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FUNCTIONAL CONJUGATION OF RBC ELECTROPHORETIC MOBILITY WITH BUCCAL CELLS AND MORPHOLOGICAL CHANGES OF ADRENAL GLANDS IN EXPERIMENT

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Abstract. In this paper we studied the RBC electrophoretic mobility, cytomorphological and cytogenetic parameters of buccal epithelial cells and morphology of adrenal glands under the influence of adrenalin which was injected to simulate a stress in rat body. It's established that an initial increase of the activity of brain substance neuroendocrinocytes (during the first hour) with further increase of the activity of the adrenal cortex (from 1 day) are combined with the change of RBC electrophoretic mobility index: at first it decreases and then it increases. Besides the cells with signs of cytotoxic changes appear. The absence of cells with karyopyknosis in stressed animal bodies indicates that the mechanisms of natural resistance of buccal epithelial cells preserve. It's associated with the development of adaptive processes.

Keywords: adrenal glands, RBC electrophoretic mobility, buccal epithelium, stress.

The study of endocrine system is of grand importance in the contemporary stage of medical science. This system provides the regulation of vital actions in a human body [1]. Adrenal glands are one of the most considerable components of the system because they are responsible for homeostasis sustentation during the development of stress reaction and pathologies [2]. Stress is a pronounced adaptive reaction but at the same time it may be the reason of neurotic, cardiovascular, endocrine, immune and other diseases [3]. At that, the morphological changes of adrenal glands in a pathogenic pathway of various diseases are insufficiently studied [4]. A clear picture of morphofunctional changes in adrenal glands is absent and it balks progress in diagnosis objectivization and in patient treatment. The study of functional state of adrenal glands in pathology and during therapy is difficult. It's based on hormonal state analysis and as a rule the patients are treated with medicaments while making analysis. That's why the research of any information technologies is of current importance. Such technologies should be easy to realize and they should reflect the objective state of adrenal glands. The RBC electrophoretic mobility may be used as an index which would characterize the activity of adrenal cortex. It was discovered during the experiment that there are regular variations of RBC electrophoretic properties which represents a response to stress and to the development of various pathologies. It's connected with the activation of sympathoadrenal and hypophysialadrenal systems [5, 6]. It's necessary to take into account that the activation of hypophysialadrenal system influences the immunological homeostasis of the organism [7, 8]. If the exposure is long-term it provokes the development of thymicolymphaticus involution and of cytotoxical changes in cells. It's shown that the genotoxic effects may be analyzed with use of buccal epithelial cells which permits to get information about genetic changes in human cells with minimal invasive intervention [9].

The aim of the work was to study the functional conjugation of adrenal gland morphology with RBC electrophoretic mobility and with cytomorphologic and cytogenetic indices of buccal epithelial cells in experimental stress.

Materials and Methods

60 white non-pedigree pubescent rats weighing 180-200g were examined. The research were carried out according to principles established in Universal Declaration of animal rights as well as according to the Order of the Ministry of the Health of Russia №119n of April 1, 2016 "Approval of the suitable laboratory practice".

The animals were separated into 2 equal groups. For stress modeling, the animals of the 1st group received a single intraperitoneal adrenalin hydrochloride solution (0,1 mg/kg). The intact animals were the 2nd, control group. The blood for analysis was taken from the sublingual vein in 1 hour, 1 day and 7 days after the adrenalin injection.

The RBC electrophoretic mobility level was measured by microelectrophoresis method by registering the 100 mkm washed rbc transmission time in tris HCL buffer with pH 7,4 and amperage 12mA [6]. The histologic specimens for optical microscopy were prepared by fixing in 10% neutral formalin solution for 72–96 hours, dehydration in alcohols with increasing concentration and subsequent paraffin-embedding. The 7 μm thick slices were obtained with SM2000R microtome (Leica, Germany) and stained with haemotoxylin and eosin. Specimen examination was carried out with DM1000 microscope (Leica, Germany), video images were captured using DFC290 camcorder (Leica, Germany).

The buccal epithelium analysis was made in 7 days after the adrenalin injection. It's caused by the moment of the exit of basal cells (there the micronuclei are generated) to the surface which is used in the analysis [10]. The scrapings of buccal mucosa were made with a spatula, the epithelial cells cytosmears were painted with Giemsa stain. The specimen analysis was made with microscope AXIOSTAR PLUS (Karl Zeiss, Germany), zoom 16×40 and 16×100 [11].

Statistical analysis was performed using BIOSTAT software. Student's t-test was used for comparison of two groups. The differences were considered to be significant at $p < 0.05$.

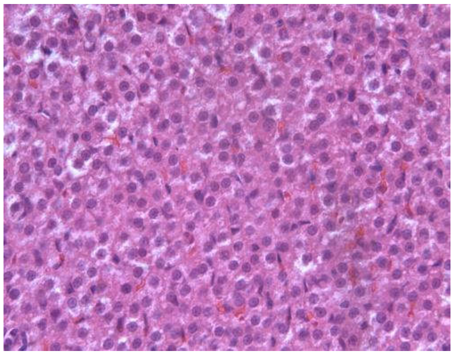
Results and Discussion

The analysis of histological samples of the structure of rat adrenal glands showed that adrenal glands of the intact animals had clearly defined cortex and medullary substance. The organ was encapsulated in thin connective tissue. The adrenal cortexes were clearly zoned. In zona glomerulosa, the small-sized endocrinocytes were assembled into spherical structures of coiled glomeruli. In zona fasciculate, the bigger polygonal endocrinocytes formed parallel cords. The nuclei of the endocrinocytes were big with well-defined nucleoli and optimal ratio eu- to heterochromatin. The border of zona reticularis and zona glomerulosa was comprised by a zone of small cells with big nuclei and more basophilic cytoplasm. This intermediate (sudanophobic) zone is responsible for the regeneration of cortical substance. Numerous hemocapillaries were located between the cell cords (Fig. 1A). The vessels of the medullary substance were plethoric (Fig. 1B).

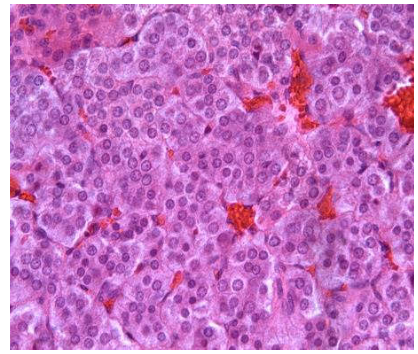
No significant morphological changes of adrenal glands in both "Adrenaline" and intact animal groups were observed one hour after the adrenalin injection. The organ retained clearly zoned. The epinephros capsule was not defibrated. Vacuolization was registered in both cortical (zona fasciculata) and medullar endocrinocytes. The intermediate zone was well-defined. The hemocapillaries of cortex and medullary substance were plethoric. As for cortex substance, it was well defined, especially in reticular zone. (Fig. 1C, D)

One day after adrenalin injection substantial hemocapillary expansion and plethora enhancement were registered. The vacuolization of endocrinocytes in cortical (zona fasciculata and zona glomerulosa) and medullar substance was observed. The border of zona glomerulosa and reticular zone lost its clarity. Local vacuolization of cells in zona glomerulosa and hemocapillary plethora were still detected one week after adrenalin administration (Fig. 1 E, F).

The RBC electrophoretic mobility index decreased after the adrenalin injection (Table 1). The maximal decrease was observed from the first till the 24th hour of the experiment. But it restored step by step up to the 7th day of the experiment. However, the index did not achieve the value of the control animal group.



A



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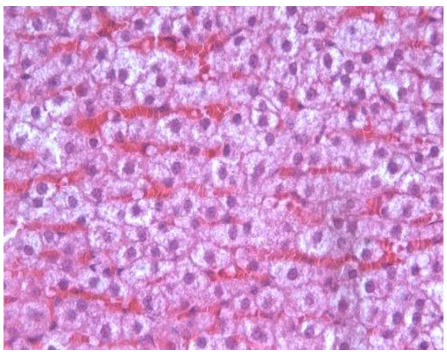
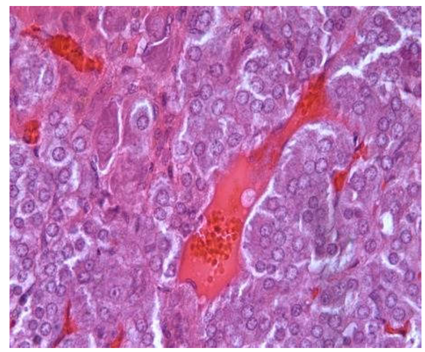


C



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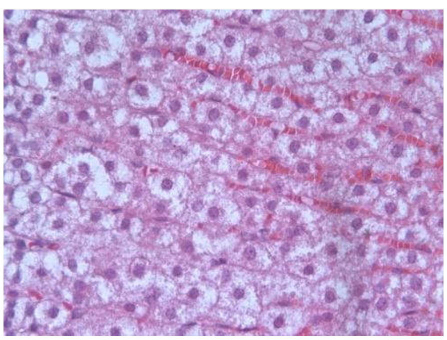
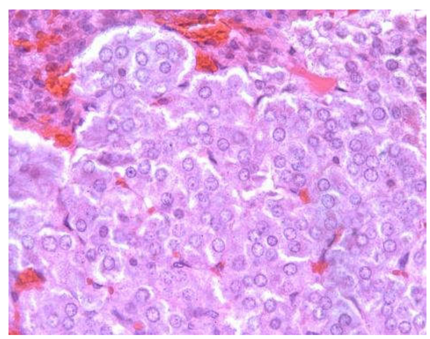


E



F

Fig. 1. The structure of cortical substance (zona fasciculata) (A, C, E) and adrenal medulla (B, D, F) of adrenal glands. A, B – intact animals. C, D – 1 hour after adrenalin injection. E, F – 7 day after adrenalin injection. Hematoxylin and eosin staining. Magnification, 400×

Table 1

Dynamic of RBC electrophoretic mobility index change ($\mu m\ cm\ B^{-1}c^{-1}$)

Kind of influence	Period after the influence		
	60 min	1 day	1 week
Adrenalin (single)	0,72±0,08*	0,83±0,09*	0,99±0,07*
Control	1,11±0,02	1,17±0,03	1,21±0,03

Notice: * statistically different from intact group, p<0.05.

Table 2

The particularities of buccal cell cytomorphology in experimented and control group (average number of cells, ‰)

Cytomorphologic indices	Experimented group	Control group
Normal value	685,21±70,59*	720,28±68,38
Cytogenetic markers		
Micronuclei	59,42±5,19*	51,67±6,49
Nucleus destruction markers		
cells with chromatin condensation	33,71±4,42*	30,65±4,58
cells with perinuclear vacuole	17,46±2,25*	12,21±2,28
Nucleous destruction termination markers		
karyorhexis	32,74±4,11	29,72±4,91
karyopyknosis	72,25±7,05	73,23±7,11
karyolysis к	99,21±6,39	82,24±6,25

Notice: * statistically different from intact group, $p < 0.05$.

The cytomorphologic researches of the animals revealed that the buccal cells had one or several micronuclei (Table 2).

The micronuclei represented round or oval blow or lilac masses with a smooth edge. The received values are certainly higher than those of the control group by 15%. Single cells with fragmented nuclei were recognized. Their contours are wrong, without any space orientation.

Cells with initial stage of nucleus destruction, cells with chromatin condensation (corrugated nucleus with condensed chromatin) and cells with renal cell vacuole are recognized very often in cytograms. The animals of the adrenalin administrated groups had more cells with condensed chromatin by 10%, more renal cell vacuole by 43% than the animals of the control group $p < 0,05$).

The analysis of results makes evident that adrenalin provokes the activation of adrenocortical system. The vacuolization of endocrinocytes in zona fasciculata and zona glomerulosa and vacuolated cytoplasm of neuroendocrinocytes means that the hormone number increases. It may be connected with their intensive use during the alteration of the organism [12]. But if adrenalin is injected to rats the staging in organism reaction and in its structural-functional organization is evident. At first the morphological markers of medulla neuroendocrinocyte increase (up to the first hour) and then the adrenal cortexes activity increases (up to the first day). It's probably connected with initial secretion of catecholamines and with the increase of the ability to synthesize corticosteroids. The plethora in cortical layer and renal medulla of adrenal glands means that the systemic response against the alteration is activated [13].

The morphological changes which were discovered during the experiment were correlated with the change of RBC electrophoretic mobility. The results of RBC electrophoretic mobility research were identic with findings received earlier: the decrease of RBC electrophoretic mobility is associated with the increase of cell adrenoreactivity whereas the RBC electrophoretic mobility increase is combined with the increase of cortisol concentration [14, 15].

The higher level of glucocorticoids may provoke the increase of chromosome aberrations [16]. The revealed forms of buccal cell necrosis up to the 7th day of the experiment make evident the destructive changes of nucleus membrane. The result of cell destruction necrotic process is karyolysis. The karyolysis is preceded with the renal cell vacuole appearance or cell vacuolization [10]. The research did not discover any statistically significant difference in karyolysis cell frequency between the animals of experimented and control groups. However, the share of cells with initial signs of necrosis in the adrenalin administrated group increases. The increase is statistically significant and it confirms indirectly the development of stress.

So all the remarked changes in adrenal glands denote that at first the functional activity of adrenal gland medulla adrenocorticocytes increases. It's combined with the decrease of RBC electrophoretic mobility followed by functional tension of adrenal cortex and by intensification of glucocorticoid secretion. For its turn glucocorticoids provoke the increase of RBC electrophoretic mobility up to the 7th day of the experiment. The described picture of adrenal gland histological state may be characterized as a period of adaptation with the persistence of voltage phase [17]. At the same time the effectiveness of immune system functioning may decrease. It's associated with immunodepressive effect of cortisol higher concentration. As a result the number of epitheliocytes with cytogenetic disturbances (cells with micronuclei, cells with atypical nucleus) and with early stage of nucleus destruction (concentration of chromatin) increased. At that the absence of cells with karyopyknosis (a natural form of buccal cell apoptosis) in experimented group animal bodies means that the mechanism of buccal cell autarcesis remains.

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References

DEDOV I.I., FADEEV V.V., MELNICHENKO G.A. Adrenal Insufficiency. M.: Knowledge, 2002. 320 p.

LUNGA P., HERBERT J. 17β-Qestradiol modulates glucocorticoid, neural and behavioural adaptation to repeated restrain stress in semale rats // J. Neuroendocrinol. 2004; 16(9): 776–785 DOI:10.1111/j.1365-2826.2004.01234.x

SOLODKOVA O. A., ZENKINA V. G., CARADINE V.S. Effect of the extract of cucumaria Japanese on the structure of adrenal gland of white rats under cold stress // Fundamental research. 2012; 8: 419–423.

SAPIN M.R., MILYUKOV V.E., DOLGOV E.N., BOGDANOV A.V. Modern concepts of the structure and functions of adrenal // Clinical and experimental morphology. 2012;1:14–20

DERYUGINA A.V., SHUMILOV A.V., FILIPPENKO E.S., GALKINA Y.V., SIMUTIS I.S., BOYARINOV G. A. Functional and biochemical parameters of erythrocytes during mexicor treatment in posttraumatic period after experimental blood loss and combined traumatic brain injury // Bulletin of experimental biology and medicine. 2017; 164(7): 34–37. DOI: 10.1007/s10517-017-3918-4

DERYUGINA A.V., OSHEVENSKIY L.V., TALAMANOVA M.N., SHABALIN M.A., KRYLOV V.N., TSVETKOV A.I., GLYAVIN M.Y. Electrokinetic and biochemical changes in erythrocytes under the action of terahertz range electromagnetic waves // Biophysics. 2017; 62(6): 914–918. DOI: 10.1134/S0006350917060033

DUNN A.J., ANDO T., BROWN R. F., BERG R.D. HPA axis activation and neurochemical responses to bacterial translocation from the gastrointestinal tract // Ann. N Y Acad. Sci. 2003; 992: 21–29. DOI: 10.1111/j.1749-6632.2003.tb03134.x

STRAUB R.H, BESEDOVSKY H. Integrated evolutionary, immunological, and neuroendocrine framework for the pathogenesis of chronic disabling inflammatory diseases // FASEB J. 2003; 17(15): 2176–2183. http://dx.doi.org/10.1096/fj.03-0433hyp

HOLLAND N., BOLOGNESI C., KIRSCH-VOLDERS M., BONASSI S., ZEIGER E., KNASMUELLER S., FENECH M. The micronucleus assay in human buccal cells as a tool for biomonitoring DNA damage. The HUMN project perspective on current status and knowledge gaps // Mutat Res. 2008; 659(1–2): 93–108. http://dx.doi.org/10.1016/j.mrrev.2008.03.007

KALAEV V.N., NECHAEVA M.S., KALAEVA E.A. The micro-Nuclear test of the human oral cavity. Voronezh: Publishing house Voronezh state University; 2016. 136 p.

DERYUGINA A.V., IVASHCHENKO M.N., IGNATYEV P.S., SAMODELKIN A.G., BELOV A.A., GUSHCHIN V.A. The evaluation of genotoxic effects in buccal epithelium under disorders of adaption status of organism // Klinicheskaya Laboratornaya Diagnostika. 2018; 63(5): 290–292. DOI: 10.18821/0869-2084-2018-63-5-290-292

GUBINA-VAKULIK G.I., ANDREEV A.V., KOLOUSOVA N.D. Pathological changes in the adrenal glands of rats after acute postnatal hypoxia // Kazan medical journal. 2013; 94(5): 615–621.

SVETLITSKAYA O.I., YUDINA O.A. Morphological characteristics of lesions of the internal organs in acute respiratory distress syndrome viral and bacterial etiology // Bulletin of Vitebsk state medical University. 2018; 17(2): 55–62.

DERYUGINA A.V., IVASHCHENKO M.N., IGNATIEV P.S., LODYANOY M.S., SAMODELKIN A.G. Alterations in the phase portrait and electrophoretic mobility of erythrocytes in various diseases // Sovremennye tehnologii v medicine. 2019; 11(2): 63–68, https://doi. org/10.17691/stm2019.11.2.09

SHUMILOVA A.V., DERYUGINA A.V., GORDLEEVA S. YU., BOYARINOV G.A. Cytoflavin action on electro-kinetic and aggregation indices of erythrocytes in the post-traumatic period of cerebrocranial injury in experiment //Eksperimental'naya i Klinicheskaya Farmakologiya. 2018; 81(3): 20-23. DOI: 10.30906/0869-2092-2018-81-3-20-23

KAMSHILOVA T.B., MIKRJAKOV V.R., MIKRYAKOV D.V. Effect of analog of cortisol and the stress of traffic in the incidence of micronuclei in peripheral blood erythrocytes of sterlet // Biology of inland waters. 2013; 2: 94–96.

POLINA Y.V., NAUMOVA L.I. Gestionale condition of the adrenal glands under stress // Arkhangelsk medical journal. 2012; 7(4): 208–209.