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Expression of preprotachykinin-B, the gene that encodes neurokinin B, in the rat uterus

Francisco M. Pinto [a], Cristina G. Cintado [a], Philippe Devillier [b], M. Luz Candenas [a,*]

[a] *Centro de Investigaciones Científicas Isla de La Cartuja, Instituto de Investigaciones Químicas, Avda. Americo Vespucio s / n, 41092 Sevilla, Spain*
[b] *Laboratoire de Pharmacologie-Toxicologie, Centre Hospitalier Universitaire, 45, rue Cognacq Jay, 51092 Reims, France*

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Abstract

Neurokinin B, a peptide belonging to the tachykinin family, is undetectable in peripheral tissues from nonpregnant animals. In the present study, we analysed the expression of the preprotachykinin-B (PPT-B) gene, which encodes neurokinin B, in the rat uterus. Preprotachykinin-B mRNA was expressed in the uterus and its levels varied greatly depending upon the hormonal conditions. This is consistent with a role of this tachykinin in the regulation of uterine functions. © 2001 Elsevier Science B.V. All rights reserved.

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The tachykinins represent a family of neuropeptides, including substance P, neurokinin A and neurokinin B, that interact with three distinct types of receptors termed NK_1, NK_2 and NK_3 (Regoli et al., 1994). Recent advances in our knowledge of the physiological role played by tachykinins have focused on tachykinin NK_1 and NK_2 receptors. Conversely, little is still known about the physiological significance of the tachykinin NK_3 receptor, particularly at the peripheral level. This is mainly due to the observation that neurokinin B, the preferred endogenous agonist for this tachykinin receptor, is undetectable in peripheral tissues (Moussaoui et al., 1992; Patacchini et al., 2000). Accumulating evidence suggests that neurokinin B and the tachykinin NK_3 receptor could play a role in regulating reproductive functions (for recent review, see Patak et al., 2000). The tachykinin NK_3 receptor is expressed in the rat uterus and its expression and function vary under different hormonal conditions (Magraner et al., 1998; Pinto et al., 1999; Patak et al., 2000; Hamlin et al., 2000). Moreover, Page et al. (2000) have shown that the human and rat placenta secretes neurokinin B and suggested that this neuropeptide can mediate or be an indicator of pre-eclampsia, one of the principal causes of maternal morbidity during pregnancy. The present study was undertaken to analyse the expression of preprotachykinin-B (PPT-B) or tachykinin-3 (TAC-3), the gene that encodes neurokinin B in the rat uterus under different hormonal conditions.

Virgin female Wistar rats (Charles River, Criffa, Spain) were maintained in an air-conditioned room at 22 °C under controlled lighting (12-h light/12-h dark). Vaginal smears were examined microscopically to assess the stage of the oestrous cycle. Uteri were obtained from: (1) young (3-month old) rats at the oestrus stage of the ovarian cycle; (2) old (30-month old) nonregularly cycling animals; (3) 3-month-old ovariectomised rats treated with progesterone (1 mg/kg/day for 3 days) and (4) 3-month-old ovariectomised rats treated with oestrogen (50 μg/kg/day for 2 days). Bilateral ovariectomy and hormonal treatment were performed as previously described (Pinto et al., 1999). Number of animals (n) was four per group. Isolation of total uterine RNA, synthesis of cDNA and endpoint reverse transcription-polymerase chain reaction (RT-PCR) studies were performed as described by Magraner et al. (1998). The PCR products were separated by agarose gel electrophoresis and stained with ethidium bromide. Real-time PCR (iCycler, Bio-Rad laboratories, CA, USA) was used to quantify the expression of the preprotachykinin-B gene, using SYBR green detection (Molecular Probes, Leiden, The Netherlands). β-actin was used as endogenous control for variations in cDNA amounts. The specific primer pairs used were: (a) rat preprotachykinin-B, forward 5′-TGATCTCTCTCTGCTACCTCCAC-3′ and re-

* Corresponding author. Tel.: +34-95-4489565; fax: +34-95-4460565.
E-mail address: mluz@cica.es (M.L. Candenas).

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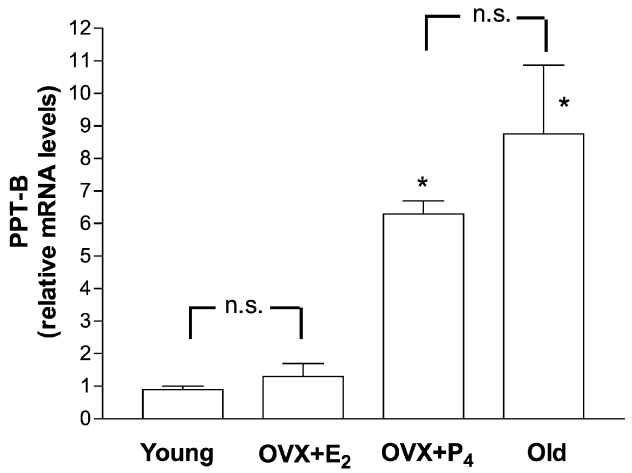

Fig. 1. Expression of preprotachykinin-B (PPT-B) mRNA in the rat uterus under different hormonal conditions. Uteri were obtained from 3-month-old oestrus rats (Young), ovariectomised rats treated with oestrogen ($OVX+E_2$), ovariectomised rats treated with progesterone ($OVX+P_4$), or 30-month-old rats (Old) ($n=4$ animals per group). Expression of preprotachykinin-B and β-actin genes was quantified by real-time PCR. Values for preprotachykinin-B mRNA (means ± S.E.M.) are shown in arbitrary units, relative to β-actin mRNA expression. $^{*}P<0.05$, significant differences versus young and $OVX+E_2$ groups; n.s., not significant; one-way ANOVA.

verse 5′-CCCTGTCTTTATGATGCAGTCC-3′, according to the published mRNA sequence and (b) rat β-actin, forward 5′-CCTAGCACCATGAAGATCAA-3′ and reverse 5′-TTTCTGCGCAAGTTAGGTTTT-3′, based on the published sequence of the rat β-actin gene. The parameters used for PCR amplification were 94 °C for 10 s, 60 °C for 20 s and 72 °C for 30 s, for 36 cycles. Values are expressed as means ± S.E.M. Statistical analysis of differences was assessed by one-way analysis of variance (ANOVA) followed by Tukey's multiple comparison test (GraphPad Prism 3.0, CA, USA). A probability level of $P<0.05$ was regarded as significant.

By using endpoint RT-PCR and agarose gel electrophoresis, we detected the presence of single bands of the expected size of cDNAs encoding preprotachykinin-B (300 bp) and β-actin (227 bp). The identity of the amplified fragments was confirmed by DNA sequence analysis. Quantitative real-time PCR showed that relative to preprotachykinin-B mRNA levels in young oestrus rats, mRNA levels of this transcript were similar in ovariectomised rats treated with oestrogen, about six-fold higher in ovariectomised rats treated with progesterone, and about nine-fold higher in old rats (Fig. 1). To our knowledge, the present study shows for the first time that preprotachykinin-B, the gene that encodes neurokinin B, is expressed in the rat uterus. The strong down-regulation of neurokinin B (this study) and the tachykinin NK_3 receptor (Pinto et al., 1999) by oestrogen is consistent with a role for this ligand–receptor pair in the regulation of uterine functions and support the existence of a link between oestrogen and the neurokinin B/tachykinin NK_3 receptor activation pathway.

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