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Neuroendocrine Integration of Hypothalamic GnRH Neurons

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Hypothalamic gonadotropin-releasing hormone (GnRH) is a pivotal regulator for controling gonadotropin secretion from the anterior pituitary. The regulation of GnRH secretion is known to be profoundly influenced by steroid feedback inputs and central 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) We examined the effect 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 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 hr on the day of P treatment. Change in GnRH receptor mRNA level in the MBH was differential: E clearly up-regulated, but P substantially down-regulated E-induced GnRH receptor mRNA levels. 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) To elucidate the autocrine role of GnRH, we analyzed the intracellular calcium levels, $[Ca^{2+}]i$ in immortalized GT1-1 cells 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 $[Ca^{2+}]i$ in a dose-dependent manner. Prior treatment of antide, a GnRH antagonist, reinstated $[Ca^{2+}]i$ oscillation by nullifying buserelin-induced inhibition of $[Ca^{2+}]i$. The autocrine regulation of GnRH also operated at the

levels of GnRH transcription and translational efficiency. 3) In vitro treatment of GT1-1 cells with 12-O-tetradecanoyl phorbol-13-acetate (TPA) induced neurite outgrowth, but suppressed cell proliferation. A prior treatment of calphostin C, a specific protein kinase C inhibitor, blocked the neurite outgrowth indicating that protein kinase C signal pathway participates in the neurite outgrowth and differentiation. We previously reported that GABA is able to up-regulate $[Ca^{2+}]i$ in intact GT1-1 cells. It is then of interest to test how GABA modulates $[Ca^{2+}]i$ level in the case of TPA-induced neurite outgrown cells. Confocal calcium imaging analysis revealed that GABA-induced $[Ca^{2+}]i$ was reversed from excitatory to inhibitory in TPA-induced differentiated GT1-1 cells, suggesting the functional plasticity of GnRH neurons. With TPA-induced differentiation model we analyzed several genes which are specifically involved in the differentiation and/or proliferation processes using differential display PCR procedures. 4) A series of Northern blot analysis and RT-PCR showed that GnRH primary transcripts and its splicing intermediates were expressed in a variety of tissues, but the mature GnRH mRNA was detected only in the GnRH-producing cells including the preoptic area. In vitro splicing study using Hela nuclear extract indicates that introns B and C, but not intron A were efficiently spliced. There exists the splicing enhancer sequences located in exon III and exon IV. It appears that excision of intron A from the primary transcript was a prerequisite for the mature GnRH mRNA synthesis. Collectively these results may provide an insight into the molecular mechanism underlying neuroendocrine regulation of GnRH neurons in the hypothalamus.