D109

## Cloning and Characterization of cDNAs Encoding Mammalian Gonadotropin-Releasing Hormone (GnRH) and Chicken GnRH-II Precursors from the Bullfrog (*Rana catesbeiana*)

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We have isolated the cDNAs, encoding the mammalian gonadotropin-releasing hormone (mGnRH) and chicken gonadotropin-releasing hormone II (cGnRH-II) precursors, repectively, from bullfrog (Rana catesbeiana) brain. The first cDNA consists of 652 bp, containing an open-reading frame of 270 nucleotides, that codes for the bullfrog mGnRH precursor. The second cDNA consists of 1075 bp, containing an open-reading frame of 255 nucleotides, that codes for the bullfrog cGnRH-II precursor. In addition, we have identified another cDNA, representing an alternative splice variant of the bullfrog cGnRH-II precursor mRNA, containing an additional 24 nucleotides in the GnRH-associated peptide (GAP) coding region that increases the length of the cGnRH-II-associated peptide from 48 to 56 amino acids. All bullfrog GnRH precursors have a similar molecular architecture as observed in other GnRH precursors, consisting of a signal peptide, followed by the GnRH decapeptide, a conserved carboxy-terminal amidation and proteolytical processing site, and the GAP. The amino acid identity of the bullfrog mGnRH precursor is 60% and less than 40% with the mGnRH precursors of Xenopus and mammals, respectively. The bullfrog cGnRH-II precursor shares 50% to 60% amino acid identity with that of its nonmammalian counterparts, but shares only 25% with that of its mammalian counterparts. Northern blot analysis revealed a single mGnRH precursor mRNA species of ~0.6 kilobases, expressed only in bullfrog forebrain, and a single cGnRH-II precursor mRNA species of ~1.2 kilobases, expressed only in bullfrog hindbrain. Genomic Southern blot analysis revealed that both bullfrog GnRH genes are present as single copy gene. This is the first report on the molecular cloning of the cGnRH-II precursor cDNA from an amphibian.

D110

## Ecdysone and Serotonin Stimulate Neurite Outgrowth of Differenting Neurons in the Deutocerebrum of the Silk Moth *Bombyx mori*

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We performed culture of differenting deutocerebral neurons dissociated from 5-stage pupae of the silk moth *Bombyx mori*. The primary cultures leaded to classification of these neurons into several morphological types, including monopolar, bipolar and multipolar cells. In this primary culture, most of these cells survive for about two weeks. We also examined the effects of 20-hydroxyecdysone (20-HE) and 5-hydroxytryptamine (5-HT, serotonin) to stimulate neurite outgrowth of the differentiating deutocerebral neurons. Our results showed that both 20-HE and 5-HT stimulate to increase lengthy extension and number of process brances from the cell bodies. Whereas the 20-HE stimulates remarkable increase of neurite outgrowth and also decreases survival days of these neuronal cells, the 5-HT stimulates a little increase of neurite outgrowth and does not affect the survival of those neurons.