論　文　要　旨

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表　　題　ラット下垂体前葉における細胞間情報伝達物質としてのレチノイン酸 に関する分子細胞学的機能解析
Retinoic acid as a cell–cell communication factor in rat anterior pituitary gland: molecular and cellular biological studies

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表題　　ラット下垂体前葉における細胞間情報伝達物質としてのレチノイン酸に関する分子細胞学的機能解析

Retinoic acid as a cell–cell communication factor in rat anterior pituitary gland: molecular and cellular biological studies

１　研究目的

Retinoic acid (RA), a lipid soluble molecule derived from vitamin A, is an important signaling molecule in embryonic development and adult tissue. RA is generated from retinal by the retinaldehyde dehydrogenases (RALDHs) which include RALDH1, RALDH2, and RALDH3 and acts on neighboring cells in an autocrine or paracrine manner. Our research group showed that RALDHs are produced in development and adult anterior pituitary glands of rats. These findings suggest that RA is generated by RALDHs and may play a role as a paracrine and/or autocrine signaling molecule in the anterior pituitary gland. RA was reported to stimulate growth hormone (GH) gene expression in the anterior pituitary cells. However, current evidence is still unclear on the role of RA in the anterior pituitary gland. The aim of this study is to assess the novel functions of RA in anterior pituitary gland of rat.

２　研究方法

Experiment I

The aim of this experiment is to analyze the possible effects of RA on growth hormone-releasing hormone receptor (Ghrh-r), growth hormone secretagogue receptor (Ghs-r) and somatostatin receptor (Sst-r) gene expression and growth hormone release in isolated anterior pituitary cells of rats. The cells were isolated from anterior pituitary of 8-10 weeks old male Wistar rats. The cells (5×10^5 cells) were exposed to ATRA, Am80 or PA024 at indicated concentrations and periods. The total RNA was isolated from cultured cells and cDNA was synthesized by reverse transcription. The expression of target genes was measured by means of real-time PCR. To analyze the effect of RA on GH release, the anterior pituitary cells were seeded into 24-well plates at a concentration of 1×10^5 cells/well and exposed to ATRA (10^{-6} M) for 72 h. After treatment with ATRA, the cells were washed with fresh medium, after which the media were incubated. Media were changed, GHRH or ghrelin was then added to media at a concentration of 10^{-9} M and the cells were incubated. GH concentrations in the media were measured by a Rat/Mouse GH ELISA Kit. And then, GH release from cultured anterior pituitary cells was measured by enzyme-linked immunosorbent assay.

Experiment II

The aim of this experiment is to analyze the effect of RA on midkine (MK) gene expression in isolated anterior pituitary gland of rats. To localize MK mRNA in the gland, in situ hybridization was first performed. MK-expressing cell was characterized by immunohistochemistry using RALDH1 antibody. Using isolated anterior pituitary cells of rats, I examined the effect of RA on gene expression of MK.

３　研究成果

Experiment I

Quantitative real-time PCR revealed that treatment with all-trans retinoic acid (ATRA; 10^{-6} M) for 24 h increased gene expression levels of Ghrh-r and Ghs-r; however, expressions of Sst-r2 and Sst-r5 were unchanged. ATRA gradually increased Ghrh-r and Ghs-r mRNA expressions from 24 to 72 h. Exposure to ATRA at 10^{-6} M for 72 h increased gene expression of Ghrh-r and Ghs-r by 3.5-fold and 3.3-fold, respectively, as compared with control. These genes expressions were significantly and dose-dependently higher after ATRA treatment for 72 h. Combination treatment with the RAR-agonist Am80 and RXR-agonist PA024 mimicked the effects of ATRA on Ghrh-r and Ghs-r expressions. Exposure of isolated pituitary cells to ATRA had no effect on basal GH release. In contrast, ATRA increased growth hormone-releasing hormone (GHRH)- and ghrelin-stimulated GH release from cultured anterior pituitary cells.

Experiment II

In situ hybridization revealed that MK-expressing cells were small and frequently aggregated in small clusters. Double-staining with in situ hybridization and immunohistochemical techniques showed that MK mRNA was expressed in RALDH1-producing cells in the anterior pituitary gland. Using isolated anterior pituitary cells of rats, quantitative real-time PCR revealed that MK expression after exposure to 10^{-6} M of retinal or ATRA was 1.8-fold and 2.1-fold that of control. Moreover, MK mRNA transcription after exposure to Am80 was 2.0-fold that of control. Am80 treatment completely mimicked the effect of ATRA on MK mRNA transcription.

４　考察

Experiment I

In the present study, I demonstrated that ATRA induced Ghrh-r gene expression in isolated anterior pituitary cells of rats. This result is consistent with the findings of a previous study, which showed up-regulation by RA of Ghrh-r mRNA levels in a rat pituitary cell line. The present study also found that ATRA induced Ghs-r gene expression in pituitary cells. In addition, Ghrh-r and Ghs-r expressions were stimulated by ATRA in a dose- and time-dependent manner. ATRA did not stimulate gene expression of Sst-r2 or Sst-r5. Combination treatment with Am80 and PA024 completely mimicked the effect of ATRA on Ghrh-r and Ghs-r mRNA expression. The present results indicate that RA directly promotes these genes expression via RAR and RXR.

GH release from somatotrophs is stimulated by GHRH and ghrelin, which are ligands for

GHRH-R and GHS-R, respectively. Exposure of cultured pituitary cells to ATRA had no effect on basal GH release. Interestingly, ATRA enhanced GHRH- and ghrelin-induced GH release from cultured cells. These findings suggest that RA maintains pituitary responsiveness to GHRH and ghrelin.

Experiment II

In the present study, MK mRNA was detected in RALDH1-immunopositive cells. Our group previously detected MK mRNA in FS cells. MK expression after exposure to 10^{-6} M of retinal or ATRA was about 2-fold that of control. There is a report indicating that rat RALDH1 catalyzes oxidation of all-trans-retinal to ATRA. ATRA controls gene transcription via the nuclear receptor RAR; however, retinal does not substantially bind RAR. Our previous study demonstrated that RALDH1-producing cells are FS cells and lactotrophs. Taken together, it was suggested that locally generated RA induces MK gene expression in FS cells in autocrine/paracrine manner. In a previous study, we reported that the MK receptor PTPRZ1 was expressed in somatotrophs and corticotrophs in rat anterior pituitary gland. We hypothesized that MK produced in FS cells acts locally on these hormone-producing cells via PTPRZ1 in the anterior pituitary. In Experiment I, RA increased Ghrh-r and Ghs-r gene expression and promoted GHRH- and ghrelin-induced GH release from isolated rat anterior pituitary cells. Past and present evidence suggests that RA and MK function as autocrine and paracrine signaling molecules in the anterior pituitary gland. Future studies should attempt to clarify the roles of RA and MK in cell–cell interaction in the gland.

5　結論

This series of studies suggests that RA plays a role as an autocrine and paracrine signaling molecule in the anterior pituitary gland. The results of these studies will lead to further research on the functions of RA in cell-cell interaction in the anterior pituitary gland.