主論文の要約

Histological analysis for neuronal pathway mediating energetic regulation of the reproductive function

(栄養による生殖機能制御を担う神経経路の組織学的解析)

By

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Energy deficiency causes reproductive dysfunction in several mammals. For example, chronic food restriction delays the onset of puberty and causes cessation of reproductive cycles in females of several mammalian species including human, sheep, cattle, pig and rats. It has been considered that the energy substance, such as blood glucose was sensed in certain cells for regulating reproductive function and food intake in the several mammals. The ependymocytes of the hindbrain were considered as one of the energy censor cells to regulate gonadotropin secretion. They have pancreatic glucokinase (GK) and glucose transporter (GLUT) 2, which are key factors for glucose sensing, and directly respond to the changes in extracellular glucose levels in vitro. The fourth ventricular (4V) injection of the 2-deoxy-D-glucose (2DG), a glucose metabolism inhibitor and alloxan, a GK inhibitor suppressed pulsatile luteinizing hormone (LH) releases in the rats. It is likely that noradrenergic (NA) neurons and/or corticotrophin-releasing hormone (CRH) neurons mediate the mechanisms relaying the low energetic signals to kisspeptin neurons in the hypothalamic arcuate nucleus (ARC) and anteroventral-periventricular nucleus (AVPV) to regulate GnRH/LH pulse and surge. The ARC kisspeptin neurons, co-expressing neurokinin B (NKB) and dynorphin A, are referred to as KNDy neurons, and are postulated to play an essential role in GnRH/LH pulsatile secretion. Therefore, this study hypothesized that the ependymocytes have connection with the ARC and AVPV kisspeptin neurons to relay the energetic signals for control LH pulse and surge via NA neurons and CRH neurons. In this dissertation, two experiments are described in Chapter 3 and 4.

The aim of Chapter 3 is to elucidate the neuronal connection of ependymocytes to the ARC and AVPV kisspeptin neurons via NA neurons and CRH neurons. Wheat-germ agglutinin (WGA), a trans synaptic tracer, is injected into the fourth ventricle (4V) of the adult female heterozygous *Kiss1*-tdTomato rats to investigate neuronal pathway originating from hindbrain ependymocytes. The WGA immunoreactivities were also found in

tdTomato positive kisspeptin neurons in the ARC and AVPV. The WGA immunoreactivities were co-localized with dopamine β-hydroxylase (DBH), a marker of NA neurons (A1-A7) and CRH immunoreactivities in the PVN and SON in the WGA-injected rats. The vimentin-positive ependymal fibers in the medulla oblongata crossed with DBH-positive dendrite. These results suggest that the hindbrain ependymocytes have neuronal connections with the kisspeptin neurons, most probably via hindbrain noradrenergic and CRH neurons to relay low energetic signals for regulation of reproduction.

In Chapter 4, purinergic cells in the hindbrain of the adult female rat were investigated, because 4V injection of a purinergic receptor antagonist, pyridoxal-phosphate-6-azophenyl-2',4'-disulphonic acid (PPADS), suppressed LH pulses in the OVX+Low E2 rats (dissertation of Mika Kinoshita 2004). In Chapter 4, immunohistochemistry of P2X2 receptor and vesicular nucleotide transporter (VNUT), a marker for ATP release, were performed to detect ATP releasing and/or receiving cells in the hindbrain. VNUT immunoreactivities clearly merged with DBH immunoreactivities in the area postrema, A2 and A6 region, but few VNUT immunoreactivity was found in the hindbrain ependymocytes. P2X2 immunoreactivities were found in the DBH immunopositive cells in the area postrema and fivers in the nucleus of solitary tract (NTS). Thus, taken together, these findings suggested that hindbrain NA neurons in the area postrema, A2 and A6 region released ATP for control of LH pulses.

In conclusion, these findings suggested that the hindbrain ependymocytes have the connection with the ARC and AVPV kisspeptin neurons for relaying energetic signals to suppress LH pulse and surge via NA and CRH neuronal pathway. Therefore, this dissertation suggested that hindbrain purinergic signaling from NA neurons in the area postrema, A2 and A6 region is involved in control of LH pulses and blood glucose levels via HPA axis.