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Original Contribution

Heme-induced contractile dysfunction in Human cardiomyocytes caused by oxidant damage to thick filament proteins

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ABSTRACT

Intracellular free heme predisposes to oxidant-mediated tissue damage. We hypothesized that free heme causes alterations in myocardial contractility via disturbed structure and/or regulation of the contractile proteins. Isometric force production and its Ca^{2+}-sensitivity (pCa_{50}) were monitored in permeabilized human ventricular cardiomyocytes. Heme exposure altered cardiomyocyte morphology and evoked robust decreases in Ca^{2+}-activated maximal active force (F_o) while increasing Ca^{2+}-independent passive force ($F_{passive}$). Heme treatments, either alone or in combination with H_2O_2, did not affect pCa_{50}. The increase in $F_{passive}$ started at 3 μM heme exposure and could be partially reversed by the antioxidant dithiothreitol. Protein sulfhydryl (SH) groups of thick myofilament content decreased and sulfenic acid formation increased after treatment with heme. Partial restoration in the SH group content was observed in a protein running at 140 kDa after treatment with dithiothreitol, but not in other proteins, such as filamin C, myosin heavy chain, cardiac myosin binding protein C, and α-actinin. Importantly, binding of heme to hemopexin or alpha-1-microglobulin prevented its effects on cardiomyocyte contractility, suggesting an allosteric effect. In line with this, free heme directly bound to myosin light chain 1 in human cardiomyocytes. Our observations suggest that free heme modifies cardiac contractile proteins via posttranslational protein modifications and via binding to myosin light chain 1, leading to severe contractile dysfunction. This may contribute to systolic and diastolic cardiac dysfunctions in hemolytic diseases, heart failure, and myocardial ischemia–reperfusion injury.

1. Introduction

Oxidative stress, characterized by excess production of reactive oxygen species (ROS), has been implicated in several cardiovascular pathologies including chronic heart failure [1–3], myocardial ischemia–reperfusion injury [4], and cardiovascular complications of hemolytic diseases [5,6]. High levels of ROS cause lipid peroxidation, DNA damage, mitochondrial dysfunction, and oxidation of myocardial proteins [7]. ROS production can modify protein function by oxidative posttranslational modifications such as protein sulfhydryl (SH) group oxidation to disulfide bonds and/or to sulfenic acid [8]. Nevertheless, the molecular mechanisms leading to impaired myocardial function during oxidative stress are obscure, and effective antioxidant therapies are lacking.

Abbreviations: A1M, Alpha-1-microglobulin; BSA, Bovine serum albumin; cMyBPC, Cardiac myosin binding protein C; CPK, Creatinine phosphokinase; DMF, Dimethylformamide; DTDP, 2,2'-Dithiodipyridine; DTT, Dithiothreitol; ECL, Enhanced chemiluminescence; EGTA, Ethylene glycol tetra-acetic acid; *F*, Isometric force; *Fo*, Force at saturating [Ca^{2+}]; $F_{passive}$, Passive force; F_{total}, Total peak isometric force; HO-1, Heme oxygenase 1; IC_{50}, Half maximal inhibitory concentration; LDH, Lactate dehydrogenase; MHC, Myosin heavy chain; MLC-1, Myosin light chain 1; MLC-2, Myosin light chain 2; pCa_{50}, Ca^{2+} sensitivity of isometric force production; PMSF, Phenylmethylsulfonyl fluoride; ROS, Reactive oxygen species; SCD, Sickle cell disease; SH, Sulfhydryl groups

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Heme, a complex of iron with protoporphyrin IX, is an essential component of hemoproteins involved in cellular oxidation–reduction reactions in all aerobic organisms [9]. In the heart, heme plays prominent roles as a catalytic subunit of mitochondrial electron transport chain complexes, myoglobin, antioxidant enzymes, and cytochrome p450 [10]. On the other hand, free heme is potentially toxic [11–13] and due to its hydrophobic nature it can diffuse through cell membranes [14–16]. Hence, it is not surprising that cellular levels of heme are tightly controlled by a fine balance between its mitochondrial biosynthesis and incorporation into hemoproteins or catabolism by heme oxygenase 1 (HO-1) [17]. Earlier investigations have reported increased myocardial heme levels in failing human hearts [18]. In neonatal rat primary myocyte cultures, heme-modified cell morphology reduced the beating rate and led to the release of markers of myocardial damage, lactate dehydrogenase (LDH) and creatinine phosphokinase (CPK) [19]. A specific association between heme and cardiac myosin was also demonstrated in the hearts of guinea pigs and in rabbit skeletal muscles [20]. In another study on porcine skeletal muscles, the formation of both reducible and nonreducible cross-links between myosin molecules and an activated hemoprotein was demonstrated [21]. Moreover, heme also interfered with actin polymerization through binding to F-actin in rabbit skeletal muscles [22].

Myocardial performance depends on the mechanical properties of cardiomyocyte sarcomeres [23] and ultimately on two closely interacting myofibrillar protein systems: the contractile machinery (generating cardiac contractions and relaxations) and the cytoskeleton (providing the necessary structural background) [24,25]. Accordingly, systolic and diastolic ventricular functions are coordinated through Ca^{2+}-regulated active [26,27] and Ca^{2+}-independent passive characteristics of myofilament proteins [28,29]. Thick myofilaments play an important role in systolic function (myosin heavy chain, myosin light chain 1, and cardiac myosin binding protein C) and during the diastole (cardiac myosin binding protein C and titin) [30]. Importantly, hemolytic diseases (e.g., sickle cell disease and thalassemia) are associated with systolic and diastolic dysfunction [5,6,31,32], and in mouse models, administration of the heme scavenger hemopexin decreased ROS production and restored cardiovascular function in a murine model of hemolytic diseases [33]. These data suggest that myocardial proteins can be affected by free heme through the formation of ROS. Nevertheless, it is currently unknown how heme-mediated ROS production affects myofilament proteins in human cardiomyocytes and, consequently, systolic and diastolic function.

Here, we investigated the effects of free heme on myocardial contractility in permeabilized left ventricular cardiomyocytes of the human heart in vitro. To this end, the mechanical properties of cardiomyocytes were investigated before and after test incubations in the presence of heme and/or hydrogen peroxide (H_2O_2), catalase, superoxide dismutase (SOD), hemopexin, and alpha-1-microglobulin (A1M). These results were interpreted in view of additional tests in the presence of agents with known oxidant and/or antioxidant properties: 2,2'-dithiodipyridine (DTDP) and/or dithiothreitol (DTT), respectively. Our results suggest that heme binds to myosin light chain 1 (MLC-1) and then evokes oxidation of SH groups to disulfide bonds and to sulfenic acid in various myofilament proteins, resulting in cardiomyocyte contractile dysfunction.

2. Methods

2.1. Ethical approval

The experiments on human tissues performed in this study complied with the Helsinki Declaration of the World Medical Association and were approved by the Institutional Ethical Committee at the University of Debrecen, Hungary (No. DEOEC RKEB/IKEB 2553-2006) and by the Hungarian Ministry of Health (No. 323- 8/2005-1018EKU).

2.2. Myocardial tissue samples

Frozen left ventricular myocardial tissue samples were obtained from four unused human donor hearts (53- and 57-year-old males and 41- and 46-year old females). The donors did not have any signs of cardiac abnormalities and had not received any medication (except short-term mannitol, noradrenaline, and dopamine). The cause of death was cerebral contusion and cerebral hemorrhage due to accident or subarachnoid hemorrhage after a stroke.

Frozen tissue blocks were first defrosted and mechanically disrupted by a homogenizer in cell isolation solution (1 mM $MgCl_2$, 100 mM KCl, 2 mM EGTA, 4 mM ATP, and 10 mM imidazole, pH 7.0). The suspension was incubated in this solution, supplemented with 0.5% Triton X-100 (Sigma, St. Louis, MO, USA) for 5 min to permeabilize all the membranous structures. Then the preparations were washed theee times (centrifugation at 1300 rpm for 1 min) and subsequently kept at 4 °C for a maximum of 24 h.

2.3. Force measurements in single-myocyte-sized preparations

A permeabilized single cardiomyocyte was mounted between two thin needles with silicone adhesive (DAP Aquarium, Baltimore, USA) while viewed under an inverted microscope (Axiovert 135, Zeiss, Germany).[34,35] One needle was attached to a force transducer element (SensoNor, Horten, Norway) and the other to an electromagnetic motor (Aurora Scientific Inc., Aurora, Canada). The measurements were performed at 15°C and the average sarcomere length was adjusted to 2.3 μm as described previously [36]. The compositions of the relaxing and activating solutions used during force measurements were calculated as described previously [37,38]. The pCa (-log[Ca^{2+}]) values of the relaxing and activating solutions (pH 7.2) were 9 and 4.75, respectively. Solutions with intermediate free [Ca^{2+}] levels were obtained by mixing activating and relaxing solutions. All the solutions for force measurements contained 6.41 mM $MgCl_2$, 37.11 mM MgATP, 7 mM EGTA, and 10 mM NN-bis (2-hydroxyethyl)-2-aminoethanesulfonic acid (BES), pH 7.2; protease inhibitors: 0.5 mM phenylmethylsulfonyl fluoride (PMSF), 40 μM Leupeptin, and 10 μM E-64. The ionic equivalent was adjusted to 150 mM with KCl, resulting in an ionic strength of 186 mM.

The isometric force production was measured at varying Ca^{2+}concentrations. When a steady force level was reached, cardiomyocyte length was reduced by 20% within 2 ms and then quickly restretched. As a result, the force first dropped from the peak isometric level to zero (difference=total peak isometric force, F_{total}) and then started to redevelop. About 8 s after the onset of force redevelopment, the cardiomyocyte was returned to the relaxing solution, where a shortening to 80% of the original length with a long slack duration (8 s) was performed to determine $F_{passive}$. F_o was calculated by subtracting $F_{passive}$ from F_{total} for each Ca^{2+} concentration.

After the first activation at pCa 4.75, the resting sarcomere length was readjusted to 2.3 μm, if necessary. The second activation at pCa 4.75 was used to calculate the maximal isometric force (F_o). The cells were subsequently exposed to a series of test solutions with various concentrations of heme for 20 min in the presence or in the absence of H_2O_2 at pCa 9.0, and subsequently pCa 4.75 without heme, to assess the concentration dependence of heme on F_o. To test reversibility, some preparations were also