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Kisspeptin, GPR54, and GnRH neurons: Interactions in the male mouse

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A novel neuropeptide, kisspeptin, binds to the receptor GPR54, to influence the initiation and regulation of puberty in mammals. It appears that the activation of GPR54 stimulates the release of gonadotropin releasing hormone (GnRH) from specialized neurons, resulting in the activation of the reproductive system. To further understand the role of the kisspeptin system in GnRH secretion, levels of GPR54 and GnRH mRNA were compared in hypothalamic tissue before, during, and after puberty in male mice. Transgenic male mice that express green fluorescent protein (GFP) in their GnRH neurons were used at 20 (prepubertal), 30 (pubertal) and 60 (postpubertal) days postnatal. RNA was isolated from brain areas known to contain GnRH neurons. Isolated RNA was reverse transcribed and will be subjected to real-time, quantitative PCR. Relative amounts of mRNA from GPR54 and GnRH will be compared with a housekeeping gene, RPII using standard curves at each of the three ages. Currently, we have used real-time PCR to detect amplicons, of approximately 60 base pairs, for each group down to the attogram (10⁻¹⁸) level. We have also determined that the ABI 7000 machine and SYBR-green as a detector are optimal for our purposes. We are currently performing PCRs for each of the three genes of interest. Amounts of GPR54 and GnRH in older animals will be expressed relative to amounts from the 20 day postnatal animals. Similar techniques will be used to study the expression of GPR54 and GnRH mRNA at the level of the single GnRH neuron, as well as the effect of steroids on the system.