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Distribution of Kappa-Opioid Receptor (KOR) mRNA in the Hypothalamus of Female Rats: An Analysis on KOR Functions in the GnRH Pulse Generation

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Pulsatile GnRH release is essential for the maintenance of fertility in mammalian species. Previous experiments have shown the possibility of hypothalamic arcuate nucleus (ARC) to be equipped with the GnRH pulse generator. In particular, ARC neurons expressing kisspeptin, neuronkinin B (NKB) and dynorphin (Dyn) are called KNDy neurons, which are considered to play a key role in generating GnRH pulses. Dyn has been reported to inhibit GnRH/LH release or hypothalamic MUA that might be the GnRH pulse generator activity. The peptide, therefore, could be involved in mediating negative feedback action of steroids or pulse generation. Kappa opioid receptor (KOR) is the receptor for Dyn, so that clarification of KOR neuron character is crucial to understand how Dyn-KOR cascade is working to generate GnRH pulses. The aim of the present study is to answer the following 3 questions: 1) Where are KOR neurons located; 2) What is the function of KOR neurons; and 3) What is the character of KOR neurons. To answer the first question, KOR mRNA levels were quantitatively determined by the real-time RT-PCR in several hypothalamic areas including the anteroventral periventricular nucleus (AVPV), preoptic area (POA), paraventricular nucleus (PVN), ventromedial hypothalamic nucleus (VMH), lateral hypothalamic area (LHA) and ARC. Expression of the KOR mRNA is highest in the PVN, followed by POA. In cortex, cerebellum or pituitary, KOR mRNA is nearly undetectable. In the ARC, our second strategy is to visualize KOR protein by transgenic rats. The sequence of fluorescence protein, venus, is now being inserted to the rat genome together with Oprk1 (KOR gene) promoter. We would determine the distribution of KOR neurons by venus fluorescence in the transgenic rats. We decided to take this strategy to determine the KOR neurons in the brain because KOR is a G-protein-coupled receptor (GPCR), which share sequence homology with other GPCRs. It, therefore, is hard to distinguish KOR from other GPCRs with immunohistochemistry with an antibody. The experiment is now going on to have a good construct to be injected to rat fertilized eggs.