Ultrastructural Organization of Capillaries in Various Compartments of the Dog Heart in Artificial Immersion Hypothermia

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Similarity of the structural characteristics of perfusion capacity of right-atrial and left-ventricular capillaries of the dog heart in health were detected, the capillaries differing significantly by size and by the majority of the morphometrical values. Exposure of animals to hypothermia induces different ultrastructural responses of the capillaries in two compartments of the heart: the perfusion characteristics of the right atrium deteriorate and the morphometrical parameters of endotheliocytes change, while in the left ventricle the population composition of endothelial cells changes significantly (the percentage of cells of the main type reduces, while the share of dark cells increases).

Key Words: *hypothermia; dog myocardium; capillaries; capillary perfusion capacity; ultrastructure*

Comparative analysis of morphological characteristics of various cardiac compartments and their intraoperative restructuring led to accumulation of a vast scope of information, mainly about cardiomyocytes (CMC). Normally atrial CMC are less differentiated cells than ventricular ones, but they are characterized by a high level of development of organelles, involved in intracellular syntheses and secretory process [5]. Atrial CMC exhibit a higher resistance to destructive factors of surgical stress at all stages of cardiosurgical interventions and more rapidly restore their structure than ventricular cells [8].

In contrast to CMC, the ultrastructural organization of capillary system of the atria and ventricles remains virtually not studied in health or under conditions of exposure to pathophysiological stimuli. Some studies demonstrated a lesser content of reserve (nonfunctioning) capillaries in the left *vs.* right heart [10]; differences in the restructuring of the secretory system of ventricular and atrial endothelial cells under conditions of artificial hypothermia were detected [1]. It remains unknown whether there are some other differences in these compartments.

Comparative ultrastructural and stereomorphometric analysis of right-atrial and left-ventricular capillaries was carried out by transmission electron microscopy under normal conditions and after surface (immersion) exposure of animals to hypothermia (22-24°C).

MATERIALS AND METHODS

The study was carried out on biopsy specimens of the right atrium and left ventricle of 14 mongrel dogs of both sexes (9-12 kg). Six animals served as controls (they were sacrificed at normal body temperature, 36-37°C); the remaining 8 dogs were

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exposed to artificial immersion surface cooling to 22-24°C under deep narcosis. Biopsy specimens were collected and immediately washed in phosphate buffer (pH 7.35), after which they were plunged into a fixative (2% paraformaldehyde and 2.5% glutar aldehyde). After 24 h biopsy specimens were divided into 1-mm^3 specimens and again plunged in the fixative (for 32 h), after which they were washed in several portions of PBS, treated during 2.5 h in 1% OsO_4 in PBS, dehydrated in ascending alcohols and acetone, and embedded in epon and araldite mixture. Ultrathin sections were contrasted by uranylacetate and lead citrate and analyzed in a JEM 100 CX electron microscope.

Quantitative ultrastructural analysis was carried out by a universal protocol. Total number of capillary profiles in a section was counted at the initial ×5000 magnification of the negative images. Capillaries with open and slit lumen were distinguished. Capillaries with slit lumen were subdivided into 2 groups: vessels with the lumen narrowed for functional and "pathological" reasons. The former group included capillaries with shrunk lumen and capillaries closed by an endotheliocyte (EC) nucleus, protruding deeply into the intravascular space. Group 2 were capillaries with the lumen obstructed by aggregated blood cells, sharply swollen EC cytoplasm or its vesicular fragments. Total number of EC profiles, forming the inner lining of capillaries, was evaluated at the initial ×10 000 magnification. The percentage of each of the five morphological variants of cells (main type, clear, dark, edematous, and hyperosmium) was evaluated in the total EC population [11]; at least 100 cells per animal were analyzed. The geometrical characteristics of right-atrial and left-ventricular capillaries were estimated by the formulas suggested previously [14] in modification [3] suggested for morphometrical analysis of components of the capillary system of the organ. The significance of differences was evaluated using Student's *t* test.

RESULTS

The overwhelming majority of left-ventricular and right-atrial capillaries in the controls had open lumen and were easily perfused by blood. The intravascular space was filled by clear plasma of even granular structure and by erythrocytes with high electron density of the matrix (Fig. 1, *a*). The group of capillaries with slit lumen included mainly shrunk capillaries and capillaries obstructed by an EC nucleus protruding into the intravascular space; in other words, these capillaries were excluded from the bloodflow by functional mechanisms. Pathological stenosis of capillary lumen was extremely rare in both cardiac compartments (Table 1).

Total diameter of the capillaries, diameter and area of the lumen, mean thickness and area of endothelial lining, and the summary factor of both contours shapes in the intact dog right-atrial myocardium were significantly ($p<0.05$) greater than in left-ventricular capillaries. The lengths of the capillary basal and luminal contours were similar in the two cardiac compartments (Table 2).

All five morphological variants of EC were detected in the capillary endothelium of control dogs: main type, clear, dark, edematous, and hyperosmium (Fig. 1, *b*). However the percentage of cells of the main type was significantly less in the right atrium than in the left ventricle, while the counts of clear and dark EC were higher (Table 3). The content of edematous and hyperosmium EC in the two cardiac compartments differed negligibly.

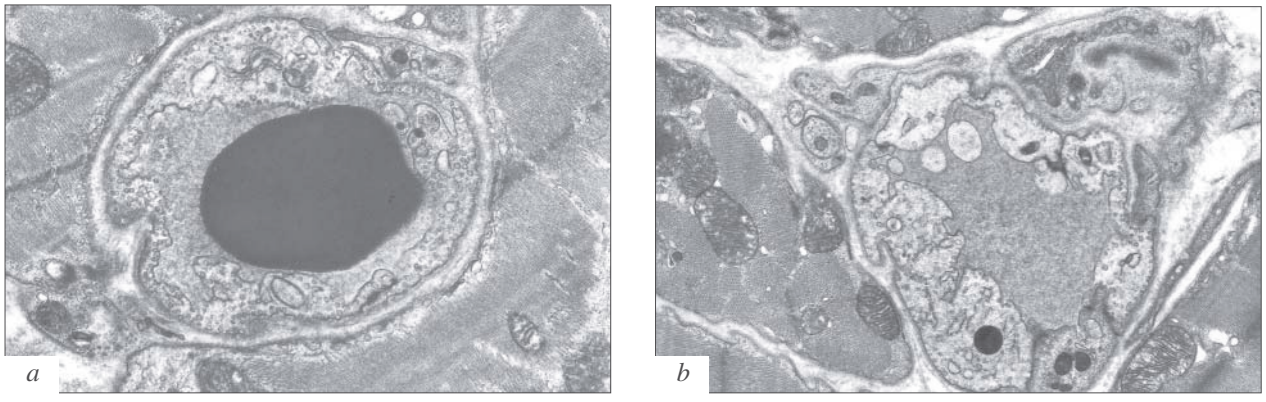

Fig. 1. Ultrastructure of exchange capillaries of the coronary bed in the intact dog myocardium. *a*) open left-ventricular capillary with intravascular space filled by clear granular plasma and containing an erythrocyte with high electron density of the matrix (×13,200); *b*) morphological variants of cells of the main types (clear and dark), forming the inner lining of capillaries in the right-atrial myocardium (×10,000).

TABLE 1. Characteristics of Perfusion Capacity of Exchange Capillaries of the Dog Right-Atrial and Left-Ventricular Myocardia Normally and after Surface Hypothermia of 22-24°C (*M*±*m*)

Group	Cardiac compartment	Capillaries (% of total number in section)		Mechanisms of capillary exclusion from bloodflow (% of total number in section)	
		open	closed	functional	abnormal
Control (T_E=36.6°C)	Right atrium (*n*=6)	86.51±1.44	13.49±1.44	10.65±1.27	2.84±0.46
	Left ventricle (*n*=6)	87.80±4.41	12.20±4.41	10.37±3.13	1.83±1.36
Hypothermia (T_E=22-24°C)	Right atrium (*n*=8)	73.36±3.82*	26.64±3.82*	18.73±2.69*	7.91±2.07*
	Left ventricle (*n*=6)	82.60±2.33	17.40±2.33	16.15±2.53	1.30±0.66+

Note. Here and in Tables 2, 3: T_E: temperature in the esophagus, $p<0.05$ *vs.* *control group, +right atrium.

Deep hypothermia (22-24°C) led to changes in the morphological characteristics of perfusion capacity of the right-atrial capillaries in comparison with the control (Fig. 2). The number of open capillaries decreased significantly, while the percentage of capillaries closed functionally and because of pathological narrowing of the lumen increased (Table 1). The number of open and closed capillaries in the left ventricle virtually did not change in comparison with the control (Table 1). The plasma in the lumens of the majority of open capillaries looked unevenly granular, sometimes floccular in both compartments of the heart. In addition to erythrocytes with normal structures, there were dumbbell-shaped, club-shaped, or irregularly shaped blood cells (Fig. 3, *a*).

Surface cooling of the body led to changes in some morphometrical characteristics of the capillaries only in the right atrium. The thickness and area of capillary endothelial lining increased significantly in comparison with the control. Capillary morphology in the left ventricle did not differ much from the control, but some characteristics differed significantly from those in the right atrium (Table 2). For example, total capillary diameter, thickness and area of the endothelium and length of the basal capillary contour remained lesser in the left ventricle after hypothermic exposure in comparison with the right atrium.

The population composition of EC in exchange capillaries changed exclusively in the left ventricle in response to hypothermia. The percentage of cells of the main type decreased significantly (by 36%) in the capillaries of this cardiac compartment, while the content of dark EC increased 1.7 times (Table 3; Fig. 3, *b*). Quantitative proportion of five morphological EC variants in the right atrium did not differ much from the control (Table 3).

Analysis indicated that the capillary compartment of the microcirculatory network of the right atrium and left ventricle were actively perfused by blood. Another proof of this fact was a low percentage of closed capillaries (mainly excluded from the bloodflow by functional mechanisms), this reflecting the changeability of their working status essential for local regulation of tissue homeostasis

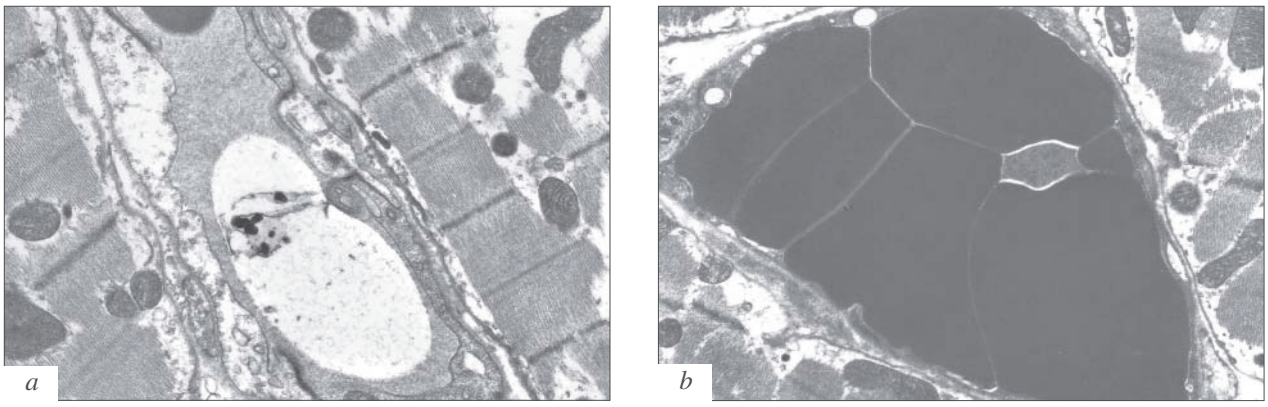

Fig. 2. Ultrastructure of exchange capillaries of the dog right-atrial myocardium after deep (22-24°C) surface hypothermia. *a*) large vesicle-like structure with myelin-like bodies in the capillary lumen (×13,200); *b*) erythrocyte aggregation blocking the intravascular space of the capillary (×10,000).

[9]. Right-atrial capillaries were larger, their walls thicker, but their basal and luminal surfaces were less twisted than in the left ventricle, the length of their contours being the same. This result can be interpreted as a lesser specialization of atrial exchange capillaries for realization of the transport function in comparison with ventricular capillaries, as it was hypothesized that the efficiency of oxygen delivery to a tissue site increased with increase in the number of capillaries and reduction of their diameter [4]. However the initial percentage of dark cells in the right-atrial capillary endothelium was significantly ($p<0.05$) higher than in the left ventricle. Previous data, demonstrating the presence of the most pronounced transcytosis system in the dark EC (of all EC variants) [11], suggest that low adaptation of atrial capillaries to realization of convection transport regulated by the capillary geometry, can be compensated for by a high level of dissipation transfer of macromolecules in them.

The conditions of deep hypothermia are inessential for the perfusion capacity of ventricular capillaries, while in the atrium hypothermia stimulated exclusion of rather many capillaries from the bloodflow. This suggests revising the assumption on the biphasic response of coronary microvessels to temperature reduction, manifesting by vasoconstriction under conditions of 25-28°C hypothermia and vasodilatation in response to hypothermia below 25°C. It seems that this reaction is observed only in the left ventricle. In the right atrium transition to the level of deep hypothermia caused no attenuation of vasospastic reaction of myocardial capillaries and even promoted activation of pathological modes of intravascular space narrowing. Presumably, the paradoxical response of right-atrial capillaries to 22-24°C hypothermia is explained by specific organization of their EC secretory system, with a higher (in comparison with the ventricle) incidence of specific endothelial granules and their more intense exocytosis under the effect of cardiosurgical stress factors [1]. The granules contain a reserve of endothelin-I (regulating vasoconstriction) and P-selectin (a potent adhesion molecule whose activation can reduce the athrombogenicity of the EC luminal surface [2]). Massive release of these bodies' contents into the blood can lead to a sharp increase of vasomotor tone and stimulation of thrombogenesis.

The mechanism responsible for opposite morphometrical changes in the capillaries of two cardiac compartments consists presumably in swelling of the "working" atrial EC types, as thickening and enlargement of the inner lining of the capillaries of this compartment are not associated with changes

TABLE 2. Morphometric Characteristics of Exchange Capillaries of the Right-Atrial and Left-Ventricular Myocardia in Dogs Normally and after Surface Hypothermia (22-24°C; *M*±*m*)

Group	Heart compartment	Total diameter of capillary, μ	Diameter of capillary lumen, μ	Thickness of capillary endothelium, μ	Length of capillary basal contour, μ	Length of capillary luminal contour, μ	Area of capillary endothelium, μ^2	Area of capillary lumen, μ^2	Summary factor of capillary shape (F)
Control (T_E=36.6°C)	Right atrium (*n*=6)	5.47±0.14	4.22±0.16	0.46±0.01	21.49±0.33	20.02±0.50	9.73±0.38	14.99±1.14	0.38±0.01
	Left ventricle (*n*=6)	4.81±0.14+	3.55±0.19+	0.41±0.01+	19.94±0.93	19.81±1.34	8.29±0.43+	10.71±1.08+	0.34±0.02+
Hypothermia (T_E=22-24°C)	Right atrium (*n*=8)	5.51±0.20	3.97±0.25	0.53±0.02*	22.06±0.96	20.12±1.03	11.46±0.68*	13.82±1.61	0.36±0.01
	Left ventricle (*n*=6)	4.91±0.13+	3.79±0.11	0.39±0.04+	19.57±0.51+	19.76±0.72	7.73±0.76+	11.99±0.69	0.36±0.01

TABLE 3. Population Composition of EC in Right-Atrial and Left-Ventricular Myocardial Capillaries of Dogs in Health and after Deep (22-24°C) Surface Hypothermia (*M*±*m*; % of Total Number)

Group	Heart compartment	EC type				
		main type	clear	dark	edematous	hyperosmium
Control (T_E=36.6°C)	Right atrium (*n*=6)	43.37±2.02	24.11±2.31	29.58±2.24	2.61±0.59	0.33±0.27
	Left ventricle (*n*=6)	65.17±3.02^{+}	15.17±3.23^{+}	17.50±2.95^{+}	1.16±0.79	1.00±0.52
Hypothermia (T_E=22-24°C)	Right atrium (*n*=8)	40.55±3.05	23.62±1.91	30.56±2.85	3.41±0.65	1.86±1.34
	Left ventricle (*n*=6)	41.50±2.68*	8.83±1.60^{+}	47.17±3.04*$^{+}$	1.33±0.80	1.17±0.65

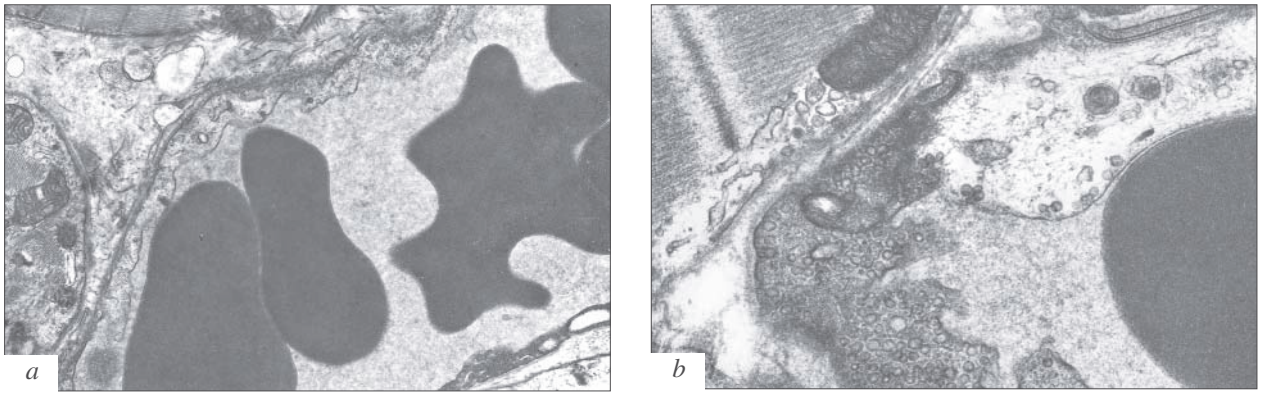

Fig. 3. Ultrastructure of exchange left-ventricular myocardial capillaries in dogs exposed to deep (22-24°C) surface hypothermia. *a*) erythrocytes with high density of matrix of normal and peculiar shape, detected in capillary lumen (×10,000); *b*) dark EC with numerous micropinocytous vesicles in the capillary endothelial lining (×26,000).

in the endothelial population composition and a significant increase in the percentage of edematous cells.

In contrast to the right atrium, deep (22-24°C) hypothermia caused shifts in the left-ventricular EC population composition in comparison with the control animals: the percentage of dark EC increased significantly, while the share of cells of the main type decreased. Presumably, the changes in the cytoplasm matrix and cytoskeleton, condensation of lipid components of intracellular membranes serve as the substrate for this EC restructuring, which is regarded as a manifestation of the favorable effect of hypothermia [6,7].

Hence, normally the ultrastructure of right-atrial and left-ventricular capillaries reflects different specialization of the exchange component of the coronary bed [12,13], providing intricate intracellular processes in CMC, different in the two cardiac compartments. For the left ventricle hypothermia is a factor mainly triggering the adaptive restructuring of capillary ultrastructure, while in the right atrium the adaptive processes are paralleled by destructive ones. These data suggest that the mechanisms, providing lasting retention of the trophic status of the cardiac compartment with the maximally developed morphological substrate of the main (contractile) myocardial function, are involved in the pathogenesis of high tolerance of hypothermia by ventricular capillaries.

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