ACTIVITY OF SALIVARY GLAND ENZYMES AND ITS IMPORTANCE IN SIALODIAGNOSTICS

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Abstract

The homeostasis of enzymes and the secretion of enzymes by the salivary glands were studied when rats were exposed to gamma radiation.

The results obtained show that gamma irradiation sharply reduces the secretion of the enzymes amylase and pepsinogen in the salivary glands.

Changes in the enzymes amylase and pepsinogen in the blood and salivary glands under the influence of gamma radiation in one direction indicate the importance of the salivary glands in enzyme homeostasis.

Keywords: Salivary glands, enzymes, secretion, recreation, gamma radiation, state of homeostasis, enzyme, homeostasis, gamma radiation, salivary glands.

Introduction

The salivary glands participate in maintaining the enzymatic homeostasis of the body through exocrine, endocrine and recretory activity; the state of enzymatic homeostasis can be determined by studying the composition of saliva. Three pairs of large (pre-nasal, submandibular and sublingual) and many small (located on the tongue, molars, lip, tongue, hard and soft palate) salivary glands open into the oral cavity. Histologically, the human salivary gland has a multi-branched tubular-alveolar [44] structure. They consist of secretory (glandulocytes) and myoepithelial parts. Depending on the content of the secreted saliva, glandular cells are divided into serous (serocytes) and mucous (mucocytes). They form three types of glands - serous, mucous and mixed.

Saliva contains high-molecular compounds, including proteins. Salivary proteins consist of albumins, globulins (1.4-6.4 g/l) and free amino acids (0.2-0.4 g/l). It has been established that the protein composition of saliva depends on the nature of the effect that induces secretion. When tickling the sympathetic nerve, salivation slows down, but its salivation and amylolytic activity increase. When tickling the parasympathetic nerve, opposite changes are observed [29].

The nature of salivary proteins has not been sufficiently studied. It has been found that salivary proteins can move through the blood [20, 26].

Secretion of proteins through the digestive glands is not a secretory process, but their transudation from the blood through the vascular wall [45].

The oral cavity is the "entry gate" of nutrients, water and other substances into the body. The first endogenous fluid that these products encounter in the body is the oral fluid, called mixed saliva [29]. Oral fluid has many properties, mainly digestive properties [8, 10]. Digestion in the oral cavity is the initial stage of a complex conveyor biotechnology of digestion. Food entering the oral cavity undergoes mechanical, physicochemical and chemical processing. Saliva is very important in these processes, it moistens, dilutes, dissolves the nutrient and its components, hydrolyzes the polymer substrate under the action of enzymes. Carbohydralase [29], which breaks down polysaccharides in saliva, especially in the saliva of the parotid gland: α-amylase, breaks down polysaccharides (starch, glycogen) first into dextrins, then into maltose and glucose. Salivary amylase has an optimal pH of 6.5-7.0 and loses activity when the pH decreases below 3-3.5. α-amylase attaches to the substrate (starch, oligosaccharides) for the degrading effect of reduced pH endurance increases. This is of great importance in the mechanism of maintaining salivary amylase activity in the stomach and small intestine [25]. A short 12-15 second stay of food in the oral cavity is not enough for salivary carbohydrolases to hydrolyze poly- and disaccharides; they exert their effect on polysaccharides until the nutrients reach gastric hydrochloric acid [21, 30]. Thus, these salivary enzymes are important not only in the oral cavity, but also in the stomach.

The amylase content in the saliva of one-month-old children is low, and at the age of 1-2 years it is equal to the activity in the saliva of adults [43]. The increase in salivary amylase activity is uneven, associated with teething in children and reaches a maximum at the age of 2-7 years.

Salivary amylase was separated into isoenzymes by the isoelectric focusing method [55] and two amylase fractions were determined. Some authors have formulated amylases of the parotid salivary gland, pancreas and human blood serum in connection with their different electrophoretic activity, catalytic activity, thermal stability and activation energy. The optimum pH of the pancreas is 7.2, pH of parotid gland amylase is 6.8. Secretion of saliva and its amylolytic activity are significantly reduced during the period of deficiency of secretory activity of chronic pancreatitis [35]. Salivary amylase can be its own, pancreatic or hepatic, and their ratio varies depending on the state of the cell membrane and the functional state of the gland, the

amount of amylase in the blood at a certain point in time and other reasons [54, 58, 59, 64, 69, 73].

Ligation of the excretory duct of the parotid gland leads to a decrease in amylase activity in the glandular tissue. The secretion of amylase in the parotid gland is influenced by the sympathetic nervous system, the gland's adrenergic receptors are of the alpha type [47]. Parasympathetic (innervation) stimulation induces non-granular secretion of amylase, compensating for the consumption of amylase [48]. Other enzymes, in addition to amylase, are also secreted in saliva, but they have not received much attention from researchers. Mixed saliva has some proteolytic activity (glandylain, salivalin, cathepsin), their participation in proteolysis is small [29]. Kodirov Sh.K. [18] showed in an in vitro study that a protein incubated for a certain time in saliva is more easily hydrolyzed by pepsin and trypsin. Another study [16] showed that breast milk breaks down into small pieces when in contact with the infant's saliva, and then when exposed to gastric juice.

BAEE-esterase activity of mixed human saliva was determined in normal subjects and in patients with periodontitis. According to the results of the examination, BAEE activity was detected in 38% of healthy individuals and 41.2% of patients with periodontitis. An increase in antitryptic activity by 1.5 times was established in periodontitis [9].

Kallikrein-like protease exists in the form of four isoenzymes. Unlike salivain and glandulain, kallikrein-like peptidase has a significantly higher proteolytic activity. Both exocrine and endocrine secretion of kallikrein is enhanced by exposure to high temperatures [49].

The importance of kallikrein is manifested in its hypotensive effect, causing functional hyperemia and increasing capillary permeability. When kallikrein is introduced into the stomach of rats, it inhibits the secretion of amylase in saliva, reduces the activity of DNase and BAEE esterase [32].

In the parenchymatous cells of the salivary gland of the cat's jaw, the presence of three different enzymes was found: acid phosphatase, peroxidase and kallikrein. When tickling the sympathetic nerve of the mandibular gland, an increase in the amount of secreted juice, a decrease in the content of protein, acid phosphatase and kallikrein in it, and a sharp increase in the activity of peroxidases were observed [51].

Andijan physiologists proved that pepsinogen secreted by saliva is produced in the stomach [20, 21].

There are different opinions on the release of alkaline phosphatase into saliva in various pathological conditions. Kovalyuk I.S. and Smolyar N.I. [17] found that alkaline and acid phosphatases decrease in caries. Other authors [11] found that the

activity of alkaline and acid phosphatases increases twofold in women working with pesticides at high temperatures and humidity. Barabash R.D. According to [15], alkaline phosphatase is actually produced only in the minor salivary glands located in the mucous membrane. Alkaline phosphatase is high in the saliva of hypersthenic women [13]. Other enzymes have also been found in saliva: mucinase, collagenase, carbonic anhydrase, transaminase, aldolase, ribonuclease. Saliva contains many proteins that interact with the microbial colony. Salivary lysozyme, lactoferrin, peroxidase, and immunoglobulin A are well known. These proteins have an antimicrobial effect in vitro [57].

Human saliva has an antiviral effect [54]. Saliva contains the enzyme lysozyme (molecular weight 19,000–15,000), which has bactericidal and anticaries properties. The enzymatic activity of lysozyme is manifested in the hydrolysis of α-(1-4)-glycosidic bonds in polysaccharides (and mucopolysaccharides) that retain formic acid. Mucopolysaccharides are present in the cell membrane and chitin of the microorganism. Due to its enzymatic properties, lysozyme is called muromidase. The high amount of lysozyme in saliva and tears, the oral cavity and barrier organs indicates the participation of lysozyme in the creation of a barrier against bacteria and natural antimicrobial immunity [12]. Saliva contains from 200 µg/ml to 500 µg/ml of lysozyme, which is 3-3.5 times higher than this activity in chicken protein of humans and dogs. Muromidase activity in mixed human saliva can be seen even at a dilution of 1:80. Lysozyme activity in the frontal gland of hamsters is 25-100 times higher than in other organs [4].

A small amount of lipases is secreted in saliva [41], and their significance in general lipolysis is very insignificant [29]. Lipase from the glands of the tongue of rats differs from lipase of the pancreas in that it does not adapt to the composition and amount of the diet [47]. Neurotransmitter control of lipase and amylase secretion in the glands of the tongue, mainly cholinergic ones, has been discovered [52]. Compared with thin women, lipoa is secreted in greater quantities into the mixed saliva of overweight women [39]. A number of studies are devoted to changes in the secretion of saliva and the content of enzymes when the body is in various functional states. Performing static work reduces the secretion of saliva, lowers its protein content and viscosity. Under the influence of physical work, the secretion of the salivary glands decreases and the amylolytic activity of saliva increases [18, 31, 35]. With an increase in the ergometric load by a certain value, a decrease in the secretion of saliva, sodium, potassium, chlorides, the flow rate of pepsinogen and an increase in the flow rate of lipase were observed [31]. Before the start of dynamic or static work, salivation at the start

increases, and during muscular work this activity is inhibited. Amylolytic activity and viscosity of saliva changed during emotional stress and physical exercise [48].

Under the influence of hypokinesia, destructive changes occur, with moderate hyperkinesis - reactive shifts in some links of the mechanisms controlling the digestive organs. Under the influence of hyperkinesis, the activity of phosphatases in the nervous structures of tissues and organs first decreases and then normalizes. At the same time, the amount of catecholamines decreases reliably. With hypokinesia, on the contrary, the amount of catecholamines increases sharply. Changes in the opposite direction are observed in the activity of acetylcholinesterase. Smirnov A.V. and co-authors [40] studied the effect of immobilization stress on the structure of the nucleus accumbens, placing 21-day-old white rats in narrow cages for 15 days. It was found that control rats of the corresponding age, placed in normal, spacious cages, had sufficiently mature neurons in the motor nuclei of the vagus nerve. Neurons are densely located in the nuclei of the solitary tract, their sizes are determined as small compared to those in the motor zone. On the 15th day of immobilization stress, pyknotic neurons with sharp basophilic cytoplasm were observed in the motor nucleus. On the 15th day, it was found that the neuropil components of the solitary tract structure did not mature compared to the control. Thus, under conditions of immobilization stress, histological changes were observed in the motor nuclei of the vagus nerve and the nuclei of the solitary tract of growing rats. The effect of 20, 60, 90 and 120-day hypokinesia on lipase secretion, incretion and restoration of the pancreas in rats was studied. According to the results of the study, significant changes in the direction of BPD were observed after 60, 90 and 120 days of hypokinesia - the lipase content in the pancreas decreased, its amount in the salivary gland, gastric mucosa and blood increased; 20-day hypokinesia did not affect the above indicators [55].

7-day hypokinesia causes specific reactions in organs associated with movement (bones, skeletal muscles, myocardium) and non-specific changes in other organs, such as the digestive tract. Histochemical decrease in glycoproteins in the salivary glands, stomach, small and large intestines and increase in leucine aminopeptidases, acid and alkaline phosphatases in the intestine are observed. Increase in plasma corticosterone levels is also observed. Similar histochemical modifications were observed in adrenectomized rats, in which plasma corticosterone levels were significantly reduced. Therefore, the secretion of these glycoproteins and enzymes does not depend on the reaction of glycoproteins [17].

Nociceptive effects on experimental animals reduce the secretion of enzymes into saliva. After the cessation of the nociceptive effect, the activity and output of salivary enzymes increase, and the duration of their aftereffect varies [14]. Static stress reduces

amylolytic activity in rat submandibular and sublingual salivary gland homogenates, tryptic activity of the parotid glands, acid and alkaline phosphatase activity in all three glands, and pepsinogen content [18].

Ten-day hypokinesia increases the activity of amylolytic, tryptic and alkaline phosphatase, as well as pepsinogen content in rat salivary gland homogenates [18].

Under the influence of hypokinesia, a decrease in the weight and mass of the anterior part of the ear, especially the submandibular and sublingual salivary glands, depends on morphofunctional changes in the body and is directly related to the duration of hypokinesia [2].

Secretion of amylase in the salivary glands increases under the influence of hypokinesia. This indicator has maximum values on the 5th day of hypokinesia of the parotid gland and on the 20th day of hypokinesia of the submandibular and sublingual salivary glands [3].

The parameters of the internal environment of the body are reflected in the amount and composition of saliva. This is especially evident in water-electrolyte homeostasis-dehydration in the form of decreased salivation and an oral thirst period [37, 45], which, in turn, induces behavior that overcomes ectophagy of water deficiency. The ratio of electrolytes in saliva directly depends on their amount in blood plasma [10].

Environmental factors such as temperature, air humidity, heat and ultraviolet radiation of the Sun, affecting the body, together form climatic effects.

One of the climatic factors affecting the climate is high temperature, which is of great importance. Under its influence, water-salt metabolism is disrupted, resulting in significant changes in the activity of the cardiovascular system, digestive system, excretory system, as well as in the morphological composition and properties of blood [34, 45]. High external temperature and insolation primarily affect the mechanisms of thermoregulation, which redistribute water and mineral salts in the internal environments of the body and require the removal of water from the body. Dehydration in these cases is not only a trigger for physiological adaptation mechanisms in the body, but also determines their further direction [29].

It has been proven that the salivary glands participate in the water-salt metabolism in the body under conditions of high temperatures outside [37, 45]. With a lack of water, the secretion of saliva decreases sharply; 10-15 hours after drinking water, blood viscosity first normalizes, and then the secretion of saliva returns to its previous level. General histological, histochemical, morphological and morphometric studies conducted on adult rats show that morphological changes in the body depend on the degree of heating. The liver, pancreas and large salivary glands were examined in rats. They found that the area of the actively working capillaries of these organs serves as a

reliable indicator of the degree of heating. The active area of the capillaries initially increases during the period of excitation, then increases during heat stroke, and after death caused by heat stroke, the active area of the capillaries reaches its maximum level. In addition to this indicator, each organ has its own symptoms that determine the degree of heating of the body. Histochemical methods were used to determine the absence of glycogen and a decrease in thermolabile enzymes in the liver of heated animals. An increase in the number of exocrine cells and a decrease in the nuclear-cytoplasmic ratio were observed in the pancreas. It was found that the concentration of DNA in the nuclei increases, while RNA decreases in the cytoplasm. It was found that the size of serocytes in the salivary glands decreases and their nuclear-cytoplasmic ratio increases. The ratio of the volumes of the stroma and parenchyma of the salivary glands is greater than that of the pancreas, which in turn indicates the participation of the salivary glands in the body's response to high-temperature adaptation [7, 38].

Macroscopic, microscopic and morphometric experiments on adult male rats have shown that acute heating at 450°C in special thermal chambers results in: 1) edema of the connective tissue surrounding the maxillary gland and swelling of the capsule and stroma of the gland; 2) the number and degranulation of fat cells were observed; 3) thinning of collagen, reticular and elastic fibers; 4) venous hyperemia, an increase in the working capillaries and an expansion of their total cross-sectional area, blood stasis; 5) a decrease in the size of acini, especially serous exocrinocytes, serous cells, vacuolization of their cytoplasm; 6) an increase in the nuclear-cytoplasmic ratio due to an increase in the nucleus of exocrinocytes and a decrease in the size of the cytoplasm; 7) a displacement of the epithelial cover in the excretory ducts; 8) dynamic changes in the volume ratio of stroma and parenchyma: during the period of acute heating and in animals that died as a result, an increase in the volume of the gland's stroma and a decrease in the parenchyma were observed. Some of these changes indicate the participation of the jaw gland in the body's adaptation to acute heating, others indicate its altering effect of heating [6].

Makhmudov E.S. [33] showed that when the external environment changes, the internal environment of the body is controlled by releasing a large amount of fluid and electrolytes from inside the cell into the cellular tissue, while the total amount of water in the body does not change.

The amount of alpha-amylase in saliva, especially norepinephrine, serves as an indicator reflecting the level of catecholamines in the blood plasma under various stresses (physical stress, rest and a written test) [50]. During acute psychosocial stress, alpha-amylase activity increases, and an increase in the level of alpha-amylase

in saliva indicates the stability of the circadian rhythm and reflects the level of cortisol in saliva. These data suggest that alpha-amylase can act as an indicator of the sympathoadrenal medullary system.

The homeostatic function of the salivary gland consists not only in the removal of endogenous and exogenous substances from the blood, but also in the endocretion of its own products into the blood [22, 25, 28]. Physiologically active substances synthesized in the salivary gland, including α-amylase, circulate in the blood. The salivary glands, like other digestive glands, have both exocrine and endocrine properties [44].

The salivary glands, like other digestive glands, participate in the homeostasis of the body, removing products secreted in the juice produced by it into the internal environment, and separating metabolites circulating in the blood in the juice, participating in the excretory activity of digestion [8, 27, 28, 29]. The implementation of this process depends on the permeability of the hematosalivary barrier, as well as the amount of secretion, on a number of factors: concentration and osmotic gradient of substances; solubility of substances in lipoid membranes; size of secreted substances, etc. Of course, these factors are important for the removal and restoration of small and large molecular substances. These enzymes are the subject of our research. That is why there are several common aspects of the secrets of their recreation in digestive juices, including saliva. In the manual of VSM Biohimmak IBL – Hamburg group of companies [36], devoted to the description of steroids in saliva, several "mechanisms of penetration of blood components into salivary ducts through the membrane barrier" are mentioned: 1) – penetration of low-molecular weight substances (< 1900 D) between acinar cells; 2) – substances with a molecular weight < 400 D are filtered through the pores of cell membranes; 3) – selective transport through the cell membrane - a) passive diffusion of lipophilic molecules (e.g. steroids); b) active transport through protein channels (e.g. peptides); c) penetration of pinocytosis along the pinocytosis pathway, sequential endo-, then -exocytosis (e.g. "large enzyme-type peptides"). Consequently, selective transport of enzymes and hormones during their own recreation can be carried out according to three (a, b, c) types [29]. Glandulocytes secrete virtually any substance that has entered the glandulocyte from the intercellular fluid and blood, including endogenous substances, products of the activity of glands or other tissues. The juice may contain endocrine secretions of other glands or components of the juice of this gland; in fact, the same product of the glandulocytes can be secreted again, which is sometimes called resecretion. This can be considered as one of the types of recreation, since not only

secretory products are non-recreational, but also substances that circulate with the blood flow and enter the gland from the paraglandulocyte fluid [27].

Recreation is one of the mechanisms of stabilization of the cytoplasm composition, by means of which the products of various cellular activities are transported from the paracellular environment to various cells in certain quantities. Recreation is apparently the result of the evolutionary development of excretory activity, the most ancient activity of cells, their homeostasis, in particular, the evolution of participation at the level of multicellular organisms in ensuring the homeostasis of enzymes and hormones in the internal circulatory environment [29]. In addition, it is becoming increasingly obvious that the synthesis and secretion of physiologically active substances in them is a function not only of endocrine and neuroendocrine cells, but also of cells of other tissues and systems of the body [16]. Thus, it is easy to predict the presence in the endocrine secretion of glands of almost any substance circulating in the bloodstream. However, their transport to different types of glandulocytes occurs in unequal quantities, which is apparently determined by the type of membrane transporters [29]. Briefly, G. F. and his colleagues, based on many years of observation, came to the conclusion that enzyme secretion is a universal feature of exocrine digestive and non-digestive (sweat, milk) glands [3, 58, 70]. The salivary glands also have restorative capacity [27, 29]. Recent studies have confirmed that the salivary glands secrete not only exogenous and endogenous substances, but also high-molecular enzymes [20]. Thus, "pepsin", discovered in saliva by Munch in 1876, was considered an "excretor" by I. A. Bendersky. Uspensky Yu. N. [42] write that not only "his" but also pancreatic amylase is secreted in saliva. Kamakin N. F. [15] obtained experimental data on the secretion of pepsinogen by the salivary glands. Kodirov Sh. K. [18] showed the restoration of amylase, pepsinogen, trypsin, trypsin inhibitor, acid and alkaline phosphatase in the salivary gland in acute and chronic experiments conducted on dogs, as well as in observations of healthy and sick people.

Separate detection of pancreatic (R) and salivary (S) amylases in blood serum, saliva and oral fluid, firstly, confirmed the literature data on the presence of two types of amylase in the blood serum of one person [24], secondly, not only saliva (S), but also the pancreas (R) showed that α-amylase was also present. This proves the presence of pancreatic amylase in saliva, which is recreated from the blood by the salivary glands. It should be noted that the interpretation of the correlations of the concentration of physiologically active substances in the blood and saliva leaves the secretory activity of the digestive glands insufficiently distinguished from the debit of secretory products. This fully applies to the importance of determining not only their concentration in saliva, but also the rate of its flow. Meanwhile, the volume of saliva,

which is often the leading factor in the flow rate, depends even in the case of the standardized saliva method for many reasons. Thus, the volume of saliva depends largely on the hydration of the body [37, 45] and decreases sharply even with mild dehydration. Under these conditions, the concentration of many processed substances in saliva increases, and the speed of their flow may decrease.

Content:

The extreme variability of saliva parameters, depending on many factors, has already been repeatedly emphasized above. N. to the previously provided information. F. We can add the research materials of Kamakin's colleagues. Thus, it was noted that girls have more pepsinogen in their saliva than boys. The saliva of hypersthenics has an increased content of alkaline phosphatase [13]. In overweight women, lipase is secreted more into mixed saliva due to its deficiency [39]. It is assumed that these data reflect the features of metabolism in the "saliva diagnostic mirror" and ultimately reflect its physiological mechanisms. In general, it should be recognized that the study of the enzyme spectrum of saliva in clinical and laboratory practice is carried out less often than the study of enzymes of the stomach and duodenum, blood serum. It is difficult to agree with the validity of such an attitude to the gland, its secretion with its quantity affects the smallest changes in the external world and the internal environment of the body and is of particular importance in the formation of its physiological foundations. It can be concluded that the content of enzymes in saliva depends on the functional state of the body. The amount of saliva I.P. It is easy to imagine that if Pavlov had a high level of information content, his qualitative indicators, including enzymatic activity, should have been no less informative, and the reflex mechanism of saliva has great significance in its transformation.

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