

Original article

Ischemia depletes dystrophin and inhibits protein synthesis in the canine heart: Mechanisms of myocardial ischemic injury

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Abstract

In this study we tested our previous hypothesis that ischemia is a multifactorial injurious event involving all components of the myocyte simultaneously. This hypothesis was based on ultrastructural findings and was now tested again by protein analysis of sarcolemmal structural proteins and of markers of transcriptional and translational activities. This knowledge may help to clarify the cellular mechanisms involved in progression of acute ischemic myocardial injury and reperfusion. Therefore, we investigated all three intracellular/extracellular linkage systems of the sarcolemma using antibodies against dystrophin, β -dystroglycan, γ -sarcoglycan, vinculin, β 1-integrin, laminin, and spectrin. In addition, antibodies were used to evaluate membrane permeability (albumin), transcriptional efficacy (non-snRNP splicing factor SC-35), and translational capacity (phosphorylated p70 ribosomal protein S6 kinase). Tissue samples were obtained from a canine model of regional myocardial ischemia (90 min or 4.5 h) with or without reperfusion. Immunofluorescence microscopy and Western blotting revealed that the rank order of sensitivity was the following: dystrophin, β -dystroglycan, γ -sarcoglycan, vinculin, spectrin, integrin and laminin. Different levels of dystrophin loss indicate reversible/irreversible injury as established by albumin uptake and electron microscopy. Dystrophin depletion closely coincided with generally depressed transcription and translation. These changes occurred simultaneously in a time-dependent manner and persisted during reperfusion. In conclusion, damage of the different structural proteins results in membrane destabilization and disruption of the contractile apparatus from the sarcolemma. These changes, concomitantly associated with disturbances in transcription and translation, are major mechanisms determining the transition to irreversibility of myocardial ischemic injury and confirm our hypothesis that ischemia is a multifactorial injurious event involving all components of the cardiac myocyte.

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1. Introduction

Among other cellular events, the transition of reversible to irreversible acute ischemic injury is characterized by cell swelling and disturbance of the membrane associated proteins [1,2].

Three distinctive groups of structural proteins that link the extracellular and intracellular milieu and confer structural stability to the cell membrane are present in cardiac myocytes: the dystrophin-associated protein complex; the vinculin–integrin link; and the spectrin-based submembranous

cytoskeleton [3]. Several studies described involvement of these membrane-related proteins in the disruption of the plasma membrane [4–8] but a systematic comparative study of the sensitivity to ischemia of all three protein systems in the same model of ischemia is still lacking.

However, not only sarcolemmal disturbances but also damage of mitochondria, nuclei and sarcomeres concomitantly occur in the ischemic myocyte. Therefore, several years ago, based on studies with the electron microscope of ischemic myocardium, we hypothesized that ischemia is a multifactorial phenomenon involving simultaneously all cellular components of the cardiac myocyte [9]. In the present study, using protein analysis by confocal microscopy, we further tested this hypothesis by comparing the changes in sarcolemmal

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structure with disturbances in protein synthesis capacity as a fundamental activity of the cell important for homeostasis and survival. We used Sc-35, a non-snRNP factor essential in spliceosome assembly and splicing [10,11] as a marker of transcriptional capacity. To test for translation, we investigated p70 ribosomal protein S6 kinase, the major physiological ribosomal protein S6 kinase in mammalian cells [12]. Phosphorylation of S6 ribosomal protein correlates well with the rate of mRNA translation and, therefore, it reflects the translational efficacy of the cell [13].

The major final conclusion of this study is that severe damage of the sarcolemma and reduction of transcription and translation are simultaneous events and correlate well with cell survival or cell death in myocardial ischemic injury.

2. Material and methods

All experiments described here were carried out in Dr. Lucchesi's laboratory. The procedures used in this study are in accordance with the guidelines of the University of Michigan University Committee on the Use and Care of Animals and conform to the standards in The Guide for Care and Use of Laboratory Animals (NIH no. 86–23). Veterinary care was provided by the University of Michigan Unit for Laboratory Animal Medicine.

2.1. Surgical preparation

Purpose-bred beagle dogs, weighing 9–12 kg, were anesthetized with sodium pentobarbital (30 mg/kg, i.v.). The animals were intubated and ventilated with room air using a Harvard respirator (Harvard Apparatus, Inc., Holliston, MA), adjusted to deliver a tidal volume of 30 ml/kg at a frequency of 12–14 breaths/min. A catheter was inserted into the right jugular and right femoral veins. Blood pressure was recorded from the right femoral artery using a calibrated Millar™ Mikro-tip catheter (Millar Instruments, Inc., Houston, TX) interfaced with a Grass Model 7-polygraph recorder (Grass Instruments Division, Astro-Med Inc., West Warwick, RI). A standard limb lead II electrocardiograph was recorded continuously to monitor heart rate.

The heart was exposed through a left thoracotomy at the fourth intercostal space and suspended in a pericardial cradle. A 1-cm segment of the left circumflex (LCX) coronary artery was exposed proximal to the first obtuse marginal branch and instrumented with a Transonic™ ultrasonic flow probe (Model 1.5RB, Transonic Systems Inc., Ithaca, NY) for continuous monitoring of phasic coronary artery blood flow.

A Silastic™ umbilical tape was placed around the LCX coronary artery and through a polyethylene sleeve to create a snare occluder. A ligature stenosis was formed by interposing a blunt 18-gauge hypodermic needle between the snare and the vessel. Traction upon the umbilical tape resulted in occlusion of the LCX coronary artery at which point the hypodermic needle was withdrawn and the resulting hyperemic

response was determined. The tension on the snare occluded was adjusted to the point where the hyperemic response to a brief 10-s occlusion was reduced by 30%. All hemodynamic parameters were recorded throughout the experiment on a Grass Instruments multi-channel recorder interfaced with a PC computer running PO-NEH-MAH data acquisition software.

Separate groups of animals were studied and are described as follows:

Group I ($n = 4$): Animals were exposed to 90 min of regional myocardial ischemia by occlusion of the LCX coronary artery (without reperfusion) at which point the experiment was terminated by electrical induction of ventricular fibrillation initiated by application of the poles from a 9 V Ni/Cd battery to the apex of the heart.

Group II ($n = 5$): The animals were managed as those in Group I, but occlusion of the LCX coronary artery was maintained for 4.5 h, followed by euthanasia as described above.

Group III ($n = 5$): The animals were subjected to 90 min of regional myocardial ischemia after which reperfusion of the LCX coronary artery was restored by removal of the occlusive ligature. The hearts were reperfused for 3 h after which euthanasia was performed by electrical induction of ventricular fibrillation.

Additionally, samples from non-operated animals (Normal dogs; $n = 7$), and from sham operated animals ($n = 3$) were also studied and used as controls.

2.2. Preparation of tissue samples

At the conclusion of the *in vivo* study the hearts were removed and cleared of blood by washing rapidly in cold 0.9% sodium chloride solution. The heart was cut and the transverse section taken through the posterior papillary muscle was selected for isolation of transmural (endocardium to epicardium) tissue blocks ($6 \times 6 \times 6$ mm) from the non-ischemic region, and from LV myocardium including the infarct zone were removed for confocal microscopy or Western blot. These were immediately frozen in liquid nitrogen, and stored at -80°C . Small samples for electron microscopy were placed in plastic tubes containing buffered 3% glutaraldehyde solution and stored in a refrigerator at 4°C .

Samples were shipped via Federal Express to Bad Nauheim, Germany, in special containers containing either dry ice or with cold packs. Sufficient refrigeration was provided to maintain the tissue samples at the appropriate temperature for a period of 7 days.

2.3. Electron microscopy

Tissue samples fixed in buffered 3% glutaraldehyde were embedded in epoxy resin following routine procedures. Ultrathin sections were stained with uranyl acetate and lead citrate and evaluated and photographed using a Philips CM 10 electron microscope to determine the degree of ischemic injury.

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