

Effect of Steroid Hormones on the Expression of c-Fos, CREB, ATF, and HSP70 in Rat Uterus

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=Abstract =

Steroid hormone is known to cause the dynamic changes of mammalian uterus during reproductive cycle. However there is little information about the effect of estrogen (E) and progesterone (P) on the expression of various transcription factors involved in gene expression. Thus the present study was designed to demonstrate E and/or P-induced expression of c-Fos, CREB, ATF and HSP70 in rat uterus.

Rats, ovariectomized (OVX) for two weeks, were divided into 6 experimental groups, 1) OVX, 2) OVX+V, 3) OVX+E, 4) OVX+P, 5) OVX+E+V, 6) OVX+E+P, and western blotting assay for nuclear extract and immunohistochemical staining were carried out for each experimental group.

Treatment of E (10 µg) showed to increase the expression of c-Fos, CREB, ATF, and HSP70, and maximal expression was occurred at 3-6 hr after E administration. P (1mg) also increased, but much less than E, the expression of c-Fos, ATF, and HSP70. However, P did not reveal any effect on the expression CREB. P treatment 4 hr after E injection decreased c-Fos, CREB, and ATF expression, but did not show any change in the E-induced HSP70 expression. In immunohistochemical study c-Fos-, CREB-, and ATF-immunoreactivities were confined to the cells of luminal epithelium of uterine endometrium. These results suggest that proliferation and differentiation of rat uterus during reproductive cycle may mediated via expression of transcription factors, such as c-Fos, CREB, ATF, and HSP70.

Key Word: Uterus, Ovariectomy, Steroid hormone, Transcription factor, Western blotting, Immunohistochemistry

(estrous cycle) ,

가 .

(hypothalamus-pituitary-gonadal axis) estrogen progesterone (Finn & Martin, 1969; Cook & Hunter, 1978; Kim & Cho, 1982). (endometrium) (luminal epithelial cell), (glandular epithelial cell) (stroma cell) , estrogen progesterone (gonadotrophins)

proto-oncogene c-fos가 . c-fos , ions,

immediate early gene (IEG) (Curran & Morgans, 1987). c-Fos c-jun heterodimer DNA AP-1 site transcription factor CREB (cyclic AMP responsive element binding protein), ATF (activation transcription factor) c-Fos c-Jun dimer (Pennypacker et al., 1995).

estrogen c-fos c-fos promoter estrogen ERE (estrogen responsive element) 가 (Hyder & Stancel, 1994).

estrogen c-fos promoter c-fos 가 (late response effect) . c-fos, c-jun,

ATF, CREB

transcription

factors

estrogen

heat shock protein (HSP)

protein

c-Fos

estrogen

HSPs

c-Fos가

가 가

steroid hormone

가 transcription factor

hsp-70

western blotting assay

immunohistochemistry

(1)

Sprague-Dawley strain .

2

2 estrogen (5 ug estradiol in 10 % ethanol/saline + 5 ug estadiol benzoate (EB) in sesame oil) progesterone (1 mg in sesame oil)

. 1) OVX, 2) OVX + vehicle, 3) OVX + E, 4) OVX + P, 5) OVX + E + P 3 .

(2) Western Blotting

decapitation uterus nuclear protein extraction Hagenduchle (1992) . uterus homogenization buffer

(15 mM Tris pH 7.5, 60 mM KCl, 15 mM NaCl, 0.15 mM spermine, 0.15 mM spermidine, 14 mM -mercaptoethanol, 0.5% NP-40, 10 μM TPCK, 0.5 mM PMSF, 0.3

M sucrose) 50-100 μm mesh filtration 0.9M sucrose pad

3,500 rpm 4 10 nucleus pellet . Low salt buffer (20 mM Tris pH 8.0, 25% glycerol, 1.5 mM MgCl₂, 0.2 mM EDTA, 0.5 mM DTT, 0.02

M KCl, 0.2 mM PMSF) stirring high salt buffer (low salt buffer 0.02 M KCl 1.2 M KCl) final conc. 0.3M 가 1 stirring

. 17,000 rpm 4 30 nuclear protein Lowery method Western blotting . 10%

SDS-PAGE semi-dry electrophoretic transfer method gel NC paper . 1 1:1000 24

biotinylated second antibody, avidin labelled alkaline phosphatase 2 . chemiluninescent substrate CSPD(Tropix)

, Amersham hyperfilm 2 .

(3)

ethyl ether (Junsei)

0.1M phosphate buffer (pH 7.4) 4%

paraformaldehyde .

4 . 20% sucrose

4 ° C overnight . OCT compound

(Reichert Jung cryostat) 25 μ m

0.01M phosphate buffered saline (pH 7.4) 2-3

dish free-floating method gelatin-coated slide

peroxidase 3% H₂O₂ 10

PBS 10 3 , normal goat

serum 30 . 1 4 ° C

overnight - . 1

Triton X-100 0.4% 가 PBS 가 (PBST x) 1:500

. 1 PBST x 30 3 ,

2 biotinylated goat anti-rabbit immunoglobulin G (Vector) 1:100

1 . PBST x 3 peroxidase-labelled avidin (Vector)

1:500 1

. 3,3'-diaminobenzidine (Sigma) 20mg 100ml

PBS 0.005% 가 가 5

. 1 PBS

, 1

1 1: 500 1ml

antigens 10 μ g 가 1 .

Estrogen c-Fos HSP70
 Western Blotting c-Fos 1 3-6
 12 (Fig.
 1), CREB, ATF HSP70
 estrogen 1 3-6 12
 (Fig. 2).
 c-Fos estrogen progesterone
 가 estrogen 가 (18),
 estrogen 24 progesterone estrogen
 24 saline c-Fos
 (Fig. 3). CREB progesterone estrogen
 가 . estrogen 24
 progesterone (X+E+V)
 (Fig. 4). ATF estrogen 가 progesterone
 가 . Estrogen progesterone
 CREB
 (Fig. 5). HSP70 estrogen progesterone
 가 가
 가 (Fig. 6). estrogen
 protein 가
 luminal epithelium (Fig. 7).

. estrogen progesterone
 (Yochim & DeFeo, 1963; Cook & Hunter, 1978) .

가 . Nelson (1930) estrogen follicular phase
 progesterone
 Marcus (1967) Finn (1969) estrogen . luteal
 phase progesterone
 . estrogen
 (Smith & Biggers, 1968).
 follicular phase estrogen luteal phase progesterone estrogen
 stromal cell 가 .

.
 c-fos (growth factor) Go stage
 S phase (Kelly et al.,
 1983; Greenberg & Ziff, 1984)
 가 (Muller et al., 1984). c-fos

. c-Fos (c-fos protein) IEG c-jun (c-Jun)
 heterodimeric transcription factor complex (Halazonetis et al., 1988; Kouzarides
 & Ziff, 1988; Rauscher et al., 1988) DNA promoter AP-1 site
 (probably late response gene) (Kouzarides
 & Ziff, 1988; Gentz et al., 1989). c-Fos 가 FRAs
 (Fos Related Antigens) c-Jun 가 Jun-B, Jun-D
 (Cohen & Curran, 1988; Ryder et al., 1988), dimer

-TGACTCA- DNA sequence AP-1 site . promoter AP-1 site
 AP-1 DNA binding complex transcription factor가 c-Fos
 c-Jun dimer cyclic AMP responsive
 element binding protein (CREB), activator transcription factor (ATF) c-Fos
 c-Jun heterodimer AP-1 site CRE
 (Hai & Curran, 1991; Pennypacker et al., 1995).

estrogen progesterone
 (O'Malley et al., 1983),
 estrogen (Quarmby & Korach,
 1984). estrogen 가
 가 protooncogene

. c-fos promoter
 estrogen-estrogen receptor complex ERE (estrogen responsive element)가
 (Hyder & Stancel, 1994), northern assay estrogen
 c-fos c-jun (Insel, 1990;

Weisz et al., 1990; Zheng et al., 1996). estrogen
 heat shock protein (HSP) mRNA가 (Mobbs et al., 1990).
 estrogen HS-70 c-fos
 heat shock c-fos가 (Dragnow et al., 1989)

estrogen HSP c-Fos 가 .
 estrogen c-Fos 1-3
 . CREB
 ATF c-Fos가 CREB ATF heterodimer

late response gene promoter
 .
 protein kinase A protein kinase C
 (cross talk) .

c-fos promoter ERE가
 (Hyder & Stancel, 1994) CREB ATF promoter ERE가

가
 . estrogen 24 c-Fos가 가
 c-Fos Fos-related antigen (FRA) (Cohen &
 Curran, 1988; Ryder et al., 1988). HSP70 HSP90
 3-6 . nuclear protein
 progesterone c-Fos 가
 progesterone estrogen
 estrogen progesterone GnRH
 c-Fos가 (Hoffman et al., 1990; Lee et al., 1992)
 . X+E+P X+E+V c-Fos
 가 (additive effect)가
 progesterone 24 estrogen c-Fos가
 CREB ATF

가

transcription factor HSP70

.

1) estrogen c-Fos, CREB, ATF, HSP70 가 ,

1 3-6 .

2) progesterone c-Fos HSP70 가 , CREB

ATF .

3) progesterone estrogen 가 c-Fos, CREB, ATF

.

4) c-Fos, CREB, ATF luminal epithelium

estrogen .

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Legends for Figures

- Fig. 1. Temporal pattern of c-Fos induction by estrogen. After estrogen treatment rats were sacrificed at the time interval indicated above and nuclear extract were analyzed for c-Fos immunoreactivity using western blotting analysis. c-Fos levels are expressed as ADUs over the control without estrogen treatment. Estrogen (10 μ g)
- Fig. 2. Time course of estrogen-induced HSP70 expression in rat uterus.
- Fig. 3. Levels of c-Fos in rat uterus induced by various steroid hormonal milieu. Two weeks after ovariectomy, estrogen (10 μ g) or progesterone (1 mg) was subcutaneously introduced and sacrificed 3 hours later (X+E, X+P). 24 hours after estrogen administration, progesterone was injected to X+E treated groups and sacrificed 3 hours later (X+E+P).
- Fig. 4. Effect of estrogen and/or progesterone on CREB induction in rat uterus.
- Fig. 5. Effect of estrogen and/or progesterone on ATF induction in rat uterus.
- Fig. 6. Effect of estrogen and/or progesterone on HSP70 induction in rat uterus.
- Fig. 7. Immunohistochemical localization of estrogen-induced c-Fos, CREB, and ATF immunoreactivity in rat uterus. CTL, control group. $\times 100$