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Expression of proopiomelanocortin, proenkephalin and prodynorphin genes in porcine theca and granulosa cells

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Abstract

Previous studies have demonstrated the presence of endogenous opioid peptides (EOP) in the ovary and suggested their implication in local interactions within ovarian structures. Nevertheless, data pertaining to the expression of genes, coding for the opioid precursors, in ovarian cells are still rudimentary and not available for the pig. The study was undertaken to test whether genes of the opioid precursors – proopiomelanocortin (POMC), proenkephalin (PENK) and prodynorphin (PDYN) – are expressed in non-treated and gonadotropin-treated theca and granulosa cells isolated from ovarian follicles of the pig. The cells were isolated from small (days 15–16 of the estrous cycle) and large (days 19–20) porcine follicles. Dispersed cells were cultured in Eagle's medium under the water saturated atmosphere of 95% air and 5% CO_2, in the presence or absence of respective gonadotropin; theca cells with LH (100 ng/ml) and granulosa cells with FSH (100 ng/ml). Following 24 h-incubation, the cells were harvested and the total RNA was isolated. The expression of genes coding for opioid precursors was estimated by the semi-quantitative RT-PCR technique involving co-amplification of the target cDNA (POMC, PENK or PDYN) and control cDNA (β-actin or 18S rRNA). Specificities of PCR products were confirmed by Southern analysis and sequencing. In theca cells the expression of opioid precursors appeared to be gonadotropin-dependent except for PENK in the cells isolated from large follicles. In turn, granulosa cells exhibited the expression of POMC and PENK genes independently on treatment with FSH. This gonadotropin induced the expression of PDYN gene in granulosa cells isolated from small and large follicles and significantly increased POMC mRNA content in the cells from the large ones. The present studies indicate that porcine follicular cells (especially granulosa cells) may produce opioid peptides and that gonadotropins may modulate gene expression of their precursors in these

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cells. Moreover, our results support a participation of opioid peptides in the local regulations within ovarian follicle.

Keywords: Proopiomelanocortin; Proenkephalin; Prodynorphin; Theca cells; Granulosa cells; Ovarian follicle

1. Introduction

Endogenous opioid peptides (EOP) originate from three protein precursors – proopiomelanocortin (POMC), proenkephalin (PENK) and prodynorphin (PDYN) – as a result of their post-translational processing. EOP basically act through three major types of opioid receptors (μ, δ or κ). β-Endorphin – arising from POMC – preferentially acts through μ opioid receptors, proenkephalin derivatives (Met- and Leu-enkaphalins) – δ receptors and PDYN products (*e.g.* neoendorphins and dynorphins) – κ receptors (Okrasa, 1997; Evans, 2004).

Many data have been accumulated, which indicate an involvement of EOP in the regulation of reproductive processes. Their modulatory effects may occur at all levels of the hypothalamo-pituitary-gonadal axis. Beside their well-known influence on GnRH and LH secretion (Barb et al., 1991; Okrasa et al., 1995), they may exert a direct action within reproductive tissues, including the ovary. The opioid receptors were found on porcine ovarian cells (Hamada et al., 1995; Slomczynska et al., 1997), and opioids appeared to affect steroidogenesis in ovarian cells of the pig (Gregoraszczuk and Slomczynska, 1998; Przala et al., 1999; Kaminski et al., 1999, 2000, 2001) and other species (Facchinetti et al., 1986; Varsano et al., 1990; Kato et al., 1993), as well as the maturation of oocytes (O, 1990; Dell'Aquila et al., 2002). The opioid effects on steroidogenesis in theca and granulosa cells – composing two discrete layers of ovarian follicle wall – were observed in several studies (Facchinetti et al., 1986; Gregoraszczuk and Slomczynska, 1998; Kaminski et al., 2000, 2003, 2004). Recently, Kaminski et al. (2003, 2004) have reported that the steroidogenesis in porcine theca and granulosa cells derived from large follicles might be altered by specific agonists of all major types of opioid receptors (μ, δ and κ).

Studies performed on different species documented the expression of genes coding for the three opioid precursors in the ovary – POMC (Melner et al., 1986; Jin et al., 1988; Sanders et al., 1990), PENK (Kilpatrick and Rosenthal, 1986; Jin et al., 1988; Rosen et al., 1990) and PDYN (Douglass et al., 1987; Kaynard et al., 1992) – as well as the presence of their products in various tissues and fluids of the female reproductive tract (Lim et al., 1983; Ehrenreich et al., 1985; Petraglia et al., 1985; Aleem et al., 1986; Lolait et al., 1986; Cupo et al., 1987; Kew et al., 1989; Li et al., 1991; Lovegren et al., 1991; Slomczynska et al., 1997; Kaminski et al., 2000; Przala et al., 2001; Okrasa et al., 2003). β-Endorphin (Kaminski et al., 2000) and PDYN derivatives (Slomczynska et al., 1997) were established in porcine follicular fluid. In addition, β-endorphin secretion from porcine theca and granulosa cells was demonstrated *in vitro* by Kaminski et al. (2000, 2001). Studies of Melner et al. (1986) revealed that the content of POMC mRNA in rat granulosa cells is up-regulated by gonadotropins and androgens. Correspondingly, β-endorphin secretion by porcine granulosa cells was markedly stimulated by FSH (Kaminski et al., 2000). These data collectively suggest that EOP are implicated in local interactions (paracrine and/or autocrine) within ovarian follicles. Nevertheless, the information pertaining to the expression of genes, coding for the opioid precursors, in ovarian follicle cells was based on fragmentary studies and is still rudimentary.