

Luteinizing Hormone(LH)

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Expression of Luteinizing Hormone(LH) and Its Receptor Gene in Uterus from Cycling Rats

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ABSTRACT

There is increasing evidence for the expression of rat LH gene in several extrapituitary sites including testis and ovary. We also have demonstrated that the local LH expression in the rat epididymis and uterus, the major accessory sex organs in male and female reproductive system, respectively. The present study was undertaken to elucidate whether the gene for LH receptor is expressed in rat uterus and whether the expressions of uterine LH and its receptor are differentially regulated during estrous cycle. Presence of the transcripts for rat LH receptor in the rat uterine tissue were confirmed by touchdown reverse transcription-polymerase chain reaction(RT-PCR). In LH semi-quantitative RT-PCR, the highest expression level was shown in estrus stage. The level of LH receptor transcripts was also fluctuated during estrous cycle. In ovariectomized rats(OVX + Oil), the expressions of both uterine LH and LH-R were markedly reduced when compared to those from normal rats. Supplement with estradiol 17 to the ovariectomized rats(OVX + E2) restored the expression levels of LH and its receptor to the levels in uteri from normal rats. Our findings indicated that 1) LH and its receptor gene are expressed in the rat uterus from cycling rats, 2) the expression of uterine LH and its receptor is mainly, if not all, under the control of ovarian sex steroid(s). These results suggested that the uterine LH may act as a local regulator with auto and/or paracrine manner, though the possibility that the pituitary LH may act directly on the regulation of uterine functions could not be discarded.

가
가 1.
(endometrium)
가
1~3.
follicle stimulating hormone(FSH) luteinizing hormone(LH)
LH 가 4~6,
LH가 . LH 7.
가
LH 가 LH
1) LH
, 2) LH
(ovaryectomy, OVX)

1.
3-5 (Sprague-Dawley strain)
(12 , 12) (ad libitum)
7 , 2
ether
1 sesame oil(Sigma) estradiol-17 (235 ug/ml, Sigma) silastic
capsule(Dow -Corning, 12mm, 1.55mm, 3.125mm) . 48
RNA

2. RNA DNA

(1) Total RNA

RNA acid phenol-guanidium isothiocyanate-chloroform Trizol
(GIBCO-BRL) 8. pellet 75% ethanol
0.1% DEPC-water UV spectrophotometer

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

RNA (SuperScript RT RNase H-; GIBCO-BRL) cDNA

pair polymerase(Takara) denaturation 1 (2% agarose gel) 5' primer 3' primer oligomer PCR denaturation(940C, 30) 1 extension(720C, 10) touchdown cycle 0.30C ethidium bromide Table 1 LH (PCR), exon PCR), elongation(720C, 1) 1 PCR Tm + 20C 40 cycle PCR primer 20 base Taq DNA 2 35 1

(3) PCR DNA sequencing

RT-PCR cDNA dideoxy chain termination -PCR 가 DNA fragment PCR sequencing kit()

cDNA alternative splicing LH RNA LH 460 bp isoform 가 (Fig. 1). LH transcript가 RT-PCR 20 30 bp fragment LH estrus stage 가 diestrus stage 가 (Fig. 2). LH proestrus estrus stage PCR cDNA PCR-sequencing primer 가 diestrus stage (data not shown), RNA 가 exon genomic DNA cDNA LH LH (OVX + Oil) LH (Fig. 3). (OVX + E)

follicle stimulating hormone(FSH) gonadotropin-releasing hormone(GnRH) luteinizing hormone(LH) 가 FSH LH

.1 가 , insulin-like growth factor(IGF) cytokine

.11 가 , LH high affinity, low capacity LH/hCG LH

LH/hCG 가 cDNA (endometrium) (myometrium) 가

.12 alternative splicing

.13,14

가 가 , 가

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Table 1. Sequences of the PCR primers used in the present study.

ID	Sequences	Product size (bp)	Ref.
LH I (sense)	GTGCCGGCCTGTCAACGCAAC	306	5
II (antisense)	CAGCTCATTGGTTGAGTCCTG		
LH-R* I (sense)	GAACTCCAATGTGCTCCAG	460	9
II (antisense)	GAGCCATCCTCCGAGCATAA		
L 27 I (sense)	GAACATTGATGATGGCACCTCC	308	10
II (antisense)	ACCATTTGTTCTTCCCTGTCTTG		

The directions of the sequence are all 5' to 3'. LH-R*, LH receptor.

Figure legends

Figure 1. Presence of the transcripts for LH receptor in the rat uterus. PCR and electrophoresis were carried out as described in Materials and Methods. m, DNA size marker; O, ovary; T, testis; U, uterus; (-), negative control.

Figure 2. Changes in the level of LH and LH receptor transcripts in the rat uterus during estrous cycle. D, diestrus; P, proestrus; E, estrus; M, metestrus; (-), negative control.

Figure 3. Effects of steroid deprivation and estradiol supplement on the expression of the uterine LH and LH receptor. 1, OVX + oil group; 2, OVX + E2 group; (-), negative control.