Ovarian expression of alpha (1)- and beta (2)-adrenoceptors and p75 neurotrophin receptors in rats with steroid-induced polycystic ovaries

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Abstract

Polycystic ovary syndrome (PCOS) is the main cause of infertility in women. Despite extensive research aimed at identifying the pathogenetic mechanism underlying this condition, the aetiology of the disease is still unknown. Evidence from studies on women with PCOS and on an experimental rat polycystic ovary (PCO) model suggests that the sympathetic regulatory drive to the ovary may be unbalanced. The present study was designed to investigate this hypothesis.

Accordingly, we used the well-defined rat PCO model, where PCO is induced by a single intramuscular (i.m.) injection of estradiol valerate (EV), and compared the model with oil-injected controls. We studied the ovarian expression of the α1- and β2-adrenoceptors (ARs), the neurotrophin receptor p75 (p75NTR), and the sympathetic marker tyrosine hydroxylase (TH) at two time points: 30 and 60 days after EV injection.

Our data demonstrate for the first time that all of the α1-AR subtypes are expressed in normal rat ovaries at both the mRNA and the protein levels. Furthermore, the expression of the α1-AR subtypes was differentially modulated in a time- and subtype-dependent manner in rats with steroid-induced PCO. The ovaries in rats with steroid-induced PCO are characterised by an early overexpression of these molecules and p75NTR, while the β2-AR was downregulated. An increase in the expression of ovarian TH after EV injection was also detected, suggesting a structural and functional remodelling of ovarian sympathetic innervation in PCO rats.

Our evidence strongly indicates that the role of the sympathetic nervous system is crucial in the pathogenesis of EV-induced PCO. Overall, our findings suggest that therapeutical approaches aimed at down-regulating the sympathetic tone to the ovary could be useful in the prevention and clinical treatment of PCOS.

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1. Introduction

Polycystic ovary syndrome (PCOS) is recognised as the primary cause of infertility in women. It is a complex disease, characterised by ovulatory failure, hyperandrogenism, variable levels of gonadotropins, and large cystic follicles (Tsilchorozidou et al., 2004). Women with PCOS also have a higher risk of developing hypertension and insulin resistance.

The precise aetiology of the disease is so far still unknown, but there are indications that human PCOS is associated with hyperactivity in the sympathetic nervous system. Findings that support the involvement of the sympathetic nervous system in the pathophysiology of
PCOS is that the catecholaminergic nerve fibres in the polycystic ovaries of women with PCOS are more dense than in normal ovaries (Heider et al., 2001; Semenova, 1969), and that the metabolism of norepinephrine (NE) in adolescents suffering from the disease is impaired (Lobo, 1988; Lobo et al., 1983; Shoupe and Lobo, 1984).

Studies on an animal model of the disease indicate that PCO induced by a single intramuscular (i.m.) injection of estradiol valerate (EV) (Brawer et al., 1986) is characterised by profound changes in catecholamine homeostasis in the ovaries. These changes start before cysts develop and persist after cysts are formed (Barria et al., 1993; Lara et al., 1993, 2000). Characteristic features of EV-induced PCO in rodents are early increases in ovarian concentrations of NE, enhanced release of NE from ovarian nerve terminals, increased activity of the catecholamine synthesis-limiting enzyme tyrosine hydroxylase (TH), and down-regulation of β2-adrenoceptors (ARs) in theca interstitial cells (Barria et al., 1993; Lara et al., 1993, 2000). An enhanced steroidosal responsiveness to β2-adrenergic stimulation (Lara et al., 2002), as well as the reverse of this response by the ablation of the sympathetic input to ovarian endocrine cells, have also been demonstrated (Barria et al., 1993).

The development and function of ovarian sympathetic innervation depend on the ability of the ovaries to produce nerve growth factor (NGF) (Lara et al., 1990). It has been demonstrated that the development of ovarian follicular cysts is preceded by an increased synthesis of ovarian NGF and low-affinity p75 neurotrophin receptor (p75NTR) mRNA in rats with steroid-induced PCO (Lara et al., 2000). Furthermore, blockade of intra-ovarian NGF actions restores the normal structural and functional features of the ovary in the steroid-induced rat PCO model (Lara et al., 2000). Thus, it can be inferred that the hyperactivation of ovarian sympathetic input in rats with steroid-induced PCO is related to an overproduction of NGF.

Nevertheless, this hypothesis does not clarify the findings of dysfunction in ovarian functionality such as the increased, sympathetically mediated expression of ovarian estradiol found in the rat model of PCO (Barria et al., 1993). It is therefore possible that other pathophysiological mechanisms linked to dysfunctions in the sympathetic signalling to the ovaries are active in the development and maintenance of steroid-induced PCO.

The expression and role of other ovarian ARs such as the α1-AR have not, to our knowledge, previously been investigated in the normal rat ovary or in the ovary of rats with steroid-induced PCO. α1-ARs are members of the G protein-coupled receptors and play critical roles in the regulation of a variety of physiological processes (Civantos Calzada and Aleixandre de Artinano, 2001). Within this classification, there are three subtypes: α1A, α1B, and α1D (Civantos Calzada and Aleixandre de Artinano, 2001), and the α1C-AR subtype has been reported to be implicated in the maintenance of vascular basal tone, the α1G-AR subtypes has been said to participate in the response to exogenous agonists, and the α1D-AR subtype is a predominant mediator of arterial vasoconstriction. It can be hypothesised that these receptors can be down- or up-regulated because of high sympathetic activity in the ovaries of PCO rats compared with controls.

The aim of the present study was to elucidate the expression and role of the α1-AR subtypes in normal and EV-induced rat ovaries, as well as broaden our knowledge of AR and p75NTR expression in rats with steroid-induced PCO. Accordingly, we used the well-established EV-induced rat PCO model and studied the ovarian expression of TH, the α1-AR subtypes, β2-AR, and p75NTR at 30 and 60 days after a single i.m. injection of EV. The time point of 30 days after EV injection was chosen since typical polycystic morphological changes start to appear by this time, and the time point of 60 days after the injection was chosen since the PCO picture is fully developed by this time (Brawer et al., 1986; Stener-Victorin et al., 2000, 2003).

2. Materials and methods

2.1. Animals

Thirty-four virgin 8-week-old cycling Wistar–Kyoto rats weighing 200±10 g were purchased from B&K Universal AB (Sweden). They were housed four to each cage at a controlled temperature of 22 °C with a 12-h light/12-h dark cycle for at least 1 week before and throughout the experimental periods with free access to pelleted food and tap water. The experiments were carried out according to the principles and procedures outlined in the National Institute of Health (NIH) Guide for the Care and Use of Laboratory Animals. The study was approved by the Animal Ethics Committee of Göteborg University.

2.2. Study procedure

Eight rats in the first experiment—PCO 30 days—and 10 rats in the second experiment—PCO 60 days—were each given a single i.m. injection of 4 mg of EV (Riedeldehaen, Germany) in 0.2 ml of oil (arachids oleum; Apoteket AB, Umeå, Sweden) to induce PCO (PCO group). Sixteen rats received a single i.m. injection of 0.2 ml of oil only (control group). Thirty days after EV injection is the time point when persistent oestrus, a permanent polycystic condition, and a characteristic pattern of abnormal plasma gonadotropin develop (Brawer et al., 1986; Stener-Victorin et al., 2000, 2003). True cystic follicles appear 60 days after EV injection, and PCO is fully developed (Brawer et al., 1986; Stener-Victorin et al., 2000, 2003).

Oestrus cyclicity was monitored daily with a vaginal smear obtained between 8:00 and 11:00 a.m. starting 10—
14 days prior to the experiments. All rats were killed by decapitation in oestrus—in the first experiment, 30 days after EV injection, and in the second experiment, 60 days after EV injection. Before decapitation, the rats were anaesthetised with 125 mg/kg body weight of thiobutabarbital sodium (Inactin, RBI, Natick, MA, USA). After the rats were decapitated, the ovaries were removed and cleaned of adherent connective fat tissue. One ovary was rapidly frozen by submersion in liquid nitrogen and stored at −80 °C until used for determination of mRNA and protein expression, and one ovary was processed for immunofluorescence analysis as described in Section 2.3.

2.3. Tyrosine hydroxylase immunofluorescence

One ovary per rat was removed immediately after the rats were killed and placed in a 4% formaldehyde solution in 0.1 M PBS (pH 7.4). After fixation for 2 days, the ovaries were placed in a 20% sucrose solution in PBS (pH 7.4) for 1 day and then sectioned (section thickness 15 μm) on a freezing microtome and processed for TH immunofluorescence. The following specific antiserum was used: rabbit anti-rat TH antibody (AB5986; Chemicon International, CA, USA). For immunofluorescence, slides were preincubated with 10% BSA and 10% normal goat serum in PBS solution containing 0.1% saponin for 2 h and then incubated overnight at 4 °C with primary antibody. To assess specificity in control slides, the primary antibody was replaced by purified rabbit IgG. After washing with PBS 0.1% saponin, the slides were incubated for 3 h at room temperature with fluorescein isothiocyanate-conjugated anti-rabbit IgG. Sections were examined under a fluorescence microscope.

2.4. Real-time PCR for adrenoreceptors

Total RNA from the ovary was extracted using RNeasy Mini kits (Qiagen, Hilden, Germany). The polymerase chain reaction (PCR) was analysed using the ABI Prism 7700 Sequence Detection System (PE Applied Biosystems, Stockholm, Sweden) and FAM-labeled probe specific for the α1a-AR (Rn00567861m1), α1b-AR (ADRA A1B-EX 152027A02), α1d-AR (Rn00577931m1), and β2-adrenoceptor (Rn00560650s1) (PE Applied Biosystems). Designed primers and a VIC-labelled probe for glycer-aldehyde-3-phosphate dehydrogenase (GAPDH) (NM 031144) were included in the reactions as an internal standard. The cDNA was amplified under the following conditions: 1 cycle at 50 °C for 2 min and at 95 °C for 10 min, followed by 40 cycles at 95 °C for 15 s and at 60 °C for 1 min. The amount of mRNA of each gene was calculated using the standard curve method (following the instructions in User Bulletin no. 2; PE Applied Biosystems) and adjusted for the expression of GAPDH. The data from the two different experiments have been plotted together and presented as percentage of control groups (see Fig. 2).

2.5. Reverse transcriptase PCR-ELISA for p75NTR

The expression of p75NTR-mRNA was evaluated using the enzyme-linked immunosorbent assay (ELISA) protocol for reverse transcriptase polymerase chain reaction (RT-PCR) as previously described by Tirassa et al. (2000). The total RNA was extracted from the ovaries using the method of Chomczynski and Sacchi (1987) as modified in the TRizol Kit (Invitrogen AB, Lidingö, Sweden). Complementary DNA was synthesised from 1 μg of total RNA using 250 ng of oligo (dT)12-18 primer 200 U of M-MLV reverse transcriptase (Promega Italia, Milan, Italy) in 20 μl of total volume reaction. The p75NTR and GAPDH genes were co-amplified in a single-tube PCR reaction (35 cycles: 1 min at 95 °C; 1 min at 55 °C; 2 min at 72 °C) using 5′-biotinylated specific primers to generate biotinylated PCR products detectable by digoxygenin-labelled probes in an immunoenzymatic assay. The primer/probe sequences are the following: p75NTR biotinylated forward: 5′ CGTGGTCTCCTGCGCAGGACA 3′; p75NTR biotinylated reverse: 5′ GAGATGGCCTGTGCTGTG 3′; p75NTR digoxygenin-labelled probe: 5′ ACAGCGAGCCAATGGAGAATA-GACAGG 3′; GAPDH biotinylated forward: 5′ CACCC- CATGGAGAGGCCC 3′; GAPDH biotinylated reverse: 5′ GATGGATGCTTGGCCAGG 3′; GAPDH digoxygenin-labelled probe: 5′ ACAATCTTGGATGGTAGTTCAT-TTCTGC 3′. The amounts of the amplified products were measured at an optical density (OD) of 450/690 nm with a Dynatech ELISA Reader 5000. A GAPDH level of OD 450/690 nm was used to normalise the relative differences in sample size, differences in integrity of the individual RNA, and variations in the efficiency of reverse transcription. For exact methodological details, see Tirassa et al. (2000). Data from the two experiments have been plotted together and presented as percentage of the control groups.

2.6. Western blotting analysis for the proteins of the ARs and p75NTR

Commercially available antibodies were used for Western blotting detection of the α1d-AR [α1d-AR [C-19]: sc-1477; Santa Cruz, California, USA], α1b-AR [α1b-AR [C-18]: sc-1476; Santa Cruz, California, USA], α1a-AR [α1a-AR [H-142]: sc-10721; Santa Cruz, California, USA], β2-AR [β2-AR [M-20]: sc-1570; Santa Cruz, California, USA], and β-actin (sc-8432; Santa Cruz, California, USA). The Western blotting detection of p75NTR was carried out using monoclonal anti-p75 antibody (clone 192) (Chandler et al., 1984) purified in our laboratory. Tissue samples were homogenised in lysis buffer (0.01 M Tris–HCl buffer, pH 7.6, containing 0.1 M NaCl, 1 mM EDTA, 1 mM EGTA, 2 mM PMSF, 50 μM leupeptin, 100
μg/ml pepstatin, and 100 μg/ml aprotinin) at 4 °C. After 8000×g centrifugation for 20 min, the supernatants were used for Western blotting. Samples (30 μg of total protein) were dissolved with loading buffer (0.1 M Tris–HCl buffer, pH 6.8, containing 0.2 M DTT, 4% SDS, 20% glycerol, and 0.1% bromophenol blue), separated by 12.5% SDS-PAGE, and electrophoretically transferred to PVDF membrane for 3 h. The membranes were incubated for 40 min at room temperature with blocking buffer (10% non-fat dry milk, 10 mM Tris, pH 7.5, 100 mM NaCl, and 0.1% Tween-20). Membranes were washed three times for 10 min each at room temperature in TTBS and incubated for 1 h with either horseradish peroxidase-conjugated anti-rabbit IgG, horseradish peroxidase-conjugated anti-goat IgG, or horseradish peroxidase-conjugated anti-mouse IgG as the secondary antibody. The blots were developed with ECL (Amersham Bioscience) as the chromophore. Similar results were obtained in five independent Western blot runs. A Macintosh computer and the public domain NIH Image program (developed at the U.S. National Institutes of Health and available on the Internet at http://rsb.info.nih.gov/nih-image/) were used to evaluate band incubation for 1 h at room temperature with primary antibodies. Membranes were washed three times for 10 min each at room temperature in TTBS and incubated for 1 h with either horseradish peroxidase-conjugated anti-rabbit IgG, horseradish peroxidase-conjugated anti-goat IgG, or horseradish peroxidase-conjugated anti-mouse IgG as the secondary antibody. The blots were developed with ECL (Amersham Bioscience) as the chromophore. Similar results were obtained in five independent Western blot runs. A Macintosh computer and the public domain NIH Image program (developed at the U.S. National Institutes of Health and available on the Internet at http://rsb.info.nih.gov/nih-image/) were used to evaluate band
density, which was expressed as arbitrary units of grey level of five different gel runs per blot. The NIH Image program determines the optical density of the bands using a grey scale thresholding operation. The optical density of h-actin bands was used as a normalizing factor. For each gel per blot, the normalized values for 30 and 60 days of PCO groups were then expressed as percentage of relative normalized controls and used for statistical evaluation.

2.7. Statistical analyses

All statistical evaluations were performed using the StatView package for Macintosh (Abacus Concepts, Berkeley, CA, USA) and data were expressed as means±S.E.M. α1-ARs and β2-AR mRNA and p75NTR mRNA concentrations in the ovaries and optical density data from Western blot analyses were evaluated using the analysis of variance (ANOVA), and the groups were tested using multiple comparisons with the correction of Fisher PSD. A p-value less than 0.05 was considered significant.

3. Results

3.1. Ovarian mRNA and protein of α1-ARs and β2-AR in EV-induced PCO

The expression of α1-AR mRNA in the ovary was moderately increased in the PCO group compared to the

Fig. 3. Concentrations of ovarian α1d-AR mRNA and protein in control and PCO ovaries. Panel A: RT-PCR for α1d-AR mRNA. Results from two different experiments (30 and 60 days PCO; see Materials and methods section for details) are expressed as percentage of relative control groups. *p<0.05 vs. control group (ANOVA). Panel B: Densitometric scanning of five different blots showing α1d-AR protein levels. Results from two different experiments are expressed as percentage of relative control groups. *p<0.05 vs. control group. Representative Western blots are shown in panel C. α1d-AR protein is expressed as a monomer with a molecular weight of approximately 80 kDa.

Fig. 4. Ovarian β2-AR mRNA and protein contents in control and PCO ovaries. Panel A: RT-PCR for β2-AR mRNA. Results from two different experiments (30 and 60 days PCO; see Materials and methods section for details) are expressed as percentage of relative control groups. *p<0.05 vs. control group (ANOVA). Panel B: Densitometric scanning of five different blots showing β2-AR protein contents. Results from two different experiments are expressed as percentage of relative control groups. *p<0.05 vs. control group. Representative Western blots are shown in panel C. β2-AR protein is expressed as a monomer with a molecular weight of approximately 80 kDa.
control group at 30 days and even higher at 60 days after EV injection ($p<0.05$) (Fig. 1A). The $\alpha_{1a}$-AR protein levels in the PCO group was higher at 30 days after EV injection ($p<0.05$) when compared with the control group (Fig. 1B and C). No significant differences between control and PCO groups were found in $\alpha_{1a}$-AR protein levels at 60 days (Fig. 1B and C).

The expression of $\alpha_{1b}$-AR in the PCO group was enhanced at 30 days ($p<0.05$) and returned to baseline levels at 60 days after PCO induction (Fig. 2A). As shown in Fig. 2B and C, the $\alpha_{1b}$-AR protein levels were higher at 30 days ($p<0.05$) and lower at 60 days ($p<0.05$) in the PCO group compared with the control group after an EV injection.

Ovarian content of $\alpha_{1d}$-AR mRNA in the PCO group was higher than in the control group at 30 days ($p<0.05$) and returned to baseline levels at 60 days after PCO induction (Fig. 3A). $\alpha_{1d}$-AR protein levels were higher at 30 days in the PCO group compared to the control group, while no differences were found at 60 days after the EV injection (Fig. 3B and C).

The expression of $\beta_2$-AR mRNA in the ovary in the PCO group was not different from the control level at 30 days and decreased below control levels at 60 days after an EV injection (Fig. 4A), while ovarian $\beta_2$-AR protein levels were down-regulated at both 30 and 60 days after injection with EV in the PCO group compared with the control group (Fig. 4B and C).

### 3.2. Tyrosine hydroxylase is up-regulated in EV-induced PCO

As illustrated in Fig. 5A and B, immunostaining for TH was detected in the control group at both 30 and 60 days after treatment. Staining was mainly detected in the thecal layer of the ovarian follicles. Overexpression of TH was found in the PCO group at both 30 and 60 days after EV injection, when compared with the control group (Fig. 5C and D). Positive TH was found in cells, and fibres were located around ovarian follicles. No TH staining was detected when non-specific IgG were used as primary antibodies (Fig. 5E and F).

### 3.3. Ovarian $p75^{NTR}$ mRNA and protein are increased in EV-induced PCO

Ovarian $p75^{NTR}$ mRNA expression was augmented in the PCO group compared with the control group ($p<0.05$) only 60 days after EV injection (Fig. 6A). Western blotting analysis revealed that at both 30 and 60 days after EV
4. Discussion

This study demonstrates that all α₁-AR subtypes are expressed in normal rat ovaries and that the expression of these subtypes was differentially modulated in a time- and subtype-dependent manner in rats with EV-induced PCO. The expression of ovarian β₂-ARs decreased and the expression of ovarian TH increased at both 30 and 60 days after EV injection compared with baseline. The up-regulation of the ovarian expression of p75NTR in rats with EV-induced PCO was detectable 30 days after EV injection and remained higher than normal 60 days after EV injection. These data support the evidence of high ovarian sympathetic activity in the EV-induced rat PCO model.

The distribution of all α₁-AR subtype proteins in adult rat tissues such as the heart, liver, kidney, aorta, prostate, adrenals, and brain has been recently investigated (Shen et al., 2000). The expression of all α₁-AR subtypes at both the mRNA and protein level in the adult rat ovary represents, to the best of our knowledge, a novel finding. As mentioned before, it has been reported that the α₁₁₂-AR subtype is implicated in the maintenance of vascular basal tone, that α₁b-AR subtypes participate in the response to exogenous agonists, and that the α₁d-AR subtype is a predominant mediator of arterial vasomotor response (Mann and Aikawa, 2001). Although our data do not allow speculations on the relative abundance and the physiological function of the different α₁-AR subtypes in the rat ovary, we found that the expression of the three α₁-AR subtypes was affected by EV injection in the present rat PCO model. Indeed, the ovarian α₁b-AR protein levels was enhanced after 30 days, and was still found to be moderately high 60 days after EV injection, while α₁b-AR and α₁d-AR protein levels were up-regulated only at 30 days and returned to baseline (α₁₁₀-AR) or were below baseline levels (α₁₁₀-AR) 60 days after EV injection. The expression of ovarian β₂-AR was previously found to be down-regulated in steroid-induced PCO, both 30 and 60 days after EV injection (Barria et al., 1993; Lara et al., 1993). This effect can be attributed to higher sympathetic activity in the ovaries and high concentrations of NE (Barria et al., 1993; Lara et al., 1993). Since it has been demonstrated that the expression of ovarian β₂-AR in rats with PCO is under sympathetic control (Barria et al., 1993), a reasonable hypothesis is that the sympathetic nervous system also regulates α₁-AR expression. Recent findings confirm this hypothesis (Izzo et al., 1990).

Changes in the metabolism of catecholamine were found in human PCOS (Shoupe and Lobo, 1984) as well as in rats with EV-induced PCO, evidenced by higher levels of TH enzyme in the ovaries and higher sympathetic activity (Lara et al., 1993). Data from the present study provide additional evidence for the current belief that TH immunoreactivity is primarily located in intrinsic cells of the normal ovary. The data also indicate an increase in not only cell-associated but also nerve fibre-associated staining, which occurs both 30 and 60 days after EV injection. These observations suggest that functional activity of ovarian sympathetic tone is enhanced, and most probably also that a sympathetic structural rearrangement with higher sympathetic innervation in the ovary has taken place in rats with EV-induced PCO. The early rise in concentrations of intra-ovarian NGF in EV-induced PCO (Lara et al., 2000; Stener-Victorin et al., 2000) and evidence that NGF plays a regulatory role in TH

Fig. 6. Concentrations of p75NTR mRNA and protein increase in rat PCO ovaries. Panel A: Ovarian p75NTR mRNA expression in control and PCO rats measured by RT-PCR-ELISA. Results from two different experiments (30 and 60 days PCO; see Materials and methods section for details) are expressed as percentage of relative control groups. *p<0.05 vs. control group (ANOVA). Panel B: Densitometric scanning of blots (n=5) showing the amount of p75NTR proteins in control and PCO rats. Results from two different experiments are expressed as percentage of relative control groups. *p<0.05 vs. control group. Panel C: Representative Western blots showing the effects of steroid-induced PCO on the expression of p75NTR in rat ovaries (A). p75NTR protein is expressed as a monomer with a molecular weight of 75 kDa.
synthesis in sympathetic neurons (Rush et al., 1997) support this hypothesis.

Many studies have found that NGF plays a crucial role in the development, survival, transmission, and connectivity of the mature sympathetic nervous system (Levi-Montalcini, 1987; Rush et al., 1997). NGF-driven sympathetic hyperinnervation of target organs has been described for the cardiovascular system (Hassankhani et al., 1995; Tuttle et al., 1995), airways (Hoyle et al., 1998), lymphoid organs (Carlson et al., 1998), pancreas (Edwards et al., 1989), and lower urinary tract (Steers et al., 1999). Evidence of increased ovarian sympathetic tone as one of the main pathogenetic mechanisms involved in the development of EV-induced PCO has been proposed by Lara et al. (2000).

The present study demonstrates that concentrations of ovarian p75NTR mRNA and protein increase at both 30 and 60 days after EV injection. Elevated levels of NGF have been found to induce morphological and neurochemical alterations in sympathetic neurons, including axonal sprouting and increased levels of p75NTR mRNA (Schmidt et al., 2000). Injection of EV resulted in higher intra-ovarian synthesis of NGF and p75NTR after 30 days, an effect that was still measurable after 60 days, the last time-point checked. This time period coincided with the time necessary for the sympathetic tone in the ovary to increase, which precedes the appearance of follicular cysts (Lara et al., 2000). It is known that p75NTR aids the development of specific populations of sympathetic neurons (Lee et al., 1994) and that this receptor is responsible for the responsiveness of adult sympathetic neurons to target-derived NGF (Cowen and Gavazzi, 1998). It is therefore possible that the activation of p75NTR by NGF is part of an important mechanism in the pathogenesis of PCO.

In conclusion, PCO that has been induced in rats by a single injection of EV modulates intra-ovarian sympathetic markers, such as all α1-AR subtypes, β2-AR, TH enzyme, and p75NTR. Most of these molecules undergo significant changes in their ovarian expression during the first 30 days after EV injection. The evidence obtained strongly points to the crucial role of the sympathetic nervous system and the regulatory action of the NGF/NGF receptor system in the pathogenesis of EV-induced PCO. Further studies on how NGF modulates sympathetic activity would be useful in devising new therapeutic approaches for human PCOS.

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