ELSEVIER

European Journal of Pharmacology 324 (1997) 125–127

ejp

Short communication

Regulation by oestrogens of tachykinin NK_3 receptor expression in the rat uterus

Francisco M. Pinto [a,b,*], Josefina Magraner [a,c], Pilar Ausina [a], Elsa Anselmi [c], Julio D. Martín [a], M. Luz Candenas [a]

[a] *Instituto de Investigaciones Científicas Isla de La Cartuja, Avenida Americo Vespuccio s / n, 41092 Sevilla, Spain*
[b] *Departamento de Genética, Facultad de Biología, Universidad de La Laguna, Tenerife, Spain*
[c] *Departament de Farmacología, Universitat de València, Avenida Vicent Andrés Estellés s / n, 46100 Burjassot, Valencia, Spain*

Received 21 February 1997; accepted 25 February 1997

Abstract

The expression of the tachykinin NK_3 receptor and its regulation by ovarian steroids were analyzed by reverse transcription-polymerase chain reaction (RT-PCR) in uteri from ovariectomized rats. A single transcript corresponding to the 325-bp product expected for the tachykinin NK_3 receptor was detected in uteri from olive oil-treated (control) ovariectomized rats. The level of tachykinin NK_3 receptor mRNA in progesterone-treated animals was similar to that observed in uteri from control ones. Tachykinin NK_3 receptor mRNA levels were significantly smaller in uteri from oestrogen-treated ovariectomized rats, with approximately a 32-fold decrease. These findings suggest that oestrogen, but not progesterone, regulates the expression of tachykinin NK_3 receptors in the rat uterus.

Keywords: Tachykinin NK_3 receptor, expression; Polymerase chain reaction; Uterus, rat

1. Introduction

Neurokinin B is a member of a family of neuropeptides called the tachykinins distinguished by a conserved carboxyl-terminal sequence Phe-X-Gly-Leu-Met-NH_2. In addition to neurokinin B, the mammalian tachykinins include substance P, neurokinin A, neuropeptide K and neuropeptide γ. Tachykinins interact with three distinct types of receptors termed NK_1, NK_2 and NK_3 and neurokinin B is the preferred endogenous agonist of the tachykinin NK_3 receptor (Regoli et al., 1994). The recent cloning of the tachykinin NK_3 receptor (Shigemoto et al., 1990; Buell et al., 1992) offers a new approach to investigating the tissue localization and the modulation of expression of this receptor molecule. These molecular studies have shown that the tachykinin NK_3 receptor is expressed predominantly in the central nervous system being undetectable or present in smaller amounts in peripheral tissues (Shigemoto et al., 1990; Tsuchida et al., 1990; Buell et al., 1992). In the oestrogen-treated rat uterus substance P, neurokinin A and neurokinin B induce contractile responses which seem not to involve activation of tachykinin NK_3 receptors (Pennefather et al., 1993). However, functional and radioligand binding studies by Barr et al. (1991) demonstrated the presence of this tachykinin receptor type in uteri from rats in the dioestrous stage of the oestrous cycle. The present work was undertaken to study the expression of the tachykinin NK_3 receptor in the rat uterus and whether this expression is modulated by ovarian steroids.

2. Materials and methods

2.1. Animals

Virgin female Wistar rats (200–250 g) were purchased from Charles River (Iffa Credo, Spain). Bilateral ovariectomy was performed 2 weeks before experiments under ether anaesthesia. Oestrogen (17β-oestradiol benzoate) and progesterone were dissolved in olive oil and injected intraperitoneally. The dose of oestrogen was 50 μg/kg per day for 2 days. The dose of progesterone was 1 mg/kg per day for 3 days. Control rats were treated with olive oil. Rats were killed by decapitation 24 h after the last injection. The apparent 'oestrous' or 'dioestrous' stage after oestrogen or progesterone treatment, respectively, was deter-

* Corresponding author at address a. Tel.: (34-5) 448-9565; fax: (34-5) 446-0565; e-mail: mluz@cica.es

PII S0014-2999(97)00150-7

mined by microscopic examination of a vaginal smear before killing. Each experimental condition, i.e., olive oil, progesterone and oestrogen treatment, was assayed on five different animals.

2.2. RNA isolation

The total RNA of approximately 20 mg of uterine tissue was isolated according to the method of Chomczynski and Sacchi (1987). The RNA pellet was resuspended in 50 μl of RNase-free water and quantified by spectrophotometric measurement at 260 nm. The integrity of the purified RNA collected by this method was confirmed by visualization of the 28S and 18S ribosomal RNA bands after the electrophoresis of RNA through a 1% agarose-formaldehyde gel. To eliminate contaminating genomic DNA, total RNA samples were treated with DNase I (Pharmacia Biotech). RNA samples (10 μg each) were resuspended in diethylpyrocarbonate-treated water and stored at −70°C until use.

2.3. Reverse transcription-polymerase chain reaction (RT-PCR)

RNA (5 μg) was reverse transcribed using random hexamers according to Pharmacia instructions (First-strand cDNA synthesis kit). The resulting cDNA samples were amplified by polymerase chain reaction (PCR) with the following specific primer pairs: rat tachykinin NK_3 receptor, forward 5′-CATTCTCACTGCGATCTACC-3′ and reverse 5′-CTTCTTGCGGCTGGATTTGG-3′; rat β-actin, forward 5′-CCTAGCACCATGA-AGATCAA-3′ and reverse 5′-TTTCTGCGCAAGTTAGGTTTT-3′. The predicted sizes of the amplified tachykinin NK_3 receptor and β-actin PCR products were 325 and 227 bp, respectively. PCR mixes contained 0.2 μM primers, 1.5 U of *Taq* polymerase (Pharmacia), the buffer supplied, 2.5 mM $MgCl_2$, 200 μM dNTPs and cDNA in 25 μl. Each experiment also contained two negative controls (one with the RT reaction containing no added RNA and the other one containing non-retrotranscribed RNA). Following heating at 94°C for 2 min, the parameters used for PCR amplification were as follows: denaturation, 30 s at 94°C; annealing, 30 s at 58°C; extension, 30 s at 72°C. Cycle numbers were 35 for the tachykinin NK_3 receptor and 24 for β-actin. PCR products were separated by gel electrophoresis on 1.7% agarose, stained with ethidium bromide and visualized and photographed under a UV transilluminator (Photodyne). Semiquantitative analysis of tachykinin NK_3 receptor PCR products was achieved by comparing the relative amounts of the target sequence in different samples of uteri from untreated, oestrogen- or progesterone-treated ovariectomized rats. NK_3 receptor mRNA expression levels were normalized to the β-actin mRNA levels used to control the efficiency of RT-PCR among the samples. Equal aliquots of the normalized RT solutions were serially diluted in a 1:2 ratio and amplified for a fixed number of cycles, to ensure analysis of products in the exponential range of amplification. In this portion of the amplification curve, the amount of PCR product derived from a given amount of total RNA in a sample is directly proportional to the concentration of target mRNA in the sample (Cullinan-Bove and Koos, 1993). Nucleotide sequences were determined using the fmol sequencing system (Promega), as previously described (Oterino et al., 1996).

3. Results

PCR amplification of uterine cDNA revealed single transcripts corresponding to the expected product sizes

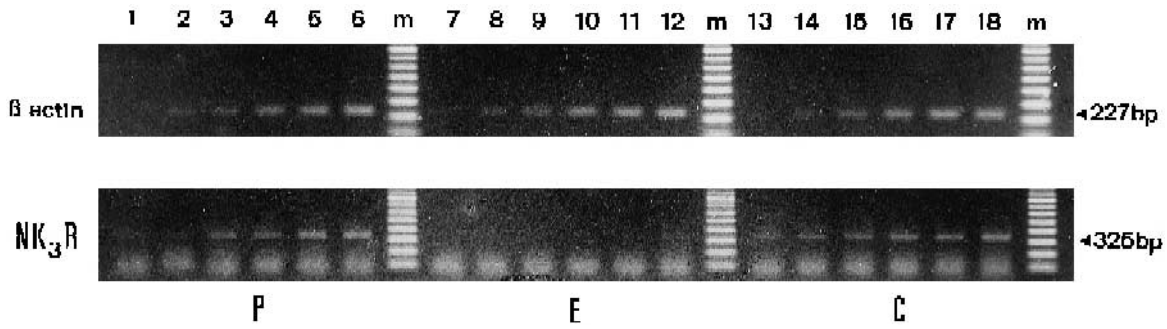

Fig. 1. Agarose gels showing products of reverse transcriptase-polymerase chain reaction (RT-PCR) assay for rat uterine cDNA from progesterone-treated (P, lanes 1–6), oestrogen-treated (E, lanes 7–12) and olive oil-treated (control, C, lanes 13–18) ovariectomized animals. After normalization to β-actin mRNA levels, equal aliquots of the RT solution were serially diluted in a 1:2 ratio and amplified for 24 (β-actin) or 35 (NK_3 receptor) cycles with β-actin- and NK_3 receptor-specific primers. Lanes 1, 7 and 13 represent the more diluted samples in each series. The observation of a steadily declining yield of product at each dilution step confirmed that the comparison of the two samples was made in the exponential portion of the amplification curve. The amount of NK_3 receptor mRNA was assessed in comparison with the amount of the coamplified β-actin fragment. RT-PCR revealed the presence of single transcripts corresponding to the size predicted for the NK_3 receptor (325 bp) and for β-actin (227 bp). A very low abundance transcript for the NK_3 receptor was detected in oestrogen treatment (lanes 7–12), compared with the olive oil controls (lanes 13–18) or the progesterone treatment (lanes 1–6). Lanes m, molecular size standards. Data are representative of typical results in 15 different animals.

encoding cDNA for the tachykinin NK_3 receptor (325 bp) and β-actin (227 bp). The identity of PCR products was confirmed by nucleotide sequence analysis. The sequence of the amplified fragment for the tachykinin NK_3 receptor was identical to that previously published for the rat brain tachykinin NK_3 receptor (nucleotide positions 918–1242, Shigemoto et al., 1990). In all experiments, the two negative controls yielded no detectable products, indicating that (i) all reagents were free of target sequence contamination; and (ii) the RT-PCR products do not come from contaminating genomic DNA. Fig. 1 illustrates a typical example of RT-PCR products from control (olive-oil-treated), oestrogen- or progesterone-treated uteri from ovariectomized rats. As can be observed, the tachykinin NK_3 receptor signal was very faint in the oestrogen-treated rat uterus. The tachykinin NK_3 receptor mRNA level in uteri from untreated animals was at least 32-fold that of uteri from oestrogen-treated rats. Fig. 1 also shows that the level of tachykinin NK_3 receptor mRNA in progesterone-treated ovariectomized animals was similar to that observed in uteri from untreated ones.

4. Discussion

Tachykinin NK_3 receptor mRNA levels were significantly higher in uteri from untreated or progesterone-treated ovariectomized rats than in uteri from oestrogen-treated animals. This suggests that oestrogens inhibit the expression of the tachykinin NK_3 receptor in the rat uterus. The observation that the mRNA levels for this tachykinin receptor type were similar in untreated and progesterone-treated rats suggests that progesterone does not participate in the regulation of the expression of the tachykinin NK_3 receptor.

Substance P and neurokinin A are located in nerves, mostly of sensory origin, that innervate the female reproductive tract in virtually all mammalian species examined (Papka and Shew, 1994). However, little is known about the neurokinin receptor subtypes expressed in the uterus, their regulation and their role in uterine function. Reports in the literature suggest that the effects of tachykinins in peripheral tissues are preferentially mediated by activation of the tachykinin NK_1 and/or NK_2 receptor types (Regoli et al., 1994; Pennefather et al., 1993). Tachykinin NK_3 receptors are widely distributed in the central nervous system (Tsuchida et al., 1990, Buell et al., 1992). However, they are present in smaller amounts or absent in peripheral tissues (Tsuchida et al., 1990; Shigemoto et al., 1990; Buell et al., 1992), and their contribution to the peripheral effect of tachykinins is less clear. In addition, little information is available on the mechanisms responsible for regulating tachykinin NK_3 receptor gene expression (Krause et al., 1994). To our knowledge, only studies by Krause et al. (1994) have demonstrated that NK_3 receptor gene expression is upregulated following nociceptive activation. The present study shows that the tachykinin NK_3 receptor is expressed in uteri from either untreated or progesterone-treated ovariectomized rats. Our data also show that tachykinin NK_3 receptor mRNA almost completely disappeared in ovariectomized rat uteri under oestrogen dominance. This suggests that tachykinin NK_3 receptors could be involved in regulating myometrial activity as well as reproductive functions in females. Further studies are needed to clarify their physiological significance.

Acknowledgements

Supported by the M.E.C. (Spain, grant PB 92-0487). The authors are very grateful to Prof. V.M. Cabrera for his invaluable help. F.M.P. is the recipient of a fellowship from Gobierno de Canarias.

References

Barr, A.J., Watson, S.P., Lopez Bernal, A., Nimmo, A.J., 1991. The presence of NK_3 tachykinin receptors on rat uterus. Eur. J. Pharmacol. 203, 287–290.

Buell, G., Schulz, M.F., Arkinstall, S.J., Maury, K., Missotten, M., Adami, N., Talabot, F., Kawashima, E., 1992. Molecular characterisation, expression and localisation of human neurokinin-3 receptor. FEBS Lett. 299, 90–95.

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

Cullinan-Bove, K., Koos, R.D., 1993. Vascular endothelial growth factor/vascular permeability factor expression in the rat uterus: rapid stimulation by estrogen correlates with estrogen-induced increases in uterine capillary permeability and growth. Endocrinology 133, 829–837.

Krause, J.E., Blount, P., Sachais, B.S., 1994. Molecular biology of receptors. Structures, expression and regulatory mechanisms. In: Buck, S.H. (Ed.), The Tachykinin Receptors. Humana Press, Totowa, NJ, pp. 165–218.

Oterino, A., Monton, F.I., Cabrera, V.M., Pinto, F.M., González, A.M., Lavilla, N.R., 1996. Arginine-164-tryptophan substitution in Connexin 32 associated with X-linked dominant Charcot-Marie-Tooth disease. J. Med. Genet. 33, 413–415.

Papka, R.E., Shew, R.L., 1994. Neural input to the uterus and influence on uterine contractility. In: Garfield, R.E., Tabb, T.N. (Eds.), Control of Uterine Contractility. CRC Press, Boca Raton, FL, 375–399.

Pennefather, J.N., Zeng, X.P., Gould, D., Hall, S., Burcher, E., 1993. Mammalian tachykinins stimulate rat uterus by activating NK-2 receptors. Peptides 14, 169–174.

Regoli, D., Boudon, A., Fauchere, J.L., 1994. Receptors and antagonists for substance P and related peptides. Pharmacol. Rev. 46, 551–599.

Shigemoto, R., Yokota, Y., Tsuchida, K., Nakanishi, S., 1990. Cloning and expression of a rat neuromedin K receptor cDNA. J. Biol. Chem. 265, 623–628.

Tsuchida, K., Shigemoto, R., Yokota, Y., Nakanishi, S., 1990. Tissue distribution and quantitation of the mRNAs for three rat tachykinin receptors. Eur. J. Biochem. 193, 751–757.