MITOCHONDRIAL OLIGOMYCIN-SENSITIVE ATPASE DURING ISOPROTERENOL-INDUCED CELL INJURY OF MYOCARDIUM

Dairo A. Rendón, Luis F. López.*

RESUMEN

LA ATPASA MITOCONDRIAL SENSIBLE A LA OLIGOMICINA DURANTE UNA LESIÓN CELULAR DEL MIOCARDIO INDUCIDA POR EL ISOPROTERENOL

En el presente trabajo se investigó la actividad enzimática de la ATPasa mitocondrial sensible a la oligomicina durante una lesión celular del miocardio inducida por el isoproterenol, usando homogenados de corazón de rata y una técnica potenciométrica. La actividad enzimática de la ATPasa mitocondrial sensible a la oligomicina, al igual que la acción inhibidora de la oligomicina sobre esta enzima, no muestra alteraciones significativas durante el tratamiento con isoproterenol. Estos resultados no concuerdan con la hipótesis sobre posibles modificaciones en la configuración activa de la ATPasa mitocondrial durante una lesión celular del miocardio inducida por el isoproterenol.

SUMMARY

The enzymatic activity of the mitochondrial oligomycin-sensitive ATPase was investigated during isoproterenol-induced cell injury of myocardium, using rat heart homogenates and a potentiometric method. The enzymatic activity of the oligomycin-sensitive ATPase and the inhibitory action of oligomycin do not show significant alterations upon treatment with isoproterenol. These results are inconsistent with the hypothesis that modifications in the active configuration of the mitochondrial ATPase take place during isoproterenol-induced injury of myocardium.

RESUME

L'ATPASE SENSIBLE À L' OLIGOMYCINE MITOCHONDRIALE AU COURS DE L' ISCHÉMIE MYOCARDIQUE DUE À ISOPROTÉRENOL.

On a étudié l'activité enzymatique de 1'ATPase mitochondriale sensible à l'oligomycine lors des altérations des cellules myocardiques, produites par isoprotérenol. Pour cette étude on a employé des homogénats de coeur de rats et une technique potentiométrique. L'activité potentiométrique de 1'ATPase mitochondriale sensible à l'ologomycine, de même que l'action inhibitrice de l'oligomycine sur cette enzyme, ne montre pas d'altérations significatives pendant le traitement avec isoprotérenol. Ces résultats ne concordent pas avec l'hypothèse de possibles modifications de la configuration active de 1'ATPase lors d'altérations des cellules myocardiques dues à l'isoprotérenol.

Palabras clave: Isoproterenol. ATPasa mitocondrial. Cardiomiopatía.
Key words: Isoproterenol. Mitochondrial ATPase. Cardiomyopathy.

*** To whom correspondence and reprint requests should be addressed.**
Universidad Nacional de Colombia-Sede Medellín. Laboratorio de Biofísica-Departamento de Física. AA 3840, Medellín-Colombia. Tel: 57-4-260 7575 ext 327. Fax: 57-4-260 4489
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INTRODUCTION

It has been shown that isoproterenol induces myocardial cell injury similar to that reported for myocardial infarction, myocardial ischemia, cardiac stress, and chagasic cardiomyopathy.[1-4] Several studies have investigated the molecular-cellular mechanisms of isoproterenol-induced cell injury of myocardium[5,6] (and the references in[6]). Among these, the study by Chagoya de Sánchez and co-workers[5] is a thorough study, that establishes a long-term, integrated model of isoproterenol-induced myocardial cell damage (encompassing structural, biochemical and physiological aspects). In these studies, different constituents of mitochondrial structure have been implicated in isoproterenol-induced cell injury, including the F_oF_1-ATPase (the mitochondrial oligomycin-sensitive ATPase or mitochondrial ATPase), which is directly responsible for the ATP synthesis supported by respiration, which supplies, under normal conditions, more than 80% of the energy demand of mammalian cells (for reviews see[7,8]).

The F_0F_1-ATPase is an oligomeric protein of the inner mitochondrial membrane constituted by a hydrophilic complex (F_1), which contains the catalytic site(s) for ATP synthesis or hydrolysis, connected to a hydrophobic protein complex (F_0), inserted in the lipid membrane, whose function is the coupling of catalytic activities of F_1 to transmembrane proton translocation. The behavior of this mitochondrial complex during isoproterenol-induced cell injury has been studied.[6,9] The two later studies favor the hypothesis that isoproterenol causes structural and functional alterations of mitochondrial ATPase.

The present study was aimed at studying further the mitochondrial oligomycin-sensitive ATPase to establish whether physical alterations occur in this enzyme during isoproterenol-induced cell injury of the myocardium. The main reason to study further the mitochondrial ATPase during isoproterenol treatment is the fact that isoproterenol-treatment induces ischemic alterations in the myocardium,[2,10,11] thus mitochondria from ischemic tissues could be altered during isolation procedures.[12] In this regard, kinetic parameters of mitochondrial ATPase measured in isolated mitochondria or submitochondrial particles from isoproterenol-treated tissues could potentially display alterations due to experimental artefacts rather than to physiological alterations taking place "*in vivo*" during isoproterenol treatment.

MATERIALS AND METHODS

Preparation of cardiac homogenates

Male Sprague/Dawley rats, weighing 200 g, were sacrificed by cervical dislocation, their hearts were rapidly excised, placed in cold sucrose (0.29 M) during three minutes, squeezed with a manual press (0.8 mm hole-diameter) and gently homogenized (3.5 ml of homogenization medium/1 g heart) in 0.17 M KCL, 10 mM EDTA, 0.1% bovine serum albumin (BSA), and 10 mM Tris-HCL; pH 7.45 (HCL or KOH). Cardiac homogenates from isoproterenol-treated rats were prepared as described above, using rats sacrificed 22 after receiving a subcutaneous injection of isoproterenol-HCL (1 mg/0.2 ml of saline).

Determination of mitochondrial ATPase activity.

Inorganic phosphate ($H_2PO_4^-$ or P_i), originated from ATP hydrolysis was estimated using a potentiometric method.[13] Briefly, P_i formation was calculated from the change in pH (DpH) caused by the dissociation of $H_2PO_4^-$ into HPO_4^{2-} and H^+ ($ATP^{4-} \rightarrow ADP^{3-} + H_2PO_4^-$; $H_2PO_4^- \rightleftharpoons HPO_4^{2-} + H^+$) after calibration of the system with KH_2PO_4. The assay medium was 100 mM sucrose, 75 mM KCL, and 3 mM Tris. HCL; pH 7.5 (HCl or KOH).

Incubations were carried out in thermostated glass vessels under magnetic stirring. The rate of pH change was measured at 37°C using a pH-meter connected to a computer providing an online display of rate values. Velocities of ATP hydrolysis were obtained in nmol P_i/mg of protein/min. The duration of the assay was approximately 10 min. Control experiments indicated that DpH was linear with time for over 15 min. Optimal concentrations for 2,4-dinitrophenol (DNP) and oligomycin were determined in control experiments. The rate of hydrolysis for ATP was titrated with DNP, and the optimal DNP concentration was the concentration that yielded the maximum rate of ATP hydrolysis. To obtain the optimal concentration for oligomycin, the rate of hydrolysis for ATP in the absence/presence of DNP was determined in heart homogenates preincubated during 2 minutes with different oli-

gomycin concentrations. The optimal oligomycin concentration was the minimum concentration that abolished further stimulation of ATP hydrolysis by DNP.

Before each experiment, this selected oligomycin concentration (0.8 μg/mg protein) was checked to ensure complete block of DNP-stimulated enzyme activity. Ethanol (0.23%) was added with oligomycin into the assay medium.

In order to estimate mitochondrial ATPase activity, the velocities of ATP hydrolysis after three consecutive additions were calculated *(Figure 1)*: (i) with ATP (v_o); (ii) with 2,4-dinitrophenol (v_1); (iii) with oligomycin (v_2). The mitochondrial ATPase activity was calculated as the difference between v_1 and v_2. Some considerations about this estimate (v_1-v_2) are given in the results section below. Protein was determined by the biuret method,[14] using BSA as standard.

All reagents were obtained from Sigma (St. Louis, Mo). They were selected to contain the smallest contamination by Na^+, Mg^{2+}, and Ca^{2+}. The reaction mixture contained small amounts of sodium, calcium, and magnesium, which originated from heart homogenates and from some impurities of the reagents. These amounts were determined by atomic absorption spectroscopy and they were 1.07 x 10^{-4}M, 1.11 x 10^{-5} M, and 8.75 x 10^{-6} M for Na^+, Mg^{2+}, and Ca^{2+}, respectively.

RESULTS

Figure 1 shows a typical trace in the determination of mitochondrial ATPase activity. After addition of ATP, all heart ATPases begin to change the pH of the solution. However, DNP stimulates only heart mitochondrial ATPase located on the inner mitochondrial membrane and is oligomycin sensitive. After oligomycin, the remaining activity is due to non-mitochondrial ATPases and may even be due to the soluble F_1 factor. The mitochondrial ATPase activity was calculated as the difference between the enzymatic activity after DNP (velocity v_1) minus the activity after oligomycin (velocity v_2). The difference v_1-v_2 is the mitochondrial ATPase activity if oligomycin inhibits only the mitochondrial ATPase. Two non-mitochondrial ATPases, the Ca^{2+}/Mg^{2+} ATPase and the Na^{2+}/K^+-ATPase, are oligomycin-sensitive too.[15-24] However, the following two considerations ensure that we measured only the mitochondrial ATPase: (i) the optimal oligomycin concentration (0.8 μg/mg protein) used in the present investigation is not high enough to inhibit these two non-mitochondrial oligomycin-sensitive ATPases, because these two ATPases are inhibited only by high oligomycin concentrations;[15,16,20] (ii) in the present study the assay was performed in a (Na^+, Mg^{2+}, Ca^{2+})-free system. In the absence of Mg^{2+} and Ca^{2+}, the Ca^{2+}/Mg^{2+} ATPase does not hydrolyze ATP,[15,16] neither does the Na^{2+}/K^+-ATPase in the absence of Na^+ and Mg^{2+}.[25] These two considerations guarantee that in the present experimental conditions the difference v_1-v_2 corresponds to the mitochondrial ATPase activity.

Figure 2 shows mitochondrial oligomycin-sensitive ATPase activity from normal and isoproterenol-treated rats. Mitochondrial ATPase activity from normal rats is approximately equal to the activity in homogenates from isoproterenol-treated rats. This observation indicates that during isoproterenol treatment no important physiologi-

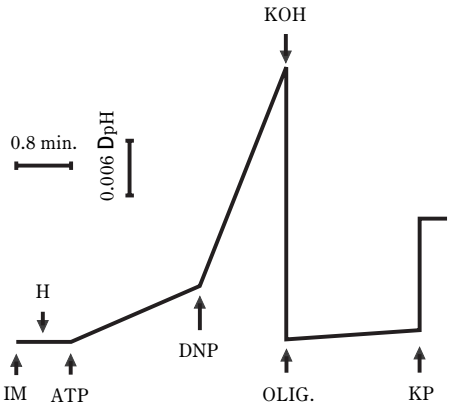

FIG. 1: A typical trace in the determination of the enzymatic activity of mitochondrial oligomycin-sensitive ATPase by a potentiometric method. Adenosine 5'-triphosphate (ATP, 1 mM), 2,4-dinitrophenol (DNP, 100 mM), rat heart homogenate (H, 1 mg/3.5 ml incubation medium), incubation medium (IM, 3.5 ml), KH_2PO_4 (KP, 200 nmol/3.5 ml incubation medium), KOH (1.7-2ml 0.2 M), and oligomycin (OLIG, 0.8 mg/mg protein) were added as indicated. Rat heart homogenates were incubated during 2 minutes before adding ATP. KH_2PO_4 was added as trace calibration. KOH was added to get more space on the computer screen for further pH recordings.

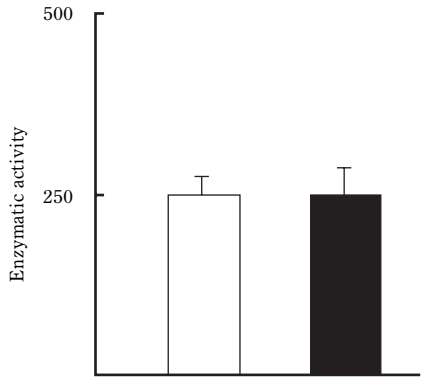

FIG. 2: Enzymatic activity of mitochondrial oligomycin-sensitive ATPase during isoproterenol treatment. The results are given in nmol Pi/mg of protein/min. Normal animals (white bars) and treated animals (black bars). All data are averages ± SD (n = 5).

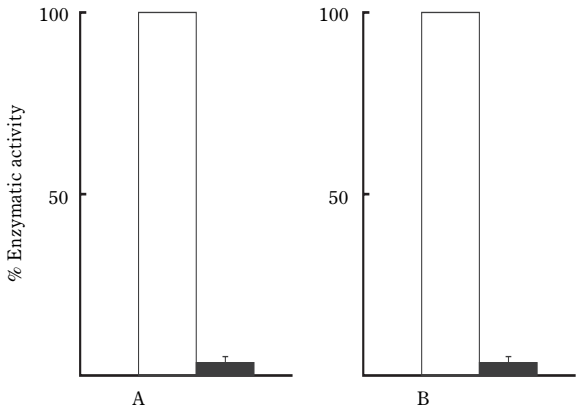

FIG. 3: Inhibitory action of oligomycin on heart ATPases. Enzymatic activities without oligomycin (white bars) were taken as 100%. Enzymatic activities with 0.8 mg oligomycin/mg protein (black bars). (A) Normal animals, (B) treated animals. The results are expressed as the percentage (±SD, n = 5) of the control values obtained in the absence of oligomycin (265.40 nmol P_i/mg of protein/min for normal animals and 264.51 nmol P_i/mg protein/min for treated animals).

cal alterations affecting enzyme activity take place in mitochondrial ATPase.

Figure 3 shows the inhibitory action of oligomycin on heart ATPase activities. It is clear that isoproterenol treatment does not modify this inhibitory action indicating that this treatment does not alter the sensitivity of heart mitochondrial ATPase to oligomycin.

Discussion

It is important to note that, in the present study, cardiac homogenates were used rather than isolated mitochondria because ischemic abnormalities have been documented in heart[2,10,11] during isoproterenol treatment. Mitochondrial isolation may influence mitochondrial structure and function, especially when ischemic tissues are used.[12] For this reason it is possible that additional alterations on kinetic parameters of mitochondrial oligomycin-sensitive ATPase might take place when isolated mitochondria or submitochondrial particles are used.

In the present investigation, Mg^{2+} was not used in the assay buffer to determine mitochondrial ATPase activity (see Materials and Methods). Experimental conditions without magnesium ions are more physiological to assay the activity of this enzyme. Normal total magnesium content in heart mitochondria is very high,[26] and a part of this magnesium is tightly bound to mitochondrial ATPase.[27]

Figure 2 shows enzymatic activity of mitochondrial oligomycin-sensitive ATPase. These data display no difference between mitochondrial ATPase activity in controls *vs* isoproterenol-treated homogenates. Thus, these results clearly show that isoproterenol does not induce functional alterations on the enzyme. *Figure 3* shows the inhibitory action of oligomycin on heart ATPase activities. From these results it is clear that isoproterenol treatment does not alter this inhibitory action. Taken together, these two observations indicate that there are no considerable functional and physical alterations of mitochondrial oligomycin-sensitive ATPase during isoproterenol-induced cell injury of the myocardium. It is important to point out that Curti and co-workers[6] reported a considerable increase of the inhibitory action of oligomycin on mitochondrial ATPase during isoproterenol treatment. It was mainly based on this increase that the investigators[6] suggested physical modifications on isoproterenol-treated mitochondrial ATPase. They consider that, during the isoproterenol-induction process, conformation changes occurred in the hydrophilic sector (F_1 moiety) of the mitochondrial ATPase, and that these changes could be transmitted to the hydrophobic protein, the oligomycin-sensi-

tive conferring protein (OSCP), thereby explaining the increased action of oligomycin on the isoproterenol-treated enzyme.

The results of the present study disagree with the main conclusion of Curti et al.[6] and Capozza et al.[9] In those studies isolated mitochondria, or submitochondrial particles, were used to study kinetic parameters of the mitochondrial oligomycin-sensitive ATPase. When mitochondria are isolated from ischemic tissues they may suffer additional alterations.[12] As a result, it is possible that kinetic alterations of the mitochondrial ATPase, during isoproterenol treatment, observed by Curti et al.[6] and Capozza et al,[9] could be an experimental artefact, because it has been reported that ischemic abnormalities take place in the heart during isoproterenol treatment.[2,10,11]

CONCLUSIONS

When rat heart homogenates are used the enzymatic activity of mitochondrial oligomycin-sensitive ATPase as well as the inhibitory action of oligomycin were not significantly modified by isoproterenol treatment. From these observations it is concluded that functional or physical alterations of the mitochondrial ATPase do not take place during that treatment. Observed alterations of kinetic parameters of isoproterenol-treated mitochondrial ATPase reported in other studies can be interpreted as experimental artefact. The reason for this interpretation is based on the fact that in those studies, isolated mitochondria or submitochondrial particles were used. When these cellular structures are isolated from isoproterenol-ischemic tissues they may undergo additional alterations.

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