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Isolation and characterization of neurons with a gonadotropin-releasing hormone (GnRH) phenotype from human foetal hypothalamus

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GnRH neurons are a peculiar hypothalamic subpopulation crucially involved in the control of the reproductive axis. As well established in several animal models, the kisspeptin (KISS1)/KISS1 receptor (KSS1R) system plays a master role in the control of GnRH neurons, however, investigations in humans are strongly hampered by the anatomical distribution of these neurons, scattered within the preoptic area of the hypothalamus. This study was aimed at establishing a human hypothalamic primary cell culture with GnRH neuron features. Brains were recovered from 11-12 week-old human fetuses, then hypothalamic tissue, lining the 3rd ventricle, was dissected and processed for cell culture isolation. The primary cultures obtained were first characterized using flow cytometry and showed a mixed composition with the majority of cells (92±8 %) positive for the neuronal marker MAP2 and a low percentage of cells positive for the glial marker GFAP (13.5±9 %). Interestingly, among the neuronal population about 80% were GnRH-positive cells (77.8±20 %). Gene expression profiling, immunofluorescence and western blot analyses confirmed that these cells expressed GnRH, as well as KISS1R. Hence, electrophisiological studies were performed to investigate if these cells responded to kisspeptin (Kp) stimulation. Using the voltage-clamp technique, we found that Kp (100nM or 1μM) induced a clear depolarizing response. Moreover, depolarizing effects of Kp involved transient receptor potential channels (TRPC), as expected by KISS1R activation (1). This is the first human hypothalamic cellular model with a GnRH neuron phenotype, representing a new tool for the investigation of human GnRH neuron biology.

References

[1] Zhang C et al. (2008) Kisspeptin depolarizes gonadotropin-releasing hormone neurons through activation of TRPC-Like cationic channels. J Neurosci 28:4423-34.

Keywords
Human hypothalamic cells, KISS1/KISS1R signaling, GnRH neuron electrophysiology.