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PROTEOLISIS STATUS IN THE ORAL MUCOSA OF RATS AFTER “MILD” STRESS

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Abstract

Background. Stress is one of the main causes of the development of most diseases. The role of proteolysis in the pathogenesis of post-stress reactions is not sufficiently elucidated.

Aim. To investigate the effect of “mild” stress on the state of proteolysis in the oral mucosa (OM).

Methods. Rats were subjected to “mild” stress by holding them at −20 °C for 5 minutes. In the OM, in blood serum, liver, kidneys and pancreas, proteolysis activity was determined by the rate of hydrolysis of two substrates: casein and BAEE (benzoyl-arginine ethyl ether).

Results. It has been established that OM has a high proteolytic activity, which begins to decrease already 5 hours after stress. In blood serum, on the contrary, the activity of proteolysis (substrate casein) increases after stress. The activity of BAEE-esterase does not change significantly after stress.

Conclusion. The level of proteolysis in OM is several times higher than the corresponding indicator for blood serum and rat liver. "Mild" stress causes an increase in the level of proteolysis in the blood serum as early as 5 hours after stress, while the level of proteolysis in the OM after stress tends to decrease. Activation of proteolysis in the blood after stress requires antiprotease actions using protease inhibitors.

Keywords: stress; proteolysis; oral cavity.

Introduction

Stress is one of the main causes of pathological conditions in the human body [1, 2]. Oxidative stress, which is a consequence of the excitation of the sympathetic nervous system [3, 4], and activation of the pituitary-adrenal system [2], play a crucial role in the pathogenesis of post-stress reactions.

Unfortunately, very little attention is paid to the excitation of the parasympathetic nervous system, which results in the activation of proteolytic systems that form biologically active substances that significantly increase the permeability of histo-hematological and intestinal barriers [5, 6].

The aim of our study was to determine the effect of “mild” stress on the state of proteolysis in the oral mucosa (OM). The state of proteolysis was assessed by the nature of changes in the hydrolysis activity of substrates such as casein and benzoyl-arginine ethyl ether (BAEE). The latter substrate is cleaved by trypsin-like proteases, among which kallikreins play a prominent role, resulting in the formation of kinins [7-9].

Materials and research methods

The experiments were conducted on Wistar rats (males, live weight 220±10 g), which were divided into 3 groups: 1st – control (intact rats), 2nd and 3rd groups – with “mild” stress, which was caused in rats by exposure to a temperature of −20 °C for 5 minutes. The animals were euthanized under thiopental anesthesia 5 hours after stress (2nd group) and 24 hours later (3rd group) by total bleeding from the heart. Blood serum was obtained, the oral mucosa, submandibular glands, liver, and kidneys were isolated, and the biomaterial was stored at −18 °C.

In tissue homogenate and blood serum, the activity of proteolytic enzymes was determined by the Kunitz method [10], using casein as a substrate, as well as the activity of trypsin-like proteases based on the hydrolysis of BAEE [7]. The enzymes that hydrolyze BAEE include kallikreins, which form kinins [7]. The protein content was determined by the Lowry method [10].

Statistical processing of the obtained results was carried out using generally accepted methods [10].

Results and discussion

The results of determining the proteolysis activity by casein hydrolysis at pH 7.6 were expressed in mg/minute of incubation at a temperature of +30 ° C. The activity of BAEE esterase was determined by spectrophotometric methods and expressed in nmol/minute of incubation.

The specific activity of proteolysis enzymes was determined per 1 mg of protein.

Fig. 1 presents the results of determining the activity of proteolysis (substrate casein), from which it is clear that the submandibular gland has the highest proteolytic activity, and the blood serum has the lowest. The oral mucosa is second only to the submandibular gland in terms of the level of proteolysis of casein.

Fig. 2 presents the results of determining the activity of BAEE esterase in various tissues of rats. It is seen that according to this indicator, OM takes the first place.

Fig. 3 presents the results of determining proteolysis indicators in serum and in OM homogenates of rats that received stress. As can be seen from these data, the level of BAEE-esterase activity in serum and in OM does not change under conditions of “mild” stress. At the same time, the activity of casein proteolysis significantly increases in serum after 5 hours and increases even more after 24 hours. In OM, the level of proteolysis decreases after stress (significantly after 24 hours).

Our results indicate that the main source of proteolytic enzymes may be the submandibular gland, in which the activity of casein hydrolysis at pH 7.6 is 25 times higher than this indicator in blood serum and almost 8-10 times higher than in other tissues.

It is not excluded that a possible route of transport of proteases from the submandibular gland into the blood may be their secretion into the oral cavity and absorption through the oral mucosa into the blood [7].

Given the significant role of proteolysis in pathological processes [5, 6], it can be considered advisable to use protease inhibitors to prevent complications that occur after stress.

Conclusion

1. The level of proteolysis in OM is several times higher than the corresponding indicator for blood serum and rat liver.

2. "Mild" stress causes an increase in the level of proteolysis in the blood serum as early as 5 hours after stress, while the level of proteolysis in the OM after stress tends to

decrease.

3. Activation of proteolysis in the blood after stress requires antiprotease actions using protease inhibitors.

Table. The effect of "mild" stress on proteolysis activity (substrate casein, pH 7.6) and BAEE-esterase activity in the oral mucosa (OM) of rats

Groups	Proteolysis		BAEE-esterase	
	Activity, μg/min·g	Specific activity, μg/min·mg protein	Activity, nmol/min·g	Specific activity, nmol/min·mg protein
1. Control	5,84±0,72	0,149±0,018	248,2±39,1	7,41±0,95
2. Stress, 5 hours	4,44±0,69 p>0,05	0,112±0,016 p>0,05	244,3±35,1 p>0,3	6,71±0,90 p>0,3
3. Stress, 24 hours	4,40±0,70 p>0,05	0,100±0,012 p<0,05	316,8±35,9 p>0,1	7,04±1,05 p>0,5

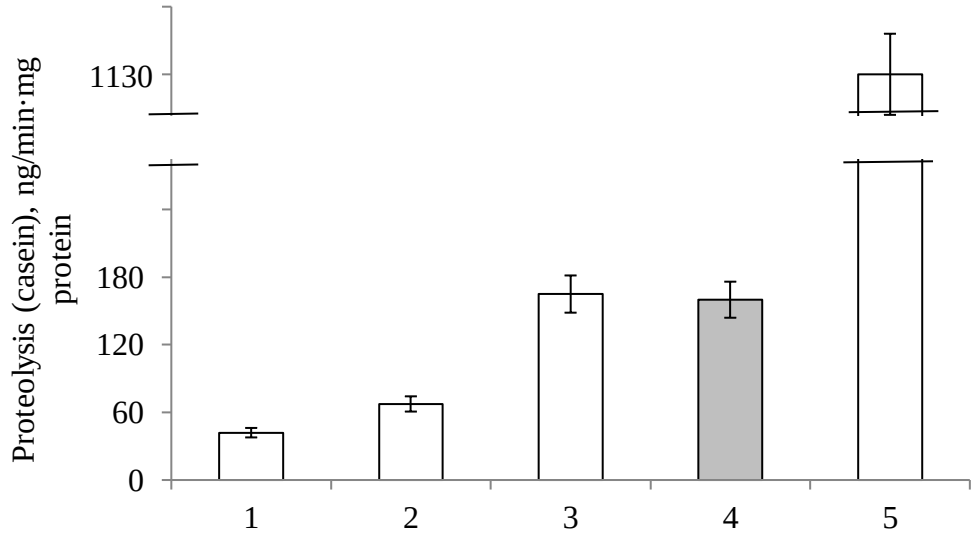

Fig. 1. Specific proteolysis activity in the rat body (1 – blood serum, 2 – liver, 3 – kidneys, 4 – OM, 5 – submandibular gland

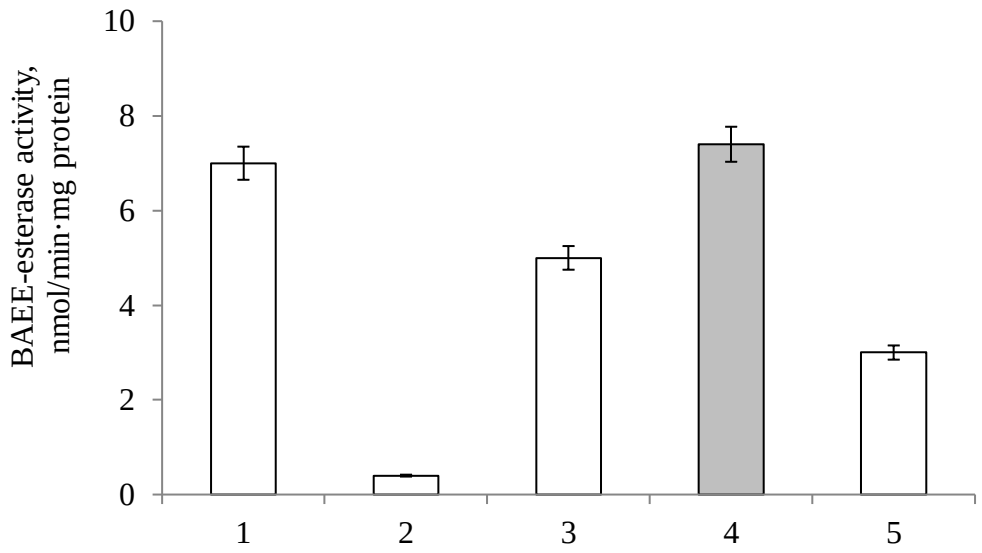

Fig. 2. BAEE-esterase activity in the rat organism (1 – blood serum, 2 – liver, 3 – kidneys, 4 – OM, 5 – submandibular gland

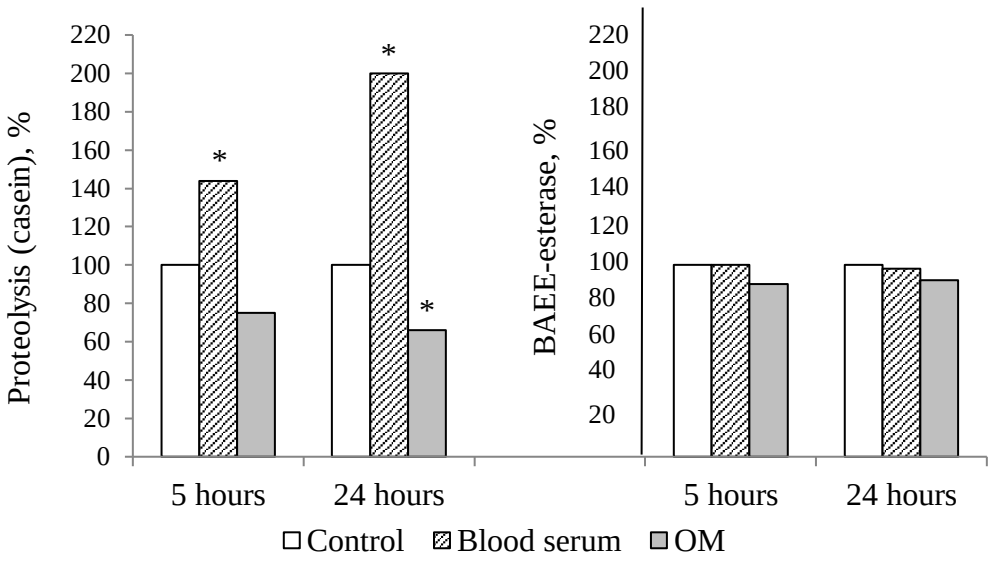

Fig. 3. Relative activity of proteolysis and BAEE esterase in blood serum and oral mucosa (OM) of rats after “mild” stress

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Data Availability Statement

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