EFFECT OF STRESS ON HORMONE LEVELS IN THE BLOOD SERUM OF RATS WITH ALIMENTARY OBESITY

Vladimir Vladimirovich Shotaev¹, Karina Ruslanovna Doshkhokloeva², Petimat Hozh-Ahmedovna Vakhidova³, Ruslan Aleksandrovich Dudkin⁴*, Daria Yurievna Kostiukova⁵, Stella Muratovna Ionova⁵, Marizhat Kamilovna Burzhunova⁶, Antonina Viktorovna Tsybulina⁵

1. Department of Therapy, Faculty of Medicine, North-Ossetian State Medical Academy, Vladikavkaz, Russia.
2. Department of Therapy, Faculty of Pediatrics, Rostov State Medical University, Rostov-on-Don, Russia.
3. Department of Therapy, Faculty of Pediatrics, Stavropol State Medical University, Stavropol, Russia.
4. Department of Therapy, Faculty of Medicine, North-Caucasus State Academy, Cherkessk, Russia.
5. Department of Therapy, Medical Institute, Tula State Medical University, Tula, Russia.
6. Department of Therapy, Faculty of Medicine, Saratov State Medical University named after V.I. Razumovsky, Saratov, Russia.

ARTICLE INFO

Received: 17 March 2024
Received in revised form: 11 June 2024
Accepted: 14 June 2024
Available online: 28 June 2024

Keywords: Obesity, Stress, Leptin, Adiponectin, Visfatin

ABSTRACT

Nowadays obesity is considered a complex chronic disease that poses a serious threat to health due to its associated cardiovascular and metabolic complications. One of the key regulators that can trigger the development of pathological processes in obesity is adipose tissue hormones – adipokines, among which leptin, adiponectin, and visfatin should be highlighted. To assess the degree of influence of a stable emotional stress state on the secretory activity of adipose tissue in the blood serum of obese rats, changes in the level of adipose tissue hormones - leptin, adiponectin, and visfatin were analyzed. It has been shown that a decrease in serum leptin levels in conditions of chronic stress can cause eating disorders, and the level of the hormone visfatin depends not only on the amount of adipose tissue. The results of the study suggest that chronic stress on the background of obesity accelerates the development of insulin resistance.


Introduction

Chronic stress, along with burdened heredity, a sedentary lifestyle, and an unbalanced diet, is one of the causes of obesity [1, 2]. It is well known that under stress, excessive release of the hormone cortisol occurs, which not only plays an important role in the adaptation of the human body but also contributes to an increase in appetite [3, 4]. Consequently, if the body experiences stressful effects for a long time, a vicious circle arises, leading over time to the development of obesity [5, 6].

Obesity is today considered a complex chronic disease that poses a serious threat to health due to its associated cardiovascular and metabolic complications [7, 8]. One of the key regulators that can trigger the development of pathological processes in obesity is adipose tissue hormones – adipokines, among which leptin, adiponectin, and visfatin should be distinguished [9, 10].
The main physiological role of leptin in the body is to regulate energy metabolism and body weight [11]. Leptin is often called the satiety hormone [12]. It is believed that it acts in the hypothalamus as a blocker of the synthesis and release of neuropeptide Y, which causes hunger [13]. Adiponectin, unlike leptin, performs a protective function, increasing tissue sensitivity to insulin, and has cardioprotective effects [14].

The physiological role of the hormone visfatin is being actively studied, and there is evidence that indicates its involvement in the development of cardiovascular pathology and metabolic changes in obesity [15-17]. Given that this hormone acts like insulin, it can be assumed that in obesity it is involved in the pathogenesis of insulin resistance, diabetes mellitus, and its complications [18]. Adipokines play an important role in the development of early vascular and metabolic complications, but there is no clear evidence of this to date [19]. Thus, the work aimed to evaluate the effect of a stable emotional stress state on the level of adipose tissue hormones: leptin, adiponectin, and visfatin in the blood serum of rats on a model of alimentary obesity.

Materials and Methods

To simulate obesity, a fat component was added to the vivarium diet for 3 months: 3 g of pork fat daily with an initial body weight of 200 g of rats. Stable emotional stress was induced by immobilization, placing animals in special narrow pencil cases daily for 60 minutes for 10 days [20]. All animals were divided into 4 groups (10 animals each):
- group 1 (control): rats that were on a vivarium diet (state of physiological norm);
- group 2 (stress): intact animals subjected to chronic immobilization stress;
- group 3 (obesity + stress): obese animals subjected to chronic immobilization stress;
- group 4 (obesity): animals with alimentary obesity.

At the end of the experiment, rats of all groups were decapitated using a guillotine, and peripheral blood was taken [21]. To assess the degree of stress exposure, the serum level of corticosterone (an analog of cortisol in humans) was measured using the enzyme immunoassay on the BioTekELx80 ELISA analyzer (USA), and the mass of the adrenal glands was recorded.

Since the activation of the process of free radical oxidation in cells occurs during intensive or prolonged stress exposure to the body, the indicators of the system "lipid peroxidation - antioxidant protection" (LP–AOP) were determined [22]. The intensity of LP was judged by a change in the level of its main product – malonic dialdehyde (MDA), the content of which was determined by reaction with 2-thiobarbituric acid [23]. The activity of superoxide dismutase (SOD), a key enzyme in antioxidant systems, was studied based on the degree of inhibition of the quercetin oxidation reaction [24]. The catalase activity (preventing the accumulation of hydrogen peroxide formed during the dismutation of the superoxide anion) was evaluated using a spectrophotometric method based on the ability of hydrogen peroxide to form a stable colored complex with molybdenum salts [25]. The extinction of the analyzed solutions was measured using an IFA analyzer, BioTekELx80 (USA). To confirm the correctness of the choice of a model of alimentary obesity, body weight was monitored throughout the experiment, and after decapitation, the animals were dissected and weighed for internal organs, and the percentage of visceral adipose tissue was estimated [26].

The levels of total cholesterol (TC), high and low-density lipoproteins (HDL, LDL), triacylglycerides (TG), glucose, amylase, and lipase enzymes were determined in blood serum using a BS-200 biochemical analyzer (China) using Randox reagents (Great Britain) and Diasens (Belarus) [27]. The content of total phospholipids in liver tissue was assessed using a method based on the detection of inorganic phosphorus formed during acid hydrolysis of phospholipids [28]. The levels of adipose tissue hormones (leptin, adiponectin, visfatin) were evaluated by enzyme immunoassay on a Chem Well analyzer (USA) using DRG test systems (Germany) [29]. The obtained data were statistically processed using the Statistica 12.0 software (USA). The normality of the distribution of indicators was checked using the Shapiro–Wilk test. The Student's t-test for independent samples or the nonparametric Mann–Whitney test were used for intergroup comparison. The results are presented in the form of an average value and a standard error of the average. The critical significance level (p) in testing statistical hypotheses in this study was assumed to be 0.05.

Results and Discussion

It was revealed that in rats of groups 2 and 3, the mass of the adrenal glands compared with the control was significantly increased (2.5 and 2 times, respectively). This fact can be explained as compensatory hypertrophy associated with the fact that the adrenal glands under chronic stress experienced significant functional stress [30]. In the same groups, the content of the main physiologically active hormone corticosterone, the main indicator of the rat body’s response to a stress effect similar to cortisol in humans, significantly decreased in the blood serum of rats (Table 1).

The data presented in Table 1 indicate the fact of depletion of stress regulation mechanisms, which is also noted by other researchers [31, 32]. When analyzing the indicators of the LP–AOP system compared with those in the control group, an increase in the content of MDA (p ≤ 0.05) in the blood serum of rats of all experimental groups was found. At the same time, a decrease in the activity of SOD and catalase was noted both in animals subjected to sustained emotional stress and in rats of the group 3 (Table 1).
A significant role in the occurrence of the observed changes could be played by developing metabolic acidosis, impaired oxygen transport, and a decrease in the production of major macroregion compounds [33]. At the same time, the decrease in the efficiency of the AOP system is most likely caused not only by a drop in the level of antioxidants or inhibition of anti-peroxide enzymes but also by blocking the processes of hydrogen supply under the action of, for example, metabolic poisons or other factors [34, 35].

In the group of experimentally obese rats, body weight monitoring showed an excess of this indicator by 18.8%, kidney weight increased by 26.0%, spleen by 33.0, and the amount of visceral fat by 80.0%. There was a significant (p < 0.05) decrease in the content of total phospholipids in liver tissue (by 47.0%) relative to the control. The TC level was 1,930 ± 0.174 mmol/L, which was 30.0% higher than their control values (1,468 ± 0.172 mmol/L). The obtained data can be explained by relative phospholipid deficiency due to impaired metabolic activity of the liver in obesity [36, 37].

Analysis of biochemical blood parameters showed that only in group 3 there was an increase in the levels of TC (0.98 ± 0.07 mmol/L in the control, 1.58 ± 0.07 mmol/L in the experimental group), LDL (0.1 ± 0.02 and 0.16 ± 0.02 mmol/L, respectively), and HDL (0.5 ± 0.055 and 0.8 ± 0.060 mmol/L, respectively) (p ≤ 0.05). Most likely, this is due to the regulatory action of gluocorticoids, which are known to increase the production of lipids by hepatocytes by enhancing the expression of the fatty acid synthase gene [38]. In the same group, a significant increase in glucose levels was observed compared with the control: 3.6 ± 0.18 mmol/L and 4.9 ± 0.20 mmol/L.

Data on the content of lipase and amylase enzymes are presented in Table 2. Under conditions of "pure" stress (group 2) and its combined effects with obesity, an increase in blood lipase and amylase levels (p < 0.05) was observed. This may indicate the onset of an inflammatory process in pancreatic tissue [39]. As is known, the development of pancreatitis is usually caused by the activation of pancreatic juice enzymes due to stress-induced regurgitation into the ducts of the contents of the duodenum, including bile [40, 41].

Table 2. Changes in the lipase and amylase activity in the blood serum under stable emotional stress of rats with alimentary obesity

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Group 1 (n = 10)</th>
<th>Group 2 (n = 10)</th>
<th>Group 3 (n = 10)</th>
<th>Group 4 (n = 10)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Amylase, U/L</td>
<td>973.8 ± 33.1</td>
<td>1470.6 ± 129.4</td>
<td>1789.2 ± 148.5</td>
<td>1076.5 ± 21.3</td>
</tr>
<tr>
<td>Lipase, U/L</td>
<td>23.8 ± 0.5</td>
<td>155.3 ± 14.2</td>
<td>211.2 ± 59.2</td>
<td>21.3 ± 2.6</td>
</tr>
</tbody>
</table>

* – p < 0.05

The study of the content of adipose tissue hormones showed that the level of leptin in blood serum significantly decreased in rats of groups 2 and 3, while in group 4 its amount was slightly increased (p ≥ 0.05) (Figure 1a). This fact in the latter case is explained by an increase in the volume of adipose tissue and an associated increase in the secretion of this hormone [42, 43]. In experimental individuals of all groups, there was a unidirectional tendency to decrease the amount of adiponectin in the blood (Figure 1b). There was also a negative correlation with a weak link between adiponectin and glucose levels in group 3. Chronic stress in the presence of alimentary obesity may accelerate the development of insulin resistance since it is known that a decrease in the blood content of adiponectin is directly related to a decrease in insulin sensitivity [44]. This assumption requires concrete confirmation within the framework of a more complete and focused study.
Figure 1. Changes in the hormone levels of leptin (a), adiponectin (b), and vistafatin (c) in the blood serum of rats with alimentary obesity under chronic emotional stress (n = 10 for each group). * – reliable differences from control (p < 0.05)

Interestingly, an increase in the level of visfatin in the blood was detected in rats from all experimental groups. It is especially clearly manifested in obesity and stress on the background of obesity (p < 0.05) (Figure 1c). Most likely, this fact is due to an increase in the amount of adipose tissue [45]. In conditions of chronic asthma, the concentration of visfatin in the blood serum was also increased, which may be due to more complex biochemical reactions during its metabolism [46]. There was also a positive correlation between corticosterone and visfatin levels in group 3. Probably, under stress, there is an increase in the expression of the visfatin gene by glucocorticoids. This is a possible mechanism involved in the secretion of hormones by adipose tissue [47-50].

Conclusion

Thus, the analysis of the obtained results allows us to conclude that a decrease in the level of leptin in the blood under conditions of chronic stress may be a factor that disrupts eating behavior. At this stage of research concerning the content of adiponectin in the blood, it can be assumed that chronic stress on the background of obesity accelerates the development of insulin resistance in tissues and organs of the body. The level of the hormone visfatin depends not only on the amount of adipose tissue, its secretion is based on a more complex mechanism and may be associated with increased expression of the visfatin gene by glucocorticoids.

Acknowledgments: None

Conflict of interest: None

Financial support: None

Ethics statement: The protocol for experiments with laboratory animals complied with the requirements of the European Convention for the Protection of Vertebrate Animals used for Experimental and other Scientific Purposes.

References


