Yitzhak Koch

Nurit Ben-Aroya
Alon Chen
Naomi Coslovsky
Yonatan Ganor
Shai Rahimipour
Tamar Schirman-Hildesheim

GnRH: A multifunctional neuropeptide

Department of Neurobiology

Tel. 972 8 934 2790 **Fax.** 972 8 934 4131
E-mail: y.koch@weizmann.ac.il

Gonadotropin-releasing hormone-I (GnRH-I) is a decapeptide that plays a pivotal role as the physiological regulator of mammalian reproduction. Today, more than a dozen forms of GnRH are known in lower vertebrates. One of these forms [His^5, Trp^7, Tyr^8] GnRH, named GnRH-II is expressed in all vertebrate classes. Lately (1998), we and two other research groups have demonstrated GnRH-II in the brain of several mammals. In contrast to GnRH-I, GnRH-II is localized mainly in the midbrain. The structural conservation of GnRH-II for over 500 million years of evolution suggests that its functions are of utmost importance.

Regulation of GnRH gene expression

We have demonstrated two human neuronal cell lines, TE-671 medulloblastoma and LAN-1 neuroblastoma cells, that coexpress mRNA encoding GnRH-I and GnRH-II. We used the TE-671 cell line to demonstrate regulation of the human GnRH-II gene by cAMP, and found that GnRH-II mRNA is strongly up-regulated (~6-fold) by (Bu)^{2}cAMP, and that more GnRH-II was released into the medium. TE-671 cells that were stimulated by (Bu)^{2}cAMP also demonstrated morphological changes. We identified a putative cAMP response element consensus site in the GnRH-II promoter. Treatment of transfected TE-671 cells with (Bu)^{2}cAMP resulted in a strong activation of the GnRH-II promoter compared with a modest activation of the GnRH-I promoter. To determine the functionality of this putative cAMP response element site, we mutated this site and demonstrated a diminished activity of the GnRH-II promoter.

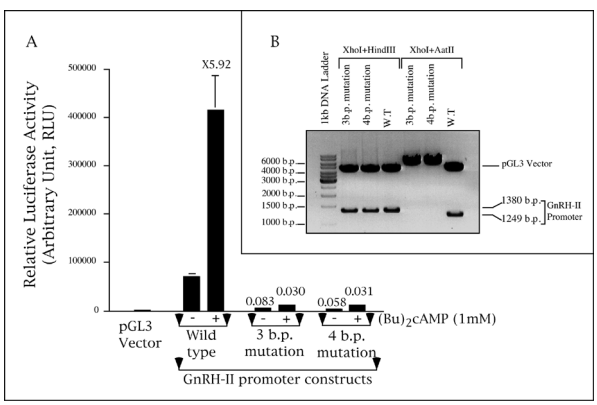

Fig. 1 *Mutations in the CRE site of the GnRH-II promoter. (A) Responsiveness to cAMP of the wild-type and the mutated promoters. (B) Note that mutations at the CRE site prevent recognition by the restriction enzyme Aat-II.*

Neuropeptides in breast

The presence of neuropeptides in the milk of several species, in concentrations that exceed those in maternal plasma, has been reported. These findings imply that milk is not just a nutrient source but also a carrier of substances that can play a role in the developmental physiology of the neonate. Recently our group has demonstrated the expression of the GnRH in the mammary gland of pregnant and lactating rats.

We examined the possibility that the genes of several neuropeptides (galanin, somatostatin, vasoactive intestinal peptide, TRH, GH-releasing hormone, cholecystokinin, neurotensin, oxytocin, and relaxin) are expressed in the rat mammary gland. RNA was extracted from the mammary glands of female rats during different stages of reproduction as well as from other tissues. Following RT reaction, the resulting cDNA were amplified by radioactive PCR using specific oligonucleotide primers. Among all the neuropeptides that were examined, somatostatin was the only one that was found to be expressed in the mammary gland of lactating rats. Somatostatin immunoreactivity was found in the epithelial cells that compose the secretory alveoli and in the secretory material. In addition, we have found that the mammary glands express the PC-1 proteinase gene that process prosomatostatin to generate somatostatin-14, but do not express furin, the enzyme that is responsible for somatostatin-28 production. This finding substantiates our previous studies that demonstrated that only somatostatin-14 is present in milk. The GnRH gene has been previously demonstrated to be expressed in the mammary gland, and in this study somatostatin was the only neuropeptide (out of ten) that was found to be produced by the mammary gland. The observation that only selected neuropeptides are being produced by the lactating mammary gland suggest that these neuropeptides have important functions in the biology of the suckling neonate and probably also in the development and function of the breast.

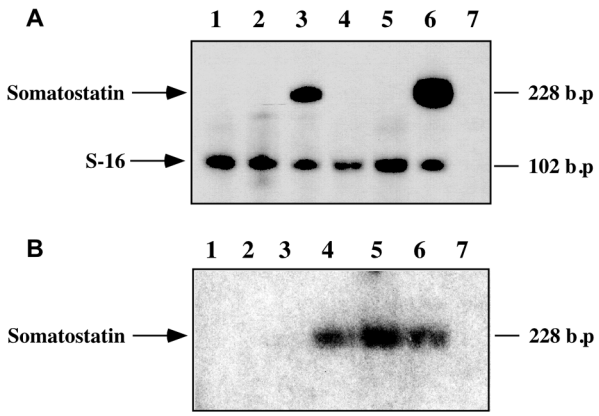

Fig. 2 *(A) Somatostatin (SOM) transcripts are present in the hypothalamus (lane 6) and in the mammary gland (MG) of lactating rats (lane 3), but not in the MG of rats in other physiological states. (B) PC-1, the enzyme that cleaves the prosomatostatin to generate SOM-14, is expressed not only in the small intestine (lane 3), but also in the MG of lactating rats (lane 2), whereas Furin that generates SOM-28 is not expressed in the MG.*

GnRH and cancer

Synthetic GnRH analogs, have attracted considerable scientific and industrial interest because of their application in the treatment of endocrine-based diseases (prostate and breast cancer), and their possible use as contraceptive pills. The mechanism of action of GnRH analogs in these diseases is believed to be related to estrogen deprivation due to pituitary desensitization as well as to the direct inhibitory effects of GnRH on cancer cell proliferation.

We synthesized the analog [D-Lys6(1,3,8-trihydroxy -6-carboxyanthraquinone)] GnRH ([D-Lys6(Emo)]GnRH) and found that it binds to GnRH receptors (IC50 = 0.25 nM), induces LH release (ED50 = 27 pM), and is devoid of any toxicity. This analog also proved to be a very potent agonist in vivo. Six hours after its administration to rats, serum LH concentrations were substantially higher than those of rats treated with a 10-fold higher dose of the parent peptide. Moreover, chronic treatment of adult male rats with this analog for one week resulted in decreased testes and prostate weight. The prolonged activity of [D-Lys6(Emo)]GnRH may be attributed to its emodic acid moiety which can be bound to human serum albumin.

Selected Publications

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Acknowledgements

Yitzhak Koch is the incumbent of the Adlai E. Stevenson III Professorial Chair of Endocrinology and Reproductive Biology. This research was supported by grants from the Israeli Science Foundation, GIF, and CaPCURE.