ROLE OF GnRH AND ITS RECEPTOR IN REPRODUCTIVE BIOLOGY AND MEDICINE

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Introduction

Gonadotropin hormone-releasing hormone (GnRH) is a key regulator of mammalian reproduction.[1] After its release from the hypothalamus, this decapeptide is transported via the portal circulation to the anterior pituitary where it stimulates the synthesis and secretion of follicle-stimulating hormone (FSH) and luteinizing hormone (LH), two gonadotropins that play pivotal roles in the regulation of reproductive performance in both males and females. Numerous GnRH analogs are now being used in many areas of reproductive medicine, such as assisted reproduction. Recently, a distinct gene encoding a second form of GnRH, termed GnRH-II, to distinguish it from the classical mammalian form (GnRH-I), has been reported in the human and other primates.[2,3] GnRH-II, which was first identified in the chicken (cGNRH-II), has been identified in a wide variety of tissues in the human. To date, the biological function(s) of GnRH-II in the human have not been defined.

Methods and Materials

Human granulosa-luteal cells (hGLCs) were obtained from the UBC Human In Vitro Fertilization (IVF) Program. The use of these cells for in vitro studies were approved by the UBC Clinical Screening Committee for Research and Other Studies Involving Human Subjects. Unlike studies in animals in which granulosa cells from different stages of differentiation can be used to assess hormonal actions, most investigators of the human ovary have used highly differentiated granulosa cells obtained from women undergoing IVF. These cells are capable of undergoing spontaneous luteinization in vitro; they are commonly referred to as luteinized granulosa cells or granulosa-luteal cells. Since the regulation of ovarian hormone production can be relatively species-specific, hGLCs are recognized as a meritorious model for the study of the human ovarian function. Standard molecular biology methodologies and techniques were used in these studies as detailed in our publications.[4-9] Statistical significance of the data was determined by analysis of variance; dose-response curves and half-maximal effective doses (ED_{50}) were calculated using an iterative nonlinear least squares regression.

Results and Discussion

In addition to its role in the pituitary, GnRH has been implicated as an autocrine/paracrine regulator in several extrapituitary tissues, including the gonads.[10-13] In the human ovary, we have shown that GnRH directly inhibits progesterone production in granulosa-luteal cells.[4,5] We have recently reported the expression of both GnRH-I and GnRH-II, as well as their mutual receptor, in human granulosa-luteal cells.[4,6] Different signaling pathways, including coupling of the GnRHR to a pertussis toxin-insensitive G protein ($G_q/_{11\alpha}$), $G_{s\alpha}$ or $G_{i\alpha}$ may be activated by GnRH.[14-22]

The detection of two distinct forms of GnRH in human tissues suggests that multiple forms of the GnRHR exist. Indeed, two GnRHR subtypes with distinct ligand binding affinities and cellular distribution in the goldfish pituitary and brain have recently have identified.[23] Although a gene encoding a second form of GnRHR in the human has been reported, it is believed that this gene encodes an antisense mRNA transcript and that the type II GnRHR is vestigial in the human.[24] Thus, it is accepted that GnRH-I and GnRH-II interact with the same transmembrane G-protein-coupled receptor (i.e. type I GnRHR).

We have isolated and characterized the human GnRHR gene, which consists of three exons and two introns.[25] Exon I contains the 5' untranslated region and part of the open reading frame encompassing transmembrane domain (TM) I-III and a portion of TMIV. Exon II codes for part of TMIV and TMV, while Exon III encodes for the remainder of the open reading frame and the 3' untranslated region. The intron-exon organization of the human[26] and murine[27] GnRHR genes were found to be similar, but structure and length of the 5' untranslated regions as well as the 5' flanking regions are different, indicating that the GnRHR genes, among vertebrates, are regulated in different manners.

Our analysis of the 5' end of human GnRHR gene revealed the presence of five consensus TATA boxes residing in close proximity to one another in a cluster-like arrangement. Primer extension experiments revealed five transcription initiation sites.[26] The finding of multiple putative promoters suggests that the human GnRHR gene is complex and is highly regulated. In fact, we have recently reported the differential use of various regions of the 5'-flanking region of human GnRHR in regulating pituitary and ovarian expression.[28] In addition, a distal promoter was located immediately 5' to a minor CAP site at 1673 found in human pituitary[29], again showing the potential of differential regulation by using multiple promoter elements. The possibility of alternative use of promoters and

transcription start sites may explain the differences exhibited in GnRHR expression levels and hence different GnRH binding affinities detected in different tissues.

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