생후 발달과정동안 남성 생식기의 Efferent Ductules에서 Monocarboxylate Transporters (MCTs)와 Basigin의 발현 양상과 에스트로젠 수용체 α에 의한 MCT1 발현 조절 연구

이기호* · 최인호** · 정정수*** · 장종수****

을지대학교 생화학교실*, 영남대학교 생명과학부**, 충북대학교 축산학과***,
한국방송통신대학교 농학과****

Expression of Monocarboxylate Transporters (MCTs) and Basigin and Estrogen Receptor α (ERα) - Mediated Regulation of MCT1 Expression in The Efferent Ductules of Male Reproductive Tract During Postnatal Development

K. H. Lee*, I. H. Choi**, C. S. Chung*** and J. S. Chang****

Department of Biochemistry-Molecular Biology and Center of Antiaging, Eulji University*, School of Biotechnology, Yeungnam University**, Department of Animal Science, Chungbuk National University***, Department of Agricultural Sciences, Korea National Open University****

요 약

본 연구에서는 남성 생식 기관인 efferent ductules (ED)에서 monocarboxylate transporter (MCT) isoform과 Basigin (Bsg)의 mRNA 발현을 탐구하고, MCT1의 발현이 에스트로젠 수용체 α에 의해 조절되는지를 연구하였다. 생후 여러 연령대의 백서 ED에서 MCT isoform과 Bsg의 mRNA 발현 여부는 real-time PCR에 의해 조사되었고, 정상 생쥐와 에스트로젠 수용체 α knockout 생쥐를 사용하여 에스트로젠 수용체 α에 의한 MCT1의 발현 조절 여부는 immunohistochemistry 방법에 의해 간접적으로 알아 보았다. 본 연구 결과는 백서의 ED에서 MCT1, 2, 3, 4와 8 그리고 Bsg 유전자의 발현은 연령에 따라 다르게 나타나며, MCT1의 발현이 ED의 ciliated 세포의 basolateral 지역에서 발현되고 어떤 측면에서 ERα에 의해 조절되어 질 수 있음을 보여 주었다. 따라서 본 연구 결과는 MCT가 monocarboxylate의 세포 내, 외부로 운반을 조절함으로써 남성 생식기의 일부인 ED의 역할을 조절하는데 관여함을 시사한다.

(**색인어** : Efferent ductules, Monocarboxylate transporter, Basigin, 에스트로젠 수용체 α, 에스트로젠)

Ⅰ. INTRODUCTION

The male reproductive tract is consisted of the testis and the excurrent ducts. The efferent ductules (ED) are a part of the excurrent ducts, and are a conduit connecting the testis and the head of epididymis. As an embryologic homology, the ED have morphological and functional similarities with proximal tubules in the kidney (Clulow et al., 1994). The ED are the places

Corresponding author : Ki-Ho Lee, Department of Biochemistry and Molecular Biology, College of Medicine, Eulji University, 143-5 Yongdoo-dong, Joong-goo, Daejeon, 301-110, Korea.
Tel : 82-42-259-1643, Fax : 82-42-259-1649, E-mail : kiholee@eulji.ac.kr

where majority of the testicular fluid is reabsorbed, resulting in an increase in sperm concentration (Clulow et al., 1994; Clulow et al., 1998). Other than fluid reabsorption, the ED play roles in transport of sperm and the testicular fluid, secretion and/or absorption of ions and proteins, and reabsorption of testicular proteins (Clulow et al., 1994).

The ED have an epithelial layer, supported by smooth muscle and connective tissues. The epithelium of the ED has two cell types, ciliated and nonciliated cells. The nonciliated cells, a dominant cell type in the epithelium of the ED, are characterized by the presence of apical microvilli (Ilio and Hess, 1994). These cells have distinct features which are specialized for the uptake of materials and fluid from the lumen (Ilio and Hess, 1994; Lee et al., 2000). The ciliated cells have apical cilia, protruding into lumen, and relatively high number of mitochondria (Ilio and Hess, 1994). Ciliated cells appear to function in movement of fluid and spermatozoa through the duct, with less extensive for fluid reabsorption (Ilio and Hess, 1994).

Monocarboxylate transporters (MCTs) facilitate the influx and efflux of monocarboxylate, such as lactate, pyruvate, and ketone bodies, across plasma membrane in various organs (Garcial et al., 1994; Halestrap and Price, 1999; Bonen et al., 2006). To date, 14 isoforms of MCT family have been identified (Halestrap and Price, 1999; Halestrap and Meredith, 2004). Of these MCTs, MCT1-MCT4 are true proton-linked cotransporters, while MCT8 is a facilitated transporter in monocarboxylate transport (Halestrap and Meredith, 2004). MCTs are widely expressed on the cell surface in a variety of tissues (Bonen et al., 2006). MCT1 is ubiquitously found in majority of tissues, while expression of certain MCTs is restricted in specific tissues, such as MCT3 in retinal pigment epithelium (Halestrap and Meredith, 2004). In the male reproductive tract, germ cells, Sertoli cells, and Leydig cells in the testis possesses MCT1, 2, and 4 (Branuchi et al., 2004; Nakai et al., 2006), and the presence of MCT1 and 2 has been detected in the epididymis (Garcia et al., 1995; Nakai et al., 2006). In addition, expression and basolateral localization of MCT1 has found in ciliated cells of mouse ED (Nakai et al., 2006). These results indicate that MCTs would play roles in regulation of spermatogenesis in the testis and functions of the excurrent duct in the male reproductive tract. However, detailed function of MCTs and expression of other MCT isoforms in the male reproductive tract have not been examined yet.

Basigin (Bsg), also known as CD 147 and extracellular matrix metalloproteinase inducer, is a multifunctional transmembrane glycoprotein. Basigin is expressed in various tissues and involves in diverse physiological events, including spermatogenesis in the testis (Chen et al., 2004) and tumor invasion (Zucker et al., 2001). Of proposed functions of Bsg is proper localization and expression of MCTs in retinal and kidney epithelia (Philp et al., 2003; Deora et al, 2005). Expression of Bsg in the testis is restricted in Leydig cells, while epididymis does not express Bsg (Nakai et al., 2006). In the ED, Bsg is present in ciliated cells and colocalizes with MCT1 on basolateral membrane (Nakai et al., 2006). Knockout of the Bsg gene results in aberrant localization of MCT1 on ciliated cells, along with a decrease of immunoreactivity, in the ED (Nakai et al., 2006). Together, these studies imply that MCTs and Bsg play a role in the ED of the male reproductive tract.

The regulation of MCT expression in the male reproductive tract has not been determined. The presence of estrogen receptor (ER) α and ERβ in the ED has been documented (Cooke et al., 1991; Rosenfeld et al., 1998). The level of ERα in the ED is the highest in the male reproductive tract

and 3.5 times higher than that of the uterus in female (Cooke et al., 1991). In addition, it has been demonstrated that estrogen plays important roles in regulation of ED function and maintenance of ED morphology (Hess et al., 1997; Lee et al., 2000; Lee et al., 2001). Moreover, the presence of functional ERα is required for proper function and normal morphology of the ED (Hess et al., 1997; Lee et al., 2000; Lee et al., 2001). Based on this information, it is speculated that estrogen would involve in the regulation of MCT expression in the ED through ERα.

The purpose of the present study was to determine if other MCT isoforms, rather than MCT1, express in the ED and if there was a differential expression of MCTs and Bsg in the ED during postnatal development by real-time polymerase chain reaction (PCR). In addition, we compared expression of MCT1 in the ED of wild type (WT) and estrogen receptor (ER) α knockout (αERKO) mice by immunohistochemistry to determine whether or not the changes in expression of MCT1 in the ED was associated with the presence of functional ER α.

Ⅱ. MATERIALS AND METHODS

1. Isolation and collection of the tissue

Male Sprague Dawley rats were purchased from Samtako (O San, S. Korea), and were individually caged under controlled conditions and given *ad libitum* food and water during the entire experimental period. Rats were randomly divided into 5 experimental groups; 1) 2 prepubertal groups, 1 week (n=8) and 2 weeks (n=6) of ages, 2) 1 pubertal group, 1 month (n=5) of age, and 3) 2 fully mature groups, 3 (n=5) and 6 months (n=5) of ages. At a proper age, rats were anesthetized by CO_2 stunning, and entire reproductive tracts were taken from the animals. Epididymal fat was rapidly removed, and the ED were separated from the rest of male reproductive tract. Then, the ED were washed with ice-cold PBS buffer before freezing in liquid nitrogen. Due to a limited size, the ED isolated from an age group were pooled to obtain sufficient amounts of total RNA for reverse transcription (RT) and real-time polymerase chain reaction (PCR).

Homozygous wild type (WT; C57BL65/129SVJ) and estrogen receptor α knockout (αERKO) sibling male mice were obtained from a resident breeding colony maintained at the University of Illinois at Urbana-Champaign. Four experimental groups consisting of both WT and αERKO were used at the following ages; 10 days-old WT (n=4) and αERKO (n=3), 18 days-old WT (n=3) and αERKO (n=4), 35 days-old WT (n=4) and α ERKO (n=4), and 60 days-old WT (n=3) and α ERKO (n=3). Mice were killed by cervical dislocation, and male reproductive tract was fixed for detection of MCT1 in the ED by immunohistochemistry. The kidney was used as a positive control.

2. RNA isolation and primer design for real-time PCR

Total RNA was prepared by using easy-Blue total RNA extraction solution (iNtRON Biotech., Sungnam, S. Korea) and a Polytron homogenizer (Fisher Scientific, Pittsburgh, USA). The isolated RNA pellets were dissolved in RNA storage buffer (Ambion, Austin, USA) and stored at −80 ℃ until used for real-time PCR. The purity and yield of the total RNA were determined by an UV spectrophotometer (Eppendorf, New York, USA), and the qualities of the total RNAs were checked by gel electrophoresis prior to proceeding RT reaction. Oligonucleotide primers for real-time PCR were prepared by either using Primer 3 software (http://www.bioneer.co.kr/cgi-

bin/primer/primer3.cgi; Whitehead Institute / MIT Center for Genomes Research, USA) or utilizing published information. Information and sequences of primers of MCTs and Bsg tested for the present study are summarized in Table 1.

3. Reverse transcription and real-time PCR analysis

The RT procedure was performed according to the instruction in ImProm-II™ reverse transcription system (Promega, Madison, USA). Briefly, 1 μg of total RNAs was reverse-transcribed in total volume of 20 μl using oligo-dT primer. Reverse transcription reaction was carried out at 25℃ for 5 min, 42℃ for 1 hour, and 70℃ for 15 min. We used 1 μl of cDNA from each age group as real-time PCR template in a 25 ul reaction mixture, including 0.75 U of GoTaq DNA polymerase (Promega, Madison, USA), 5 ㎕ of 5 × buffer 5, 0.2 mM dNTP, 2.5 ㎕ of 3000X SYBR Green I (BMA, Rockland, USA), and 10 pmol of each primer. The PCR program employed an initial step of 95℃ for 5 min for denaturation, followed by denaturation at 94℃, annealing, and extension step at 72℃ of cycles using PTC-200 Chromo4 real-time system (Bio-Rad Laboratories, Hercules, USA). The final extension at 72℃ for 10 min was carried out for the PCR. No RNA, no template, and no primer controls were included in the PCR. In order to visualize the PCR results, the PCR products were subjected to electrophoresis on 1.2% agarose gel. The image of each gel was photographed under

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

MCTs	Forward primer sequence (5' - 3')	Reverse primer sequence (5' - 3')	Product size (bp)	GenBank access number	Tm (℃)
MCT 1	AGAAGTCAGCCTTCC TCCTTT (1071 - 1091)	CCACAAGCCCAGTAT GTGTAT (1444 - 1464)	394	NM_012716	60
MCT 2	GGCCTTCGGTAGGATT AATAG (1095 - 1115)	ATGCCTGATGATAACA CGACT (1441 - 1461)	367	X97445	60
MCT 3	GCTCTGAAGAACTAT GAAATCA (1352 - 1373)	GTGAACAGGGTCTAA CATATTG (1757 - 1778)	427	AF059258	53
MCT 4	TGCGGCCCTACTCTGT CTAC (881 - 900)	TCTTCCCGATGCAGA AGAAG (1017 - 1036)	369	AF178954	60
MCT 8	TCCCTTCCTCATCAAA ATGC (759 - 775)	CGTAGGGGACGAAGT AACCA (1017 - 1036)	281	AJ496570	60
Bsg	CAGGATCAAGGTGGG AAAGA (330 - 349)	CACTTGGGCTGGGAT AAGAA (944 - 963)	634	NM_012783	60
GAP-DH	CCCCTGGCCAAGGTC ATCCATGACAACTTT (540 - 569)	GGCCATGAGGTCCAC CACCCTGTTGCTGTA (1023 - 1052)	513	X02231	60

MCTs : monocarboxylate transporters ; Bsg : Basigin, aka. CD147 and EMMPRIN
Numbers in parenthesis of primer sequences indicate the positions of bases in GenBank sequences.

UV using an image documentation system (Vilber Lourmat, Marne-la-Vallée, France). In this assay, we included GAPDH, which served as an internal PCR control.

4. Immunohistochemistry

The male reproductive tract was fixed in Bouin's fixative for 8 hours. Then the ED were careful dissected from other parts of the reproductive tract. The ED were dehydrated, cleaned, and infiltrated with paraffin. Paraffin-embedded ED were sectioned at 4 μm thickness. The sections were deparaffinized and rehydrated in an ethanol series of baths. Then, the sections were microwaved for 10 min in 0.01 M citrate buffer, pH 6.0, for antigen retrieval, and treated with 0.3% hydrogen peroxide in methanol for 10 min to inactivate endogenous peroxidase. After washing in PBS, sections were incubated in 10% normal goat serum to prevent nonspecific binding. We used a 1:100 dilution of a chicken anti-MCT1 antibody (Chemicon International, Temecula, CA) as a primary antibody. After overnight incubation at 4℃ in a humidified chamber, slides were washed with PBS and treated with HRP-labeled goat anti-chicken IgG antibody (Kirkegaard and Perry Laboratories, Gaithersburg, MD) at 1:200 dilution for 1 hr at room temperature in a humidified chamber. Then, section were treated with a mixture of 3,3'-diaminobenzidine (DAB; Sigma, St. Louis, MO), 0.05 M Tris-HCl buffer, and 5% hydrogen peroxide to detect the peroxidase and counterstained with hematoxylin, dehydrated, and mounted. For the negative control, either normal serum at the same dilution as the primary antibody or PBS was placed on the section. The immunostaining was evaluated with digitalized images captured with Olympus-CoolSNAP cf color/OL camera (Olympus America, Melville, NY) using RSImage version 1.1 software (Roper Scientific, Duluth, GA). The photographic images were processed in PhotoShop software (Adobe Systems, San Jose, CA).

5. Data presentation and statistical analysis

We repeated the RT reaction and PCR for each age group at five times to obtain a mean and a standard deviation. The difference in threshold cycles ($\triangle$Ct values) for each MCT isoform at different ages was normalized with those of GAPDH. Data of mRNA abundance of each MCT isoform were expressed relative to 1 week of age as arbitrary units. Comparisons of expression differences among the age groups for each MCT molecule were made using one-way ANOVA followed by Tukey's test. In all cases, results were considered significant if $P < 0.05$.

Ⅲ. RESULTS

1. Expression of MCT1 and MCT2 in the efferent ductules of rats during post-natal development

Expression of MCT1 and MCT2 mRNAs in the ED of rats is shown in Fig. 1. A significant increase of MCT1 mRNA expression was detected at 2 weeks of age, compared with its at 1 week of age (Fig. 1A). At 1 month of age, abundance of MCT1 mRNA in the ED was decreased to the level of 1 week of age, followed by further reduction of MCT1 mRNA levels at 3 and 6 months of age (Fig. 1A). Expression of MCT2 mRNA was detected in the ED of all age groups (Fig. 1B). Dramatic increases, about 5 folds, of MCT2 mRNA level were found at 2 weeks and 1 month of ages (Fig. 1B). At 3 months of age, abundance of MCT2 mRNA was transiently decrease, but still significantly higher than its at 1 week of age (Fig. 1B). A further decrease of MCT2 mRNA level, close to its at 1 week of age, was found

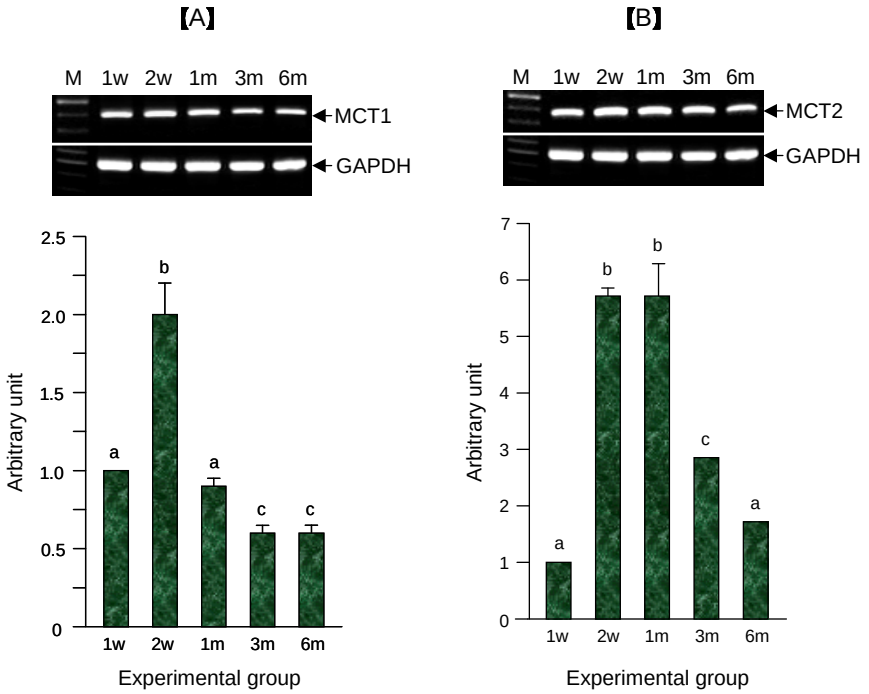

Fig. 1. mRNA expression of MCT1 [A] and MCT2 [B] in the efferent ductules of male rats during postnatal development. 1w : 1 week, 2 w : 2 weeks, 1m : 1 month, 3m : 3 months, and 6m : 6 months of age. M : 100bp marker. a-c : means with the different letters are significantly different ($P < 0.05$). MCT : monocarboxylate transporter.

in the ED at 6 months of age (Fig. 1B)

2. Expression of MCT3 and MCT4 in the efferent ductules of rats during postnatal development

Expression pattern of MCT3 and MCT4 mRNA was an age-dependent as shown in Fig. 2. In case of MCT3, the highest level of mRNA expression was found in the ED at 1 week of age (Fig. 2A). Significant decreases of MCT3 mRNA levels were observed as animals became aged (Fig. 2A). The lowest abundance of MCT3 mRNA was detected in the ED at 3 months of age, followed by a slight increase but statistically not significant at 6 months of age (Fig. 2A). In contrast to MCT3, expression of MCT4 mRNA was increased according to the age (Fig. 2B). The lowest level of MCT4 mRNA was found in the ED at 1 and 2 weeks of ages (Fig. 2B). However, the level of MCT4 mRNA was significantly increased at 1 month of age, with further increases at 3 and 6 months of ages (Fig. 2B)

3. Expression of MCT8 and Bsg in the efferent ductules of rats during postnatal development

The existence of MCT8 mRNA was detected in the ED of postnatally developing rats (Fig. 3A). A significant increase of MCT8 mRNA abundance was found at 2 weeks of age, compared with its at 1 week of age (Fig. 3A). However, at 1 month of age, the level of MCT8 mRNA was significantly decreased close to the level at 1 week of age (Fig. 3A). Abundance of MCT8 mRNA in the ED at 3 and 6 months of age was significantly lower than other age groups

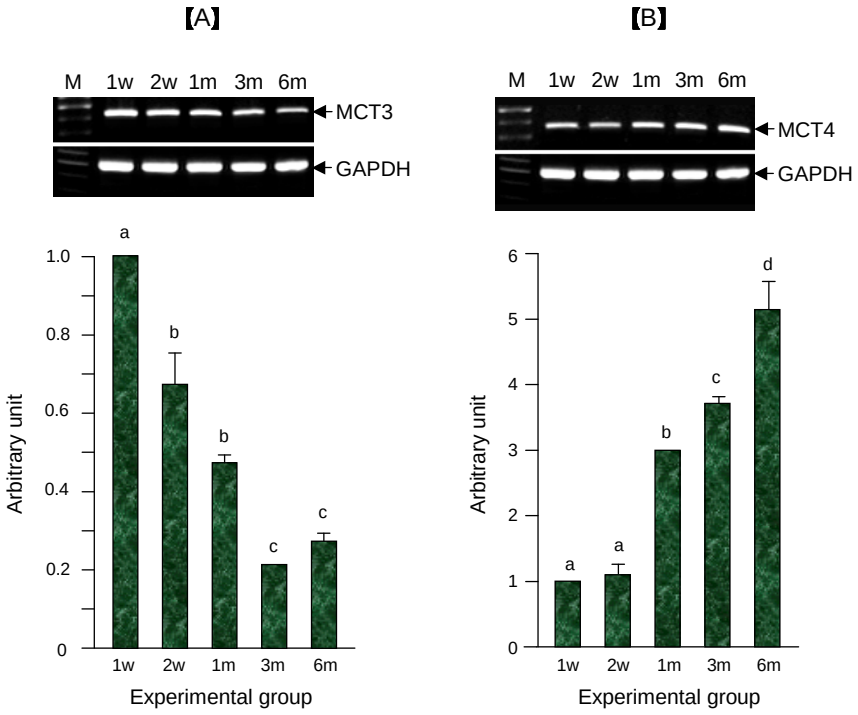

Fig. 2. mRNA expression of MCT3 [A] and MCT4 [B] in the efferent ductules of male rats during postnatal development. 1w : 1 week, 2 w : 2 weeks, 1m : 1 month, 3m : 3 months, and 6m : 6 months of age. M : 100bp marker. a-d : means with the different letters are significantly different ($P < 0.05$). MCT : monocarboxylate transporter.

(Fig. 3A). As expected, Bsg mRNA was detected in the ED of rats (Fig. 3B). The highest level of Bsg mRNA was found at 2 weeks of age, followed by gradual decreases of Bsg mRNA levels as animals became aged (Fig. 3B). However, abundance of Bsg mRNA was not significant among 1, 3, and 6 months of ages (Fig. 3B).

4. Immunohistochemistry of MCT1 in the efferent ductules of WT and αERKO mice during postnatal development

Positive immuno-reactivity for MCT1 was detected in the ED of WT and αERKO mice (Fig. 4). Very strong immunostaining for MCT1 was localized at basolateral membrane of ciliated cells in the ED (Fig. 4). Also, immuno-positive ciliated cells had somewhat weak staining for MCT1 in basolateral cytoplasm. Nonciliated cells and connective tissues in the ED were immuno-negative for MCT1 (Fig. 4). In WT mice, a positive immunostaining for MCT1 in the ED was observed at all age groups (Fig. 4A, C, E, and G). At 10 days of age, a few ciliated cells were positive for MCT1 immunostaining (Fig. 4A). An increase of number of immuno-positive cells for MCT1 was observed at 18 days of age (Fig. 4C). Most of ciliated cells at 35 days of age were positively stained for MCT1 (Fig. 4E), and eventually all of ciliated cells were immuno-positive for MCT1 at 60 days of age (Fig. 4G). In ERKO mice, no positive immuno-staining for MCT1 was observed in the ED at 10 days of old (Fig. 4B). However, immuno-reactivity for MCT1 was detected on basolateral region of ciliated cells at 18 days of age, even though not

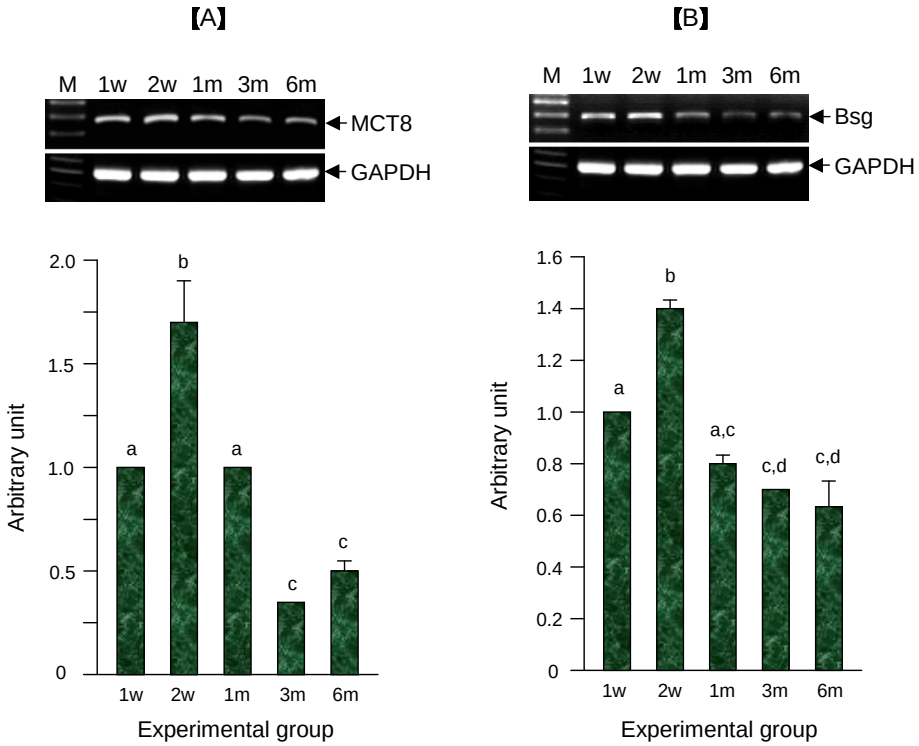

Fig. 3. mRNA expression of MCT8 [A] and Bsg [B] in the efferent ductules of male rats during postnatal development. 1w : 1 week, 2 w : 2 weeks, 1m : 1 month, 3m : 3 months, and 6m : 6 months of age. M : 100bp marker. a-c : means with the different letters are significantly different ($P < 0.05$). MCT : monocarboxylate transporter. Bsg : Basigin.

all of ciliated cells were positive (Fig. 4D). At 35 days of age, a number of immuno-positive cells for MCT1 in αERKO mice were less than those in WT mice, and intensity of immuno-reactivity along basolateral side for MCT1 was visibly lower than WT mice (Fig. 4F). Compared with WT mice, immuno-reactivity and a number of ciliated cells for MCT1 in αERKO mice were vividly decreased at 60 days of age (Fig. 4H). In kidney as a positive control, the basal cytoplasm of epithelial cells lining the initial part of the proximal convoluted tubules was intensively immuno-stained for MCT (Fig. 4I). Negative control replaced with serum or PBS in place of antibody showed no immuno-reactivity for MCT1 in the ED (Fig. 4J).

Ⅳ. DISCUSSION

This is the first study to demonstrate the presence of various MCT isoforms in the ED of male reproductive tract. It is shown that MCT isoforms and Bsg are differentially expressed according to the ages during postnatal development. In addition, the present study provides, in part, an evidence of ERα-mediated regulation of MCT1 expression in the ED, as seen in αERKO mice.

Expression and distribution of several MCT isoforms in a tissue or an organ have been demonstrated in a number of studies (Graham et al., 2007; Enoki et al., 2006; Bonen et al., 2006). In male reproductive tract, the testis possesses MCT1, 2, and 4, and the expression of these MCTs shows cell type-specificity (Branuchi

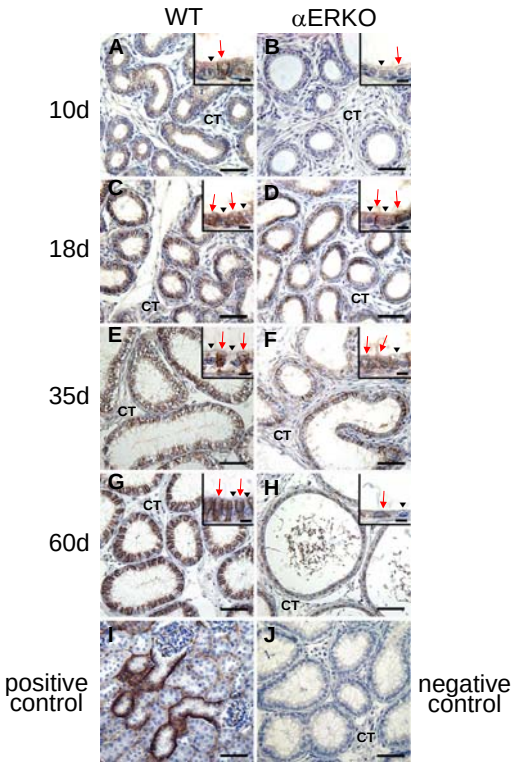

Fig. 4. Immunohistochemical analysis of MCT1 in the efferent ductules of WT and α ERKO mice during postnatal development. A positive immuno-reaction for MCT1 shows in the ED of WT mice throughout postnatal development (A, C, E, and G). In the ED of αERKO mice, immunoreactivity for MCT1 is detected at 18 and 35 days of ages, followed by visible decrease of intensity at 60 days of age (B, D, F and H). Positive immuno-reaction for MCT1 is localized in basolateral region of ciliated cells (red arrows), while non-ciliated cells (black arrow heads) are immuno-negative for MCT1. In kidney, a positive control, a strong immuno-reactivity for MCT1 is localized in the basal region of epithelia of the proximal convoluted tubules (I). In negative control, replacement with normal serum or PBS in the place of MCT1 antibody shows no immuno-staining in the efferent ductules of mice (J). 10 d : 10 days, 18 d : 18 days, 35 d : 35 days, and 60 d : 60 days of age. CT : connective tissues. Bar = 50 μm. Bar in insert = 5 μm.

et al., 2004). For example, MCT1 and 4 are expressed in pachytene spermatocytes and round spermatids, while expression of MCT2 is restricted in round spermatids (Branuchi et al., 2004). MCT1 is also expressed in the head and tail epididymis (Koehler-Stec et al., 1998; Nakai et al., 2006), and the expression of MCT2 is detected in the epididymal fat (Koehler-Stec et al., 1998). In the ED, Nakai et al. (2006) showed the presence of MCT1 in basolateral region of ciliated cells of the mouse ED. Existence of several MCT isoform mRNAs in the ED detected from the current study implies a complexity of monocarboxylate movement through epithelial cells, because each MCT has a different substrate-specificity (Halestrap and Price, 1999) and a functional characteristic (Halestrap and Meredith, 2004). A functional role of MCTs in the ED have not been determined yet. Because the ED are a major site at which more than 90% of the testicular fluid is reabsorbed (Clulow et al., 1994), it is proposed that MCTs might involve in the regulation of fluid reabsorption in the ED. MCT1-4 are proton-coupled cotransporters, while MCT8 is proton-independent thyroid hormone faciliatated transporter (Halestrap and Meredith, 2004). Lee et al. (2001) have demonstrated that fluid movement in the ED is coupled with active Na^+, Cl^-, H^+, and HCO_3^- transports. Thus, it is possible that MCTs, working together with other ion transporters, would participate in the control of fluid movement in the ED. A functional study with selective inhibitors for MCTs would provide information of physiological role of MCTs in the ED.

Immunohistochemical localization for MCT1 in ciliated cells of the ED in the present study is in agreement with the finding from Nakai et al. (2006). Our study also demonstrated increases of a number of immuno-positive cells and intensity of immunoreactivity for MCT1 in the ED of WT mice during postnatal development. Interestingly, a lack of functional ERα in the ED led to

aberrant expression pattern of MCT1, indicating that the present of ERα is, at least, necessary for proper expression of MCT1 in the ED. However, we could not rule out a possibility that a decrease of MCT1 immuno-intensity in αERKO mice would be due to a reduction of epithelial height in the ED, as a consequence of morphological abnormality to a lack of functional ERα (Hess et al., 1997; Lee et al., 2000). Information on the mechanism underlying ERα-mediated regulation of MCT1 expression in the ED is not available at this point. It is also notable that expression pattern of MCT1 mRNA in the rat ED and MCT1 protein in the WT mouse ED are different according to developmental ages. Such disparity would be due to species difference and/or post-transcriptional regulation on MCT1 expression in the ED. Future studies should be conducted to clarify regulatory effect of ERα on MCT1 expression in the ED. In addition, a quantitative analysis of MCT1 protein, such as western blotting, in the ED could provide an answer for the discrepancy on expression patterns of MCT1 mRNA and protein.

In designing the present study, experimental age groups were selected based on the time at which the Sertoli cell junction in the testis is formed, as an indicative of active testicular secretion. The formation of the junctional complex occurs around 2 weeks of age in rat (Gondos and Berndston, 1993). Thus, it seems that the testicular fluid begins to emerge in the excurrent ducts at 2 weeks of age and is actively secreted at 1 month of age after completion of the junction. It is expected that such changes in the testicular fluid secretion would influence function of the excurrent ducts by regulating gene expression of various molecules during postnatal development. The present study showed differential expression of several MCTs and Bsg in the ED during postnatal development. It is well documented that Bsg acts as a chaperone for proper localization and function of MCT1 and 4 to the cell surface (Kirk et al., 2000). The absence of Bsg leads to reduction of MCT1, 3, and 4 expression in the retinal pigment epithelium and neural retina (Philp et al., 2003) and mis-localization, apical rather than basolateral localization, of MCT1 in the ED (Nakai et al., 2006). Thus, it is not surprising that expression patterns of MCT1 and Bsg were closely similar, as observed in the present study. However, it is also recognizable that expression pattern of MCT4 mRNA did not correlate with its of Bsg, indicating the existence of another regulatory mechanism for MCT4 mRNA expression, other than Bsg. In addition, the present study showed that expression patterns of MCT3 and MCT4 mRNA was somewhat negatively correlated. Such relationship between MCT3 and MCT4 has not been reported in any tissue or organ yet. It is speculated that MCT3 and MCT4 would compensate their roles in the ED during postnatal development. A detailed research should be followed in future to determine the association of MCT3 and MCT4.

Detection of MCT8 mRNA in the ED from the current study was unexpected because the presence of MCT8 mRNA has not been reported in the male reproductive tract, other than neuron cells (Heuer et al., 2005) and liver, kidney, and brain (Friesema et al., 2003). It is known that MCT8 functions as active thyroid hormone transporter (Friesema et al., 2003). A potential role of thyroid hormone on the ED has been suggested with destruction of normal morphology in the ED induced by congenital hypothyroidism (Wistuba et al., 2007). A function of thyroid hormone and MCT8 in the ED has not been elucidated at this point. Further examination of link between thyroid hormone and the ED function is suggested for future studies.

In conclusion, the present study has demonstrated the presence and expression of Bsg and

several MCT isoforms in the rat ED. The expression of these molecules is differentially regulated in the ED during postnatal development. In addition, expression of MCT1 in the ED seems to be regulated through ERα, in part. Additional studies are proposed to resolve regulatory mechanisms and factor(s) for the expression of MCTs in the ED.

Ⅴ. ABSTRACT

The purpose of the present study was to determine if monocarboxylate transporter (MCT) isoforms and Basigin (Bsg) are expressed in the efferent ductules (ED) and if MCT1 expression is under estrogen receptor (ER) α-regulation in the ED of male reproductive tract. The presence of MCT isoforms and Bsg mRNAs was detected by real-time polymerization chain reaction (PCR), and ERα-mediated regulation of MCT1 expression in the ED was indirectly determined by immunohistochemistry. Current study found differential expression of MCT isoforms (MCT1, 2, 3, 4, and 8) and Bsg mRNAs in rat ED according to postnatal ages. In addition, comparison of MCT1 expression in the ED between wild type and ER α knockout mice at different postnatal ages showed basolateral localization of MCT1 in ciliated cells of the ED and, in part, ERα-mediated regulation of MCT1 expression. It is suggested that MCTs would play a role in regulation of function of the ED.

(**Key words** : Efferent ductules, Monocarboxylate transporter, Basigin, Estrogen receptor α, Estrogen)

Ⅵ. ACKNOWLEDGEMENT

This study was funded in part by the Biogreen 21 program (20050401-034-712), Rural Development Administration.

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(접수일자 : 2007. 5. 10. / 채택일자 : 2007. 6. 20.)