主論文の要約

Brain mechanism regulating puberty onset in female rats

(性成熟を制御する中枢メカニズム)

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In recent years, the consumption of animal product such as meat, milk and related products rapidly increases because of the increase in world populations and economic growth in developing countries. Consequently, the number of livestock increases in the modern farm in order to meet the demands. On the other hand, livestock farming is facing several problems such as the late onset of puberty and poor estrus expression, or silent heat, which lead to a decrease in the lifetime productivity of livestock. Thus, the timing of puberty is a significant reproductive trait in livestock and the mechanism underlying puberty onset is worth to study. The present study focuses on a central mechanism controlling puberty onset in female mammals.

Increasing evidence shows that puberty onset is largely dependent on body weight rather than chronological age. The present study aimed to determine central mechanism controlling puberty onset in rats under the food manipulation, because energetic cues regulate body growth and puberty onset. Puberty onset is considered to be timed by an increase in pulsatile gonadotropin-releasing hormone (GnRH)/gonadotropin secretion. Currently, it becomes widely accepted that kisspeptin/neurokinin B/dynorphin A-expressing neurons (KNDy neurons), located in the arcuate nucleus of the hypothalamus (ARC) play a crucial role in controlling pulsatile secretion of GnRH/gonadotropin that leads to puberty onset and reproductive function. GnRH/gonadotropin secretion is strongly suppressed by ovarian estrogen in females during the prepubertal period. Nevertheless, to date, the central mechanism controlling pubertal onset still remains elusive.

The first part of the present study investigated the mechanism involved in the energetic control of puberty onset. To this end, I examined the effects of chronic food restriction during the prepubertal period and the resumption of *ad libitum* feeding for 24 and 48 h on estrous cyclicity, *Kiss1* (kisspeptin gene), *Tac3* (neurokinin B gene) and *Pdyn* (dynorphin A gene) expression in the hypothalamus, luteinizing hormone (LH) secretion and follicular

development in female rats. When animals weighed 75 g, they were subjected to a restricted feeding to retard growth to 70-80 g by 49 days of age. Then, animals were subjected to *ad libitum* feeding or remained food-restricted. The growth-retarded rats did not show puberty onset associated with suppression of both *Kiss1* and *Pdyn* expression in the ARC. 24-h *ad libitum* feeding increased tonic LH secretion and the number of Graafian and non-Graafian tertiary follicles with an increase in the numbers of ARC *Kiss1*- and *Pdyn*-expressing cells. 48-h *ad libitum* feeding induced the vaginal proestrus and a surge-like LH increase with an increase in *Kiss1*-expressing cells in the anteroventral periventricular nucleus (AVPV). These results suggest that the negative energy balance causes pubertal failure with suppression of ARC *Kiss1* and *Pdyn* expression and then subsequent gonadotropin release and ovarian function, while the positive energetic cues trigger puberty onset via an increase in ARC *Kiss1* and *Pdyn* expression and thus gonadotropin secretion and follicular development in female rats.

The second part of the present study investigated the effects of estrogen on *Kiss1*, *Tac3* and *Pdyn* expression in the ARC and AVPV of chronic growth-retarded female rats. The growth-retarded rats were ovariectomized (OVX) and half of them were treated with estradiol (OVX+E2). After the 5-day recovery period, half of the animals were subjected to *ad libitum* feeding for 24 h or the other half remained food-restricted. In ovary-intact and OVX+E2 growth-retarded rats, both ARC *Kiss1* and *Pdyn* expression was suppressed under the food restriction and was up-regulated by the resumption of *ad libitum* feeding. On the other hand, ARC *Kiss1* expression was up-regulated in both food-restricted and *ad libitum* feeding conditions, whereas ARC *Pdyn* expression was suppressed under the food restriction and was up-regulated by the resumption of *ad libitum* feeding in OVX rats. In summary, the present study suggests that underlying mechanisms of prepubertal restraint of ARC *Kiss1* and *Pdyn* expression under the negative energy balance differ from each other: ARC *Kiss1* expression is suppressed by negative energy balance in an estrogen-dependent manner, whereas ARC *Pdyn*

expression is suppressed by negative energy balance in an estrogen-independent manner in female rats.

The last part of the present study tested whether recovery of *Kiss1* gene expression by using an adeno-associated virus (AAV) vector could rescue the *Kiss1* knockout (KO) rats from the ovarian quiescence. The *Kiss1* KO rats received AAV-*Kiss1* exhibited follicular development from the secondary to the tertiary stage in the ovary. Follicular cysts, but not corpora lutea, were detected in the ovary, because of the lack of LH surge in *Kiss1* KO rats. These results suggest that kisspeptin biosynthesis in the ARC KNDy neurons is fundamental to initiate follicular development in female rats.

Taken together, these results obtained from the present study suggest that the completion of ARC KNDy gene expression by an increase in *Kiss1* and *Pdyn* expression controlling GnRH/gonadotrophin pulses to trigger puberty onset, and that estrogen plays a key role in prepubertal suppression of *Kiss1*, but not *Pdyn*, in the ARC KNDy neurons of female rats. The present study showed energetic cues rather than chronological ages to define the puberty onset via affecting the ARC *Kiss1* and *Pdyn* expression and the ARC KNDy neurons are particularly important to induce pulsatile GnRH/gonadotropin release to promote the follicular development and steroidogenesis that causes puberty onset and subsequently LH surge and ovulation.