Effects of Nesfatin-1 on the Metabolism of Glycolipids in the Lateral Ventricle of Rats and its Relationship with the Expression of AMPK in Hypothalamus

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ABSTRACT
Objective: To investigate the effect of lateral ventricle injection inhibitor nesfatin-1 on glycolipid metabolism and its relationship with hypothalamic AMPK expression. Methods: Nesfatin-1 was injected into the lateral ventricle of rat by the brain stereotactic technique (nesfatin-1 group), and the sham operation group was injected with an equal volume of artificial cerebrospinal fluid and the control group had no interference factor. The rats were kept for 4 weeks and an automatic biochemical analyzer was used to detect blood sugar and blood lipid levels. The expression of AMPK mRNA in rat hypothalamus was detected by Western blot. The expression of AMPKα and phosphorylated AMPKα (pAMPKα) in the rat hypothalamus was detected by Western blot. Results: (1) According to the results of biochemical tests, compared to the control group, the level of FPG and TG in experimental group were significantly decreased (P <0.05), while the level of TC, HDL and LDL were not significant change (P >0.05); (2) According to the results of RT-PCR and Western blotting, compared to control group, the expression of AMPK mRNA and protein level in experimental group were significantly lower (P <0.05) and shown no significant difference (P >0.05) with sham-operated group. Conclusion Nesfatin-1 may play an important role in the process of neuroregulation on glycolipid metabolism, the mechanism may be related to the AMPK expression level of hypothalamus.

Keywords: Nesfatin-1, Glycolipid Metabolism, AMP-Activated Protein kinase, Hypothalamus

Introduction
The visceral function is regulated by the nervous system. The hypothalamus is the regulation center of energy balance. There are receptors for various energy changes, which can receive signals from peripheral nerves, hormones and nutrients, and regulate feeding and energy metabolism through sympathetic/parasympathetic nerves. Nesfatin-1 is a peptide consisting of 82 amino acids at the N-terminus of nucleobindin-2 (NUCB2) and plays an important role in the regulation of feeding, obesity and energy metabolism. ATP-activated protein kinase (AMPK) is widely present in eukaryotic cells. A large body of evidence suggests that AMPK, which is present in the hypothalamic region, mediates the role of signaling molecules in feeding and energy regulation, but the relationship between AMPK and nesfatin-1 is not yet clear.

To elucidate the role of nesfatin-1 in the regulation of glucose and lipid metabolism in the brain and its relationship with AMPK in the hypothalamus, this study intends to inject nesfatin-1 into the lateral ventricle of rats by means of brain stereotactic technique to detect changes in blood glucose and lipid levels and the expression level of AMPK in the thalamus; To explore the hypothalamic mechanism of nesfatin-1 regulating glucose and lipid metabolism and provide experimental evidence for the in-depth study of the neuroregulatory mechanism of glycolipid metabolism.

1. Materials and Methods.
1.1 Animals and group
Forty-five male Spraque-Dawley (SD) rats weighing 250-300 g were randomly divided into experimental group, sham operation group and control group. All animals followed the “Qingdao University Laboratory Animal Protection and Use Management Methods.” All experiments were in accordance with the Qingdao Animal Center Standard. The nesfatin-1 group rats (n = 15) were anesthetized with 10% chloral hydrate (4...
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ml/kg, intraperitoneal injection) and fixed on the stereotaxic instrument. A 23-gauge stainless steel cannula was placed at the lateral ventricle (0.8 mm posterolateral, 1.5 mm lateral, 3.5 mm deep) according to the stereotaxic map of the rat brain [3], and nesfatin-1 was injected at a rate of 0.25 per hour for six hours. Sham group (n = 15) was injected with an equal volume of artificial cerebrospinal fluid (NaCl 126 mmol/L; CaCl$_2$ 2.0 mmol/L; H$_2$O 2.0 mmol/L; MgCl$_2$ 3.5 mmol/L; MgSO$_4$ 1.0 mmol/L; NaHCO$_3$ 26.0 mmol/L; NaH$_2$PO$_4$ 1.25 mmol/L; glucose 10.0 mmol/L; pH 7.35-7.45). The control group (n = 15) did not exert any interfering factors.

1.2 Blood glucolipid levels detection and tissue specimen processing
Rats were fed for 4 weeks after injection of nesfatin-1 in the right lateral ventricle. The rats were anesthetized with chloral hydrate (4 ml/kg, intraperitoneal injection) and fixed on the stereotaxic instrument. After the rats were fixed, the rats were sacrificed with a 1 ml syringe at the strongest point of the left sternal border of the rat, and the blood was collected and the plasma was separated. Fasting blood glucose (FPG), triglyceride (TG), total cholesterol (TC), high-density lipoprotein cholesterol (HDL) and low-density lipoprotein cholesterol (LDL) were measured using an automatic biochemical analyzer. Immediately after the blood was collected from the heart, the brain was decapitated, and the hypothalamus was separated and stored in liquid nitrogen for storage.

1.3 RT-PCR method for detection of AMPK mRNA expression in rat hypothalamus
The hypothalamic tissue was ground into powder in liquid nitrogen, and total RNA was extracted by Trizol method. RNA was precipitated by adding chloroform and isopropanol in sequence. After washing, it was dissolved in RNase-free water, and the concentration was measured and used. The RNA was reverse transcribed into cDNA, and the AMPK mRNA expression level of each sample was detected by real-time PCR. The reaction conditions were pre-denaturation at 95 °C for 10 min; denaturation at 95 °C for 15 s, annealing at 58 °C for 25 s, extension at 72 °C for 30 s, and 40 cycles. The CT values of each group of samples were obtained by software analysis, and the relative expression levels of each group were expressed by $2^{-\Delta\Delta CT}$ values. The following primers were used in the real-time PCR. Primer sequences for AMPK were:

<table>
<thead>
<tr>
<th>Primer</th>
<th>Sequence</th>
<th>Status</th>
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<tbody>
<tr>
<td>forward primer</td>
<td>5'-CTCTGAGGGCCACCAAGAAAC-3'</td>
<td>5'-GGTTGGTGGTGACCGCAGAGG-3'</td>
</tr>
<tr>
<td>reverse primer</td>
<td>5'-GGTGGTGTTGACCGCAGAGG-3'</td>
<td>5'-CTCTGAGGGCCACCAAGAAAC-3'</td>
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1.4 Western blotting method for detection of the expression levels of AMPKα and phosphorylated AMPKα (pAMPKα) in rat hypothalamus
The hypothalamic tissue was ground to a powder in liquid nitrogen and 100 microliters of lysate was added per 20 mg of tissue. The protein concentration was determined by BCA kit, and the protein was separated by SDS-PAGE. The membrane was subjected to 90 mA for 1.5 h. The PVDF membrane was placed in AMPK primary antibody at 4 °C overnight, and the secondary antibody was added and incubated for 1 h at room temperature. The PVDF membrane was incubated with the chemiluminescent substrate according to the instructions of the ECL luminescence kit, and exposed to X-film exposure. The gel imaging analysis system measured and analyzed the protein band densitometry. The ratio of the gray value of the AMPK protein to the gray value of the reference gene β-actin represents the relative amount of AMPK protein in the sample.

1.5 Statistical analysis
Data were processed by SPSS 17.0 statistical software. One-way analysis of variance followed by post hoc Bonferroni’s test was used to compare multi-set experimental data. Measurement data were expressed as mean± SD. P < 0.05 was used as a statistically significant difference.

2. Results
2.1 Rat blood glucolipid levels
The results of automatic biochemical analyzer showed that the FPG and TG of the nesfatin-1 group were significantly decreased compared with the control group (P <0.05, Figure 1). However, there was no significant change in TC, HDL and LDL levels (P > 0.05, Figure 1). There was no significant difference between the sham group and the control group (P > 0.05, Figure 1).

![Figure 1 Rat blood glucolipid levels](http://www.ijSciences.com)

*P<0.05 vs. control group

2.2 AMPK expression level in rat hypothalamus
The rat hypothalamic tissue was detected by real-time PCR after injection of nesfatin-1 into the right lateral ventricle of rats. Compared with the control group, the expression level of AMPK mRNA in the nesfati-1 group was significantly lower (P < 0.05, Figure...
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2. Western blotting results showed that the AMPK subunit size was 63 kD. Compared with the control group, the expression levels of AMPK and pAMPK in the nesfatin-1 group were significantly lower (P < 0.05, Figure 3). There was no significant difference in AMPK mRNA and protein expression levels between the sham group and the control group (P > 0.05).

Nesfatin-1 is a peptide located at the N-terminus of NUCB2. Intraventricular injection of nesfatin-1 has a significant inhibitory effect on feeding. The intermediate fragment (M30, 24-53 residues) may be the key to the feeding inhibition of nesfatin-1. Peripheral feeding signals can also lead to changes in the expression of nesfatin-1 in the hypothalamus, and elevated expression of nesfatin-1 is associated with decreased food intake and weight loss. Another study showed that short-term perfusion of central nesfatin-1 increased insulin sensitivity (IS) in rats and inhibited hepatic gluconeogenesis. In this study, the recombinant protein of Nesfatin-1(1-82) was slowly injected into the lateral ventricle by stereotactic technique. After 4 weeks, the biochemical tests showed that the FPG and TG were significantly decreased. There were no significant changes in TC, HDL and LDL, suggesting that nesfatin-1 may play an important role in the regulation of glycolipid metabolism. Nesfatin-1 regulates visceral function signaling pathways in the hypothalamus. Studies have shown that nesfatin-1 knockdown enhances hepatic gluconeogenesis in rats associated with mTOR-STAT3 signaling pathway. It has also been reported that nesfatin-1 may act through the POMC pathway and may not be related to the leptin signaling pathway. AMPK plays an important role in the regulation of energy metabolism, mainly expressed in skeletal muscle, liver, pancreas, adipose tissue and central nervous system. Its main function is to regulate the production capacity and energy metabolism according to the needs of the body. Hypothalamic AMPK is an intermediary for regulating signaling pathways in feeding behavior. Ingestion inhibitors inhibit the activity of AMPK in the hypothalamus, which in turn reduces food intake.

The results of this study showed that the decrease of blood glucose and lipid levels induced by Nesfatin-1 in the lateral ventricle may be related to the decrease of AMPK mRNA and protein expression in hypothalamus. It is speculated that AMPK in hypothalamus is closely related to the regulation of food intake and energy metabolism by nesfatin-1. However, the exact
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mechanism needs to be further studied, such as the thalamic signal pathway and the role of visceral sympathetic and parasympathetic nerves. It was also reported [18] that the expression level of NUCB2/Nesfatin-1 in the hypothalamus of diet-induced obesity (DIO) rats was significantly decreased, which may play an important role in the neuro-modulation of glycolipid metabolism.

In summary, this study further confirmed the role of nesfatin-1 in the regulation of glycolipid metabolism by lateral ventricle injection, and its mechanism may be related to the expression level of AMPK in the hypothalamus. To elucidate this mechanism, we provide a basis for the study of neuromodulation mechanisms of feeding and substance metabolism.

References