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Expressional Comparison of Glucose Cotransporter Isoforms in the Rat Epididymis During Postnatal Development

Dong Mok Lee*, Hee Jung Seo**, Chan Wok Son**, Yong Ho Lee***, Inho Choi*, Tae Hoon Chun****, Yong Pil Cheon****** and Ki Ho Lee**

School of Biotechnology, Yeungnam University, Gyeongsan, S. Korea*, Department of Biochemistry and Molecular Biology and Medical Sciences Research Institute, Eulji University, Daejeon, S. Korea**, Department of Biomedical Science, Catholic University of Daegu, Gyeongsan, S. Korea***, School of Life Sciences and Biotechnology, Korea University, Seoul, S. Korea****, School of Life Sciences and Chemistry, Institute of Basic Sciences, Sungshin Women's University, Seoul, S. Korea*****

ABSTRACT

Glucose is a major source of metabolic fuel and lipid and protein syntheses. Transport of glucose into the cell is regulated by an action of glucose transport-associated transporters, especially solute carriers 2A (*Slc2a*, protein symbol GLUT). The present study was focused on examination of mRNA expression of various *Slc2a* isoforms in the epididymis during postnatal development. Total RNAs isolated from different epididymal segments (caput, corpus, and caudal epididymis) were utilized for real-time polymerase chain reaction analyses. Results showed that *Slc2a 1*, *3*, *4*, *5*, and *8* were expressed in the entire epididymal regions. In addition, the abundance of these *Slc2a* isoforms' transcripts was different within each epididymal regions. Moreover, the present study showed differential expression of these *Slc2a* isoforms among different epididymal segments according to postnatal ages. The current study suggests that glucose transport in the epididymis via various *Slc2a* isoforms would be necessary for maintenance of the epididymal functions.

(**Key words :** Epididymis, Glucose cotransporter, Male reproduction, Postnatal development, Gene expression)

INTRODUCTION

The epididymis is a part of the excurrent duct of the male reproductive tract and is divided into three regions, head (caput), body (corpus), and tail (cauda), based on their morphological features and physiological functions (Cornwall, 2009; Cosentino and Cockett, 1986). The epididymis is a coiled tubular structure which has a lumen inside surrounded by a single layer of epithelium (Cornwall, 2009; Cosentino and Cockett, 1986). The primary cell type of the epithelium is the principle cell which is responsible for secretion of various proteins into the lumen (Cornwall, 2009). However, other cell types also participate in regulation and maintenance of epididymal functions (Kujala et al., 2007; Pietrement et al., 2006; Seiler et al., 1999). The main function of the epididymis is maturation of spermatozoa produced from the testis (Cornwall, 2009). Moving throughout the epididymis leads to acquirement of fertilizing capacity of spermatozoa (Cornwall, 2009). In addition, the epididymis plays other important functional roles, including storage of spermatozoa, reabsorption of luminal fluid, and acidification of luminal compartment for sperm quiescence (Cornwall, 2009). Hence, it is important to understand how the epididymis maintains its functions for male fertility.

Glucose is a major substance utilized commonly by most of mammalian cells to generate energy in the form of ATP and to synthesize protein and lipid (Zhao and Keating, 2007). Because blood glucose levels in mammals must be maintained within a narrow range, movement of glucose into the cell should be precisely controlled by homeostatic mechanisms (Zhao and Keating, 2007). Generally, transport of extracellular glucose into the cell is regulated by a passive and/or active transport processes (Widdas, 1988; Zhoa and Keating, 2007). A passive, facilitative transport of glucose is simply driven by gradient differences of

Corresponding author : Ki Ho Lee, Department of Biochemistry and Molecular Biology, Eulji University, 143-5 Yongdoodong, Joonggoo, Daejeon 301-746, S. Korea . Tel: 82-42-259-1643; Fax: 82-42-259-1649; E-mail: kiholee@eulji.ac.kr

glucose concentration across the plasma membrane (Widdas, 1988). Active transport of glucose is primarily used by a secondary active transport mechanism which absorbs glucose against its electrochemical gradient (Zhao and Keating, 2007). This active glucose transport uses Na^+ concentration gradient established by Na^+-K^+ ATPase and is chiefly occurred in the epithelial cell brush border of the small intestine and in the proximal convoluted tubules of the kidney (Zhao and Keating, 2007). Thus, the regulation of glucose transport across cell membrane is chiefly governed by complex but coordinated actions of a number of glucose transport-associated transporters.

The passive glucose transport process is mediated by the family of facilitative glucose transporters, solute carriers 2A (*Slc2a*, protein symbol GLUT), while Na^+-dependent glucose transport is achieved by the family of Na^+/glucose cotransporters, solute carriers 5A (*Slc5a*, protein symbol SGLT). Until now, there are 13 members of *Slc2a* family and 6 members of *Slc5a* family identified in mammalian (Wright and Turk, 2004; Zhao and Keating, 2007). Even though members of *Slc2a* genes are structurally related each other, most of GLUT proteins have different kinetics and efficiencies for glucose and hexose transport (Zhao and Keating, 2007). For example, *Slc2a4* has a high affinity for glucose, while *Slc2a5* has a high affinity for fructose and a very low affinity for glucose (Wood and Trayhurn, 2003; Zhao and Keating, 2007). In addition, each *Slc2a* isoforms has a tissue- and/or cell-specific distribution, even one or more isoforms are expressed in the same tissue at different developmental time points (Zhao and Keating, 2007). For example, there are 7 different *Slc2a* isoforms present in the skeletal muscle and 4 different *Slc2a* isoforms expressed in the kidney, at least (Wood and Trayhurn, 2003). In the testis of the male reproductive tract, predominant expression of *Slc2a8* has been demonstrated by Chen et al. (2003) and Zhao et al. (2004). Others have also shown that *Slc2a1*, *Slc2a5*, and *Slc2a7* are localized in the testis (Burant et al., 1992; Davidson et al., 1992; Li et al., 2004; Ulisse et al., 1992). Even though Schürmann et al. (2002) have shown the presence of GLUT8 at the acrosomal region of mature spermatozoa within the epididymis, the expression of other *Slc2a* isoforms in the epididymis has not been determined in detail.

Our preliminary study showed the expression of five *Slc2a* transcripts, *Slc2a1*, *3*, *4*, *5*, and *8*, of 13 *Slc2a* isoforms in the caput epididymis at 1 month of age (data not shown here). Based on these findings, in the present study, we attempted to detect expression of these *Slc2a* isoforms in the epididymal segments of the male reproductive tract using real-time PCR analysis. In addition, we tried to determine expression pattern of *Slc2a* isoforms in the epididymis during postnatal development.

MATERIALS AND METHODS

1. Animals and tissue collection

Male Sprague Dawley rats were purchased from Samtako (O San, S. Korea). In the current study, we used 4 different postnatal age groups, 1 week (n=8), 2 weeks (n=7), 1 month (n=5), and 3 months (n=4), in a total of 24 rats. Prepubertal male rats at 1 week and 2 weeks of ages were obtained from pregnant female Spragure Dawley rats purchased from Samtako. Animals were kept under controlled conditions and given *ad libitum* food and water for entire experimental period. Once reaching at proper ages, animals were anesthetized by CO_2 stunning, and male reproductive tract were isolated. The epididymis was separated from the testis and vas deferens and further dissected into 3 parts, caput, corpus, and caudal epididymis in cold PBS buffer. Tissues were quickly rinsed in a new cold PBS buffer and frozen in liquid nitrogen. These tissue samples were stored in -80^oC until used to isolate total RNAs.

2. Total RNA isolation and cDNA preparation

Total RNAs were isolated from frozen epididymal tissues using easy-Blue total RNA extraction solution (iNtRON Biotech, Sungnam, S. Korea) and a polytron homogenizer (Fisher Scientific, Pittsburgh, USA). After phenol-chloroform extraction, the RNA pellets were dissolved in RNA storage buffer (Ambion, Austin, USA) and kept in -80^oC until used for reverse-transcription (RT) reaction to generate the first strand of cDNA. The qualities and purity of total RNAs were evaluated by an UV spectrophotometer (Eppendorf, New York, USA) and gel electrophoresis, respectively. The RT reaction was performed according to the instruction in ImProm-II^{TM} reverse transcription system (Promega, Madison, USA). Briefly, 1 μg of total RNA was used for RT reaction in total volume of 20 μl with oligo-dT primer (Promega, Madison, USA). The RT reaction was carried out at 25^oC for 5 min, 42^oC for 1 hr, and 70^oC for 15 min.

3. Real-time polymerase chain reaction (real-time PCR)

The real-time PCR was performed in a mixture of 1 μl of first-stranded cDNA, 0.75 U of GoTaq DNA polymerase

(Promega, Madison, USA), 5 µl of 5X buffer, 0.2 mM of dNTPs (Promega, Madison, USA), 2.5 µl of 3000X SYBR Green dye (BMA, Rockland, USA), and 10 pmols of forward and reverse primers. A total volume of the mixture for each real-time PCR was 25 µl. The PCR program employed an initial step of pre-denaturation at 95°C for 5 min, followed by denaturation at 94°C for 30 sec, annealing at T_m (Table 1) for 30 sec, and extension at 72°C for 30 sec of cycles. Glyceraldehyde-3-phosphate dehydrogenase (*Gapdh*) was served as an internal PCR control. Primer sequences and PCR conditions are summarized in Table 1. Oligonucleotide primers for PCR were obtained from published information and/or by utilizing commercially available primer design software. The sizes of PCR products were checked up by fractionation on 1.2% agarose gel.

4. Data presentation and statistical analysis

We prepared three separated cDNAs from an experimental animal and performed real-time PCR for each cDNA to obtain a mean and a standard deviation of an experimental group. That is, a total of 24 real-time PCRs were carried out to acquire a mean and a standard deviation of a *Slc2a* isoform in a given epididymal part. The expression levels of *Slc2a* mRNAs were compared with those of *Gapdh* and were presented as relative expression ration between *Slc2a* and *Gapdh* mRNAs. Mean differences among 4 experimental age groups were analyzed using one-way ANOVA, followed by a post-hoc test, Tukey's test. In all cases, statistical significances were considered when $p < 0.05$.

RESULTS

1. Expression of *Slc2a1* in the epididymal segments during postnatal development

The expression of *Slc2a1* was detected in all segments of the epididymis at all experimental ages (Fig. 1). In the caput epididymis, a level of *Slc2a1* at 2 weeks of age was significantly higher than that at 1 week of age (Fig. 1A). However, expression levels of *Slc2a1* at 1 month and 3 months of ages were significantly lower than that at 1 week of age (Fig. 1A). Unlike the caput epididymis, levels of *Slc2a1* mRNAs in the corpus epididymis were significantly increased according to postnatal age (Fig. 1B). Especially, a tremendous increase of *Slc2a1* mRNA level at 3 months of age was notable (Fig. 1B). In the caudal epididymis, compared with 1 week of

Table 1. Primer sequences and conditions for real-time PCR

Gene (GenBank access number)	Primer sequence	Expected PCR Size (bps)	Tm (℃)
Slc2a1 (BC061873)	F : GCCTGAGACCAGTTGAAAGCAC (2188-2209) R : CTGCTTAGGTAAAGTTACAGGAG (2457-2479)	292	60
Slc2a3 (NM_017102.2)	F : AACAGAAAGGAGGAAGACCA (643-662) R : CGCAGCCGAGGGGAAGAACA (1253-1272)	630	58
Slc2a4 (NM_012751)	F : GTCATCAACGCCCCACAGAA (270-289) R : GAGAAGATGGCCACGGAGAGAG (385-406)	137	65
Slc2a5 (D13871)	F : TGGTGAATAACTTGGGCAGA (314-333) R : GAGAAGCCGATGAGGAGAAG (1069-1088)	775	60
Slc2a8 (AB033418)	F : TAACCTCACTTGACTGGGGG (1899-1918) R : CACTGAGACCAGGGAAGAGC (2092-2111)	213	60
Gapdh (X02231)	F : CCCCTGGCCAAGGTCATCCATGACAACTTT (540-569) R : GGCCATGAGGTCCACCACCCTGTTGCTGTA (1023-1052)	513	60

Slc2a1 : glucose transporter (GLUT) 1; *Slc2a3* : GLUT3; *Slc2a4* : GLUT4; *Slc2a5* : GLUT5; *Slc2a8* : GLUT8; and *Gapdh* : glyceraldehyde-3-phosphate dehydrogenase.

Numbers in parentheses of primer sequence indicate the positions of nucleotides in GenBank sequence.

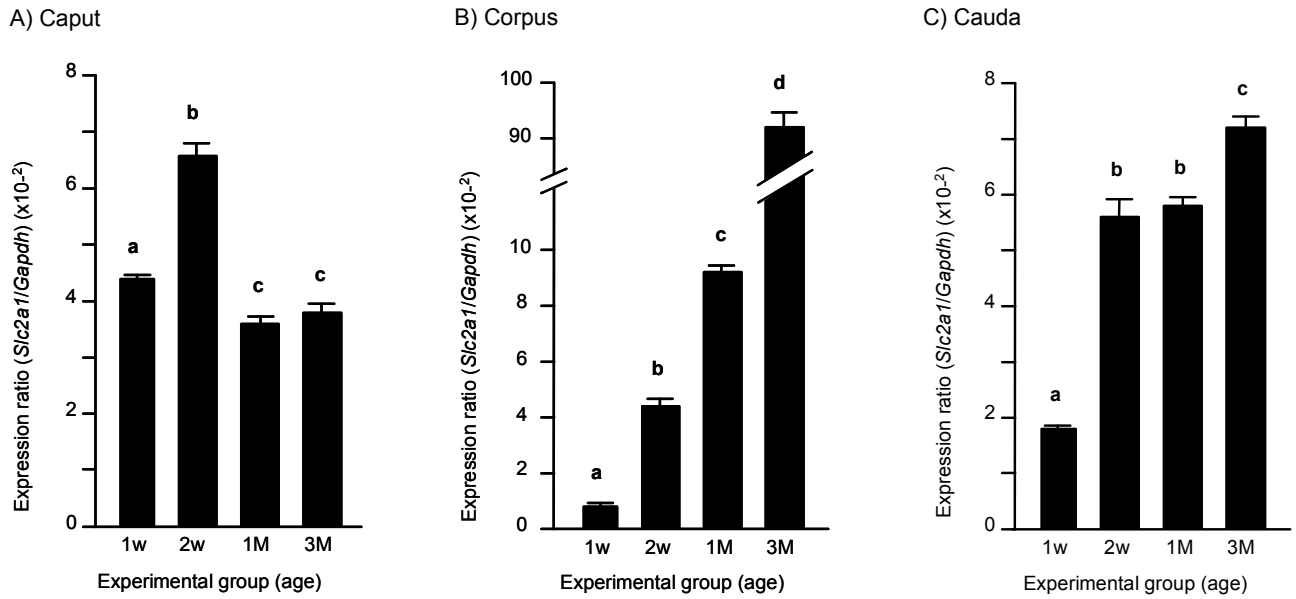

Fig. 1. Expression pattern of *Slc2a1* mRNA in the rat epididymis during postnatal development. The graphs are showing relative expression ratios between *Slc2a1* and *Gapdh* transcripts in the caput (A), corpus (B), and caudal (C) epididymis. 1w : 1 week of age; 2w : 2 weeks of age; 1M : 1 month of age; and 3M : 3 months of age. Different letters indicate significant differences among means ($p < 0.05$).

age, expression levels of *Slc2a1* mRNAs were significantly increased at 2 weeks and 1 month of ages (Fig. 1C). There was no significant change of *Slc2a1* mRNA level between 2 weeks and 1 month of ages (Fig. 1C). A further significant increase of *Slc2a1* transcript level was detected at 3 months of age (Fig. 1C).

2. Differential expression of *Slc2a3* in the rat epididymis during postnatal development

Expression patterns of *Slc2a3* mRNA in the epididymal regions are shown in Fig. 2. In the caput epididymis, a significant increase of *Slc2a3* mRNA level was found at 2 weeks of age, compared with that at 1 week of age (Fig. 2A). The highest abundance of *Slc2a3* mRNA was observed at 1 month of age, followed by a significant reduction to the lowest level of *Slc2a3* mRNA at 3 months of age (Fig. 2A). In the corpus epididymis, the abundance of *Slc2a3* mRNA was significantly increased according to postnatal age, the lowest level at 1 week of age and the highest level at 3 months of age (Fig. 2B). In the caudal epididymis, expression level of *Slc2a3* transcript at 2 weeks of age was significantly higher than that at 1 week of age (Fig. 2C). However, the level of *Slc2a3* mRNA was significantly decreased at 1 month of age, compared with that at 1 week of age (Fig. 2C). A further significant reduction of *Slc2a3* mRNA level was found at 3 months of age (Fig. 2C).

3. Detection of *Slc2a4* expression in the epididymis at different postnatal ages

Differential expression of *Slc2a4* in the epididymis during postnatal development is shown in Fig. 3. In the caput epididymis, expression of *Slc2a4* was relatively high at 1 week of age, and a significant increase of *Slc2a4* mRNA level was followed at 2 weeks of age (Fig. 3A). However, a significant decrease of *Slc2a4* expression was observed at 1 month of age, compared with that at 1 week of age (Fig. 3A). The lowest level of *Slc2a4* mRNA was detected at 3 months of age (Fig. 3A). In the corpus epididymis, a drastic increase of *Slc2a4* mRNA level was found at 2 weeks of age, compared with that at 1 week of age (Fig. 3B). Moreover, the level of *Slc2a4* mRNA at 1 month of age was significantly higher than that of 2 weeks of age, followed by a transient decrease of *Slc2a* mRNA abundance to the lowest level at 3 months of age (Fig. 3B). In the caudal epididymis, the level of *Slc2a4* mRNA was

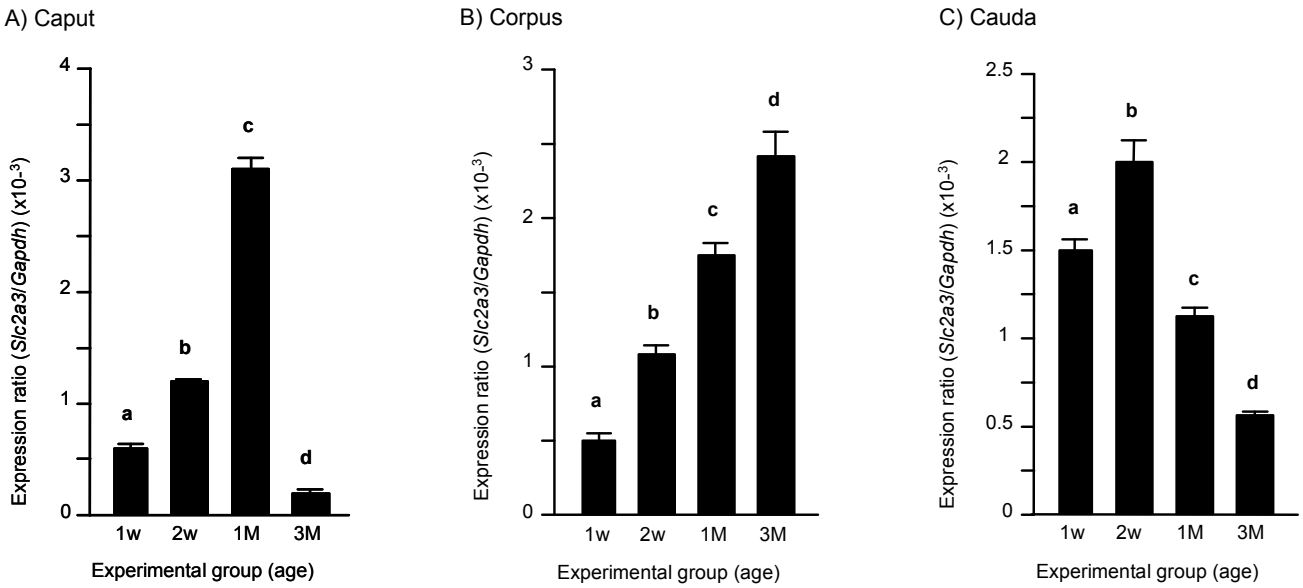

Fig. 2. Expression pattern of *Slc2a3* mRNA in the rat epididymis during postnatal development. The graphs are showing relative expression ratios between *Slc2a3* and *Gapdh* transcripts in the caput (A), corpus (B), and caudal (C) epididymis. 1w : 1 week of age; 2w : 2 weeks of age; 1M : 1 month of age; and 3M : 3 months of age. Different letters indicate significant differences among means ($p < 0.05$).

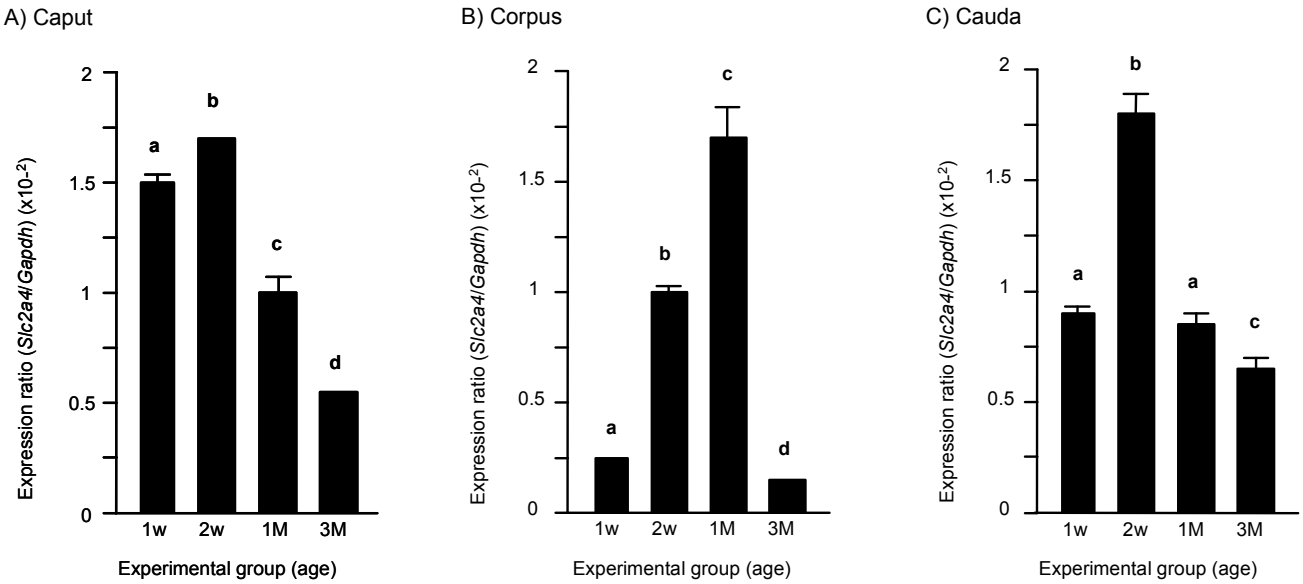

Fig. 3. Expression pattern of *Slc2a4* mRNA in the rat epididymis during postnatal development. The graphs are showing relative expression ratios between *Slc2a4* and *Gapdh* transcripts in the caput (A), corpus (B), and caudal (C) epididymis. 1w : 1 week of age; 2w : 2 weeks of age; 1M : 1 month of age; and 3M : 3 months of age. Different letters indicate significant differences among means ($p < 0.05$).

significantly increased at 2 weeks of age, compared with that at 1 week of age (Fig. 3C). However, the abundance of *Slc2a4* transcript at 1 month of age was decreased to the level at 1 week of age, and a significant decrease of *Slc2a4* mRNA level was found at 3 months of age, compared with that at 1 week of age (Fig. 3C).

4. Comparison of *Slc2a5* expression in different epididymal regions during postnatal development

Expression profile of *Slc2a5* among the rat epididymal regions during postnatal development is shown in Fig. 4. In the caput epididymis, the level of *Slc2a5* mRNA was not changed until 1 month of age (Fig. 4A). However, the abundance of *Slc2a5* transcript was significantly decreased at 3 months of age, compared those at the earlier ages (Fig. 4A). Unlike the case of the caput epididymis, expression of *Slc2a5* in the corpus epididymis at 1 week of age was almost undetectable (Fig. 4B). However, the levels of *Slc2a5* mRNA were significantly increased according to postnatal age, with the highest level at 3 months of age (Fig. 4B). In the caudal epididymis, the expression of *Slc2a5* was extremely low, except at 1 month of age at which the level of *Slc2a5* mRNA was significantly increased (Fig. 4C).

5. Expression patterns of *Slc2a8* in the rat epididymis at different postnatal ages

The expression of *Slc2a8* was observed in the entire epididymal regions during postnatal development (Fig. 5). Expression levels of *Slc2a8* in the caput epididymis were significantly reduced according to postnatal ages, the highest level at 1 week of age and the lowest level at 3 months of age (Fig. 5A). In the corpus epididymis, however, a significant increase of *Slc2a8* mRNA level was found at 2 weeks of age, compared with that at 1 week of age (Fig. 5B). At 1 month of age, the abundance of *Slc2a8* transcript was significantly lower than that at 1 week of age, and a further significant decrease of *Slc2a8* mRNA level was observed at 3 months of age (Fig. 5B). Similarly, in the caudal epididymis, a significant increase of *Slc2a8* mRNA level was detected at 2 weeks of age, followed by a significant decrease of *Slc2a8* mRNA abundance at 1 month of age (Fig. 5C). There was no significant change of *Slc2a8* mRNA level between 1 month and 3 months of ages (Fig. 5C).

DISCUSSION

The present study showed the presence of a number of *Slc2a* mRNAs in the epididymis. Also, data from the current study demonstrated that mRNA expression levels of *Slc2a* isoforms were different within each epididymal segment. Moreover, our present study revealed differential expression of these *Slc2a* isoforms among epididymal segments according to postnatal ages.

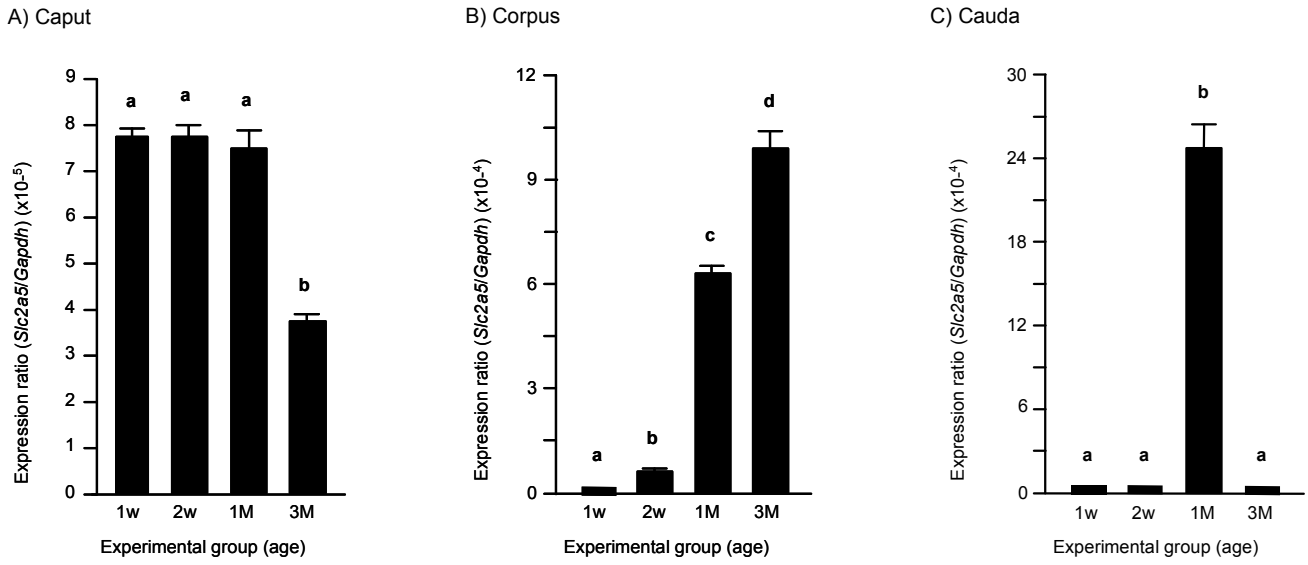

Fig. 4. Expression pattern of *Slc2a5* mRNA in the rat epididymis during postnatal development. The graphs are showing relative expression ratios between *Slc2a5* and *Gapdh* transcripts in the caput (A), corpus (B), and caudal (C) epididymis. 1w : 1 week of age; 2w : 2 weeks of age; 1M : 1 month of age; and 3M : 3 months of age. Different letters indicate significant differences among means ($p < 0.05$).

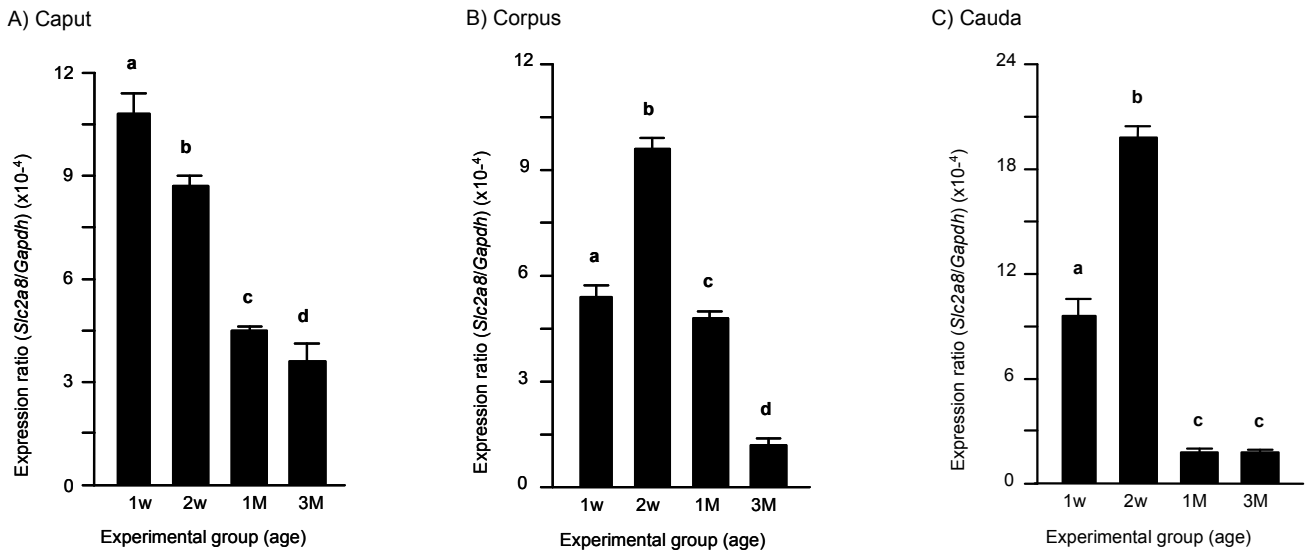

Fig. 5. Expression pattern of *Slc2a8* mRNA in the rat epididymis during postnatal development. The graphs are showing relative expression ratios between *Slc2a8* and *Gapdh* transcripts in the caput (A), corpus (B), and caudal (C) epididymis. 1w : 1 week of age; 2w : 2 weeks of age; 1M : 1 month of age; and 3M : 3 months of age. Different letters indicate significant differences among means ($p < 0.05$).

Expression and localization of *Slc2a* isoforms in mammalian tissues have been widely examined by a number of researches. The *Slc2a1* ubiquitously expressed functions basal glucose uptake for the cells (Mueckler et al., 1985; Zhao et al., 1993). Expression of *Slc2a3* is specifically localized in the brain and nerve cells, even though a strong expression of *Slc2a3* is found in the testis and spermatozoa (Haber et al., 1993; Shepherd et al., 1992). The *Slc2a4* is highly expressed in insulin-sensitive tissues, including adipose tissues and skeletal muscle (Abe et al., 1997; Duhlmeier et al., 2005; Gonzalez and McGraw, 2006). In the male reproductive tract, a strong expression of *Slc2a4* has been detected in the epididymal fat (Stephens et al., 1992). In contrast to *Slc2a1*, *3*, and *4* which mediate glucose transport, *Slc2a5* exhibits transport activity for fructose and is localized in intestine, kidney, and testis, as well as adipose tissues and skeletal muscle to a lesser extent (Corpe et al., 2002; Davidson et al., 1992; Rand et al., 1993). The presence of *Slc2a8* has been demonstrated in the testis, brain, adipocytes, as well as spermatozoa within the epididymis (Schürmann et al., 2002; Wood and Trayhurn, 2003; Zhao and Keating, 2007). Based on these observations, it is suggested that transport of glucose and hexose in a specific tissue is regulated by a combined and cooperative action of *Slc2a* isoforms. Even though the presence of *Slc2a* isoforms in the testis has been well determined, the expression of *Slc2a* isoforms in the other male reproductive tissues has not caught attention. Our present study demonstrates that at least 5 different *Slc2a* isoforms are expressed in the epididymis. In addition, the current study shows differential mRNA abundance of these *Slc2a* isoforms among the epididymal segments. However, we can not rule out a possibility which other *Slc2a* isoforms would be expressed in the epididymal regions. Thus, an additional study would be necessary to find out new types of *Slc2a* isoforms present in the epididymis.

The present study has demonstrated differential expression patterns of *Slc2a* isoforms among the epididymal segments during the postnatal development. A number of researches have shown the expressional regulation of *Slc2a* isoforms by various factors. In the testis, the expression of *Slc2a1* in Sertoli cells is regulated by follicle-stimulating hormone (FSH), interleukin-1β (IL1β), and basic fibroblast growth factor (bFGF) (Galardo et al., 2008). In addition, the expression of *Slc2a1* in Sertoli cells is stimulated by thyroid hormone, especially triiodothyronine (T_3) (Carosa et al., 2005; Ulisse et al., 1992). The expression of *Slc2a8* in Leydig cells is up-regulated by human chorionic gonadotropin (hCG) and insulin-like growth factor-I (IGF-I) and down-regulated by cytokines, murine interleukin-1α (mIL-1α), murine tumor

necrosis factor-α (mTNF-α), and murine interferon-γ (mIFN-γ) (Chen et al., 2003). Moreover, testosterone and estrogen involve in regulation of GLUT expression in the testis (Doege et al., 2000; Nualart et al., 2009). The epididymis contains luteinizing hormone (LH) receptor (Lei et al., 2003), thyroid hormone receptor (Del Rio et al., 2000), androgen and estrogen receptors (Yamashita, 2004), and TNF receptor (Kajihara et al., 2006). Thus, it is speculated that the expression of *Slc2a* isoforms detected from the current study would be under control of various intra- and/or inter-gonadal factors. A relationship between expression patterns of these regulatory factors' receptors and *Slc2a* isoforms during postnatal developmental period should be determined in a future study.

The functions of GLUTs include basal glucose uptake, transport of fructose, insulin-regulated glucose transport, and fuel supply of spermatozoa (Zhao and Keating, 2007). Most importantly, these functions of GLUTs depend on their cellular localization and specificity to substrates (Zhao and Keating, 2007). The epididymis is a metabolically very active tissue which plays a number of important functions for male reproduction, such as maturation of spermatozoa, storage of sperms, secretion and absorption of protons, and endocytotic absorption and secretion of luminal proteins and factors (Cornwall, 2009). The epididymal epithelium has several different cell types, including principal, narrow, basal, apical, and clear cells (Cornwall, 2009). Except the principal cells, the functions of other cell types have not been well defined (Cornwall, 2009). Because the cellular localization of GLUTs in the epididymis has not been determined, the roles of these GLUTs in the epididymis would not be explainable at this time. However, it is reasonably considered that glucose transported via GLUTs would be required to maintain the basal metabolism of the epididymis. It is also suggested that GLUT-delivered glucose would be utilized for synthesis of epididymal proteins secreted into the lumen for sperm maturation. The cellular composition among the epididymal segments varies at different ages during postnatal development (Setty and Jehan, 1977). Thus, it is possible that formation of such epididymal compartmentation during postnatal period would associate with differential expression of *Slc2a* isoforms among the epididymal segments during postnatal development, at least in part, as shown in the present study. Examination of the localization of GLUTs within the epididymal segments would provide valuable information to estimate possible roles of GLUTs in the epididymis.

A particular role of GLUTs in the epididymis has not been elucidated at current point. However, the present study suggests that differential expression of several *Slc2a* isoforms in the epididymis during postnatal development would relate with proper sperm maturation in the epididymis, so thus male fertility.

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