ROLE OF CYCLIC NUCLEOTIDES IN THE REGULATION OF MITOCHONDRIAL CALCIUM UPTAKE AND EFFLUX KINETICS

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SUMMARY

The effects of cyclic AMP and/or cyclic GMP upon various mitochondrial parameters were investigated. It was found that these nucleotides were unable to promote either inhibition of Ca^{2+} uptake and/or efflux or phosphorylation of ADP. These results are in contrast with those of other investigators, and suggest that cyclic nucleotides do not chemically mediate mitochondrial activity.

INTRODUCTION

By means of an energy dependent process, mitochondria are capable of regulating intracellular Ca^{2+} homeostasis. Rasmussen has speculated that rises in cytoplasmic Ca^{2+} concentration accompany various cyclic AMP mediated processes (1). Recently several investigators have reported cyclic AMP and/or cyclic GMP stimulation of Ca^{2+} efflux from isolated mitochondria (2-4). Particularly, Matlib and O'Brien reported that this process is atractyloside insensitive, implicating that it is not due to an interaction with the adenine nucleotide translocase such as an exchange of Ca^{2+} for cyclic nucleotides (3). Furthermore, this effect was present only when the concentration of cyclic AMP was between 1 and 4 μM.

The present study was undertaken to further substantiate these results. Instead, experimental evidence suggests that cyclic AMP and cyclic GMP are ineffectual in promoting Ca^{2+} efflux. The kinetics of Ca^{2+} and O_2 uptake were utilized in determining the above result.

Abbreviations

ADP - adenosine-5'-diphosphate
cyclic AMP - cyclic adenosine 3¦5' monophosphate
cyclic GMP - cyclic guanosine 3¦5' monophosphate

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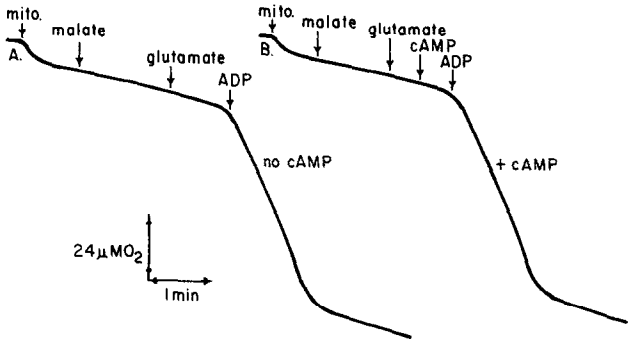

Figure 1. An experiment demonstrating O_2 uptake under control conditions (a) and in the presence of cyclic AMP or cyclic GMP (b). Rat liver mitochondria were suspended in the reaction medium in the presence of 10 mM glutamate, 10 mM malate, 10 mM Tris-PO_4, 15 mM $MgCl_2$, and 0.5 mM ADP or 1 mM $CaCl_2$. Protein concentration was 2.5 mg/ml . Upon addition of cyclic AMP and/or cyclic GMP, no changes in respiratory control ratios were observed.

METHODS

Mitochondria were isolated from adult rat livers in a medium consisting of 0.225 M mannitol, 0.075 M sucrose, 100 μM ethylenedinitrilotetracetic acid (EDTA), and Bovine Serum Albumin (BSA) as 1 mg/ml. BSA and EDTA were eliminated in two final washings.

Ca^{2+} uptake was measured by monitoring the change in optical density of murexide at 540-507 nm in a dual wavelength spectrophotometer (5). The reaction medium consisted of 0.075 M sucrose, 0.225 M mannitol, and 20 mM Tris-HCl. Succinate was utilized as a substrate in the presence of rotenone. All other additions and concentrations are as indicated on the individual figures.

O_2 uptake was measured polarographically by utilizing a Clark electrode in a medium of 0.225 M mannitol, 0.075 M sucrose, 10 mM Tris-PO_4, and 10 mM Tris-HCl. Succinate in the presence of rotenone and glutamate was tested as respiratory substrates. ADP and Ca^{2+} were utilized in stimulating respiration. All other additions and concentrations are indicated on the individual figures.

Titrations of cyclic AMP and cyclic GMP concentrations were carried out in the ranges of 1-413 μM.

Mitochondrial protein was determined by a biuret reaction.

RESULTS

Control Data. Mitochondria in the presence of Mg^{2+}, glutamate, and malate exhibited mean respiratory control ratios (RCR) of 7.9 ± 0.80. When succinate

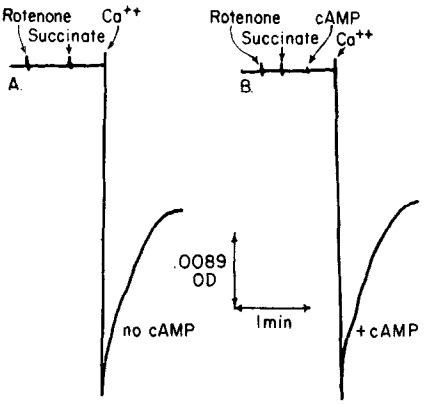

Figure 2. An experiment demonstrating Ca^{2+} uptake under control conditions (a) and in the presence of cyclic AMP or cyclic GMP (b). Rat liver mitochondria were suspended in the reaction medium in the presence of 3 mM Tris-PO_4, 4 mM succinate, 4 μM rotenone, 32 μM murexide and 250 μM $CaCl_2$. Protein concentration was 2.5 $mg \cdot ml^{-1}$. Upon addition of cyclic AMP or cyclic GMP, no changes in initial kinetics of Ca^{2+} uptake were observed.

was tested as a substrate in the presence of Mg^{2+} and rotenone the mean RCR was 9.3 ± 0.70. These values were similar when either ADP or Ca^{2+} was used to stimulate respiration. See Figure 1a.

The mean Ca^{2+} uptake rate in the presence of inorganic phosphate was 300 ± 70 nmoles Ca^{2+}/min/mg protein. See Figure 2a.

The Effect of cAMP and cGMP upon O_2 Uptake: When cyclic AMP or cyclic GMP was added to a suspension of mitochondria actively accumulating Ca^{2+} or phosphorylating ADP, no changes in O_2 uptake were observed. This was true whether the cyclic nucleotide was added preceding or following addition of ADP or Ca^{2+}. See Figure 1b. No effect upon state 4 respiration was noticed.

The Effect of cAMP and cGMP upon Ca^{2+} Transport: When cyclic AMP or cyclic GMP was added to a suspension of mitochondria, efflux of Ca^{2+} did not occur.

The absence or presence of 5 mM ATP or various concentrations of K^+ in the reaction medium did not alter the above results.

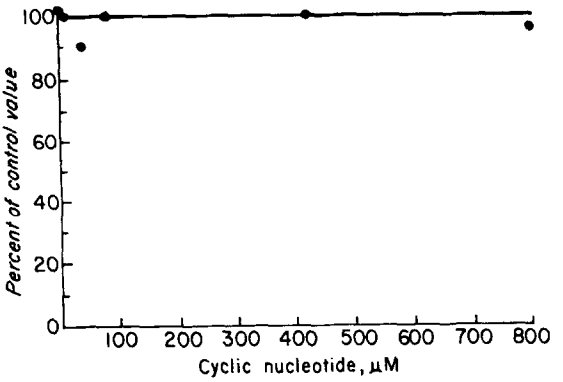

Figure 3. A titration of cyclic AMP or cyclic GMP as a function of percent of control values of Ca^{2+} uptake. For all concentrations of the nucleotides between 1-813 μM, no stimulation of Ca^{2+} efflux or uptake occurred. These results were unaffected whether the nucleotide was added preceding or following additions of Ca^{2+}.

A titration of cyclic AMP and cyclic GMP concentration from 1-413 μM produced no variation in the initial kinetics of Ca^{2+} uptake. See Figures 2b and 3. This was true whether the cyclic nucleotide was added preceding or following addition of Ca^{2+}. Ratios of cyclic AMP to cyclic GMP in proportions of 1:2, 2:1, and 1:1 were also ineffectual in promoting Ca^{2+} efflux.

DISCUSSION

The results of this investigation indicate that in concentrations of 1-413 μM, cyclic AMP or cyclic GMP does not stimulate mitochondrial Ca^{2+} efflux or uptake. The presence or absence of phosphate does not alter these findings.

The fact that O_2 uptake after the addition of cyclic AMP or cyclic GMP was unaffected when either ADP or Ca^{2+} was used to stimulate respiration further supports these conclusions.

This communication is in contrast with previous investigations (2-4) in which it is reported that Ca^{2+} efflux is strongly facilitated by the presence of cyclic AMP or cyclic GMP. Matlib and O'Brien have attempted to explain these results by suggesting that cyclic AMP causes Ca^{2+} efflux as a result of phos-

phorylation of a membrane component (3).

The apparent discrepancy between the above results and those obtained by other investigators is possibly attributable to differences in mitochondrial preparation, purity, i.e., contamination by other cellular components and enzyme systems.

Since cyclic AMP or cyclic GMP has no effect on mitochondrial Ca^{2+} efflux or uptake, they could not provide a control mechanism for in vivo release of Ca^{2+} from mitochondria as is suggested by Borle (2) and others (3,4).

Acknowledgments

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