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INFLUENCE OF "MILD" STRESS ON THE LEVEL OF BIOCHEMICAL MARKERS OF PATHOLOGY IN THE SUBMANDIBULAR GLANDS OF RATS

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Abstract

Background. Stress is one of the important causes of the development of pathological processes in the body. The salivary glands play a certain role in the pathogenesis of post-stress pathological reactions

Aim. To determine the state of the submandibular gland of rats after "mild" stress (−20 °C, 5 minutes) by such indicators as proteolysis, kallikrein-kinin system, and oxidation.

Methods. The state of proteolysis was assessed by the rate of casein hydrolysis (pH 7,6) according to the Kunitz method, the state of the kallikrein-kinin system was determined by the rate of BAEE (benzoyl-arginine ethyl ether) hydrolysis, and the oxidation activity was determined by the rate of ascorbic acid oxidation.

Results. It has been established that in rats after stress, the level of proteolysis increases (significantly after 24 hours) and the level of oxidation increases very strongly (almost 6 times). The activity of BAEE esterase does not significantly respond to "mild" stress.

Conclusion. The submandibular gland is sensitive to "mild" stress, increasing the activity of oxidation and proteolysis processes.

Keywords: stress; submandibular gland; proteolysis; oxidation; kallikrein.

Introduction

Stress is one of the main causes of almost all human diseases [1]. As a result of the action of stress-inducing factors (stressors), the cerebral cortex is excited, which sequentially causes the excitation of the sympathetic, parasympathetic, and endocrine systems [2]. Excessive production of adrenaline (noradrenaline), acetylcholine, corticosteroids, thyroid hormones, regulatory proteins of the liver, kidneys, and digestive glands creates conditions for the occurrence of serious metabolic disorders and physiological functions [3].

However, the moderate production of the above factors, which occurs under the influence of “mild” stress (short-term effect of a stressor that is not strong in its indicators), can be a mobilizing stimulus for the body [4].

Mobilization of the animal organism causes mobilization of metabolic systems (increased levels of glucose, free fatty acids, amino acids, antioxidants, and other physiologically active factors) [5, 6].

As shown by the research of Prof. A. P. Levytsky [7], a significant role in the processes of mobilization of the body is played by the salivary glands, in particular, the submandibular gland. It has been established that the submandibular gland produces a large number of various hydrolytic enzymes, in particular, proteolytic, which affect the state of histo-hematic barriers, the regulation of blood circulation, and the activation of the immune system [7].

The goal of our work was to determine the impact of “mild” stress on the state of the submandibular gland based on such biochemical indicators as the level of proteolysis, the activity of the kallikrein-kinin system, and the activity of oxidative processes.

Materials and research methods

The experiments were conducted on Wistar rats (males, 180-220 g), in which “mild” stress was reproduced by exposing the rats to a temperature of −20 °C for 5 minutes. After 5 and 24 hours, the rats were euthanized under thiopental anesthesia by total bleeding from the heart. The submandibular glands were isolated and the level of proteolysis in the homogenate of the latter was determined by the rate of casein hydrolysis (pH 7,6) (Kunitz method) [8], the state of the kallikrein-kinin system by the rate of BAEE (benzoyl-arginine ethyl ester) hydrolysis [9].

The state of oxidative processes was determined by the rate of oxidation of ascorbic acid [10]. The protein content in the homogenates was determined by the Lowry method [8]. Statistical processing of the experimental results was carried out by standard methods.

Results and discussion

Table 1 presents the results of determining the rate of casein hydrolysis at pH 7,6 by the Kunitz method. As can be seen from these data, "mild" stress causes activation of proteolysis (as measured by specific activity) 24 hours after stress.

Table 1. The effect of stress on proteolysis activity (casein, pH 7,6)
in the submandibular gland of rats

№ group	Group	Activity, ng/min·g	Specific activity, ng/min·mg protein
1	Control	8700±12205	1129±150
2	Stress , 5 hours	92334±13402 p>0,3	1099±138 p>0,3
3	Stress, 24 hours	116100±15107 p>0,05	1575±159 p<0,05

Table 2 shows that "mild" stress does not significantly affect the activity of BAEE hydrolysis.

Table 2. The effect of stress on BAEE-esterase activity
in the submandibular gland of rats

№ group	Group	Activity, nmol/min·g	Specific activity, nmol/min·mg protein
1	Control	212,4±31,2	3,01±0,55
2	Stress , 5 hours	235,7±29,8 p>0,3	2,91±0,49 p>0,3
3	Stress, 24 hours	197,3±30,2 p>0,3	2,62±0,50 p>0,3

Table 3 presents the results of determining the rate of ascorbic acid oxidation. It can be seen that "mild" stress significantly (by 6 times) increases the rate of ascorbic acid oxidation, which indicates a significant activation of oxidative processes in the submandibular gland (oxidative stress).

Table 3. The effect of stress on the rate of ascorbic acid oxidation in the submandibular gland of rats

№ group	Group	Activity, μg/min·g	Specific activity, μg/min·mg protein
1	Control	3,40±0,48	0,047±0,009
2	Stress , 5 hours	5,40±0,64 p>0,05	0,074±0,018 p>0,05
3	Stress, 24 hours	20,46±1,80 p<0,01	0,290±0,051 p<0,01

The figure shows the relative activity of all three biochemical markers of pathology.

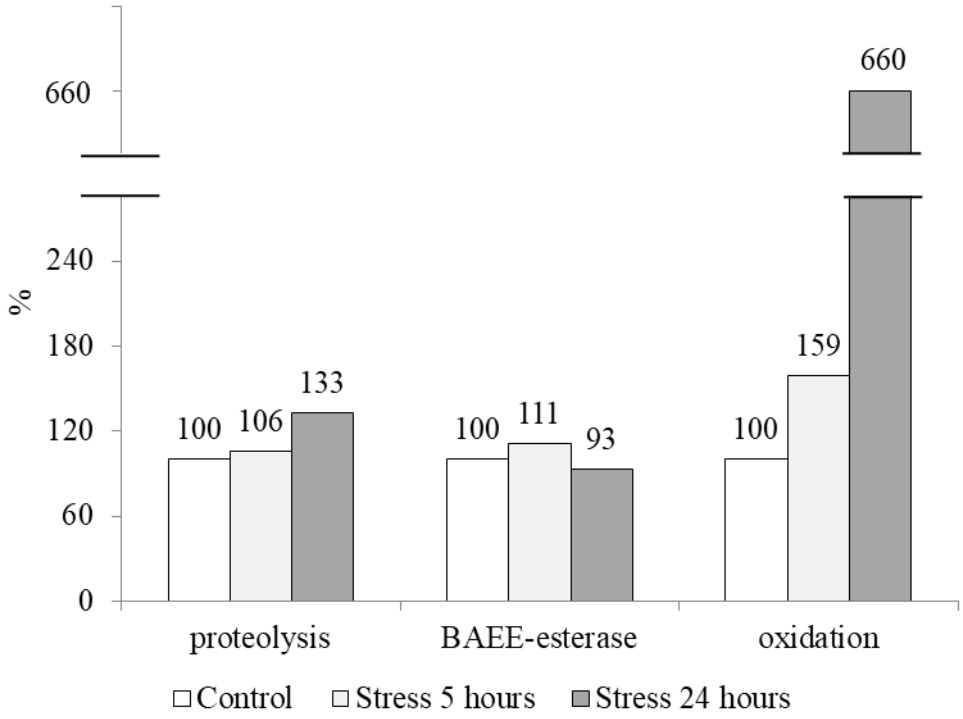

Fig. Relative levels of pathology markers in the submandibular gland of rats after stress (5 and 24 hours)

Conclusion

"Mild" stress causes a significant increase in oxidative processes in rats, which may lead to an increase in proteolysis in the submandibular gland.

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Informed Consent Statement

Informed consent was obtained from all subjects involved in the study.

Data Availability Statement

All information is publicly available and data regarding this particular patient can be obtained upon request from corresponding senior author.

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