EFFECT OF MALONYL-CoA ON CALCIUM UPTAKE AND PYRIDINE NUCLEOTIDE REDOX STATE IN RAT LIVER MITOCHONDRIA

Paul E. WOLKOWICZ and Jeanie McMillin WOOD

Departments of Medicine (Section of Cardiovascular Sciences) and *Biochemistry Baylor College of Medicine, Houston, TX 77030, USA*

Received 8 February 1979

1. Introduction

A possible role for Ca^{2+} in the regulation of intracellular metabolic events has been proposed [1–3]. Pyruvate dehydrogenase phosphatase is stimulated [4] and pyruvate carboxylase inhibited [5] by mitochondrial Ca^{2+}. The effect of glucagon to increase gluconeogenesis and to stimulate ketogenesis is significantly diminished by cellular Ca^{2+} depletion [6]. In addition, malonyl-CoA concentrations decrease with glucagon treatment and increase in meal-fed rats [7–9]. Recent evidence suggests that malonyl-CoA specifically inhibits liver mitochondrial carnitine palmitoyltransferase I [10]. Palmitoyl-CoA has been shown to inhibit Ca^{2+} uptake and to effect Ca^{2+} release from cardiac mitochondria [11]. Possible regulation of Ca^{2+} flux by the redox state of mitochondrial pyridine nucleotides has also been suggested [12]. In these studies, 4 μM palmitoyl-CoA inhibited Ca^{2+} uptake by rat liver mitochondria. When carnitine was added, Ca^{2+} uptake rates returned to normal, but addition of malonyl-CoA reduced the rates to levels seen with palmitoyl-CoA along (K_i 3 μM, malonyl-CoA). Ca^{2+} release and pyridine nucleotide oxidation were induced by palmitoyl-CoA and these effects were modified by carnitine and malonyl-CoA. A possible regulation of mitochondrial Ca^{2+} movements by cellular lipid metabolites is suggested.

2. Material and methods

Rat liver mitochondria were isolated by the method in [13] in a medium containing 220 mM mannitol, 70 mM sucrose, 2 mM *N*-2-hydroxyethylpiperazine-*N'*-2 ethanesulfonic acid (Hepes) and 1% bovine serum albumin (fraction V, Sigma) (pH 7.2). Mitochondrial protein was measured by the biuret method [14] and respiratory activity determined on a model 53 oxygen monitor (Yellow Springs Instruments, OH).

Ca^{2+} uptake and release was monitored using an Aminco DW-2 ultraviolet/visible dual wavelength spectrophotometer. Mitochondria (3 mg) were incubated at 30°C in a medium containing 100 μM tetramethylmurexide (K and K Labs, Plainview, NY), 1.67 mM succinate, 10 μg rotenone, 73 mM KCl in 0.25 M sucrose, 10 mM Hepes (pH 7.2) to 3 ml final vol. For uptake studies, additions were made as indicated in the figure legends. Ca^{2+} uptake was initiated by addition of 67 μM Ca^{2+} into the cuvette. The wavelength pair, 518–542 nm, was employed to measure ΔA in the Ca^{2+} : dye complex.

In the release studies, preincubation was carried out for 3 min either with no additions or with 1 mM L-carnitine ± 60 μM malonyl-CoA included in the assay medium. In these experiments Ca^{2+} release was effected by injection of 8 μM palmitoyl-CoA. The oxidation state of NADH was monitored under the same incubation conditions as Ca^{2+} release except that tetramethylmurexide was omitted. The wavelength pair, 340–370 nm, was employed to follow redox changes in pyridine nucleotides.

3. Results and discussion

The ability of 4 μM palmitoyl-CoA to inhibit Ca^{2+} uptake by liver mitochondria is shown in fig.1A. In

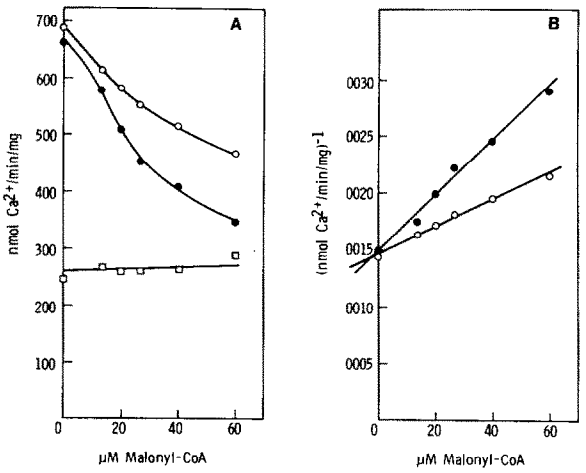


Fig.1. The effect of malonyl-CoA on respiration-dependent calcium uptake by rat liver mitochondria. Ca^{2+} uptake by 3 mg rat liver mitochondria was initiated as in section 2. (A) Effect of malonyl-CoA on Ca^{2+} uptake in the presence of 4 μM palmitoyl-CoA. Mitochondria were preincubated for 3 min before Ca^{2+} addition in the presence of 4 μM palmitoyl-CoA and varying concentrations of malonyl-CoA, average of 2 separate experiments (□——□). Mitochondria were preincubated for 1 min (●——●) and 3 min (○——○) before Ca^{2+} addition in the presence of 4 μM palmitoyl-CoA + 1 mM L-carnitine and varying concentrations of malonyl-CoA, average of 3 separate experiments each. (B) Dixon plot of malonyl-CoA inhibition of Ca^{2+} uptake in the presence of 4 μM palmitoyl-CoA and 1 mM carnitine. (●——●) Mitochondria preincubated for 1 min before initiation of Ca^{2+} uptake. (○——○) Mitochondria preincubated for 3 min before initiation of Ca^{2+} uptake. App. K_i 3.0 μM.

Table 1
Effect of malonyl-CoA on respiration-dependent Ca^{2+} uptake by rat liver mitochondria (nmol Ca^{2+}/min/mg)[a]

	No addition	+ Palmitoylcarnitine	
		10 μM	20 μM
Control			
+ malonyl-CoA	632	625	576
20 μM	611	619	567
80 μM	597	605	609

[a] Results are the average of 2–4 separate determinations

After a 1 min preincubation of 3 mg rat liver mitochondria in the absence or presence of palmitoylcarnitine ± malonyl-CoA as indicated, Ca^{2+} uptake was initiated by addition of 67 μM Ca^{2+} into the cuvette. The % standard deviation of experimental points determined in the presence of malonyl-CoA compared to control uptake values was < 3.9% where no further addition was made and < 4.8% in the presence of either 10 μM or 20 μM palmitoylcarnitine

the absence of exogenous carnitine, palmitoyl-CoA depresses the rate of mitochondrial Ca^{2+} uptake by ~60%. This depression is not affected by increasing malonyl-CoA concentrations.

Similar experiments were carried out in the presence of 4 μM palmitoyl-CoA + 1 mM carnitine (fig.1A). In the absence of added malonyl-CoA, no inhibitory effect of palmitoyl-CoA in the presence of carnitine is observed when compared to control uptake rates (table 1) at either 1 or 3 min preincubation times. When malonyl-CoA is also included in the uptake medium, both a time and concentration-dependent effect on Ca^{2+} uptake rates is observed. Since malonyl-CoA has been suggested to act competitively to inhibit the carnitine acyltransferase reaction [10], the shorter preincubation time would be expected to result in relatively higher concentrations of palmitoyl-CoA associated with mitochondrial membranes. Indeed, this appears to be the case as shown in fig.1A, where 1 min preincubation demonstrates a more dramatic inhibition of Ca^{2+} uptake in the presence of malonyl-CoA when compared to the 3 min preincubation. As the malonyl-CoA concentration is increased, Ca^{2+} uptake rates begin to approximate the rates observed with 4 μM palmitoyl-CoA alone. A Dixon plot of the data derived from fig.1A is consistent with competitive inhibition kinetics for Ca^{2+} uptake and the observed K_i for malonyl-CoA is 3 μM, a value within the physiological range (fig.1B) [7,8].

A bimodal nature for the carnitine acyltransferase reaction has been postulated [15–17]. Although the data in fig.1 implicate the site of malonyl-CoA inhibition as the external transferase (I), further experiments were carried out to investigate inhibition at the site of the inner membrane transferase (II).

Addition of 20 μM or 80 μM malonyl-CoA to rat liver mitochondria incubated in control uptake medium has no effect on the rates of Ca^{2+} uptake (table 1). Similarly, when mitochondria were preincubated with either 10 μM or 20 μM palmitoylcarnitine to provide substrate to carnitine acyltransferase II, malonyl-CoA produces no significant alteration in Ca^{2+} uptake rates

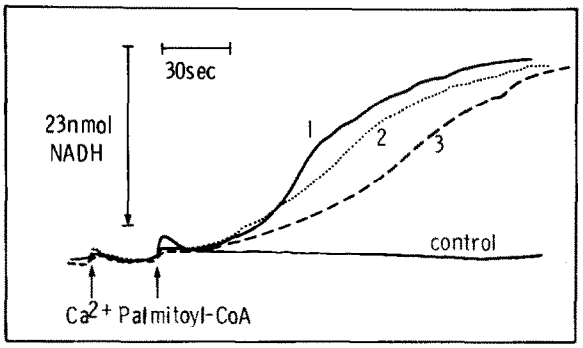
(table 1). This result is in agreement with the report [10] that malonyl-CoA acts as a specific inhibitor of the external transferase.

Palmitoyl-CoA has been shown to effect Ca^{2+} efflux from cardiac mitochondria [11]. The possibility that inhibition of the carnitine acyltransferase reaction by malonyl-CoA could affect the release of Ca^{2+} from hepatic mitochondria was tested. Mitochondria were allowed to accumulate Ca^{2+} in the absence or presence of 1.0 mM l-carnitine ± malonyl-CoA, after which 8 μM palmitoyl-CoA was introduced into the cuvette. Addition of palmitoyl-CoA in the absence of carnitine causes rapid release of Ca^{2+} from the mitochondria (fig.2, lower panel). In results not shown, preincubation of mitochondria with lower concentrations of palmitoyl-CoA (3-6 μM) caused decreased Ca^{2+} uptake and premature release of any Ca^{2+} accumulated.

When carnitine is included in the incubation medium, addition of palmitoyl-CoA again produces release of Ca^{2+} but the time to release is increased ~2-fold. Addition of 60 μM malonyl-CoA in the presence of carnitine causes a Ca^{2+} release pattern similar to that seen with palmitoyl-CoA alone. These results may be explained by an increase in the unreactive concentration of palmitoyl-CoA.

Recently, it has been proposed that Ca^{2+} uptake and release from mitochondria may be related to the redox state of the mitochondrial pyridine nucleotides [12]. Although exogenous palmitoyl-CoA is not able to cross into the mitochondrial matrix space and thus directly affect internal metabolism, the possibility that the efflux of Ca^{2+} from mitochondria results from, or is concomitant with, oxidation of pyridine nucleotides was tested.

Palmitoyl-CoA was added after Ca^{2+} accumulation by mitochondria was complete and redox changes followed (fig.2, upper panel). Palmitoyl-CoA alone causes rapid oxidation of mitochondrial pyridine



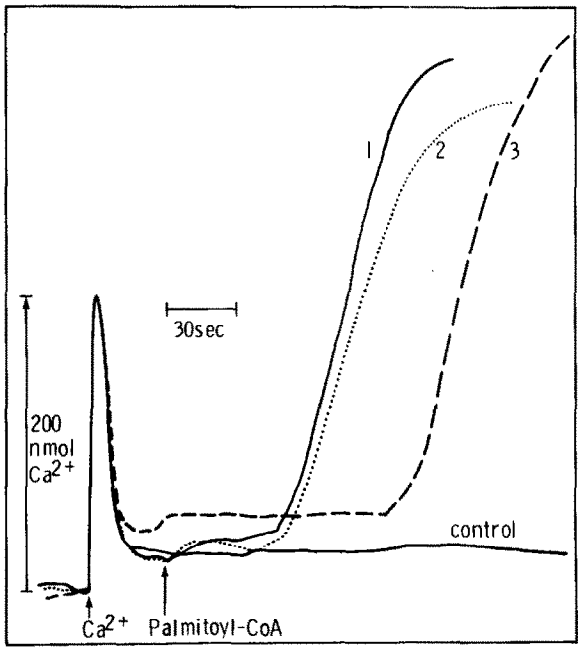


Fig.2. (upper panel). The effect of malonyl-CoA on Ca^{2+}-induced changes in the reduction state of pyridine nucleotides in the presence of palmitoyl-CoA. Control, lower trace (——): pyridine nucleotide absorption when either 8 μM palmitoyl-CoA or 67 μM Ca^{2+} were added separately to 3 mg rat liver mitochondria. In subsequent traces, following uptake of 67 μM Ca^{2+}, 8 μM palmitoyl-CoA was added to effect Ca^{2+} release. 1. Upper trace (——): oxidation of pyridine nucleotides following addition of 8 μM palmitoyl-CoA alone. 2. (· · ·): oxidation of pyridine nucleotides in the presence of 1 mM carnitine and 60 μM malonyl-CoA. 3. (– – –): oxidation of pyridine nucleotides in the presence of 1 mM carnitine.

Fig.2. (lower panel). The effect of malonyl-CoA on Ca^{2+} efflux from mitochondria. Control, lower trace (——): Ca^{2+} : dye absorbance when either 8 μM palmitoyl-CoA or 67 μM Ca^{2+} were added separately to 3 mg rat liver mitochondria. In subsequent traces, following uptake of 67 μM Ca^{2+}, 8 μM palmitoyl-CoA was added to effect of Ca^{2+} release. 1. (——): Ca^{2+} : dye ΔA following addition of palmitoyl-CoA alone. 2. (· · ·): Ca^{2+} : dye ΔA in the presence of 1 mM carnitine and 60 μM malonyl-CoA. 3. (– – –): Ca^{2+} : dye ΔA in the presence of 1 mM carnitine.

nucleotides. Separate addition of either palmitoyl-CoA or Ca^{2+} does not result in a similar effect. In the presence of carnitine, the time required to cause complete NADH oxidation is prolonged as is Ca^{2+} release. Inclusion of malonyl-CoA and carnitine in the assay medium results in rapid oxidation of pyridine nucleotides following palmitoyl-CoA addition, approximating that seen with palmitoyl-CoA alone.

A role for pyridine nucleotides as controlling factors in mitochondrial Ca^{2+} metabolism requires the interaction of intramitochondrial metabolic events in the mediation of Ca^{2+} fluxes [12]. The data presented suggest that events occurring at the external surface of the mitochondrial membrane may also act to control cellular Ca^{2+} movements. Palmitoyl-CoA on the external surface of the mitochondrial membrane may affect a membrane-associated pool of pyridine nucleotide, the latter being generated by Ca^{2+} addition [18]. However, it is not clear if the oxidation events observed are occurring at internal sites on the mitochondrial membranes, or extra-mitochondrially, following release of NADH into the medium. The latter possibility concerning Ca^{2+}-induced release of mitochondrial pyridine nucleotides has been reported [18] albeit at much higher Ca^{2+} concentrations.

A role for lipid metabolites in the control of Ca^{2+}-mediated enzymatic responses is attractive and may offer some insight into the postulated role of Ca^{2+} as a second messenger [1].

Acknowledgements

The technical assistance of Mr Lonnie Franks during the course of these experiments and the expert typing of the manuscript by Mrs Sandra Haley are gratefully acknowledged.

References

[1] Rasmussen, H., Jensen, P., Lake, W., Friedman, N. and Goodman, D. B. P. (1975) Adv. Cycl. Nucl. Res. 5, 375–394.
[2] Bygrave, F. L. (1967) Nature 214, 667–671.
[3] Gewers, W. and Krebs, H. A. (1966) Biochem. J. 98, 720–735.
[4] Chiang, P. K. and Sacktor, B. (1975) J. Biol. Chem. 250, 3399–3408.
[5] Foldes, M. and Barritt, G. J. (1977) J. Biol. Chem. 252, 5372–5380.
[6] Soler-Argilaga, C., Russell, R. L., Werner, H. V. and Heimberg, M. (1978) Biochem. Biophys. Res. Commun. 85, 249–256.
[7] Guynn, R. W., Veloso, D. and Veech, R. L. (1972) J. Biol. Chem. 247, 7325–7331.
[8] Cook, G. A., Nielsen, R. C., Hawkins, R. A., Mehlman, M. A., Lakshmanan, M. R. and Veech, R. L. (1977) J. Biol. Chem. 252, 4421–4424.
[9] McGarry, J. D., Stark, M. J. and Foster, D. W. (1978) J. Biol. Chem. 253, 8291–8293.
[10] McGarry, J. D., Leatherman, G. F. and Foster, D. W. (1978) J. Biol. Chem. 253, 4128–4136.
[11] Asimakis, G. K. and Sordahl, L. A. (1977) Arch. Biochem. Biophys. 179, 200–210.
[12] Lehninger, A. L., Vercesi, A. and Bababunmi, E. A. (1978) Proc. Natl. Acad. Sci. USA 75, 1690–1694.
[13] Schneider, W. C. (1948) J. Biol. Chem. 176, 259–266.
[14] Layne, E. (1957) Methods Enzymol. 3, 450–451.
[15] Fritz, I. B. and Yue, K. T. N. (1963) J. Lipid Res. 4, 279–288.
[16] Yates, D. W. and Garland, P. B. (1970) Biochem. J. 119, 547–552.
[17] Hoppel, C. L. and Tomec, R. J. (1972) J. Biol. Chem. 247, 832–841.
[18] Vinogradov, A., Scarpa, A. and Chance, B. (1972) Arch. Biochem. Biophys. 152, 647.