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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 α_1- and β_2-adrenoceptors (ARs), the neurotrophin receptor p75 ($p75^{NTR}$), 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 α_1-AR subtypes are expressed in normal rat ovaries at both the mRNA and the protein levels. Furthermore, the expression of the α_1-AR subtypes was differentially modulated in a time- and subtype-dependent manner in rats with EV-induced PCO. The ovaries in rats with steroid-induced PCO are characterised by an early overexpression of these molecules and $p75^{NTR}$, while the β_2-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.

Keywords: Polycystic ovary syndrome; Polycystic ovaries; Sympathetic activity; Alpha-adrenoceptors; Beta-adrenoceptors; NGF; $p75^{NTR}$; Anovulation

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

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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 β_2-adrenoceptors (ARs) in theca interstitial cells (Barria et al., 1993; Lara et al., 1993, 2000). An enhanced steroidal responsiveness to β_2-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 ($p75^{NTR}$) 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 α_1-AR have not, to our knowledge, previously been investigated in the normal rat ovary or in the ovary of rats with steroid-induced PCO. α_1-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: α_{1a}, α_{1b}, and α_{1d} (Civantos Calzada and Aleixandre de Artinano, 2001), and the α_{1a}-AR subtype has been reported to be implicated in the maintenance of vascular basal tone, the α_{1b}-AR subtypes has been said to participate in the response to exogenous agonists, and the α_{1d}-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 α_1-AR subtypes in normal and EV-induced rat PCO ovaries, as well as broaden our knowledge of AR and $p75^{NTR}$ 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 α_1-AR subtypes, β_2-AR, and $p75^{NTR}$ 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–