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学 位 論 文 の 要 約
（研 究 成 果 の ま と め）

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学位論文名　Expression of prosaposin and its receptors in the rat cerebellum after kainic acid injection

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学位論文の要約

Background: Prosaposin (PSAP) is the precursor of four small non-enzymatic glycoproteins, terme dsaposins A, B, C, and D . In addition, intact PSAP is widely expressed in various tissues. GPR 37 and GPR37-like 1 (GPR37L1) are two orphan G-protein-coupled receptors (GPCRs) that have enhanced expression in the rat brain. Recent reports demonstrate that these two related receptors c ould be stimulated by prosaposin and its active peptide fragment- prosaptide (Meyer et al., 2013). Kainic acid (KA) (2-carboxy-4-isopropenyl-pyrrolidin-3-ylacetic acid), a glutamate analog, is a po werful neurotoxic agent that stimulates excitatory neurotransmitter release . In this study, we exa mined the expression of PSAP and its two receptors, GPR37 and GPR37L1, in rat cerebellum usi ng the same KA-injected rat model.

Materials and methods: Ten-week-old, 220–260g-weight, male Wistar rats were used in the KA-i njected (5 mg/kg) rat model. Tissues were obtained at indicated time points and prepared for imm unoblot analysis, immunohistochemistry (IHC), immunofluorescence (IF), and in situ hybridization (ISH), respectively.

Results: immunoblotting showed PSAP was recognized by the specific antibody and one band of approximately 65 kDa was detected that corresponding to previously reported results (Gao et al., 2013a). Expression was increased markedly at one and three days post-KA injection compared to the control. IHC and IF showed specific PSAP dot-like immunostaining was observed predominant ly in the cytoplasm of Purkinje cells, and not in the nucleus. In the molecular layer, some intern eurons were also stained by the anti-PSAP antibody, and the intensity increased after KA injection. ISH analysis confirmed that the damage caused by KA resulted in increased expression of intact PSAP (AS3: Pro+9) rather than saposin precursor expression (AS4: Pro+0). Immunoblotting revea led attenuation of GPR37 expression after KA injection. Although the decrease on day 1 was not significant, there was a sharp decrease in expression by day 3. IHC showed that GPR37 expressi

on was highly enriched in the cytoplasm of Purkinje cells. The labeling was also seen in Purkinje cell dendrites and interneurons in the molecular layer. However, the intensity did not differ between KA-treated and control animals.

Discussion: PSAP is a bi-functional protein that is a precursor protein of saposin A-D, and is released extracellularly as full-length protein, acting as a trophic factor in the nervous system (Gao et al., 2013b). The results showing that PSAP expression is upregulated after KA injection are similar to results of previous studies that showed that PSAP expression is increased under conditions of cellular stress . Therefore, the results suggest that the PSAP may exert protective effects on cerebellar neurons against KA damage. ISH results suggest that KA induces neurons to produce PASP. These results are supported by a previous study that showed that cerebral ischemia negatively impacted lysosomal processing of PSAP .

GPR37 and GPR37L1 are orphan GPCPs that exhibit distant similarity to the endothelin receptors. Their high level expression and widespread distribution in the central nervous system suggest that they play important roles . In this study, we observed the single band at approximately 50KDa. Although the predicted molecular weight of GPR37 is 67KDa (Marazziti et al., 1997), we attribute this size difference to the different GPR37 fragment targets. In addition, immunoblotting revealed that GPR37 expression was decreased three days after KA injection; however, IHC did not revealed any expression changes. One possible explanation for this is that paraformaldehyde fixation and paraffin embedding may prevent some protein from being detected.

GPR37L1 is named for its similarity to GPR37. However, less is known about GPR37L1 compared to GPR37, which is likely due to lack of a specific antibody for experimentation. Unfortunately, we could not acquire consistent data for GPR37L1 despite using three separate antibodies.

In summary, this study revealed the relationship between PSAP and its receptors, GPR37 and GPR37L1 in rat cerebellum after KA-induced cytotoxicity. However, many questions remain regarding this model. Despite its preliminary nature, the current study provides important information for future research on the role of PSAP in neurodegenerative process.

References:

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