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Evidence for net uptake and efflux of mitochondrial coenzyme A

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Coenzyme A transport was studied by determining [^{14}C]CoA associated with isolated rat heart mitochondria. HPLC analysis of a mitochondrial extract obtained following incubation with [^{14}C]CoA revealed an increase in [^{14}C]CoA. In the presence of pyruvate or α-ketoglutarate, [^{14}C]CoA associated with mitochondria was converted to acetyl- or succinyl-[^{14}C]CoA, respectively, demonstrating the intramitochondrial localization of transported CoA. Net uptake of CoA was demonstrated by the findings that the increase in mitochondrial content of CoA following incubation with CoA was equal to the values of CoA uptake obtained from experiments using [^{14}C]CoA. Sequestration of intramitochondrial CoA as metabolically inert derivatives with maleate stimulated CoA uptake, supporting the concept of unidirectional CoA uptake rather than exchange. Altering the membrane electrochemical gradient with valinomycin, nigericin, calcium, phosphate or a combination of phosphate and calcium caused efflux of endogenous CoA. The largest efflux was observed with valinomycin or a combination of Ca^{2+} and P_i. The Ca^{2+} and P_i-induced CoA efflux was effectively prevented by succinate or pyruvate. The results suggest that the uptake process, which is dependent on the membrane electrical gradient can be reversed by dissipating the electrical gradient. The relevance of CoA efflux induced by Ca^{2+} and P_i is discussed with respect to reperfusion injury following ischemic damage. Other factors regulating the maintenance of CoA within the mitochondrial matrix include the matrix pH and the acylation state of CoA.

Introduction

Coenzyme A (CoA) is an essential factor in a large number of acylation reactions in the cell including those involved in energy production [1]. In liver cells, all the enzymes responsible for synthesis of CoA are found in the cytosol. In addition, liver cell mitochondria contain the final two enzymes of the CoA synthetic pathway suggesting that intramitochondrial synthesis of CoA is possible [2,3]. In myocardial cells, the complete CoA synthetic pathway appears to be extramitochondrial [4]. Following synthesis in cardiac cells, CoA is transported into mitochondria [5] which contain nearly 90% of the total cellular CoA [6]. Thus mitochondria of myocardial cells may derive their CoA from the cytosolic compartment or, if a pathway similar to that in the liver exists in the heart, from the mitochondrial synthetic pathway. The mitochondrial transport of CoA is driven by the membrane electrical gradient and is regulated by the intramitochondrial pH [7]. Since synthesis of CoA is subject to feedback inhibition by free CoA [8,9], transport into mitochondria could serve as a mechanism for decreasing cytosolic CoA and thereby relieving the inhibition of CoA synthesis. Also, efflux could be mediated by the transporter and this could potentially be the first step in the degradation of CoA since CoA-degrading enzymes are thought to be extramitochondrial [10,11].

Transport of substances into mitochondria can occur either by uniport or by exchange. Phosphate and pyruvate enter mitochondria on uniport systems, dicarboxylic acids and tricarboxylic acids are transported on electroneutral exchange systems, and adenine nucleotides, glutamate and aspartate are transported by electrophoretic exchange mechanisms (for review, see Ref. 12). From the information available on the transport of CoA into mitochondria, it is not clear whether this system involves net influx of CoA or exchange for intramitochondrial CoA. The importance of the distinction lies in the implications of each of these processes, i.e., while a uniport results in a net increase, an

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exchange mechanism provides a mean of equilibration of the internal and external pools of the compound in being studied. Although the previous communications on CoA transport showed that CoA was accumulated by mitochondria, they did not show that the transported CoA was within the mitochondrial matrix. In the present study, we investigated whether the exogenous CoA associated with mitochondria was within the mitochondrial matrix and whether influx of [^{14}C]CoA resulted in increased CoA levels in mitochondria. Since a previous study suggested that CoA efflux may occur in the absence of an electrochemical gradient [7], the ability of mitochondria to retain CoA was also studied.

Experimental procedures

Isolation of mitochondria

Mitochondria were isolated from hearts of male Sprague-Dawley rats by a modification of the method of Chance and Hagihara [13] as described by Idell-Wenger et al. [14]. The isolation medium contained 0.225 M mannitol, 0.75 M sucrose and 0.1 mM EDTA (pH 7.4) (MSE buffer). The heart tissue was minced and incubated with the proteinase nagarse (nagarse 2 mg/g heart weight) for 1 min prior to the homogenization step. The final mitochondrial pellet was suspended in 150 mM KCl/10 mM Mops (pH 7.4). Preparations having respiratory control ratios greater than 6 using pyruvate and malate as substrates were used.

Measurement of CoA uptake

Isolated mitochondria were incubated in a medium containing: 7% dextran, 30 mM Tes, 2 mM $MgCl_2$, 80 mM KCl, 5 mM dithiotheritol, 10 mM L-tartarate (pH 7.4). [^{14}C]CoA, synthesized from [^{14}C]pantothenic acid using *Brevibacterium ammoniagenes* by the method of Shibata et al. [15] as described in a previous communication [7] was used as a tracer to measure CoA uptake. The specific activity of the synthesized [^{14}C]CoA was approx. 50 nCi/nmol. Unless mentioned otherwise, 100 μM CoA (1 nCi/nmol) was used in all uptake experiments. Incubations were initiated by addition of mitochondria (1–1.5 mg/ml, unless otherwise specified) and were terminated by centrifuging an aliquot through silicone oil into a layer of 14% perchloric acid (PCA) as described previously [5]. [^{14}C]Sucrose was used as an extramitochondrial marker in parallel incubations. All experiments were carried out at 30°C.

Measurement of CoA efflux

Efflux of endogenous CoA was also studied in isolated mitochondria. Samples were incubated in the presence or absence of various compounds including DMSO (control for valinomycin and nigericin), 10 μM valinomycin, 10 μM nigericin, and various concentrations of phosphate and calcium. At various time intervals, aliquots were centrifuged and the supernatant was removed. The tube was rinsed carefully (so that the mitochondrial pellet remained intact) with 5 mM DTT and the pellet resuspended in 1 ml 5 mM DTT. The supernatant and the pellet suspension were boiled for 10 min, centrifuged and the supernatants assayed for CoA.

HPLC analysis

Samples were incubated with 100 μM [^{14}C]CoA (approx. 400000 cpm/ml) under various conditions (described in the legends to figures) and the reaction was stopped at various time intervals by centrifuging a 0.5 ml aliquot of the incubation mixture through silicone oil into 200 μl 14% PCA/10 mM dithiotheritol. Radioactivity in aliquots of the supernatant (100 μl) and mitochondrial pellet (25 μl) layers was determined to calculate CoA uptake. The supernatant was then aspirated and the portion of the tube above the oil layer rinsed with 5 ml distilled water without disturbing the oil and PCA layers. Most of the oil was then aspirated and 200 μl 1 M K_2CO_3/500 mM Mops added to neutralize the PCA. The tube was vortexed thoroughly to resuspend the pellet, centrifuged and a 100 μl aliquot of the supernatant injected into an HPLC system to separate the acyl-CoAs by the method of Corkey et al. [16]. The HPLC system used was a Beckman 332 component system equipped with an Altex C_{18} column (4.6 mm × 250 mm, 5 μm particle size) and a Beckman guard column (4.6 mm × 45 mm). A gradient mobile phase was used at a total flow rate of 1.0 ml/min (Solution A, 100 mM potassium phosphate (pH 5.3), Solution B, methanol). The mobile phase composition was changed from 10% Solution B to 25% Solution B linearly over 80 min. Eluent fractions were collected (2 ml/fraction) and radioactivity determined by liquid scintillation counting. Absorbance was continuously monitored at 254 nm. Under these conditions, the following elution times for standards were obtained : CoA, 34–36 min, succinyl CoA, 44–46 min and acetyl CoA, 54–56 min. Radioactivity in a 100 μl aliquot of the neutralized PCA extract was also determined. In most samples, 80–100% of the radioactivity was recovered as CoA and its metabolites.

Other procedures

Coenzyme A was assayed fluorometrically using the α-ketoglutarate dehydrogenase reaction [17]. Proteins were determined using the method described by Bradford [18]. Electrical and pH gradients were determined as described previously [7].

Results

Location of transported CoA

Incubation of mitochondria with [^{14}C]CoA resulted in accumulation of radioactivity in a time dependent

manner in a peak corresponding to CoA (Fig. 1A). In the presence of pyruvate (Fig. 1B) or α-ketoglutarate (Fig. 1C), [^{14}C]CoA associated with mitochondria remained relatively constant with time while the increase in radioactivity occurred in the peaks corresponding to acetyl CoA or succinyl CoA, respectively (Figs. 1B and 1C). The radioactivity present as [^{14}C]CoA in the presence of these substrates reflects the inaccessibility of this pool to the matrix enzymes and therefore probably represents surface bound CoA. The relative constancy of the size of the [^{14}C]CoA pool over extended periods of time further supports the concept that this pool is bound CoA. On the other hand, [^{14}C]CoA that is esterified in the presence of pyruvate or α-ketoglutarate increases with time in parallel with the increase in total radioactivity. This represents the [^{14}C]CoA within the mitochondrial matrix. In a different series of experiments, when 10 μM carboxyatractyloside, which has been shown to prevent binding of CoA [5], was added to the incubation buffer, it was observed that relatively less radioactivity was recovered as [^{14}C]CoA in the absence or presence of pyruvate and α-ketoglutarate at all the time points studied (Figs. 1D, E and F). However, the radioactivity converted to acyl-[^{14}C]CoA was not affected significantly by carboxyatractyloside (Fig. 1). These data provide evidence that a large proportion of the carboxyatractyloside-insensitive component of mitochondrial CoA accumulation is within the mitochondrial matrix.

Efflux of coenzyme A

Although the above results provide evidence for influx of exogenously added radioactive CoA into mitochondria, it was not clear whether the process represented net influx or whether it was an exchange process such that the total mitochondrial CoA did not increase. This was investigated by determining the mitochondrial CoA content enzymatically following incubation of mitochondria with CoA. In the presence of added CoA, a time-dependent increase in the mito-

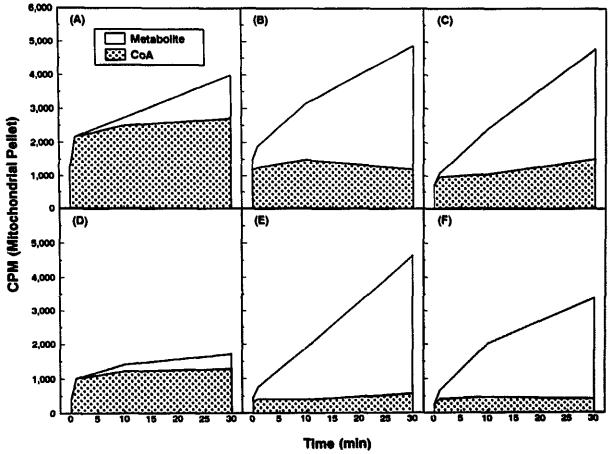

Fig. 1. Chemical forms of [^{14}C]CoA associated with mitochondria in the presence (panels A, B and C) or absence (panels D, E and F) of 10 μM carboxyatractyloside under different incubation conditions are expressed as a function of time. Isolated rat heart mitochondria were incubated with 100 μM CoA and a tracer amount of [^{14}C]CoA (approx. 400000 cpm/ml, specific activity of CoA in the incubation medium was approx. 1 nCi/nmol). Mitochondria were incubated without added substrate (panels A and D), with 2.5 mM pyruvate (panels B and E) or with 3 mM α-ketoglutarate (panels C and F). Incubation was initiated with the addition of mitochondria (approx. 1–1.5 mg/ml of incubation medium). A 0.5 ml aliquot of the incubation mix was centrifuged through silicone oil into 14% perchloric acid (PCA) to terminate the reaction. The supernatant and oil layers were aspirated, the PCA layer neutralized and 100 μl of the neutral extract subjected to HPLC analysis. For details of the gradient system used, see Experimental procedures. The metabolite in panels A and D eluted at 18 min. The CoA metabolite in panels B and E was identified as acetyl CoA and that in panels C and F as succinyl CoA. These eluted at 55 min and 45 min, respectively, while standard CoA eluted at 36 min. Recovery of counts/min injected into the HPLC as CoA and its metabolites was typically in the range of 80–100%.

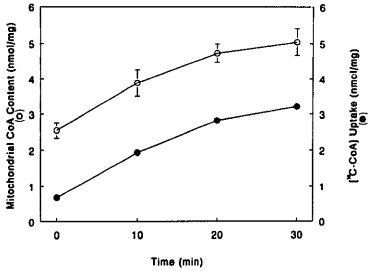

Fig. 2. Mitochondrial CoA uptake determined by using [^{14}C]CoA (closed circles) or by determining total CoA content following incubation with CoA (open circles).

chondrial CoA content was observed. The magnitude of the increase was identical to the values of uptake obtained by uptake of [^{14}C]CoA (Fig. 2). Using both these approaches, a plateau was observed at approx. 20–25 min following the start of incubation suggesting a decrease in the net uptake of CoA.

In order to confirm the above findings, a more sensitive approach was taken to determine whether the observed uptake reflected net uptake exclusively or whether there was an efflux component as well. Mitochondria were incubated with [^{14}C]CoA, the reaction was stopped by centrifugation through oil and the specific activity of CoA was determined in the supernatant and the pellet. In support of the suggestion that CoA uptake reflects net uptake, the specific activity of

TABLE I

Specific activity of CoA in mitochondria and the postmitochondrial supernatant following incubation with [^{14}C]CoA

Rat heart mitochondria (approx. 5 mg/ml) were incubated with 100 μM [^{14}C]CoA. Aliquots were taken at the times indicated and mitochondria were separated from the incubation mixture by centrifugation. The supernatant was aspirated, boiled, radioactivity determined and CoA assayed. The mitochondrial pellet was rinsed with and resuspended in 15 mM DTT, boiled, radioactivity determined and assayed for CoA. The values represent the mean of two different batches of mitochondria using the same incubation medium. Similar trends were seen in other experiments carried out as above.

Time (min)	Specific activity (cpm/nmol)	
	mitochondria	supernatant
0	778	3394
10	1600	3390
20	2181	3280
30	2250	3297

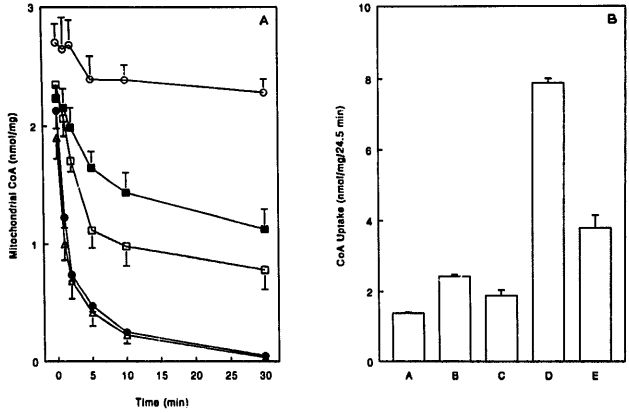

Fig. 3. Effect of maleate on mitochondrial CoA content (A) and [^{14}C]CoA uptake (B). Isolated rat heart mitochondria were incubated without any substrate (○——○), with 3 mM α-ketoglutarate (□——□), 3 mM maleate (●——●), 3 mM α-ketoglutarate+3 mM maleate (△——△) or α-ketoglutarate, maleate and 1 mM butyl malonate (■——■) and free CoA was determined enzymatically. CoA uptake was carried out as described in Experimental procedures in the absence of substrate (A), with 3 mM α-ketoglutarate (B), 3 mM maleate (C), α-ketoglutarate+maleate (D) or with α-ketoglutarate, maleate and 1 mM butyl malonate (E). Values represent means±S.E. of three or four experiments.

CoA in the mitochondria increased while the specific activity of CoA in the supernatant remained constant with time (Table I).

The results thus far suggested that CoA influx into mitochondria was a uniport system rather than exchange. If this were true, then decreasing the available intramitochondrial CoA would not be expected to affect the ability of mitochondria to accumulate CoA. Mitochondrial CoA was decreased by incubating mitochondria with maleate, the *cis* isomer of fumarate which has been shown to sequester mitochondrial free CoA as the thioether and thioester derivatives [19,20]. The reaction is catalyzed by a transferase which is present in the heart, kidney and other organs but is absent in the liver [21,22]. In the presence of maleate, free CoA in heart mitochondria decreased significantly in the first few minutes following incubation and by 30 min following the start of incubation, free CoA could not be detected (Fig. 3). In agreement with results reported previously [19], CoA could not be recovered by alkaline hydrolysis. When CoA uptake was studied, maleate failed to inhibit CoA uptake. On the contrary, in the presence of α-ketoglutarate maleate caused a 4–5-fold stimulation of CoA uptake (Fig. 3). Identical results were obtained in the presence of other oxidizable substrates including pyruvate, succinate, malate, and palmitoyl carnitine. The effect of maleate was attenuated in the presence of butyl malonate, an inhibitor of mitochondrial dicarboxylate transport [23] (Fig. 3). Similar studies carried out in isolated liver mitochondria did not demonstrate any decrease in CoA levels in the presence of maleate nor a stimulation of CoA uptake (Fig. 4). HPLC analysis of radioactivity in the mitochondrial pellet carried out on samples obtained from myocardial tissue and incubated with maleate revealed that, as in the case of incubations with pyruvate or α-ketoglutarate, radioactivity in the peak corresponding to CoA in samples incubated with maleate remained relatively constant with time (Fig. 5). A large proportion of the time-dependent accumulation of radioactivity was seen in two peaks neither of which corresponded to succinyl-CoA. One of these eluted in the void volume and the other at approx. 58 min. The identity of these peaks was not pursued further. The peaks probably represent the maleyl thioether and thioester derivatives of CoA which have been described previously [19,20].

Effect of ionophores on mitochondrial CoA

The concentration of CoA in mitochondria is approx. 2 mM and the concentration used in our studies on CoA transport was 100 μM. Thus CoA accumulated in the mitochondria against a 20-fold concentration gradient and, as reported [7], was primarily driven

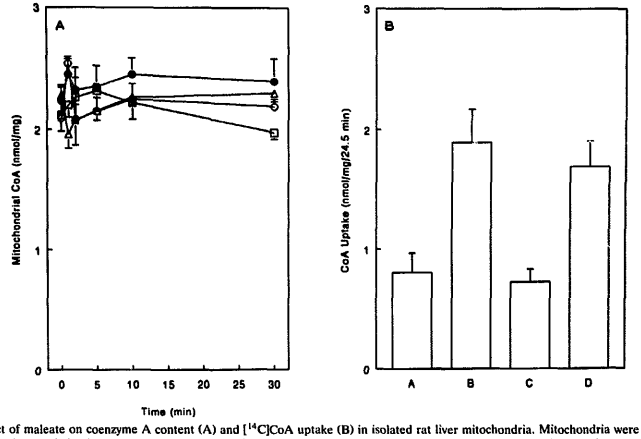

Fig. 4. Effect of maleate on coenzyme A content (A) and [^{14}C]CoA uptake (B) in isolated rat liver mitochondria. Mitochondria were incubated without any substrate (○), with 3 mM α-ketoglutarate (□), 3 mM maleate (●) or 3 mM α-ketoglutarate + 3 mM maleate (△). CoA uptake was carried out as described in Experimental procedures in the absence of substrate (A), with 3 mM α-ketoglutarate (B), 3 mM maleate (C) or α-ketoglutarate + maleate (D). Values represent means ± S.E. of three experiments.

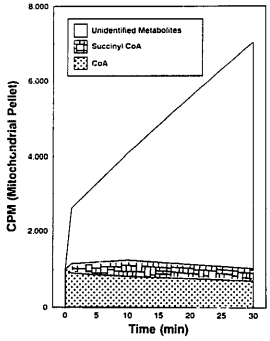

Fig. 5. Distribution of radioactivity in mitochondria following incubation with 3 mM maleate + 3 mM α-ketoglutarate. See legend to Fig. 1 for details. The unidentified metabolites in this figure represent the sum of two unidentified peaks, one of which eluted in the void volume and the other at approx. 58 min.

by the electrical gradient. It was thus of interest to determine whether abolishing the electrical gradient would induce the efflux of mitochondrial CoA. Also, since the intramitochondrial pH was shown to be an important determinant of CoA uptake [7], it was of interest to determine if altering the intramitochondrial pH caused CoA efflux. Mitochondria were thus incubated in the presence or absence of ionophores. At various time intervals following incubation, aliquots were taken, mitochondria separated from the medium by centrifugation and CoA determined in the supernatant and pellet. Samples were also taken simultaneously to determine total CoA in the incubation mixture. As shown in Fig. 6, in the presence of valinomycin and nigericin, mitochondrial CoA decreased with time; this loss was accounted for by an increase in the supernatant CoA while total CoA remained unchanged.

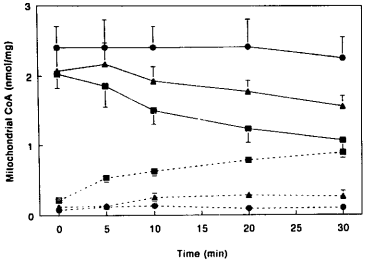

Fig. 6. Effect of valinomycin and nigericin on mitochondrial CoA content. Mitochondria were incubated without any additions (●), with 10 μM nigericin (▲) or 10 μM valinomycin (■). Details of the experiment are provided in Experimental procedures.

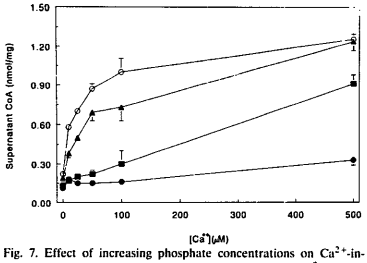

Fig. 7. Effect of increasing phosphate concentrations on Ca^{2+}-induced CoA efflux. Mitochondria were incubated with Ca^{2+} in the absence of phosphate (●), with 1 mM (■), 5 mM (▲) or 20 mM (○) phosphate. Mitochondria were incubated with the respective agents and samples were taken at the start and 20 min following the start of incubation and centrifuged. CoA content of the postmitochondrial supernatant was determined enzymatically.

Effects of phosphate and calcium on mitochondrial endogenous CoA

Phosphate is known to dissipate the membrane pH gradient in normal mitochondria. However, in mitochondria loaded with calcium, addition of phosphate causes release of calcium which is accompanied by dissipation of the electrical gradient. In view of the findings that dissipation of the membrane electrochemical gradient caused efflux of CoA and since an increase in the concentrations of these ions is known to occur during reperfusion following ischemic damage, it was of interest to determine whether incubation of mitochondria with phosphate with or without calcium could cause efflux of CoA. As described in Experimental procedures, mitochondria were incubated with increasing concentrations of Ca^{2+} in the absence of added substrate and CoA assayed in the supernatant and the pellet. The results of these experiments are shown in Fig. 7. While lower concentrations of calcium did not have significant effects on the endogenous CoA levels, a small decrease in CoA associated with the mitochondrial pellet (and a corresponding increase in CoA appearing in the supernatant) was observed at relatively high Ca^{2+} concentrations. Upon incubation with increasing concentrations of inorganic phosphate, a time-dependent efflux of mitochondrial CoA was observed only at the higher concentrations. However,

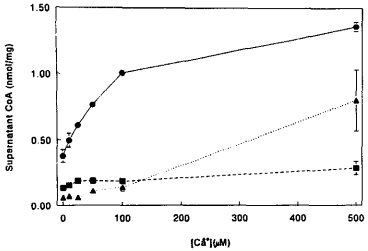

Fig. 8. Effect of 10 mM succinate or 5 mM pyruvate on Ca^{2+}- and P_i-induced CoA efflux. Mitochondria were incubated with 20 mM P_i and different concentrations of Ca^{2+} in the absence of substrate (●——●), presence of succinate (■- - - - -■) or pyruvate (▲·····▲). Supernatant CoA levels were determined following alkaline hydrolysis to hydrolyze any acyl esters of CoA.

in the presence of phosphate, significant CoA efflux was observed at relatively low concentrations of Ca^{2+} (Fig. 7). These results can be reconciled with the hypothesis that dissipation of the membrane electrical gradient affects the ability of mitochondria to retain CoA. Further support for the hypothesis was provided by experiments in which the ability of substrates at preventing the Ca^{2+}- and P_i-induced efflux was studied. The presence of 10 mM succinate in the incubation medium almost completely prevented CoA efflux (Fig. 8). Similarly, pyruvate attenuated the efflux of CoA induced by Ca^{2+} and P_i.

Discussion

The conversion of CoA to acetyl-CoA (in the presence of pyruvate) or succinyl-CoA (in the presence of α-ketoglutarate) is mediated by the matrix enzymes pyruvate dehydrogenase (PDH) or α-ketoglutarate dehydrogenase (α-KGDH), respectively. The finding that following incubation with [^{14}C]CoA, a large proportion of radioactive CoA associated with the mitochondrial pellet was readily converted to these two acyl-CoA derivatives in the presence of pyruvate or α-ketoglutarate respectively, provides evidence that CoA is transported into the mitochondrial matrix. The mitochondrial matrix space does not change appreciably with time suggesting that breakage of mitochondria under these conditions is minimal. This is further supported by observations that the postmitochondrial supernatant obtained after incubation of mitochondria for upto 30 min does not contain appreciable amounts of α-ketoglutarate dehydrogenase (unpublished observations). Another observation from these experiments was that in the presence of acyl donors free radioactive CoA associated with mitochondria remained relatively constant. At least part of this PDH and α-KGDH inaccessible and relatively constant pool represents binding of CoA to surface sites since the presence of carboxyatractyloside decreased (but did not abolish) free CoA associated with the mitochondrial pellet but did not affect the amount of [^{14}C]CoA which could be converted to acetyl-CoA or succinyl-CoA. To summarize, the following pools of [^{14}C]CoA can be discerned: (a) a carboxyatractyloside-sensitive, surface pool which probably represents CoA bound to the adenine nucleotide translocase; (b) a carboxyatractyloside-insensitive pool, which may be CoA bound to CoA-recognizing sites other than the nucleotide translocase and (c) a matrix pool which can be converted to acyl-CoA esters.

The results from previous studies suggested that CoA enters mitochondria on a uniport system driven by the electrical gradient. Further evidence in favor of this hypothesis was obtained by the experiments with maleate i.e. despite complete sequestration of mitochondrial CoA, uptake was not inhibited. On the contrary, dramatic stimulation was observed. The increase in radioactivity associated with mitochondria in the presence of maleate appears to be the manifestation of a matrix event. This is supported by the following findings: (a) in the presence of maleate, endogenous (matrix) CoA was rapidly converted to a nonhydrolyzable form; (b) [^{14}C]CoA was converted to ^{14}C-labeled compounds which are probably maleyl derivatives of CoA as described by Pacanis and co-workers [19,20] whose formation is catalyzed by succinyl-CoA : 3-keto-acid transferase, an enzyme present in significant quantities in heart mitochondria [21]; (c) in isolated liver mitochondria, maleate affected neither CoA levels nor CoA uptake (the lack of effect on CoA content being due to lack of the transferase) and (d) the effect of maleate was abolished by butyl malonate which inhibits maleate entry into mitochondria. Based on these results, it appears unlikely that exogenous CoA exchanges for intramitochondrial CoA since, if it did, sequestration of CoA with maleate would decrease influx rather than stimulate it. Mitochondrial carnitine exchange, for instance, is inhibited by depletion of intramitochondrial carnitine [24]. The results may also be interpreted as suggesting that CoA influx is subject to feedback regulation by free CoA. By converting free CoA to inert CoA derivatives, mitochondrial free CoA is decreased, thus feedback inhibition may be relieved and CoA uptake is stimulated. Alternatively, decreasing the mitochondrial CoA concentration may stimulate CoA influx by affecting the CoA concentration gradient.

Although the CoA transport process may be important for cellular homeostasis of CoA (see Introduction), supporting evidence is not yet available. An additional role for the CoA transport system in fatty acid

metabolism is proposed. It is known that activation of long chain fatty acids occurs in the cytosol of myocardial cells, the reactions are mediated by acyl CoA synthetase [25]. It is also known that one of the means by which the synthetase is regulated is the concentration of free cytosolic CoA such that when the cytosolic CoA decreases, fatty acid activation decreases leading to decreased fatty acid oxidation [26]. Myocardial cells contain approx. 14 μM CoA [6]. Based on the intracellular volume being 0.3 ml/g wet heart weight [27], 1 gram heart would contain approx. 50 nmol CoA in the cytosolic compartment. If it were assumed that 50% of the total cytosolic CoA was free CoA, uptake of as little as 0.1 nmol CoA/mg protein by mitochondria, which would translate to uptake of 5.5 nmol/g heart weight (based on 55 mg mitochondria/g wet heart weight) [6], would decrease the cytosolic CoA by approx. 20% within 1 min. Such a decrease in cytosolic CoA would significantly affect the ability of cells to activate fatty acids and mitochondrial transport of CoA could thereby regulate fatty acid oxidation. Our studies have indicated that mitochondria are capable of transporting as much as 4 nmol/mg per 30 min or 0.13 nmol/mg per min and that the K_m for CoA is 18 μM [5,7]. These calculations are based on time averaged values and the initial rates of uptake are even higher. Thus, mitochondrial CoA transport could be a viable means of regulating fatty acid oxidation. This hypothesis is currently being investigated.

In the absence of an electrical gradient, there is an large (20:1) outward concentration gradient of CoA. The effects of valinomycin or the combination of calcium and phosphate can be explained by the ability of these treatments to dissipate the membrane electrical gradient. Calcium alone is known to dissipate the electrical gradient in the absence of any additions over extended periods of time [28]. However, in the presence of phosphate, diamide or palmitoyl-CoA, calcium-induced dissipation of the membrane electrical gradient is virtually instantaneous [28]. Also, in the presence of phosphate and calcium, the mitochondrial membrane becomes leaky such that small molecules are released from the matrix. It is unclear whether the efflux of CoA observed in this study in the presence of Ca^{2+} and P_i was due to specific carrier-mediated efflux due to dissipation of the electrical gradient or whether it was due to nonspecific leakage. The effectiveness of succinate and pyruvate at preventing CoA efflux argue against a nonspecific leak.

Whatever the mechanism of $Ca^{2+} + P_i$-induced CoA release, these findings have significant pathophysiological implications. In hearts subjected to global ischemia, the concentration of inorganic phosphate can increase from 1 mM to as high as 20 mM, due to breakdown of high energy phosphates [29]. Further, if ischemic hearts are subjected to reperfusion with calcium-containing buffer, large quantities of calcium accumulate in the tissue in both, the cytosolic and mitochondrial compartments [30,31]. Although the experimental conditions used in this study are far less complex than those which occur in tissue ischemia, at least some of the manifestations of ischemia, i.e., elevated Ca^{2+} and P_i are represented in our experiments. The Ca^{+}-induced efflux observed in the presence of phosphate occurred at pathological concentrations suggesting that CoA efflux from mitochondria may occur during reperfusion following myocardial ischemia. Moreover, the efflux of mitochondrial CoA observed in this study is within the time frame of irreversible ischemic/reperfusion damage. Given the importance of CoA in cellular metabolism, an alteration in cellular CoA distribution could have detrimental effects on cell function. Whether or not CoA efflux from mitochondria occurs when hearts are subjected to ischemia is currently under investigation.

Acknowledgments

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