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Protein Content of Human Saliva in Various Psycho-emotional States

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Abstract—The protein composition of human saliva depends on psycho-emotional state of individuals. Depression was accompanied by decrease of proteins of molecular masses ranging from 20 to 200 kD, whereas emotionally positive intellectual activity caused the opposite effect. It is suggested that human saliva may be used as an experimental model for the development of diagnostics of various psycho-physiological states.

Key words: proteins, saliva, electrophoresis, humans

In the beginning of the 20th century Russian physiologist I. P. Pavlov, using an animal model, provided convincing evidence that psycho-emotional state can regulate salivation processes [1]. Almost a century later it was demonstrated that change in psycho-emotional state in man may also influence biochemical composition of saliva. For example, some types of stress influenced saliva concentration of monoamine oxidase A inhibitor [2], kallikrein [3], and activity some antioxidant enzymes [4]. The content of secretory immunoglobulin A also depended on mood of investigated subjects [5].

Human saliva contains proteins exhibiting various biological activities. Some of them are involved in food digestion (amylase, maltase, peptidases, phosphatases, etc.), others (immunoglobulins, lysozyme, lactoferrin, histatins, staterins, cystatins, mucins, etc.) have protective functions in the oral cavity and gastrointestinal tract. Some proteins are also involved in the regulation of the cardiovascular system (kallikrein, histamine, renin, tonin), hemopoiesis (erythropoietin, thymocyte transforming factor, colony stimulating factor, etc.), and the nervous system (neuroleukin, nerve growth factor, etc.). Saliva also contains epidermal and mesodermal growth factors [6]. It has been shown that some somatic diseases affect the concentration of individual saliva proteins and the evaluation of the content of individual proteins and their biological activity may have certain diagnostic value [7]. The dependence of protein saliva content on a psycho-emotional state of human subjects has not been investigated yet.

In the present study we investigated the effect of psycho-emotional state on saliva protein content in the range of molecular masses from 20 to 200 kD.

MATERIALS AND METHODS

Samples of mixed saliva (i.e., the biological fluid formed during natural mixing of secretions of various salivary glands in the oral cavity) were used for analysis. These samples were obtained from human subjects subdivided into three groups: I) relatively neutral (normal) psycho-emotional state (lack of strong emotions) (n = 40); II) emotionally positive intellectual activity (n = 10); III) marked depression (n = 40). The first group was formed by volunteers, recruits, and students, and saliva was collected during medical examination. The second group was formed by researchers under conditions of creative activity (saliva was collected during discussion of results of scientific experiments). The third group was formed by patients of psychiatric clinics (saliva was collected at the day of their admission before the beginning of medical treatment). Psycho-emotional state of all examined subjects was evaluated by a psychiatrist.

Saliva samples (100 μl each) were centrifuged at 10,000 rpm for 10 min; the resulting supernatant was stored at −20°C until analysis.

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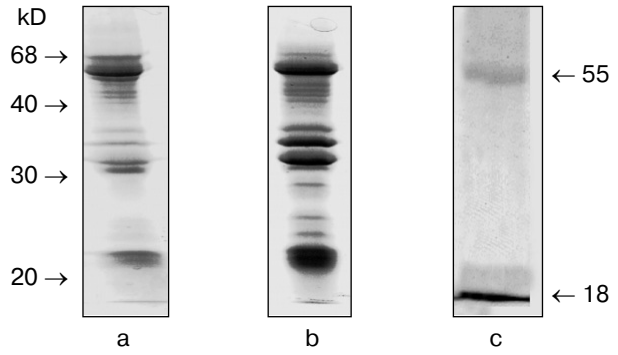

Electrophoretic analysis of saliva proteins from human subjects in various psycho-emotional states: a) relatively neutral (normal) psycho-emotional state; b) emotionally positive intellectual activity; c) depression. Molecular masses of individual protein markers are shown on the left

Saliva protein content was analyzed by electrophoresis in 12% polyacrylamide gel according to the method of Laemmli [8]. Before analysis saliva samples were diluted 1.5 times with 0.1 M Tris-HCl buffer, pH 7.5, containing 2% sodium dodecyl sulfate, 2% mercaptoethanol, 0.02% bromophenol blue, and 20% glycerol. The mixture was incubated 10 min at room temperature.

Results of electrophoresis were quantified by intensity of the protein band of ~55 kD at $\lambda = 650$ nm using a DM-1 medical densitometer.

RESULTS AND DISCUSSION

Saliva protein electrophoregrams of each group of the human subjects were characterized by some protein patterns typical for the particular group.

Saliva of the first group (characterized by relatively neutral psycho-emotional state) contained up to 20 protein fractions (figure, panel (a)). The protein fraction with molecular mass of 55 kD predominated. The mean value of absorbance of this fraction was 0.17 ± 0.01 unit.

Electrophoresis of saliva samples of the second group (characterized by emotionally positive intellectual activity) revealed up to 30 proteins (figure, panel (b)). Electrophoretic pattern of this group was characterized by significant increase of protein content with molecular mass <60 kD. The mean value of absorbance of 55 kD protein band was 0.30 ± 0.06 unit ($p < 0.01$).

Electrophoretic pattern of saliva proteins from the depressed psychiatric patients (third group) was characterized by significant reduction of proteins over the whole range of molecular masses studied (figure, panel (c)). The mean value of absorbance of the 55 kD protein band was 0.11 ± 0.01 unit ($p < 0.01$).

There were insignificant variations of protein fractions within each group, but they did not affect characteristic pattern of saliva protein content typical for these groups.

The data suggest that psycho-emotional state influences saliva protein content.

It is possible that the psycho-dependent change of saliva protein content is controlled by the vegetative nervous system regulating salivary gland functioning. However, comprehensive understanding of this process requires studies of independent effects of sympathetic and parasympathetic systems on regulation of protein content of secretions from each type of large salivary (parotid, submaxillary, and sublingual) glands.

It is also important to study functions of proteins, which are changed in various psycho-emotional states. It would be interesting to determine whether psycho-emotional states have any effects on synthesis and chemical modification of proteins in secretory cells of the salivary glands.

The results of the present work may have certain importance for the study of effects of various psycho-emotional states on protein content of various biological body fluids of human subjects and also for laboratory diagnostics of different psycho-emotional states of patients. Saliva is a convenient model system for the development of new laboratory tests. It can be simply and easily collected from patients without any psychological trauma or skin damage. Electrophoresis does not take much time and is also characterized by high sensitivity.

REFERENCES

1. Pavlov, I. P. (1923) *The Twenty Year Experience of Study of Higher Nervous Activity (Behavior) of Animals* [in Russian], Petrograd.
2. Clow, A., Doyle, A., Hucklebridge, F., Carroll, D., Ring, C., Shrimpton, J., Willemsen, G., and Evans, P. D. (1998) *J. Neural. Transm. Suppl.*, **52**, 71-77.
3. Smith-Hanrahan, C. (1997) *Can. J. Physiol. Pharmacol.*, **75**, 301-304.
4. Lukash, I. A., Vnukov, V. V., Kucherenko, A. O., Ananyan, A. A., Milutina, N. P., and Prokofiev, V. N. (1997) *Fiziol. Cheloveka*, **23**, 106-109.
5. Hucklebridge, F., Lambert, S., Clow, A., Warburton, D. M., Evans, P. D., and Sherwood, N. (2000) *Biol. Psychol.*, **53**, 25-35.
6. Sukmansky, O. I. (1991) *Biologically Active Compounds* [in Russian], Zdorov'e, Kiev.
7. Grigoriev, I. V., and Chirkin, A. A. (1998) *Klin. Lab. Diagn.*, **6**, 18-20.
8. Laemmli, U. K. (1970) *Nature*, **227**, 680-684.