THE PARTICULARITIES OF ORGANISM STRESS REACTION DEVELOPMENT UNDER THE INFLUENCE OF BEE VENOM

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Abstract. The research of stress reaction under the influence of bee venom and immobilization on rat is made in this paper. It is demonstrated that the stress reaction is developed under the influence of both factors. However, it's shown that the second phase of stress reaction connected with hypothalamo-pituitary-adrenal axis activation and glucocorticoid concentration increase predominates in case of bee venom injection. The revealed prolongation of the second, compensatory phase may define the organism resistance increase after the bee venom injection and bee venom therapeutic action.

Keywords: bee venom, compensatory reactions, stress.

Introduction

The researches of bee venom as a therapeutic agent in spite of the centuries-old history did not lose their importance at the present day when the use of synthetic remedies is not always so efficiently and it has a lot of drug side effects. It's a common knowledge that bee venom has analgesic and painkilling characteristics. But it should be added that it's used in cardiovascular diseases therapy and for endocrine system treatment, as a radioprotector and as an antihypoxic remedy (Habermann, 1972; Krasnikova et al., 2019).

By making grounding of bee venom therapy, it's necessary to note that the injected dose of bee venom determines in many respects the therapeutic effect. A stress reaction which drives to stress realizing the organism system activation develops in organism under the bee venom influence. At that it's well-known that stress is a nonspecific response (nonspecific response component) of a living system on different extremal stimuli outgoing from as outside as inside the system and threatening with homeostasis disorder (Baroboy, 2006). The revelation of stress response specific component under the influence of bee venom in comparison with other stress influence is not represented in literature for the moment.

It was demonstrated before that the analysis of RBC electrophoretic mobility, an index which represents a function of cell membranes, may serve as a marker of stress reaction (Deryugina et al., 2017b). So the first phase of stress (alarm reaction) is caused by the catecholamine «emergency» hormones emission and it's shown in RBC electrophoretic mobility decrease. The second phase is a stage of increased resistance with predomination of not stress reactions but adaptive responses which provoke the exit from stress and it's shown in RBC electrophoretic mobility increase. Besides the hypertrophy of suprarenal cortex which develops independently of the stress sort is one of the classical factors of stress trinary (Selye, 1979).

The purpose of paper is the research of RBC electrophoretic mobility and epinephroi morphology modeling the stress by bee venom injection and immobilization of animals in experiment.

Material and methods

60 white female rats with weight 180-200g were examined for the experimental part of the research. The researches were made in accordance with rules of execution of works and use of experimental animals established by Euro-

pean Convention for the Protection of Vertebrate Animals used for Experimental and Other Scientific Purposes of March 18, 1986; and by normative documents represented in "Guide for care and use of laboratory animals. ILAR publication, 1996, National Academy Press".

The animals were separated into 4 equal groups. Bee venom was injected intraperitoneally (i.p.) in dose 0,5 mg/kg to the animals of the first group. The rats which received physiological solution at the same volume were a control (second) group. Immobilisation stress was created by fixations of the rats on plates for 4 limbs on the back during 3 hours (3 group). Intact rats (4 group) were a group of control for immobilization.

Blood and epinephroi morphology were analyzed in 1 hour, then in 1 and 7 days after the stress influence. The blood for analysis was taken from the sublingual vein.

The RBC electrophoretic mobility was determined with microelectrophoretic technique (Deryugina et al., 2017a). A washed erythrocytes dredge was prepared for RBC electrophoretic mobility measuring. The washed erythrocytes were created by three-time centrifugation (200 g, 10 min, 0,85% NaCl). The suspension was made with tris hcl buffer, pH = 7,4 and after that the RBC electrophoretic mobility was measured by registering the 100 mkm rbc transmission time in tris hcl buffer with pH 7,4 and amperage 12 мА. The RBC electrophoretic mobility was determined by formula: U = S/TH. when S is distance which the cells covered, T is time of cell moving in the distance of S, H – energy gradient. The energy gradient was determined by the formula: $H=I/g\chi$, when I – amperage, g – chamber cross section, χ – media electric conductivity

Histological analysis of epinephroi. The experimental material was fixed in 10% neutral formaline then it was dehydrated in high-proof alcohols and then it was poured in paraffin. The paraffin cuts 7 mkm thick were manufactured with microtome SM 2000R (Leica, Germany) and colored by haemotoxyline and eosin. The histologic specimen observation were made with microscope DM1000 (Leica, Germany), the registration of video image were made with camcorder DFC290 (Leica, Germany).

The cortisol concentration was determined with immunoenzyme method.

Spreadsheet Microsoft Excel 2007 and program Statistica 6.0 were used for statistical processing of finding. When it was proven with Shapiro –Wilk test that the findings belonged to normal distribution the values of mean absolute error and standard deviation was determined. Student's t-test was used for comparison of two groups.

Results

The findings showed that the bee venom injection provoked the RBC electrophoretic mobility decrease up to 60 min of observation and a considerable increase of this index up to the end of the first day. During the 1 week of registration the index overlevel relative to control group value kept (table 1). The immobilization of animals determined a considerable RBC electrophoretic mobility decrease with consequent less strongly pronounced increase of this index in comparison with bee venom influence.

So homotypic two-phase reaction of cells on stress is determined. The reaction is shown in RBC electrophoretic mobility changes: its decrease (the first phase) and then increase (the second phase). It correlates with findings of previous researches (Deryugina et al., 2017b). In our opinion, quantitative differences in phase expressiveness are very important. The immobilization provoked the development of the first phase of RBC electrophoretic mobility change. A more prolonged second phase of RBC electrophoretic mobility change was typical for the bee venom group. It showed in a considerable index increase the value increased considerably the reference value.

When the analysis of the correlation between RBC electrophoretic mobility change and content of adrenal cortex hormone (cortisol) in blood plasma under the influence of stress was made it was evident that the increase of RBC electrophoretic mobility correlated with the increase of cortisol concentration (table 2). The analysis was made by immunoenzyme method.

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Table 1

Trend of development of RBC electrophoretic mobility (mkm·cm/V·c) during different kind of influence

Kind of influence	Period after the influence		
	60 min	1 day	1 week
Bee venom	0,85±0,03*	1,21±0,04*	1,11±0,04*
Physiological solution (control for bee venom)	0,99±0,04	1,07±0,03	1,00±0,03
Immobilization	0,69±0,07*	1,14±0,04*	1,12±0,05
Intacts (control for immobilization)	1,09±0,04	1,06±0,02	1,10±0,04

Notice: * – statistically significant differences ($p < 0,05$) with control group animals

Table 2

Change of RBC electrophoretic mobility and cortisol level in blood after the bee venom intraperitoneal injection

Kind of influence	Analyzed indices	
	RBC electrophoretic mobility (mkm·cm/V·c)	Cortisol concentration (nmol/l)
Intact rats	1,06±0,01	47,77±5,12
Bee venom	1,21±0,02*	120,12±6,19*
Immobilization	1,14±0,04*	87,32±6,97*

Notice: * – statistically significant differences ($p < 0,05$) with values before the influence.

The findings make evident the correlation between RBC electrophoretic mobility and adrenal cortex activity. This thesis is proved with morphological research of epinephroi under the influence of analyzed stressors.

The clear difference between cortical substance and medullary substance is discovered by analyzing the histologic specimen in intact animal group. The zonality of adrenal cortexes is very clearly traced. The endocrinocytes of cortical substance and medullary substance had nuclei with well-defined nucleoli. Little vacuoles were recognized in the cytoplasm. Many hemocapillaries were observed between cells. Plethora was observed in the medullary substance (Fig. 1. A)

The epinephroi histological picture of control group animals did not differ from intact group animal picture. A non-considerable enforcement of medullary substance vacuolization as well as a feebly marked plethora of cortical substance hemocapillaries were observed after 60 minutes of observation (Fig. 1. B, C)

The immobilization of animals as well as bee venom intraperitoneal injection made a considerable influence on epinephroi. In 60 minutes after the influence the plethora of cortical substance hemocapillaries was observed, the vacuolization of medullary substance endocrynocytes which is more marked in bee venom injection animal group enforced. The vacuolization of cytoplasm was observed in cortical substance zona fasciculata cells (Fig. 1. D, G).

Morphological changes of epinephroi of experimented group animals increased in one day. A visual increase of cortical substance dimension (mainly due to zona fasciculata) was observed. The borders between zones of a cortical substance were a little indistinct. The dimensions of endocrynocytes in this zone were much increased, the vacuolization of their cytoplasm was observed. The hemocapillaries were enlarged, the plethora was marked. These changes were best defined in bee venom animal group. The changes of medullary substance endocrynocytes kept (Fig. 1. E, H).

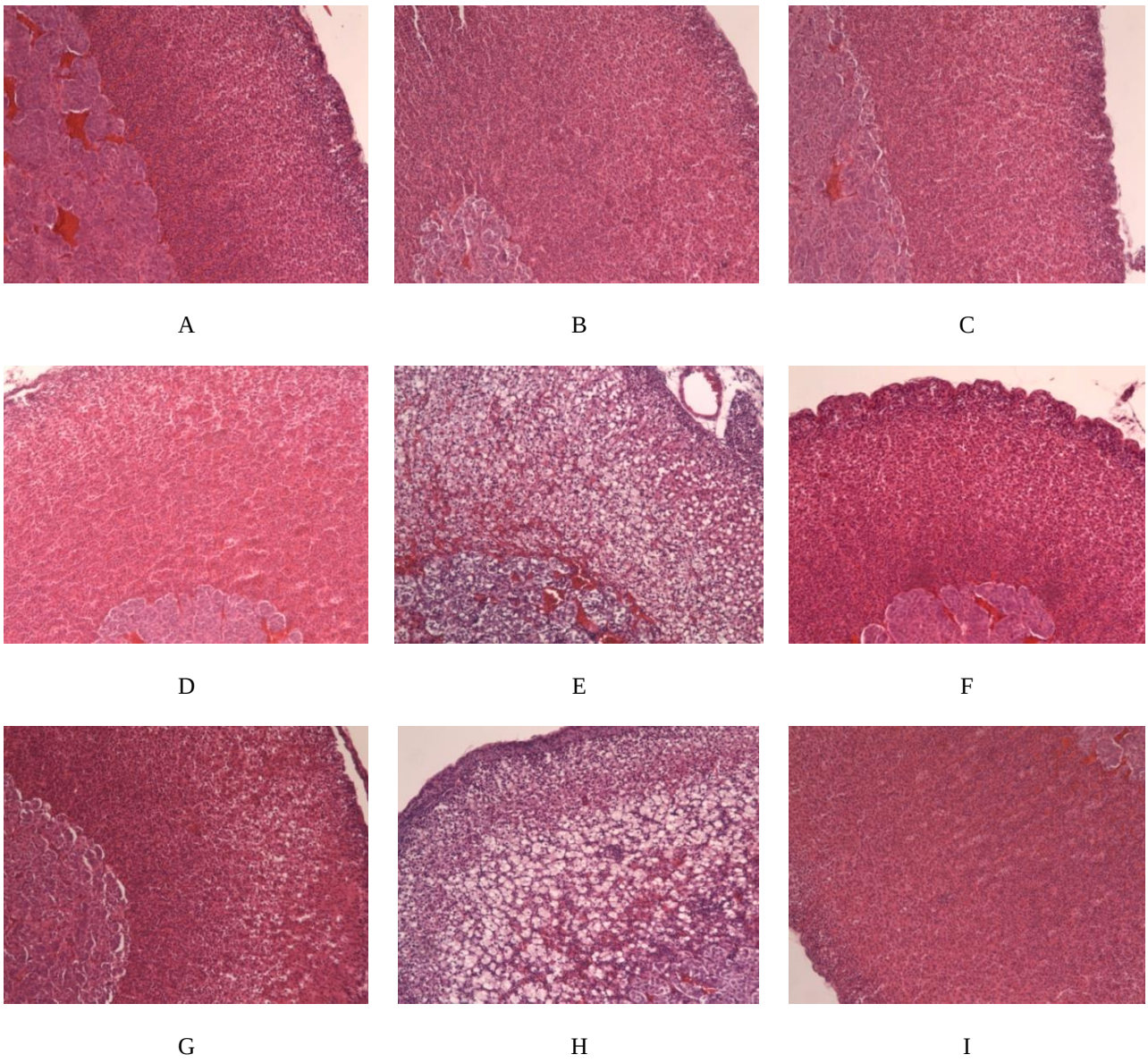

Fig. 1. Adrenal gland structure. A - intact animals. B, C – in 1 hour and 1 day after the introduction of physiological solution. D, E, F – in 1 hour, 1 day and 1 week after immobilization. G, H, I – 1 hour, 1 day and 1 week after the introduction of bee venom.100 magnification. Painting with hematoxylin and eosin

After a week, the histological picture of epinephroi of experimental animals approached that of intact ones. Though the bee venom animals had a little plethora of cortical substance hemocapillaries and bad-defined vacuolization of zona fasciculata endocrynocytes (Fig. 1. F, I).

Discussion

So the primary RBC electrophoretic mobility decrease during stress influence which was registered is connected with activation of epinephroi medullary substance. It's a common knowledge that the concentration of adrenalin in plasma during the reaction on stress increases tens of times already in several minutes. In particular, it happens in 1-5 minutes after the intraperitoneal injection of bee venom to dogs (Vick, 1972). During the stress reaction development catecholamines stimulate the increase of adrenal cortex hormone level in blood by ac-

tivating the ACTH emission. While catecholamines reflect the appearance of a short time releaser the long time effect is typical for corticosteroids (Rao, Raju 2000). It defines an increase of resistance with predomination of stress-but adaptive reactions (Dallman et al., 1992; Baroboy, 2006).

It should be noted that the second phase of stress reaction under the bee venom influence is considerably longer in comparison with that of immobilization one.

Conclusion

Thus the well-known therapeutic property of bee venom may be caused by the development of relatively long compensatory reactions in the organism (second phase) which drive to the increase of circulating glucocorticoids level which action is directed to the elimination of pathogen stress factor.

Conflict of interest statement

Nothing declared

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