생후 발달과정동안 숫 백서의 Efferent Ductules에서 Connexins 발현 양상 연구

이기호* · 최인호** · 정정수*** · 장종수**** · 김지영*****

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

Expression Profiling of Connexins in the Efferent Ductules of Male Rats During Postnatal Development

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

Department of Biochemistry-Molecular Biology and Antiageing Research Center, College of Medicine, Eulji University*, School of Biotechnology, Yeungnam University**, Department of Animal Science, Chungbuk National University***, Department of Agricultural Sciences, Korea National Open University****, Department of Biochemistry, College of Medicine, Kyunghee University*****

요 약

본 연구의 목적은 생후 발달과정에서 숫 백서 생식기의 일부인 efferent ductules (ED)에서 다양한 connexin 이형질체의 발현과 양상을 알아보았다. 1주, 2주, 1개월, 3개월 그리고 6개월령의 백서의 ED로부터 total RNA을 분리하였으며, semi-quantitative RT-PCR 방법을 사용하여 총 14개 connexin 이형질체의 mRNA 발현 양상을 알아보았다. 백서의 ED에서 연구된 14개 중 6개 connexin 이형질체의 발현이 보여졌으며, connexin 26과 30의 mRNA 발현은 연령에 따라 심한 변화를 보여주었다. Connexin 31.1의 발현은 1달령의 ED에서 가장 낮게 나타났으며, connexin 37과 45는 주로 초기 발달 과정에서 높은 수준으로 발현됨을 보여주었다. 또한, connexin 43의 발현은 1주령에서 가장 낮았으며 2주령 이후에는 큰 변화없이 일정한 수준을 유지하였다. 본 연구는 백서의 ED에서 다양한 종류의 connexin 이형질체가 발현되며, 이러한 발현은 생후 연령에 따라 서로 다르게 조절됨을 보여준다.

(**Key words** : Efferent ductules, Connexin, Male fertility, mRNA expression, Gap junction)

Ⅰ. INTRODUCTION

Male fertility is precisely controlled at various ways. Sperms produced from the testis become mature and acquire fertilizing capacity throughout traveling the excurrent system, including the efferent ductules (ED) and the epididymis. Even though a mechanism of sperm maturation in the excurrent system is not well understood, a number of evidences have demonstrated the important role of the ED and the epididymis to maintain successful male fertility.

The ED are tubules that connect the testis and the epididymis of male reproductive tract. As a

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

conduit for the testicular fluid and spermatozoa produced from the testis to the epididymis, the ED are consisted of a layer of epithelium surrounded by thin smooth muscle and connective tissue layer (Ilio and Hess, 1994). There are two types of cells in the epithelium of the ED, ciliated and nonciliated cells, and the later is the dominant one in the ED (Ilio and Hess, 1994; Lee et al., 2000). Beside of their known function of sperm and the testicular fluids transport, the ED also secrete various ions and protein, absorb testicular protein, and reabsorb most of the testicular fluid (Ilio and Hess, 1994; Clulow et al., 1994; Clulow et al., 1998). It is known that reabsorption of more than 90% of the testicular fluid from the ED results in concentrating luminal sperm prior to entry into the epididymis, and thus creating adequate microenvironment for proper sperm maturation in the epididymis(Clulow et al., 1994). It is believed that nonciliated cells in the ED are chiefly responsible in absorption of the testicular fluid (Ilio and Hess, 1994; Lee et al., 2000).

Local cell-cell interactions are essential in the regulation and maintenance of physiological function of tissues in mammalian. For example, in male reproductive tract, direct contact-dependent junctional communication among various cell types in the testis is critical to control spermatogenesis (Mruk and Cheng, 2004). There are various specialized proteins involving in formation of direct cellular junctions, such as integrin, occludin, connexin, and etc (Pointis et al., 2005). Among several forms of cell-cell junctional complexes, gap junctions are specialized channels that directly link the cytoplasm of neighboring cells. Gap junctions are made of connexins and mediate the direct exchange of small molecules and ions between contacting cells (Sáez et al., 2005). Up to date, at least 20 connexin genes in rodents have been identified (Söhl and Willecke, 2004). Each connexin expresses in tissue- or cell-type specific manner. Some connexins are widely expressed in various cell types, while expression of others is limited in certain cells and/or organs. In addition, multiple connexin isoforms co-exist within same tissue (Pointis et al., 2005). Even various connexins are present in same cell, suggesting the formation of different types of channels with more than one type of connexin (Pointis et al., 2005). A number of researches have shown that expression of connexin is regulated by cell-type specific and developmental manners, such as connexin 43 in Leydig cells in the testis and connexins 26 and 43 in the epididymis (Pointis et. al., 2005; Pointis and Segretain, 2005). Even though the presence and developmental expression of connexin isoforms in the testis and the epididymis have been revealed, the expression of connexins in the ED has not been studied yet.

Thus, in this study, we attempted to evaluate the expression of various connexin isoforms in the ED of rats. In addition, the present study was conducted to determine the differential expression of connexins in the ED of rats at different ages, 1 week, 2 weeks, 1 month, 3 months, and 6 months, during postnatally developing period.

Ⅱ. MATERIALS AND METHODS

1. Isolation and collection of the tissue from animals

Male Sprague Dawley rats were purchased from Samtako (O San, S. Korea). The animals were individually housed under controlled conditions and given *ad libitum* food and water until reaching proper ages. In designing the present study, we selected two prepubertal groups, 1 week and 2 weeks of ages, one pubertal group, 1 month of age, and two fully mature groups, 3 and 6 months of ages. The formation of the Sertoli cell junction, an indicative of active secretion of the testicular fluid, occurs between 10 and 16 days of age

(Gondos and Berndston, 1993). Thus, the secretion of the testicular fluid from the rat testis seems to emerge around 2 weeks of age. In addition, patchytene spermatocytes, the first germ cell type expressing P450 aromatase, appears around 2 weeks of age (Carreau et al., 2001). At 1 month of age, the testis actively secrets the testicular fluid, and spermatozoa, expressing P450 aromatase activity, are formed and released. Two fully mature groups at 3 and 6 months of ages were chosen for a comparison purpose. When experimental animals became 1 week (N = 10), 2 weeks (N = 7), 1 month (N = 6), 3 months (N = 5), and 6 months (N = 5) of ages after the birth, rats were anesthetized by CO_2 stunning. Entire male reproductive tracts were removed from the animals, and the testes were separated from the rest of male reproductive tract. Fat surrounding the ED were rapidly trimmed away, and the ED were washed with ice-cold PBS buffer before being frozen in liquid nitrogen. Due to small size of the ED, the ED collected from an age group were pooled to obtain sufficient amounts of RNA for reverse transcription (RT) and polymerase chain reaction (PCR).

2. RNA isolation and primer design for semi-quantitative RT-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 semi-quantitative RT-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 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 connexins tested for the present study are summarized in Table 1.

3. Semi-quantitative RT-PCR analysis

The RT and PCR procedures were performed according to the instructions in ImProm-II™ reverse transcription system (Promega, Madison, USA) and GoTaq DNA polymerase (Promega, Madison, USA), respectively. Briefly, 2μg of isolated total RNAs were reverse-transcribed in total volume of 20μl 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 for PCR. 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. The PCR conditions for connexin molecules expressing in the ED were summarized in Table 1. The final extension at 72 ℃ for 10 min was carried out for the PCR. The PCR products were subjected to electrophoresis on 1.2% agarose gel. The image of each gel was photographed under 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. Data presentation and statistical analysis

We repeated the RT reaction and PCR for each age group at least three times to obtain a mean and a standard deviation. The optical densities of the RT-PCR products were measured and quantified using the NIH image software (public domain). The density values of the PCR products were normalized by comparison to abundance of GAPDH. The ratios of mRNA expression levels of connexin molecules

Table 1. Primer sequences, expected product sizes, and PCR conditions of connexin isoforms tested for semi-quantitative RT-PCR

Cxs	Forward primer sequence (5'−3')	Reverse primer sequence (5'−3')	Product size (bp)	PCR condition (number of cycles)	GenBank accession number	Note
Cx 26	CATTGCCTGGCTGTT AAGC (958−976)	AGCATAGTCACACCA GCACG (1198−1217)	260	94℃, 1 min 55℃, 1:30 min 72℃, 45 sec (40)	X51615	
Cx 30	GGCCTACTACAGACA TGA (551−568)	TGAAGCAGTCCACGA GA (794−810)	260	94℃, 1 min 55℃, 2 min 72℃, 3 min (40)	AF170284	
Cx 30.3	AGTGGATGCAGGTGT GTATCC (1551−1571)	CAGATGACCACTTAG CTTGGC (1892−1912)	362	94℃, 1 min 55℃, 1:30 min 72℃, 45 sec (40)	X76168	N.D.
Cx 31	TGTTCTACACACGCT CTGGC (520−539)	TGGTACTGCTTCCAG ATCACC (824−844)	325	94℃, 1 min 55℃, 1:30 min 72℃, 45 sec (40)	M59936	N.D.
Cx 31.1	GTCTGCTATGATGAG TTCTTCCC (379−401)	CACCAAGTAGAGCAG CTCCA (794−813)	435	94℃, 1 min 55℃, 2 min 72℃, 3 min (45)	M76533	
Cx 32	ATATGACTCTCCAGC ACCGG (710−729)	CTAGGCATGGATAAT GCTGCC (1118−1137)	409	94℃, 1 min 55℃, 1:30 min 72℃, 45 sec (40)	M23565	N.D.
Cx 33	TGAAGAAGCAAGAG GAGGAGG (905−925)	TGACGAAGAGACACA GGAAGG (1360−1380)	476	94℃, 1 min 55℃, 1:30 min 72℃, 45 sec (40)	M76534	N.D.
Cx 36	GCAGAGAGAACGCC GGTACT (424−443)	CTTGGACCTTGCTGCT GTGC (660−679)	256	94℃, 1 min 55℃, 1:30 min 72℃, 45 sec (40)	NM_019281	N.D.
Cx 37	AGTGTGTGTACCTTG GATGCC (1147−1167)	CAGCACACTTAGCCA AGAGC (1350−1369)	223	94℃, 1 min 55℃, 1:30 min 72℃, 45 sec (40)	M76532	
Cx 40	GACAAGCACCAGCTT CTTGG (730−749)	AGAGAAGGTGCTGAG GAAGG (1152−1171)	442	94℃, 1 min 55℃, 1:30 min 72℃, 45 sec (40)	M83092	N.D.
Cx 43	CAAGGTGAAAATGA GGGG (630−647)	AGACATAGGCGAGAG TGGAG (1028−1047)	417	94℃, 45 sec 52℃, 45 sec 72℃, 45 sec (28)	M19317	
Cx 45	GTCCTAACTGCGGTA GGA (101−118)	CAACTCAGTGTACTG GAT (896−913)	813	94℃, 1 min 55℃, 2 min 72℃, 3 min (40)	AF536559	
Cx 46	CTCAGAAGTCAGGCA CAAGC (950−969)	ACCAAGGCACTCTCC TCTAAGC (1303−1324)	375	94℃, 1 min 55℃, 1:30 min 72℃, 45 sec (40)	X57970	N.D.
Cx 50	GACAACCGATGACA ATCGG (1267−1285)	ACCTTGACAGGCCCT ACACA (1517−1536)	270	94℃, 1 min 55℃, 1:30 min 72℃, 45 sec (40)	AB078344	N.D.
GA-PDH	CCCCTGGCCAAGGTC ATCCATGACAACTTT (540−569)	GGCCATGAGGTCCAC CACCCTGTTGCTGTA (1023−1052)	513	94℃, 30 sec 70℃, 30 sec 72℃, 1 min (25)	X02231	

N.D. : not detected ; Cxs : connexins
Numbers in parentheses of primer sequences indicate the positions of bases in GenBank sequences.

to that of GAPDH were expressed as arbitrary units. Comparisons of measurements among the age groups for each connexin 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. Profile of expression of connexin isoforms in the ED of postnatally developing rats

Expression of total 14 connexin molecules in the rat ED was examined in the present study (Table 1). Among those connexin isoforms, the presence of 6 connexin molecules were detected in the ED, including connexins 26, 30, 31.1, 37, 43, and 45, while 8 connexin isoforms, including connexins 30.3, 31, 32, 33, 36, 40, 46, and 50, were not detected in the ED of postnatally developing rats (Table 1).

2. Expression patterns of connexins 26 and 30 in the ED of developing rats

The PCR results of connexins 26 and 30 are shown in Fig. 1. Expression of connexin 26 was detected in the ED of all experimental age groups (Fig. 1A). Compared to that at 1 week of age, mRNA level of connexin 26 was significantly increased in the ED of rat at 2 weeks of age, followed by transient decreases at 1 month and 3 months of ages (Fig. 1A). A slight increase of connexin 26 mRNA level in the ED was observed at 6 months of age. The level of connexin 30 mRNA was significantly decreased at 2 weeks of age, compared to that at 1 week of age (Fig. 1B). However, the mRNA level of connexin 30

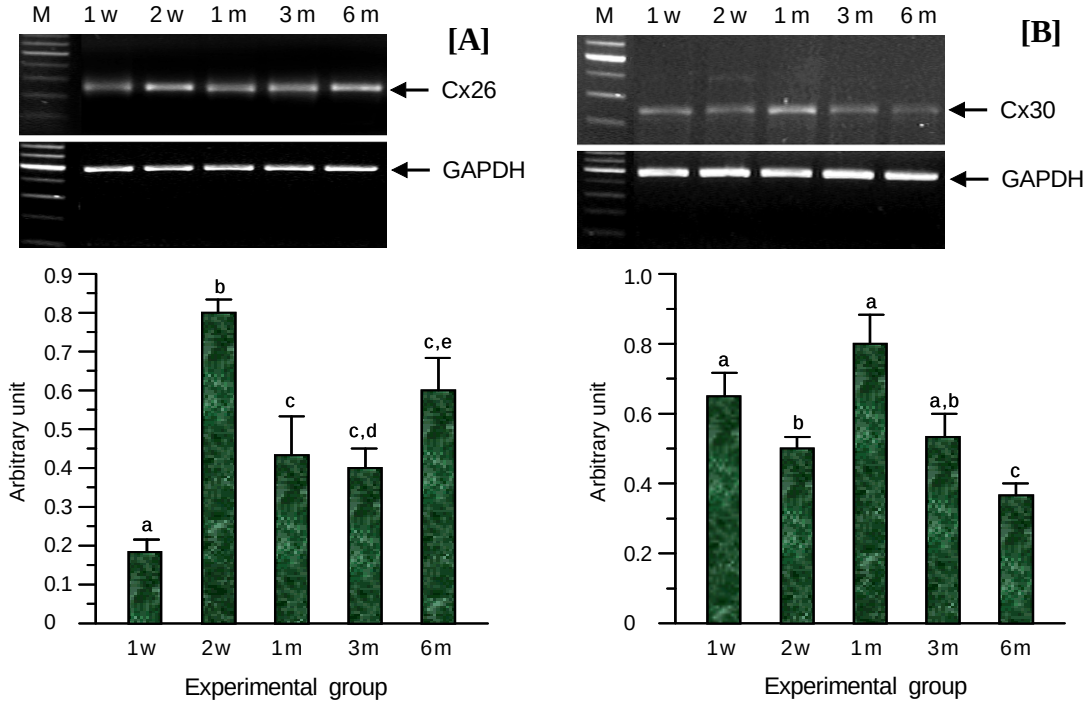

Fig. 1. mRNA expression of connexin 26 [A] and 30 [B] in the efferent ductules of rats during postnatal development.

1w : 1 week, 2 w : 2 weeks, 1m : 1 month, 3 m : 3 months, and 6 m : 6 months of age.

a-e : Means with the same letters are not significantly different (P > 0.05). Cx : connexin.

at 1 month of age was increased to the level at 1 week of age, followed by a transient decrease at 3 months of age and a further decrease at 6 months of age (Fig. 1B).

3. Expression patterns of connexins 31.1 and 37 in the ED of developing rats

The mRNA expression of connexin 31.1 at 1 week of age was the highest among experimental groups (Fig. 2A). There was a sharp decrease of mRNA level of connexin 31.1 at 1 month of age, while other age groups showed no significant difference at the level of connexin 31.1 mRNA, compared to that at 1 week of age (Fig. 2A). The levels of connexin 37 mRNAs at 1 week and 2 weeks of ages were higher than other age groups (Fig. 2B). After 2 weeks of age, the expression of connexin 37 in the ED was dramatically decreased and remained at low level until 6 months of age (Fig. 2B)

4. Expression patterns of connexins 43 and 45 in the ED of developing rats

Unlike connexins 31.1 and 37, the lowest level of connexin 43 mRNA was found in the ED of 1 week of age (Fig. 3A). At 2 weeks of age, mRNA abundance of connexin 43 was significantly increased and consistently remained at high level until 6 months of age (Fig. 3A). In contrast to the expression pattern of connexin 43 mRNA, the level of connexin 45 mRNA expression was decreased as animals became aged (Fig. 3B). The highest level of connexin 45 mRNA was detected in the ED of 1 week of age, followed by a decrease at 2 weeks of age at statistically no significant level (Fig. 3B). Further reduction of connexin 45 mRNA level was observed at 1 month of age, and at 3 and 6 months of age, connexin 45 mRNA was hardly detected in the ED (Fig. 3B).

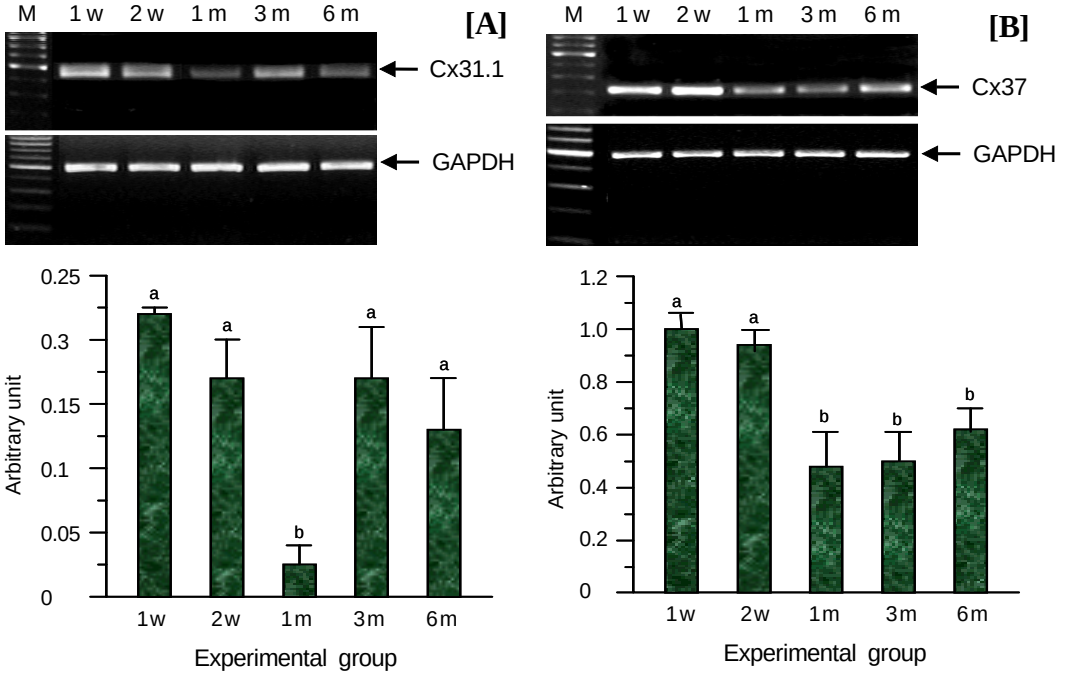

Fig. 2. mRNA expression of connexin 31.1 and 37 in the efferent ductules of rats during postnatal development.

1w : 1 week, 2 w : 2 weeks, 1 m : 1 month, 3 m : 3 months, and 6 m : 6 months of age.
a-b : Means with the same letters are not significantly different ($P > 0.05$). Cx : connexin.

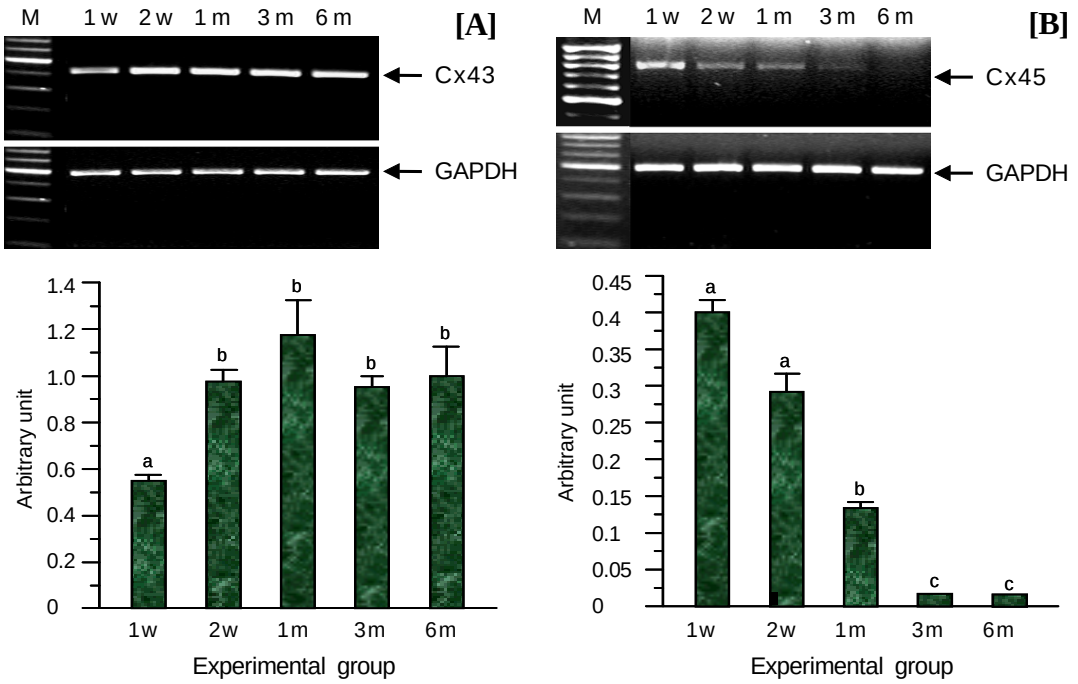

Fig. 3. mRNA expression of connexin 43 and 45 in the efferent ductules of rats during postnatal development.

1w : 1 week, 2 w : 2 weeks, 1 m : 1 month, 3 m : 3 months, and 6 m : 6 months of age.
a-c : Means with the same letters are not significantly different($P > 0.05$). Cx : connexin.

Ⅳ. DISCUSSION

Gap junction is consisted of connexin isoforms. It has been known that belt-like gap junction is present in the adluminal area between the nonciliated cells of the ED revealed by freeze-fracture (Nagano and Suzuki, 1980). However, to date, no information was available to demonstrate the expression of connexin isoforms in the ED. The aim of present study was to determine the mRNA expression of various connexin isoforms in the rat ED. In addition, we attempted to show differential expression pattern of connexin mRNAs according to ages. Results from the present study clearly demonstrated the existence of a variety of connexin isoforms in the ED of rat. Also, our results revealed that mRNA expression of connexin isoforms in the ED is developmentally regulated.

Expression of connexins is regulated by a variety of molecules, including hormones, transcription factors, and other factors (Oyamada et al., 2005). In male reproductive tract, expression of connexin 43 in the Sertoli cells is upregulated by follicle-stimulating hormone (FSH) and retinoid and downregulated by thyroid hormones, testosterone, and estradiol (Pointis and Segretain, 2005). In addition, human chorionic gonadotropin induces a reduction of connexin 43 expression in the Leydig cells (You et al., 2000). In the epididymis, thyroid hormone and testosterone regulate expression of connexin 43 (St-Pierre, et al., 2003; Cyr, et al., 1996). Moreover, expression of connexin isoforms in the prostate, a sex hormone-dependent tissue, is controlled by androgen and estrogen (Habermann et al., 2001; Prinsac et al., 2001). Therefore, because the ED has known to be a target of androgen and estrogen (Hess et al., 1997; Lee et al., 2000; Lee et al., 2001; Bentvelsen et al., 1995), it is possible that expression of connexin isoforms in the ED would be under the regulation of

testosterone and estrogen, as well as other paracrine and endocrine factors. Further studies are suggested to determine effects of estrogen and androgen on the regulation of connexin expression in the ED.

Developmental expression of multiple connexin isoforms has been demonstrated in various tissues. In Leydig cells, as well as Sertoli cells, in the testis, expression of connexin 43 is regulated in an age-dependent manner (Perez-Armendariz, et al., 2001; Barvo-Moreno, et al., 2001). Expression of multiple connexin isoforms in the epididymis is segment-specific and age-dependent (Dufresne et al., 2003). Our present study also showed differential expression of connexin isoforms in the ED depending on postnatal development. Expression of some connexins was high at early development, while expression of other connexins was high at late development. In addition, fluctuation of gene expression of other connexins was observed in the present study. These results indicate that mRNA expression of connexin isoforms in the ED is tightly and complicatedly regulated. It is not clear which a factor(s) involves in the regulation of expression of connexin isoforms in the ED at age-dependent manner. However, it is reasonable to speculate that the testicular factor(s) should directly and/or indirectly affect the expression of connexins in the ED. Among the testicular factors, testosterone and estrogen could be candidate molecules engaged in such differential expression of connexins in the ED according to age. Testosterone production from the rat testis increases as aged (Paz et al., 1980). Also, it is believed that concentration of estrogen in the testicular fluid would increase according to age, because the activity of P450 aromatase, an enzyme involving in the irreversible conversion of testosterone to estrogen, in the testis increase with aging (Carreau et al., 2001). Because the ED are a sex hormone-dependent tissue, changes of concentration of testosterone and estrogen with aging would influence gene expression in the ED, such as connexins in the present study. In addition, we could not rule out other testicular and endocrine factors affecting activities of transcription factors, which regulate the expression of connexin isoforms. Furthermore, we could not exclude a possibility of inadequate PCR conditions for connexin isoforms undetected from the present study. Detailed researches are required to determine a molecular mechanism of age-dependent differential expression of connexin isoforms in the ED.

Multiple connexins co-exist within the same tissue, and more than one isoform could form a gap junction channel. Our present study revealed that more than 2 connexin isoforms co-exist in the ED at same age. Thus, it is absolutely possible that gap junction channel present in the ED could be either homomeric composed of the same connexin and/or heteromeric composed of the different connexins. However, which connexin isoforms form homomeric or heteromeric channels in the ED during postnatal development remains in question. In addition, even though belt-like gap junction is present between neighboring nonciliated cells in the ED (Nagano and Suzuki, 1980), precise localization of connexin isoforms in the ED has not been determined. *In situ* hybridization or immunohistochemistry of connexin isoforms should be performed to localize connexins mRNAs or proteins, respectively. Although regulation of connexin gene expression is mainly determined at transcriptional level, other mechanisms may influence the level of expression. Indeed, expression of connexins 26, 32, and 43 is regulated at translational level (Oyamada et al., 2005). Thus, a quantitative study should be followed in future to determine if mRNA expression of connexin isoforms detected in the ED from the present study corresponds to protein expression.

In the testis, at least 11 connexin isoforms are associated with Sertoli cells, germ cells, and Leydig cells, involving in regulation of steroidogenesis and spermatogenesis (Lee et al., 2007). In addition, age-dependent and stage-dependent expression of connexin 43 has been reported in the testis (Risley et al., 1992). It is believed that cell-cell

communication between different cell types through gap junctions in the testis is important for the regulation of spermatogenesis (Risley, 2000). Our present study revealed differential expression of various connexin isoforms in the rat ED at different ages. However, the importance of such differential expression of connexins in age-dependent manner is not apparent at this point. The ED play an important role on male fertility by reabsorption of most of the testicular fluid and ions and secretion of various proteins and ions (Ilio and Hess, 1994; Clulow et al., 1998). Like other tissues, it is believed that cell-cell communication between different and/or same cell types is crucial for maintenance of functions of the ED. Thus, it is speculated that differential expression of connexin isoforms in the ED would relate to the regulation of the ED functions. Future studies are suggested to evaluate functional change of the ED by affecting expression of connexins.

In conclusion, our results clearly demonstrate for the first time the presence of several connexin isoforms in the ED of rats. In addition, our present study shows differential expression of connexin isoforms in the ED during postnatal development in the rat.

Ⅴ. ABSTRACT

The purpose of this study was to evaluate the presence and differential expression of connexin isoforms in the efferent ductules (ED) of male rat reproductive tract during postnatal development. Total RNA was isolated from the ED collected from rats at 1 week, 2 weeks, 1 month, 3 months, and 6 months of ages. Expression of six connexin mRNAs of 14 isoforms tested was detected by semi-quantitative RT-PCR. Fluctuation of mRNA levels of connexins 26 and 30 was found according to ages. A significant decrease of connexin 31.1 mRNA level was observed in the ED at 1 month of age. The highest levels of connexin 37 and 45 mRNAs were detected in the ED of early developmental period, while the expression of connexin 43 was the lowest at 1 week of age. The present study demonstrates differential regulation on expression of connexin isoforms in the rat ED in age-dependent manner.

Ⅵ. ACKNOWLEDGEMENT

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Ⅶ. REFERENCES

1. Bentvelsen, F. M., Brinkmann, A. O., van der Schoot, P., van der Linder, J. E., van der Kwast, T. H., Boersma, W. J., Schroder, F. H. and Nijman, J. M. 1995. Developmental pattern and regulation by androgens of androgen receptor expression in the urogenital tract of the rat. Mol. Cell. Endocrinol. 22:245-253.
2. Bravo-Moreno, J. F., Diaz-Sanchez, V., Montoya-Flores, J. G., Lamoyi, E., Saez, J. C. and Perez-Armendariz, E. M. 2001. Expression of connexin 43 in mouse Leydig, Sertoli, and germinal cells at different stages of postnatal development. Anat. Rec. 264:13-24.
3. Carreau, S., Bourquiba, S., Lambard, S., Galeraud-Denis, I., Genissel, C., Bilinska, B., Benahmed, M. and Levallet, J. 2001. Aromatase expression in male germ cells. J. Steroid Biochem. Mol. Biol. 79:203-208.
4. Clulow, J., Jones, R. and Hansen, L. 1994. Micropuncture and cannulation studies of fluid composition and transport in the ductuli efferentes testis of the rat: comparisons with the homologous metanephric proximal tubule. Exp. Physiol. 79: 915-928.
5. Clulow J., Jones, R. C., Hansen, L. A. and Man, S. Y. 1998. Fluid and electrolyte reabsorption in the ductuli efferentes testes. J. Reprod. Fertil. Suppl. 53:1-4.
6. Cyr, D. G., Hermo, L. and Laird, D. W. 1996. Immunocytochemical localization and regulation of connexin 43 in the adult rat epididymis. Endocrinology.

137:1474-1484.

7. Dufresne, J., Finnson, K. W., Gregory, M. and Cyr, D. G. 2003. Expression of multiple connexins in the rat epididymis indicates a complex regulation of gap junctional communication. Am. J. Physiol. 284:C33-C43.
8. Gondos, B. and Berndston, W. E. 1993. Postnatal and pubertal development. in the sertoli cell, L. D. russel. and Griswold, M. D. (Eds.), cache river press, clearwater, U.S.A. p. 115.
9. Habermann, H., Chang, W. Y., Birch, L., Mehta, P. and Prins, G. S. 2001. Developmental exposure to estrogens alters epithelial cell adhesion and gap junction proteins in the adult rat prostate. Endocrinology. 142:359-369.
10. Hess, R. A., Bunick, D., Lee, K. -H., Bahr, J., Taylor, J. A., Korach, K. S. and Lubahn, D. B. 1997. A role for oestrogens in the male reproductive system. Nature. 390:509-512.
11. Ilio, K. and Hess, R. 1994. Structure and function of the ductuli efferentes: A review. Microsc. Res. Tech. 29:432-467.
12. Lee, K. H., Hess, R. A., Bahr, J. M., Lubahn, D. B., Taylor, J. and Bunick, D. 2000. Estrogen receptor α has a functional role in the mouse rete testis and efferent ductules. Biol. Reprod. 63:1873- 1880.
13. Lee, K.H., Finnigan-Bunick, C., Bahr., J. and Bunick, D. 2001. Estrogen regulation of ion transporter messenger RNA levels in mouse efferent ductules are mediated differentially through estrogen receptor (ER) α and ERβ. Biol. Reprod. 65:1534-1541.
14. Lee, N. P., Yeung, W. S. and Luk, J. M. 2007. Junction interaction in the seminiferous epithelium: regulatory roles of connexin-based gap junction. Front Biosci. 12:1552-1562.
15. Mruk, D. D. and Cheng, C. Y. 2004. Sertoli-Sertoli and Sertoli-germ cell interactions and their significance in germ cell movement in the seminiferous epithelium during spermatogenesis. Endocr. Rev. 25:747-806.
16. Nagano, T. and Suzuki, F. 1980. Belt-like gap junctions in the ductuli efferentes of some mammalian testes. Arch. Histol. Jpn. 43:185-189.
17. Oyamada, M., Oyamda, Y. and Takamatsu, T. 2005. Regulation of connexin expression. Biochim. Biophys. Acta. 1719:6-23.
18. Paz, G. F., Winter, J. S., Reyer, F. I. and Faiman, C. 1980. Developmental pattern of testosterone production by the rat testis. Steroids. 36:675-688.
19. Perez-Armendariz, E. M., Lamoyi, E., Mason, J. I., Cisneros-Armas, D., Luu-The, V. and Bravo Moreno, J. F. 2001. Developmental regulation of connexin 43 expression in fetal mouse testicular cells. Anat. Rec. 264:237-246.
20. Pointis, G. and Segretain D. 2005. Role of connexin-based gap junction channels in testis. Trends Endocrinol. Metabol. 16:300-306.
21. Pointis, G., Fiorini, C., Defamie, N. and Segretain D. 2005. Gap junctional communication in the male reproductive system. Biochim. Biophys. Acta. 1719:102-116.
22. Prinsac, G. S., Birch, L., Habermann, H., Chang, W. Y., Tebeau, C., Putz, O. and Bieberich, C. 2001. Influence of neonatal estrogens on rat prostate development. Reprod. Fertil. Dev. 13:241-252.
23. Risley, M. S. 2000. Connexin gene expression in seminiferous tubules of the Sprague-Dawley rat. Biol. Reprod. 62:748-754.
24. Risley, M. S., Tan, I .P., Roy, C. and Saez, J. C. 1992. Cell-, age- and stage- dependent distribution of connexin 43 gap junctions in testes. J. Cell. Sci. 103:81-96.
25. Sáez, J. C., Retamal, M. A., Basilio, D., Bukauskas, F. F. and Bennett, M. V. L. 2005. Connexin-based gap junction hemichannels: Gating mechanisms. Biochim. Biophys. Acta. 1711:215-224.
26. Söhl, G. and Willecke, K. 2004. Gap junctions and the connexin protein family. Cardiovasc. Res. 62:228-232.
27. St-Pierre, N., Dufresne, J., Rooney, A. A. and Cyr, D. G. 2003. Neonatal hypothyroidism alters the localization of gap junctional protein connexin 43 in the testis and messenger RNA levels in the epididymis of the rat. Biol. Reprod. 68:1232-1240.
28. You, S., Li, W. and Lin, T. 2000. Expression and regulation of connexin 43 in rat Leydig cells. J. Endocrinol. 166:447-453.

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