主論文の要約

The role of hypothalamic arcuate nucleus KNDy neurons in control of reproduction in cows

（ウシの繁殖制御における視床下部弓状核 KNDy ニューロンの役割）

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The importance of cow production efficiency is increasing all over the world to meet our increasing demands for animal protein and milk. In Egypt, the total number of cows in 2015 was 6.5 million heads. Egyptians prefer beef to other types of meat including poultry and lamb. Beef production in Egypt, as an example for the subtropical developing countries, is not sufficient for the increasing population with more than 90 million in 2016. Many factors are contributed to low productivity of cows. Anovulatory disorders are considered to be the main problem that leads to low fertility that negatively affects the productivity of the breeding cows. Understanding the physiological mechanisms involved in these reproductive disorders is essential to improve the current therapeutic protocols used to treat infertility in cows.

In general, reproduction is controlled by hypothalamo-hypophyseal-gonadal axis in mammals. Gonadotropin-releasing hormone (GnRH) released from the hypothalamus into the portal vein stimulates the release of two gonadotropins from the pituitary gland: luteinizing hormone (LH) and follicle-stimulating hormone (FSH). The pulse and surge modes of GnRH release are reflected to the peripheral LH level to form pulse and surge modes of LH secretion, respectively. Pulse and surge modes of GnRH/LH release play pivotal roles to stimulate the follicular development, steroidogenesis, and ovulation. In farm animals, especially in cows, understanding the central neuroendocrine mechanisms that regulate follicular development through GnRH/gonadotropin release is a fundamental issue to establish more appropriate protocols that stimulate the follicular development and ovulation. Hormonal treatment that stimulates the endogenous release of GnRH/gonadotropin is one of the possible therapeutic approaches to solve the infertility caused by anovulation in cows.

Kisspeptin, a peptide hormone encoded by the *KISS1* gene, act as the main regulator

of the reproductive axis by stimulating GnRH neuronal activity. Kisspeptin induces GnRH release via stimulation of kisspeptin receptor, G protein-coupled receptor 54 (GPR54), expressed on the GnRH neurons, and GnRH subsequently stimulates gonadotropins secretion. In rodents and small ruminants, two populations of kisspeptin neurons were identified in the hypothalamus. One is a caudal population that is located in the hypothalamic arcuate nucleus (ARC) and the other is a rostral one that is differ among species: in the preoptic area (POA) in sheep, goats, and primates, in the anteroventral periventricular nucleus in rodents and in the periventricular nucleus in pigs.

In mammals, such as rodents and small ruminants, it is reported that two other neuropeptides, neurokinin B (NKB) and dynorphin A (Dyn) are expressed in the ARC kisspeptin neurons. Neurokinin B has a stimulatory effect while Dyn has an inhibitory effect on the pulse mode of GnRH release in small ruminants. These neurons are recently known as kisspeptin/NKB/Dyn (KNDy) neurons. In goats and rats, the KNDy neurons are suggested to be the intrinsic source of the brain mechanism controlling periodic activation of GnRH neurons, and therefore, the center controlling the follicular development via stimulation of pulsatile gonadotropin release. On the other hand, it is accepted that POA kisspeptin neurons has an essential role in controlling surge mode of GnRH release in small ruminants, monkeys and rodents. The surge mode of GnRH release stimulates the preovulatory LH surge required for ovulation.

In chapter 3 of the present dissertation, the first aim is to characterize the kisspeptin neurons in the hypothalamus of cows, and to identify the possible physiological role(s) of these kisspeptin neuronal populations. Kisspeptin-, NKB- and Dyn-containing

neurons in the bovine hypothalamus were immunohistochemically visualized. Kisspeptin neuronal cell bodies were identified in the ARC and POA. In the ARC kisspeptin neurons, either NKB or Dyn was co-localized in both the follicular and luteal phases. These results indicate the presence of KNDy neurons in the bovine ARC. Determining KNDy neurons through different phases of the estrous cycle suggests that this neuronal set is functional as a part of the GnRH pulse generator in cows. In the POA kisspeptin neuronal population, no co-localization with NKB and Dyn was detected. Interestingly, kisspeptin expressions increased in the POA during the follicular phase compared to the luteal phase, suggesting that the POA kisspeptin neurons play an important role in generation of surge mode of GnRH release in cows.

In chapter 4, to identify the role of NKB/NK3R (neurokinin-3 receptor) signaling in the regulation of GnRH release, senktide, a potent and highly selective NK3R agonist, was administrated peripherally. Continuous intravenous administration of senktide at high dose (300 nmol/min) for 2 h successfully stimulated the follicular development and gonadotropin secretion in cows. Treatment with low dose (30 nmol/min) of senktide or vehicle infusion has little to no effect on the gonadotropin secretion and follicular development. Treatment with high dose of senktide enhanced the time of ovulation in 2 out 4 cows treated in the follicular phase. None and one out of four animals treated with low dose of senktide and vehicle, respectively, showed an early ovulation in the follicular phase. Stimulation of follicular development and ovulation by continuous intravenous administration of senktide in the present chapter gives some advantages for the clinical use of this novel drug or other NK3R agonists. Senktide would be useful to treat infertility caused by anovulatory conditions in cows via stimulation of the endogenous release of GnRH/gonadotropin, and subsequent enhancement of the ovarian

functions.

In summary, this dissertation demonstrated that 1) Kisspeptin neurons are expressed in two hypothalamic regions in cows: the ARC and the POA; 2) Neurokinin B and Dyn are expressed in the ARC kisspeptin neurons in both the follicular and luteal phases; 3) In the POA kisspeptin neurons, NKB and Dyn were not expressed and the kisspeptin expression in the follicular phase was higher than in the luteal phase; and 4) Continuous peripheral administration of senktide, an NK3R agonist, stimulated gonadotropin secretion, follicular development, and ovulation in cows. Taken together, these findings suggest that KNDy neurons in the ARC are a part of GnRH pulse generator in cows. Kisspeptin neurons in the POA would be a generator of surge-mode GnRH release in cows. Senktide can be used in the veterinary field as a novel drug for treatment of cows suffered from anovulatory infertility problems. Particularly, the ARC KNDy and POA kisspeptin neurons would be potential therapeutic targets that should be investigated to overcome the anovulatory conditions in cows.