学　位　論　文　要　旨

Expression of Gonadotropin releasing hormone (GnRH), metastin and related peptides in the ovary: dynamic changes and their regulation during estrous cycle of rats

卵巣における性腺刺激ホルモン放出ホルモン（GnRH）とメタスチン及びその関連ペプチドの発現：ラット性周期中の発現変動とその調節機序

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1. Introduction: The reproductive cycle of mammalian females consists of recurring physiological changes induced by reproductive hormones. Among the various hormones, Gonadotropin-releasing hormone (GnRH, luteinizing hormone releasing hormone) is called the master hormone of reproduction. GnRH stimulates the secretion of gonadotropins (luteinizing hormone, LH and follicle stimulating hormone, FSH) at pituitary gonadotropes. LH and FSH, in turn, initiate folliculogenesis, steroidogenesis and ovulation. Recently, metastin, also called kisspeptin, the product of KISS-1 gene acting via G protein-coupled receptor 54 (GPR54), has been reported to stimulate GnRH secretion in the hypothalamus. Beside the long range control by gonadotropins, local regulation factors in the ovary are also involved in the recurrence of the cycle. Interestingly, GnRH is also synthesized in peripheral tissues, including the ovary. It was shown to cause corpus luteum regression and a relationship with follicular atresia was suggested. Our laboratory recently observed that mast cells in the mammary tissues contain GnRH immunoreactivity and that GnRH facilitates mammary involution after lactation. These results suggest that mast cells are a common producer of GnRH in various tissues. Although GnRH is expressed in the ovary, so far it is not known how the expression of GnRH is controlled and which cells produce GnRH. In the present study, changes in the GnRH expression, mast cells, metastin, and related peptides were examined in rats.

2. Variation in Ovarian GnRH: GnRH mRNA expression rate was measured through the estrous cycle of rats by real-time RT-PCR using whole ovary RNA. Estrous cycle of rats consists of diestrous 1, 2, proestrous and estrous days. There were two peaks in the variation of GnRH mRNA expression rate. One

was at 20:00 h of diestrus 2 and another was at 20:00 h of proestrus. These changes were demonstrated to be specific to the estrous cycle. The source of GnRH in the ovary was examined by means of Laser Microdissection (LMD). Corpora lutea, follicles and interstitial tissues were collected with LMD at 20:00 h of proestrus and RNA was extracted. The expression rate of GnRH mRNA was not different among these compartments. GnRH immunoreactivity was detected in all compartments of the ovary. In the granulosa layer and corpus luteum, GnRH positive cells were restricted to only a portion of the tissues. Interstitial tissues were stained diffusely, but mast cells were very strongly positive. Mast cell production of GnRH mRNA was confirmed by peritoneal mast cells. Mast cells stained with toluidine blue distributed to mainly interstitial tissues. The number of mast cells in the ovary varied during the estrous cycle with two distinct peaks in the evening of diestrus 2 and late afternoon of proestrus. The number of mast cells remained low during luteal phases, e.g. pseudopregnancy, pregnancy and lactation, and it was suppressed by prolactin administration. This variation during the estrous cycle matches with the changes in GnRH mRNA content. Because GnRH agonist (200 ng/50 µl) given into the hemi-lateral ovarian bursa increased the number of ovarian mast cells, GnRH would be a chemo-attractant for mast cell migration to the ovary. When peritoneal mast cells were incubated with GnRH agonist for three hours, the expression of GnRH mRNA was augmented. These data suggest that GnRH stimulates both the GnRH production of mast cells and the migration of mast cells into the ovary. C57BL/6-W^{sh}/W^{sh} is a mutant allele at the mouse W (*c-kit*) locus. Mice carrying this allele lack mast cells. The length of estrous cycle of C57BL/6J mice and C57BL/6-W^{sh}/W^{sh} mice is the same. Ovary weight

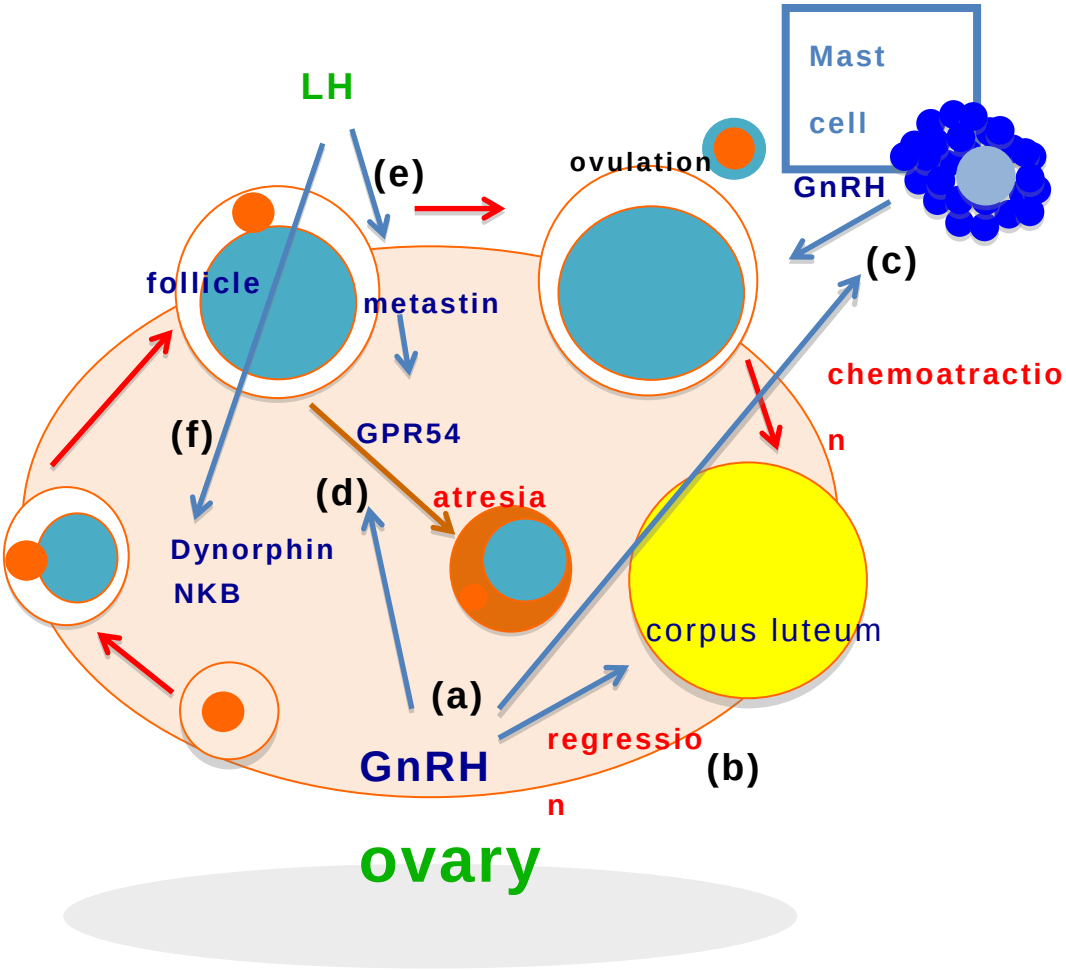
was smaller in C57BL/6-W^{sh}/W^{sh} mice. Although this difference seems to reflect the absence of mast cells and GnRH, ovarian GnRH mRNA expression was not different between the two strains. In summary, when ovarian synthesis of GnRH increases, mast cells are attracted by GnRH and migrate into the ovarian tissues. Mast cell production of GnRH is suggested to contribute, at least partly, to the variation of ovarian GnRH mRNA.

3. Expression of Metastin and Related Peptides in the Ovary: While metastin is synthesized in the ovary, its physiological implication is unknown. In the present study, detailed variations of the expression rate, source and physiological relevance of metastin and related peptides, Neurokinin B (NKB), dynorphin and GPR54, were examined in the ovary of cycling rats. Metastin and dynorphin mRNAs were dramatically increased at 20:00 h of proestrus. An increase of NKB mRNA starts simultaneously, but its peak is delayed until 2:00 h of estrus. GPR54 mRNA levels declined inversely to those of metastin. A variety of treatments were used to determine their effects. Both GnRH antagonist and pentobarbital given at noon of proestrus were expected to suppress the LH surge. These treatments did inhibit the expression of metastin mRNA at 20:00 h. The administration of hCG *in vivo* stimulated the expression of metastin and dynorphin but not NKB mRNAs. Prolactin had no affect on metastin expression. The variation of GnRH mRNA expression rate was not synchronized with that of metastin in the ovary. Furthermore, when metastin 45-54, kiss-10 (Human) or metastin 43-52, kiss-10 (Rat) was given into ovarian bursa for 6 hours on diestrus 2, the expression of GnRH mRNA was not affected. By means of LMD, metastin mRNA expression was shown to be restricted almost entirely to follicles, but NKB and dynorphin were

expressed in interstitial tissues. No apparent difference was demonstrated for GPR54 mRNA expression among components of the ovary. Immunohistochemistry demonstrated metastin distributed to almost all compartments of the ovary. So, metastin synthesized in granulosa cells should be available to surrounding tissues in the ovary. In the primary culture of granulosa cells prepared from PMSG-pretreated immature rats, hCG was clearly shown to stimulate the expression of metastin, NKB, and dynorphin mRNA. Intra-ovarian bursa administration of p234, an inhibitor of metastin action, with an osmotic-minipump for three days from proestrus occasionally induced a histological change. A distortion at the borders between the corpus luteum and surrounding tissues was observed. Progesterone production stimulated by hCG in the culture was suppressed by p234. These data demonstrate first that in response to the proestrous LH surge, granulosa cells synthesize significant amounts of metastin, but not NKB and dynorphin. They also suggest metastin has a role in the luteinization of granulosa cells. And lastly, that metastin is not responsible for GnRH mRNA expression in the ovary.

4. Conclusion: The specific variation of ovarian GnRH expression during the estrous cycle was demonstrated. Our laboratory previously observed that GnRH stimulates luteal apoptosis in the afternoon of diestrus 2. So, the first peak of GnRH mRNA expression would be responsible for luteal regression during the estrous cycle. The second peak on proestrus suggests a relationship with follicular atresia, as reported by others. Although the precise physiological role of GnRH in the ovary is still unknown, the present study suggests a new function of GnRH as a chemo-attractant for mast cells. Mast

cell migration into the ovary varies according to GnRH expression. Mast cells are thought to have a specific role on the regression of the corpus luteum and follicles. It was clarified that metastin is synthesized in granulosa cells under the control of the LH surge. Metastin, however, is not involved in the stimulation of GnRH expression in the ovary. Although NKB and dynorphin are synthesized in the granulosa cells to a lesser extent, the massive synthesis of NKB and dynorphin occurs in the interstitial tissues. It is suggested that metastin, dynorphin and NKB are involved in the ovulation and luteinization processes. The present study suggests that the metastin, NKB, dynorphin and GnRH expressed in the hypothalamus form a different network in the ovary.



Summary of results

Results of the thesis are summarized. (a) GnRH is synthesized in the ovary during the estrous cycle of rats. GnRH would induce luteal regression (b) and follicular atresia (d). GnRH also play a role of chemoattractant to call mast cells from outside the ovary (c). Mast cells synthesize GnRH. Proestrous LH surge augments metastin synthesis in the granulosa cells (e) and dynorphin production in the interstitial tissues (f). NKB synthesis is also augmented in the interstitial tissues.