Effects of Histamine on Lipid Metabolic Disorder in Mice Loaded With Restraint Stress

Rong Rong He¹,², Nan Yao¹, Min Wang¹, Xue Song Yang³, Chin Chin Yau⁴, Keiichi Abe⁴, Xin Sheng Yao¹,² and Hiroshi Kurihara¹,*

¹Institute of Traditional Chinese Medicine & Natural Products, Jinan University, Guangzhou 510632, China
²College of Traditional Chinese Materia Medica, Shenyang Pharmaceutical University, Shenyang 110016, China
³Medical College, Jinan University, Guangzhou 510632, China
⁴BRAND’S Health Science Center, Cerebos Pacific Ltd., Singapore 048423, Singapore

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Abstract. The present study was conducted to investigate the effects of histamine on the lipid metabolic disorder in mice loaded with restraint stress. When Kun Ming (KM) mice were exposed to restraint stress for 20 h, the histamine level in both plasma and cerebral regions significantly increased (P<0.01). Moreover, when a lipid emulsion (10% Intralipid®) was injected intravenously into the mice, the elimination period of plasma triglyceride was prolonged in the restraint group. Plasma triglyceride was 523±44 mg/dl at 35 min after the Intralipid® administration in the restraint stress group, while it was 436±41 mg/dl in the restrained mice given histamine at a dose of 50 mg/kg. The improved plasma triglyceride metabolism was well explained by the observations of the significantly up-regulated hepatic triglyceride lipase (HTGL) activity and mRNA expression in response to histamine. These results suggested that the effects of stress-induced histamine on lipid metabolic disorder in mice loaded with restraint stress arose from its anti-stress action and promotion of lipase activity.

Keywords: histamine, energy metabolism, restraint stress, lipid emulsion Intralipid®, triglyceride

Introduction

There have been a number of studies about the mechanisms of the symptoms caused by stress (1). Energy metabolism is important in various stress reactions (2). Generally, a source of energy is required to sustain physiological functions of the brain, central nervous system, heart, and muscles, and so on. This energy supplement is mainly from dietary glucose, lipid, or from glycogen stores and fat deposits. Moreover, Ricart-Jané’s study (3) indicated that stress caused lipid energy-substrate redistribution between tissues, which was required for the fight-or-flight response. Other studies also indicated that stress limited the supply of energy, which lead to poor utilization of biological energy sources, neurological or tissue dysfunctions, and resulted in tiredness and various other physiological disorders (4, 5). Stress is also well known for its direct influences on the central nervous system (6). A large number of experimental evidences supported that the histaminergic system performs its physiological functions through H₁, H₂, and H₃ receptors (7). Recent studies suggested that the activities of histaminergic neurons are likely to increase the response to stress (8). On one hand, proper stress can enhance biosynthesis and the release of histamine (9). On the other hand, histamine has been implicated in a variety of responses to stress as well as noradrenergic neurons activity, prolactin, adrenocorticotropic, renin secretion, dopamine releases, and metabolism in different brain regions (10 – 14). Therefore, histamine plays an important role in mediating the activity of both the central nervous system and endocrine system (15).

Although the effects of stress in histaminergic nervous and the endocrine system have been widely reported (16), less attention has been paid to the influence of histamine on utilizing blood lipids in stress-loaded animals. Thus, we demonstrated the relationship between
stress and blood lipid metabolism using Intralipid® as a tracer for the simple fat emulsion clearance test in mice exposed to restraint stress. Intralipid®, an artificial lipid emulsion used to supply energy and essential fatty acids, is commonly used in clinical practice (17) and also as a tracer for blood triglyceride (18).

Materials and Methods

Materials

Histamine was purchased from Wako Pure Chemical Industries (Osaka). It was dissolved in phosphate-buffered saline (PBS) solution immediately before use. The 20% Intralipid® (lipid emulsion including 20% soybean oil, 1.2% lecithin and 2.2% glycerol) was purchased from Pharmacia AB Co. (Stockholm, Sweden). It was diluted with PBS to make a 10% soybean oil solution immediately before use.

Animals

Seven-week-old female Ku Ming (KM) mice were purchased from the Center for Laboratory Animal Science Research of Southern Medical University (Guangzhou, China). All mice were kept in a specific pathogen-free animal room under the controlled conditions of room temperature at 23 ± 1°C with a 12-h light-dark cycle (lights on from 06:00 to 18:00), and they were provided with standard laboratory diet and water. The animals were handled in accordance with a protocol approved by the animal care committee of Jinan University. All the animal experiments were conducted in accordance with the Guide for the Care and Use of Laboratory Animals as adopted and promulgated by the United States National Institutes of Health.

Measurement of histamine levels in plasma and brain regions

The effects of restraint stress on histamine secretion in plasma and brain regions were investigated. In the starved control group, food and water were deprived for 20 h. In the restrained group, mice were confined to an oval metal restraint cage for 20 h before the assay. Animals were handled in accordance with a protocol approved by the animal care committee of Jinan University. All the animal experiments were conducted in accordance with the Guide for the Care and Use of Laboratory Animals as adopted and promulgated by the United States National Institutes of Health.

Plasma lipids tolerance test procedure

The effects of restraint on triglyceride metabolism were investigated. Mice were divided into three groups. In the normal control group, no treatment was given to the mice. In the starved group, mice were deprived of food and water for 20 h. In the restrained and starved group, mice were confined to an oval metal restraint cage for 20 h before the assay. Intralipid® was injected through the tail vein at a dose of 0.1 ml/10 g of body weight. On the other hand, we investigated the effect of histamine on plasma triglyceride elimination. In the stress control and histamine groups, mice were confined to an oval metal restraint cage for 20 h while being starved before the assay. PBS solution or histamine were injected through the tail vein at a dose of 0.1 ml/10 g of body weight 35 min before the administration of Intralipid® to mice that had been restrained for 20 h while being starved. In the starved control group, only PBS solution was administered. Blood samples were taken from the heart under anesthesia with diethyl ether and kept in a tube containing 2% sodium heparin. Then, the blood samples were centrifuged at 5,000 rpm for 5 min at 4°C, and the supernatant was collected and stored at −20°C until the assay. Plasma triglyceride was measured by the glycerol kinase/glycerol-3-phosphate oxidase (GK-GPO) method (20). The assay kit was purchased from International Reagents Corporation.
Measurement of hepatic triglyceride lipase (HTGL)

Histamine (Sigma, St. Louis, MO, USA) was injected into two groups of mice through the tail vein at a dose of 50 mg/kg of body weight. One group was sacrificed at 30 min after the histamine injection, and another group was sacrificed at 20 h after the histamine injection. The hepatic tissue was quickly dissected out during the PBS solution perfusion and then weighed. A 100-mg sample of tissue was removed immediately from each liver and kept in PBS solution under an ice-cold condition. Then, the tissues were minced with scissors and homogenized with a homogenizer at the maximum setting for 30 s under an ice-cold condition in 1 ml of solution (containing 0.25 M sucrose, 1 mM ethylenediaminetetraacetic acid (EDTA), 10 mM Trizma hydrochloric acid (Tris·HCl), and 12 mM deoxycholate, pH 7.4). The homogenate was collected by centrifuging at 12,000 rpm 4°C for 20 min; it was diluted with 4 volumes of the homogenization solution and then decanted and immediately stored at −20°C until the HTGL activity measurement. The HTGL activity assay was carried out using the BALB-DTNB method as described previously by Kurooka and Kitamura (21).

Reverse transcription polymerase chain reaction (RT-PCR) analysis of HTGL mRNA expression

The mice were sacrificed at different times after the histamine was injected through the tail vein at a dose of 50 mg/kg. The hepatic tissue was quickly dissected out during the PBS solution perfusion. Total RNAs were extracted from the liver samples by using Trizol (Invitrogen, Carlsbad, CA, USA). A 3-μg amount of total RNA was reverse-transcribed at 42°C for 1 h in a 20-μl reaction mixture containing mouse Moloney leukemia virus reverse transcriptase (Tiangen, Beijing, China) with oligo (dT) primers (Tiangen) followed by RT-PCR amplification. Thereafter, cDNA was amplified together with Taq polymerase (Tiangen), using specific primers with 35 cycles at 94°C for 30 s, an annealing temperature of 58°C for 50 s, and then 72°C for 50 s, with final incubation at 72°C for 7 min. The RT-PCR primers for mouse HTGL mRNA were (F) 5′-TGGACGGAAGAAACAAG-3′ and (R) 5′-GGAGTCAATGAAGAGGTGC-3′, and the product size was 495 base pairs. The primers for the mouse housekeeping gene β-actin (β-actin) mRNA were (F) 5′-GAGGGAATTGTGCGTGAC-3′ and (R) 5′-GCTGGAAGGGTGACAGTGAG-3′, and the product size was 446 base pairs. The RT-PCR products were fractionated on a 1% agarose gel and visualized by ethidium bromide staining. The band intensity of ethidium bromide fluorescence was measured by using an image analysis system (Bio-Rad, Hercules, CA, USA), quantified by a Bandscan (Glyko, Novato, CA, USA) and expressed as the ratio to β-actin.

Statistical analyses

The data were each presented as a mean ± S.E.M. Significant differences between the two groups were analyzed with Student’s t-test. One-way analysis of variance (ANOVA) was applied to analyze differences in data of biochemical parameters among the experimental groups, followed by Dunnett’s test for pair-wise multiple comparisons. Differences were considered as statistically significant at P<0.05.

Results

Effect of restraint stress on histamine

We investigated the effects of restraint stress on the concentrations of histamine in plasma and brain regions by HPLC with a fluorescence detector. As shown in Table 1, the basal value of histamine obtained from the control mice was 5 ± 0.6 pmol/ml in plasma. When the mice were exposed to restraint stress for 20 h, the average plasma histamine value was significantly increased to 74 ± 9 pmol/ml (P<0.01). On the other hand, the histamine level was 596 ± 47 pmol/ml in the midbrain and was significantly increased compared with the level in the non-stress control mice (332 ± 43 pmol/ml, P<0.01). However, the effect of restraint on histamine was weaker in the cortex. The results suggested that the histaminergic neuron might play an important role in stress responses.

Effect of restraint stress on plasma triglyceride elimination

The metabolism of lipids was found to respond remarkably to the restraint stress in this study. Figure 1A shows the chronological changes of the triglyceride elimination rate in plasma after the injection of

| Table 1. Histamine concentration in the plasma and brain regions of KM mice exposed to restraint stress |
|---|---|---|---|
| Group | Plasma (pmol/ml) | Cortex (pmol/g) | Midbrain (pmol/g) |
| Starved control | 5 ± 0.6 | 159 ± 13 | 332 ± 43 |
| Restraint stress | 74 ± 9** | 182 ± 18 | 596 ± 47** |

In the starved control group, food and water were deprived for 20 h. In the restraint stress group, each mouse was starved and individually confined to an oval restraint cage for 20 h before the assay. The results represent the mean ± S.E.M. of the values obtained from 7 KM mice in each experimental group. **P<0.01: significantly different from the starved control mice as determined by Student’s t-test.
The average level was $422 \pm 40$ mg/dl at 35 min after the injection of Intralipid®. When the mice starved for 20 h, the elimination rate was higher at 35 min and the average level was $350 \pm 39$ mg/dl as shown in Fig. 1B. The plasma triglyceride level was $523 \pm 44$ mg/dl at 35 min ($P<0.01$), and the clearance rate was lower in the mice that were exposed to stress for 20 h than the starved mice tested in the same way (Fig. 1C). The results suggested a good correlation between the elimination of triglyceride in blood and the response to stress. It also indicated that plasma triglyceride metabolism was definitely disrupted by the stress and decreased the utilization of triglyceride as an energy source.

**Effect of histamine on plasma triglyceride elimination in restrained mice**

In this study, we also investigated the effect of histamine administered through the tail vein on the elimination of plasma triglyceride in restrained mice. As shown in Fig. 2, when mice were fixed in the restraint cage for 20 h, the amount of plasma triglyceride level at 35 min after the injection of Intralipid® increased from $350 \pm 39$ to $523 \pm 44$ mg/dl ($P<0.01$). The concentration of plasma triglyceride was $463 \pm 47$ mg/dl in the low-dose histamine (25 mg/kg) group, $436 \pm 41$ mg/dl in the middle-dose histamine (50 mg/kg) group, and $423 \pm 43$ mg/dl in the high-dose histamine (100 mg/kg) group, showing an improvement by 11.5, 16.7 ($P<0.01$), and 19.2 ($P<0.01$), respectively, compared with the stress control group. Results indicated that plasma triglyceride metabolism in these mice under restraint stress was improved by the histamine, and the effects were dose dependent.

**Effects of histamine on HTGL activity in hepatic tissue**

Figure 3 shows the alteration of HTGL levels in hepatic tissue after administration of histamine. The basal value was $1421 \pm 357$ U/g in normal mice. The amount of HTGL in the starved control mice was $1603 \pm 263$ U/g. When histamine was given for 30 min, the HTGL activity was $1674 \pm 352$ U/g. However, when treated with histamine for 20 h, the HTGL activity significantly increased to $2147 \pm 304$ U/g compared with the basal value ($P<0.01$). The results showed that histamine moderately affects the HTGL levels in hepatic tissue.
Effects of histamine on the mRNA expression of HTGL in hepatic tissue

The mRNA expression of HTGL in mouse liver was determined by RT-PCR. Figure 4 shows the chronological changes of mRNA expression after the injection of histamine. Compared with the normal control group, there was no significant increase of HTGL mRNA expression in the starved group. As shown in Fig. 4A, compared with the starved control, the expression significantly up-regulated after 50 mg/kg histamine was injected ($P<0.01$). When the histamine was given for 30 min or 20 h, HTGL mRNA expressions were both remarkably up-regulated (Fig. 4B).

Discussion

Compared with the starved control mice, we found that the plasma triglyceride elimination rate was definitely prolonged in the restraint stress group. Generally, stress loading requires a large quantity of carbohydrates and fatty acids as an energy source from liver glycogen and adipose tissues (18). Researchers demonstrated that stress reduces production of insulin and causes energy metabolic decline (19). As the decline in energy metabolism is a cause of fatigue from stress (20), this means improvement of the energy metabolism can promote fatigue recovery and stress relaxation. These studies supported our results by suggesting that stress limits the supply of energy to organs, leading to poor utilization of biological energy. Therefore, the elevated levels of plasma lipids in the restrained mice may be a reflection of the degraded tissue function, while the decline in the elimination rate of plasma lipids may be a sign of stress.

On the other hand, we investigated the effects of stress on histamine levels in plasma and brain. When mice were fixed in a restraint cage for 20 h, plasma histamine concentration significantly increased and showed an upward tendency in the cerebrum. Studies indicated that the histamine was mainly released from mast cells and enterochromaffin-like cells (21). Neuropeptides, such as substance P and neurokinin A, might stimulate histamine release from mast cells (22), and these neuropeptides were mediated by stress (23). All the above suggested that histamine was implicated in the host stress response. Previous studies indicated that stress enhanced the production of hormones, which was likely required for tolerating the life-threatening effect of stress (24). Studies also demonstrated various stressors had effects on the autonomic nervous system, and the activity of histaminergic neurons is likely to be increased in response to stress (25). However, less attention was paid to the role of stress-induced histamine in plasma lipid utilization. In the present experiment, we investigated the effects of histamine on the basic metabolism of plasma lipids in mice loaded with restraint stress and found that loading with histamine remarkably increased the elimination rate of plasma triglyceride. The effect of histamine seemed to be
improving lipid utilization as an energy source. Since the direct influences of histamine on the mechanism of lipid utilization has not yet been systematically investigated, HTGL activity measurement was performed to record the changes of liver lipase following histamine treatment. HTGL is the rate-limiting enzyme for hydrolysis of triglyceride and thus controls the uptake of fatty acids into tissues (26). The present results showed that the lipase level in hepatic tissue improved by 33.9% (P<0.01) after peripheral loading with 50 mg/kg of histamine for 20 h. When the same dose of histamine was given for 30 min, the HTGL activity was not increased significantly, but HTGL mRNA expression was significantly up-regulated (P<0.01). Therefore, histamine promoted lipid metabolism by increasing lipase activity, which might be due to the markedly up-regulated lipase mRNA expression in response to histamine.

Our results also implicated that histaminergic neurons may be one of the factors controlling the stress-induced physiological stress-response. Many researchers indicated that there is innervation of histaminergic neurons in a number of brain regions (27) and this might be important in the response capacity of animals to stress (28). Histaminergic neurons could modulate energy imbalance during stress through central histaminergic neuron receptors (29). The histamine H3 receptor has been demonstrated in association with stress-induced brain activity (30, 31). Some reports suggested that there are changes of histamine contents and histamine H1 and H3 receptors in the brains of mice subjected to food-deprived stress and revealed that there is a discrepancy between the levels of H1 and H3 receptors in the acute and chronic phases of stress conditions (8). Also, histamine nerve systems played a crucial role in maintaining homeostatic energy balance (32, 33). In our studies, restraint stress increases histamine turnover in the brain, and in turn, histamine up-regulates HTGL expression in hepatic tissue. The latter may optimize lipid metabolism, improve metabolic dysfunction, and increase utilizing triglyceride as an energy source. Therefore, the effect of histamine may be also partly related to the alleviation of the adverse effects of stress on bio-homeostasis.

Based on our findings, restraint-induced adaptability of plasma histamine improves stress-suppressed energy metabolism. These results suggested that the effects of histamine on lipid metabolic disorder in mice loaded with restraint stress arose from its anti-stress activity and promotion of lipase activity.

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