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Rapid Report

The effect of aging and acetyl-L-carnitine on the activity of the phosphate carrier and on the phospholipid composition in rat heart mitochondria

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The effect of aging and treatment with acetyl-L-carnitine on the activity of the phosphate carrier and on the phospholipid composition in rat heart mitochondria was studied. It was found that the activity of the phosphate carrier was reduced by aging. Treatment of aged rats with acetyl-L-carnitine reversed this effect. The mitochondrial level of cardiolipin was decreased with aging. Treatment of aged rats with acetyl-L-carnitine restored the level of cardiolipin to that of young rats. It is proposed that acetyl-L-carnitine may restore the correct phospholipid composition (cardiolipin level) of the mitochondrial membrane, altered by aging, thereby restoring the activity of the phosphate carrier.

Aging has a profound effect on cardiac performance. At mitochondrial level aging causes changes in biochemical pathways involved in the energy metabolism [1]. The transport of metabolites in heart mitochondria may represent a crucial step in the regulation of energy metabolism. Age-linked changes in the activity of several anion carrier systems present in heart mitochondria have been reported [2–5].

The transport of phosphate in mitochondria is mediated by a specific carrier [6]. This carrier protein has been isolated and purified and its activity reconstituted in liposomes [7–8]. These reconstitution experiments have shown that cardiolipin is essential for the activity of the phosphate carrier [9,10]. Compositional changes in the lipid of cardiac mitochondrial membranes may occur with aging [11]. The transport of phosphate in mitochondria may be important for several biochemical reactions involved in the energy metabolism, such as the synthesis of ATP during the oxidative phosphorylation and the uptake of essential Krebs-cycle intermediates [6]. Acetyl-L-carnitine is a natural biomolecule which acts by stimulating the energy metabolism [12–14], although its molecular mechanism of action is still not well known. However, many different effects of acute treatment of aged rats with this compound have been recently reported [15–17]. In this work, the effect of aging and acetyl-L-carnitine on the transport of phosphate and on the phospholipid composition in rat heart mitochondria was studied.

Male Fisher rats of 5 and 28 months were used throughout these studies. In each experiments, two rats of 5 months and two rats of 28 months were injected intraperitoneally with 300 mg/kg b.w. of acetyl-L-carnitine [15] and killed three hours after. Rat heart mitochondria were prepared as described previously [19]. The transport of phosphate in mitochondria was measured at 0°C essentially as described in Ref. 18. Mitochondrial osmotic volume changes were measured by the apparent absorbance changes at 540 nm, at 25°C. Phospholipids were analyzed by HPLC as described in Ref. 20. Protein concentration was measured by the usual biuret method.

Fig. 1 illustrates the results of four separate experiments on the time-course of phosphate transport in heart mitochondria from young, aged and aged plus acetyl-L-carnitine treated rats. The transport activity of the phosphate carrier was greatly reduced by aging. Treatment of aged rats with acetyl-L-carnitine restored

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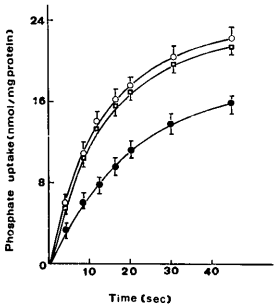

Fig. 1. Time-course of phosphate transport in heart mitochondria from young, aged, and aged plus acetyl-L-carnitine treated rats. Mitochondria (around 1 mg of protein/ml) were preincubated in a reaction medium containing in 1 ml: 100 mM sucrose, 50 mM KCl, 20 mM Tris-HCl, 0.5 mM EDTA, 1 mM *n*-butyl malonate and 2 μg/ml rotenone. Final pH 7.4. After 2 min of equilibration phosphate transport was initiated by adding radioactive phosphate and stopped after time *t* by rapid addition of 0.2 mM mersalyl. ○, Mitochondria from young rats; ●, mitochondria from aged rats; □, mitochondria from aged rats treated with acetyl-L-carnitine. Values are expressed as means ± S.E. for four separate experiments.

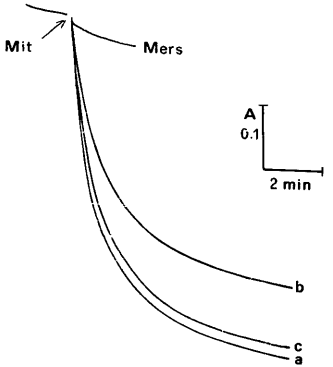

Fig. 2. Swelling of heart mitochondria from young, aged, and aged plus acetyl-L-carnitine treated rats in solution of ammonium phosphate. Mitochondria (0.5 mg of protein) were suspended in 3 ml solution of 125 mM NH_4PO_3, containing in addition 5 mM Hepes, 0.5 mM EDTA and 3 μg of rotenone. Final pH 7.4. When present, mersalyl was added at 0.2 mM concentration. The reaction was initiated by the addition of mitochondria to the reaction medium. The experiment shown is representative of four experiments which gave similar results. Trace a, mitochondria from young rats; trace b, mitochondria from aged rats; trace c, mitochondria from aged rats treated with acetyl-L-carnitine.

the activity of the phosphate carrier to the level of young rats. Acetyl-L-carnitine had no effect on the activity of the phosphate carrier in mitochondria isolated from young rats (results not shown).

The effect of aging and acetyl-L-carnitine on the transport of phosphate in rat heart mitochondria was also studied by swelling experiments. A typical experiment, reported in Fig. 2, shows that mitochondria from young and aged rats underwent large-amplitude swelling when suspended in isoosmotic solution of ammonium phosphate. Both the rate and the final extent of the swelling in ammonium phosphate were markedly decreased in mitochondria from aged rats as compared to the values obtained in mitochondria from young rats. Treatment of aged rats with acetyl-L-carnitine almost completely reversed this effect.

It has been reported that cardiolipin, a phospholipid located on the inner mitochondrial membrane, is required for the optimal functioning of the isolated phosphate carrier protein, reconstituted in liposomes [9,10]. Moreover, the transport of phosphate in rat heart mitochondria was found to be sensitive to doxorubicin, an antitumoral agent known to form specific complexes with cardiolipin molecules [21,22]. If this in vitro requirement of cardiolipin for the activity of the phosphate carrier reflects the in vivo situation, then changes in the cardiolipin level in the mitochondrial inner membrane, may affect the activity of this carrier protein. The results reported in Table I, show that the level of cardiolipin, which is reduced in aged rats, is brought back to the level of young rats by pretreatment of aged rats with acetyl-L-carnitine. Thus, the most obvious explanation of the effect of acetyl-L-carnitine on the activity of the phosphate carrier is the restora-

TABLE I

The effect of aging and acetyl-L-carnitine treatment on phospholipid composition in rat heart mitochondria as determined by HPLC

Each value represents the mean ± S.E. obtained for five separate experiments with two rats of each group. DPG = cardiolipin, PE = phosphatidylethanolamine; PI = phosphatidylinositol; PS = phosphatidylserine; PC = phosphatidylcholine. [a] $P < 0.01$ vs. young rats; [b] $P < 0.02$ vs. young rats.

Phospholipid	Distribution (mol%)			
	young	treated-young	aged	treated-aged
DPG	14.1 ± 1.1	15.2 ± 1.3	9.8 ± 1.0 [a]	14.6 ± 1.2
PE	35.0 ± 1.7	34.8 ± 1.5	32.4 ± 1.7	32.8 ± 1.8
PI	1.4 ± 0.4	1.6 ± 0.3	1.5 ± 0.3	1.8 ± 0.5
PS	2.4 ± 0.4	2.5 ± 0.5	3.1 ± 0.7	2.6 ± 0.5
PC	47.1 ± 1.5	45.9 ± 1.4	53.2 ± 1.6 [b]	48.2 ± 1.7

tion of the correct lipid microenvironment (cardiolipin level) of this carrier protein in the inner mitochondrial membrane, altered by aging. It should be noted that treatment of young rats with acetyl-L-carnitine had no effect either on the activity of the mitochondrial phosphate carrier or on the level of cardiolipin. This suggests that the observed effects of acetyl-L-carnitine are related to changes produced by aging.

Cardiolipin is known to be required for the optimal functioning of other carrier systems [23,24] and of membrane associated enzymes [25,26]. It is, therefore, possible that the observed effect of acetyl-L-carnitine on the activity of the phosphate carrier is not confined only to this transporting system. Experiments are underway to verify this possibility.

Aging is known to be associated with a decline in heart performance. The transport of phosphate in heart mitochondria may be involved in the regulation of the synthesis of ATP during the oxidative phosphorylation. Thus, the observed effects of acetyl-L-carnitine on the mitochondrial level of cardiolipin and thereby on the activity of the phosphate carrier (and probably of other cardiolipin-dependent anion transporters and enzymes) may lead to an improvement of heart performance in aged animals.

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