

Luteinizing Hormone(LH)

*
,

*
.

Expression of Luteinizing Hormone(LH) Gene in Rat Uterus and Epididymis

Sung Ho Lee* and Youngki Lee

Dept. of Biology, Sangmyung University, Seoul, Korea*, Dept. of Histology, College of Medicine, Cheju National University, Cheju, Korea

* : 7, () 110-743
: (02) 396-6133, : (02) 2287-5139, e-mail: shlee@pine.sangmyung.ac.kr

ABSTRACT

Recent studies clearly demonstrated that the novel expression of LH gene in the rat testis, and suggested the local action of the LH-like molecule. The present study was performed to analyze the expression of LH genes in the rat accessory reproductive organs. Expression of LH subunit genes in the rat uterus and epididymis was demonstrated by reverse transcription-polymerase chain reaction(RT-PCR) and specific LH radioimmunoassay(RIA). The LH β transcripts in these organs contained the published cDNA structure, the pituitary type exons 1-3, which encoded the entire LH β polypeptide. Presence of the transcripts for the α -subunit in the rat reproductive tissues were also confirmed by RT-PCR. In the LH RIA, significant levels of LH were detected in crude extracts from the rat ovary, uterus and epididymis. The competition curves with increasing amount of tissue extracts were parallel with those of standard peptide, indicating that the immunoreactive LH-like materials in these tissues are similar to authentic pituitary LH molecule. In rat epididymis, the highest amount of immunoreactive LH were detected in corpus area. Our findings demonstrated that the genes for LH subunits are expressed in the rat accessory reproductive organs, and suggested that these extrapituitary LH may act as a local regulator with auto and/or paracrine manner.

Key words : LH, Gene expression, Uterus, Epididymis, Local regulator

gonadotropin-releasing hormone(GnRH)
gonadotropin, follicle stimulating hormone(FSH) luteinizing
hormone(LH) . FSH LH 가
, LH 가
(Zhang *et al.*, 1995a,b; Lee, 1998).
LH LH가 steroidogenesis
(El-Gehani *et al.*, 1998), LH가 가
가 LH
human Chorionic Gonadotropin(hCG) progesterone .
(Griffin and Ojeda, 1995),
LH-like molecule 가
LH 가

1.

25

(Sprague-Dawley strain)

pregnant mare's serum gonadotropin(PMSG; 15 IU/rat, Sigma)

(Lee *et al.*, 1994).

(4)

RNA

LH

-70°C

2.

(Radioimmunoassay, RIA)

LH 1 N acetic acid(10 vol)

1 N HCl acidify . Chloramine-T I¹²⁵(NEN)

LH reference peptide(NIDDK, Bethesda, USA) anti-rat LH antiserum(1:200,000)

(72 hour protocol)

(Lee *et al.*, 1994).

3. RNA DNA

(1) Total RNA

RNA acid phenol- guanidium isothiocyanate- chloroform (Chomzynski and Sacchi, 1987) Trizol (GIBCO-BRL)

pellet 75% ethanol 0.1% DEPC- water

UV spectrophotometer

(2) Reverse transcription-polymerase chain reaction(RT - PCR)

RNA reverse transcriptase(SuperScript RT RNase H⁻; GIBCO-BRL)

cDNA . LH exon 20 base pair

5' 3' primer oligomer (), cDNA Taq

DNA polymerase(Takara) PCR . PCR 94°C 2

denaturation 1 denaturation(94°C, 30), annealing(54°C, 30), elongation(72°C,

1) 35 1 extension(72°C, 10)

(2% agarose gel) ethidium bromide . PCR

primer LH subunit 5'-GT GCCGGCCT GT CAACGCAAC-3'(sense)

5'-CAGCTCATTGGTTGAGTCCTG-3'(antisense) , -subunit 5'-ATACTTCTCCAAG

CTGGGTGC-3'(sense) 5'-CGACACTCAGTGCCATCGCAG-3'(antisense) (Godine *et al.*,

1982; Zhang *et al.*, 1995b).

(3) PCR DNA sequencing

RT-PCR cDNA

가

DNA fragment

dideoxy chain termination-PCR

PCR sequencing kit

()

LH

transcript RT-PCR , RNA LH subunit
 bp (Fig. 1A). - subunit transcript cDNA 306
 294 bp cDNA가 PCR (Fig. 1B). PCR cDNA
 PCR-sequencing - subunit
 (data not shown). LH ,
 LH RIA , LH standard curve
 competition curve immunoreactive LH-like
 가 (Fig. 2). LH 1/10
 , homogenate LH RIA
 corpus LH 가
 (41.5 pg/mg), caudal(27.8 pg/mg) caput(24.2 pg/mg) (Fig. 3).

LH transcript

(Zhang *et al.*, 1995a; Lee, 1998),

LH RIA LH - subunit
 subunit 가 . LH가
 LH , LH
 LH
 10 ,
 10 GnRH 가
 GnRH .
 gonadotropin

(i) steroidogenesis , (ii) , (iii) (oocyte maturation) , (iv) , (v) (apoptosis)
(Hsueh *et al.*, 1994; Richards, 1994; Gnnessi *et al.*, 1997).

가 , (seminal vesicle), GnRH가
(Khodr and Siler-Khodr, 1980; Izumi *et al.*, 1985; Ikeda *et al.*, 1996),
GnRH chorionic gonadotropin 가 (Siler-Khodr *et al.*,
1986). GnRH-LH 가 .

GnRH가 LH가
- GnRH LH (endocrine)
GnRH LH autocrine paracrine 가
. LH GnRH
(circuitry) , LH
GnRH
. LH ,
LH가 (excretion) 가 .

LH FSH thyroid stimulating hormone(TSH) chorionic
gonadotropin glycoprotein hormone family (Albanese *et al.*, 1996).
- subunit , - subunit가
- subunit가 (Ryan *et al.*, 1988).
LH subunit 3
5' untranslated region(UTR) (3.2, 2.4, 0.86 kb)가
(0.8 kb) (Zhang *et al.*, 1995b). LH
가 ,
(round type spermatid) (spermiogenesis)
(Zhang *et al.*, 1995b).
LH pregnant mare's serum gonadotropin(PMSG)
(Lee, 1998), LH .
LH LH

LH

LH subunit (RT-PCR)

LH

LH β subunit exon primer RT-PCR

306 bp band가 , LH, FSH, TSH

hCG -subunit PCR

LH standard curve , curve가

sigmoid immunoreactive LH가

LH가

Albanese C, Colin IM, Crowley WF, Ito M, Oestell RG, Weiss J, Jameson JL. The gonadotropin genes : evolution of distinct mechanisms for hormonal control. *Recent Prog Horm Res* 1996; 51: 23-58.

Chomzynski P, Sacchi N. Single-step method of RNA isolation by acid guanidium thiocyanate-phenol-chloroform extraction. *Anal Biochem* 1987; 162: 156-59.

El-Gehani F, Zhang FP, Pakarinen P, Rannikko A, Huhtaniemi I. Gonadotropin-independent regulation of steroidogenesis in the fetal rat testis. *Biol Reprod* 1998; 58: 116-123.

Godine JE, Chin WW, Habener JF. Alpha subunit of rat pituitary glycoprotein hormones: primary structure of the precursor determined from the nucleotide sequence of cloned cDNAs. *J Biol Chem* 1982; 257: 8368-71.

Gnessi L, Fabbri A, Spera G. Gonadal peptides as mediators of development and functional control of the testis : An integrated system with hormones and local environment. *Endocr Rev* 1997; 18: 541-609.

Griffin JE, Ojeda SR. Textbook of endocrinology. 3rd ed. UK: Oxford University Press; 1995.

Hsueh AJW, Billig H, Tsafiriri A. Ovarian follicle atresia : A hormonally controled apoptotic process. *Endocr Rev* 1994; 15: 707-24.

Ikeda M, Taga M, Sakakibara H, Minaguchi H, Ginsburg E, Vonderhaar BK. Gene expression of gonadotropin-releasing hormone in early pregnant rat and steroid hormone exposed mouse uteri. *J Endocrinol Invest* 1996; 19(11): 708-13.

Khodr GS, Siler-Khodr TM. Placental luteinizing hormone releasing factor and its synthesis. *Science* 1980; 207: 315-7.

- Lee SH. Expression of luteinizing hormone(LH) subunit genes in the rat ovary. *Kor J Fertil Steril* 1998; 25(2): 199-205.
- Lee SH, Song ES, Yu SK, Kim C, Lee DK, Choi WS, Kim K. Temporal changes in ovarian gonadotropin releasing hormone mRNA levels by gonadotropins in the rat. *Mol. Cells* 1994; 4: 39-44.
- Richards J. Hormonal control of gene expression in the ovary. *Endocr Rev* 1994; 15: 725-51.
- Ryan RJ, Chalesworth MC, McCormick DJ, Milius RP, Keutmann HT. The glycoprotein hormones : Recent studies of structure-function relationships. *FASEB J* 1988; 2: 2661-9.
- Siler-Khodr TM, Khodr GS, Valenzuela G, Rhode J. Gonadotropin-releasing hormone effects on placental hormones during gestation : I. Alpha-human chorionic gonadotropin, human chorionic gonadotropin and human chorionic somatomammotropin. *Biol Reprod* 1986; 34(2): 245-54.
- Zhang FP, Rannikko A, Huhtaniemi I. Isolation and characterization of testis-specific cDNAs for luteinizing hormone β -subunit in the rat. *Biochem Biophys Res Commun* 1995a; 210: 858-65.
- Zhang FP, Markkula M, Toppari J, Huhtaniemi I. Novel expression of luteinizing hormone subunit genes in the rat testis. *Endocrinology* 1995b; 136: 2904-12.

Figure legends

Figure 1. Detection of the transcripts for rat LH subunits in the rat reproductive tissues by RT-PCR. PCR and electrophoresis were carried out as described in Materials and Methods. **A**; Amplification of cDNA fragments coding the LH β regions commonly found in the rat pituitary and testis. **B**; Amplification of cDNA fragments from the β -subunit transcripts. M, DNA size marker; P, pituitary; T, testis; E, epididymis; O, ovary; U, uterus; (-), negative control.

Figure 2. LH RIA parallelism with increasing amounts of LH standard and the tissue extracts, indicating the presence of immunoreactive LH-like molecules in the rat ovary. In this experiment, ovaries and uteri from immature rats(Day 25) and epididymis from adult rats(4 months old) were used.

Figure 3. LH RIA for measuring the rat epididymal LH contents. Caput, corpus and caudal part of epididymis from adult rats(4 months old) were homogenized, centrifuged and the resulting supernatant were used in RIA.