学位論文要旨

Relationship and function of gonadotropin-releasing hormone and related bioactive peptides in rat granulosa cells

ラットの顆粒層細胞におけるゴナドトロピン放出ホルモンと関連生理活性ペプチドの機能と相関

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Introduction: Mammalian ovary contains follicles in various stages and corpus luteum. In the ovary of rats, a set of follicles starts to grow in each estrous cycle and ovulation recurs with a short interval. After ovulation, theca and granulosa cells of the follicle proliferate and become corpus luteum. This process is called luteinization and it accompanies tissue remodeling, the shift of steroidogenic pathway, exit cell cycle and changes in responsiveness to pituitary hormones. While ovulation and luteinization is induced by luteinizing hormone (LH) surge in the afternoon of proestrus, it is becoming clearer that various local hormones in the ovary also contribute to the periodical changes in the ovary. Interestingly hypothalamic neuro-peptides are also expressed in the ovary. Gonadotropin releasing hormone (GnRH) augments annexin A5 (ANXA5) expression in the pituitary gonadotropes and also in other peripheral tissues including the ovary. GnRH is expressed in the ovary, but its physiological function in the ovary is still obscure. GnRH related neuro-peptides seen in the hypothalamus, kisspeptin, dynorphin and neurokinin B (NKB), are also expressed in granulosa cells. Hence, it is great interest to know the relationship and function of GnRH and related neuro-peptides in granulosa cells, especially during luteinization. In the present study, it was examined whether GnRH is involved in the process of luteinization and the relationship between GnRH, kisspeptin, dynorphin, and NKB in the granulosa-luteal cells. Finally, a function of ANXA5 in the granulosa cells during luteinization was also studied.

Involvement of GnRH in luteinization process induced by hCG: In the present study, ovarian GnRH was examined whether it is involved in the process of luteinization using immature rat model. Follicular growth was induced by pregnant mare serum gonadotropin (PMSG, 15 IU/0.15 ml) given to 25-day old female rats. Luteinization was induced by human chorionic gonadotropin (hCG, 20 IU/0.2 ml) administration on day 27 after PMSG administration. Plasma level of progesterone was increased by hCG with a peak at 6 hr. ANXA5, a biomarker of GnRH action, expression in the granulosa cells was increased after

hCG administration. GnRH mRNA was increased in the ovary 3 hrs after hCG administration. Primary culture of granulosa cells was established by liberating cells from large follicles obtained 2 day after the PMSG treatment. The majority of cells was proved to express 3β-HSD by immunocytochemistry. Progesterone synthesis was augmented by hCG in a dose-dependent manner. GnRH mRNA was also increased by 0.01 IU hCG in the primary culture of granulosa cells and GnRH agonist (GnRHa, des-Gly10 [Pro9]-GnRH ethylamide) increased ANXA5 mRNA expression. GnRH (10^{-7} M) or GnRHa (10^{-8} M) suppressed hCG stimulated progesterone synthesis during 3 hrs incubation, revealing GnRH stimulation is rather suppressive to progesterone synthesis. Interestingly, concomitant administration of GnRHa and hCG clearly increased LH receptor mRNA expression and decreased follicle-stimulating hormone receptor. As these changes are the characteristics of luteinization, GnRH is suggested to have a cooperative role with LH in the differentiation of granulosa cells to luteal cells. GnRHa also affected the expression of genes related to differentiation (p21, p27, FOXO1 and prolactin receptor). GnRH and hCG affected synergistically on these genes (p27, FOXO1 and prolactin receptor). Present data clearly show that GnRH is involved in the effect of hCG on the transformation of granulosa cells to luteal cells. ANXA5 is suggested to have a role under GnRH receptor.

Relationship between GnRH, kisspeptin, dynorphin and NKB in the granulosa cells: Kisspeptin, dynorphin and NKB mRNA are all augmented by LH surge in the ovary during the estrous cycle of rats. The relationship between these peptides in the granulosa cells and the effect on progesterone production were examined. Kisspeptin and dynorphin mRNA expression in the ovary were augmented by 3 hrs after hCG administration on day 27. NKB mRNA was gradually increased but not significant. GnRH, kisspeptin, dynorphin and NKB mRNA expression were all stimulated by hCG also in the primary culture of granulosa cells until 3 hrs. Progesterone production stimulated by hCG was suppressed by concomitant

incubation with kiss-10 (bioactive peptide of kisspeptin), dynorphin A and NKB. Inter-relationship between neuro-peptides was examined on mRNA expression rate and results suggest a sequence of events after LH surge. GnRH would stimulate the expression of kisspeptin, dynorphin and later NKB mRNA. Then NKB would suppress at least GnRH and kisspeptin mRNA expression. Immunohistochemistry showed kisspeptin in granulosa cells after 6 hrs of hCG administration. Kisspeptin receptor (GPR54), NKB receptor (Tachykinin receptor 3) and dynorphin receptor (kappa opioid receptor) mRNA were confirmed in granulosa cells. Functional relationship among neuro-peptides in granulosa cells and changes in the expression of the neuro-peptides are suggested to relate to the differentiation of granulosa cells, namely luteinization (Fig. 1).

GnRH-ANXA5 function in granulosa cells: It is known that the expression of ANXA5 in the rat corpus luteum is suppressed by prolactin during pseudopregnancy and increased by GnRH when luteolysis occurs. ANXA5 is not seen in the granulosa cells and it appears in luteal cells. Granulosa cells were incubated with GnRHa with or without 0.01 IU hCG. GnRHa increased ANXA5 mRNA expression at 3 and 6 hrs but the stimulating effect gradually decreased and disappeared until 24 hrs. Although hCG stimulated GnRH expression in early phase of incubation, ANXA5 mRNA was suppressed by concomitant administration of hCG in granulosa cell culture. Even though ANXA5 is induced by hCG treatment as shown by immunohistochemistry, changes induced by hCG in granulosa cells seem to become suppressive for ANXA5 expression. Recombinant ANXA5 (10^{-9}M) did not show any effect on progesterone production of granulosa cells. However, when hCG was given first, the suppressive effect of ANXA5 appeared. These data suggest that changes induced by hCG would sensitize granulosa cells to inhibitory action of ANXA5 on progesterone production. On the other hand, GnRHa and ANXA5 reduced cell number of granulosa cells not treated with hCG. Recombinant ANXA5 decreased while anti-ANXA5 increased cell growth during 24 hrs incubation of granulosa cells.

The reduction of cells by ANXA5 was shown to accompany the increase of terminal deoxynucleotidyl transferase nick end labeling (TUNEL) positive cells suggesting induction of apoptosis. Pro-apoptotic function of ANXA5 on granulosa cells disappeared after hCG treatment. It was clearly demonstrated that granulosa cells would change by hCG that is evaluated by responses to GnRH and ANXA5.

Conclusion: GnRH action was assumed by the expression of ANXA5 in granulosa cells after hCG stimulation. Actually, GnRH expression was augmented by hCG in early phase of hCG action. GnRH was demonstrated to be involved in the luteinization process by hCG. GnRH related neuro-peptides, kisspeptin, prodynorphin and NKB were all stimulated by hCG. NKB was suggested to cease these reactions by suppressing each expression. ANXA5 was shown to be pro-apoptotic on granulosa cells and to be suppressive on progesterone production of luteal cells. The former response is suggested to relate to follicular atresia and the latter to luteinization (fig.2). Present study shows a network of ovarian neuro-peptides in different way from that seen in the hypothalamus. They are suggested to have a role to initiate luteinization. GnRH-ANXA5 would be a novel mechanism for regulation of granulosa and luteal cells in the ovary.

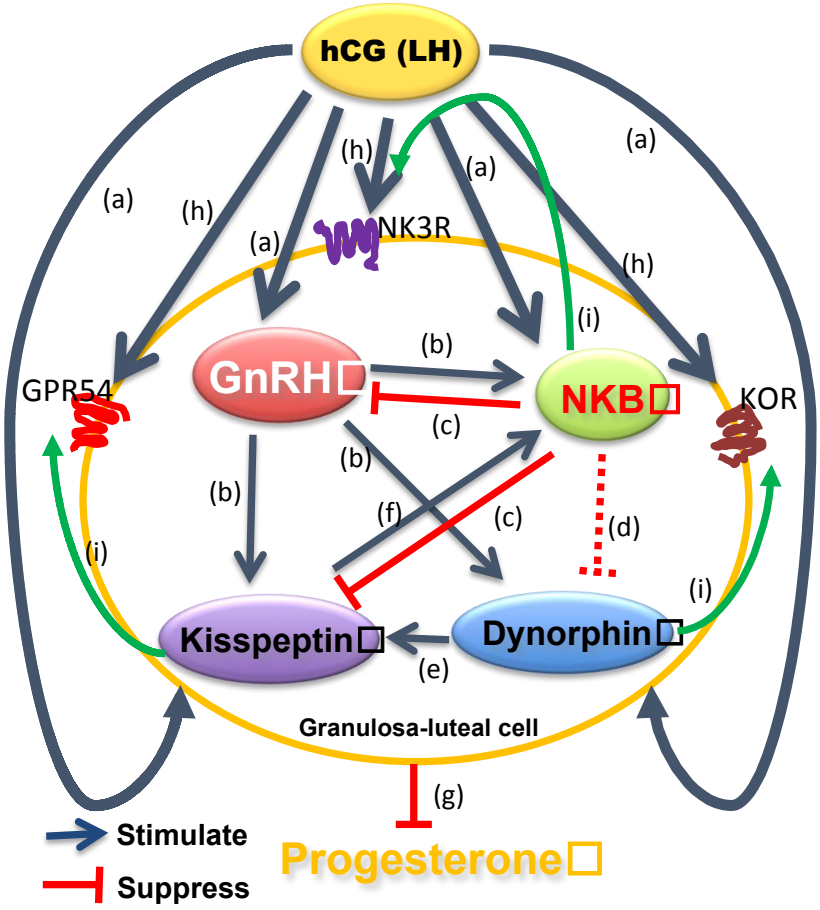

Fig. 1 Summary of interaction

(a) hCG stimulates GnRH, kisspeptin, dynorphin, and NKB mRNA expression in granulosa cell. (b) GnRH increases kisspeptin, dynorphin, and NKB mRNA expression. (c) NKB decreases GnRH and kisspeptin mRNA expression. (d) NKB may also suppress dynorphin mRNA expression. (e) Dynorphin increases kisspeptin mRNA expression. (f) Kisspeptin increases NKB mRNA expression. (g) All of peptides suppress progesterone production stimulated by hCG. (h) hCG increases GPR54, NK3R., and KOR. (i) kisspeptin, dynorphin, and NKB will bind their receptors.

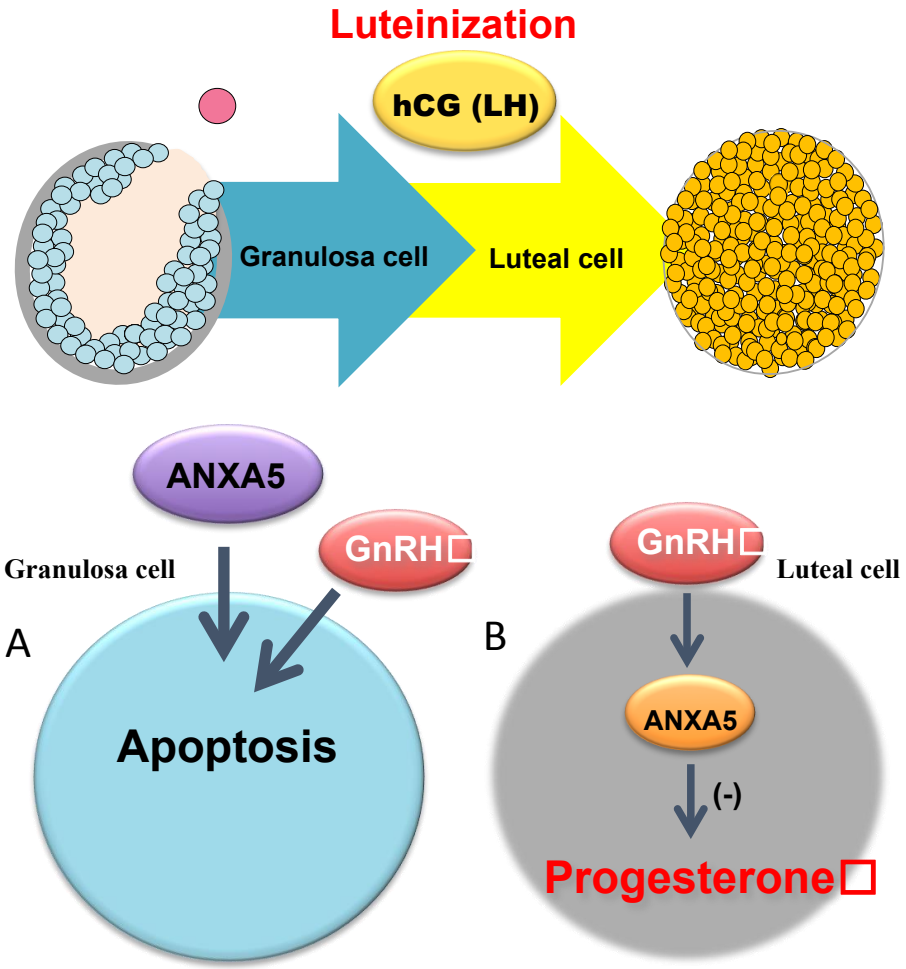

Fig. 2 Changes in response to GnRH and ANXA5 by luteinization

(A) GnRH and ANXA5 stimulates granulosa cell apoptosis. (B) GnRH and ANXA5 has suppressive effects on progesterone production in early phase of luteinization.