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THE ROLE OF MELATONIN IN REGULATION OF PERMEABILITY OF MICROHEMOCIRCULATION PATHWAYS

(Experimental research. First Communication)

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Abstract

Comprehensive morphological, morphometric, and fluorescent microscopic methods of research in small laboratory animals (rats, mice, hamsters) were applied to study the state of permeability in mesentery microhemocirculation pathways, loose connective tissues, as well as hamster's cheek pouch under conditions of single intravascular administration of melatonin. All experimental animals were administered rather low doses of melatonin similar to those determined in nocturnal blood serum of intact mammals.

Morphometric studies allowed to reveal that the single intravascular administration of melatonin was not accompanied by the increase of microvessels permeability for colloid coal and FITC-labeled gamma-globulin. However, the histohematic barrier of microcirculation pathways appeared to be permeable for such a relatively low-molecular compound as fluorescein (uranin). It was ascertained by morphometric and fluorescent-microscopic methods of research that such an important extravascular factor for regulation of transcapillary exchange and microvessel permeability as tissue basophiles is not engaged. Thus, after single administration of rather low concentrations of melatonin, almost no signs of degranulation were observed in tissue basophiles; whereas the quantitative fluorescent microscopy allowed to determine high indices of histamine and serotonin compared to the control group. It is not excluded that under conditions of our experiment there were involved both the receptor mechanisms of direct inhibiting influence of melatonin towards the processes of tissue basophiles proliferation and secretion of histamine and serotonin by these cells. It should be specially emphasized that in case of single intravascular administration of melatonin the cytoarchitectonics of all investigated connective tissue derivates was preserved.

Therefore, the revealed evidence of enhanced permeability of microvessels for low-molecular compounds should be considered from the point of view of physiology: as a possible mode of functioning at the level of microcirculation pathways of melatonin-dependent mechanism for regulation of transcapillary exchange.

Keywords: melatonin, microcirculatory bed, vascular permeability, tissue basophiles.

Introduction

Despite the considerable advance achieved in present-day endocrinology, the biological significance and mechanisms of action exerted by numerous hormones are insufficiently studied. In a majority, the activity of endocrine glands is subject to precise seasonal and circadian rhythmicity. In this respect, melatonin is of no less importance in maintenance of many integrative functions of the organism which are also subject to cyclic processes.

Endocrine system functioning at all the levels of its structural organization is in dynamic equilibrium, thus ensuring the hormonal homeostasis of an organism. Therefore, to our mind, the search for new functional "loops", including melatonin-dependent ones ensuring the activity of cardio-

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vascular system at all the levels of its structural organization is a rather promising direction of modern medicine.

It should be mentioned that the majority of studies report data on cardioprotective and vasoprotective potential under conditions reproducing different pathology states: experimentally induced myocardial ischemia, cold, oxidative and immobilization stress, etc. [*Jou M. et al., 2004; Lotufo C. et al., 2006; Howard J., 2007; Silva C. et al., 2007; Ceyran H. et al., 2008; Petrosillo G. et al., 2008; Sahna E. et al., 2008; Tengattini S. et al., 2008*].

As a rule, the authors used rather high concentrations of melatonin exceeding from 3 to 8 order of magnitude those maximum concentrations of melatonin, which are registered at night in blood serum of experimental animals [*Battista P., Condon W., 1986; Lemus-Wilson A. et al., 1995; Diaz-Trelles R. et al., 2000; Silva C. et al., 2007*].

Moreover, levels applied by researchers were administered to laboratory animals not only as one-time dose but repeatedly as well. At the same time, in our opinion, the biological effects of melatonin should not be realized using its "therapeutic" doses; on the contrary, - rather low concentrations of epiphyseal hormone should be used being maximum approximated to those in blood serum and plasma of mammals.

One should specially emphasize that biological effects of melatonin were studied at the level of the initial chain of cardiovascular system: large- and medium calibre arteries. Administration of melatonin to patients with hypertension was accompanied by relaxation of smooth-muscle elements thus bringing forth vasodilation of great vessels and decreased blood pressure [*Anisimov V., 2006*].

The goal of the present investigation was to study the morphofunctional state of microcirculation pathways under conditions of minimal concentrations of melatonin administered to small laboratory animals.

While arranging the experiment, the circadian rhythms of melatonin were taken into consideration as its concentrations in blood serum of mammals varies in nocturnal and daytime within the wide range.

Material and Methods

Experiments were performed in puberal male animals: 68 white rats, 30 white mice, and 38 hamsters. The following objects were studied: mesentery and loose connective tissue of rats, mice and hamsters, as well as hamster's cheek pouch.

In each series (under experiment conditions in each animal species) the animals were divided into the following groups: Control, Experimental Group I: animals were withdrawn from the test in 20 *min* after melatonin administration; Experimental Group II: animals were withdrawn from the test in 2 hours; Experimental Group III: animals were withdrawn from the test in 8 hours. Melatonin (Sigma, USA) was administered intravascular as a single nocturnal dose of 110 *pg/ml*. The choice of time interval for melatonin administration was based on the results of chronobiological studies, in which the circadian rhythms of melatonin in animals were rather clear-cut. The maximum level of melatonin in blood serum was determined in sample at night and made 70-110 *pg/ml*. Therefore, administration of melatonin at the above-mentioned dose will correspond to only 2-fold increase of melatonin levels in blood serum of mammals. Administration of melatonin at mentioned concentration in daytime was not expedient from the point of view of methodical procedures, as daytime concentration of melatonin in blood serum of mammals was rather low and varied in the range of 10-20 *pg/ml*. Therefore, due to melatonin administration in daytime its level in blood serum approximately corresponded to physiological concentrations of melatonin registered at night (80-110 *pg/m*). Control animals were administered only the saline in an amount equal to melatonin vehicle, i.e. 0.1 *ml*. The animals were kept in a vivarium under conditions of adequate ration and were withdrawn from the experiment with the compliance of all standards of bioethics and humane experimental animals care. Planar preparations were obtained from mesentery and loose connective tissue. Cheek pouches of hamsters were fixed in formalin and after appropriate processing in ethanol of

increasing concentrations were embedded in paraffin. The slices and film preparations were stained by hematoxylin and eosin, azure II eosin, toluidine blue, and azane by Heidenhain.

Upon determination of vascular permeability in mesentery and loose connective tissue of experimental animals, the following series were arranged: animals of the Experimental Groups I and II were administered appropriately colloid coal and FITC-labeled homologous gamma-globulin; animals of the Experimental Group III were administered fluorescein sodium salt.

In animals of the first series, 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 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 and 10 fields of vision of loose connective tissue. The total number of ink-labeled microvessels was determined taking into account 4-stage permeability thereof: (1) presence of 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.

In rats of the first series the state of vascular permeability was determined with the help of a single intravascular administration of FITC-labeled gamma-globulin (obtained from N.F. Gamalei Institute of Epidemiology and Microbiology, Russia) diluted as 1:32. Antiserum was administered in 0.1 *ml*.

In rats of the third series the study on histohematic barrier permeability of mesentery and loose connective tissue of rats, mice and hamsters was performed by means of the single intravenous administration of 5% sodium fluorescein solution (Fluorescein sodium salt/uranin, Fluca-Sigma, USA), as 0.5 *ml* per *kg* of animal body weight. The choice of a dose level was done according to commonly accepted method for determination of histohematic barriers with the use of uranin [*Ushevskaya L., 1963*).

Histamine and serotonin content in tissue basophiles was determined in film specimens of mesentery with the help of a fluorescent method, using ortho-phthaleic aldehyde and paraform-aldehyde (Sigma, USA). With this purpose, the slide glasses were placed in glass chambers, at the bottom of which there was poured 200 *mg* crystalline ortho-phthaleic aldehyde and 2 *g* paraform-aldehyde, appropriately. The chambers were covered with the hood and placed into the termostate at 80ºC for 1 hour. The quantitative determination of histamine was done taking into account 20 tissue basophiles in each preparation; luminescent microscopes Boeca (Germany), Lyumam-P-3 (Russia) and the fluorometric nozzle FMEL-1A were used. Optical filters with the wavelength of 445 and 495 *nm* were adjusted to the nozzle and corresponded to maximum histamine and serotonin fluorescence. The amounts of histamine and serotonin were expressed in conventional units of fluorescence (CUF).

In all studied series, the content of ionized calcium was determined in mesentery and loose connective tissue. After the preliminary washing-out in 3-4 portions of distilled water, the preparations were viewed with the help of luminescent microscope Lyumam-P-3. Ligtfilters FS-1 and ZhS-18 were introduced in the microscope beforehand, as the maximum absorption for *Ca*-chlortetracycline complex equals to 380 *nm* [*Vladimirov Yu., Dobretsov G., 1980*]. With the help of a disk adjusted to the fluorimetric nozzle FMEL-IA that is a part of a luminescent microscope Lyumam–I-3, there was mantled the interference lightfilter with the wavelength of 540 *nm*, which corresponds to maximum fluorescence of *Ca*-chlortetracycline complex, as well as all necessary lens and a probe. The quantitative evaluation of fluorescence was done in cytoplasm

of 50 tissue basophiles taking into account the intensity of the background luminescence. Ionized *Ca* content was expressed in conventional units of fluorescence (CUF). Data obtained due to statistical processing were the indicator of ionized *Ca* mean content in 1 tissue basophile. Intervascular tissue basophiles were counted in 50 fields of vision in each preparation with the lens magnification 20-fold. 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 statistical analysis was performed using Student's criteria.

Results

The cytoarchitectonics of mesentery, loose connective tissue, and cheek pouch of hamsters were studied with the use of generally accepted morphological methods and practically never differed from those in animals of the control group. In particular, we registered no visual structural changes in endotheliocytes of arterioles, venules, and capillaries. Only in arterioles, venules and capillaries of the arteriolar link there were observed signs of moderate widening in inter-endothelial spaces; this latter was manifested as less compact orientation of adjacent endotheliocytes alongside the entire length of microvessels. After staining with azane by Heidenhain there were observed the phenomena of connective tissue edema especially in perivascular sites.

In 20 minutes post intravascular administration of melatonin, the content of labeled microvessels significantly decreased. Thus, the total content of ink-labeled microvessels in animals of the Experimental Group 1 decreased 2.9-fold compared to the content in rats of Control Group. The decrease in total number of labeled microvessels in mesentery of rats from Experimental Group 1 occurred due to decreased number of microvessels with the 1st and 2nd degree marks (Table 1; Figure 1 c,d,e,f). In 2 and 8 hours after melatonin administration, the indices of microvessel permeability did not differ from those in mesentery of rats in Control Group. It should be mentioned that in animals of Control Group and the three Experimental Groups no microvessels with the highest degree of permeability (4th degree mark) were determined.

In the next series of the experiment, the state of vascular permeability in mesentery of rats was determined with the help of intravascular administration of FITC-labeled gamma-globulin to rats of Control and Experimental Groups.

In no test groups the intravascular administration

Table 1.

The state of mesentery microvessels permeability in rats under melatonin administration

Study Groups (n=16)	Distribution of microvessels according to degree of permeability for colloid coal particles		
	1st degree	2nd degree	3rd degree
Control	6.9 ± 0.6	3.2 ± 0.3	1.6 ± 0.2
Experimental I	2.4 ± 0.2 P < 0.0005	1.6 ± 0.09 P < 0.0005	not revealed
Experimental II	6.15 ± 0.2 0.25 > P > 0.1	3.7 ± 0.1 0.1 > P > 0.05	1.3 ± 0.4 0.4 > P > 0.25
Experimental III	6.4 ± 0.25 0.25 > P > 0.1	3.5 ± 0.2 0.25 > P > 0.1	1.9 ± 0.2 0.25 > P > 0.1

P was determined by comparison of indices in Experimental Group with those in Control.

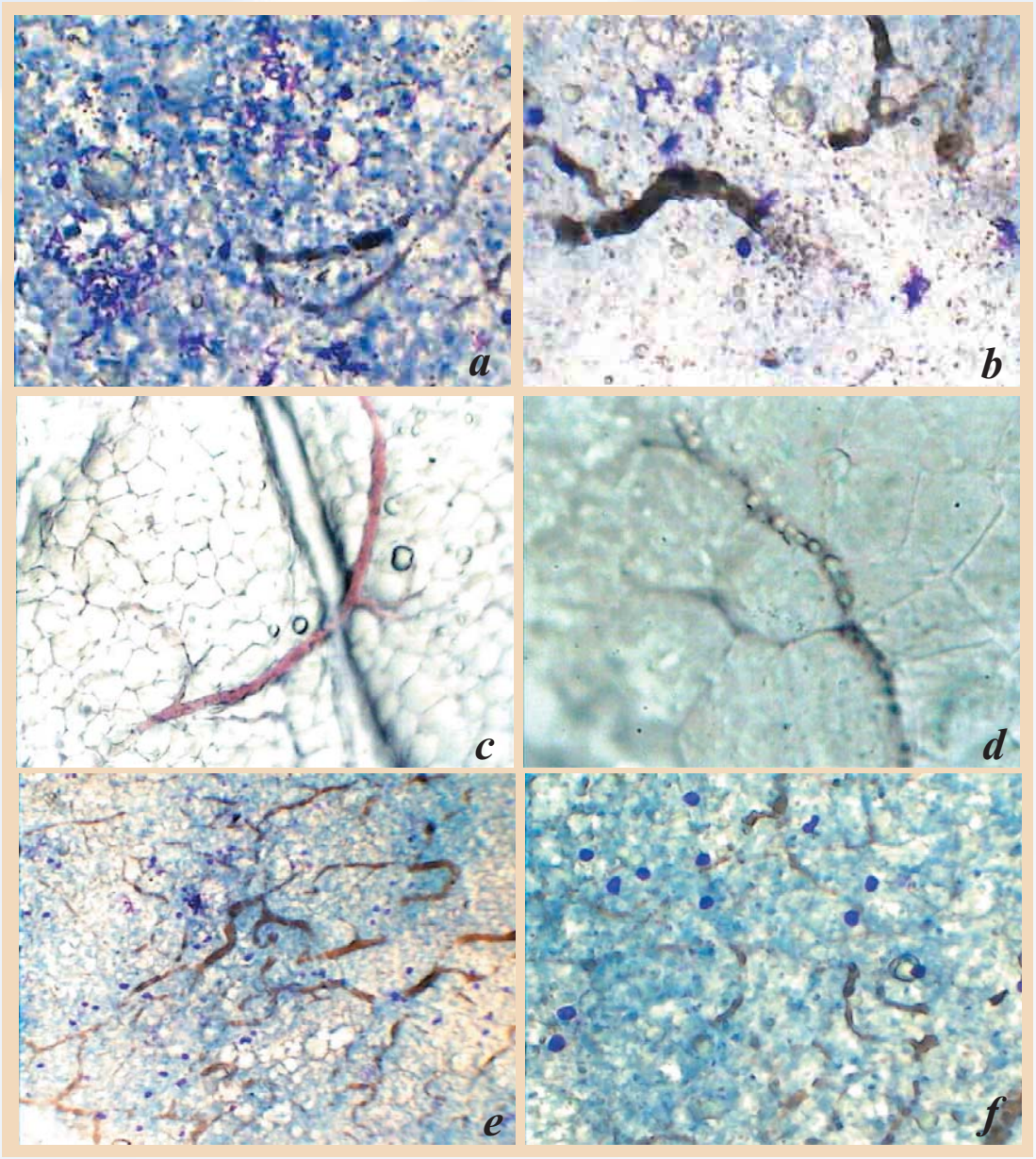

Figure 1. *The state of mesentery microvessel permeability for particles of colloid coal under conditions of melatonin administration to experimental animals.*

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 (obj. 10, oc. 10).

b) Control Group. In sites of increased permeability of capillary walls (3rd degree marks) there occurs complete degranulation of certain tissue basophiles. Subsequent staining of the preparation with azure II eosin (obj. 40, oc. 7).

c) No signs of increased vascular permeability for colloid coal particles. Microparticles present only on inner surface of venules (obj. 10, oc. 10).

d) Experiment Group I. No signs of increased vascular permeability with deposition of colloid coal particles on the surface of erythrocytes (obj. 40, oc. 7).

e) Experiment Group I. Capillary network is revealed with the signs of increased vascular permeability (1st and 2nd degree marks), with perivascular-oriented tissue basophiles. Subsequent staining azure II-eosin (obj. 10, oc. 10).

f) Experiment Group I. Singular capillaries are revealed with the 1st and 2nd degree marks. Perivascular-oriented tissue basophiles without degranulation signs. Subsequent staining with azure II-eosin (obj. 40, oc. 7).

of labeled gamma-globulin was accompanied with its release into perivascular space, thus signifying that under condition of melatonin administration the histohematic barrier was practically impermeable for plasma proteins of globular origin nature. The specific fluorescence was exclusively observed in the lumen of mesentery microvessels. In the lumen of arterioles, capillaries and venules labeled anti-*IgG* globulins were determined on the surface of small lymphocytes (reception on the surface of B-lymphocytar populations) and erythrocytes (surface adhesion). The intravascular specific fluorescence bore both diffuse and focal character (Figure 2 a,b).

In the 3rd series of experiment determination of vascular permeability was performed by administration of fluorescein sodium salt (uranin) to animals of Control and Experimental Groups. Single administration of uranin to animals of the Experimental Group I was followed by the release of colloid coal particles outside the lumen of mesentery and loose connective tissue. This fact was manifested as homogeneous and/or large-lump specific fluorescence of connective-tissue base particularly in perivascular spaces (Figure 2c,d). Relatively high vascular permeability was observed in microvessels of arteriolar link. In microvessels

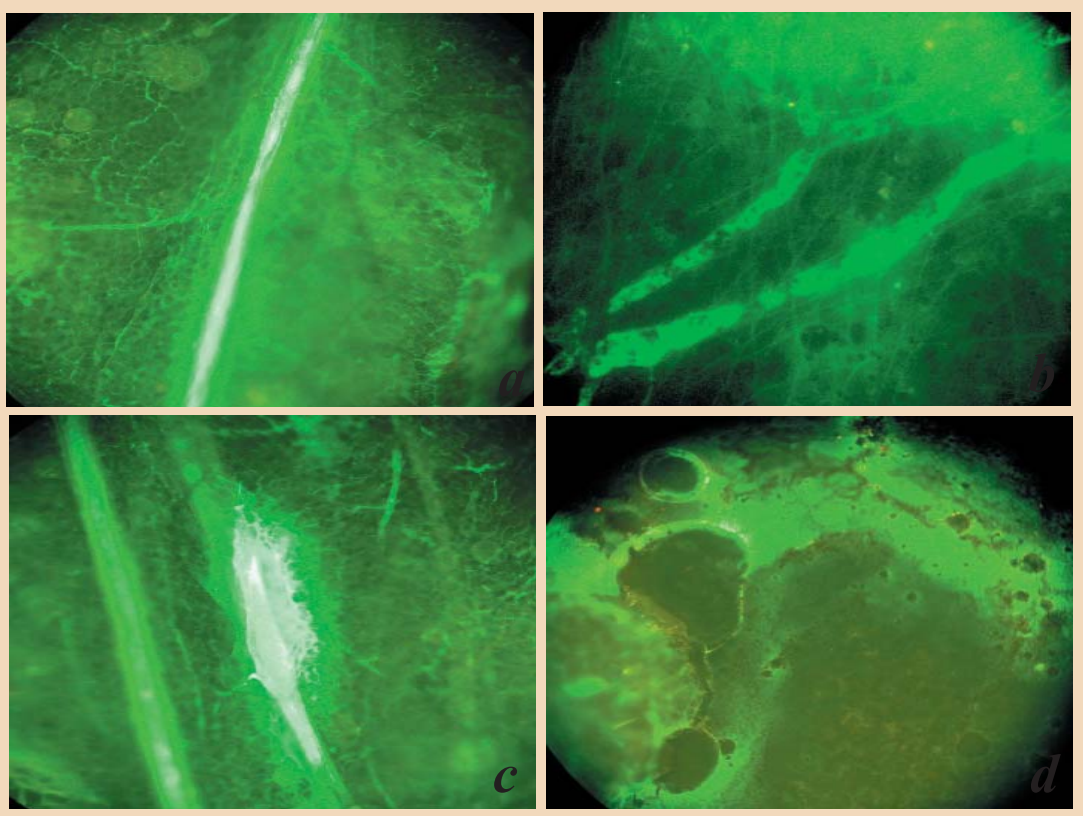

Figure 2. *Permeability of mesentery microvessels of rats for homologous gamma-globulin and fluorescein 20 min after melatonin administration (Experiment Group I). Fluorescent microscopy.*

***a**) No signs of increased vascular permeability for homologous gamma-globulin. The specific fluorescence is observed in lumen of venules and capillaries. Intravascular administration of FITC-labeled gamma-globulin (obj. 4, oc. 10).*

***b**) Image detail of preparation **a**. Gamma-globulin is present in lumen of capillaries, on the surface of blood cells (obj. 4, oc. 10).*

***c**) Microvessels with signs of increased permeability for fluorescein. Specific fluorescence is determined along the venule in perivascular connective tissue. Intravascular administration of fluorescein (uranin) (obj. 4, oc. 10).*

***d**) Specific fluorescence is determined in cytoplasm of most endotheliocytes and in pericapillary sites of connective tissue. Intravascular administration of fluorescein (uranin) (obj. 4, oc. 10).*

of venular link (venules, capillaries) the specific fluorescence bore less disseminated character and was revealed in both lumen of microvessels and in a perivascular space. In 2 and 8 hours after melatonin administration, the permeability of mesentery permeability was normalized.

Thus, under melatonin administration the histohematic barrier of microvessels in mesentery and loose connective tissue of rats was impermeable for such large disperse particles as particles of colloid coal and for blood high-molecular proteins such as gamma-globulins. At the same time, 20 *min* after melatonin administration the signs of increased permeability of microvessels emerged for relatively lower molecular compounds; this latter was revealed due to intravascular administration of uranin.

At the next stage of our study, morphometric and fluorescent microscopy investigations were carried out with the aim to reveal topical extravascular factors performing processes of transcapillary exchange regulation and vascular permeability. Investigation was performed to study the morphofunctional state of tissue basophiles as an important extravascular factor, which regulates permeability of microhemocirculation pathways.

The necessity of a study on the functional state of tissue basophiles (mastocytes) was dictated by the following circumstance. It is considered to be an old-established fact that sustaining of local homeostasis by tissue basophiles is occurring due to producing, depositing and eliminating a wide range of regulatory type biologically active substances: cytokines, histamine, serotonin, melatonin, etc. [*Rudzit K., 1962; Vinogradov V., Vorobjova N., 1973; Serov V., Shekhter A., 1981*]. The accumulation of histamine in perivascular space as a result of tissue basophiles degranulation is fraught with dilatation of arterioles, precapillaries, and venules thus bringing forth the enhancement of vascular permeability at the level of microhemocirculation pathways [*Gorizontova M., Chernukh A., 1975; Aleksandrov P. et al., 1976*].

As revealed by the morphometric analysis, in mesentery of Control Group rats there were determined approximately similar indices of tissue basophiles without the signs of degranulation and cells with the 1st and 2nd degree degranulation (summary indicator). No signs of complete degranulation of tissue basophiles (3rd degree degranulation) were observed in either Control or Experimental Groups (Table 2).

In mesentery of rats involved in Experiment Group 1, tissue basophiles without any signs of degranulation dominated in 20 *min* after melatonin administration. Similar cells were found to be omnipresent: both in pericapillary compartments and sites localized remotely from microvessels.

Table 2.

The number of mesentery tissue basophiles with different degree of degranulation under conditions of melatonin administration to rats

Study Groups (n=16)	Distribution of cells according to degree of degranulation			
	Without degranulation	1st degree	2nd degree	3rd degree
Control	14.5 ± 1.3	10.4 ± 1.1	6.5 ± 0.7	not revealed
Experimental I	24.2 ± 1.0 P < 0.0005	3.7 ± 0.3 P < 0.0005	1.8 ± 0.2 P < 0.0005	not revealed
Experimental II	15.9 ± 2.4 0.4 > P > 0.25	10.1 ± 1.5 P > 0.4	6.1 ± 1.2 0.4 > P > 0.25	not revealed
Experimental III	13.8 ± 0.6 0.4 > P > 0.25	11.0 ± 1.4 0.4 > P > 0.25	6.2 ± 0.2 0.4 > P > 0.25	not revealed

P was determined by comparison of indices in Experimental Groups with those in Control.

Thus, in mesentery of rats embraced in Experimental Group I the number of tissue basophiles, in which signs of degranulation failed to be found, exceeded the number of cells with the 1st and 2nd degrees of degranulation (summary indicator) 4.4-fold and the number of tissue basophiles without the signs of degranulation in mesentery of animals of Control Group 1.7-fold. In mesentery of animals of Experimental Groups II and III indices of tissue basophiles degranulation practically did not differ from those of Control Group.

Table 3 reflects the results of the quantitative fluorescent microscopy analysis performed for determination of histamine and serotonin in tissue basophiles.

As obvious from Table 3, the content of histamine and serotonin in tissue basophiles of mesentery in rats of Experimental Group I was markedly increased. In 2 and 8 hours post melatonin administration the level of histamine and serotonin was normalized. The specific fluorescence in cytoplasm of tissue basophiles of mesentery in rats of Control Group had a dual character: small-granular and homogenous, whereas in mesentery cells of Experimental Group I rats the intense specific fluorescence was registered, being of exceptionally homogenous character; this latter signified to rather high contents of histamine and serotonin in cytoplasm of tissue basophiles (Figure 3b,d). In animals of Control Group as well as in those of Experimental Groups II and III due to partial degranulation of tissue basophiles the specific fluorescence as separate granules of histamine and serotonin was determined in perivascular spaces, along the arterioles, capillaries and, especially, venules. Only singular granules were determined in perivascular spaces of animals of Experimental Group I on the background of a decrease in number of tissue basophiles with the 1st and 2nd degrees of degranulation.

It should be specially mentioned that similar morphofunctional shifts were observed in mesentery and loose connective tissue of experimental mice and hamsters, as well as cheek pouches of hamsters. This latter to a certain degree signifies in favour of information value of our research findings.

The results of fluorometric analysis performed for revealing ionized calcium in mesentery cells of experimental animals are presented in Table 3 and Figure 4b. As evident from the Table, 20 *min* after melatonin administration to intact rats the specific fluorescence indices of chlortetracyclin-calcium complex significantly decreased in tissue basophiles. The indices of fluorescence in mesentery tissue basophiles of rats from Experimental Group I were 2.0 times lower than those in Controls. The marked attenuation of a specific lumines-

Table 3.

The content of histamine, serotonin, and ionized calcium in mesentery tissue basophiles of rats under conditions of melatonin administration

Study Groups (n=16)	Indices under study in conventional units of fluorescence		
	histamine	serotonin	Ca^{2+}
Control	20.4 ± 2.3	28.6 ± 2.1	56.3 ± 6.5
Experimental I	34.4 ± 2.7 0.005 > P > 0.0005	37.2 ± 3.4 0.025 > P > 0.01	27.5 ± 4.7 0.005 > P > 0.0005
Experimental II	22.8 ± 1.7 0.25 > P > 0.1	32.1 ± 3.8 0.25 > P > 0.1	43.75 ± 7.7 0.25 > P > 0.1
Experimental III	19.2 ± 2.0 0.4 > P > 0.25	29.5 ± 2.2 0.4 > P > 0.25	50.9 ± 4.0 0.25 > P > 0.1

P was determined by comparison of indices in Experimental Groups with those in Control.

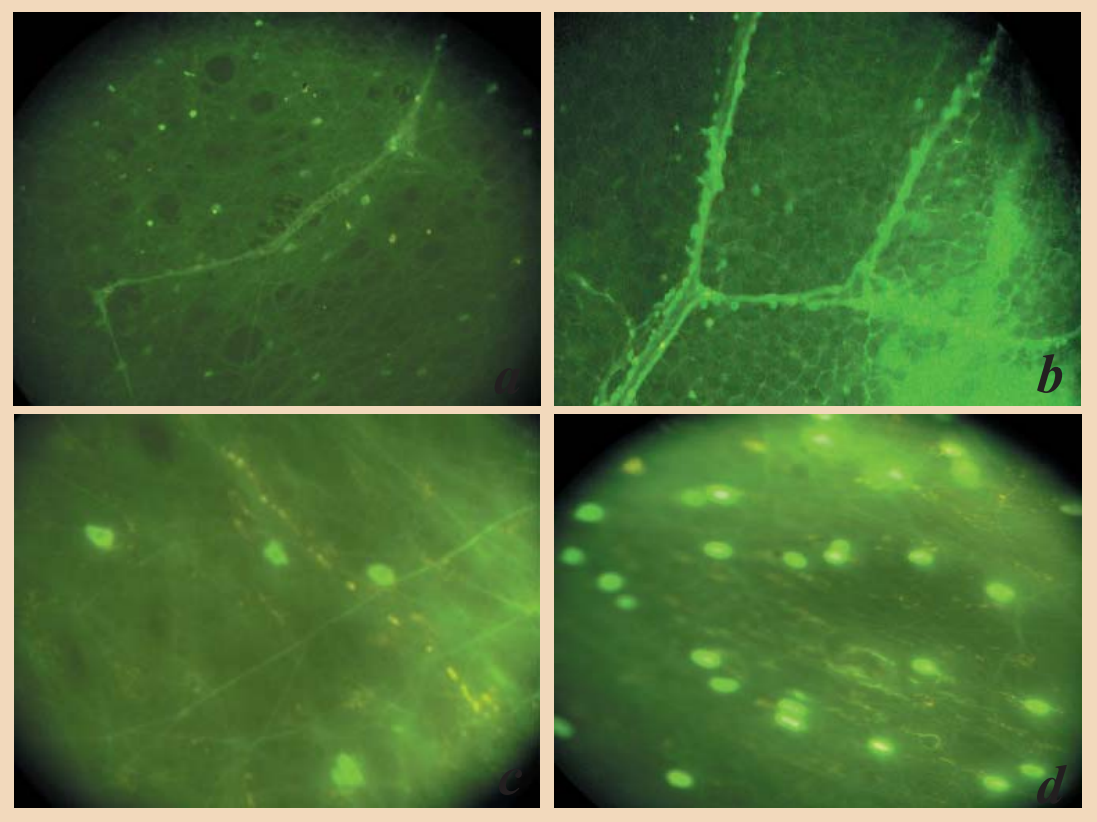

Figure 3. *Topical peculiarities of histamine and serotonin distribution in mesentery tissue basophiles of rats under melatonin administration. Fluorescent microscopy.*

a) Control Group. Moderate fluorescence of serotonine in cytoplasm of pericapillary-oriented tissue bazophiles.

b) Experiment Group I. Intense specific fluorescence of serotonin in tissue basophiles oriented along the capillaries (obj. 4, oc. 10).

c) Control Group. Singular perivascular-oriented tissue basophiles with signs of histamine moderate fluorescencein cytoplasm (obj. 4, oc. 10).

d) Experiment Group I. Intense specific fluorescence of histamine in cytoplasm of most tissues basophiles (obj. 40, oc. 7).

cence/fluorescence was revealed in cytoplasm of fibroblastic type cells (Figure 4d). However, in cytoplasm of endothelial cells of mesentery arterioles, venules and capillaries rather high degree of specific fluorescence intensity was determined compared to the Control Group. Alongside with this as stated above in tissue basophiles of Experimental Group I relatively high indices of histamine and serotonin were registered.

It is known that in the mammalian organism universal mechanisms are engaged ensuring the processes of synthesis and secretion of a wide range of biological active substances, including those of mediatory and hormonal origin which are secured due to optimal concentrations of intracellular/extracellular calcium under obligatory maintenance of regional ionic balance. In this concern, we have the following interpretation of the revealed fact on inverse dependence of high levels of histamine and serotonin, on the one hand, and the low content of ionized calcium in tissue basophiles, on the other hand. Under conditions of melatonin administration, relatively high indices of histamine and serotonin in mesentery tissue basophiles were not due to their enhanced synthesis, but a consequence of impaired processes ensuring

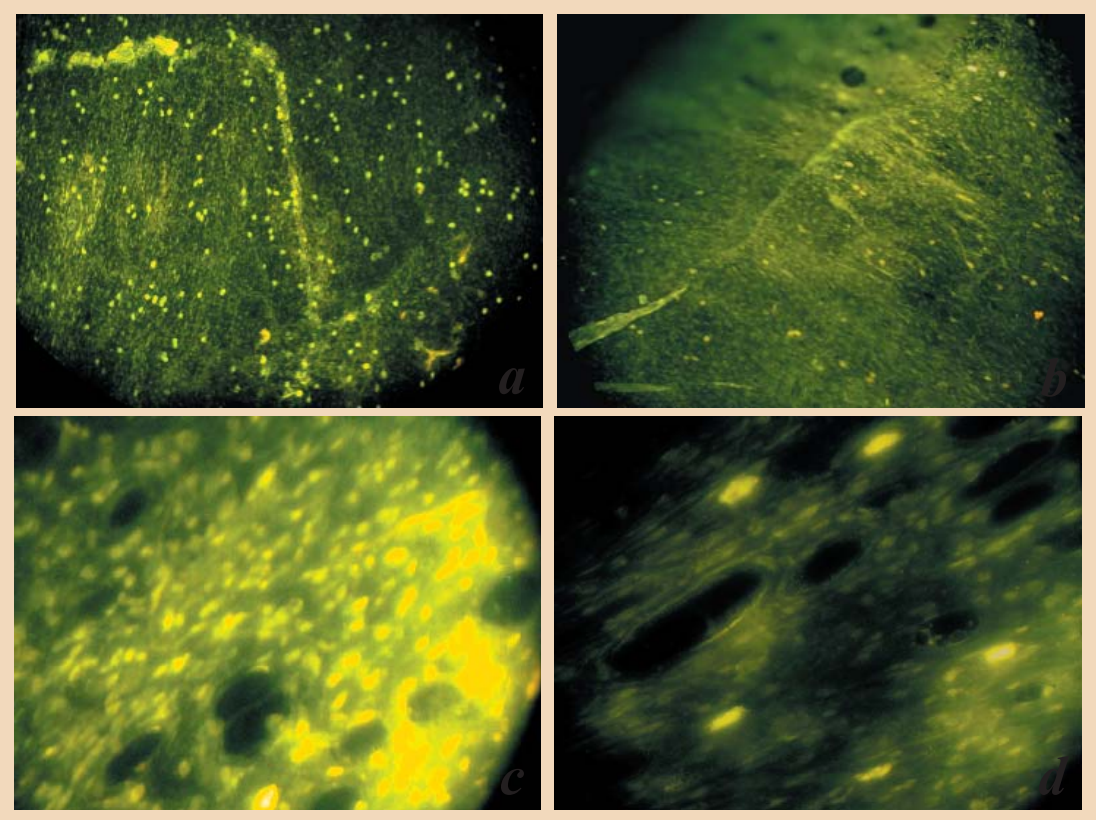

Figure 4. *Topical peculiarities of chlortetracycline-calcium complex in cytoplasm of rats mesentery cells under melatonin administration. Fluorescent microscopy. Chlortetracycline-calcium probe processing.*
a) Control Group. Intense fluorescence of chlortetracycline-calcium complex in cytoplasm of most perivascular-oriented tissue basophiles (obj.4, oc. 10).
b) Experiment Group I. Marked decrease of intense specific fluorescence in cytoplasm of tissue basophiles signifying to low content of ionized calcium (obj. 4, oc. 10).
c) Control Group. Intense fluorescence of chlortetracycline-calcium complex in cytoplasm of most fibroblasts and fibrocytes (obj. 40, oc. 7).
d) Experiment Group I. Moderate and low fluorescence of chlortetracycline-calcium complex in cytoplasm of fibroblasts and fibrocytes (obj. 40, oc. 7).

their release into pericapillary space. To this latter, though indirectly, signified the fact of significantly decreased number of cells without signs of degranulation, in which we registered low indices of ionized calcium.

Undoubtedly, the mentioned assumption requires a special investigation aimed at studies on exquisite mechanisms of impairment in regional calcium metabolism and ionic balance under conditions of rather low concentrations of melatonin administered to experimental animals. We consider it untimely to interpret the obtained findings on an increase of ionized calcium levels in endotheliocytes of mesentery microvessels and loose connective tissues of experimental animals. It is not ruled out that melatonin produces modulating influence on processes of biological active substances production ensuring under conditions of normal organism functioning the maintenance of vascular-tissue homeostasis at the level of microcirculation pathways.

Discussion

The single intravascular administration of rather low doses of melatonin to experimental animals was accompanied by the marked decrease

of mesentery microvessels permeability for relatively large disperse particles of colloid coal. Based on our findings, it is concluded that of no less importance for the process of vascular permeability decrease is the role of tissue basophiles which are considered as a pivotal extravascular factor for regulation of histohematic barriers and, in particular, the local blood flow at the level of microhemocirculation. To our mind, the decrease of vascular permeability for colloid ink particles is associated with the functional state of tissue basophiles that occurs upon administration of melatonin.

Thus, at the initial stages of the experiment (in 20 *min* after melatonin administration), there were observed almost no signs of tissue basophiles degranulation and release of histamine and serotonin granules into pericapillary space; this latter having an important role for regulation of transcapillary exchange and vascular permeability. In favour of this circumstance signifies also the fact that in animals of Experimental Group I on the background of a decrease in number of degranulated tissue basophiles there occurs an increase of cells without the signs of degranulation; in this latter rather high content of histamine and serotonin is determined. The hematosynovial barrier was also impermeable for plasma proteins with relatively high molecular weight, as registered in our experiments upon administration of FITC-labeled homologous gamma-globulin to experimental animals. Alongside with this single intravascular administration of melatonin was followed by an increase of microhemocirculation pathways permeability for relatively low-molecular compounds; to this latter signified positive results of fluorescent microscopy studies under conditions of uranin (sodium fluoresceinate) intravascular administration.

Thus, based on research performed, we can draw a conclusion, according to which administration of melatonin at concentration similar to that in rats nocturnal blood serum is accompanied with an increase of microvessels permeability exceptionally for low-molecular compounds. Apparently, this circumstance signifies in favour of occurrence of melatonin-dependent mechanism for regulation of vascular permeability at the level of microhemocirculation pathways in the mammalian organism. It should be mentioned that such important factors of extravascular regulation of vascular permeability as histamine and serotonin are not engaged in this process. The revealed shifts evidently bear a transient, temporary character, as in relatively late period of observation (in 2 and 8 *hours*) indices of both vascular permeability and tissue basophiles functional state are normalized. What are the possible mechanisms underlying the revealed shifts?

Firstly, the immediate influence of administered melatonin to structural components of microvessels walls cannot be excluded and first of all the effect to endotheliocytes and smooth muscle cells. There is evidence according to which melatonin produces the effect to large arterial vessels thus bringing forth a decrease of blood pressure in patients with hypertension [Anisimov V., 2006]. It is not excluded that under normal functioning of the organism the effect of melatonin is spread out to the smooth muscle cells of arterioles as well. In favour of the proposed supposition also signify the results of our investigation, according to which the signs of increased vascular permeability in the system of microhemocirculatory bed are most frequently traced precisely in arterioles.

Secondly, there is evidence according to which melatonin possesses the ability to markedly decrease free radicals levels, including also NO in endotheliocytes of experimental animals under a number of induced pathological processes accompanied by the marked increase of free radical activity.

Probably, melatonin under normal and pathological conditions actively participates in processes of balanced production and inhibition of free radicals in cellular components of microvessel walls. At the same time, it should be specially mentioned that mechanisms of the probable action of endogenously produced melatonin to integral components of the cardiovascular system should not base only on findings obtained under

pathology conditions. In this latter case, i.e. when different pathology states are reproduced in experiment, new adaptation mechanisms can be engaged, the activity of which under normal functioning of integrative systems of the organism remains without any demand.

In this concern to our mind, it seems rather promising to perform investigation aimed to study the activity of iNOS and to determine NO levels in endotheliocytes of the microhemocirculatory bed of intact animals under conditions of melatonin administration at rather low concentrations. Probably, under conditions of physiological activity of the organism melatonin activates processes of NO topical synthesis in endotheliocytes thus bringing to vasodilation of microvessels in arteriolar link of microhemocirculation pathways. Similar studies will be performed in the nearest future.

Thirdly, one cannot exclude the influence of exogeneously-administered melatonin towards tissue basophiles; moreover, it is known that melatonin easily penetrates through the existing hematohistocytar barriers. Probably, under conditions when the organism normally functions at the intercellular and especially at cellular level, in tissue basophiles there are engaged physiological mechanisms functioning according to reciprocal principle: between melatonin, on the one hand, and histamine and serotonin, on the other hand.

Indeed, data is available, according to which not only serotonin is revealed as a precursor of melatonin in tissue basophiles but also melatonin itself is found [*Kwetnoi I. et al., 1999*]; this evidence supports the proposed concept on probability of reciprocal mechanisms for inter-regulation of serotonin and melatonin synthesis and inhibition processes in tissue basophiles.

In conclusion, we consider it necessary to dwell on still another melatonin-dependent mechanism of functional activity in tissue basophiles, which is apparently the fundamental one and might be engaged under conditions of our experiment as well. To our mind, it seems expedient to present main relevant statements set forth in a rather informative paper of G. Izzo and co-authors reflecting the highest scientific and methodological level of the investigation [I*zzo G. et al., 2004*]. The authors studied the effect of melatonin to tissue basophiles of mammals, amphibians, and frogs under conditions of estradiol-induced proliferation of mentioned cells. It is of interest that the authors used doses of melatonin similar to those tested in our studies. In control series of the experiment G. Izzo and co-authors demonstrated that estradiol caused proliferation of tissue basophiles in experimental animals and the increased synthesis of histamine and serotonin followed. Preliminary administration of melatonin *in vivo* and *in vitro* was accompanied by inhibition of these processes, i.e. melatonin possessed the expressed antiproliferative action towards tissue basophiles. Under estradiol-induced stimulation of tissue basophiles, the antiproliferative effect of melatonin had a transient character. The probable mechanisms of antiproliferative action exerted by melatonin under estradiol-induced activity of tissue basophiles were also discussed. In particular, the authors assumed a direct influence of melatonin to tissue basophiles by means of a receptor transcription RBL-2H3 of rats tissue basophiles. These results convincingly signify to the direct effect of melatonin to tissue basophiles through autologous receptors.

One cannot rule out that under conditions of our experiment the receptor mechanisms of direct inhibiting effect of melatonin to processes of tissue basophiles proliferation were engaged as well as those of histamine and serotonin secretion.

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