
EXPERIMENTAL PAPERS

Influence of Chronic Lead Intoxication on Functional Characteristics and Isoform Composition of Left Ventricular Myosin in the Rat Heart

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Abstract—The chronic lead intoxication was modelled by injecting outbred rats intraperitoneally with lead acetate 3 times a week for 5 weeks. Using an in vitro motility assay, it was shown that lead intoxication causes changes in in vitro actin–myosin interaction, specifically, a decrease in the maximum sliding velocity of native thin filaments over myosin isolated from the left ventricular myocardium of the rat heart. No statistically significant changes were found in the calcium sensitivity and cooperativity of the “*p*Ca–velocity” curve, characteristics of the motile filament fraction, and isometric force. Using electrophoretic separation of proteins, a shift in the ratio of myosin heavy chains toward β -chains with a lower ATPase activity was found.

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Cardiovascular diseases are the main cause of death in the world [1], and the impact of heavy metals contributes to the incidence and exacerbation of these pathologies [2]. Vast human subpopulations, including the workers of industrial enterprises and people living in the zone of their technogenic influence, are exposed to heavy metals that pollute the surrounding atmosphere [2, 3]. Lead is the most abundant xenobiotic in the human habitat. It is present in air, house dust, soil, water, food, and varied consumption items. Environmental lead accumulation is characterized by stability, creating conditions for toxic exposures many years after the cessation of industrial emissions.

Lead accumulates in human and animal bodies, especially in bones, where its half-life averages decades. It has been shown that there is no safe level of lead in the blood, and its toxic effects are present at levels much lower than supposed previously [2, 4–8].

The literature abounds in data on a toxic effect of lead on all systems of the body, including the cardiovascular [5, 9–17]. Meanwhile, the known published data on the impact of lead on mechanical activity of the myocardium are scarce, do not cover all its aspects, and are, in part, contradictory. The cardiotoxic effect of lead has not been studied at the level of isolated proteins, despite the suggestion that heavy metals affect directly the

proteins involved in muscle contraction.

In the ventricular myocardium, there are three myosin isoforms: V1, V2, and V3 [18]. The amino acid sequence of isoforms V1 and V3 of mammalian cardiac myosin is $\geq 93\%$ identical [19]. Isoforms V1 ($\alpha\alpha$) and V3 ($\beta\beta$) represent homodimers containing two α - or β -myosin heavy chains (MHC), respectively, while V2 ($\alpha\beta$) is a heterodimer composed of one α - and one β -MHC [20].

In the ventricle of small mammals (e.g., mice and rats), α -MHC is dominant [21–23]. However, gene expression of MHC isoforms in the heart can change in response to the effect of thyroid hormones, hemodynamic load, or varied pathological stimuli, including congestive heart failure; in the myocardium of rodents, these processes can proceed faster and to a larger extent than in humans [22].

Mechanical characteristics of the actomyosin complex depend on the isoforms of cardiac myosin [20]. Using an *in vitro* motility assay, it was found that the V1 isoform moves actin 2–3 times faster than the V3 isoform [23–25] because V1 has a higher cross-bridge cycling rate [23]. The isometric force developed by the V1 and V3 isomyosin heads is species-specific: in larger animals (rabbits and pigs) the force developed by the V3 myosin head is higher than in V1, and in small mammals (rats and mice), the forces are indistinguishable [23, 24, 26].

To study the cardiotoxic effect of lead salts, we studied the functional characteristics and isoform composition of myosin isolated from the left ventricle of rats. The sliding velocity of thin filaments over myosin was determined by an *in vitro* motility assay; the ratio of MHCs in the myocardium of the control rat group and the group of rats with chronic lead intoxication was investigated using the electrophoretic separation of proteins.

MATERIALS AND METHODS

Intoxication model

The experiment was carried out on outbred white male rats aged 4 months and weighing 300 g at the beginning of the experiment. All rats were kept under standard conditions, breathed unfiltered air, and were provided with a standard balanced diet. Chronic lead intoxication (“Pb”

group) was simulated by intraperitoneal injection of a lead acetate solution, 3 times a week for 5 weeks (up to 15 injections). A single dose was 12.5 mg of lead per 1 kg of body weight. The control group of rats (“C” group) received the same volume of sterile distilled water according to a similar scheme [27, 28].

Experiments were planned and carried out in accordance with the principles of the Basel Declaration and were approved by the Ethics Committee of the Institute of Immunology and Physiology of the Ural Branch of the Russian Academy of Sciences.

Protein extraction

Myosin was isolated from the left ventricle of the rat heart using a standard method [29]. Actin was obtained from rabbit skeletal muscles according to the standard procedure [30], and after polymerization was labeled with TRITC-phalloidin (Sigma-Aldrich, USA) by an addition of 2 mM ATP, 4 mM $MgCl_2$, and 100 mM KCl. Cardiac troponin was isolated from the pig hearts as described elsewhere [31]. Recombinant human tropomyosin was obtained as described in [32]. The use of contractile and regulatory proteins isolated from different species in an *in vitro* motility assay is a common practice [26, 33]. Regulated thin filaments were reconstituted from actin, troponin and tropomyosin by mixing these proteins at the following concentrations: 400 nM F-actin labeled with rhodamine-phalloidin, 100 nM troponin and 100 nM tropomyosin at 4°C in a buffer containing (in mM) 25 KCl, 25 imidazole, 4 $MgCl_2$, 1 EGTA, and 10 DTT (pH 7.5). Native thin filaments were extracted from the rat left ventricle according to the Spiess protocol [34]. The protein composition of the reconstituted and native thin filaments was checked by electrophoresis in polyacrylamide gel with sodium dodecyl sulfate [35].

In vitro motility assay

An *in vitro* motility assay was used to determine the sliding velocity of native and reconstituted thin filaments, consisting of actin, troponin and tropomyosin, over myosins extracted from the left ventricle of rats of the “C” and “Pb” groups at various calcium concentrations in solution. Cal-

Table 1. Characteristics of “*p*Ca–velocity” relationship in the left ventricle of rats of groups “C” and “Pb”*

	V_{\max} , $\mu\text{m/s}$	$p\text{Ca}50$	h
C	4.07 ± 0.13	6.63 ± 0.02	2.3 ± 0.3
Pb	$3.27 \pm 0.12^*$	6.58 ± 0.03	2.2 ± 0.3

V_{\max} —maximum sliding velocity of native thin filaments over myosin in the left ventricle, $p\text{Ca}50$ —calcium sensitivity, h —Hill cooperativity coefficient. Data presented as *mean* \pm *SD*. Upper index (*) marks the values that are different vs. control ($p < 0.05$, Mann–Whitney U test).

cium ions interact with a regulated thin filament in a flow cell solution. Calcium binds to troponin C and leads thereby to conformational changes in the thin filament, due to which myosin-binding sites on actin globules open. A mechanical actin–myosin interaction can be evaluated by the characteristics of how the filaments move and stop. Changes in these characteristics, depending on the calcium concentration in a solution with the regulated thin filaments, make it possible to study the mechanisms of calcium regulation of the actin–myosin interaction [33]. Experiments in an *in vitro* motility assay were carried out at 30°C as described previously [26]. Fluorescently labeled actin filaments in an experimental flow cell were visualized using the Axiovert 200 inverted epifluorescence microscope (Carl Zeiss, Germany) equipped with a 100 \times /1.45 Plan-Fluar oil alpha-objective. The movement of thin filaments over the myosin-coated surface was recorded using the EMCCD iXon-897BV camera (Andor Technology, UK), while their velocity was analyzed using the GMim-Pro software [36]. The dependence of the sliding velocity of thin filaments on the calcium concentration was analyzed using the Hill equation:

$$V = V_{\max} (1 + 10^{h(p\text{Ca} - p\text{Ca}50)})^{-1}, \quad (1)$$

where V and V_{\max} are the velocity and maximum velocity at a saturating calcium concentration, respectively, $p\text{Ca}50$ (calcium sensitivity) is the $p\text{Ca}$ value, at which a half of the maximum velocity is achieved, and h is the Hill cooperativity coefficient.

*Determining the relative isometric force developed by myosin in an *in vitro* motility assay*

To determine the isometric force in an *in vitro* motility assay, we used the “mixture protocol”, in which the myosin under study was mixed with

noncycling NEM-modified myosin [37]. The latter, when attached to actin, prevents thin filament sliding and serves as a load. The fraction of NEM-modified myosin, needed to stop filament sliding, is a measure of the relative force that the myosin under study can develop.

Electrophoresis

Isoforms of rat myocardial MHC were determined by electrophoresis in a polyacrylamide gel with sodium dodecyl sulfate [38]. After the completion of electrophoresis, the gel was stained with Coomassie Brilliant Blue R250, then washed from the stain and scanned with a densitometer (BioRad, USA) to determine the percentage of α - and β -MHCs.

Statistical analysis

The statistical significance of the intergroup differences between the mean values of all the obtained parameters was assessed using the non-parametric Mann–Whitney U test; the differences were considered statistically significant at $p < 0.05$. Data are expressed as *mean* \pm *SD*.

RESULTS

Under the influence of chronic lead intoxication, the maximum sliding velocity of native thin filaments over the left-ventricular myosin decreased compared to the control group from 4.07 ± 0.13 to 3.27 ± 0.12 $\mu\text{m/s}$ (Table 1), i.e. by 20% (Fig. 1). The calcium sensitivity and Hill cooperativity coefficient were not significantly distinguishable between groups “C” and “Pb”.

The characteristics of the dependence of the fraction of motile filaments on the calcium concentration (the maximum value of the fraction, $p\text{Ca}50$, Hill cooperativity coefficient) were not significantly different (Table 1, Fig. 2).

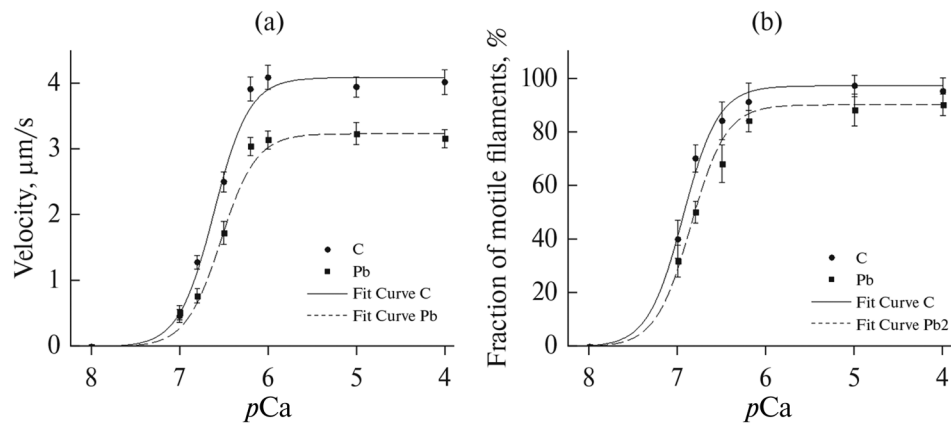


Fig. 1. Dependence of the sliding velocity of native thin filaments (a) and the fraction of motile filaments (b) over left-ventricular myosin in rats of groups "C" and "Pb" on calcium concentration in solution. pCa is a negative decimal logarithm of calcium concentration. The regression curve fits the Hill equation. Velocity is represented as *mean* \pm *SD* for 4 experiments.

Lead intoxication reduces the maximum sliding velocity of native thin filaments over myosin, but does not affect calcium sensitivity and the Hill cooperativity coefficient. The curve of the dependence of the fraction of motile filaments on the calcium concentration shows no significant differences in all parameters, but there is a clear tendency toward a decrease in the fraction under the influence of lead.

Under the influence of chronic lead intoxication, there was a significant shift in the ratio of MHCs toward an increase in the content of β -chains with a lower ATPase activity ($55 \pm 10\%$ α -MHC and $45 \pm 10\%$ β -MHC in group "Pb", and $86 \pm 4\%$ α -MHC and $14 \pm 4\%$ β -MHC in group "C").

To stop the motility of reconstituted thin filaments over the left-ventricular myosin in groups "C" and "Pb", $34 \pm 4\%$ and $42 \pm 6\%$ of NEM-modified myosin were required, respectively. This difference was statistically nonsignificant (Fig. 3).

DISCUSSION

Studies of the influence of chronic lead intoxication on isolated multicellular preparations of the left ventricle showed that the force of isometric contraction of papillary muscles did not change when changing calcium cycle parameters in cardiomyocytes [39, 40].

Previously, we studied the characteristics of the actin–myosin interaction and contraction of multicellular preparations (trabeculae and papil-

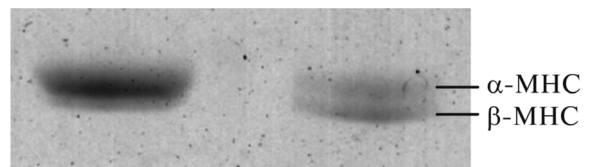


Fig. 2. Representative electrophoregram to demonstrate a ratio of α - and β -MHCs in the left ventricle of rats in groups "C" (left) and "Pb" (right). Group "C" (left) – $86 \pm 4\%$ α -MHC and $14 \pm 4\%$ β -MHC. Group "Pb" (right) – $55 \pm 10\%$ α -MHC and $45 \pm 10\%$ β -MHC.

lary muscles) of the right ventricle when exposed to lead ions. It was shown that the maximum sliding velocity of reconstituted thin filaments over right-ventricular myosin decreased under the influence of lead intoxication [27], and the ratio of MHC isoforms shifted toward the slower β -chains [28]. In multicellular preparations of the right ventricle of the same rats that were used in our study, we found an increase in the cross-sectional area of trabeculae and papillary muscles, a decrease in passive (diastolic) mechanical tension, a decrease in the maximum rate of isometric force development and the rate of isotonic shortening of papillary muscles [27].

In our experiments using native thin filaments and myosin from the left ventricle, a similar tendency was observed, namely the maximum velocity of actin–myosin interaction dropped under the influence of chronic lead intoxication by 20% compared to the control group. This was explained by a shift in the α -MHC/ β -MHC ratio: an 31% increase in the content of β -chains with a

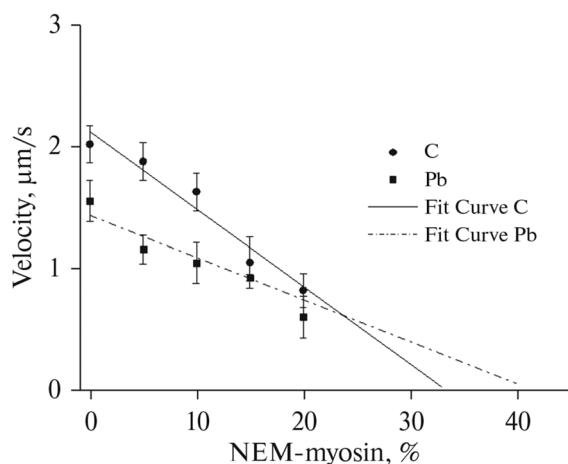


Fig. 3. Dependence of the sliding velocity of reconstituted thin filaments over left-ventricular myosin in rat groups “C” and “Pb” on NEM-modified myosin concentration. Velocity is represented as *mean* ± *SD* for 4 experiments. Differences in the values of NEM-modified myosin amount needed to stop motility in rat groups “C” ($34 \pm 4\%$) and “Pb” ($42 \pm 6\%$) were statistically insignificant.

lower ATPase activity and a lower actin-myosin cross-bridge cycling rate. Such a change can be an adaptive energy-saving molecular mechanism in cardiac pathologies [25, 41]. The characteristics of the fraction of motile filaments were statistically indistinguishable; however, there was a tendency toward a decrease in this parameter during lead intoxication.

The differences between groups “C” and “Pb” in the NEM-modified myosin amount required to completely stop the motility of thin reconstituted filaments were statistically insignificant, which can be explained by the absence of differences in the force developed by myosin isoforms in small mammals (rats and mice) [23, 24, 26], although there was a small tendency toward an increase in the isometric force in the left-ventricular myosin of group “Pb”. This effect may be worth a deeper investigation.

Thus, when studying the functional characteristics and myosin isoform composition in the left ventricle of rats with chronic lead intoxication, we revealed a decrease in the rate of actin-myosin interaction and a shift in the ratio of MHCs toward β -chain. These results, along with the aforementioned changes in the contractile characteristics of multicellular right-ventricular preparations of the same rats [27, 28], may result

from the development of myocardial hypertrophy caused by pressure overload [28, 42, 43]. The assumption on possible myocardial hypertrophy due to lead intoxication indirectly confirms the increase in the relative heart weight in these rats, as calculated per 100 g of body weight, and in the amplitude of the QRS complex on the electrocardiogram [28], as well as an increase in pressure and the thickness of cardiomyocytes of the left ventricle in rats intoxicated with lower lead doses [44]. Thus, with a professional and environmentally conditioned lead load on the organism, there arises an increased risk of cardiovascular pathology, which may be associated, among other things, with myocardial contractile dysfunction at the molecular level.

AUTHORS' CONTRIBUTION

Idea and design of the experiment: L.V.N. and O.P.G.; data collection and processing: O.P.G. and S.R.N.; manuscript writing and editing: O.P.G. and L.V.N.

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CONFLICT OF INTEREST

The authors declare that they have neither evident nor potential conflict of interest associated with the publication of this article.

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