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学位論文題目	Brain Agouti-Related Peptide and Sympathetic Nervous System Regulate Tumor Necrosis Factor-α mRNA Expression in White Adipose Tissue
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論文内容の要旨

BACKGROUND AND OBJECTIVE—White adipose tissue (WAT) not only stores excess calories in the form of triacylglycerol, but also serves as an endocrine organ, which secretes hormones such as leptin and adiponectin, proinflammatory cytokines and chemokines. Chronic inflammation in obese WAT is characterized by excessive proinflammatory cytokine production and associated with the insulin resistance. Tumor necrosis factor-α (TNF-α) is a crucial proinflammatory cytokine that is considered to contribute to insulin resistance in obese rodents and humans. Macrophage chemoattractant protein-1 (MCP-1), also increased in obese WAT, plays an important role in the recruitment of macrophages into WAT. The increased production of TNF-α has been believed as a metabolic consequence of obesity and macrophage infiltration into the hypertrophied WAT.

Previous studies have shown that central melanocortin system, consisting of α-melanocyte stimulating hormone (α-MSH) and agouti-related peptide (AgRP), which reciprocally regulate the melanocortin receptor (MCR), controls lipid metabolism in WAT and food intake. Activation of the brain MCR increases lipolysis in WAT via the sympathetic nerves innervating the tissue, while inhibition of MCR increases gene expression of lipogenic enzyme in the tissue. Moreover, the sympathetic nervous system has been shown to regulate the expressions of TNF-α and other proinflammatory cytokines in lymphocytes, peritoneal and bone marrow-derived macrophages and macrophage clonal cell line. However, it remains unclear whether the brain MCR and sympathetic nervous system control the production of proinflammatory cytokines in WAT.

In the present study, the author examined the regulatory role of the brain MCR in TNF-α and MCP-1 mRNA expressions in WAT. Furthermore, she examined the role of the sympathetic nervous system and norepinephrine (NE) in those mRNA expressions in WAT in lean mice. She also examined the role of the sympathetic nervous system and NE in the mRNA expressions in high-fat diet (HFD)-induced obese (DIO) mice.

RESEARCH DESIGN AND METHODS— AgRP or MCR agonist Melanotan (MT-II) was injected into the lateral cerebroventricle (icv) in free moving C57Bl/6J mice. Six hours (h) later, epididymal (epi) and inguinal (ing) WAT were collected, and mRNA amounts of TNF-α, MCP-1 and macrophage markers including F4/80, CD11c and CD206 were determined by quantitative real-time PCR. Sympathetic nerve activity in WAT was assessed by NE turnover method. NE content was measured by high-performance liquid chromatography (HPLC). To explore the role of sympathetic nerve in TNF-α and MCP-1 mRNA expressions in WAT, the author conducted the following experiments: 1) surgical sympathetic denervation of epiWAT; 2) subcutaneous injection of β-adrenergic antagonist propranolol into mice; 3) measurement of TNF-α, MCP-1 and macrophage markers mRNA expressions in WAT in β_1, β_2, and β_3-adrenergic receptors-deficient mice (β-less mice). In addition, she investigated the effects of NE on TNF-α mRNA expression in epiWAT explants, as well as mature adipocytes and stromal vascular

fraction (SVF) cells in epiWAT. Finally, she compared the gene expressions in DIO mice with those of β-less mice, and explored the role of sympathetic nervous system in TNF-α mRNA expression by examining the effect of NE on it in epiWAT isolated from DIO mice.

RESULTS— Icv injection of AgRP increased TNF-α mRNA expression in epiWAT, but not ingWAT, at 6 h after the injection. Expressions of MCP-1 and macrophage markers did not change after AgRP injection in either epi or ingWAT. MT-II injection did also not change those mRNA expressions in WATs.

AgRP injection did not alter plasma glucose, free-fatty acids, insulin, corticosterone, epinephrine or norepinephrine levles. In contrast, AgRP injection suppressed sympathetic nerve activity innervating epiWAT, but not ingWAT. Surgical sympathetic denervation of epiWAT increased TNF-α mRNA expression in the denervated epiWAT. Systemic administration of propranolol increased TNF-α mRNA expression in epiWAT.

The author examined the effect of NE on TNF-α mRNA expression in vitro in epiWAT explants isolated from C57Bl/6J mice. NE decreased TNF-α mRNA expression in epiWAT explants, and propranolol inhibited the effect of NE. β-adrenergic receptor agonist isoproterenol and adenylate cyclase activator forskolin (FSK) suppressed TNF-α mRNA expression in epiWAT explants. An activator of protein kinase A (PKA), 6-Bnz-cAMP, but not activator of exchange protein directly activated by cAMP (Epac), 8-pCPT-2'-O-Me-cAMP, suppressed TNF-α mRNA expression in epiWAT explants. The effects of specific β-adrenergic receptor antagonists and agonist revealed that β_2-adrenergic receptor involves the NE-induced suppression of TNF-α mRNA expression in epiWAT, while β_3-adrenergic receptor stimulates lipolytic activity in WAT in response to NE.

The author next examined the effects of NE on TNF-α mRNA expression in mature adipocytes and SVF cells in epiWAT after treatment with lipopolysaccharide (LPS). TNF-α mRNA expression was higher in SVF cells than that in mature adipocytes, and NE suppressed TNF-α mRNA expression in SVF cells but not in mature adipocytes. Macrophage may be a target of NE in SVF fraction, because β_2-adrenergic receptor and TNF-α mRNA expressions are significantly higher in macrophage marker CD11b-positive ($CD11b^+$) cells than that in CD11b-negative ($CD11b^-$) cells.

She examined TNF-α expression in β-less mice, and compared with that in DIO mice. Plasma TNF-α concentration significantly increased in β-less mice with a similar extent in DIO mice, although DIO mice have significantly heavier weights of body and WAT. β-less mice increased TNF-α mRNA expression in both epi and ingWAT, comparable to that in DIO mice, except a larger increase in epiWAT of DIO mice than that of β-less mice. MCP-1 did not change or only slightly increased the mRNA expression in epiWAT and ingWAT in β-less mice, and β-less mice did not alter F4/80, CD11c or CD206 mRNA expression in WATs. Thus, β-less mice increased TNF-α mRNA expression in both epi and ingWAT, similar to those in DIO mice, without increase in the recruitment of macrophages.

She finally examined the role of the sympathetic nervous system in TNF-α expression

in WAT of DIO mice. NE but not FSK, failed to suppress TNF-α mRNA expression as well as to increase lipolysis in epiWAT in DIO mice. β_2 and β_3-adrenergic receptor mRNA expressions significantly decreased in both epi and ingWAT in DIO mice. These results suggest that β-adrenergic receptor signaling is impaired in WAT in DIO mice.

CONCLUSIONS— The brain AgRP and sympathetic nervous system regulate TNF-α mRNA expression in WAT via β_2-adrenergic receptor and PKA signaling pathway. In contrast, NE can not suppress TNF-α mRNA expression in WAT in DIO mice. Thus, the sympathetic nervous system inhibits TNF-α mRNA expression in WAT, while it stimulates the release of free-fatty acids, which are an inducer of TNF-α production as well as energy source for other tissues. Her data also suggest that impairment of the β-adrenergic receptor signaling contributes to the increase in TNF-α mRNA expression in WAT in DIO mice.

博士論文の審査結果の要旨

白色脂肪組織におけるTNF-αの過剰産生は、インシュリン抵抗性の発症と深く関わることが知られている。これまで、肥満した脂肪組織におけるTNF-αの過剰産生は、脂肪組織の肥大とそれによって引き起こされるマクロファージの浸潤によると考えられてきた。しかしながら、脳がTNF-αの産生にどのような調節作用を営むかは不明である。本研究において、摂食促進神経ペプチドAgouti-related peptide (AgRP)をC57BL/6Jマウス（オス）の脳室内に投与し、脂肪組織でのTNF-α mRNA発現に及ぼす効果を調べた。また、調節機構を明らかにするために交感神経の作用を調べた。

AgRPをマウス脳室内に投与すると、副睾丸脂肪組織においてTNF-αのmRNA 発現が亢進した。マクロファージマーカーの発現は変化しなかった。また、AgRPを脳室内に投与すると、副睾丸脂肪組織を支配する交感神経の活動が選択的に抑制された。外科的神経切除、β-アドレナリン (Ad)受容体拮抗薬の投与、β-Ad受容体遺伝子欠損マウスを用いて交感神経の調節作用を調べた。その結果、交感神経は、β受容体を介してTNF-α発現を抑制すること、AgRPは交感神経作用を抑制することによってTNF-αの発現を高めることが分かった。β-Ad受容体遺伝子欠損マウスでは、極く少ししか肥満していないにも関わらず、高脂肪食誘導性肥満（DIO）マウスと同程度に、脂肪組織におけるTNF-αのmRNA 発現及び血中TNF-α濃度が亢進していた。

TNF-α のmRNA発現に及ぼす交感神経の調節機構を調べるため、ノルエピネフリン（NE）をex vivoで副睾丸脂肪組織に直接作用させ、その効果を調べた。NE、β-Ad受容体作動薬イソプロテレノール（ISO)、forskolin（FSK）及びprotein kinase A (PKA)活性化剤によって、脂肪組織におけるTNF-α のmRNA発現が抑制された。NEの効果は、β2-Ad受容体の選択的拮抗薬によって抑制された。副睾丸脂肪組織からCD11b（+/+）細胞であるマクロファージを単離し、β-Ad受容体、TNF-α のmRNA発現をCD11b（-/-）細胞と比較した。脂肪組織マクロファージでは、β2-Ad受容体とTNF-α のmRNAが高発現していた。以上の実験結果から、NEは、脂肪組織に存在するマクロファージのβ2-Ad受容体—PKA経路を介してTNF-αの発現を抑制することが示唆される。

最後に、DIOマウスの脂肪組織において、NEがどのような効果を及ぼすかを調べた。FSKによってTNF-αのmRNA 発現は低下したが、NEでは抑制されなかった。また、β2-Ad受容体mRNA発現も低下していた。このことから、DIOマウスの脂肪組織では、NE作用はβ受容体レベルにおいて障害されると考えられる。

以上の実験結果から、脂肪組織におけるTNF-αの過剰産生は、脂肪組織の肥大とそれによって引き起こされるマクロファージの浸潤によるだけでなく、脳—交感神経—β2受容体経路の異常によっても引き起こされることが明らかとなった。

本研究は、白色脂肪組織でのTNF-α mRNA発現に及ぼす脳及び交感神経の調節作用に着目し、交感神経—β受容体経路の異常が脂肪組織でのTNF-α 過剰産生に関与することを初めて明らかにしたものであり、審査の結果、博士論文に値すると審査委員全員が一致して判断した。