Effect of oxidative phosphorylation uncoupler FCCP and F1F0-ATPase inhibitor oligomycin on the electromechanical activity of human myocardium

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Abstract

Purpose: The purpose of the study was to determine the influence of oxidative phosphorylation uncoupler FCCP (carbonyl cyanide p-trifluoromethoxy-phenylhydrazone) and F_1F_0-ATPase inhibitor oligomycin on the parameters of electromechanical activity in human myocardium.

Material and methods: The experiments were performed on isolated human ventricle strips from patients undergoing cardiac corrective open heart surgery. Effect of investigative agents was registered using conventional method of registration of cardiac electromechanical activity.

Results: FCCP (10^{-9}, 10^{-8}, 10^{-7}, 10^{-6} mol/L) caused the gradual reduction of contraction force (P). The maximal decrement of P (to 8.3±3.1% (n=5) vs control), was achieved at 10^{-6} mol/L FCCP concentration. The duration of action potential at 50% of repolarization (AP_{50}) was decreased only at 10^{-7} and 10^{-6} mol/L FCCP concentrations, i.e. to 94.3±1.9% and 55.5±3.1% (n=4), respectively, vs control. Oligomycin ($2x10^{-5}$ mol/L) alone decreased P only to 77.8±5.1 % (n=5) and slightly reduced AP50 to 94.2±6.2% (n=4) vs control. Application of FCCP on top of oligomycin decreased P at the smaller extent than under the action of FCCP alone: the highest concentration of FCCP (10^{-6} mol/L) reduced P to 21.1±4.5% (n=5) vs effect of oligomycin. The duration of AP_{50} was also less shortened after application of FCCP in the presence of oligomycin. The highest concentration of FCCP (10^{-6} mol/L) reduced AP_{50} to 73.5±10.1% (n=4) vs effect of oligomycin.

Conclusions: In conclusion, our data show that the inhibition of F_1F_0-ATPase reduces the impairment of electromechanical activity caused by oxidative phosphorylation uncoupler FCCP in human myocardium.

Key words: human myocardium, contraction force, action potential duration, FCCP, oligomycin.

Introduction

Cardiac contractility strongly depends on mitochondria, which supply ATP for ionic channels, ATPases and participate in calcium homeostasis. The most part of cellular ATP (80--90%) is generated by mitochondrial oxidative phosphorylation, which comprises the electron transport chain (complexes I to IV) and F_1F_0-ATPase (ATP synthase). Under normal physiological conditions F_1F_0-ATPase generates ATP from ADP using the proton gradient, established by the electron transport chain of the inner mitochondrial membrane. Under these conditions, the mitochondrial ATP synthase provides the cell with ATP, which is then used in diverse cell functions. ATP level in the myocyte is a critical key for normal cardiac function as ATP is used by actomyosin ATPase and various sarcolemmal, as well as sarcoplasmic reticulum, ion channels (L-type Ca^{2+} channel, Ca^{2+} ATPases, ATP sensitive K^+ channel, Na^+-K^+ ATPase) during contraction and relaxation. Under oxygen deficiency conditions, i.e. during ischemia, mitochondrial electrochemical gradient collapses, and F_1F_0-ATPase starts hydrolyzing ATP for the proton gradient recovery. Then F_1F_0-ATPase instead of producer becomes the main consumer of ATP in failing cardiomyocytes and contributes to the heart failure progression [1-4]. Experimental observations demonstrated that this ATPase consumed 35-50% of the overall high-energy phosphates during heart ischemia [1,5]. Studies showed that inhibition of F_1F_0-ATPase could reduce this undesirable effect and protect ischemic myocardium from ATP depletion [1,6,7]. For that purpose could be used an inhibitor oligomycin, a macrocyclic compound produced by actinomycetes, which binds to the F0

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***Table 1*. Clinical characteristics of the patients**

Patient No	Sex	Age	Tissue	NYHA	Medication	Comorbidities	Operation
1	M	74	endo	III	Hep, AC, ACE, N, D	Hypertonic disease	AoVpr, TVa
2	F	70	endo	IV	AC, N, D	Strume diffuse-nodose	AoVpr, MVpr
3	F	74	endo	III	AC, ß, ACE, N	Diabetes mellitus I	CABG, AoVpr, MVpr.
4	M	42	endo	III	Hep, ß, ACE, D	Hypertonic disease	CABG, MVpr.
5	F	60	endo	III	Hep, AC, ACE, D	Hypertonic disease	AoVpr, TVa, MVa
6	F	72	endo	III	ß, ACE, N, D	Hypertonic disease	AoVpr
7	M	73	endo	IV	AC, ACE, D	Hypertonic disease, diabetes mellitus I	CABG, TVa, MVa
8	F	66	endo	III	ß, N, D	-	CABG, AoVpr, MVa, TVa
9	F	81	endo	III	Hep, ß,	-	AoVpr
10	M	43	endo	III	ACE	Hypertonic disease	CABG, Bentall procedure

Abbrevations: M, male; F, female; endocardium; NYHA, New Your Heart Association Clases I to IV; AoVpr, aortic valve prosthesis; MVpr, mitral valve prosthesis; MVa, mitral valve annuloplastic, TVa, Tricuspid valve annuloplastic; CABG, coronary artery bypass grafting. Medication: Hep, heparin; AC, anticoagulant; ß, beta AR blocker; ACE, angiotensin converting enzyme inhibitor; N, nitrates; D, diuretics

domain and blocks proton flow through the F_1F_0-ATPase [8]. In the experimental models the decrease of mitochondrial potential can be evoked by mitochondria uncoupling of oxidative phosphorylation with the agents such as FCCP (carbonyl cyanide p-trifluoromethoxy-phenylhydrazone) which inner mitochondrial membrane makes permeable to protons [3], or with an inhibitors of the respiratory chain complexes. Our previous studies with rat hearts showed that oligomycin significantly reduced myocardial injury evoked by inhibitors of complexes III or IV of the mitochondrial respiratory chain [9], or by mitochondrial uncoupling [10]. The aim of the present study was to investigate the effect of oxidative phosphorylation uncoupler FCCP and F_1F_0-ATPase inhibitor oligomycin on the electromechanical activity of human myocardium.

Materials and methods

The experiments were performed on human ventricular muscle strips (0.25-1 cm^2) that were resected from patients undergoing open-heart surgery (general anesthesia) – mid sternal longitudinal sternotomy – just before cannulating heart and instituting cardiopulmonary bypass. The investigations were approved by the institutional Ethics Committees and conform to the European Community guiding principles. Clinical characteristics of the patients are shown in *Tab. 1*. A pharmacological pretreatment was stopped 24 h before surgery. In addition, all patients received sedatives, anesthesia. The pieces of human tissue were transported in cold (10°C) St. Thomas cardioplegic solution composed of (in mmol/L): NaCl 110, KCl 16, $CaCl_2$ 1.2, $MgCl_2$ 16, glucose 5, Hepes 10, pH 7.4 (adjusted with NaOH). After transportation, muscles were placed in an experimental chamber and perfused (for 30 min) with oxygenated Tyrode solution (pO_2 580-600 mmHg) composed of (in mmol/L): NaCl 137, KCl 5.4, $CaCl_2$ 1.8, $MgCl_2$ 0.9, glucose 5, Hepes 10, pH 7.4 (adjusted with NaOH). Perfusion was kept at a rate of 6 ml/min and temperature was continuously monitored at 36±0.5°C. All preparations were continuously paced at frequencies of 1 Hz with pulses of 2-5 ms duration and twice the diastolic threshold. Isometric contraction was recorded using a linear force-displacement transducer (Harvard Apparatus, USA). Transmembrane action potentials were recorded with glass microelectrodes filled with 3 mol/L KCl (resistance 7-10 MΩ). The microelectrodes were connected to the input stage of a high-impedance amplifier (MEZ-7101; Nihon Kohden US, Inc., Foothill Ranch, CA). The amplified signals were displayed on a dual-beam oscilloscope (C1-69) and sampled at 10 kHz using a 16-bit analog-to-digital converter (PCL816; Advantech) [11]. The data was recorded and analyzed with specialized computer program.

The effect of oxidative phosphorylation uncoupler FCCP and F1F0-ATPase inhibitor oligomycin was registered after an equilibration period of 40-50 min. In the first group of experiments (n=5) we determined the effect of FCCP (10^{-9}, 10^{-8}, 10^{-7}, 10^{-6} mol/L) on contraction force (P) and action potential duration at 50% of repolarization (AP50) of human myocardial strips. In the second group (n=5) – the effect of FCCP on these parameters was investigated in the oligomycin treated ventricular strips. The experiments were performed as follows: after 30 min perfusion of ventricular strips with a Tyrode solution containing oligomycin, the increasing concentrations of FCCP were added to this solution and the perfusion with each concentration was continued for a 20 min. We used $2x10^{-5}$ mol/L of oligomycin, i.e. concentration, which induced inhibition of mitochondrial F_1F_0-ATPase activity [12].

Changes of the parameters were expressed in percentage: under the influence of FCCP or oligomycin alone – in respect to control (Tyrode solution), and under the influence of FCCP in the presence of oligomycin – in respect to the effect of oligomycin. All values were presented as means ±S.E.M. The significance of data was assessed using Student's t-test and the results were considered significant at $p<0.05$.

All agents used in experiments were from "Sigma" (USA).

Results

It was established that under control conditions, i.e. at perfusion of human ventricle strips with Tyrode solution, an average of contraction force was 1.21±0.3 mN (n=10) and action potential duration (AP_{50}) – 214.56±29.87 ms (n=9).

***Figure 1*. Time-course of the effect of FCCP on the contraction force of human ventricle strip, slow speed recordings. Arrows indicate the moment administration of FCCP (concentrations in mol/L are shown above arrows)**

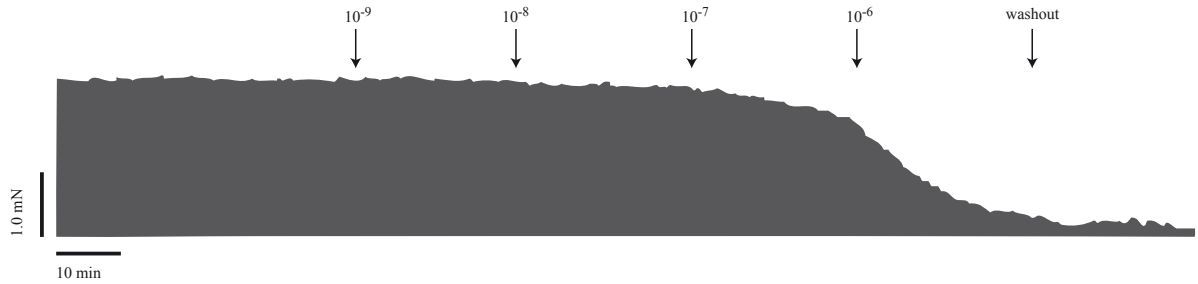


***Figure 2*. Effect of FCCP, an uncoupler oxidative phosphorylation, and oligomycin, an inhibitor of F1F0-ATPase, on the contraction and action potential of human ventricle strips. A – the action of FCCP (10^{-9}, 10^{-8}, 10^{-7}, 10^{-6} mol/L) alone on contraction (a) and action potential (a'); B – the action of oligomycin ($2x10^{-5}$ mol/L) alone and with FCCP (10^{-6} mol/L) on contraction (b) and action potential (b')**

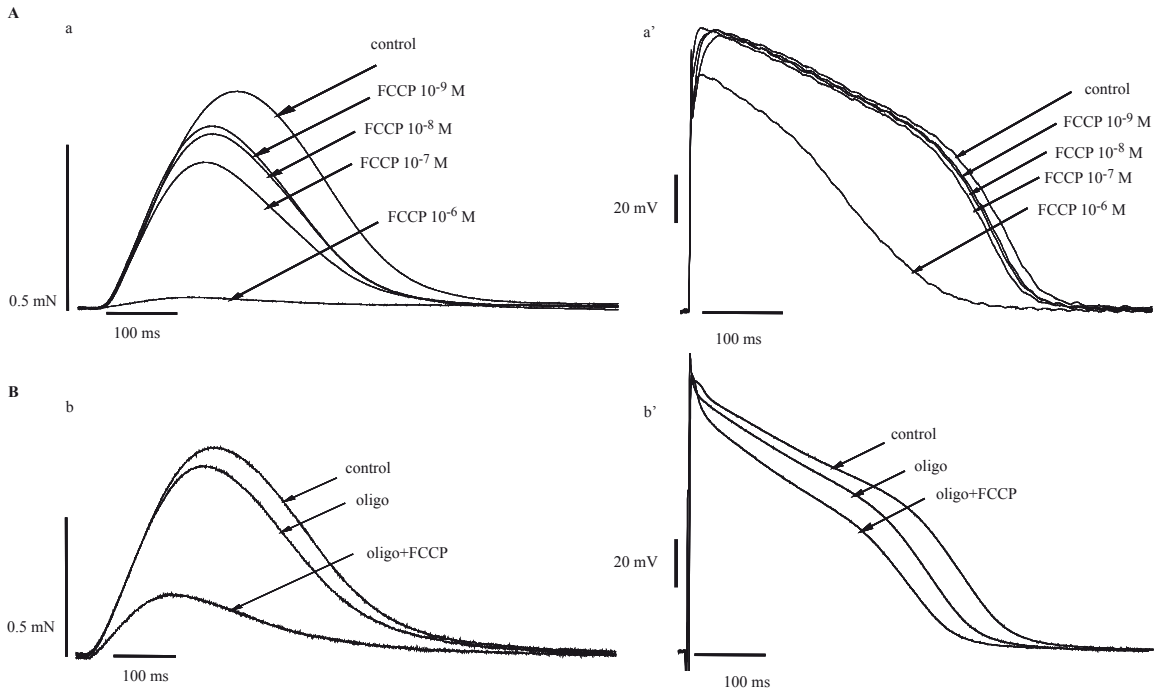
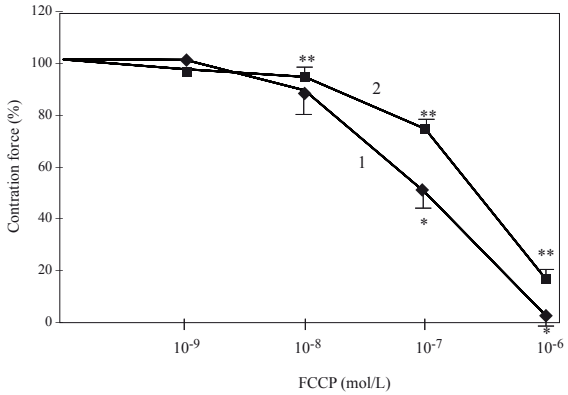


Fig. 1 depicts a representative example of the time-course of the effect of FCCP. In this example up to 10^{-8} mol/L of FCCP (of about 40 min in the control and 20 min in the presence of 10^{-9} mol/L FCCP) the contraction force was stationary. The negligible action of FCCP started at the 10^{-8} mol/L whereas 10^{-7} and 10^{-6} mol/L of FCCP caused substantial decrease in contraction force.

Fig. 2 (A) demonstrates the original traces from a typical experiment, showing contractions (A, a) and action potentials (A, a') in control conditions, and after 20 min of the action of FCCP (10^{-9}, 10^{-8}, 10^{-7}, 10^{-6} mol/L). The averaged data of the changes in contraction force under the influence of different concentrations of this uncoupler are presented in *Fig. 3* curve 1). FCCP caused the reduction of P in a dose-dependent manner and the maximal decrement of P to 8.3±3.1% (n=5) vs control, was obtained at 10^{-6} mol/L FCCP. AP_{50} decreased with drug concentration only at 10^{-7} and 10^{-6} mol/L to 94.3±1.9% and 55.5±3.1% (n=4), respectively, vs control. At the end of experiments the perfusion of myocardial strips with the Tyrode solution did not restored P and AP_{50}, i.e. the inhibitory effect of FCCP was irreversible.

In order to test the influence of F_1F_0-ATPase inhibition on alterations of human myocardium contraction force and action potential duration caused by FCCP, myocardial strips were pretreated with oligomycin for 30 minutes before the increasing concentrations of FCCP were added. Oligomycin ($2x10^{-5}$ mol/L) alone decreased the contraction force to 77.8±5.1% (n=5), and slightly reduced the action potential duration to 94.2±6.2% (n=4) vs control. *Fig. 2* (B) demonstrates the original traces of myocardial contractions (B, b) and actions potentials (B, b') recorded during experiments under the influence of oligomycin alone and together with FCCP. *Fig. 3* shows the averaged data of action of FCCP (10^{-9}, 10^{-8}, 10^{-7}, 10^{-6} mol/L) together with oligomycin ($2x10^{-5}$ mol/L) (curve 2) on the contraction force of human ventricular strips. It was established that in the presence of oligomycin FCCP decreased P at the smaller extent than

***Figure 3*. Effect of FCCP alone (curve 1) and with oligomycin ($2x10^{-5}$ mol/L) (curve 2) on the contraction force of human ventricle strips. Data are given as mean ±S.E.M., * p<0.05, vs control , ** p<0.05, vs oligomycin effect**



under the action of FCCP alone: the highest concentration of FCCP (10^{-6} mol/L) P reduced to 21.1±4.5% (n=5) vs effect of oligomycin. The duration of AP_{50} under the same experimental conditions was also less shortened, as compare with the FCCP action without oligomycin, i.e. AP_{50} at 10^{-6} mol/L FCCP in the presence of oligomycin decreased to 73.5±10.1% (n=4) vs effect of oligomycin.

Discussion

The present study describes the effect of mitochondrial oxidative phosphorylation uncoupler FCCP and F_1F_0-ATPase inhibitor oligomycin on the electromechanical activity parameters of human ventricular myocardium. Our experiments show that under the influence of FCCP the contraction force and duration of action potential decreased in a dose response manner. One of the major consequences of uncoupling of oxidative phosphorylation by FCCP is a decrease of intracellular ATP and creatine phosphate pools in myocardial cells [13-15]. The depletion of high-energy phosphates leads to impaired functioning of all energy-dependent systems, which regulate ion movement and the contraction of myocardial cells, i.e. sarcolemmal L-type Ca^{2+} channels, Ca^{2+} pump, Na^+-K^+ pump dependent Na^+-Ca^{2+} exchange, ATP-sensitive potassium channels and sarcoplasmic reticulum Ca^{2+}-ATPase (SERCA2), phospholamban, ryanodine receptors. This results in an elevation of the intracellular concentration of Ca^{2+} and Na^+ ions, development of diastolic contracture, diminishing of myocardial contraction force and heart failure [13]. Our data and a numerous other experimental investigations corroborate this explanation [9,13,16]. It has been shown that L-type Ca^{2+} current is decreased under conditions of metabolic inhibition in guinea pig and rabbit ventricular myocytes [14,16]. The decrease of ATP evokes shortening of action potential duration not only for the reduction of L-type Ca^{2+} current but also for an increase of K^+ current through the ATP dependent K^+ channels [17,18].

Our experimental data show that F_1F_0-ATPase inhibition with oligomycin significantly slowed the decrease of contraction force and reduced shortening of action potential duration evoked by FCCP in human myocardium. It is necessary to point out that oligomycin is nonselective inhibitor of F_1F_0-ATPase and inhibits both synthase and hydrolase activities of that enzyme [1,8]. This might explain our observation that oligomycin alone caused a moderate decrease of contraction force and action potential duration of human ventricular muscle. However, it has been shown that during ischemia oligomycin, as well as aurovertin, an inhibitor of F_1F_0-ATPase, attenuated the rate of ATP depletion in myocardium of rats and dogs [1,6]. Grover et al. demonstrated that oligomycin, or specific inhibitor of F_1F_0-ATPase BMS-199264, which selectively blocks hydrolase activity and has no or weak effect on synthase activity, significantly increased ATP concentration during ischemia in rat cardiomyocytes [8]. Recent studies demonstrated similar effect of oligomycin on electromechanical activity in rat myocardium at metabolic inhibition with anoxia, antimycin A, or FCCP, i.e. the inhibition of F_1F_0-ATPase by oligomycin not only slowed the diminishing of myocardial contraction, but also delayed the development of contracture [9,10].

It is known that human as well as larger animals express relatively high amounts of IF1 protein, a selective inhibitor of ATP hydrolase activity of F_1F_0-ATPase [1,19]. Fall of mitochondrial electrochemical gradient and intracellular pH to 6.7 as might be seen during ischemia causes reversibly binding of IF1 protein to F_1F_0-ATPase and blocking ATP hydrolase activity [20]. However, it was demonstrated that IF1 does not completely inhibit the hydrolase activity; therefore, further inhibition will confer the added benefit [8,21]. This suggests that during metabolic inhibition human cardiac cells are not capable to stop ATP hydrolysis in full by protein IF1, and the addition F_1F_0-ATPase inhibitor, such as oligomycin, can contribute to maintenance of electromechanical activity. Our experiments with FCCP and oligomycin in human myocardium support this explanation.

In conclusion, our data show that the inhibition of F_1F_0-ATPase reduces the impairment of electromechanical activity caused by oxidative phosphorylation uncoupler FCCP in human myocardium.

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