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THE ROLE OF MELATONIN PRODUCED BY TISSUE BASOPHILES IN REGULATION OF PERMEABILITY OF MICROHEMOCIRCULATORY BED

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Abstract

The degree of hemomicrocirculatory pathways permeability and synthetic potencies of tissue basophiles under conditions of lipopolysaccharide (LPS) *E. coli* administration to laboratory animals was studied from the complex standpoint of morphology, morphometry and fluorescent microscopy. LPS *E. coli* was intraperitoneally administered to experimental animals 4 times during 4 days at the dose consentration of 10^{-9} *mg/mL.* The degree of mesentery microvessels permeability in animals of Control and Experimental Groups was determined through preliminary intravascular administration of a colloid probe with subsequent morphological analysis on the number of ink-labeled microvessels. The functional state of tissue basophiles was determined by counting degranulated forms and defining the content of histamine, serotonin, and melatonin in the cells using the method of quantitative fluorescent microscopy analysis. Coons' indirect reaction was applied in order to reveal GABA and eNOS in cellular components of microvessel walls. As demonstrated by results of the performed investigation administration of LPS *E. coli* rather low concentrations to laboratory animals was accompanied by signs of increased vascular permeability for particles of colloid coal. Among mechanisms of increased vascular permeability, an important place is devoted to such an important extravascular factor of transcapillary exchange regulation as the tissue basophiles. Thus, in animals of the Experimental Group there was a significant increase in number of degranulated tissue basophiles, in which we recorded high levels of histamine (27.8±4.4 *vs.* 18.6±2.7 in Control) and melatonin (23.4±3.5 vs. 11.01±2.9 in Control) and low levels of serotonin (9.3 ±2.8 *vs.* 22.4 ±5.2 in Control). The content of GABA significantly increased in endotheliocytes of mesentery microvessels on the background of eNOS low activity. Processes of tissue basophiles expressed degranulation were accompanied by releases of histamine and melatonin into the perivascular space.

Thus, under conditions of LPS *E. coli* administration to laboratory animals the important role of melatonin and histamine in mechanisms of enhanced permeability at the level of microhemocirculation pathways was revealed. Due to performed studies the conclusion might be drawn, according to which the increased permeability of microvessels to a large extent is conditioned by tissue-basophiles produced melatonin, as this latter is known to possess expressed vasodilatational spectrum of action at all levels of cardiovascular system structural organization.

Except the direct influence of extrapineal melatonin towards the endotheliocytes, it also modulated the vasodilatational effect through activation of GABA synthesis in the same endothelial cells. It was also established that NO-dependent mechanisms ensuring vasodilatational effect at the level of microhemcirculation was not engaged under conditions of our experiment. To our mind, eNOS low activity was conditioned by enhanced income of extrapineal melatonin into pericapillary space, as melatonin is capable to inhibit eNOS synthesis in endotheliocytes.

Keywords: synthesis of melatonin, tissue basophiles, microcirculatory bed, vascular permeability, histamine, serotonin

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INTRODUCTION

It is considered generally accepted that under the influence of different molecules, such as immunoglobulin E-specific antigen, anaphylotoxin, kinins, neuropeptides, tissue basophiles (mast cells) produce a wide range of biologically active substances.

Tissue basophiles are also considered the important extravascular factor for vascular permeability regulation in the peripheral link of cardiovascular system: in microhemocirculation pathways through synthesis and/or subsequent release of histamine and serotonin into pericapillary space.

At present aspects relevant to extrapineal synthesis of melatonin and mechanisms of its modulatory action to organs of immune, endocrine and cardiovascular systems are the subject of a wide discussion. In particular, the presence of melatonin was revealed in enterochromeafine cells of gastrointestinal tract, β-cells of pancreatic islet apparatus, secretory cells of cortical and medullar layer of adrenals, visual receptors of retina [*Kvetnoy I., 1999; Wellard J., Morgan I., 2004*].

Melatonin presence was also recorded in cells «scattered» all over the organism: neurosecretory and immune cells, including APUD-system cells as well. With the use of immunofluorescent analysis the presence of melatonin in tissue basophiles, thrombocytes, endotheliocytes, eosinophiles, monocytes, T-lymphocytes subpopulations was revealed [*Raikhlin N. et al., 1975; Finocchiaro L. et al., 1988; García-Mauriño S. et al., 1999*; *Kvetnoy I., 1999; 2002*; *Carrillo-Vico A. et al., 2004; Izzo G. et al.,* 2004*; Lardone P. et al., 2006; Silva C. et al., 2007*].

It is generally known that the only source of melatonin synthesis is serotonin that under conditions of activation transforms into melatonin. Therefore, to our mind, the assumption of I. Kvetnoy [*Kvetnoy I., 1999*] seems appropriate: all apudocytes, which are capable to produce melatonin and are presently considered the integral part of neuroendocrine system, should be regarded as the probable source of melatonin synthesis.

One should also mention the following circumstance of no small importance. Unlike pineal melatonin, apudocytes-produced melatonin is not subject to diurnal (circadian) rhythms.

The biological significance of melatonin produced in tissue basophiles never became the subect of a special study. One cannot exclude that in a mammalian organism there are subtle intra- and extravascular mechanisms, which in norm ensure processes of trans-capillary exchange, moreover that apart from the fact of melatonin revealing in tissue basophiles on the surface of these cells receptors to melatonin are detected [*Izzo G. et al., 2004*].

The current communication embraces the results of morphological, morphometric, fluorescent-morphological and immunomorphological analysis of the functional state of microvessels and tissue basophiles in intact animals and animals with a 4-day administration of endotoxin *E. coli.*

The selection of lipopolysaccharide (LPS) *E. coli* was dictated by the following consideration. It is known that administration of LPS *E. coli* to laboratory animals is accompanied by signs of increased vascular permeability at the level of microhemocirculation pathways [*McKenna T.M. et al., 1990*].

We assume that biologically active substances of tissue basophiles are engaged in mechanism of enhanced vascular permeability, as the direct influence of LPS *E. coli* towards tissue basophiles is not excluded as well.

Since GABA and NO produced in endotheliocytes have a dose-dependent vasodilatational spectrum of action at all levels of cardiovascular system structural organization, involving the microhemocirculatory bed as well, the present study was also aimed at a research on intercellular interrelation of tissue basophiles and endotheliocytes of microvessels. The research was performed for elucidating the partial share of melatonin produced in tissue basophiles, as well as GABA and NO produced by endotheliocytes of microvessels in mechanisms of induction of enhanced vascular permeability at the level of microhemocirculation pathways either in norm or under conditions of LPS *E. coli* administration to experimental animals.

MATERIAL AND METHODS

The experiments were carried out in 40 puberal white rats (body weigh = 150-170 *g*). Animals

were divided into 2 groups: Control and Experimental. Animals of the Experimental Group (18 rats) were intraperitoneally administered LPS *E. coli* (Sigma) at the concentration of 10^{-9} *mg/mL* during 4 days. Animals of the Control Group were administered only the equal amount of saline in which 0.1 *mL* LPS *E. coli* was dissolved.

The animals were kept in a vivarium under conditions of adequate ration and were withdrawn from the experiment in compliance of all standards of bioethics and humane experimental animals care. Planar film preparations were obtained from mesentery of Control and Experimental Group rats. Preparations were stained by hematoxylin and eosin, azure II eosin, toluidine blue, and azane by Heidenhain.

The state of vascular permeability was determined by deposition of colloid ink particles on the surface of microvessels according to accepted method [*Gorizontova M., Chernukh A., 1975*]. "Pelican" ink was used (Sweden). In order to reduce toxicity and remove the aggregates, the ink was heated during 15-20 *min* at 60ºC prior to administration. Upon cooling, the ink was centrifuged at 7000 *rpm* for 30 *min*. Thus obtained, the colloid ink was administered to animals as 0.15-0.2 *mL* per 100 *g* animal body weight. The total number of ink-labeled vessels was counted in 10 windows of mesentery. The total number of ink-labeled microvessels was determined taking into account 4 degrees of their permeability: (1) presence of singular particles and granules of colloid coal in a wall; (2) presence of granules and homogenous mass in certain microvessels; (3) presence of granules and homogeneous mass on a significant length of the microvessel; (4) diffuse intense deposition of ink on the surface and walls of microvessels of arteriolovenular link.

Histamine and serotonin content in tissue basophiles was determined in film specimens of mesentery with the help of a fluorescent method.

Intervascular tissue basophiles were counted in 50 fields of vision in each preparation with 20-fold lens magnification. There were counted: tissue basophiles without apparent signs of degranulation; basophiles with the 1st degree degranulation observed as a release of singular granules; basophiles with the 2nd degree degranulation or partial degranulation; basophiles with the 3rd degree degranulation i.e. complete degranulation.

The content of histamine, serotonin, and melatonin was determined in film preparations of mesentery by the quantitative immunomorphological method in Coon's indirect reaction with the help of luminescent microscope BOECO (Germany) adjusted to fluorimetric nozzle FMEL-IA (Russia); the results were expressed in conventional units of fluorescence (CUF).

For histamine measurements we used anti-histamine antibodies produced in rabbits (Sigma, USA), for serotonin: anti-serotonin antibodies produced in rabbits (Sigma, USA), for melatonin: anti-melatonin antibodies produced in rabbits (AbD-Serotec, UK), for GABA: anti-GABA antibodies produced in rabbits (Dia-Sorin, USA), for: anti-eNOS antibodies produced in rabbits (Sigma, USA). At the 2nd stage of Coon's indirect reaction upon determination of all the above-mentioned biologically active substances we used FITC-labeled anti-rabbit IgG-FITC antibodies produced in goat (Sigma). According to protocols of immunomorphological studies, in each precise case the necessary control tests were obligatory done to exclude fluorescence of non-specific character. With this aim at the 2nd stage of Coon's indirect reaction instead of FITC-labeled anti-rabbit IgG produced in goat FITC-labeld anti-human, anti-mouse and anti-rat IgG (Sigma) were applied to the preparations. To exclude autholuminescence (spontaneous luminescence of cells and tissues) all the conditions of stage-by-stage processing of preparations were observed, except application of FITC-labeled anti-rabbit IgG produced in goat at the 2nd stage.

All statistical analyses were performed using SPSS version 11.0 statistical package for Microsoft Windows (SPSS, Inc., Chicago, IL). Data are presented as mean±SEM. Correlations between variables were analyzed using Student's *t* test. The comparisons between groups were performed using *t* test.

Results

In animals of the experimental group cytoangioarchitectonics of mesentery in rats and mice was preserved and in practice did not differ from

Table 1.

The state of mesentery microvessels permeability in rats under conditions of LPS *E. coli* administration

Study Groups (n=18)	Permeability Degree		
	1st degree	2nd degree	3rd degree
Control	9.8±2.3	6.4±1.1	3.7±1.2
Experiment	12.5±2.3 0.25>*p*>0.1	17.7±4.7 0.025>*p*>0.01	10.4±2.2 0.01>*p*>0.05

Table 2.

Tissue basophiles content in mesentery according to degree of degranulation

Study Groups (n=18)	Degree of degranulation			
	without degranulation	1st degree	2nd degree	3rd degree
Control	16.5±1.7	12.3±2.1	6.8±1.9	2.4±1.6
Experiment	9.4±1.9 0.01>*p*>0.005	23.8±3.3 0.005>*p*>0.0005	12.5±2.3 0.05>*p*>0.025	8.6±2.2 0.025>*p*>0.01

that in animals of Control Group. No apparently visible structural changes in endotheliocytes of microvessels (arterioles, venules, and capillaries) visual were registered by us. However, in microvessels of venular "knee" there were observed signs of a marked widening in inter-endothelial space; this latter was expressed in less compact orientation of adjacent endotheliocytes at a significant extent of longitudinally and transversely oriented microvessels. Moreover, while staining by azane according to Heidenhain, there were observed phenomena of edema of connective tissue basis of mesentery, predominantly in perivenular and pericapillar sites. At the same time, there occurred processes of parietal orientation of neutrophils, leukocytes, eosinophiles and lymphocytes, their migration into perivascular space. The number of labeled microvessels significantly increased in mesentery of animals of experimental group intravascularly administered colloid coal (Picture 1 b).

As obvious from Table 1, the ink labeled vessels two-fold increased in number, as compared to Control Group. The increase in total number of labeled microvessels in mesentery of experimental group rats occurred exceptionally due to increase in number of microvessels of the 2nd and 3rd degree of label. Hence, the summary index of microvessels of the 2nd and 3rd degree of label was 2.8 times higher the analogous index in mesentery of Control group rats. Thus, under conditions of LPS *E. coli* to test animals, the histohematic barrier at the level of venular «knee» of microhemocirculation pathways became permeable for such large-disperse particles as particles of colloid coal.

The next stage of our research involved morphometric and fluorescent microscopy study aimed at revealing regional extravascular factors engaged in regulation of trans-capillary exchange realized through balanced level of vessel permeability at the lpathways hemomicrocirculatory bed. In this connection we performed investigation on morphofunctional state of tissue basophiles, which, are known to be an important factor regulating the permeability of microvessels in a mammalian organism.

For a long time already, it is considered that realization of vasoactive effect of tissue basophiles occurs due to production, depositing and elimination of a wide range of biologically active substances (cytokines, histamine, serotonin, etc. into the perivascular space [*Rudzit K., 1962; Vinogradov V., Vorobjova N., 1973; Serov V., Shekhter A., 1981*]. In particular, the release of histamine by tissue basophiles in perivascular space is accompanied by dilatation of microvessels with simultaneous increase of vascular permeability (*Gorizontova M., Chernukh A., 1975; Aleksandrov N et al.., 1976*]. The vasoactive effect of serotonin is strictly dose-dependent. Moreover, as the point of its application serve both endotheliocytes of microvessels, to which this mediator ex-

Table 3.

The content of histamine, melatonin and serotonin in tissue basophiles of rat mesentery under conditions of LPS *E. coli* administration

Study Groups (n=18)	Studied indices in fluorescence conventional units of (FCU)		
	Histamine	Melatonin	Serotonin
Control	18.6±2.7	11.0±2.9	22.4±5.2
Experiment	27.8±4.4 0.05>*p*>0.025	23.4±3.5 0.01>*p*>0.005	9.34±2.8 0.025>*p*>0.01

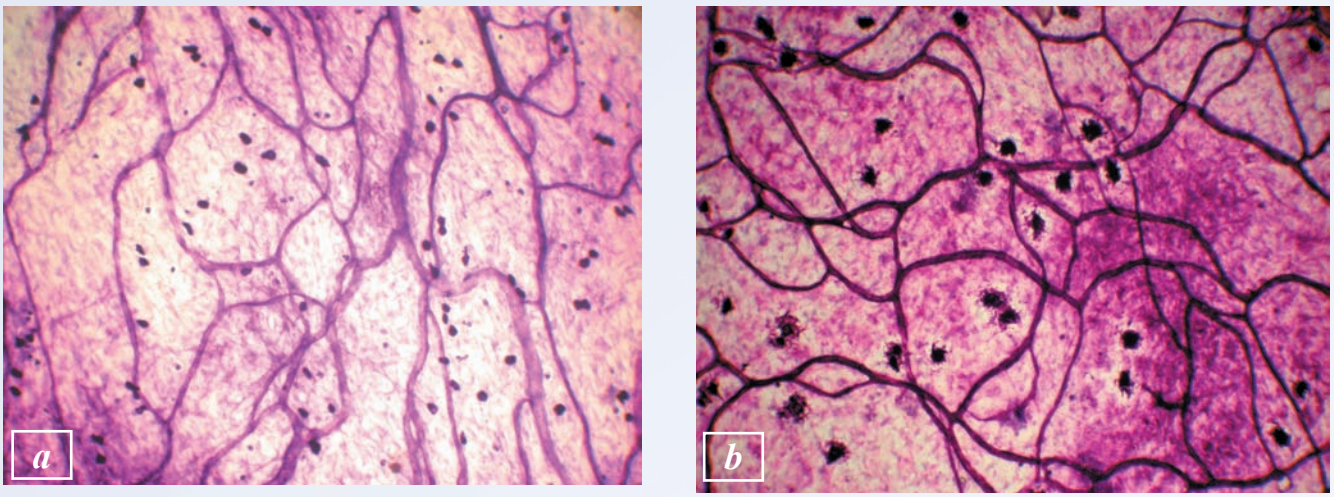
erts a vasoconstrictive effect, and smooth-muscle cells of arterioli, towards which serotonin produces diametrically opposed action that manifests in vasoconstriction of arterioli.

As demonstrated by results of the morphometric analysis (Table 2), in mesentery of Control Group rats there were determined approximately similar indices characteristic to tissue basophiles number without signs of degranulation on the one hand, and cells of the 1st and 2nd degree of degranulation (summary index), on the other hand.

In mesentery of Experimental Group rats there dominated tissue basophiles with the 2nd and 3rd degree of degranulation. Similar cells were overall revealed both in pericapillary parts and in sites remote from microvessels. Thus, indices of tissue basophiles with the 2nd and 3rd degree of degranulation exceeded the analogous indices in rats of Control Group 1.8 and 3.6 times, appropriately.

The results of performed quantitative fluorescent microscopy analysis for determination of histamine, serotonin and melatonin in tissue basophiles are presented as Table 3.

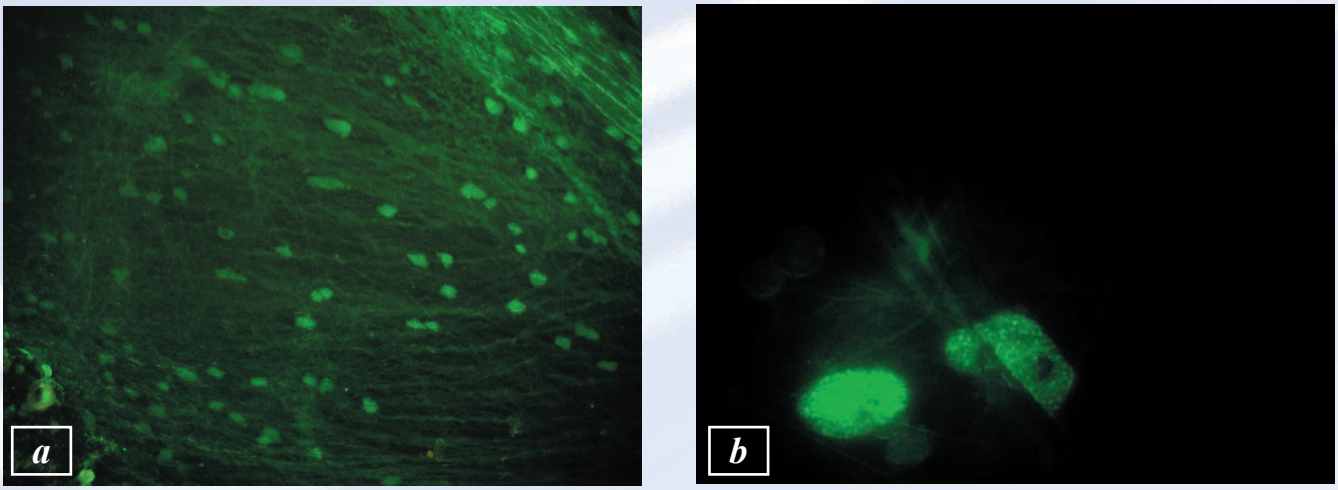
As obvious from Table 3, the content of histamine and melatonin in tissue basophiles of Experimental Group rats markedly increased. Thus, the levels of histamine and melatonin exceeded control indices 1.5 and 2.1 times, correspondingly. In animals of Experimental Group the processes of expressed degranulation were accompanied by the enhanced release of histamine and melatonin into pericapillary space. The specific fluorescence of histamine and melatonin containing granules was observed in both perivascular spaces and in the microvessels walls (Pictures 2 a, b; 3 a, b). The following circumstance is of no least importance



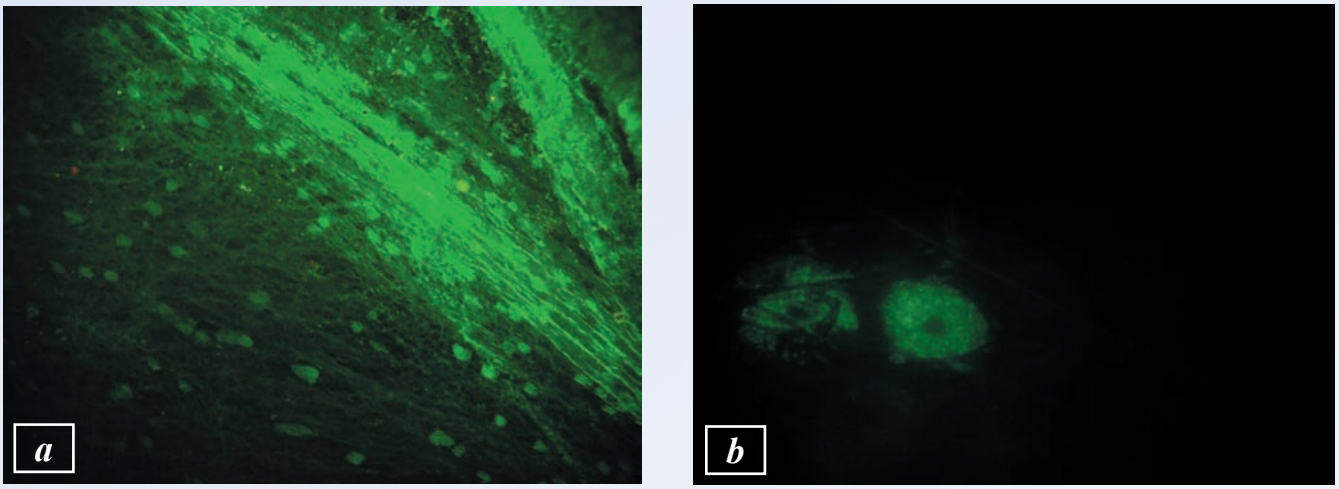
Picture 1. *The state of vascular permeability of microvessel walls in rats mesentery under conditions of LPS E. coli administration. Azure II-eosin. (obj. 20, oc. 10).*

a. *Control Group. Microvessels are identified with the 2nd degree mark on the background of partial degranulation of tissue basophiles. Subsequent staining of the preparation with azure II eosin..*

b.*Experimental Group. Microvessels with the 2nd and 3rd degree marks prevail. There are focal and/or diffuse depositions of colloid coal particles on the surface of microvessels and in perivascular spaces. In sites of increased vascular permeability the expressed degranulation of tissue basophiles is observed.*



Picture 2. *Topical characteristics of melatonin localization in tissue basophiles of rat mesentery under conditions of LPS E. coli administration. Fluorescent microscopy.*
***a**.Experimental Group. In perivascular sites and those remote from microvessels tissue basophiles with melatonin high content dominate (obj. 10, oc. 7).*
***b**.Image detail of preparation: (a) tissue basophiles are characterized with the presence of melatonin in tissue basophiles that is manifested as specific luminescence along the entire perimeter of a cell with clearly contoured dark nucleus or as an intense homogenous fluorescence at the site of cell cytoplasm and nucleus (obj. 100, oc. 10).*

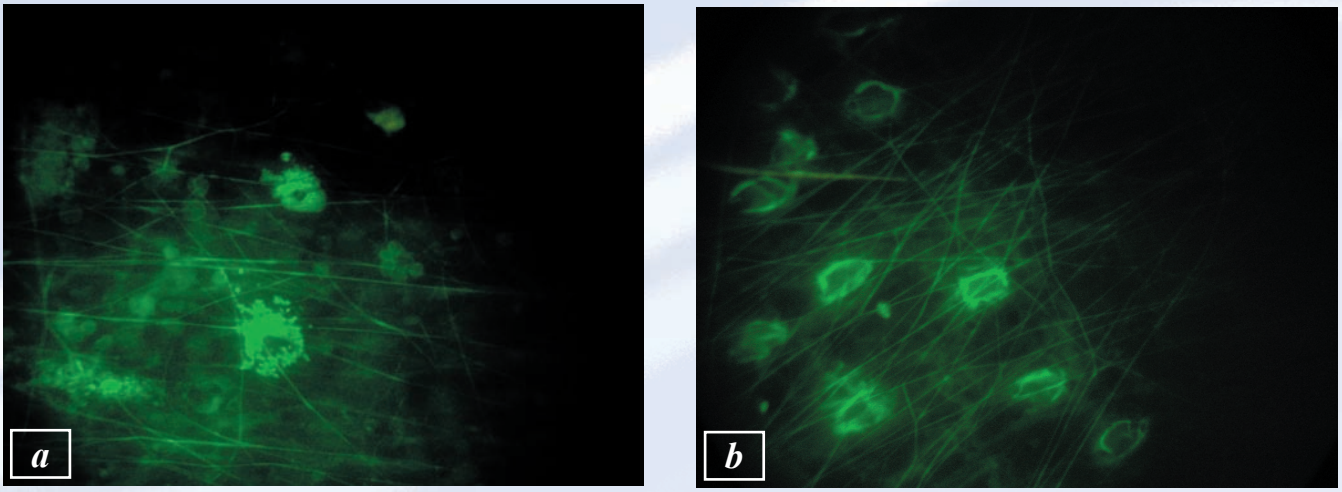


Picture 3. *The character of melatonin distribution in tissue basophiles and in pericapillary sites of rat mesentery under conditions of LPS E. coli administration. Fluorescent microscopy.*
a.*Presence of melatonin-rich tissue basophiles in pericapillary sites. Small-granular and homogenous luminescence is also determined extracellularly: in perivascular sites and on the surface of microvessels (obj. 10, oc. 10).*
b.*Image detail of preparation: (a)Presence of histamine in tissue basophiles with signs of initial and final stages of degranulation (obj. 100, oc. 10).*

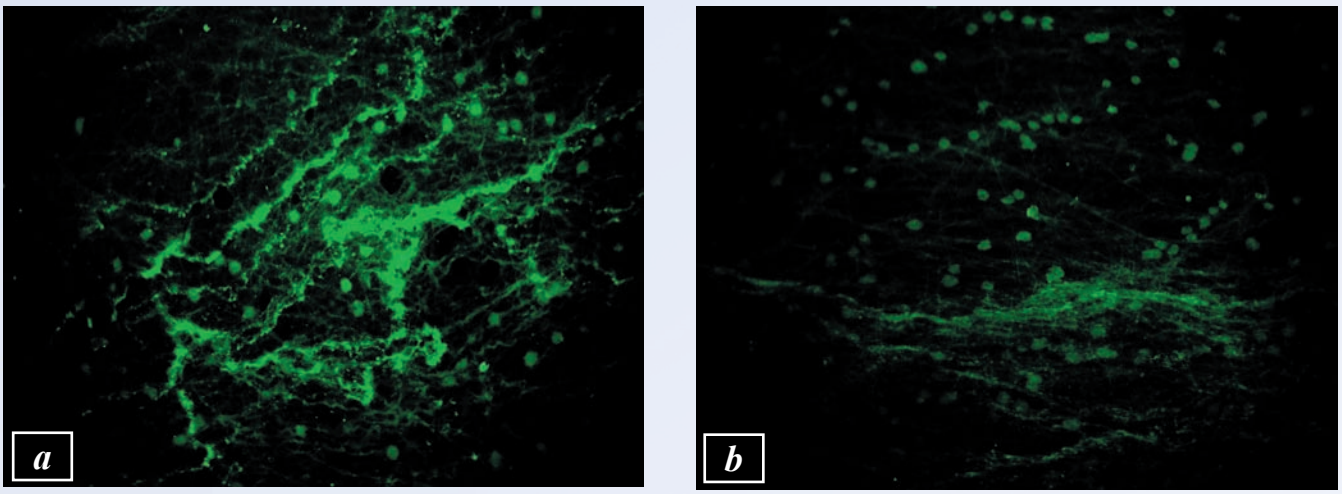
and should be noted. While the presence of histamine in tissue basophiles was predominantly determined as a specific fluorescence of small granules, which were diffusely oriented throughout the cell cytoplasm, the specific fluorescence indicating the presence of melatonin was of dual character: there was observed fluorescence of granules similar to that of histamine, as well as the specific homogenous fluorescence that was revealed as an edge in periphery parts of tissue basophiles (Picture 4 a, b).

Thus, the results of fluorescent microscopy analysis allow to draw a conclusion, according to which the processes of melatonin release from tissue basophiles occur in two ways: through both degranulation and granulolysis.

Attention should be paid to another circumstance of no small importance. On the background



Picture 4. *Topical peculiarities of melatonin distribution in tissue basophiles of rat mesentery under conditions of LPS E. coli administration. Fluorescent microscopy (obj. 100, oc. 10).*
***a**. Experimental Group. Melatonin is revealed in tissue basophiles as specific granular fluorescence. The process of histamine granules release into perivascular space is observed*
***b**. Experimental Group. Melatonin is revealed in tissue basophiles as specific homogenous luminescence in the immediate vicinity of inner surface of the cell.*



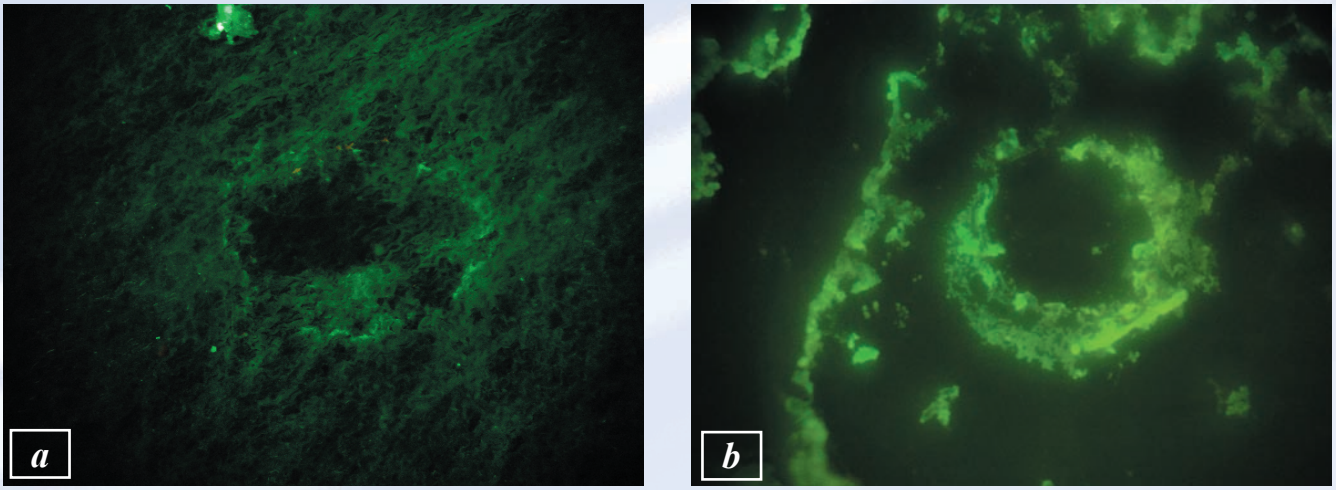
Picture 5. *Serotonin content in tissue basophiles of rat mesentery under conditions of LPS E. coli administration. Fluorescent microscopy (obj. 40, oc. 10).*
***a** Control Group. Moderate homogenous specific fluorescence in cytoplasm of most tissue basophiles.*
***b**.Experimental Group. Low level of specific luminescence in cytoplasm of in tissue basophiles.*

of the relative increase of histamine and especially melatonin content in tissue basophiles a 2.4-fold decrease of serotonin levels occurred as compared to similar index in tissue basophiles of Control Group rats. The specific fluorescence in tissue basophiles was mainly of granular character (Picture 5 b). Along with degranulation of tissue basophiles single serotonin-containing granules were revealed in both immediate vicinity of endotheliocytes of venules and capillary, and at sites of arterioli smooth-muscle cells localization.

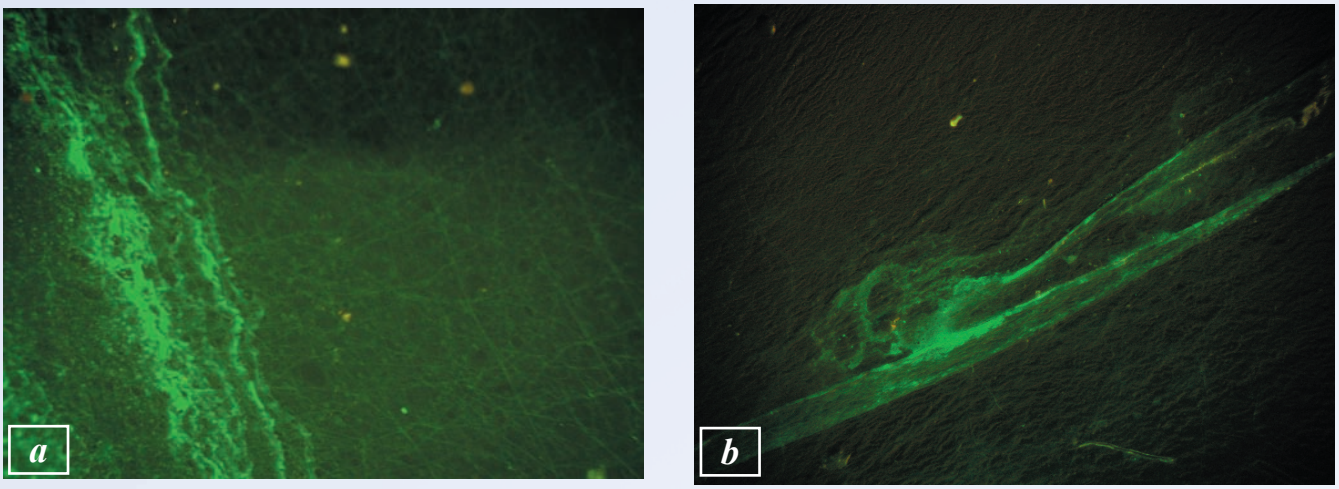
DISCUSSION

The performed morphofunctional studies allowed to reveal that fourfold administration of rather low concentrations of LPS *E. coli* to test animals was followed by an increase of microcirculatory pathways permeability; to this latter signified the increase in number of ink-labeled mesentery microvessels.

One cannot exclude that high vascular permeability is conditioned by the direct effect of exogenously administered LPS *E. coli* to structural components of microvessels, as it is considered to



Picture 6. *Topical characteristics of GABA localization in microvessel walls of rat mesentery under conditions of LPS E. coli administration. Luminescent microscopy (obj. 40, oc. 10).*
***a**.Control Group. Specific low fluorescence signifying in favour of GABA presence in the microvessel wall.*
***b**. Experimental Group. Intense specific fluorescence in structural components of arteriola and capillary.*



Picture 7. *Topical characteristics of eNOS localization in microvessel walls of rat mesentery under conditions of LPS E. coli administration. Fluorescent microscopy. (obj. 10, oc. 10).*
***a**.Control Group. Intense specific fluorescence alongside the capillary.*
***b**. Experimental Group. Evidence of eNOS activity as specific fluorescence revealed only at singular sites of capillaries as bifurcation sites.*

be established that LPS *E. coli* in a dose-dependent mode causes a significant increase of microvessels permeability through the damaging action to endotheliocytes [*McKenna T., 1990*]. However, the carried out study failed to reveal changes of dystrophic and inflammatory character in structural components of microvessel walls. Therefore, it is possible to conclude that in our study when putrescine was administered at rather low concentrations, which were 3-4 orders lower than those used by other researchers, the revealed enhanced vascular permeability does not result from a direct alterative impact of exogenous putrescine to the integral components of microvessel walls.

At the same time, the structural and functional shifts in tissue basophiles signify to the fact that such an important extravascular factor of microvessel permeability regulation as tissue basophiles under conditions of LPS *E. coli* administration is actively engaged in mechanisms of increased vascular permeability.

As we demonstrated above, processes of tissue basophiles degranulation were followed by the enhanced release of histamine into the pericapil-

lary space. Hence, under conditions of our experiment in mechanisms of increased permeability not the least role should be devoted to tissue basophiles produced histamine, the vasodilatational effect of which towards the microvessels is considered to be established.

Due to performed quantitative fluorescent microscopy studies, we revealed that under conditions of administering rather low concentrations of LPS *E. coli* to test animals the content of melatonin in tissue basophiles increased on the background of a decreased serotonin content in the same cells. It is not excluded that LPS in a dose-dependent mode directly and/or indirectly stimulates processes of melatonin synthesis from serotonin immediately in tissue basophiles.

It is established that tissue basophiles synthesize melatonin [*Kvetnojy I., 2002; Maldonaldo M. et al., 2010*]. According to cited authors on the surface of tissue basophiles there are M_1 and M_2 melatonin receptors. In available literature, there is only scarce information relevant to the influence of melatonin to the functional state of tissue basophiles. According to Cikler E. et al. (2005) and Cetinel S. et al. (2005) melatonin administered to rats under stress conditions averted the degranulation of tissue basophiles. Similar effect was obtained by other researchers as well, as they revealed that under conditions of preliminary stimulation of tissue basophiles by estradiol, administration of melatonin caused not only a decrease in number of undergranulated cells, but also was accompanied with a marked inhibition of tissue basophiles proliferation [*Izzo G. et al., 2004*].

It should be noted that in the mentioned studies the functional state of tissue basophiles was determined only based on counting both total number of basophiles and evaluating the number of undegranulated forms. In those studies information on content of such important factors of vascular permeability regulation as histamine, serotonin and melatonin in tissue basophiles is lacking. Moreover, the studies were carried out not in a normally functioning mammalian organism but in extreme situations, which preliminary ensure the processes of tissue basophiles stimulation, proliferation, and degranulation. Thus, it is not excluded that under conditions of intense proliferation of tissue basophiles caused by different provoking factors there are engaged mechanisms directed at inhibition of earlier increased vascular permeability, in particular, by inhibiting processes of synthesis of vasoactive substances of histamine, serotonin and melatonin type and their further release into the pericapillary space.

At the same time, under conditions of our experiment, i.e. upon administration of rather low concentrations of LPS *E. coli* to test animals, processes of tissue basophiles degranulation were accompanied by enhanced inflow of melatonin into the perivascular space. To our mind, melatonin plays not the last role in mechanism of increased permeability of the microcirculatory bed.

Due to our studies it is possible to suppose that endotoxins and LPS *E. coli* in the first place in the process of resident Gramm-negative microorganisms vital activity and degradation under conditions of both norm and pathology have not the last role in initiation of the increased permeability of hemomicrocirculatory bed, in the mechanism of which tissue basophiles are engaged.

In the mentioned aspect, the vasoactive role of serotonin produced in tissue basophiles should be considered from qualitatively new positions. Until present there dominated a point of view, according to which degranulation of tissue basophiles is accompanied by simultaneous enhanced inflow of histamine and serotonin into the perivascular space. However, in our studies under conditions of maximum low concentrations of LPS *E. coli* to laboratory animals the content of serotonin in tissue basophiles and perivascular part of mesentery was significantly decreased. One cannot exclude that in this specific case in tissue basophiles occur processes selectively directed towards transformation of serotonin to melatonin. The proposed supposition to a known extent has its confirmation in a number of recent investigations. There is evidence that melatonin inhibits vasoactive effects of serotonin. Thus, in model experiments with the use of rat aorta flap some authors [*Satake N. et al., 1991*] established that melatonin inhibits the constrictive activity of aorta in response to administration of serotonin. It is also elucidated [*Matheus N. et al., 2010*] that

melatonin retards activity of serotonin transporter. In this case, endotheliocytes serve as the point of application, as according to authors removal of endotheliocytes from the inner surface of aorta retards the reaction induced by melatonin as as response to serotonin administration. To our mind, realization of serotonin-induced effects to endotheliocytes of microvessels to a known extent is under control of extrapineal melatonin that is produced in the same tissue basophiles.

In our opinion, mechanisms of vasodilatational influence of extrapineal melatonin produced immediately in tissue basophiles seem rather debatable. In accessible literature, we failed to find similar investigations. It is not excluded that at the level of microcirculatory pathways mediatory functional loops based on mechanisms of short-distant interrelations of different cell populations and, in the first place, endotheliocytes and tissue basophiles are engaged.

First, it is not excluded that histamine produced in tissue basophiles potentiates synthesis of melatonin in the same cells. Similar mechanism is engaged in epiphysis [*Novak J. et al., 1997*].

Second, in favour of the proposed supposition also signify our data, according to which under conditions of LPS *E. coli in vivo* stimulation besides the enhanced release of melatonin into pericapillary space, high activity of GABA (Picture 6 b) that is known to possess the expressed vasodilatational spectrum of action as well [*Takenaka K. et al., 1995*] was recorded in cell components of microvessel walls. It is also known that a number of endogenous active factors, including melatonin as well, act as GABA modulators in the mammalian organism. Thus, under conditions of the experiment it was established that melatonin causes a decrease of arterial pressure in rats with stress-induced hypertension [*Li H. et al., 2008*]. According to authors, the mechanism of melatonin antihypertensive effect is realized due to $M_{(1)}$ and $M_{(2)}$ receptors, as well as through stimulation of GABA(A) receptors.

Third, it is not excluded that due to enhanced degranulation of tissue basophiles and melatonin inflow to perivascular space, there occurs NO activity inhibition in endotheliocytes, whereas its is known that NO likewise melatonin possesses a dose-dependent vasodilatational spectrum of action.

In our studies the release of melatonin is accompanied by a marked decrease of eNOS activities in endotheliocytes (Picture 7 b).

The obtained results are also confirmed by investigation of Silva C. et al. (2008) and Tamura E. et al. (2009), who established that melatonin decreases vascular permeability through inhibition of NO formation in endothelial cells *in vitro* and *in vivo*.

Apparently, in this precise case there are engaged reciprocal mechanisms of intercellular cooperation at the level of tissue basophiles and endotheliocytes in the aspect of selective influence to endotheliocytes of microvessels of both vasodilatational factors: melatonin and NO.

The direct effect of administered LPS *E. coli* to processes of NO synthesis in endotheliocytes should not be excluded, as it is known that LPS decreases dilatational activity of endothelial NOS [Tamura E. et al., 2009].

Based on performed research, a conclusion might be drawn: an important part in mechanisms of vascular permeability regulation at the level of microhemocirculation pathways should be devoted to melatonin produced in tissue basophiles and endotoxins of resident Gram-negative microorganisms.

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