Abstract  The epidemic of obesity has become a serious health challenge in both developed and developing countries. Since the identification of leptin, a secretory protein from the white adipose tissue, secretory proteins have become recognized as important regulators of energy metabolism. We previously identified neudesin as a novel secretory protein. We also showed that neudesin was strongly expressed in central the nervous system of embryonic and adult mice, and was expressed in various peripheral tissues of adult mice. Thus, in this review, we discuss the physiological roles of neudesin and focus on its involvement in energy metabolism. We generated neudesin knock-out (KO) mice, and showed that they were resistant to high-fat diet (HFD)-induced obesity. Neudesin KO mice were protected from HFD-induced insulin resistance and hepatic steatosis. Furthermore, neudesin KO mice showed increased energy expenditure due to enhanced sympathetic nervous activity (SNA). Thus, resistance to HFD-induced obesity in neudesin KO mice was the result of increased energy expenditure. Neudesin KO mice also showed increased heat production and fatty acid oxidation in the adipose tissue. These results suggest that neudesin might act on neurons and adipocytes to suppress SNA. In conclusion, we establish the role of neudesin as a suppressor of energy expenditure via regulating SNA. Neudesin is a novel regulator of energy metabolism and might be a target for the treatment of obesity and obesity-related metabolic syndrome.

Keywords : neudesin, obesity, sympathetic nervous activity, adipose tissue

Introduction

Obesity is the main cause of metabolic syndrome including, diabetes, dyslipidemia, and cardiovascular diseases; and the obesity epidemic has become a serious health challenge in both developed and developing countries1-3). The imbalance between energy intake and energy expenditure results in obesity. Thus, identifying novel molecules that regulate energy metabolism might contribute to the development of new anti-obesity drugs. The identification of leptin, a secretory protein from white adipose tissue (WAT), was considered a breakthrough in obesity research4). Leptin acts on the hypothalamus and strongly suppresses appetite and increases energy expenditure. Mice with a mutation in the leptin gene, called ob/ob mice, show hyperphagia and are morbidly obese. After the identification of leptin, various other secretory proteins have proven to be important for regulating energy metabolism5,7). Thus, secretory proteins are now recognized as crucial regulators of energy metabolism.

We identified novel secreted proteins in mouse cDNA, including neudesin, in the GenBank nucleotide sequence database using PSORT for subcellular localization prediction5-13). Neudesin is well conserved in vertebrates from zebrafish to humans14). Human neudesin is a protein of 172 amino acids with a conserved cytochrome 5-like heme/steroid-binding domain14,15). The domain is conserved in the membrane-associated progesterone receptor (MAPR) family; and thus neudesin is thought to be a member of the MAPR family15). Neudesin was shown to be strongly expressed in the central nervous system15), and showed neurotrophic activity in primary cultured neurons14). Neurotrophic factors promote the survival, differentiation, and maintenance of neurons and are crucial for nervous system functions17-19). In addition to its neurotrophic activity, neudesin promotes neural cell proliferation and differentiation4,15). Neudesin regulates neuronal development, and its functions have been characterized in vivo20). Byerly et al. reported that the administration of recombinant neudesin to the hypothalamus repressed appetite. Novais et al. generated neudesin knockout (KO) mice and reported that deletion of the neudesin gene resulted in increased anxious-like behavior caused by insufficient development of hippocampal neurons21). These results showed that neudesin is crucial for neuronal development and function. However, neudesin was also found to be expressed in the central nervous system22), and thus seems to be involved in other physiological functions.
in various peripheral tissues including the heart, adipose tissue, and skeletal muscle. Several reports indicated that neudesin also showed non-neuronal effects. Therefore, we generated neudesin KO mice to elucidate its physiological roles. We found that neudesin KO mice were resistant to high-fat diet (HFD)-induced obesity. Thus, neudesin was expected to be a novel regulator of energy metabolism. Regarding these results, in this review, we focus on the role of neudesin as a regulator of energy metabolism.

Neudesin expression in the central nervous system and peripheral tissues

Neudesin was originally identified as a secretory molecule that is strongly expressed in the brain and spinal cord of developing mice. Neudesin was detected in the brain of adult mice; and, more specifically, was found to be expressed in various regions of the brain including the hypothalamus, thalamus, hippocampus, and cerebral cortex. Several reports show that neudesin is a crucial factor for proper neuronal development and function. However, neudesin was also found to be expressed in various peripheral tissues including adipose tissue, the heart, skeletal muscle and kidney. We recently found that sympathetic nervous activity (SNA) could affect the expression levels of neudesin in WAT. The expression levels of neudesin in WAT were significantly increased in mice with obesity with a reduction in adipose SNA. In contrast, the expression levels of neudesin in WAT were significantly decreased in mice with cold exposure or administration of the CL-316,243 beta-3 adrenergic receptor agonist (CL). Both cold exposure and CL administration enhance SNA in WAT. These results suggest that SNA might regulate the expression levels of neudesin in WAT. We discuss the relationship between SNA and neudesin in more detail below.

Resistance of neudesin KO mice to HFD-induced obesity

We generated neudesin KO mice to elucidate the physiological roles of neudesin. These neudesin KO mice showed the expected Mendelian ratios and were apparently normal. Tibia length, which reflects growth, was similar between wild type (WT) and neudesin KO mice. However, we found that neudesin KO mice were relatively lean when fed normal chow (NC). HFD-feeding significantly increased the body weights of WT mice, whereas neudesin KO mice showed significant resistance to HFD-induced obesity. Obesity causes insulin resistance and hepatic steatosis, and the neudesin KO mice were protected from these effects. Our results indicated that neudesin is a regulator of energy metabolism in vivo. Therefore, we focused on the role of neudesin in energy metabolism.

Resistance of neudesin KO mice to HFD-induced obesity is independent of food intake

As mentioned above, the imbalance between energy intake and energy expenditure results in the development of obesity. Thus, we examined the food intake of neudesin KO mice to elucidate the role of neudesin in energy intake. Although neudesin KO mice were resistant to HFD-induced obesity, the food intake of the neudesin KO mice fed HFD was comparable to that of WT mice fed HFD. This result suggested that resistance to HFD-induced obesity in neudesin KO mice was independent of food intake. However, we also found that neudesin KO mice fed NC showed significantly decreased food intake compared to WT mice. The hypothalamus is crucial for regulating appetite. Neudesin was shown to be expressed in the paraventricular nuclei and arcuate nucleus of the hypothalamus, which are especially important for regulating appetite. Byerly et al. reported that the administration of recombinant neudesin to the hypothalamus repressed appetite. They also showed that the expression level of neudesin in the hypothalamus was decreased by the administration of brain-derived neurotrophic factor, which is recognized as an important regulator of food intake. Thus, the role of neudesin in the regulation of food intake should be examined in the future.

Neudesin KO mice show increased energy expenditure

As the resistance to HFD-induced obesity in neudesin KO mice was independent of food intake, we examined the effect of the deletion of neudesin on energy expenditure. Neudesin KO mice fed HFD showed a higher rectal temperature, although voluntary activity was comparable between WT and neudesin KO mice. Respiratory gas analysis showed that oxygen consumption was significantly increased in neudesin KO mice fed the HFD. The respiratory quotient was significantly lower and fatty acid oxidation was significantly increased in neudesin KO mice fed the HFD. These results indicate that energy expenditure was increased in neudesin KO mice fed the HFD, which could contribute to the observed resistance to HFD-induced obesity. Thus, we conclude that increased energy expenditure in neudesin KO mice contributes to its resistance to HFD-induced obesity.

Neudesin KO mice show increased SNA

We attempted to elucidate why neudesin KO mice showed increased energy expenditure. As increased SNA often results in increased energy expenditure, we examined the effect of the deletion of neudesin on SNA. Neudesin KO mice showed an increased heart rate, which is a common indicator of SNA. Noradrenaline levels in the plasma and noradrenaline content in the adipose tissue were also significantly increased in neudesin KO
mice. These results indicate that neudesin KO mice show increased SNA, which could contribute to the resistance to HFD-induced obesity observed in neudesin KO mice.

**Neudesin KO mice show increased heat production and fatty acid oxidation in adipose tissue**

Adipose tissue is crucial for regulating energy metabolism. Adipose tissue can be divided into two distinct types: white and brown. WAT stores excess energy as triglyceride (TG)\(^{34}\), whereas brown adipose tissue (BAT) dissipates energy as heat\(^{35}\). The sympathetic nervous system regulates adipose functions and the development of obesity because it stimulates lipolysis in WAT and enhances heat production and fatty acid oxidation in BAT\(^{36,37}\). Our research showed that lipolytic activity was significantly increased in the WAT of neudesin KO mice fed HFD. We examined the expression levels of UCP1, CPT1, PPAR\(\alpha\), and PGC-1\(\alpha\) in BAT. UCP1 is strongly expressed in BAT and is indispensable for thermogenesis. CPT1 catalyzes the entry of fatty acids into the mitochondria and is crucial for fatty acid oxidation. PPAR\(\alpha\) and PGC-1\(\alpha\) are the transcriptional regulators of thermogenesis and fatty acid oxidation. The expression levels of these genes in the BAT of neudesin KO mice fed HFD were significantly higher than those in WT mice. These results indicate that sympathetic activity was increased in the adipose tissue of neudesin KO mice. Thus, the energy expenditure was increased by the increased sympathetic activity in the WAT and BAT of neudesin KO mice, resulting in resistance to diet-induced obesity (DIO).

Several recent studies have shown that UCP1-positive adipocytes even appeared in WAT in response to increased SNA\(^{38-40}\). These UCP1-positive adipocytes in WAT are called “beige” or “brite” adipocytes. Recent research has demonstrated that beige adipocytes are also present even in the WAT of adult humans, raising the possibility that augmenting the function of beige adipocytes could provide an effective avenue for the treatment of obesity and its associated diseases\(^{41,42}\). As neudesin KO mice showed enhanced SNA, we expected that they would have increased browning of WAT. UCP1 was barely detected in the WAT of WT mice. However, we confirmed that UCP1 was clearly detected in the WAT of neudesin KO mice. In addition, we found that the expression levels of CPT1, PPAR\(\alpha\), and PGC-1\(\alpha\) were all significantly increased in the WAT of neudesin KO mice. Thus, neudesin KO mice certainly showed increased browning of WAT.

**Action of neudesin on neurons and adipocytes to suppress SNA**

We showed that neudesin KO mice were resistant to HFD-induced obesity owing to increased energy expenditure caused by enhanced SNA. We next tried to elucidate why deletion of neudesin would result in increased SNA. We previously reported that neudesin acted on neurons. In our recent paper, we reported that recombinant neudesin suppressed the expression levels of tyrosine hydroxylase, which encodes a rate-limiting enzyme of the synthesis of noradrenaline, in differentiated PC12 cells, that show sympathetic neuron-like properties\(^{43}\). These results suggest that neudesin could act on sympathetic neurons to suppress noradrenaline synthesis.

Neudesin could also act on non-neuronal cells. For example, we previously reported that neudesin acted on 3T3-L1 cells to suppress adipogenesis\(^{23}\). As mentioned above, neudesin was strongly expressed in the WAT; however, neudesin was not detected in the plasma in our preliminary experiment (unpublished observation). There-
fore, neudesin could directly act on adipocytes. CL treatment significantly increased glycerol release, which is an indicator of lipolytic activity, from adipocytes and recombinant neudesin suppressed the glycerol release although these results are preliminary (unpublished observation).

As explained above, neudesin could act on neurons and adipocytes to suppress SNA. Since neudesin is a secretory protein, a neudesin receptor should exist which has not yet been identified. Identifying a neudesin receptor will greatly help us to understand the mechanism of the action of neudesin.

Summary

We tried to elucidate the physiological roles of neudesin, a secretory protein identified by our group, by using neudesin KO mice. We found that neudesin KO mice were resistant to HFD-induced obesity owing to increased energy expenditure, highlighting neudesin as a novel regulator of energy metabolism. Our findings are summarized in Fig. 1. As mentioned above, the epidemic of obesity has become a serious health challenge worldwide, and the development of novel anti-obesity drugs is urgently needed. Thus, further research of neudesin might contribute to the development of new anti-obesity drugs. (Fig. 1)

Conflict of Interests

The author declares that there is no conflict of interests regarding the publication of this article.

References

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