J. Membrane Biol. 29, 205–208 (1976)
© by Springer-Verlag New York Inc. 1976

Letter to the Editor

On the Problem of the Release of Mitochondrial Calcium by Cyclic AMP

Received 26 April 1976

Borle (1974) has recently published that cyclic AMP induces a rapid and extensive release of the accumulated Ca^{++} from mitochondria isolated from livers, kidney and hearts. Shortly afterwards similar results were communicated by Matlib and O'Brien (1974) on liver and adrenal cortex mitochondria. In the abstract of a communication to a meeting, Snart (1975) has also mentioned that cyclic AMP controls the Ca^{++} content of kidney mitochondria. These observations have far-reaching physiological implications, since they indicate that cyclic AMP may act as the cellular messenger for the release of Ca^{++} from mitochondria and thus as the central agent for the regulation of the intracellular Ca^{++} homeostasis. The identification of a natural agent able, at physiological concentrations, to induce Ca^{++} release from mitochondria would provide experimental support to the suggestion that mitochondria play a major role in the regulation of the numerous Ca^{++}-dependent reactions in the cell. These results have attracted considerable interest and stimulated much experimental work during the last two years. In this letter we, unfortunately, report our failure to reproduce the basic observation that cyclic AMP induces Ca^{++} release from mitochondria under the experimental conditions described by Borle (1974), and by Matlib and O'Brien (1974), and under a variety of other experimental conditions.

Both Borle (1974) and Matlib and O'Brien (1974) have shown that cyclic AMP dramatically stimulate Ca^{++} release from Ca^{++}-loaded mitochondria. The phenomenon had the following essential characteristics:

(a) The release was rapid (less than 3 sec) and massive.

(b) It occurred in a variety of isolated mitochondria (from kidney, liver, heart and adrenal cortex). The release in adrenal cortex mitochondria was more evident than in liver mitochondria.

(c) The concentration range of cyclic AMP which induced Ca^{++} release from mitochondria was rather narrow 0.1 to 3 μM (Borle, 1974), 1 to 4 μM (Matlib & O'Brien, 1974).

(d) The effect was quite specific for cyclic AMP. Cyclic GMP had a slight effect at 10^{-5} M, whereas other nucleotides, such as dibutyryl cyclic AMP, 5′ AMP, ATP, and cyclic IMP had no effect at any concentration.

(e) The release induced by cyclic AMP was observed after Ca^{++} uptake had been driven by either respiratory substrates and ATP (Borle, 1974) or substrates alone (Matlib & O'Brien, 1974).

(f) Cyclic AMP had no effect on the Ca^{++} accumulation process in the experiments by Borle (1974) whereas an inhibitory effect was observed by Matlib and O'Brien (1974).

(g) The release of Ca^{++} induced by cyclic AMP was not accompanied by uptake of H^+. However, when the cyclic AMP-induced release of Ca^{++} was reversed, then the Ca^{++} uptake was accompanied by release of protons (Borle, 1974).

We have tested the effect of cyclic AMP on Ca^{++} release on rat liver mitochondria isolated from rat liver in the usual mannitol (0.72 M) sucrose (0.07 M), Tris-Cl (0.09 M, pH 7.4) medium, or in a similar medium, containing 0.5 mM Na-EDTA (the latter was not included in the final washing). It was also tested on rat and guinea pig heart mitochondria isolated by both the Nagarse (Pande & Blanchaer, 1971) and the polytron (Sordahl & Schwartz, 1967) methods. Mitochondria were isolated from animals obtained from different suppliers, of different sex and size, and kept under various diets. Calcium movements were measured either radiochemically after the separation of the mitochondria from the supernatant by Millipore filtration or rapid centrifugation, or spectrophotometrically by following the changes in absorbance of the metallochromic indicators murexide (Scarpa, 1972) or arsenazo III (Scarpa, 1976) in a dual wavelength spectrophotometer. Mitochondria were allowed to take up calcium in a veriety of experimental conditions: the mitochondrial concentrations in the medium were varied from 0.4 to 5 mg protein/ml; the reaction was carried out in the presence of 1 to 10 mM ATP, or in its absence; in the presence of 5 mM substrates (rotenone and succinate, glutamate and malate, or pyruvate and malate), plus or minus ATP; in the absence of permeant anions, or in the presence of 5 mM Na phosphate, or 5 mM Na acetate; in the presence or in the absence of added Mg^{++}; in the presence of initial concentrations of Ca^{++} ranging from 10 to 500 μM. Under all the above-mentioned experimental conditions, however, we

could not induce release of Ca^{++} from mitochondria upon addition of cyclic AMP. The cyclic nucleotide was added at different times during and after the uptake of Ca^{++}, at final concentrations ranging from 0.5 to 10 μM. The solutions of cyclic AMP were prepared from stocks obtained from various suppliers, and from various batches coming from the same supplier. The effect of cyclic AMP was also studied in mitochondria exhibiting various degrees of aging, or having different respiratory control ratios. It was also studied in the presence of aliquots of whole homogeate.

The effect of cyclic AMP on the release of mitochondrial Ca^{++} was tested unsuccessfully in several other laboratories (*see* Acknowledgments), and it is thus clear that the findings of Borle (1974) and Matlib and O'Brien (1974) are in sharp contrast with the lack of effect of cyclic AMP observed not only by us, but in a variety of other laboratories as well. At the moment, a clear and convincing explanation for the experimental discrepancy is lacking, but it is reasonable to conclude that the effect described by Borle (1974) and Matlib and O'Brien (1974) is very difficult to reproduce. It is obvious that particular caution should be exercised in evaluating these results from the standpoint of the intracellular Ca^{++} regulation.

We want to thank the following colleagues, who have communicated to us their inability to reproduce the release of Ca^{++} by cyclic AMP: S. Batra (Lund), J.B. Chappell (Bristol), H. Rasmussen (Philadelphia), N. Saris (Helsinki), L. Sordahl (Galveston), P. Walter (Basel).

A. Scarpa
Department of Biochemistry & Biophysics
University of Pennsylvania School of Medicine
Philadelphia, Pa. 19174

K. Malmstrom
M. Chiesi
E. Carafoli
Laboratory of Biochemistry
Swiss Federal Institute for Technology (ETH)
Zurich, Switzerland

References

Borle, A.A. 1974. Cyclic AMP stimulation of calcium efflux from kidney, liver and heart mitochondria. *J. Membrane Biol.* **16**:221

Matlib, A., O'Brien, J.P. 1974. Adenosine 3′:5′-cyclic monophosphate stimulation of calcium efflux. *Biochem. Soc. Trans.* **2**:997

Pande, S.V., Blanchaer, M. 1971. Reversible inhibition of mitochondrial adenosine diphosphate phosphorylation by long acyl coenzyme A esters. *J. Biol. Chem.* **246**:402

Scarpa, A. 1972. Spectrophotometric measurement of calcium by murexide. *Methods Enzymol.* **24**:343

Scarpa, A. 1976. Metallochromic indicators. *Methods Enzymol.* (*in press*)

Snart, R.S. 1975. Role of mitochondrial Ca^{2+} in antidiuretic hormone action. *Clin. Nephrol.* **4**:81

Sordahl, L.A., Schwartz, A. 1967. Effects of dipyridamole on heart muscle mitochondria. *Molec. Pharmacol.* **3**:509