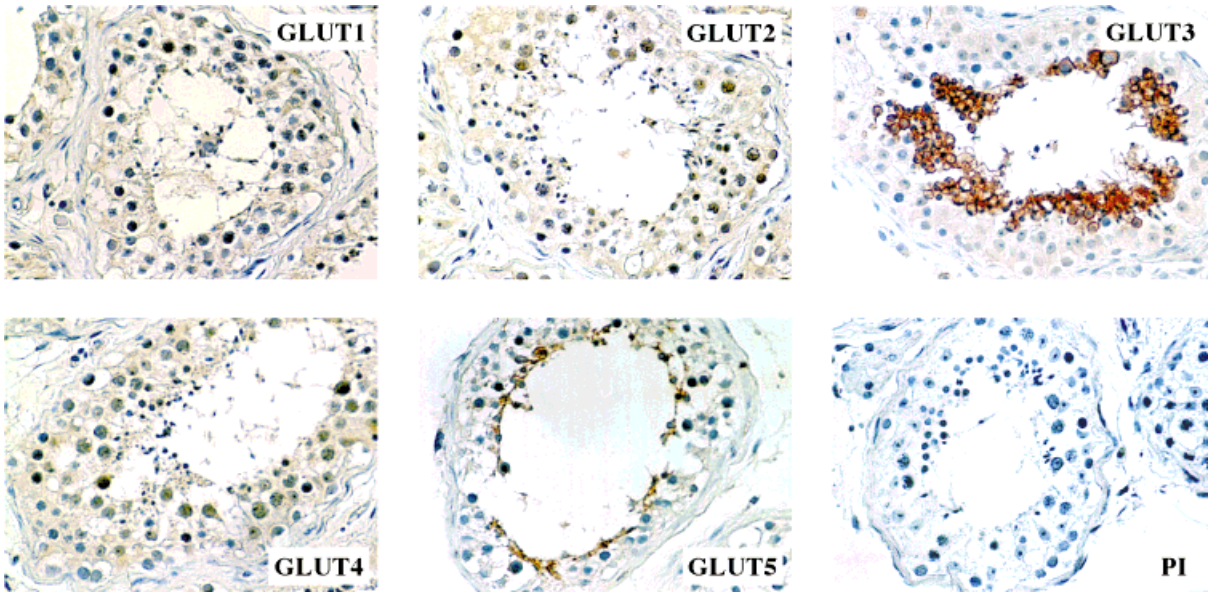

Fig. 1. Immunolocalization of hexose transporters in adult human testis. Tissue sections were incubated with GLUT1, GLUT2, GLUT3, GLUT4, and GLUT5 antibodies directed against the appropriate C-terminal sequences of the human proteins or with preimmune serum (PI), followed by incubation with a secondary antibody conjugated to horseradish peroxidase. Magnification ×200.

tails visible in the lumen of the seminiferous tubules. There was weak immunoreaction in less mature cell types, with the bulk of the immunoreactivity located intracellularly. GLUT5 immunoreactivity was strictly restricted to the most mature germinal cells lining the lumen of the seminiferous tubules (Fig. 1). No material reactive with anti-GLUT5 was observed in less mature germinal cells or Sertoli cells. GLUT1-, GLUT2-, and GLUT4-immunoreactive materials were located at the level of the plasma membrane of the testicular cells, although cytoplasmic staining was also observed (Fig. 1). The most mature germinal cells were stained with anti-GLUT1, anti-GLUT2, and anti-GLUT4. Negative controls incubated with preimmune serum or preabsorbed with the corresponding peptides did not have any positive staining, thereby confirming the specificity of the immune reaction (Fig. 1 and data not shown).

The expression of the five facilitative hexose transporters in adult human testis was confirmed by RT-PCR of adult human testis RNA by using primers specific for each transporter isoform. Each reverse transcription amplification reaction produced a single DNA band (Fig. 2). As judged by their migration compared with known size standards, the amplified DNA bands contained approximately 400, 700, 410, 490, and 400 nucleotides, the expected sizes of the amplification products for GLUT1, GLUT2, GLUT3, GLUT4, and GLUT5, respectively (Fig. 2). No amplification product was observed in samples in which the cDNA synthesis step was performed in the absence of reverse transcriptase, indicating the absence of DNA contamination in the RNA preparation. The primers used in these studies and the conditions for successful reverse transcription amplification were tested and optimized by using RNA from human tissues that express the respective transporter isoforms. GLUT1 and GLUT3 were amplified from brain RNA, GLUT2 from liver RNA, GLUT4 from skeletal muscle RNA, and GLUT5 from small intestine RNA. As assessed by the intensity of the ethidium bromide staining, the amount of amplified product from testis RNA decreased in the order GLUT5 > GLUT1 ≥ GLUT3 ≥ GLUT4 > GLUT2. To ensure that cDNA synthesis and reverse transcription amplification were equivalent among the samples, we measured the expression of the β-actin gene, which is constitutively transcribed. Beta-actin amplification was similar in all the samples, indicating that the differences observed in the

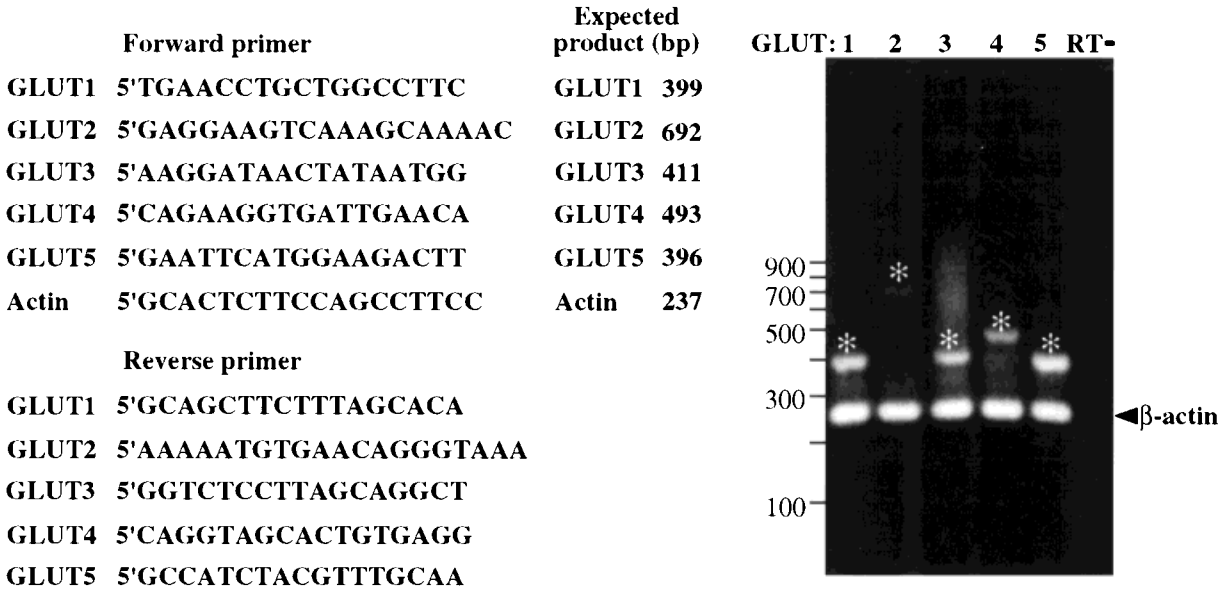

Fig. 2. Reverse transcription–polymerase chain reaction (RT-PCR) of hexose transporters expressed in adult human testis. Total RNA from human testis was subjected to RT-PCR by using primers specific for each hexose transporter (GLUT1–GLUT5), and the PCR products were fractionated on 1.5% agarose gels and visualized by staining with ethidium bromide. The asterisk above each band identifies the amplification product for each transporter. A reaction in which the cDNA synthesis step was performed in the absence of reverse transcriptase (RT−) was used to control for the presence of contaminant DNA in the RNA samples. Beta-actin was amplified to control for the efficiency of the RT-PCR (arrowhead). The numbers to the left are base pairs and indicate the migration in the agarose gel of a series of DNA 100-mer size standards.

amount of amplification product for the different hexose transporter genes reflect their relative expression in the human testis.

We then studied the expression of the different hexose transporter isoforms in human spermatozoa. Immunoblotting of membrane proteins extracted from human spermatozoa demonstrated protein bands reacting strongly with GLUT1, GLUT2, GLUT3, and GLUT5 antibodies, but weak or no immunoreactive bands were observed in samples tested with GLUT4 antibodies or with preimmune serum (Fig. 3). The GLUT1 antibodies reacted with two main protein bands that migrated with apparent M_rs of 52,000 and 60,000. Only one immunoreactive band, with an apparent M_r of 62,000, reacted with the GLUT2 antibodies, whereas the GLUT3 antibodies reacted with two main protein bands that migrated with apparent M_rs of 54,000 and 62,000. Only one protein band with an apparent M_r of 68,000 reacted with the GLUT5 antibodies. The specificity of each antibody was tested by using membrane proteins extracted from tissues and cells expressing the different transporter isoforms (GLUT1 and GLUT3, brain; GLUT2, liver; GLUT4, skeletal muscle; GLUT5, small intestine).

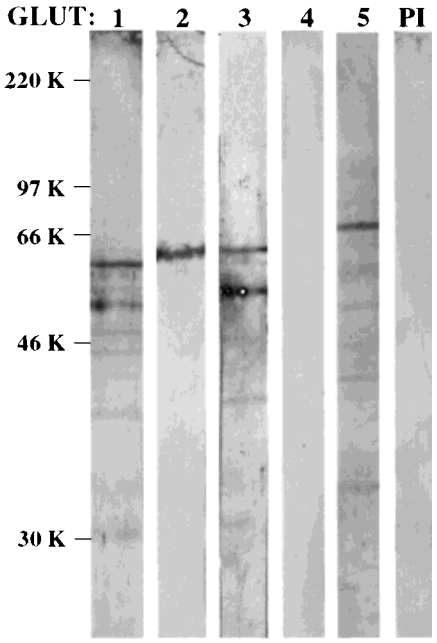

Fig. 3. Identification of hexose transporters in human spermatozoa by immunoblotting. Membrane proteins isolated from human ejaculated spermatozoa were fractionated by polyacrylamide gel electrophoresis in the presence of sodium dodecylsulfate, transferred to nitrocellulose membranes, and probed with the different GLUT antibodies (**lanes 1–5**) or with preimmune serum (PI), followed by incubation with a secondary antibody coupled to alkaline phosphatase. Sizes on the left are in kilodaltons and indicate the migration of molecular weight standards.

The presence of four facilitative hexose transporter isoforms in human spermatozoa was confirmed by immunolocalization studies using immunogold, immunoperoxidase, and immunofluorescence. The results of experiments using detection with immunogold indicated that human spermatozoa expressed the transporters GLUT1, GLUT2, GLUT3, GLUT5, and traces of GLUT4 (Fig. 4, Table 1, and data not shown). Staining with GLUT1, GLUT2, GLUT3, GLUT4, and GLUT5 antibodies resulted in the differential staining of different regions of the tail and the head of the spermatozoa, and the intensity of the reaction differed for the different transporters. Anti-GLUT3 and anti-GLUT5 showed a strong immunoreaction, anti-GLUT1 showed a moderate immunoreactivity, and only a weak signal was observed with anti-GLUT2 (Fig. 4, Table 1). A strong immunoreaction was observed in the posterior half of the sperm head with GLUT1, GLUT3, and GLUT5 antibodies, and the acrosomal region stained positively only with anti-GLUT1 (Fig. 4, Table 1). No reactivity with GLUT2 antibodies was observed at the level of the sperm head. A similar distribution pattern was observed when these experiments were repeated with secondary antibodies labeled with fluorescein isothiocyanate (Fig. 4) or immunoperoxidase (data not shown). No immunoreactive material was seen in samples treated with preimmune serum or absorbed with the peptides used to generate the antibodies, thereby showing the specificity of the reaction. Overall, the data indicate that human spermatozoa mainly express GLUT1, GLUT2, GLUT3, and GLUT5.

Similar fluorescent immunolocalization of hexose transporters in rat spermatozoa demonstrated that they express the transporters GLUT1, GLUT2, GLUT3, and GLUT5, with each transporter showing a different level and a unique pattern of expression along the tail and the head of the spermatozoa (Fig. 5, Table 1). There was clear anti-GLUT1 immunoreactivity in the principal piece of the tail and in the acrosomal region of the head, but only a weak immunoreaction was observed in the midpiece and the posterior half of the sperm head. GLUT2 immunoreactivity was observed only at the level of the acrosome in the sperm head, and it was very low to nonexistent along the sperm tail.

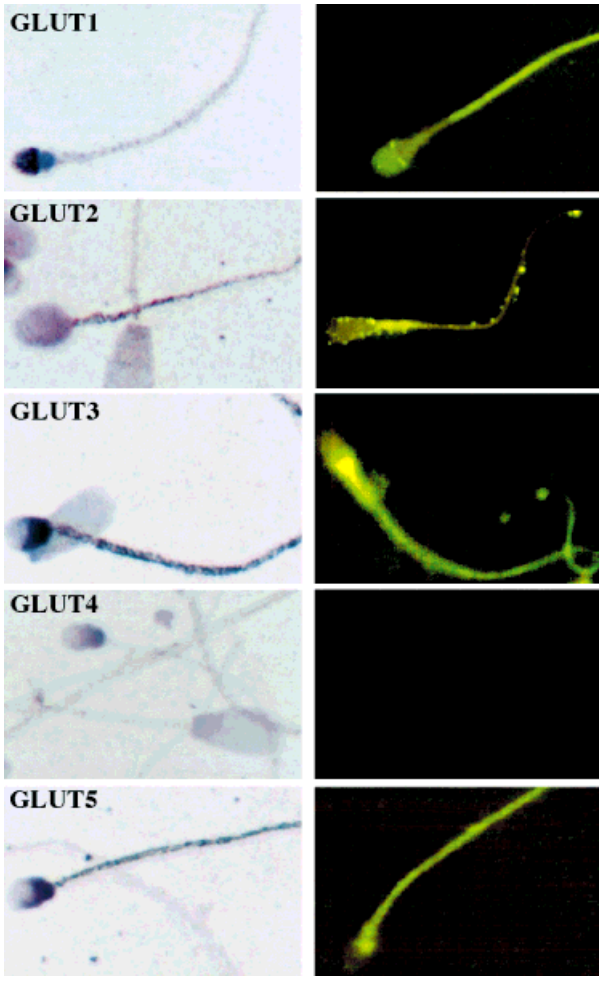

Fig. 4. Immunolocalization of hexose transporters in human spermatozoa. Spermatozoa were spread onto coated slides and probed with the different GLUT antibodies (GLUT1–GLUT5) followed by incubation with a secondary antibody coupled to colloidal gold and counterstained with azure C (left) or with an antibody coupled to fluorescein isothiocyanate (right). Magnification ×1,000.

TABLE I. Expression of Hexose Transporters in Mammalian Spermatozoa[a]

	GLUT1			GLUT2			GLUT3			GLUT4			GLUT5		
	a	b	c[b]	a	b	c	a	b	c	a	b	c	a	b	c
Head															
Anterior	+[c]	+	+	−	+	±	−	−	+	−	−	−	−	+	+
Posterior	±	−	−	−	−	±	+	−	+	−	−	−	+	+	+
Sperm tail															
Midpiece	−	−	−	+	−	±	+	+	+	−	−	−	+	+	+
Principal piece	+	+	+	−	−	−	+	±	−	−	−	−	+	±	±
End piece	+	+	+	−	−	−	±	−	−	−	−	−	−	−	−

[a]Data were obtained from immunolocalization experiments using secondary antibodies labeled with alkaline phosphatase, horseradish peroxidase, or fluorescein isothiocyanate.
[b]a, human sperm; b, rat sperm; c, bull sperm.
[c]+, Clear reaction with the respective GLUT antibody; ±, weak reactivity with the GLUT antibody; −, very weak or no reactivity with the GLUT antibody.

This contrasted with the strong anti-GLUT3 immunoreactivity observed along the sperm tail, with maximal reaction at the level of the midpiece. No anti-GLUT3 reactivity was evident in the sperm head. The data for GLUT3 were obtained with anti-rat GLUT3 antibodies because anti-human GLUT3 failed to recognize the rat protein. Similar to GLUT3, no material immunoreactive with anti-GLUT5 was evident in rat spermatozoa when using an anti-human GLUT5. When these experiments were repeated with anti-rat GLUT5, strong immunore-

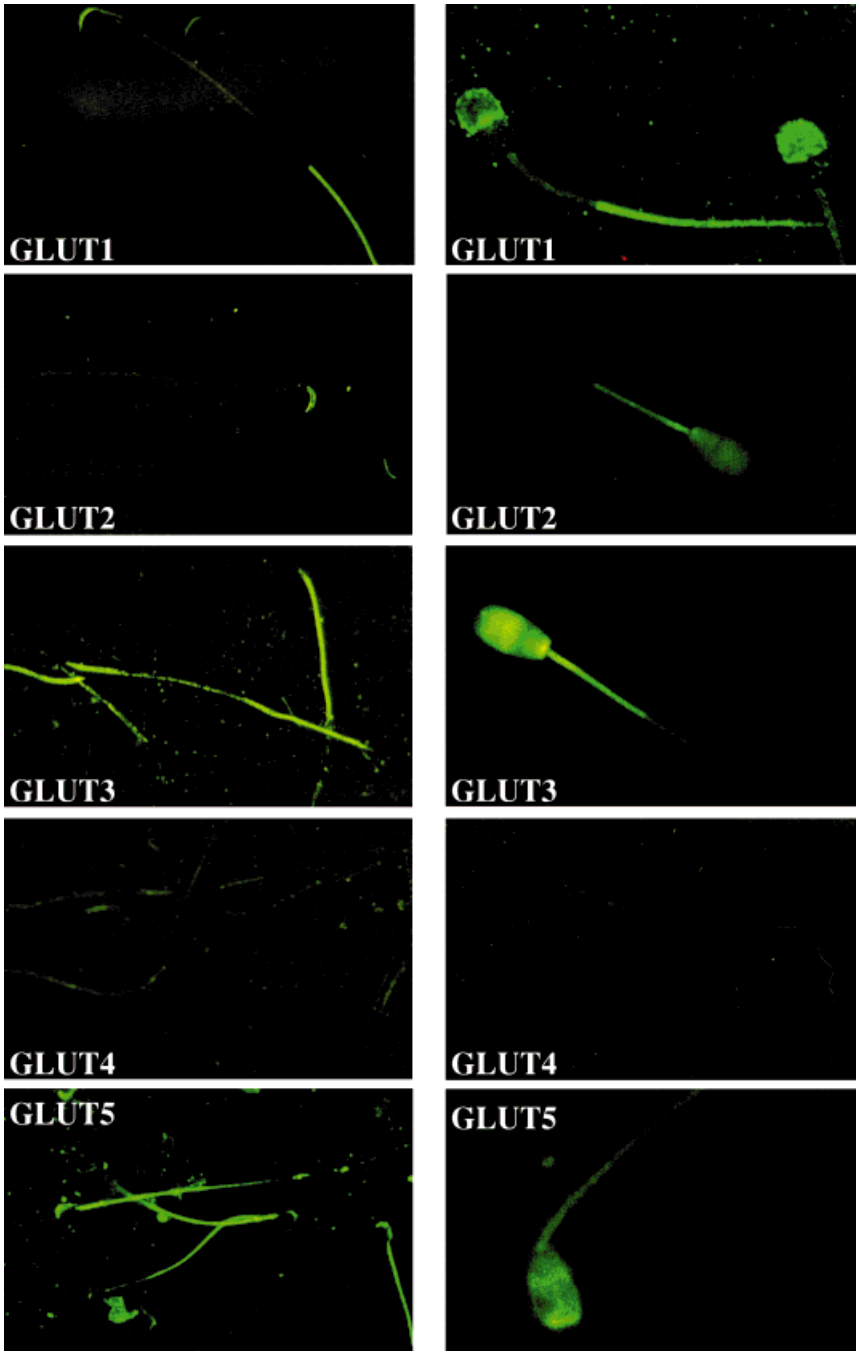

Fig. 5. Immunolocalization of hexose transporters in bull and rat spermatozoa. For immunofluorescence, the rat (left) and bull (right) spermatozoa were spread onto coated slides and reacted with GLUT antibodies (GLUT1–GLUT5) and visualized using fluorescein-coupled secondary antibodies. The GLUT1, GLUT2, and GLUT4 antipeptide antibodies were directed against the appropriate C-terminal sequences of the human proteins, and GLUT3 and GLUT5 antibodies were against the respective sequences from the rat proteins.

activity was observed along the sperm tail and the sperm head (Fig. 5). Very low anti-GLUT4 immunoreactivity was observed in these experiments, thus confirming the data obtained from human sperm. A similar pattern of expression and cellular distribution was observed in experiments using secondary antibodies labeled with horseradish peroxidase.

Bull spermatozoa were similar to human and rat sperm in that they expressed the transport-

ers GLUT1, GLUT2, GLUT3, and GLUT5, with weak or no expression of GLUT4 (Fig. 5, Table 1, and data not shown). However, the cellular distribution of some of the transporter isoforms was different from that observed in human and rat spermatozoa. Similar to human and rat, anti-GLUT1 immunoreactivity was abundant on the acrosomal region of the anterior half of the sperm head, with no reaction observed in the posterior half of the sperm head. In the sperm tail, anti-GLUT1 staining was restricted to the principal piece, with no reaction observed in the midpiece. GLUT2 and GLUT3 antibodies stained the midpiece of the tail and the complete head, and staining with anti-GLUT3 was clearly stronger than with anti-GLUT2. Staining with GLUT5 antibodies resulted in homogeneous staining of the sperm tail and the head, but compared with GLUT3 the intensity of the reaction was moderate to low. Similar to human and rat spermatozoa, anti-GLUT4 immunoreactivity was the lowest in the experiments with bull spermatozoa.

Because of the difficulties in obtaining a highly purified and homogeneous population of human sperm for analyzing the functional properties of the hexose transporters expressed by human spermatozoa, we performed hexose and vitamin C uptake studies with bull spermatozoa. Uptake assays demonstrated that transport of deoxyglucose by bull spermatozoa occurred rapidly, with half maximum uptake occurring in less than 1 min (Fig. 6A). Transport was linear for the first 30 sec and reached a plateau in about 10 min. Dose–response experiments examining uptake at 30 sec indicated that transport approached saturation at about 25 mM deoxyglucose (Fig. 6B). A detailed examination of the dose–response curve demonstrated the presence of two functional components involved in the transport of deoxyglucose, with an apparent Km of 0.8 and 5.9 mM and V_{max} of 16 and 51 nmol/min/10^8 cells, respectively (Fig. 6C). Inhibition experiments indicated that cytochalasin B inhibited the uptake of deoxyglucose by bull spermatozoa (Fig. 7D). Different concentrations of cytochalasin B are required to inhibit the functional activities of GLUT1 (Ki = 0.2 μM), GLUT2 (Ki = 7.5 μM), and GLUT3 (Ki = 2.1 μM) [Colville et al., 1993]. At 0.5 mM deoxyglucose, cytochalasin B inhibited uptake with an IC_{50} of approximately 3 μM (Fig. 7D). In bull spermatozoa, less than 20% inhibition of uptake was observed at 0.2 μM cytochalasin B, the concentration that inhibits the activity of GLUT1 by approximately 50%, but greater than 80% inhibition was observed at 10 μM cytochalasin B. Thus, the kinetic analysis and the inhibition data are consistent with the concept that both GLUT3 and GLUT1 may have important roles in the uptake of glucose by bull spermatozoa, especially in situations in which the cells are exposed to widely variable extracellular concentrations of glucose.

These studies also showed that bull spermatozoa transported fructose (Fig. 7A). Transport was linear for the first 30 sec and reached a plateau in about 20 min. Dose–response experiments examining uptake at 30 sec indicated

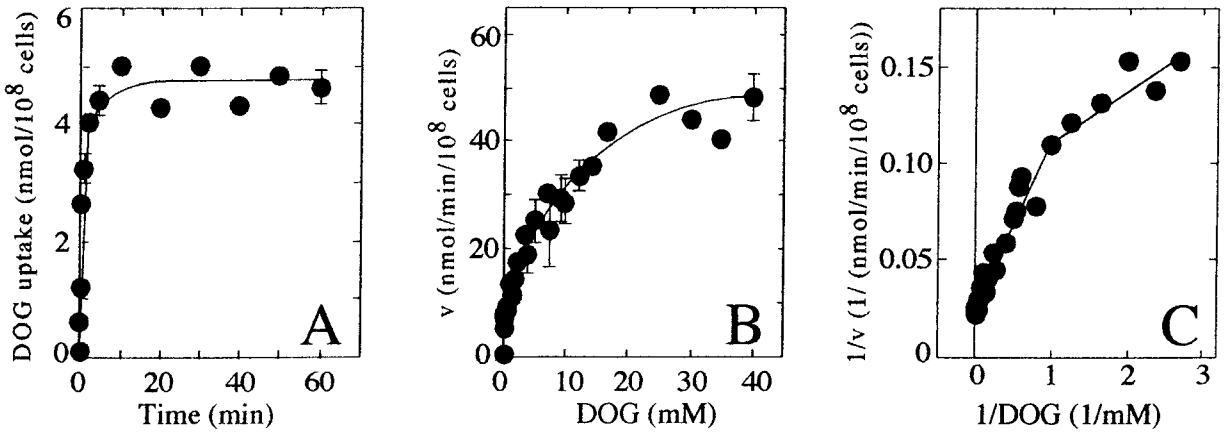

Fig. 6. Uptake of deoxyglucose by bull spermatozoa. **A:** Time course of the uptake of 0.5 mM deoxyglucose. **B:** Dose–response of the transport of deoxyglucose; 30-sec uptake assay. **C:** Double-reciprocal plot of the substrate dependence for deoxyglucose transport; 30-sec uptake assay. Data represent the mean ± SD of four samples. DOG, deoxyglucose.

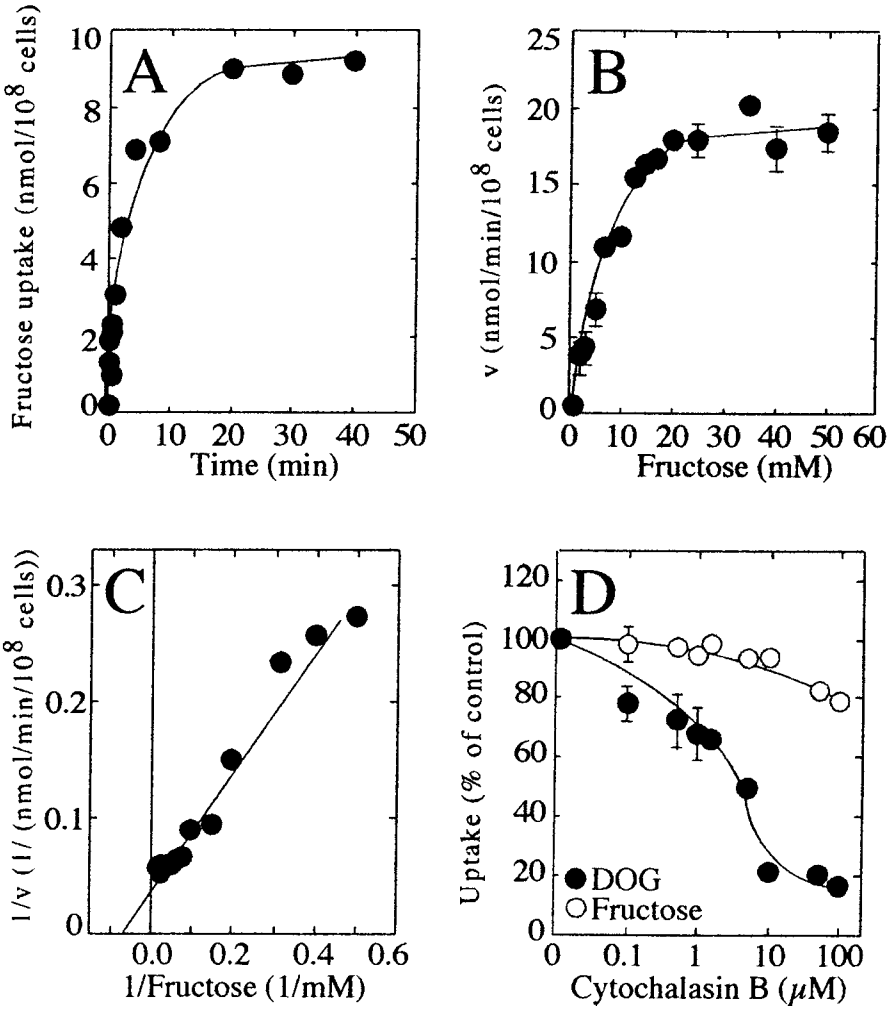

Fig. 7. Uptake of fructose by bull spermatozoa. **A:** Time course of the uptake of 0.5 mM fructose. **B:** Dose–response of the transport of fructose; 60-sec uptake assay. **C:** Double-reciprocal plot of the substrate dependence for fructose transport. **D:** Semi-log plot of the concentration dependence for inhibition of transport of deoxyglucose (solid circles) and fructose (open circles) by cytochalasin B. Data represent the mean ± SD of four samples. DOG, deoxyglucose.

that transport approached saturation at about 40 mM fructose (Fig. 7B). A detailed examination of the dose–response curve demonstrated the presence of one functional component involved in the transport of fructose, with a Km of 12 mM and a V_{max} of 28 nmol/min/10^8 cells (Fig. 7C). GLUT2 is a low-affinity transporter of fructose, with a Km for transport of about 70 mM, but the Km for transport of fructose by GLUT5 is in the range of 10–20 mM. Thus, our data point to GLUT5 as the main route of fructose in bull spermatozoa. Consistent with this notion was the finding that cytochalasin B, which inhibits GLUT2 but not GLUT5, decreased the uptake of fructose by less than 20% at concentrations that caused a total inhibition of the uptake of deoxyglucose by bull spermatozoa (Fig. 7D).

Additional studies showed that bull spermatozoa efficiently transported the oxidized from of vitamin C, dehydroascorbic acid (Fig. 8A). Short uptake experiments showed that bull spermatozoa failed to take up measurable amounts of the reduced form of vitamin C, ascorbic acid (Fig. 8A). A small amount of uptake was observed at longer incubation times, which is consistent with the slow oxidation of ascorbic acid to dehydroascorbic acid in solution [Vera et al., 1994, 1995]. Dose–response experiments examining uptake at 15 sec showed that transport of dehydroascorbic acid by bull spermatozoa was mediated by several func-

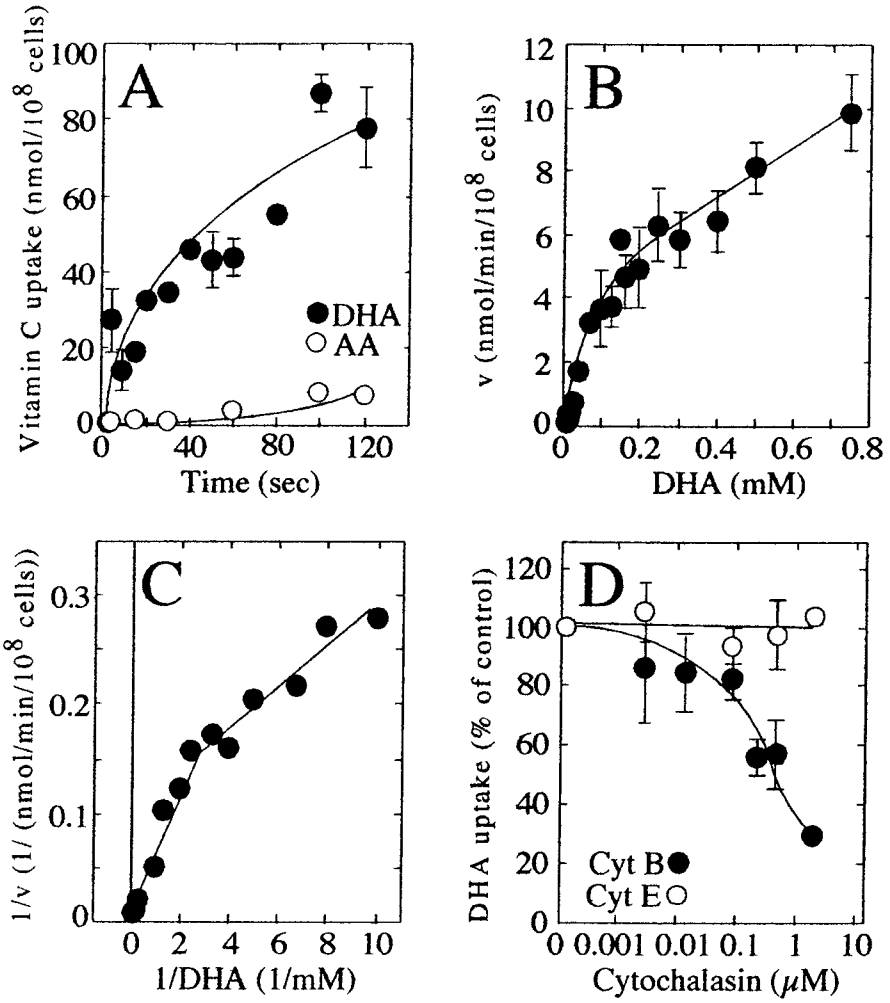

Fig. 8. Uptake of dehydroascorbic acid by bull spermatozoa. **A:** Time course of the uptake of 60 μM dehydroascorbic acid (DHA; solid circles) or ascorbic acid (AA; open circles). **B:** Dose–response of the transport of dehydroascorbic acid; 15-sec uptake assay. **C:** Double-reciprocal plot of the substrate dependence for dehydroascorbic acid transport; 15-sec uptake assay. **D:** Semi-log plot of the concentration dependence for the inhibition of dehydroascorbic acid transport by cytochalasin (Cyt) B (solid circles) or cytochalasin E (open circles). Data represent the mean ± SD of four samples.

tional components with characteristic kinetic properties. A high-affinity component was clearly evident at dehydroascorbic acid concentrations lower than 0.3 mM (Fig. 8B). The rate of uptake then increased almost linearly from 0.3 to 0.75 mM dehydroascorbic acid and approached saturation only at about 10 mM dehydroascorbic acid (Fig. 8B and data not shown). The presence of several transporters of dehydroascorbic acid in bull spermatozoa was confirmed by the nonlinearity of the Michaelis–Menten double reciprocal transformation of the transport data (Fig. 8C). The high-affinity component had an uncorrected apparent Km of 0.17 mM and a V_{max} of 9 nmol/min/10^8 cells for the transport of dehydroascorbic acid (Fig. 8C). A second, intermediate-affinity component with an estimated Km of about 0.7 mM, and a third, low-affinity component, with an estimated Km in the range of 5–6 mM for the transport of dehydroascorbic acid, were also evident from these studies. The data are consistent with previous estimates of the Km for transport of dehydroascorbic acid by the glucose transporters. GLUT1 transports dehydroascorbic acid with an estimated Km of 0.7 mM [Vera et al., 1995], whereas transport mediated by GLUT2 shows an estimated Km of 3–4 mM [Spielholz et al., 1997]. No data are available on the capacity of GLUT3 to transport dehydroascorbic acid.

Inhibition experiments indicated that cytochalasin B, but not cytochalasin E, inhibited the uptake of dehydroascorbic acid by bull spermatozoa with an IC_{50} of approximately 1 μM, which is a value intermediate between the respective Ki for inhibition of GLUT1 and GLUT3 (Fig. 8D). Approximately 25% inhibition was observed at 0.2 μM cytochalasin B, the concentration expected to inhibit transport of dehydroascorbic acid trough GLUT1 by 50%. Overall, the data are consistent with the involvement of GLUT1 and GLUT3, and perhaps GLUT2, in the transport of dehydroascorbic acid by bull spermatozoa.

DISCUSSION

Our findings indicate that human, rat, and bull spermatozoa express several members of the family of facilitative hexose transporters, GLUT1, GLUT2, GLUT3, and GLUT5, and that they localize on specific cellular compartments at the level of the sperm head and tail. Our data point to the direct participation of these transporters in the transport of glucose, fructose, and vitamin C by the spermatozoa.

The uptake data indicate that bull spermatozoa take up deoxyglucose and dehydroascorbic acid in a dose-dependent manner and that transport of both substrates was inhibited by cytochalasin B. The data directly relate to the expression of GLUT1, GLUT2, and GLUT3 by bull spermatozoa and are consistent with results of previous studies indicating that human and rat spermatids and spermatozoa take up hexoses [Glander and Dettmer, 1978a,b; Grootegoed and den Boer, 1990; Hall and Nakamura, 1981; Hiipakka and Hammerstedt, 1978a,b; Mita and Hall, 1982; Nakamura et al., 1987; Peterson and Freund, 1975; Peterson et al., 1977]. Although the available data are consistent with the concept that human spermatozoa may be able to use glucose as a normal source of energy, this is still a matter of controversy. The glucose content in the female reproductive tract is similar to that present in serum and thus could be used as a source of energy by the spermatozoa [Leese et al., 1981; Weed and Carrera, 1970]. However, the glucose content of the seminiferous tubules is very low, and the data on seminal fluid indicate a great variability on its glucose content, from near zero to 5 mM [Patel et al., 1988; Paz et al., 1977; Tauber et al., 1975]. Therefore, it is possible that the spermatozoa is subjected to widely different extracellular concentrations of glucose during its life cycle. The expression of several hexose transporters that transport glucose with different affinities may represent a functional adaptation for transporting an energy-producing substrate whose concentration varies widely.

Previous studies have established that GLUT3 and GLUT5 are the isoforms expressed in human spermatozoa [Burant et al., 1992; Haber et al., 1993]. Our more detailed studies indicate that GLUT1, GLUT2, GLUT3, GLUT5, and low levels of GLUT4 are present in human, rat, and bovine spermatozoa. The localization of GLUT3 in the midpiece of the sperm tail in the three species examined is consistent with a possible role in transporting an energy-producing substrate. GLUT3 was also present on the sperm head in human and bull spermatozoa but was restricted to the sperm tail in rat spermatozoa. This observation may represent a species-specific difference or it may be related to the fact that human and bull spermatozoa were obtained from ejaculates and rat spermatozoa were obtained from epididimal origin. GLUT1 and GLUT2 were also expressed in human, rat, and bull spermatozoa and showed a specialized cellular localization at the level of the sperm tail and the acrosome. These transporters are able to transport glucose with lower affinities than that of GLUT3 and may have an important role in conditions of increased glucose availability, whereas GLUT3 may be especially useful in a medium with a low content of glucose. The specific acrosomal localization of GLUT1, GLUT2, and GLUT3 may also reflect a functional adaptation [Fraser and Quinn, 1981].

The immunolocalization and immunoblotting data indicating the presence of GLUT5 and GLUT2 in human, rat, and bull spermatozoa are compatible with previous evidence indicating that human spermatozoa take up fructose [Glander and Dettmer, 1978a,b]. GLUT5 is present at the level of the posterior half of the head, but it is preferentially expressed at the level of the sperm tail. Fructose is produced and secreted by the seminal vesicles and is thought to be the only sugar available for respiration and maintenance of motility [Peterson and Freund, 1975]. Thus, fructose taken up by mammalian spermatozoa in the seminal fluid may be directly channeled through GLUT5 to the glycolytic machinery in the sperm midpiece [Grootegoed and den Boer, 1990]. However, the concentration of fructose in human seminal fluid

can be as high as 25 mM [Kucukkomurcu et al., 1980; Patel et al., 1977, 1988; Tauber et al., 1975], and at these high concentrations, GLUT2 could have a role in the transport of fructose by the spermatozoa. The uptake data indicating that a high-affinity fructose transporter is functionally active in bull spermatozoa and the lack of effect of cytochalasin B on transport are consistent with the concept that the transport of fructose in bull spermatozoa is mediated mainly by GLUT5.

The uptake data indicating that bull spermatozoa take up dehydroascorbic acid in a dose-dependent manner with the involvement of several functional activities with distinct kinetic properties and that transport was inhibited by cytochalasin B directly relates to the expression of GLUT1, GLUT2, and GLUT3 by bull spermatozoa. Direct evidence from *Xenopus laevis* expression studies have shown that GLUT1, GLUT2, and GLUT4 are efficient transporters of dehydroascorbic acid [Vera et al., 1993], and kinetic and inhibition data derived from studies of the transport of vitamin C in human neutrophils, erythrocytes, and leukemia cells have confirmed that GLUT1 is directly involved in the transport of dehydroascorbic acid in these cells [Rivas et al., 1997; Vera et al., 1993, 1994, 1995, 1996]. Although there is no direct evidence indicating that GLUT3 transports dehydroascorbic acid, its known functional properties indicating that it is a high-affinity transporter of glucose are consistent with the concept that it may transport dehydroascorbic acid. Moreover, the cytochalasin B inhibition data point to a direct role of GLUT3 in the uptake of dehydroascorbic acid by bull sperm. It is also possible that GLUT5, the fructose transporter present in bull spermatozoa, may transport dehydroascorbic acid. Expression studies in *Xenopus laevis* oocytes have indicated, however, that GLUT5 is unable to transport vitamin C, reduced or oxidized, under conditions in which it transports fructose efficiently (unpublished data).

Our data indicate that the expression of the different hexose transporters is regulated during testicular development in humans, with GLUT3 and GLUT5 expressed at high levels in the more mature germinal cells and the spermatozoa and GLUT1 and GLUT2 expressed at low levels in germinal and nongerminal cells. Although human, rat, and bull spermatozoa expressed low levels of GLUT4, this transporter was expressed in adult human testis. The expression of GLUT4 in adult testis is of interest because GLUT4 is a transporter that responds to insulin in cells sensitive to this growth hormone, and it has been described that testicular cells express and respond to insulin-related growth factor 1, which affects cellular glucose uptake in a manner similar to that of insulin [Cailleau et al., 1990]. The data are compatible with the notion that testicular cells are able to transport the energy-producing substrates glucose and fructose and also dehydroascorbic acid and that cells at different stages of differentiation show differential expression of specific hexose transporter isoforms.

Our findings indicate that the expression of hexose transporters by human, rat, and bull spermatozoa is not restricted to the high-affinity glucose transporter GLUT3 and the fructose transporter GLUT5, but they also express the intermediate-affinity glucose transporter GLUT1 and the low-affinity glucose/fructose transporter GLUT2. The different hexose transporter isoforms are functionally active and provide the spermatozoa with the capacity to transport glucose, fructose, and dehydroascorbic acid, which is consistent with an important role for these substrates in the normal physiology of the spermatozoa. Most importantly, the expression in spermatozoa of transporters with different kinetic characteristics may provide them with the high degree of functional flexibility required to survive in the ever changing environment of the maturing spermatozoa.

ACKNOWLEDGMENTS

We thank Dr. David W. Golde from the Memorial Sloan-Kettering Cancer Center and Dr. Luis O. Burzio from BIOS CHILE IGB for their continuous interest and support, Dr. Luis Norambuena from the Instituto de Histología y Patología, Universidad Austral de Chile, and Dr. Francisco Nualart from the Departamento de Histología y Embriología, Universidad de Concepción, for some of the testis samples.

REFERENCES

Aitken RJ, Clarkson JS (1988): Significance of reactive oxygen species and antioxidants in defining the efficacy of sperm preparation techniques. J Androl 9:367–376.

Birnbaum MJ (1989): Identification of a novel gene encoding an insulin-responsive glucose transporter protein. Cell 57:305–315.

Birnbaum MJ, Haspel HC, Rosen OM (1986): Cloning and characterization of a cDNA encoding the rat brain glucose-transporter protein. Proc Natl Acad Sci USA 83:5784–5788.

Burant CF, Takeda J, Brot-Laroche E, Bell GI, Davidson NO (1992): Fructose transporter in human spermatozoa and small intestine is GLUT5. J Biol Chem 267:14523–14526.

Cailleau J, Vermeire S, Verhoeven G (1990): Independent control of the production of insulin-like growth factor I and its binding protein by cultured testicular cells. Mol Cell Endocrinol 69:79–89.

Carruthers A (1990): Facilitated diffusion of glucose. Physiol Rev 70:1135–1176.

Chinoy NJ, Mehta RR, Seethalakshmi L, Sharma JD, Chinoy MR (1986): Effects of vitamin C deficiency on physiology of male reproductive organs of guinea pigs. Int J Fertil 31:232–239.

Colville CA, Seatter MJ, Jess TJ, Gould GW, Thomas HM (1993): Kinetic analysis of the liver-type (GLUT2) and brain-type (GLUT3) glucose transporters in Xenopus oocytes: Substrate specificities and effects of transport inhibitors. Biochem J 290:701–706.

Concha II, Velásquez FV, Martínez JM, Angulo C, Droppelmann A, Reyes AM, Slebe JC, Vera JC, Golde DW (1997): Human erythrocytes express GLUT5 and transport fructose. Blood 89:4190–4195.

Dawson EB, Harris WA, Rankin WE, Charpentier LA, McGanity WJ (1987): Effect of ascorbic acid on male fertility. Ann NY Acad Sci 498:312–323.

Dawson EB, Harris WA, Teter MC, Powell LC (1992): Effect of ascorbic acid supplementation on the sperm quality of smokers. Fertil Steril 58:1034–1039.

Fraser LR, Quinn PJ (1981): A glycolytic product is obligatory for initiation of the sperm acrosome reaction and whiplash motility required for fertilization in the mouse. J Reprod Fertil 61:25–35.

Glander H-J, Dettmer D (1978a): Monosaccharide transport across membranes of human spermatozoa I. Development of a radiochemical method of measuring monosaccharide uptake by spermatozoa. Andrologia 10:69–73.

Glander H-J, Dettmer D (1978b): Monosaccharide transport across membranes of human spermatozoa II. Basic properties of D-fructose and D-glucose uptake. Andrologia 10:273–277.

Grootegoed JA, den Boer PJ (1990): Energy metabolism of spermatids: a review. In Hamilton DW, Waites GMH (eds): "Scientific Basis for Fertility Regulation. Cellular and Molecular Events in Spermiogenesis." Cambridge: Cambridge University Press, pp 193–216.

Haber RS, Weinstein SP, O'Boyle E, Morgello S (1993): Tissue distribution of the human GLUT3 glucose transporter. Endocrinology 132:2538–2543.

Hall PF, Nakamura M (1981): Influence of temperature on hexose transport by round spermatids of rats. J Reprod Fertil 63:373–379.

Hiipakka RA, Hammerstedt RH (1978a): 2-Deoxyglucose transport and phosphorylation by bovine sperm. Biol Reprod 19:368–379.

Hiipakka RA, Hammerstedt RH (1978b): Changes in 2-deoxyglucose transport during epididymal maturation of ram sperm. Biol Reprod 19:1030–1035.

Hyne RV, Edwards KP (1985): Influence of 2-deoxy-D-glucose and energy substrates on guinea-pig sperm capacitation and acrosome reaction. J Reprod Fertil 73: 59–69.

Kayano T, Fukumoto H, Eddy RL, Fan YS, Byers MG, Shows TB, Bell GI (1988): Evidence for a family of human glucose transporter-like proteins. Sequence and gene localization of a protein expressed in fetal skeletal muscle and other tissues. J Biol Chem 263:15245–15248.

Kayano T, Burant CF, Fukumoto H, Gould GW, Fan YS, Eddy RL, Byers MG, Shows TB, Seino S, Bell GI (1990): Human facilitative glucose transporters. Isolation, functional characterization, and gene localization of cDNAs encoding an isoform (GLUT5) expressed in small intestine, kidney, muscle, and adipose tissue and an unusual glucose transporter pseudogene-like sequence (GLUT6). J Biol Chem 265:13276–13282.

Kucukkomurcu S, Delogne-Desnoeck J, Robyn C (1980): Prolactin and fructose in human seminal fluid. Int J Fertil 25:117–121.

Leese HJ, Astley NR, Lambert D (1981): Glucose and fructose utilization by rat spermatozoa within the uterine lumen. J Reprod Fertil 61:435–437.

Maher F, Simpson IA (1994): The GLUT3 glucose transporter is the predominant isoform in primary cultured neurons: Assessment by biosynthetic and photoaffinity labelling. Biochem J 301:379–384.

Mita M, Hall PF (1982): Metabolism of round spermatids from rats: Lactate as the preferred substrate. Biol Reprod 26:445–455.

Mueckler M (1994): Facilitative glucose transporters. Eur J Biochem 219:713–725.

Mueckler M, Caruso C, Baldwin SA, Panico M, Blench I, Morris HR, Allard WJ, Lienhard GE, Lodish HF (1985): Sequence and structure of a human glucose transporter. Science 229:941–945.

Nakamura M, Okinaga S, Arai K (1987): Studies of metabolism of round spermatids: Cytochalasin B binding to cell membrane in relation to glucose transport. Andrologia 19:178–182.

Patel SM, Skandhan KP, Mehta YB (1988): Seminal plasma fructose and glucose in normal and pathological conditions. Acta Eur Fertil 19:329–332.

Paz GF, Sofer A, Homonnai ZT, Kraicer PF (1977): Human semen analysis: Seminal plasma and prostatic fluid compositions and their interlations with sperm quality. Int J Fertil 22:140–147.

Peterson RN, Freund M (1975): Metabolism of human spermatozoa. In Ese H (ed): "Human Semen and Fertility Regulation in Men." St. Louis: Mosby, pp 552–556.

Peterson RN, Bundman D, Freund M (1977): Binding of cytochalasin B to hexose transport sites on human spermatozoa and inhibition of binding by purines. Biol Reprod 17:198–206.

Rand EB, Depaoli AM, Davidson NO, Bell GI, Burant CF (1993): Sequence, tissue distribution, and functional characterization of the rat fructose transporter GLUT5. Am J Physiol 264:G1169–G1176.

Rivas CI, Vera JC, Guaiquil VH, Vel´squez FV, Bórquez-Ojeda O, Cárcamo JG, Concha II, Golde DW (1997): Increased uptake and accumulation of vitamin C in human immunodeficiency virus 1-infected hematopoietic cell lines. J Biol Chem 272:5814–5820.

Rose RC (1988): Transport of ascorbic acid and other water-soluble vitamins. Biochim Biophys Acta 947:335–366.

Spielholz C, Golde DW, Houghton AN, Nualart F, Vera JC (1997): Increased facilitated transport of dehydroascorbic acid without changes in sodium-dependent ascorbate transport in human melanoma cells. Cancer Res 57:2529–2537.

Takagi H, Tanihara H, Seino Y, Yoshimura N (1994): Characterization of glucose transporter in cultured human retinal pigment ephitelial cells: Gene expression and effect of growth factors. Invest Ophthalmol Vis Sci 35:170–177.

Tauber PF, Zaneveld LJ, Propping D, Schumacher GF (1975): Components of human split ejaculates. I. Spermatozoa, fructose, immunoglobulins, albumin, lactoferrin, transferrin and other plasma proteins. J Reprod Fertil 43:249–267.

Thorens B, Sarkar HK, Kaback HR, Lodish HF (1988): Cloning and functional expression in bacteria of a novel glucose transporter present in liver, intestine, kidney, and beta-pancreatic islet cells. Cell 55:281–290.

Thorens B, Cheng ZQ, Brown D, Lodish HF (1990): Liver glucose transporter: A basolateral protein in hepatocytes and intestine and kidney cells. Am J Physiol 259:C279–C285.

Vera JC, Brito M, Zuvic T, Burzio LO (1984): Polypeptide composition of rat sperm outer dense fibers. J Biol Chem 259:5970–5977.

Vera JC, Rivas CI, Fischbarg J, Golde DW (1993): Mammalian facilitative hexose transporters mediate the transport of dehydroascorbic acid. Nature 364:79–82.

Vera JC, Rivas CI, Zhang RH, Farber CM, Golde DW (1994): Human HL-60 myeloid leukemia cells transport dehydroascorbic acid via the glucose transporters and accumulate reduced ascorbic acid. Blood 84:1628–1634.

Vera JC, Rivas CI, Velásquez FV, Zhang RH, Concha II, Golde DW (1995): Resolution of the facilitated transport of dehydroascorbic acid from its reduction-dependent intracellular accumulation. J Biol Chem 270:23706–23712.

Vera JC, Reyes AM, Cárcamo JG, Velásquez FV, Rivas CI, Zhang RH, Strobel P, Iribarren R, Scherr HI, Slebe JC, Golde DW (1996): Genistein is a natural inhibitor of hexose and dehydroascorbic acid transport through the glucose transporter, GLUT1. J Biol Chem 271:8719–8724.

Waddell ID, Zomerschoe AG, Voice MW, Burchell A (1992): Cloning and expression of a hepatic microsomal glucose transport protein. Comparison with liver plasma-membrane glucose-transport protein GLUT 2. Biochem J 286: 173–177.

Weed JC, Carrera AE (1970): Glucose content of cervical mucus. Fertil Steril 21:866–872.