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Short communication

Correlation between the alterations in the mRNA expressions of the α_1-adrenoceptor and estrogen receptor subtypes in the pregnant human uterus and cervix

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Abstract

Our present aim was to determine the association between the mRNA expressions of the estrogen and adrenoceptor subtypes in the pregnant human uterus and cervix. The presence of the mRNA expressions of all the α_1-adrenoceptor and estrogen receptor subtypes in the uterus and cervix was proved by means of a reverse transcription polymerase chain reaction method, with a predominance of the mRNAs of the α_{1B}-adrenoceptor and estrogen α receptors, respectively. The change in the mRNA expression of the estrogen receptor α correlated strongly with the change in mRNA level of the α_{1B}-adrenoceptors. We presume that the expression of the α_{1B}-adrenoceptors at 33–34 weeks in the pregnant human uterus is regulated by estrogen through the estrogen receptor α subtypes.

Keywords: α_1-adrenoceptor subtypes; Estrogen receptor subtypes; Pregnant human uterus

1. Introduction

In an earlier study, we demonstrated that the α_{1A}-adrenoceptors play the major role in the regulation of the pregnant rat uterus, especially at term (Ducza et al., 2002). Moreover, the mRNA expression of the estrogen receptor α was also active during pregnancy in the rat myometrium: maximum expression was attained on the day of implantation; a gradual decrease was then observed until the second half of pregnancy, when the expression continuously increased up to the day of labor (Minorics et al., 2004). This point to a correlation between the expressions of the mRNAs of the α_{1A}-adrenoceptor and the estrogen receptor α in the pregnant rat uterus.

Our present aim was to determine the estrogen receptor and α_1-adrenoceptor subtype gradients in the human uterus and cervix, together with the associations between the mRNA expressions of these receptor subtypes.

There are two estrogen receptors, α and β, which are encoded by separate genes (Foegh and Ramwell, 1998). Estrogen receptor α and estrogen receptor β can mediate opposing transcriptional activates, depending on the type of response element in the target gene promoters and on other cell-specific factors, such as the presence or absence of co-regulation (Dechering et al., 2000). There are also differences in the cellular distribution of estrogen receptors α and β. It is well known that estrogen receptors are expressed in the smooth muscle tissues of the myometrium, but the gradient distributions of the mRNAs of the estrogen receptors α and β in the late pregnant human uterus and cervix have not been determined. We first analyzed the distributions of the mRNAs of the estrogen receptors α and β via a reverse transcription polymerase chain reaction (RT-PCR).

Ovarial steroid hormones also regulate the expression of adrenoceptors: acute treatment with estradiol has been found to increase the number of α-adrenoceptors, while inversely, progesterone increases the number of β-adrenoceptors in

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ovariectomized rats and rabbits (Kano, 1982), but little information is available on the localization and possible correlation of adrenoceptor and estrogen receptor subtypes in the pregnant human uterus and cervix. Our studies have indicated a possible correlation between the mRNA expressions of these receptor subtypes.

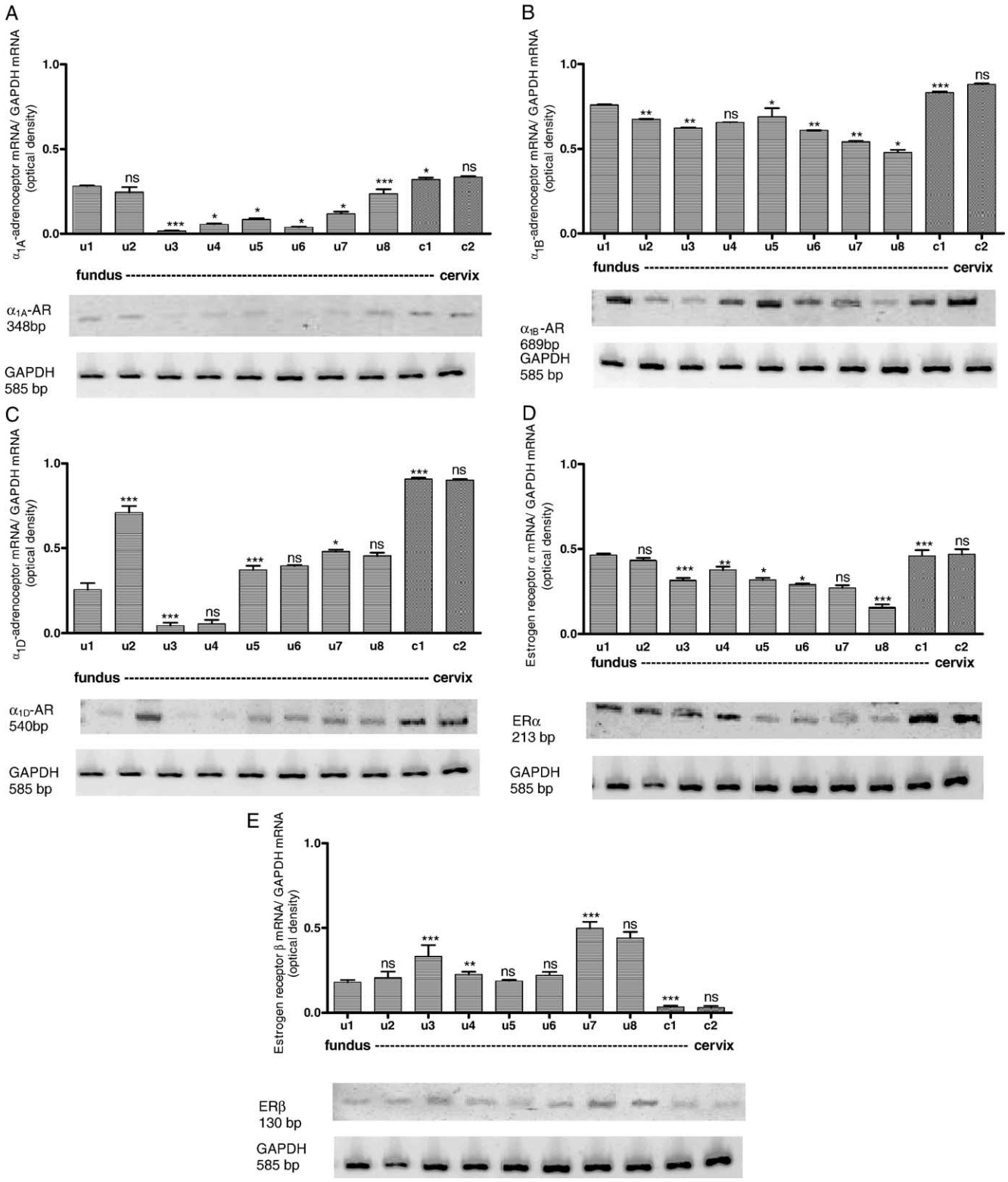

Fig. 1. The changes in mRNA expression of the α_{1A}- (A), the α_{1B}- (B) and the α_{1D}-adrenoceptors (C) , the estrogen receptor α (D) and the estrogen receptor β (E) in the human uterus and cervix at 33–34 weeks of pregnancy, measured by RT-PCR as described in Materials and methods. The relative amounts of the receptor subtype mRNAs are indicated by the optical densities of the bands. The strips of uteri were cut into 8 parts (u1–u8) and the cervical tissues into 2 parts (c1–c2). (ns = $P > 0.05$; * = $P < 0.05$; ** = $P < 0.01$; *** = $P < 0.001$ as compared with the data on the previous day). Each bar represents the mean ± S.D., $n = 6$.

2. Materials and methods

The experimentation on human uterus tissue samples was approved by the Ethics Committee of the University of Szeged (No.: IV/1813/2002).

2.1. Human uterus samples

These samples ($n=6$) originated from women (mean age 30.6, range 25–38) at 33–34 weeks of pregnancy. In our investigations, uterus strips from the fundus to the cervix were cut into 8 equal parts, and the cervix tissues were divided into 2 equal parts for RT-PCR studies. The removed uterus tissues were dissected in ice-cold saline (0.9% NaCl) containing 2 units/ml of recombinant ribonuclease inhibitor (Promega, U.K.) The tissues were frozen in liquid nitrogen and then stored at −70 °C until the extraction of the total RNA.

2.2. RT-PCR studies

2.2.1. Total RNA preparation

Total cellular RNA was isolated by extraction with acid guanidinium thiocyanate-phenol chloroform by the procedure of Chomczynski and Sacchi (1987). After precipitation with isopropanol, the RNA was washed three times with ice-cold 75% ethanol and then dried. The pellet was resuspended in 100 μl DNase and RNase-free distilled water. The RNA concentrations of the samples were determined from their absorbance at 260 nm.

2.2.2. RT-PCR

The RNA (0.5 μg) was denatured at 70 °C for 5 min in a reaction mixture containing 20 μM oligo(dT) (Invitrogen, UK), 20 U RNase inhibitor (Invitrogen), 200 μM dNTP (Sigma-Aldrich, Hungary) in 50 mM Tris–HCl, pH 8.3, 75 mM KCl, and 5 mM $MgCl_2$ in a final reaction volume of 20 μl. After the mixture had been cooled to 4 °C, 20 U M-MLV reverse transcriptase (Invitrogen, UK) and RNase H Minus (Invitrogen, UK) were added, and the mixture was incubated at 37 °C for 60 min.

The PCR was carried out with 5 μl cDNA, 25 μl ReadyMix REDTaq PCR reaction mix (Sigma-Aldrich, Hungary), 2 μl 50 pmol sense and antisense primers and 16 μl DNase- and RNase-free distilled water. The PCR was performed with a PCR Sprint thermal cycler (Hybaid Corp., UK) with the following cycle parameters: after initial denaturation at 95 °C for 2 min, the reactions were taken through 35 cycles of 30 s at 94 °C, 35 s at 45 °C and 30 s at 72 °C (estrogen receptor subtypes), while the amplification protocol of the α_1-adrenoceptor subtypes was 90 s at 94 °C, 2 min at 60 °C, and 2 min at 72 °C. The sequences of the primers were earlier reported by Tschugguel et al. (2003) and Ricci et al. (1999), respectively. Simultaneously, we performed RT-PCR for housekeeping gene glyceraldehyde-3-phosphate dehydrogenase (GAPDH) in the uterus and cervix as a positive, internal control. The RT-PCR products were separated on 2% agarose gels, stained with ethidium bromide and photographed under a UV transilluminator. Quantitative analysis was performed by densitometric scanning of the gel with Kodak EDAS290 equipment (Csertex Ltd., Hungary).

For statistical evaluations, data were analyzed by analysis of variance (ANOVA) with the Neuman–Keuls test.

3. Results

To determine the distributions of the mRNAs of the estrogen receptors α and β and the α_1-adrenoceptor subtypes in the myometrium and cervix of pregnant women, total RNAs from each tissue were reverse transcripted. The resulting cDNAs were amplified by PCR, using a set of primers specific for each estrogen and adrenoceptor cDNA sequence. The successful normalization of RNA amounts during the RT step was verified by amplification of a fragment of the reference standard, GAPDH cDNA, in all of the samples analyzed. Fig. 1 shows the respective pictures of the RT-PCR analysis.

The presence of the mRNAs of all the α_1-adrenoceptor subtypes (α_{1A}, α_{1B} and α_{1D}) in the pregnant human uterus and cervix was proved, and a predominance of the mRNA of the α_{1B}-adrenoceptor was detected. The level of the α_{1B}-adrenoceptor mRNA slowly decreased from the fundus toward the cervix (Fig. 1B). A lower expression of the α_{1D}-adrenoceptor mRNA (Fig. 1C) and a minimal amount of the α_{1A}-adrenoceptor mRNA (Fig. 1A) were observed in the investigated parts of the human myometrium. We did not observe any regularity between the expressions of the mRNAs of the α_{1A}- and α_{1D}-adrenoceptors.

The expressions of the mRNAs, of all of the α_1-adrenoceptor subtypes were significantly higher in the cervix tissues as compared with the uterus.

The mRNAs of the estrogen receptors α and β were also detected, with a predominance of the estrogen receptor α mRNA in the pregnant uterus and cervix tissues (Fig. 1D). The expression of the estrogen receptor β mRNA (Fig. 1E) was lower and contrasted slightly with that of the estrogen receptor α in the uterus tissues. The level of the estrogen receptor β mRNA was dramatically lesser in the cervix than in the uterus.

The change in the expression of the estrogen receptor α mRNA correlated strongly ($r^2=0.8012$) with the change in

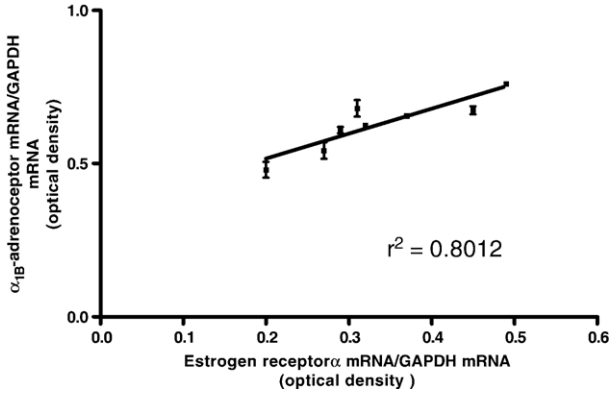

Fig. 2. Correlation between the estrogen receptor α mRNA level and the α_{1B}-adrenoceptor mRNA expression in the human uterus.

the α_{1B}-adrenoceptor mRNA level in the myometrium (Fig. 2). We did not detect any correlation between the mRNA levels of the other estrogen receptor subtypes and the adrenoceptor subtypes.

4. Discussion

The myometrial adrenergic response and receptors are modulated by the hormonal environment, e.g. by estrogen. Estrogen regulates the reproductive functions through the binding of a nuclear protein, the estrogen receptor, which belongs in a superfamily of ligand-activated transcription factors that regulate the expression of target genes by binding to specific response elements. Estrogen administration and elevated endogenous estrogen levels increase the uterine contractile response to sympathetic stimulation. This contractile response is mediated by α-adrenergic mechanisms. Marshall (1981) reported that the ovarial steroid hormones regulate the uterine contractility and appear to determine the response of the uterus to catecholamines. Roberts et al. (1981) demonstrated that estrogen increases the number, but not the affinity of myometrial α-adrenoceptors, and that this increase in receptor number may account for the enhanced α-adrenoceptor sensitivity in myometrial strips prepared from estrogen-treated animals. Moreover, the increase in α-adrenoceptors is produced by estrogen treatment; withdrawal of estrogen decreases the α-adrenoceptor number (Legrand et al., 1987).

The presence of the mRNAs of all the α_1-adrenoceptor subtypes (α_{1A}, α_{1B}, and α_{1D}) in the pregnant human uterus and cervix was proved in our studies, and a predominance of the mRNA of the α_{1B}-adrenoceptors was detected.

In the nonpregnant human uterus, it has been shown that estrogen receptor α and β proteins are co-expressed in numerous cell types, including the stroma and epithelial cells lining the glands, but only the β form is present in the endothelial cell lining of the blood vessel walls. Quantitative studies on the expression of the uterine estrogen receptor mRNA have suggested that the level of the estrogen receptor α mRNA may be 300-times higher than that of the estrogen receptor β mRNA during the proliferative phase (Scobie et al., 2002). On the other hand, sufficient information is not yet known on the distributions of the estrogen receptors α and β in the pregnant human uterus.

We determined a predominance of the estrogen receptor α mRNA at 33–34 weeks of pregnancy. The amount of estrogen receptor α mRNA continuously decreased from the fundus toward the cervix, but then increased significantly in the cervix. The expression of the estrogen receptor β mRNA was lower than that of the estrogen receptor α mRNA in both the cervix and the uterus tissues. We conclude that the effects of estrogen are mostly mediated by the estrogen receptor α subtypes in the pregnant human uterus and cervix at 33–34 weeks. Moreover, the estrogen receptor β subtypes seem to be less important than the estrogen receptor α in this period in the pregnant human uterus and cervix. Our results support those of Wu et al. (2000), who established that the estrogen receptor α is the predominant estrogen receptor subtype in the nonpregnant and the early pregnancy myometrium, but at 37–40 weeks there is a dramatic switch from estrogen receptor α to β expression in the myometrium; the estrogen receptor β is the predominant subtype in the term myometrium. In this period, the myometrial estrogen receptor β may inhibit activator protein-1 activity and thus block induction of the *cx43* gene and other labor-associated genes. Labor may ensue after a loss of myometrial estrogen receptor β expression.

We have proved that the change in the expression of estrogen receptor α mRNA correlated strongly with the change in the α_{1B}-adrenoceptor mRNA level in the myometrium at 33–34 weeks of pregnancy.

Karkanias et al. (1996) demonstrated that estradiol treatment increases the density of the α_{1B}-adrenergic receptors in the hypothalamus-preoptic area of ovariectomized female rats. This brain region is involved in the control of the reproductive function.

We presume that the α_{1B}-adrenoceptor mRNA expression is regulated by estrogen, through the estrogen receptor α subtypes. Published results allow the hypothesis that estradiol might promote a functional interaction between insulin-like growth factor-1 (IGF-1) and α_{1B}-adrenoceptor signaling, as in the hypothalamus of female rats (Quesada and Etgen, 2002). Thus, the mechanism of this heterolog regulation is multifaceted, involving changes in gene expression (of the α_{1B}-adrenoceptor), the switching of the receptor linkage to previously inactive intracellular pathways, and the promotion of cross-talk between IGF-1 and the α_{1B}-adrenoceptor.

This regulatory mechanism between the estrogen receptor α and the α_{1B}-adrenoceptor may play a role in physiological changes, e.g. the contraction in the late pregnant human uterus, which results in premature labor or labor.

References

Chomczynski, P., Sacchi, N., 1987. Single-step method of RNA isolation by acid guanidinium thiocyanate-phenol-chloroform extraction. Anal. Biochem. 162, 156–159.

Dechering, K., Boersma, C., Mosselman, S., 2000. Estrogen receptor α and β: two receptors of a kind? Curr. Med. Chem. 7, 561–576.

Ducza, E., Gáspár, R., Falkay, G., 2002. Altered levels of mRNA expression and pharmacological reactivity of alpha1-adrenergic receptor subtypes in the late-pregnant rat myometrium. Mol. Reprod. Dev. 62, 343–347.

Foegh, M.L., Ramwell, P.W., 1998. Cardiovascular effects of estrogen: implications of the discovery of the estrogen receptor subtype b. Curr. Opin. Nephrol. Hypertens. 7, 83–89.

Kano, T., 1982. Effects of estrogen and progesterone on adrenoceptors and cyclic nucleotides in rat uterus. Jpn. J. Pharmacol. 32, 535–549.

Karkanias, G.B., Ansonoff, M.A., Etgen, A.M., 1996. Estradiol regulation of alpha 1b-adrenoceptor mRNA in female rat hypothalamus-preoptic area. J. Neuroendocrinol. 8, 449–455.

Legrand, C., Maltier, P.P., Benghan-Eyene, Y., 1987. Rat myometrial adrenergic receptor in late pregnancy. Biol. Reprod. 37, 641–650.

Marshall, J.M., 1981. Effects of ovarial steroids and pregnancy in adrenergic nerves of uterus and oviduct. Am. J. Physiol. 240, C164–C174.

Minorics, R., Ducza, E., Márki, Á., Páldy, E., Falkay, G., 2004. Investigation of estrogen receptor alpha and beta mRNA expression in the pregnant rat uterus. Mol. Reprod. Dev. 68, 463–468.

Quesada, A., Etgen, A.M., 2002. Functional interactions between estrogen and insulin-like growth factor-I in the regulation of α_{1B}-adrenoceptors and female reproductive function. J. Neurosci. 22, 2401–2408.

Ricci, A., Bronzetti, E., Conterno, A., Greco, S., Mulatero, P., Schena, M., Schiavone, D., Tayebati, S.K., Veglio, F., Amenta, F., 1999. Alpha1-adrenergic receptor subtypes in human peripheral blood lymphocytes. Hypertension 33, 708–712.

Roberts, J.M., Insel, P.A., Goldfien, A., 1981. Regulation of myometrial adrenoceptors and adrenergic response by sex steroids. Mol. Pharmacol. 20, 52–58.

Scobie, G.A., Macpherson, S., Millar, M.R., Groome, N.P., Romana, P.G., Saunders, P.T.K., 2002. Human oestrogen receptors: differential expression of ER alpha and beta and identification of ER beta variants. Steroids 67, 985–992.

Tschugguel, W., Dietrich, W., Zhegu, Z., Stonek, F., Kolbus, A., Huber, J.C., 2003. Differential regulation of proteosome-dependent estrogen receptor a and b turnover in cultured human uterine artery endothelial cells. J. Clin. Endocrinol. Metab. 88, 2281–2287.

Wu, J.J., Geimonen, E., Andersen, J., 2000. Increased expression of estrogen receptor β in human uterine smooth muscle at term. Eur. J. Endocrinol. 142, 92–99.