Article

Prolactin receptor mRNA expression in oocytes and preimplantation mouse embryos

Dr Dimitris Loutradis obtained his medical degree from Athens University, Greece. He also completed his PhD thesis in the Medical School of University of Athens. He was fellow in Obstetrics and Gynecology in Harvard Medical School, Boston, Massachusetts, USA for 3 years. He established and is Director of the Division of Reproductive Medicine, 1st Department of Obstetrics and Gynecology of University of Athens, Alexandra Maternity Hospital. He received the award of the National Academy of Greece in 1996. He now holds the position of Associate Professor of Athens Medical School. He is an elected member of the Advisory committee of ESHRE 2002–2006. He has produced 98 publications in Greek medical journals and 80 publications in international peer-reviewed medical journals.

Dr Dimitris Loutradis

Erasmia Kiapekou[1], Dimitris Loutradis[1], Eleni Patsoula[1], Giorgos A Koussidis[1], Vasilis Minas[2], Ritsa Bletsa[1], Aristeidis Antsaklis[1], Stylianos Michalas[1], Antonis Makrigiannakis[2,3]
[1]1st Department of Obstetrics and Gynaecology, Alexandra Maternity Hospital, University of Athens, 11528, Athens; [2]Laboratory of Human Reproduction, Department of Obstetrics and Gynaecology, Medical School, University of Crete, Heraklion 71110, Greece
[3]Correspondence: Tel: +30 2810 392333; Fax: +30 2810 392759; e-mail: makrigia@med.uoc.gr

Abstract

Prolactin was first identified as an anterior pituitary lobe hormone, responsible for the regulation of mammary gland growth and development. Prolactin receptors have been localized in a number of peripheral tissues, including tissues involved in reproduction. Studies with knockout animals have shown that prolactin receptor deficient mice present reproductive defects, whereas prolactin promotes the developmental potential of preimplantation mouse and rat embryos *in vitro*. To better understand the role of prolactin in the process of reproduction and early embryo development in mice, the expression of the four transcript variants of prolactin receptor was examined in the first stages of mouse embryo development. Prolactin long receptor mRNA was expressed in all stages examined, that is in cumulus cells, oocytes, zygotes, 2-cell embryos, 4-cell embryos, morulae and blastocysts. Prolactin receptor type S1 mRNA was observed only in cumulus cells, while S2 mRNA was present in cumulus cells, oocytes, zygotes and 2-cell embryos. S3 mRNA was expressed only in cumulus cells and oocytes. These results indicate that different isoforms of prolactin receptors may be present in the various stages of mouse preimplantation embryo and may play an important role in the control of its growth and development.

Keywords: *in-vitro oocyte maturation, preimplantation embryos, prolactin, receptor isoforms*

Introduction

Prolactin was initially described as an anterior pituitary lobe hormone, with the ability to regulate the growth and development of the mammary gland (Stricker and Grueter, 1928). This description was based on observations of histological and weight changes of the anterior pituitary lobe in pregnant women.

Subsequently, Astwood reported the luteotrophic actions of prolactin, thus setting the basis for a series of additional studies that investigated the multiple physiological roles of prolactin (Astwood, 1941). Indeed, more than 300 biological functions of prolactin have been described, including regulation of water and electrolyte balance, growth and development, metabolism, behaviour, immune response and finally reproduction (reviewed in Bole-Feysot *et al*., 1998).

The prolactin receptors (PRLR) are single pass transmembrane proteins and a number of isoforms, which vary among species, have been characterized so far. The isoforms share common extracellular and transmembrane domains, a common portion of the cytoplasmic domain, and present different carboxy terminal domains, which occur after alternative splicing of the exons of the *PRLR* gene, thus suggesting a level of complexity for the intracellular mediation of PRL signalling (Clarke *et al*., 1993; Ormandy *et al*., 1998). Significantly, prolactin receptors were identified in various peripheral tissues, and studies in experimental animal models revealed a number of reproductive defects in mice with absence of PRLR mediated signalling, as well as mice with null mutation of the prolactin receptor gene (Ormandy *et al*., 1997). Homozygous PRLR knockout mice presented abnormal mammary gland development and reactively increased

concentrations of circulating prolactin (Kelly *et al.*, 2001). They also showed reduced ovulation, abnormal cycles and fertilization defects (Ormandy *et al.*, 1997). Additionally, these mice had abnormal luteal function with increased apoptosis of luteal cells and extensive intra-luteal inhibition of angiogenesis (Grosdemouge *et al.*, 2003). This impaired luteal function results in inadequate progesterone production, which is mandatory for the establishment of early pregnancy. Indeed, the administration of progesterone in homozygous PRLR-deficient mice rescues the implanting blastocyst and pregnancies proceed, though they do not complete (Binart *et al.*, 2000).

The direct involvement of PRL signalling in reproduction can be viewed in detail through the specific defects in the process of oocyte maturation that are imposed on PRLR-deficient mice. These defects include reduction of oocyte maturation potential, characterized by inability to proceed further from the germinal vesicle stage, the occurrence of fragmented embryos and a decrease in the number of recovered embryos (Bole-Feysot *et al.*, 1998).

Supporting the necessity of prolactin signalling, in-vitro studies have shown that when administered at adequate concentrations, prolactin promotes the developmental potential of preimplantation mouse and rabbit embryos (Yohkaichiya *et al.*, 1988; Yoshimura *et al.*, 1989, 1991; Karabulut *et al.*, 1999). This direct positive effect of prolactin on preimplantation embryos signifies a functional prolactin signalling system in the primary steps of embryo development.

In order to assess the extent to which prolactin is implicated in the process of reproduction and early embryo development in mice, the expression of PRLR mRNA transcripts was examined using reverse transcriptase polymerase chain reaction (RT-PCR) in mice cumulus cells and nude oocytes in metaphase II, as well as in the stages of zygote, 2 cells, 4 cells, morula and blastocyst.

Materials and methods

Cell collection

Mouse cumulus cells, oocytes and preimplantation embryos were obtained from 6- to 10-week-old female mice [New Zealand Black (NZB) × New Zealand White (NZW); Pasteur Institute, Athens] after hormonal ovarian stimulation with i.p. injections of 5 IU equine chorionic gonadotrophin (eCG; Sigma-Aldrich, Dorset, UK) followed by administration of 5 IU human chorionic gonadotrophin (HCG; Sigma) 48 h later.

The mice were killed by cervical dislocation and the ovaries were removed and placed in Dulbecco's phosphate-buffered saline (DPBS; Invitrogen Life Technologies, Paisley, UK), supplemented with bovine serum albumin (BSA: 9:1) (Sigma). Oocytes were collected by puncturing the ovaries 15–17 h after HCG administration. Subsequently, 60 IU hyaluronidase/ml was added in the DPBS with BSA and after 2–3 min, cumulus cells were removed and collected for the preparation of the RNA. Hyaluronidase was removed by washing the cumulus-free oocytes twice in fresh medium.

Male and female mice were placed together overnight to mate. Female mice were killed by cervical dislocation 15–17 h after HCG administration. Oviducts were removed, washed in DPBS and after puncture, zygotes were collected. Two-cell embryos were flushed from the oviducts 34–40 h after HCG administration. Zygotes were cultured in modified Ham's culture medium without hypoxanthine (Loutradis *et al.*, 1994) (Invitrogen Life Technologies) and the culture conditions were adjusted at 37°C, 95% humidity, 5% CO_2 concentration and pH 7.3. At 42–46 h after the initiation of culture, 4-cell embryos were obtained, whereas morulae and blastocysts were collected 70 and 96 h after the beginning of culture respectively. The embryos were washed several times in DPBS with BSA prior to mRNA isolation.

RNA extraction

Total RNA was extracted from groups of 30–50 cumulus cells surrounding oocytes, and groups of 10–20 zygotes, 2-cell embryos, 4-cell embryos, morulae and blastocysts. RNA was also isolated from the cumulus cells that surround the oocytes. For total RNA extraction, a commercially available kit was used (RNAeasy micro kit; Qiagen, Valencia, CA, USA) according to manufacturers' instructions. The kit includes RNase-free DNase I and carrier RNA, offering highly purified RNA.

RT-PCR reaction

The RT reaction was performed by using a commercially available kit (Retroscript kit; Ambion, Austin, TX, USA) for cDNA synthesis in the presence (RT+) and absence (RT–) of reverse transcriptase. RT was followed by two rounds of nested PCR for PRL receptor mRNA and one round of PCR for GAPDH mRNA. Nested PCR is commonly used for situations involving very low quantities of extract RNA, as arises with oocytes and embryos. Two different pairs of primers (inner, outer) were used for PRL receptors and one pair for GAPDH in both PCR. Sequences of cDNA clones for mRNA have previously been deposited in GenBank (NCBI, Bethesda, MD, USA; GenBank accession number X73372 for the PRLRL, M22958 for PRLRS1, M22959 for PRLRS2, M22957 for PRLRS3). The primer sequences of PRL receptor mRNA used in both of the PCR were designed with the Primer 3 program (Rozen and Skaletsy, 1996, 1997). The primers used for GAPDH mRNA amplification were described by Foitzik *et al.* (2003). All primers were ordered from MWG Biotech (Ebersberg, Munich, Germany) (**Table 1**). The same outer and inner sense primers were used for PRLRS1, RS2 and RS3, which are in the common cytoplasmic domain to all forms, and different antisense primers, which are in the unique region of the cytoplasmic domain.

For the first PCR, 5 µl of 10× PCR buffer, 1.5 mmol $MgCl_2$/l, 0.2 µmol of 3′ and 5′ outer primer, 0.2 mmol of each dNTP/l and 1.5 IU *Taq* polymerase were used (Invitrogen Life Technologies). Into the first PCR mastermix, 3 µl of cDNA was added to a total volume of 50