THE STIMULATORY EFFECT OF EDTA ON CARDIAC MITOCHONDRIAL RESPIRATION

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Ethylenediamine-tetra-acetic acid (EDTA) is widely used in the preparation of mitochondria and the study of mitochondrial respiration. The beneficial effect of EDTA on the respiration of mitochondria prepared from different tissues is well documented (Slater, 1957). The mechanism of this action of EDTA has been referred to its ability to remove Ca^{++} from the mitochondrial preparation (Slater, 1957). The present study is an investigation into the general validity of this mechanism.

The preparation of cardiac mitochondria and the manometric determination of the mitochondrial oxygen uptake were according to Montgomery and Webb (1956). A steady rate of mitochondrial respiration was obtained with α-ketoglutarate as substrate. Concentrations of EDTA from 3×10^{-6} to 3×10^{-3} M produced approximately the same stimulation of oxygen uptake (Fig. I). Thus a relatively small concentration of EDTA is required to produce a stimulatory effect. The stimulatory effect was pronounced when α-ketoglutarate (+101%) or pyruvate plus malate (+177%) were used as substrates but relatively small with succinate (+30%) or malate (+35%).

Some inhibition of respiration was observed when Ca^{++} (3×10^{-5} M) was added to the reaction medium. EDTA (10^{-5} M) added with Ca^{++} (3×10^{-5} M) had a distinct stimulatory effect only slightly less than when EDTA was added alone. The EDTA present with Ca^{++} would be almost entirely in the form of

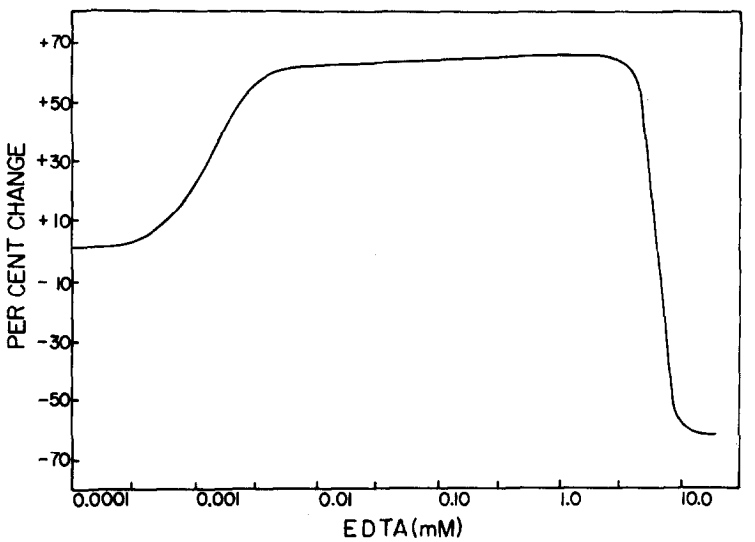

Figure 1. Stimulation of Mitochondrial Respiration at Different Concentrations of EDTA

the Ca-EDTA complex and would not be able to remove Ca^{++} from the medium or mitochondria. When the mitochondria were prepared in the presence of 10^{-2} M EDTA to remove most of the Ca^{++}, and washed twice with EDTA-free homogenization medium, the mitochondrial respiration was markedly stimulated by 10^{-5} M EDTA in the flask medium. As in the previous experiment, Ca^{++} present in three times the concentration of EDTA, did not remove the stimulation (Table I).

The presence of EDTA in the reaction medium is therefore a necessary requirement for its stimulatory action, inasmuch as mitochondria washed free of EDTA did not show stimulated respiration and increased respiration was observed when EDTA was added to this washed preparation. This, together with the fact that Ca^{++} did not remove completely the stimulatory effect of EDTA, indicates a direct stimulatory effect of EDTA on mitochondrial respiration.

The effects of EDTA on mitochondrial respiration in the presence of metal ions other than Ca^{++} are interesting. The stability constant of Mn-EDTA chelate is higher than that of the Ca-EDTA chelate, while Fe^{+++} has

the highest affinity for EDTA. As shown in Table I, Mn-EDTA was not stimulatory, stimulation not being observed until the concentration of EDTA was in excess of the Mn^{++} concentration. Fe-EDTA, however, was stimulatory. The differential effects of metal ion complexes with EDTA on mitochondrial respiration are thus evident. As Fe^{+++} has the highest affinity for EDTA and EDTA exerts its stimulatory action in the presence of Fe^{+++} ion, the removal of detrimental ions other than Ca^{++} in the medium by EDTA could scarcely offer an explanation of the stimulatory action. The prevention of the release of bound DPN from mitochondria by EDTA, as reported by Lester and Hatefi (1958), could give a more direct explanation. Hunter et al (1959) suggested that most of the effect of EDTA in preventing mitochondrial swelling and leakage

Table I

Effect of Ca^{++}, Mn^{++} and Fe^{+++} on EDTA-stimulated Mitochondrial Respiration

EDTA	Control	Metal Ions
		Ca^{++} (0.03mM)
None	69.1[a]	51.2
0.01mM	99.0	94.9
None[b]	52.5	41.4
0.01mM[b]	142.2	117.0
		Mn^{++} (0.10mM)
None	92.3	42.7
0.10mM	148.2	55.3
0.30mM	148.2	136.2
1.0 mM	148.2	149.2
		Fe^{+++} (0.10mM)
None	68.3	100.3
0.01mM	124.9	123.8

[a] Q_{O_2} with α-ketoglutarate, 5mM as substrate.

[b] Mitochondria prepared in medium containing 10mM EDTA and washed twice with EDTA-free medium.

of mitochondrial bound DPN is due to its complex formation in the mitochondrial membrane. Their suggestion may also be applied here to explain the stimulatory action of EDTA.

Abbreviation: DPN, diphosphopyridine nucleotide.

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