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On the mechanism of oligomycin inhibition of Ca^{2+}-induced mitochondrial respiration

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The addition of oligomycin in the presence of Ca^{2+} increased the ADP pool in mitochondrial suspension. It is suggested that oligomycin inhibition of Ca^{2+}-induced mitochondrial respiratory activation is the function of the increased endogenous ADP pool. Low ADP concentrations (5–20 μM) produce the same inhibitory effect as oligomycin. The increase of ADP levels in the presence of glucose plus hexokinase resulted in the inhibition of Ca^{2+}-induced respiration, while the addition of phosphoenol pyruvate plus pyruvate kinase followed by a reduction in ADP levels, reversed the oligomycin inhibitory effect. One of the essential stages of ADP accumulation in mitochondrial suspensions in the presence of oligomycin and Ca^{2+} is proposed to be the formation of ADP from AMP and ATP, effected by adenylate kinase.

Ca^{2+}-induced respiration; Oligomcyin; Adenine nucleotide; Mitochrondrion

1. INTRODUCTION

The inhibitory effect of oligomycin on Ca^{2+}-induced respiration, and correspondingly, on the system responsible for Ca^{2+}-driven ion fluxes has been extensively studied [1–3]. In particular, the correlation was revealed between the efficiency of oligomycin inhibition of Ca^{2+}-ion efflux from mitochondria and matrix total pool of adenine nucleotides, which was altered by exchange with PP_i [4] and PEP [5]. On the other hand, the addition of oligomycin to a mitochondrial suspension containing 0.5 mM ATP resulted in the increase of ADP and AMP contents [6]. These experiments were carried out in the absence of Ca^{2+}. As it was demonstrated [7–9,5,13], ADP (but not ATP and AMP) effectively abolishes the Ca^{2+}- induced processes. This paper presents evidence that the increase of the ADP pool in a mitochondrial suspension, measuring the initial stage of a process which results in the alteration of the endogenous ADP/ATP ratio is essential for the inhibitory effect of oligomycin on Ca^{2+}-induced respiration. An attempt was undertaken to investigate the initial stages of oligomycin inhibition, to reveal key reactions involved in the accumulation of ADP in a mitochondrial suspension.

Abbreviations: PEP, phosphoenol pyruvate; PK, pyruvate kinase; Ap_5A, P^1,P^5-di(adenosine-5')pentaphosphate; CAT, carboxyatractylosid.

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2. MATERIALS AND METHODS

Rat liver mitochondria were prepared by the centrifugation method as described in [10], except that EDTA was excluded during the washing step. The rate of oxygen uptake was measured polarographically using a Clark oxygen electrode of a LP-7E polarograph (Czechoslovakia) at 20°C. The ATP levels in the mitochondria suspension were determined by luciferin-luciferase method [11,12]. For these measurements, all samples were taken from the polarographic cell 3–5 min after the addition of Ca^{2+}, which corresponded to the end of respiration induction period (lag) in the absence of oligomycin. We determined this lag-period as a time during which the rate of initial respiration increased by about 25–30% after Ca^{2+} addition. The samples were fixed with a trichloroacetic acid–EDTA mixture at 3–6°C and assayed on a Luminometer 1250 (LKB, Sweden) in medium containing 100 mM Tris-acetate, 2 mM EDTA and 1 mM $MgCl_2$ (pH 7.75). To determine the ADP content in the mitochondrial suspension, part of the samples which had also been used for ATP assay, were supplemented with 1 mM PEP and 40 U/ml of PK. The ADP content was calculated as the difference between ATP content in the samples in the presence and absence of PEP plus PK. In one series of experiments the extramitochondrial ADP and ATP content was measured. In this case, prior to fixation the samples taken from the polarographic cell, immediately centrifuged for 3 min at 10 000 × *g* at 5°C to obtain the mitochondrial fraction. Aliquots of supernatant were also treated with TCA and EDTA and subsequent ADP and ATP assays were performed as above. The basic medium for polarographic studies contained 10 mM HEPES, 5 mM Tris-HCl, 5 mM succinic acid, 20 mM KCL, 2 mM H_3PO_4, 1 μM rotenon, 250 mM sucrose, 500 μM $MgCl_2$ (pH 7.0). The protein concentration in all samples was 1 mg/ml. Ca^{2+} additions were variable, ranging from 50 to 100 μM.

3. RESULTS AND DISCUSSION

We measured the endogenous contents of ADP and ATP in a mitochondrial suspension under the conditions of respiratory activation by Ca^{2+} (see section 2) in the presence and absence of oligomycin (Fig. 1). In these experiments, oligomycin produced an increase in ADP content in the mitochondrial suspension with a parallel

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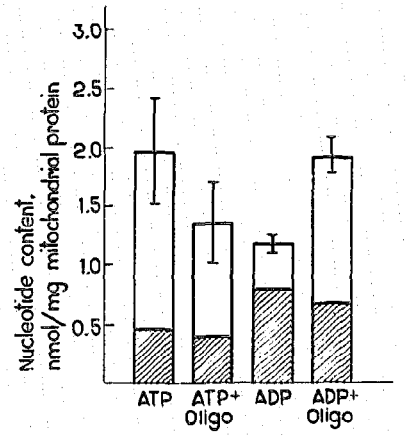

Fig. 1. ATP and ADP contents in a mitochondrial suspension in the presence and absence of oligomycin. Unhatched columns, net pool of extra- and intra-mitochondrial nucleotides; hatched columns, extramitochondrial nucleotides.

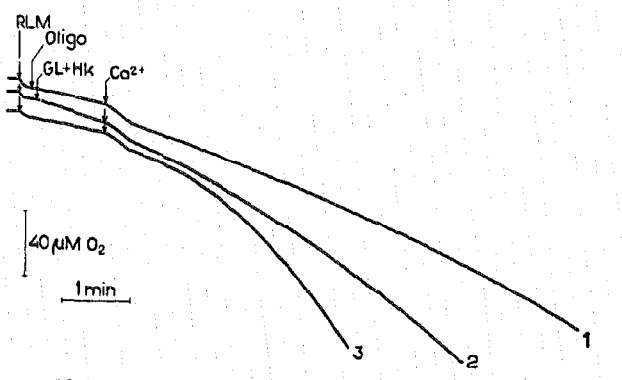

Fig. 2. Effects of oligomycin and glucose (Gl) plus hexokinase (Hk) on mitochondrial respiration stimulated by Ca^{2+}. Trace 1, oligomycin 0,7 γ/mg protein; trace 2, Gl 10 mM plus Hk 2 U/mg protein; trace 3, control.

reduction of the ATP content. This process was accompanied by an increase in the lag-period of the activation of respiration (Fig. 2, trace 1). The data of Fig. 1 show that the extramitochondrial ADP and ATP pool is sufficiently high. In Fig. 4 (trace 2) we present data demonstrating that low ADP concentrations suppress significantly the process of respiration stimulation by Ca^{2+} in phosphorylating mitochondria in the absence of oligomycin. In doing so, the increase of the total nucleotide pool results in a steady-state ADP level increase in the mitochondrial suspension. The data of Fig. 2 (trace 2) show that the enhancement of the energy expenditure after the addition of glucose plus hexokinase was accompanied by the inhibition of Ca^{2+}-induced respiration and the increase of ADP content in a mitochondrial suspension to the same extent as under the conditions of oligomycin inhibition (data not shown). It should be noted that the initial respiration rate was higher owing to the activation of the oxidative phosphorylation process in the presence of glucose plus hexokinase. No inhibition by oligomycin was observed under the condition of simultaneous PEP plus PK and oligomycin additions (Fig. 3, trace 4). All these data imply that a positive correlation may exist between the endogenous ADP content in the mitochondrial suspension and the efficiency of respiratory stimulation induced by Ca^{2+} (the time of induction period). An attempt was made to investigate the biochemical reactions leading to the increase of mitochondrial ADP content in the presence of oligomycin. Since oligomycin blocks ATP-synthase as well as ATPase, it may be assumed that ATP splitting in mitochondria was realized through biosynthetic reactions such as protein, nucleotide and lipid syntheses. All these reactions produce AMP and PP_i. Under the conditions used, AMP would be converted to ADP via the adenylate kinase reaction. The existence of such a reaction sequence may be confirmed by the fact that the adenylate kinase inhibitor, Ap_5A, sharply decreased the inhibitory effect of oligomycin (Fig. 4, trace 3). Note that this inhibitor decreased the suppression of respiration by low ADP concentrations in the absence of oligomycin (Fig. 4, trace 4). These data showed that inhibition of adenylate kinase activity induced the reduction of ADP levels in mitochondria, both in the case of ATP synthesis inhibition by oligomycin and when ATP is synthesized. The intermembraneous localization of adenylate kinase allows the suggestion that the reaction sequence leading to ADP accumulation must include nucleotide translocation via the ADP/ATP antiporter. The data of Fig. 5 (trace 3) clearly show the ability of CAT to reverse the oligomycin-induced inhibition of respiration. No substantial activation of mitochondrial

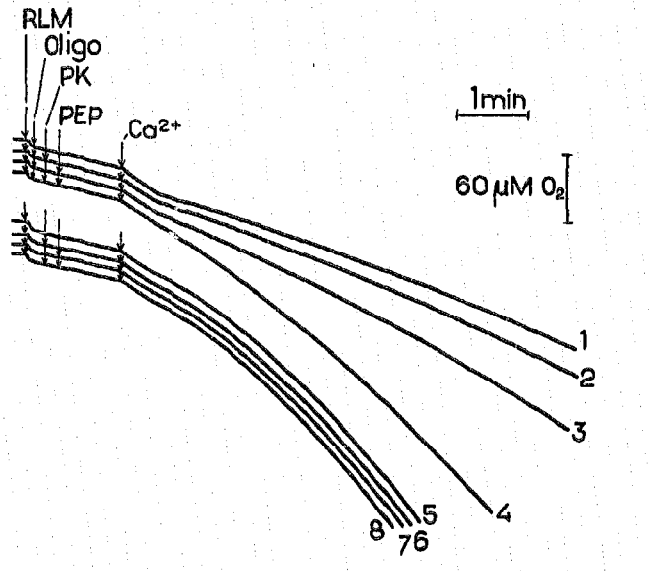

Fig. 3. Effect of oligomycin and pyruvate kinase (PK) plus phosphoenol pyruvate (PEP) on mitochondrial respiration stimulated by Ca^{2+}. Traces 1–4, oligomycin 0,7 γ/mg protein; trace 2 and 6, PK 40 U/mg protein; trace 3 and 7, PEP 1 mM; trace 4 and 8, PK+PEP; trace 5, control.

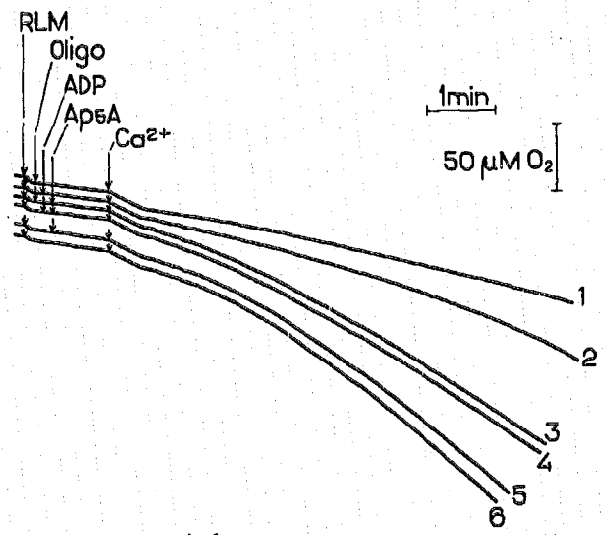

Fig. 4. Reversal by P^1,P^5-di(adenosine-5′)pentaphosphate (Ap_5A) of the inhibitory effects of oligomycin and ADP on mitochondrial respiration stimulated by Ca^{2+}. Traces 1 and 3, oligomycin 0,7 γ/mg protein; trace 2 and 4, ADP 10 μM; trace 3–5, Ap_5 A 80 μM; trace 6, control.

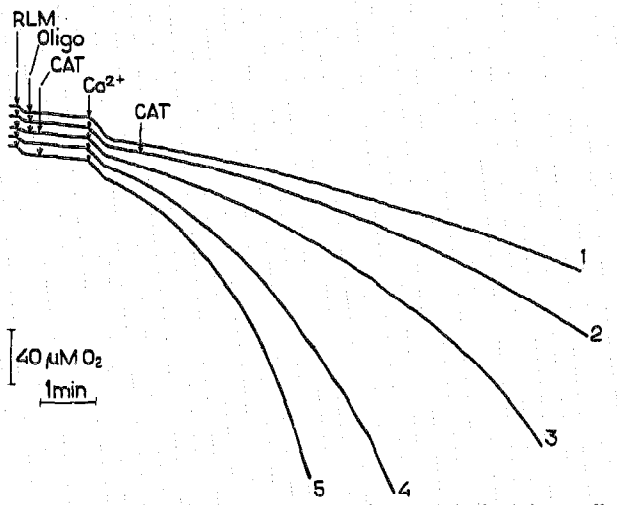

Fig. 5. Reversal by carboxyatractylosid (CAT) of the inhibitory effect of oligomycin on mitochondrial respiration stimulated by Ca^{2+}. Traces 1–3, oligomycin 0,7 γ/mg protein; traces 2,3 and 5, CAT 0,5 μM; trace 4, control.

respiration by CAT was observed in the absence of oligomycin (Fig. 5, traces 4,5). We used a low concentration of CAT (0.5 μM). It might be expected that the conformational effect of CAT (see [13,14]) manifested itself to a small degree in our experiments. It is also important that CAT added simultaneously with oligomycin diminished the inhibitory effect of the antibiotic (Fig. 5, trace 3). On the other hand, CAT, when added several minutes after oligomycin, was unable to abolish the oligomycin inhibition (Fig. 5, trace 2). Such relationships testify that, under our experimental conditions, the influence of CAT on the Ca^{2+}-induced respiration was due to its inhibitory effect on nucleotide transport, rather than to its effect on the conformational state of the ADP/ATP carrier. As mentioned above, the inhibition of ATP synthesis (oligomycin) and an acceleration of ATP utilization (glucose+hexokinase) caused the same effect, i.e. suppression of respiratory induction by Ca^{2+}. In both cases, the decrease in the ratio of ATP synthesis/utilization rates took place. So this ratio was the key factor in the regulation of Ca^{2+}-induced respiration. Our data concerning the inhibitory action of ADP (Fig. 4) indicated that the size of the total nucleotide pool could be another essential parameter.

The question arises as to the physiological role of this regulatory system in mitochondria. Such a system operates when the Ca^{2+} elevation in the cytoplasm occurs parallel with the energy expenditure increase in response to the hormonal signal. In this case, the increase in the steady-state concentration of ADP will block the uncoupling and thus will maintain the normal functioning of oxidative phosphorylation system.

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