

The Regulation of GnRH Expression

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Gonadotropin-releasing hormone (GnRH), was originally isolated and characterized from mammalian hypothalamus, and is considered to be the prime regulator of reproduction. Research in lower vertebrates has demonstrated that at least two forms of GnRH are present in the brain of each species: one regulates reproduction, whereas the second form is extrahypothalamic and its functions are still unknown. Various studies have demonstrated that the hypothalamic GnRH form, which regulates reproduction, is species-specific whereas the second form (named GnRH-II) is identical in all vertebrates in which it is expressed.

We have found that GnRH-II is expressed in the brainstem of humans. We subsequently looked for human neuronal cell lines that express GnRH-II and GnRH-I and thus may serve as model systems for studies aimed at regulation of gene expression, secretion, etc. We found two human neuronal cell lines: the TE-671 medulloblastoma and LAN-1 neuroblastoma that express GnRH-I and GnRH-II.

In two subsequent studies we probed the differential transcriptional regulation of the two forms of GnRH in TE-671. We found that GnRH-II mRNA is strongly upregulated by a cAMP analog, and identified a cAMP response element (CRE) only in the hGnRH-II promoter. Using a luciferase reporter, we found that when the CRE was mutated, even the basal activity of the GnRH-II promoter was diminished. In another study we looked into the transcriptional regulation of the GnRH-I and GnRH-II genes by estrogen, and found that they are differentially regulated. Estrogen increases hGnRH-II mRNA but decreases hGnRH-I mRNA levels. Analysis of the GnRH-II promoter sequence revealed a perfect SP1 site and a partial putative estrogen response element in TE-671 cells.

We found that normal human breast tissue expresses the two forms of GnRH, and that they are

overexpressed in the cancerous tissues of the same patient. The mechanisms by which GnRH exerts its antiproliferative effects on breast and prostate cancer cells are not fully understood. We used the atlas human cDNA expression array and found that both GnRH-I and GnRH-II inhibit the expression of mRNA encoding the 60S acidic ribosomal phosphoproteins, P1 and P2 in MCF-7 cells. These results were confirmed by RT-PCR, Western blot analysis and immunofluorescence staining. The P1 and P2 proteins interact with elongation factors EF1 and EF2 and the level of their phosphorylation is one of the regulatory mechanisms of protein elongation. These studies, therefore, suggest a putative mechanism for the direct antiproliferative effects of GnRH in breast cancer cells.

In collaboration with Dr. Mia Levite, we found GnRH-II expression in normal and leukemic T-cells. We exposed a resting mouse T-cell line to GnRH-II, used a cDNA expression array and found that the neuropeptides induced the expression of several mRNAs, including that encoding the non-integrin, 67kD laminin receptor. We found that exposure of normal or leukemic human T-cells to either forms of GnRH triggers gene transcription as well as cell surface expression of the non-integrin laminin receptor. This effect triggers adhesion of the stimulated T-cells to laminin, chemotaxis toward the chemokine SDF-1 α and augmented in vivo entry of metastatic T-lymphoma into spleen and bone marrow. Moreover, in hpg mice which do not express GnRH-I, the homing of T-cells was significantly reduced. Cetrorelix, a specific GnRH-I antagonist, blocked the GnRH-I but not the GnRH-II-induced effects.

Over the years, it has become evident that GnRH-I and recently also GnRH-II, are produced not only in the brain but also in a variety of tissues and organs such as breast, prostate, pituitary gland, ovary, T-

cells, etc but their functions in these tissues are still elusive. The two GnRH forms are biologically active peptides that are found in minute quantities and have a very short life span. The GnRH forms may thus be considered as local hormones which act at the vicinity of their production sites by an autocrine/paracrine way. Once GnRH reach the general circulation it is vastly diluted to inactive concentrations and is further degraded. Thus, GnRH-I or GnRH-II that are expressed in certain organs, are practically functioning in an isolated paradigm and therefore different mechanisms of action may have evolved in different organs. Indeed, it is well established that GnRH-I is capable of activating almost all signal transduction pathways. As described above GnRH-I and GnRH-II may elicit similar or opposite responses. Likewise, the regulation of the two GnRH genes may be different also at the transcriptional level. thus, estrogen causes an increase in GnRH-I expression and a decrease in GnRH-II expression in MCF-7 (breast cancer cells), whereas it leads to oppsit effects on the expression of both neuropeptides in TE-671 (neuronal cells). The differential regulation of the expression of the two GnRH genes as well as their diverse functions may provide a unique mechanism for establishing a fine internal feedback that regulates a biological function.

To substantiate this hypothesis, recent studies in our laboratory have demonstrated that various cell lines differ in their ability to splice the GnRH mRNA and that stimulation of these cells result in differentially regulated expression of GnRH-I and GnRH-II mRNA. We also investigated the expression of GnRH-I in the hypothalamus, pituitary and ovary throughout the rat estrous cycle. We found different circadian expression patterns of GnRH-I mRNA in different organs, implying differential regulation of this neurohormone.

Selected Publications

- Chen A., Yahalom D., Laskar-Levy O., Ben-Aroya N. and Koch Y. (2001) Two isoforms of gonadotropin-releasing hormone are co-expressed in neuronal cell lines. *Endocrinology*. 142, 830-837.
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