Cardiopulmonary Microcirculation and Gas Exchange in Acute Adrenaline-Induced Injury

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Abstract: Microcirculatory alterations lead to cell and organ dysfunction. High levels of catecholamines induce tachycardia, coronary vasospasm, microcirculatory disruption, hypoxia, ventricular fibrillation. Hypercatecholaminemia increases pulmonary capillary resistance and permeability as well.

Aim of this research is to study adrenaline-induced alterations in myocardial and lung microcirculation, and gas exchange parameters in rats.

Experiments were performed in male Wistar rats divided into 2 groups: control (n=11), and animals (n=12) treated with histotoxic-dose adrenaline. Anesthetized animals were exposed to mechanical lung ventilation at a frequency of 40 breaths/min and were sacrified 20 min after adrenaline injection. Paraffin-embedded myocardial and lung tissues were stained by hematoxylin and eosin. Myocardial and pulmonary microcirculation was studied by Sisakyan and Chilingaryan methods for revealing intraorganic MCB based on detection of phosphatase enzymes' activities (acid phosphatase and Ca^{2+}-ATP-ase, respectively) in the vessel walls. Mean capillary diameter (MCD) was calculated using ocular-micrometer. Partial pressures of arterial blood O_2, CO_2 and pH were measured.

Histological studies showed that adrenaline induced contraction band lesions of cardiomyocytes, diffuse pulmonary infiltrations, hemorrhages. Myocardial and lung microcirculation studies revealed inhomogeneous and less intensive staining, tortuous course of capillary walls and their destructive changes. Blood gas analysis data indicated hypoxemia and hypercapnia developing along with acidosis following adrenaline injection.

In conclusion, high-dose adrenaline induces acute myocardial and lung injuries, manifested in inflammatory and microcirculatory alterations, disorders of pulmonary gas exchange that may aggravate further development of cardiopulmonary pathology. The data achieved in present study may allow further adjustment of treatment strategies for stress-induced myocardial injury considering the role of lung microcirculation and gas exchange disorders in the given pathology.

Keywords: Adrenaline, Gas exchange, Heart and lung injury, Microcirculation.

INTRODUCTION

Intraorganic capillary network provides gas exchange and metabolism between the blood stream and tissue cells. Consequently, microcirculatory disorders may lead to dysfunction of cells and organs. Therefore it is of major importance to study the microcirculation bed (MCB) for better understanding of organ functioning in physiological and pathological conditions.

Stress-induced overproduction of catecholamines (CA) may provoke tachycardia, coronary vasospasm, microcirculatory disorders with subsequent hypoxia, necrosis of cardiomyocytes (CMC) and ventricular fibrillation [1]. Activated phospholipases and cytotoxic radicals of CA oxidation along with influx of Ca^{2+} cause damage of CMC membranes [2].

High amounts of CA found in pheochromocytoma as well as CA injected to experimental animals may lead to increased pulmonary capillary resistance and permeability, lung inflammation, edema, generalized hypoxia and acute lung injury (ALI) [3, 4]. This state currently in clinical conditions corresponds to acute respiratory distress syndrome (ARDS) [5]. In turn, disorders of pulmonary gas exchange, the progressing hypoxia in ALI additionally contribute to myocardial injury (non-coronarogenic).

Arachidonic acid metabolites (prostanoids) that have diverse physiological effects in lungs and heart contribute also to ALI [6] and cardiovascular pathology [7, 8], trigger acute ischemic tissue injury via activation of endotheliocytes and oxidative stress processes [9].

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Histomorphological methods of microcirculatory studies in coronary artery disease and in experimental research allow workout and clinical appliance of new diagnostic tools, treatment and prevention strategies for cardiovascular pathologies.

The aim of this research was to study the acute adrenaline-induced alterations in myocardial and pulmonary microcirculation and gas exchange in rats.

MATERIALS AND METHODS

The experiment was approved by the Institutional Ethical Committee at the Yerevan State Medical University (Yerevan, Armenia) and conforms to the National Research Council guidelines for the Care and Use of Laboratory Animals [10].

Experiments were performed in male Wistar rats (150-170 g.) divided into 2 groups: group 1 – control animals (n=11); group 2 (n=12) – animals treated with single injection 0,09 mg/kg i.v. Adrenaline (Adr) (Adrenalin-zdorovye, Ukraine) under sodium pento-barbital anesthesia (40 mg/kg, i.p.). Anesthetized animals were exposed to mechanical lung ventilation (TOPO™ Volume/Pressure Small Animal Ventilator, Kent Scientific, USA) at a frequency of 40 breaths/min and tidal volume of 2,5 ml with a warmed and humidified room air (positive end-expiratory pressure (PEEP) ventilation mode, PEEP set at 3 cmH_2O) and sacrificed in 20 min following Adr injection.

Paraffin-embedded lung and myocardial tissues were stained by hematoxylin and eosin (H&E).

Microcirculatory Bed Staining

Hearts and lungs were removed into formaldehyde fixative for 12 hr or absolute acetone for 7 days. They were then mounted on a freezing microtome and 50-60 mkm transversal sections were cut. Sections were stained using Sisakyan [11] and Chilingaryan [12] methods for revealing intraorganic MCB based on detection of phosphatase enzymes' activities (acid phosphatase and Ca^{2+}-ATP-ase, respectively) in the vessel walls. Mean capillary diameter (MCD) was calculated using ocular-micrometer to measure the diameter of capillaries in 10 fields on each of ten slides in every animal.

Arterial Gas Analysis

Arterial blood samples were collected into heparinized vessels following the ligation of carotid artery immediately before sacrifice.

Partial pressure of oxygen (pO_2), pCO_2, and pH of arterial blood (carotid artery) were detected by Roche Cobas 121b gas analyzer (Roche, Germany).

ECG

Heart beat (HB) was calculated on ECG records (ECG recorder, BIOLAM, Russia).

Statistical Analysis

Was performed using Student's *t* test (statistical significance set at $P < 0.05$). All data are presented as mean ± SD.

RESULTS

Macroscopic examination on dissection of animals demonstrated enlarged, hyperemic myocardium, as well as edema and diffuse hemorrhages of lungs following Adrenaline injection.

H&E Staining

Histopathology of the heart revealed contractures and wavy outlines of CMC following Adr injection (Figure **1**). Interrupted intercalated disks leading to

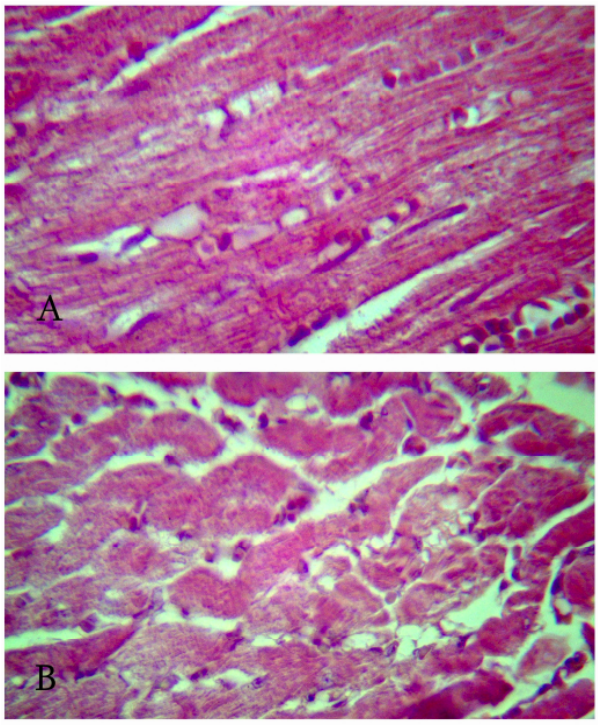

Figure 1: Light micrographs of cardiac muscle tissue stained with H&E (x400).
A) Control with normal muscle fibers, inter-muscular tissue and vasculature; **B**) Heart of an Adr-treated rat displaying massive muscle cell degeneration, inflammatory cellular response and marked stromal edema and degeneration.

CMC dyscomplexity were often found, intercellular capillaries were anemic, the myocardial stroma showed neutrophil retention (Figure **1**). Lung specimens of the same group animals exhibited pulmonary edema with interstitial and intra-alveolar involvement (Figure **2**). Eosinophilic exudate within bronchi and alveoli as well as thickening of interalveolar septa (due to hyperemia, focal erythrodiapedesis and diffuse inflammatory infiltration presenting recruitment of neutrophils) were found (Figure **2**).

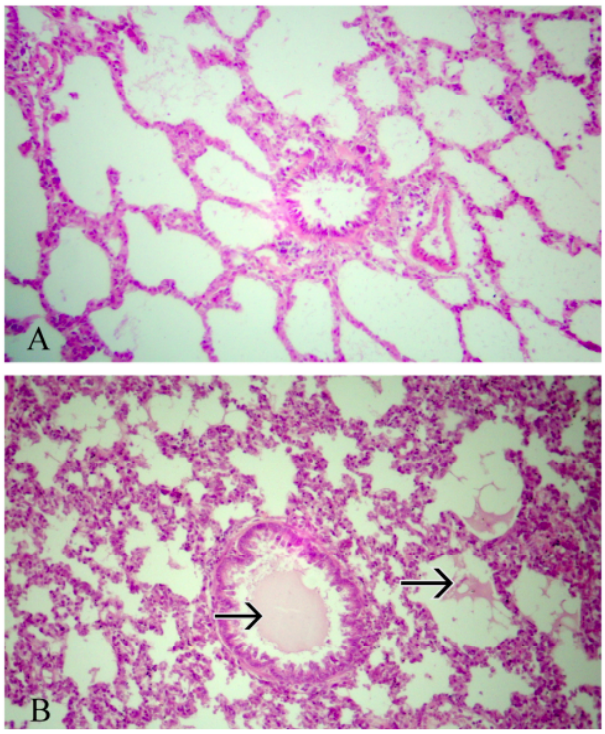

Figure 2: Lung sections stained by H&E (x100).
A) Control lung with normal structure of alveolar and bronchiolar tissues, **B**) Adr-treated animal lung with edema, inflammatory exudate in the alveolar spaces (right arrow) and bronchioles (left arrow).

Microcirculatory Bed Staining

Chilingaryan method of MCB staining in myocardium of control group showed parallel and continuous course of capillaries (Figure **3**), MCD was calculated as 6,33±0,5 mkm. Arterioles and venules could be visualized with arteriolar wall presenting circularly developed striated pattern provided by arrangement of smooth muscles (Figure **3**). Sisakyan's method could reveal the striated structure of myocardium along with location of capillaries parallel to each other and CMC (Figure **3**). Histotoxic dose adrenaline injection developed non-homogenous, weak staining of myocardial capillaries (Figure **3**) and increase of MCD by 8,6% (6,88 ± 0,44 mkm, P>0,05). Though the capillary dilation is not significant, it may, however, result in hemodynamic and functional alterations. Increase of capillary permeability led to recruitment of erythrocytes from blood stream.

Lung sections showed staining of bronchial and alveolar capillaries (Figure **4**). In contrast to myocardial microvessels, alveolar capillaries were shorter and located along the perimeter of capillary wall. Branched arterioles were discovered (Figure **4**). MCD of pulmonary capillaries was 5,82 ± 0,63 mkm. Pulmonary capillaries showed less intensive staining and interrupted course due to endotheliocyte disruption in Adr group vs. control (Figure **4**). MCD was measured as 6,5 ± 0,92 mkm (increase by 10,5%, P<0,05).

Blood Gas Analysis

Arterial gas profile showed hypoxemia (2,5-fold decrease of pO_2, P<0,001) and hypercapnia (three fold increase of pCO_2, P<0,001) development following Adr injection; blood pH changed to more acidic value (Table **1**).

Table 1: Blood Gas Profile and pH (M±SD)

Parameter	Control (n=11)	Adr (n=12)
pO_2 *(mm Hg)*	67,86±4,1	26,9±4,6*
pCO_2 *(mm Hg)*	38,15±4,29	118,12±10,59*
pH	7,36±0,1	7,08±0,19

* - P<0,001, significance of differences between Adr and Control groups.

Adrenaline injection caused significant decrease of heart beat by 87% in 1-3 min (from 284±32/min to 37±5/min, P<0,001) after adrenaline injection. Further cardiotoxic effect was accompanied with arrhythmias such as tachysystolia and extrasystoles (3-4 min follow up) leading to fibrillation and cardiac arrest in 5-7 min follow up.

DISCUSSION

Our experimental research provides the examinations of myocardial and pulmonary microcirculation and arterial gas exchange profile in acute adrenaline-induced injury.

One of the major causes of ischemic heart disease in clinical practice is coronary atherosclerosis, but myocardium with normal or mildly affected coronary arteries can be affected as well, particularly due to coronary vasospasm, microvascular obstruction,

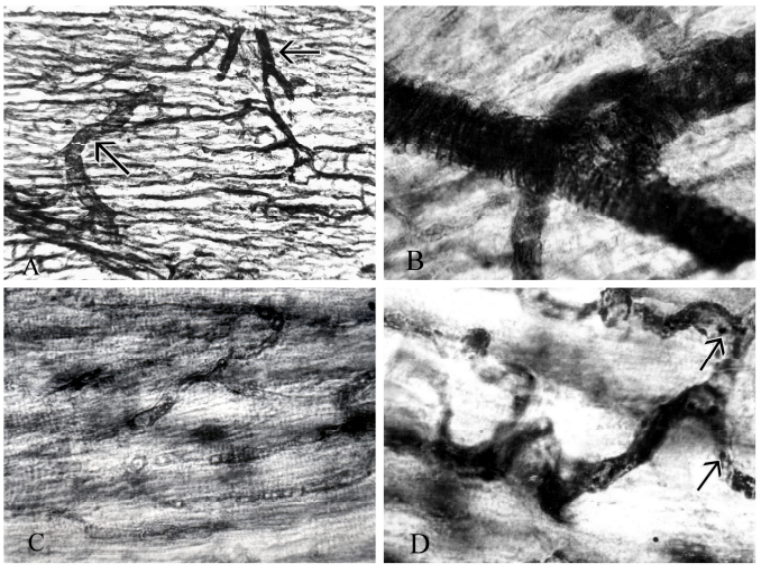

Figure 3: MCB of control myocardium (**A, B**) and Adr-treated rat myocardium (**C, D**).
A) parallel course of capillaries, branched arterioles and venules ranging between 30-50 mkm, darker and thicker staining of arterioles (right arrow) compared to venules (left arrow), Chilingaryan method (x40). **B**) Circular striations in the wall of a branched arteriole, stained by Chilingaryan method (x100). **C**) Weak staining of capillaries with RBC outlines within, visualization of CMC striations, Sisakyan method (x100); **D**) Capillaries have tortuous course, visible nuclei of endotheliocytes (arrows), inconsistent diameters with assortments of narrowed and bulged segments, Sisakyan method (x200).

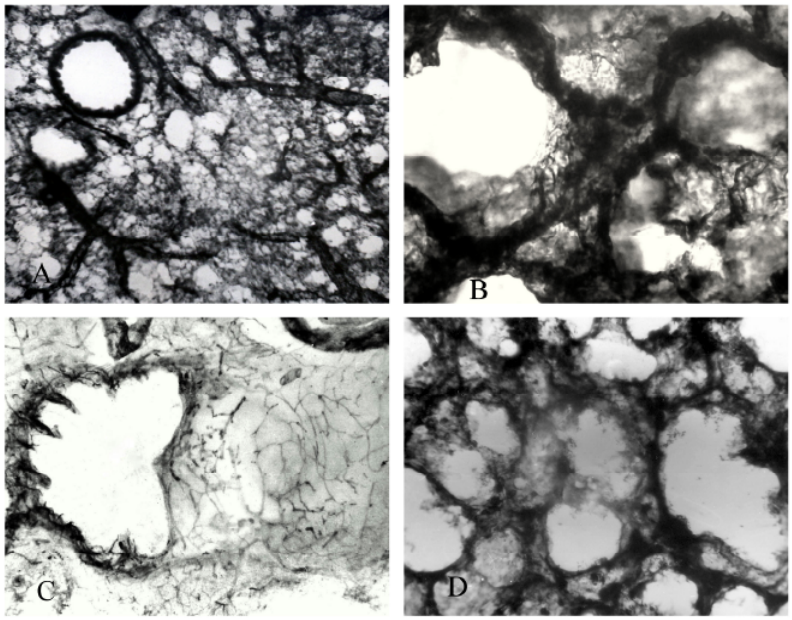

Figure 4: MCB staining in lungs of control rats (**A, B, C**) and Adr-treated animals (**D**).
A) Tree-like branching of arterioles, staining of bronchiolar capillaries in cross-section, Chilingaryan method (x40). **B**) Circular course of alveolar capillaries along the perimeter of alveoli, Chilingaryan method (x400). **C**) Cross section and a longitudinal fragment of a large bronchiole wall with stained capillaries, Chilingaryan method (x100). **D**) Pulmonary capillaries of Adr-treated animal lung stained by Sisakyan method. Interrupted and uneven staining of capillary walls, exudate retention in the alveolar spaces (x 400).

metabolic injuries secondarily developed due to myocardial capillary disorders [13, 14].

Our experiments in control animals revealed myocardial capillaries parallel to each other and CMC. This morphological pattern is physiological, since it prevents the systolic compression of capillaries, and provides increased capillary metabolic surface [15]. Almost twofold functional reserve in the microvasculature of the heart allows its adaptation to

short-term hypoxia [16], various non-coronarogenic factors (for example, high doses of adrenaline) may exert inhomogeneous pattern of myocardial injury.

As stated in the report of "Acute Lung Injury in Animals Study Group", an animal model of ALI ideally should involve one or more features of human ALI, including rapid onset (hours) after an inciting stimulus, evidence of pulmonary physiological dysfunction (*e.g.*, abnormalities of gas exchange), histological evidence of injury to the lung parenchyma (inflammatory response) and evidence of increased permeability of the alveolocapillary membrane [17]. Our results showed histological alterations, inflammatory response, and physiological dysfunction found in ALI/ARDS. In lungs, high-dose Adr treatment induced extravasation of neutrophils, erythrocytes, interstitial and intraalveolar edema, diffuse alveolar damage.

The research of Rassler and co-authors showed that infusion of CA (norepinephrine, phenylephrine, isoproterenol) over 72h may induce pulmonary remodelling (fibrosis and vascular hypertrophy) in rats. Authors concluded that both α- and β-adrenergic mechanisms contribute to such alterations. In this study cardiac hypertrophy developed presumably due to a direct adrenergic (predominantly β-adrenergic stimulation) effect rather than a consequence of pulmonary fibrosis [3].

Beta-adrenergic stimulation by adrenaline may have several deteriorate effects such as the increase of myocardial oxygen consumption, ventricular arrhythmias (particularly when the cardiac tissue is acidotic), transient hypoxemia secondary to attenuation of hypoxic pulmonary vasoconstriction, and subsequent increase in intrapulmonary, arteriovenous shunting, impaired microcirculation [18], and heart failure development [19].

Oxygen supply and development of hypoxia are predominantly dependent on lungs, and on morpho-functional state of alveolar capillaries. In contrast to capillaries in other organs, morphology and distribution of pulmonary capillaries is maximally adapted for diffusion function providing gas exchange [20, 21]. For example, these capillaries develop basket-form networks around the alveoli and cover around 75% of alveolar surface [22]. Such pattern of capillary arrangement provides lower pulmonary blood pressure required for more efficient functioning [23].

Results of our investigation showed disrupted gas exchange and acute lung injury, which are expectable, since the lung inflammation, edema and hemorrhages are mediated by structural alterations in capillary endothelium, responsible for the hemato-pulmonary barrier [24, 25]; and inflammatory fluid within alveolar space slows down the gas exchange preventing normal lung inflation [26]. As shown by several authors, hypercatecholaminemia increases pulmonary arterial pressure, and capillary wedge pressure [27, 28], which is accompanied by increase of permeability of capillary microvessels and recruitment of blood cells (leukocytes, erythrocytes) and high-molecular proteins [3].

CONCLUSION

High-dose adrenaline induces acute myocardial and lung injuries manifested in inflammatory and microcirculatory alterations, disorders of pulmonary gas exchange that may aggravate further development of cardiopulmonary pathology. The data achieved in present study may allow further adjustment of treatment strategies for stress-induced myocardial injury considering the role of lung microcirculation and gas exchange disorders in the given pathology.

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