Special Lecture I

GnRH and its regulation

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Hypothalamic gonadotropin-releasing hormone (GnRH) is a major central regulator for controling gonadotropin secretion from the anterior pituitary. The regulation of GnRH secretion is known to be profoundly influenced by steroids and neurotransmitters. Little is, however, known about their actions on GnRH gene expression. We analyzed the regulation of GnRH gene expression in the hypothalamic tissues in vivo and immortalized GT1-1 neuronal cells in vitro: 1) With competitive RT-PCR procedure, we studied the effects of steroids on GnRH and its receptor mRNA levels in discrete hypothalamic nuclei, such as the preoptic area (POA) and mediobasal hypothalamus (MBH) micropunched from the brain slices of individual rat. Treatment with estrogen (E) for 2 days to ovariectomized (OVX) rats clearly down-regulated the GnRH mRNA level in the POA, and administration of progesterone (P) to OVX+E rats unequivocally up-regulated both GnRH mRNA level in the POA and GnRH receptor mRNA level in the pituitary, when examined at 17:00 h on the day of P treatment. Changes in GnRH receptor mRNA levels in the MBH were differential: E up-regulated, but P substantially down-regulated E-induced GnRH receptor mRNA level. This finding raises the possibility that there is a reciprocal relationship between GnRH neurons and GnRH receptor-containing neurons (or glial cells) which are yet unidentified. 2) We examined the effect of GABA on GnRH and GnRH receptor mRNA levels in micropunched POA and MBH brain slices derived from OVX rats. A single intracerebroventricular injection of muscimol (10 pmol) markedly reduced serum LH level, while baclofen (10 pmol) failed to alter serum LH level. Activation of GABA-A receptor with muscimol clearly reduced GnRH mRNA level in the POA and GnRH receptor mRNA levels in the POA and MBH. No such change was observed when GABA-B receptor was activated with baclofen. These data clearly indicate that GABAergic neurotransmission negatively regulates GnRH gene expression in the hypothalamus through GABA-A type receptor. It appears that GABA presynaptically regulates GnRH neurons at the level of the MBH. 3) To elucidate the autocrine role of GnRH, we took advantage of the immortalized GnRH neuronal cell line (GT1-1). We analyzed intracellular calcium levels, [Ca]i at a single GT1-1 cell level loaded with Fura-2AM fluorescent dye. GT1-1 cells (about 50%) displayed spontaneous rhythmic oscillation with a periodity ranging from 2 to 60 sec. Application of buserelin, a superactive GnRH agonist, or

GnRH readily abolished rhythmic oscillation of [Ca]i in a dose-dependent manner. Prior treatment of antide, a GnRH antagonist, reinstated [Ca]i oscillation by nullifying buserelin-induced inhibition of [Ca]i. To determine whether such an autocrine mechanism may operate at the level of GnRH gene transcription, we employed a transfection-based transcriptional assay where GT1 cells were transfected with a 3kb of the rat GnRH promoter-derived luciferase reporter gene. Treatment of GT1 cells with native GnRH (10 μM) or buserelin (1 μM) reduced GnRH promoter-directed luciferease activity by half, while GnRH antagonist, antide (10 μM) failed to alter luciferease activity. Buserelin-induced decrease in GnRH promoter activity was in a dose- and time-related manner, which well correlated with changes in GnRH mRNA levels. Experiment with serial deletion constructs from -3.0 Kb up to -0.5 Kb of the 5' flanking sequence of the GnRH promoter showed that a putative buserelin response element may reside within -500 bp. It appears clear that autocrine inhibition of GnRH exerts, in part, at the level of transcription. Furthermore we found that post-transcriptional processing and translational efficiency are also important for autocrine regulation of GnRH in GT1-1 cells. Taken together, these results may provide an insight into the molecular mechanism underlying neuroendocrine regulation of GnRH neurons in the hypothalamus.