Effect of inhibitors of mitochondrial respiratory chain complexes on the electromechanical activity in human myocardium

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Key words: human myocardium; rotenone; antimycin A; anoxia; electromechanical activity.

Summary. The aim of the study was to investigate the effect of inhibitors of mitochondrial respiratory chain complexes I, III, and IV on the electromechanical activity in human myocardium.

Material and methods. The experiments were performed on the human myocardial strips obtained from patients with heart failure (NYHA class III or IV) using a conventional method of registration of myocardial electromechanical activity. Under the perfusion with physiological Tyrode solution (control), contraction force (P) was 0.94±0.12 mN (n=16), relaxation time (t_{50}) was 173.38±5.03 ms (n=15), action potential durations measured at 50% (AP_{50}) and 90% (AP_{90}) repolarization were 248.96±13.38 ms and 398.59±17.93 ms, respectively (n=13).

Results. The inhibition of respiratory chain complex I by rotenone (3×10^{-5} M, the highest concentration applied) decreased contraction force of human myocardium to 48.99%±14.74% (n=3) (P<0.05); AP_{50}, to 81.34%±15.81%; and AP_{90}, to 87.28%±7.25% (n=3) (P>0.05) of control level, while relaxation time and resting tension remained almost unchanged. Antimycin A, an inhibitor of complex III, applied at the highest concentration (3×10^{-4} M) reduced P to 41.66%±8.8% (n=5) (P<0.001) and marginally increased t_{50} and decreased the durations of AP. Anoxia (3 mM Na_{2}S_{2}O_{4}) that inhibits the activity of complex IV reduced the contraction force to 9.23%±3.56% (n=6) (P<0.001), AP_{50} and AP_{90} to 65.46%±9.95% and 71.07%±8.39% (n=5) (P<0.05) of control level, respectively; furthermore, the resting tension augmented (contracture developed).

Conclusions. Our results show that the inhibition of respiratory chain complex IV had the strongest inhibitory effect on the electromechanical activity of failing human myocardium.

Introduction
Heart failure is one of the most dangerous pathologies that occurs as an end stage of different heart diseases, such as hypertension, myocardial infarction or idiopathic, dilated, restrictive cardiomyopathies (1, 2). Currently, a lot is done in the diagnosis and treatment of this pathology but increasing mortality due to heart failure nevertheless is still one of the most relevant problems in the present-day medicine.

Contractility, the main function of heart, is triggered by Ca^{2+} ions and their interaction with contractile proteins of cardiomyocytes. Ca^{2+} enters the cell during depolarization via sarcolemmal slow (L-type) Ca^{2+} channels and triggers Ca^{2+} release from sarcoplasmic reticulum (SR) via ryanodine receptors (RyR) (3). Sarcolemmal Ca^{2+} pump (Ca^{2+}–ATPase), Na^{+}–Ca^{2+} exchanger, SR Ca^{2+}–ATPase, and phospholamban (PLB) also play an important role in the process of myocardial contraction–relaxation. The energy derived from hydrolysis of adenosine triphosphate (ATP) is essential for functioning of all the systems that regulate cardiac contraction–relaxation. A decrease in intracellular ATP concentration impairs the function of these energy-dependent cell systems and leads to an increase in the intracellular concentrations of Na^{+} and Ca^{2+} ions (Ca^{2+} overload), decreases myocardial contraction force, and provokes the development of heart failure, arrhythmias, and necrosis of cardiomyocytes (1, 4).

About 80% to 90% of cellular ATP is produced by mitochondrial oxidative phosphorylation; the most part of generated ATP (>60%) is consumed for the contraction–relaxation of myocardium (5). Mitochondrial F_{0}F_{1}–ATP synthase uses the proton gradient, generated by complexes I, III, and IV of the electron transport chain, to generate ATP from ADP. Altered activity of these complexes can cause a reduction in the mitochondrial electrochemical gradient and ATP synthesis (6, 7). A variety of studies demonstrated that patients suffering from idiopathic dilated cardiomyopathy or ischemia have impaired activity of mitochondrial respiratory chain complexes I (8–10), III (11–14), and IV (11, 15). Thus, impaired function of mitochondria...
could be one of the main reasons causing heart failure. However, there is a lack of integrated studies on the influence of impairment of every separate respiratory chain complex activity on the human cardiac contraction, relaxation, and action potential. Therefore, the aim of this study was to investigate the effect of inhibitors of mitochondrial respiratory chain complexes I, III, and IV on the electromechanical activity in human myocardium.

Material and methods
The experiments were performed on human left ventricular strips (0.3–1 cm²) obtained from patients (7 males aged 55.71±7.31 years and 9 females aged 59.13±3.44 years) undergoing heart surgery for aortic valve prosthesis or mitral valve anuloplasty at the Hospital of the Lithuanian University of Health Sciences (former Kaunas University of Medicine), Lithuania. The investigations with human cardiac tissue were approved by the institutional Ethics Committee of Biomedical Research (protocol No. BE-2-18; April 12, 2006) and conformed to the European Community guiding principles. Most of patients that had NYHA class III or IV heart failure were treated with various agents (β-adrenoreceptors blockers, calcium antagonists, angiotensin-convert- ing enzyme inhibitors, NO donors, diuretics, and/or antiarrhythmic drugs) before surgery. In addition, the patients received sedatives and antibiotics.

For transportation to the laboratory, the isolated pieces of human left ventricular tissue were placed in cold (10°C) St. Thomas cardioplegic solution, containing (in mM): 110 NaCl, 16 KCl, 1.2 CaCl₂, 0.9 MgCl₂, 5 glucose, 10 HEPES, pH 7.4 (adjusted with NaOH). Afterward, muscle strips of appropriate size (3.66±0.17 mm in length, 1.88±0.09 mm in thickness, 9.59±0.92 mg in weight) were cut from the pieces of heart tissue in the same solution. The preparation were then placed in an experimental chamber and superfused with oxygenated Tyrode solution, containing (in mM): 137 NaCl, 5.4 KCl, 1.8 CaCl₂, 0.9 MgCl₂, 5 glucose, 10 HEPES, pH 7.4, pO₂ 580–600 mm Hg. Constant flow was kept at the rate of 4 mL/min, and temperature was continuously monitored and kept at 36.0°C±0.5°C. Isometric contraction was recorded using a linear force-displacement transducer (Harvard Apparatus, USA), and action potentials were registered with glass microelectrodes filled with 2.5 M KCl. The cardiac samples were continuously paced with chlorine-coated silver electrodes at a frequency of 1 Hz with rectangular pulses of 2–5 ms in duration, and 3–4-fold greater amplitude than diastolic threshold. Contraction force (P), half time of relaxation (t₅₀), resting tension (contracture), action potential (AP) duration at 50% (AP₅₀), and 90% (AP₉₀) of repolarization were recorded and analyzed using specialized custom-made computer software. In control, i.e., during perfusion with Tyrode solution, values of contraction force, relaxation time, and AP durations were set at 100%. Changes in these parameters are given in percent (±standard error) of control level. Differences in resting tension are presented in relative units in regard to values of contraction force in control. For statistical evaluation, Student’s t test was used, and differences were considered statistically significant when P<0.05. Rotenone, antimycin A, and O₂ scavenger sodium dithionite (Na₂S₂O₄) were used to block activity of respiratory chain complexes I, III, and IV, respectively, and glibenclamide was used to block ATP-dependent K⁺ channels. All the drugs used in experiments were from Sigma-Aldrich.

Results
Under perfusion with Tyrode solution (control), contraction force of human ventricular strips was 0.94±0.12 mN (n=16); half time of relaxation, 173.38±5.03 ms (n=15); AP₅₀, 248.96±13.38 ms; and AP₉₀, 398.59±17.93 ms (n=13). Fig. 1 demonstrates original traces of contraction (A) and action potentials (B), and changes in contraction force (curve 1) and relaxation time (curve 2) (C) under the influence of rotenone, an inhibitor of mitochondrial respiratory chain complex I. Rotenone (10⁻⁶ M to 3×10⁻⁵ M) in a concentration-dependent manner reduced contraction force of human ventricular strips. The greatest reduction in P, i.e., to 48.99%±14.74% (n=3) of control level, was observed at the highest concentration of rotenone (3×10⁻⁵ M) (P<0.05). During reperfusion with Tyrode solution, P partly recovered to 68.95%±21.18% (n=5) (P>0.05). Rotenone had no significant effect on relaxation time of human myocardium; even at 3×10⁻⁵ M, t₅₀ was 97.13%±5.82% (n=5). However, reperfusion with rotenone-free physiologically saline increased t₅₀ to 123.47%±17.24% (n=5) (P<0.05). Rotenone caused insignificant shortening of action potentials; at 3×10⁻⁵ M of this inhibitor, AP₅₀ and AP₉₀ decreased to 81.34%±15.81% and 87.28%±7.25% (n=3), respectively (P>0.05) (Fig. 1B). The resting tension of human myocardium was not affected by rotenone.

Fig. 2 shows the effect of antimycin A, an inhibitor of mitochondrial respiratory chain complex III, on electromechanical activity of human myocardium. As shown in Fig. 2A and C (curve 1), antimycin A (3×10⁻⁶ M to 3×10⁻⁴ M) induced concentration-dependent reduction in contraction force. Under the action of 3×10⁻⁵ M and 3×10⁻⁴ M of this inhibitor, P decreased to 69.71%±8.24% (n=6) (P<0.05) and 41.66%±8.8% (n=5) (P<0.001), respectively. Antimycin A marginally increased the relaxation time (Fig. 2C, curve 2) to 106.15%±1.57% (at 3×10⁻⁵ M)
Effect of inhibitors of mitochondrial respiratory chain in human myocardium

**Fig. 1. Effect of rotenone on contraction force, action potentials, and relaxation time in human myocardium**

A and B, superimposed traces of contraction and action potentials, respectively, recorded in control and in the presence of rotenone. C, changes of contraction force (curve 1) and relaxation time (curve 2) obtained under the influence of rotenone. *P<0.05.

**Fig. 2. Effect of antimycin A on contraction force, action potentials, and relaxation time in human myocardium**

A and B, superimposed traces of contraction and action potentials, respectively, recorded in control and in the presence of antimycin A. C, changes of contraction force (curve 1) and relaxation time (curve 2) obtained under the influence of antimycin A. *P<0.05; **P<0.001.
The activity of mitochondrial respiratory chain complex IV was inhibited by anoxia. During experiments, human ventricular strips were perfused with Tyrode solution containing $3 \times 10^{-3}$ M sodium dithionite ($\text{Na}_2\text{S}_2\text{O}_4$) that scavenges $\text{O}_2$ molecules in the solution and reduces the oxygen tension to zero. Fig. 3 shows original traces of contraction (A) and action potentials (B), and change of contraction force (C) under the influence of anoxia. The inhibitory effect of anoxia on contraction force was obvious already after the first minutes of perfusion with anoxic solution, and after 50 minutes, $P$ was reduced to $9.23\% \pm 3.56\%$ (n=6; $P<0.001$). The effect of anoxia was partly reversible. On reperfusion with oxygenated Tyrode solution, contraction force was restored to $36.33\% \pm 13.44\%$ (n=6; $P<0.05$). Relaxation time $t_{50}$ of ventricular strips was not affected by anoxia (92.3\% \pm 9.74\%, n=4; $P>0.05$). In two cases, anoxia caused augmentation of resting tension. During these experiments, development of contracture started after 11\% ± 5.66 min, and its maximum value (1.75\% ± 0.94) was registered after 29\% ± 22.63 min. The inhibitory effect of anoxia was also obvious on action potentials: $\text{AP}_{50}$ and $\text{AP}_{90}$ decreased to $65.46\% \pm 9.95\%$ and $71.07\% \pm 8.39\%$, respectively (n=5; $P<0.05$). The duration of action potentials was restored nearly to control level when glibenclamide ($2 \times 10^{-5}$ M), an inhibitor of ATP dependent K+ channels, was added to anoxic solution, i.e., anoxia-shortened $\text{AP}_{50}$ and $\text{AP}_{90}$ were prolonged to $84.77\% \pm 13.7\%$ and $93.77\% \pm 8.3\%$ (n=3), respectively (Fig. 3B).

**Discussion**

Our experimental data show that inhibition of mitochondrial respiratory chain complex I by rotenone, complex III by antimycin A, and complex IV by anoxia decreased contraction force, duration of action potentials and did not affect or marginally increased relaxation time and resting tension of human myocardium. The inhibition of respiratory chain complex IV by anoxia had the most obvious inhibitory effect on these parameters.

![Fig. 3. Effect of anoxia on the contraction force and action potentials in human myocardium](image-url)

A and B, superimposed traces of contraction (A) recorded in control and anoxia (after 20 min) and action potentials (B) recorded in control, under the influence of anoxia alone and together with glibenclamide. C, change of contraction force obtained under the influence of anoxia. *$P<0.05$; **$P<0.001$. 

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It is known that mitochondrial respiratory chain complexes I, III, and IV function as proton pumps and pass protons from the matrix to the intermembrane space of mitochondria during electron transport. This process results in the generation of electrochemical potential that is necessary for ATP production (16). Electrons of oxidative substrates can pass to electron transport chain not only through complex I (NADH dehydrogenase), but also through complex II (succinate dehydrogenase). This may explain why the inhibition of complex I by rotenone in our experiments did not elicit a strong inhibitory effect on the parameters of electromechanical activity of human myocardium. Similar results were obtained by Sward et al. (17), who found that the inhibitory effect of rotenone on intracellular Ca2+ concentration, which is proportional to contraction force alterations in rat smooth muscles, was smaller than one of antimycin A.

Duchen (16) demonstrated that under the inhibition of complex IV activity, the electron transport along mitochondrial respiratory chain stops and electrochemical gradient, essential for ATP synthesis, collapses, and consequently ATP level decreases in myocardial cells. It has been demonstrated that during ischemia, when the activity of respiratory chain complex IV is blocked due to lack of O2, intracellular ATP level in ferret cardiomyocytes decreased by 90%, i.e., from 5–10 mM to <100 μM (18). Perfusion with glucose-free physiological saline containing rotenone, an inhibitor of complex I, decreased ATP level by 67% in canine ventricular myocytes (19). Li et al. found that the inhibition of respiratory chain complex IV by cyanide resulted in a rapid (within 2 min) fall in ATP level by 60% in epithelial cells of opossum kidney (20).

Sarcogemmal ATP-dependent K+ channels (KATP) that open due to reduction of intracellular ATP ([ATP]) and consequently shortening of action potentials serve as an indirect indicator of the reduction in cellular ATP content in myocardial cells (21, 22). If AP shortens due to reduction of [ATP], the blocking of KATP channels by glibenclamide has to increase AP duration. We have found that action potential duration AP90 of human myocardium shortened by anoxia to 71.07%±8.39% was then prolonged to 93.77%±8.3% by blocking of KATP by glibenclamide. Previous studies of our laboratory also have shown that action potential duration of guinea pig myocardium decreased during hypoxia, and under the action of an inhibitor of ATP-dependent K+ channels – glibenclamide – AP duration was restored (23). These data confirm the fact that ATP level in myocardial cells decreases during the inhibition of respiratory chain complex IV activity, and therefore, myocardial contraction force and action potential duration decrease in concert with it.

Decreased cellular ATP concentration results not only in opening of sarcolemmal ATP-dependent K+ channels and shortening of action potential, but also in impairment of functioning of other cellular systems dependent on ATP, including sarcogemmal L-type Ca2+ channels, Na+-K+ ATPase, Na+-Ca2+ exchanger, sarcoplasmatic reticulum Ca2+-ATPase, phospholamban, RyR channels (1, 4). Decreased ICaL leads to suppressed release of Ca2+ ions from sarcoplasmatic reticulum via RyR channels. As these Ca2+ ions are essential for formation of actin–myosin cross-bridges during muscle contraction–relaxation, the contraction force decreases as well. Impaired functioning of Na+-K+ ATPase and Na+-Ca2+ exchanger, Ca2+ pumps of sarcogemmal and sarcoplasmatic reticulum (Ca2+-ATPases) induces influx of Na+ and Ca2+ ions in the cells (Ca2+ overload develops) (4, 24). Ca2+ overload leads to an increased level of formation of actin–myosin cross-bridges. However, ATP is necessary for breakdown of these cross-bridges (interaction cycle of muscle myosin with actin requires roughly 75% of cellular ATP [25]); therefore, the relaxation of muscle slows down, i.e., relaxation time increases, contraction of cardiac cells starts to develop. In our experiments, relaxation time of human myocardium was not changed or marginally increased under inhibition of respiratory chain complexes I and III activity while contracture developed only under inhibition of complex IV by anoxia. As there was no possibility to perform such experiments on healthy human myocardium, our experiments were carried out on ventricular preparations obtained from patients with NYHA class III or IV heart failure. Our previous investigations with rat myocardium showed that inhibition of the activity of mitochondrial respiratory chain complexes significantly stronger decreased contraction force and action potential duration, increased relaxation time if compared with human myocardium, and contracture of rat myocardium developed not only under inhibition of the activity of complex IV, but also complex III (26). A number of studies have demonstrated that myocardial remodeling occurs in heart failure resulting in changes of heart activity and regulation, i.e., reduction in muscle contraction force, slowing down relaxation, reduction in the sensitivity of cardiomyocytes to Ca2+ ions and catecholamines, decrease in the content of SR Ca2+-ATPases, increase in Na+-Ca2+ exchanger activity (3, 27, 28). Remodeling may be a reason of smaller change in electromechanical activity parameters of human myocardium than in rat myocardium during inhibition of the activity of mitochondrial respiratory chain complexes. The results suggest that failing myocardium can be less sensitive to metabolic changes caused by inhibitors of oxidative phosphorylation.
Conclusions
The inhibition of mitochondrial respiratory chain complexes I, III, and IV activity (by rotenone, antimycin A, and anoxia, respectively) decreased contraction force, action potential duration, but did not affect or marginally slowed down relaxation of failing human myocardium.

The inhibition of mitochondrial respiratory chain complex IV had the strongest inhibitory effect on electromechanical activity of failing human myocardium.

Mitochondrijų kvėpavimo grandinės kompleksų slopiklių įtaka žmogaus miokardo elektromechaniniam aktyvumui

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Raktažodžiai: žmogaus miokardas, rotenonas, antimicinas A, anoksija, elektromechaninis aktyvumas.


Medžiaga ir metodai. Eksperimentiniai tyrimai atlikti su žmonių, sergančių širdies nepakankamumu (III ir IV NYHA funkcinės klasės), miokardo preparatais, naudojant standartinį miokardo elektromechaninio kvėpavimo registruojimą metodiką. Perfuzuojant preparatus fiziologinio Tyrode tirpalu (kontrolė), susitraukimo jėga (P) buvo 0,94±0,12 mM (n=16), pusinis atsipalaidavimui laikas (t50) – 173,38±5,03 ms (n=15), veikimo potencialų trukmės, esant 50 proc. (VP50) ir 90 proc. (VP90) repoliarizacijos lygiams, atitinkamai – 248,96±13,38 ms ir 398,59±17,93 ms (n=13).

Rezultatai. Kvėpavimo grandinės I komplekso slopiklio roteneno koncentracijai didėjant iki 3×10–5 M, P mažėjo iki 48,99±14,74 proc. (n=3) (p<0,05), atsipalaidavimo laikas be ramybės įtampa beveik nekito, VP50 ir VP90 sumažėjo atitinkamai iki 81,34±15,81 proc. bei 87,28±7,25 proc. (n=3) (p>0,05) palyginus su kontrolė. Antimicinas A, III komplekso aktyvumo slopiklis, didinant jo koncentraciją iki 3×10–4 M, sumažino P iki 41,66±8,8 proc. (n=5) (p<0,001), nežymiai padidino t50 bei sumažino VP trukmes. Slopinant IV komplekso aktyvumą anoksija (3 mM Na2S2O4), susitraukimo jėga, palyginus su kontrolė, sumažėjo iki 9,23±3,56 proc. (n=6) (p<0,001), VP50 ir VP90 – atitinkamai 65,46±9,95 proc. ir 71,07±8,39 proc. (n=5) (p>0,05), didėjo ramybės įtampa (vystėsi kontraktūra).

Išvados. Taigi, mūsų atliktų tyrimų duomenimis, didžiausią sabinamųjį poveikį žmonių, sergančių širdies nepakankamumu, miokardo elektromechaniniams aktyvumams sukélė mitochondrijų kvėpavimo grandinės IV komplekso aktyvumo sabinimasis.

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effect of inhibitors of mitochondrial respiratory chain in human myocardium

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