

論 文 内 容 要 旨

Investigation of pathophysiological factors and pharmacological targets for ameliorating leptin resistance in obesity

(肥満におけるレプチン抵抗性の改善を目指した病態生理学的因子及び薬理的標的因子の検討)

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A lack of physical activities and excess in food intake in modern life style play important role in the development of overweight and obesity. Yet the underlying mechanisms are still being elucidated in details. Leptin, an adipocyte-derived hormone, centrally regulates energy homeostasis by accelerating energy expenditure and suppressing food intake. However, leptin resistance is a hallmark of obese subjects. We and others have previously reported the involvement of endoplasmic reticulum (ER) stress in the pathophysiology of leptin resistance. Thus, being able to identify the factors involved in and the novel target for ameliorating leptin resistance would provide a potential therapeutic approach for obesity and related diseases. The aims of the present studies are to identify physiological factors and pharmacological targets for ameliorating leptin resistance in obesity.

First, we identified pathophysiological factors mediated leptin resistance including excess in dehydroascorbic acid (DHAA) and excess in saturated fatty acids (SFAs) in defective leptin signaling. DHAA, an oxidized form of vitamin C, was found to be diabetogenic. In the current study, we hypothesized the drawback effect of DHAA on defective leptin signaling. A human neuroblastoma cell line stably transfected with the Ob-Rb leptin receptor (SH-SY5Y-ObRb), was treated with DHAA and leptin signaling was then analyzed. Interestingly, we found that an elevated of DHAA inhibited leptin-induced STAT3 phosphorylation. We found that DHAA increased mRNA expression levels of ER stress-related genes such as glucose-regulated protein 78 (GRP78), C/EBP homologous protein (CHOP) and X-box-binding protein 1 (XBP1) splicing. These results suggested that DHAA plays important role in the development of leptin resistance through ER stress.

High-fat rich diet is associated with lipotoxicity and ER stress. Accordingly, using SH-SY5Y-ObRb cells, we found that the principle source of SFAs, palmitate inhibited leptin-induced STAT3 in neuronal cells. To further confirm the harmful effect of excess SFAs on leptin signaling, an inhibition of stearoyl-CoA desaturase-1 (SCD1), an enzyme that catalyzes the synthesis of monounsaturated fatty acids (MUFAs) from SFAs, was then evaluated. To achieve this hypothesis, disruption of SCD1 was carried out through the use of SCD1 inhibitor, CAY10566 and SCD1 knockdown, respectively. As expected, leptin-induced phosphorylation of STAT3 was inhibited in SCD1 inhibitor-treated and SCD1-knockdown SH-SY5Y-ObRb cells. Moreover, SCD1 inhibitor induced ER stress responsive gene, GRP78 in HEK293-ObRb cells. Thus, excess in SFA levels is involved in the pathophysiology of leptin resistance.

Second, we aimed to search for pharmacological targets for ameliorating leptin resistance in obesity. ER stress is known to implicate in the pathogenesis of multiple diseases including leptin resistance. Therefore, an intervention that alleviates ER stress would provide a useful therapeutic approach for obesity and ER stress-related diseases. In response to ER stress, cells activate an adaptive response termed unfolded protein response (UPR). One of the protective mechanisms of UPR is the induction of ER chaperone, i.e., glucose-regulated protein 78 (GRP78). GRP78, a major chaperone located in the lumen of the ER, protects against ER stress by promoting folding of proteins to prevent aggregation. Therefore, the induction of GRP78 is critical in maintaining normal cell functions. In the present study, using SH-SY5Y-ObRb cells we demonstrated that leptin induces GRP78 expression. In addition, leptin-induced GRP78 was not depended on its classical upstream activation pathway, IRE1-XBP1, as we could not detect IRE1 and XBP1 activation in our experimental conditions. In contrast, we showed that PI3K, LY294002, and mTOR inhibitor, rapamycin, blocked leptin's effect on the induction of GRP78. Of note, ER-apoptotic marker, CHOP, was not induced by leptin treatment in our experiments. Thus, leptin may specifically induce the expression of GRP78. Moreover, we showed that leptin protects against ER stress-induced cell death in human neuronal cell line. Therefore, leptin may induce the expression of GRP78, thereby protecting against ER stress related to obesity.

To date, the overlaps in the regulation of glucose and energy homeostasis have been reported between leptin and insulin. However, the effects of insulin on leptin's actions in hypothalamus have not yet been completely elucidated. In the current work, we found that insulin potentiates leptin-induced STAT3 phosphorylation through GRP78. The role of GRP78 in leptin's actions was also confirmed by defective leptin-induced STAT3 phosphorylation in SH-SY5Y-ObRb and HEK293-ObRb cells in which GRP78 was knocked down. Indeed, we found that the overexpression of GRP78 enhanced leptin-induced STAT3 phosphorylation. Based on the important role of GRP78 on leptin-induced signal, we also investigate the detailed mechanisms of which GRP78 modulated leptin signaling. We hypothesized that GRP78 may directly bind to ObRb long isoform of leptin receptor which leads to potentiation of leptin-induced signals. To do this, we first co-overexpressed ObRb receptor and GRP78 in HEK293T cells prior to an immunoprecipitation of ObRb. GRP78 was then analyzed by western blotting. As the result, we found that GRP78 binds to the long isoform of leptin receptor ObRb.

Our results highlight the novel pathophysiological factors of leptin resistance such as excess in SFAs and DHAA. ER stress, indeed, may play important role in the pathophysiology of leptin resistance induced by DHAA or SFAs. Moreover, we demonstrated that leptin upregulate the expression of GRP78 through the PI3K-mTOR pathway, and protects against ER stress. We also showed that GRP78 plays an important role in leptin's actions. We demonstrated that insulin enhanced the leptin-induced activation of STAT3 by inducing GRP78. To this, GRP78 would be a new mediator between leptin and insulin in the regulation of energy homeostasis. Together our data may provide useful information for further understanding of the pathophysiological factors as well as target molecule for attenuating leptin resistance in obesity.