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Letter to the Editor

New Evidence for the Role of Cyclic AMP in the Release of Mitochondrial Calcium

In a letter to the editor of this journal it was stated that the reported effect of cAMP in causing release of Ca^{2+} from mitochondria [2] could not be repeated in several laboratories [9]. The problem was reinvestigated and Borle [3] reported that in only 6% of a large number of experiments did cAMP cause Ca^{2+} release. In spite of this set-back, interest in the topic continues. A recent report on the effect of glucagon and dibutyryl cAMP on hepatocytes claimed that the observations supported the idea that cAMP causes efflux of Ca from mitochondria [6]. During hepatointoxication the rise in cAMP was postulated to modulate the rise of intracellular Ca^{2+} [1].

In heart mitochondria Na^+ seems to be the physiological mechanism for release [5] and in the small intestine, Ca^{2+}-binding protein caused release of Ca^{2+} from mitochondria [7]. However, until recently no such mechanism was known for liver. Working with rat liver and manipulating the redox potential of the medium, it was found that oxidizing conditions caused release of Ca^{2+}, whereas conditions favoring a high NADH/NAD ratio caused retention of Ca^{2+} [8]. We have been able to repeat this work using β hydroxybutyrate and acetoacetate as the redox couple (H.A. Juzu & E.S. Holdsworth, *manuscript submitted*). Since glucagon and dibutyryl cAMP can alter the ratio of β hydroxybutyrate/acetoacetate in favor of the oxidized state, this may be the cause of Ca efflux from hepatocytes treated with glucagon and dibutyryl cAMP [6]. When palmitate is the source of the ketone bodies, glucagon or dibutyryl cAMP treatment of isolated hepatocytes caused a small shift in the ratio of B hydroxybutyrate/acetoacetate from 0.91 to 0.58 after 120 min incubation [M.E.S. Neville & S.E. Jamieson, *unpublished; see also* 4].

The question then arises, does cAMP cause Ca release from mitochondria and by regulating Ca-sensitive enzymes alter the ratio of β hydroxybutyrate to acetoacetate, or does cAMP, by altering the redox state of site 1 phosphorylation in the respiratory chain, cause Ca release [8]? We have re-examined the effect of cAMP on Ca^{2+} uptake and release from rat liver mitochondria using palmitoyl CoA as substrate (H.A. Juzu & E.S. Holdsworth, *manuscript submitted*). The 5-ml medium contained 250 mM sucrose, 2.5 mM Hepes at pH 7.4, 2 mM Pi, 1 mM $MgCl_2$, 72 mM KCl, 2 mM D.L. carnitine HCl, 20 μM palmitoyl CoA, 1 mM ATP and 400 nmol $CaCl_2$. The reaction was started by adding mitochondria, (6–12 mg mitochondrial protein). A Ca^{2+}-sensitive electrode was used to follow Ca^{2+} movements. Ca^{2+} uptake was rapid, ~1 min, and was unaffected by the presence of 38 or 75 μM cAMP. In the presence of cAMP at these concentrations a significant slow release of Ca greater than the control was observed at 3 min, which continued during the 20 min of the experiment. Of the total uptake of 66 nmol/mg protein, 8–10 nmol Ca^{2+}/mg protein were released in 20 min. The effect of dibutyryl cAMP was similar, but no effect was observed with cyclic GMP. The effect of cAMP was not observed when succinate was the substrate either in the presence or absence of rotenone, as found in the earlier letters to this journal [3, 9]. By using a high performance amplifier with anti-log device, it has been possible to show that 75 μM cAMP causes release of mitochondria with their natural Ca^{2+} content, i.e., not preloaded with Ca^{2+}. During the period of Ca^{2+} release, the 20 natoms Ca^{2+}/mg of the mitochondria fell by 1 natom Ca^{2+} when incubated in the above medium (without added Ca^{2+}). Although this is a sensitive technique for following Ca^{2+} release, the system *understates* the release since the released Ca^{2+} was chelated by ATP present in the medium and the Ca^{2+} electrode only responded to ionic Ca^{2+}. The response to cAMP has been reproducible, the only occasions when no release of Ca^{2+} was obtained was when "aged" mitochondria, over 1 hr after isolation, were used. The question to be asked now is which

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comes first, the release of Ca^{2+} by cAMP or a change in NADH/NAD ratios which would cause the release.

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