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INFLUENCE OF ADENOSINE AND NAGARSE ON PALMITOYL-CoA SYNTHETASE IN RAT HEART AND LIVER MITOCHONDRIA

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SUMMARY

1. A Q_{10} of about 3 for palmitoyl-CoA synthetase (EC 6.2.1.3) in rat heart and liver mitochondria is found.

2. In heart mitochondria Nagarse (EC 3.4.4.16) destroys the ability to activate palmitate. When, however, heart mitochondria are oxidizing palmitate, they are protected from the inactivating action of Nagarse.

3. Although treatment of liver mitochondria with Nagarse causes the loss of about 95 % of the palmitoyl-CoA synthetase activity, no influence is observed on palmitate oxidation.

4. Adenosine inhibits palmitoyl-CoA synthetase in liver and heart mitochondria. Adenosine is a competitive inhibitor with respect to ATP with an apparent K_i of 0.1 mM. The residual palmitoyl-CoA synthetase in Nagarse-treated liver mitochondria is much less sensitive to adenosine.

5. 2 mM adenosine or 2 mM adenosinesulfate inhibit palmitate oxidation (in the presence of 2.5 mM ATP) in heart mitochondria 60–90 %.

6. The data obtained are consistent with the concept of a palmitoyl-CoA synthetase localized on the outside of the outer membrane of rat heart and liver mitochondria, with an additional locus of (ATP-dependent) palmitoyl-CoA synthesis in the inner membrane matrix compartment of liver mitochondria.

INTRODUCTION

It was shown in a preliminary note[1] that adenosine strongly inhibited ATP-dependent palmitoyl-CoA synthetase (acid:CoA ligase (AMP), EC 6.2.1.3) activity in rat heart and liver mitochondrial sonicates. In the present communication further details are given about the specificity of this inhibition. Furthermore, the influence of adenosine on palmitate oxidation by mitochondrial preparations is shown.

DE JONG AND HÜLSMANN[2] and PANDE AND BLANCHAER[3] independently observed that Nagarse (subtilopeptidase A, EC 3.4.4.16), often used to isolated mitochondria from cardiac muscle, acted destructively on palmitoyl-CoA synthetase in heart mitochondria. The same was found in liver mitochondrial and heart and liver microsomal preparations[2]. The available evidence indicates that the highly active palmitoyl-CoA synthetase on the outside of the outer membrane of mitochondria is

destroyed[1,4]. Whether there is a separate ATP-dependent palmitoyl-CoA synthetase in the inner membrane matrix compartment, is still a matter of discussion. VAN TOL AND HÜLSMANN[4] and SKREDE AND BREMER[5] noticed a small, but distinct, ATP-dependent long-chain fatty acid activation in the inner membrane matrix fraction of rat liver mitochondria (see also VAN DEN BERGH *et al.*[6] and LIPPEL AND BEATTIE[7]). However, in a very recent paper AAS[8] did not find conclusive evidence for activation of palmitate in the inner membrane or matrix fraction of liver mitochondria. ALLMANN *et al.*[9] performed localisation studies with beef heart mitochondria (but see ref. 10).

In this paper some experiments are presented which are in agreement with the concept of an ATP-dependent palmitoyl-CoA synthetase situated on the outside of the outer membrane of rat heart and liver mitochondria, with an additional locus of (ATP-dependent) palmitoyl-CoA synthetase in the inner membrane matrix compartment of liver mitochondria. The kinetic properties of the two differently localized enzymes in liver mitochondria provide further indirect support of two separate palmitoyl-CoA synthetases.

EXPERIMENTAL

Reagents

Nagarse was furnished by Serva Entwicklungslabor, Heidelberg. Adenosine (puriss.) and other nucleosides were purchased from Koch–Light Lab., Colnbrook, Bucks. Adenosinesulfate, 2′-AMP and 3′-AMP were obtained from Schuchardt, Munich. Cyclic 2′,3′-AMP was purchased from Sigma Chemical Co., St. Louis, Mo., whereas other nucleotides and enzymes were from C. F. Boehringer und Söhne, Mannheim. Coenzyme A and sodium pyruvate (Boehringer) were solved just before use. Bovine serum albumin (from Pentex Inc., Kankakee, Ill.) was defatted by charcoal treatment[11] and dialyzed. [1-^{14}C]Palmitic acid was supplied by The Radiochemical Centre, Amersham, diluted with potassium palmitate and complexed to albumin in a 7:1 molar ratio as described before[2]. In the same reference the source of DL-[*Me*-^{3}H]carnitine is acknowledged.

Preparations

Rat heart and liver mitochondria were isolated as described before[2]. For the experiments shown in Table IV and Figs. 4 and 5 the heart mitochondria were not subjected to the 12000 × *g* centrifugation step. Sonicates of the mitochondrial fractions were prepared at 0–5° with a MSE 100-W Ultrasonic Disintegrator (Measuring and Scientific Equipment Ltd., London), operated with a microtip at 21 kcycles/sec (amplitude 6.5 μm, peak to peak) for 60 sec/ml of suspension, at least when the mitochondrial protein concentration was below 20 mg/ml. Otherwise the sonication time was doubled.

Carnitine palmitoyltransferase (palmitoyl-CoA:carnitine *O*-palmitoyltransferase, EC 2.3.1.—) was purified from calf liver mitochondria as described by FARSTAD *et al.*[12].

Methods

Palmitoyl-CoA synthetase activity was assayed either as the formation of palmitoylhydroxamate according to PANDE AND MEAD[13,14] or as the synthesis of [^{3}H]-

palmitoylcarnitine in the presence of [^{3}H]carnitine and carnitine palmitoyltransferase. The latter method was described by FARSTAD *et al.*[12], and modified in our laboratory[2,15].

Oxygen uptake was determined in a vessel equipped with a Clark oxygen electrode (Yellow Springs Instruments Co., Yellow Springs, Ohio) and a Micrograph BD5 recorder (Kipp en Zonen, Delft) or manometrically with a differential respirometer (Gilson Medical Electronics, Villiers-le-Bel, France). Calibration of the electrode was performed with NADH and catalase (*cf.* ROBINSON AND COOPER[16]).

Protein was measured by the biuret method as described by JACOBS *et al.*[17].

Radioactivity was estimated in a Nuclear-Chicago 720 liquid scintillation counter. $^{14}CO_2$ was counted with an efficiency of about 65 % in 10 ml of the mixture of toluene, Triton X-100 and ethanol (containing PPO and POPOP) described by PATTERSON AND GREENE[18].

RESULTS AND DISCUSSION

Kinetics of the inhibition of palmitoyl-CoA synthetase by adenosine

Fig. 1 shows the inhibition of palmitoyl-CoA synthetase by various concentrations of adenosine with isolated heart mitochondria. With these mitochondria a normal Dixon plot (concentration of inhibitor *versus* the reciproke value of the activity at two ATP concentrations, *cf.* ref. 19) is obtained. The K_i for adenosine is 0.1 mM (two separate experiments). The inhibition is competitive with respect to ATP.

In preparations of liver mitochondria the Dixon plot obtained for the inhibition of palmitoyl-CoA synthetase by adenosine does not give one straight line (Fig. 2). In this case a K_i of 0.1 mM (two experiments) is found when the concentration of inhibitor is less than about 1 mM (see Fig. 2, insert). This value was reported in the preliminary note[1] and confirmed by VAN TOL AND HÜLSMANN[4], who used a different assay. The inhibition is of the competitive type with respect to ATP. A second, much higher K_i can be found by extrapolation of the values in the Dixon plot obtained for

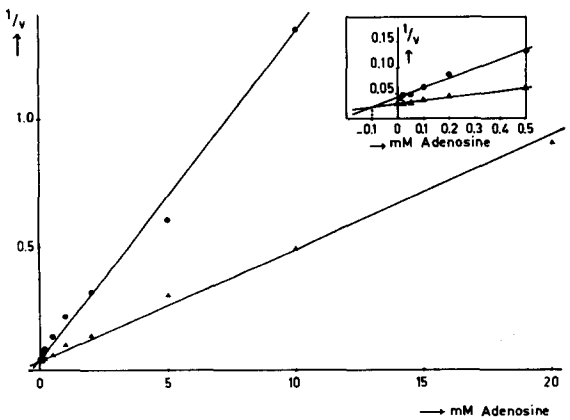


Fig. 1. The Dixon plot of inhibition by adenosine of palmitoyl-CoA synthetase in sonicated rat heart mitochondria. Palmitoyl-CoA synthetase was assayed with hydroxylamine according to PANDE AND MEAD[13,14]. The concentration of ATP was 4 mM (●—●) or 15 mM (▲—▲). 2 mM palmitate and 1.2 mM CoASH were present initially. The incubation was carried out with 0.54 mg mitochondrial protein for 30 min at 37°. V = munits/mg protein.

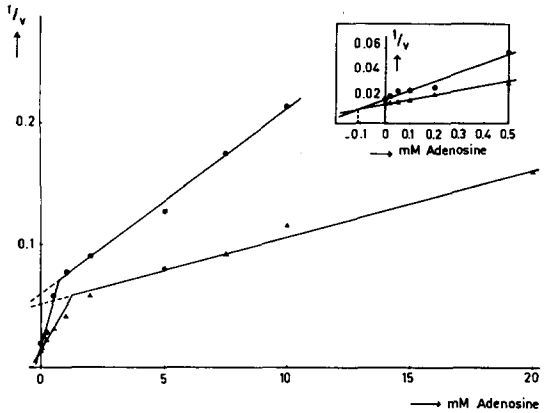


Fig. 2. The Dixon plot of inhibition of palmitoyl-CoA synthetase in sonicated rat liver mitochondria. In the assay (hydroxylamine method[13]) 0.38–0.70 mg mitochondrial protein was used. The concentration of ATP was 4 mM (●—●) or 15 mM (▲—▲). Further conditions as indicated in the legend to Fig. 1.

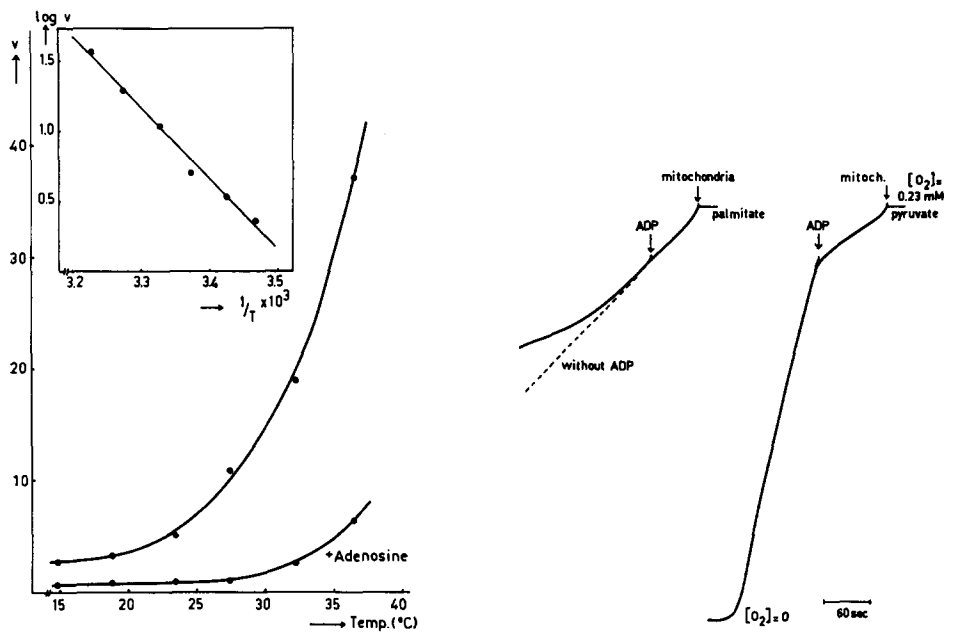


Fig. 3. Temperature dependence of palmitoyl-CoA synthetase in a heart mitochondrial sonicate. Palmitate activation was determined with the hydroxylamine method[13,14] in the presence of 2 mM potassium palmitate, 15 mM ATP, 1.2 mM CoASH and 0.26–1.0 mg mitochondrial protein. The incubation time was 30–60 min. Where indicated, the assay was carried out in the presence of 2.0 mM adenosine. The insert shows the Arrhenius plot (reciproke value of absolute temperature *versus* log (munits/mg protein)) for palmitoyl-CoA synthetase without inhibitor.

Fig. 4. Inhibition of palmitate oxidation in rat heart mitochondria by ADP. For the isolation of mitochondria see *Preparations*. Oxygen uptake was measured with a Clark oxygen electrode. The incubation medium contained 12.5 mM potassium phosphate, 50 mM KCl, 1.0 mM ATP, 2.5 mM $MgCl_2$, 0.02 mM CoASH, 0.2 mM GSH, 80 mM Tris–HCl, 4.0 mM EDTA, 0.05 mM cytochrome *c*, 0.25 mM L-malate, 0.062 mM palmitate/0.01 mM albumin and 1.0 mM L-carnitine. In the control 7.5 mM sodium pyruvate was substituted for palmitate + carnitine. Where indicated 0.98 mg mitochondrial protein (+ 25 μmoles sucrose) and 10 μmoles ADP were added. The final volume was 2.0 ml, the pH 7.4 and the temperature 30.0°.

adenosine concentrations higher than 1 mM. In liver mitochondria a palmitoyl-CoA synthetase with high activity is located on the outside of the mitochondrion[1,4]. This activity is lost on treatment of the liver mitochondria with Nagarse[1,2,4]. For Nagarse-treated liver mitochondria a K_i value of 0.2 mM has been reported[4]. When the hydroxamate assay is used instead of the carnitine assay (see *Methods*), K_i values of 2–10 mM are found. Also in this case the inhibition is competitive with respect to ATP[1,4].

When the inhibition of palmitoyl-CoA synthetase by adenosine was investigated at several temperatures a very high Q_{10} for the activation of fatty acid was found. In the literature no experiments were found concerning the temperature dependence of palmitoyl-CoA synthetase activity. Fig. 3 shows the activity of palmitoyl-CoA synthetase at different temperatures in the presence or absence of 2.0 mM adenosine

TABLE I

TEMPERATURE DEPENDENCE OF PALMITATE ACTIVATION IN HEART MITOCHONDRIAL SONICATES

Mitochondrial fractions were prepared as described before[2]. Palmitoyl-CoA synthetase was determined either with hydroxylamine[13] or with [^{3}H]carnitine[12], as modified in ref. 15. The amounts of mitochondrial protein and the incubation time for the former are mentioned in the legend to Fig. 3. In the carnitine assay 0.2–0.4 mg enzymatic protein, 1.0 mM palmitate/albumin, 2.0 mM ATP and 0.2 mM CoASH were used. The incubation time was 6 min in this case. The results were arranged in an Arrhenius plot (*cf.* Fig. 3), from which Q_{10} and energy of activation were calculated.

	Energy of activation (*kcal·mole*$^{-1}$)	Q_{10} *20–30°*	*30–40°*
Hydroxylamine assay			
Expt. I	23.6	3.98	3.64
Expt. II	19.5	3.13	2.90
[^{3}H]*Carnitine assay*			
Expt. I	18.0	2.87	2.68
Expt. II	19.3	3.03	2.88

TABLE II

INHIBITION OF PALMITOYL-CoA SYNTHETASE IN SONICATED LIVER AND HEART MITOCHONDRIA BY NUCLEOSIDES

Mitochondrial fractions were prepared as described before[2]. The hydroxylamine assay was carried out at 30° as given in the legends to Figs. 1 and 2 with 15 mM ATP and 2.0 mM nucleoside. Average values were calculated for 3–6 experiments. The specific activity of the enzyme without inhibitor was 31.3 munits/mg protein (liver) and 14.6 munits/mg protein (heart).

Nucleoside + NH_2 *group*	*Inhibition* (%)		*Nucleoside* − NH_2 *group*	*Inhibition* (%)	
	Liver	*Heart*		*Liver*	*Heart*
Adenosine	79.2	82.1	Inosine	0.2	3.3
Deoxyadenosine	25.1	27.0			
Guanosine	18.2	1.6	Xanthosine	7.1	8.9
Cytidine	24.4	4.9	Uridine	9.4	0.0
			Thymidine	10.9	8.4

in a heart mitochondrial sonicate. From the Arrhenius plot [reciproke value of absolute temperature *versus* log(activity), *cf.* ref. 19] a Q_{10} of about 3 could be calculated (Fig. 3, insert). Table I shows the data obtained with different preparations using two different assay systems to estimate long-chain fatty acid activation. This Table also gives the energy of activation for palmitoyl-CoA synthetase as calculated from the Arrhenius plots. Similar results were obtained with liver mitochondria (not shown).

The specificity of adenosine as an inhibitor of palmitoyl-CoA synthetase

In a preliminary note[1] the inhibition of palmitoyl-CoA synthetase in heart mitochondria by adenosine and some analogs was shown. Table II shows the activity of the enzyme in sonicates of liver and heart mitochondria in the presence of several nucleosides. From this Table it can be concluded that the inhibition by adenosine is somewhat more specific in heart than in liver. In liver mitochondria the inhibition of the enzyme by the analogs tested seems to be dependent on the presence of an aminogroup.

PANDE AND MEAD[13] reported the inhibition of palmitoyl-CoA synthetase in rat liver microsomes by AMP (and to a smaller extent by ADP and cyclic AMP). They mentioned an AMP inhibition of palmitate activation by the mitochondrial fraction without giving details. In Table III the inhibition of palmitoyl-CoA synthetase in heart and liver mitochondrial sonicates by a variety of phosphate esters of adenosine is shown. As can be seen from Table III the inhibition by adenosine is several times greater than that obtained by the nucleotides. Adenosinesulfate is as effective an inhibitor as adenosine. This compound could be useful in the study of the regulation of palmitate catabolism.

TABLE III

INHIBITION OF PALMITOYL-CoA SYNTHETASE IN SONICATED LIVER AND HEART MITOCHONDRIA BY ADENOSINE, ADENOSINEPHOSPHATE ESTERS AND ADENOSINESULFATE

Conditions were similar to those described in the legend to Table II.

Inhibitor (*2.0 mM*)	*Inhibition* (%)	
	Liver	*Heart*
Adenosine	79.2	82.1
5′-ADP	10.5	8.8
Cyclic 3′,5′-AMP	12.4	13.5
Cyclic 2′,3′-AMP	8.6	7.7
5′-AMP	22.8	24.2
3′-AMP	19.3	7.0
2′-AMP	9.4	8.6
Adenosine sulfate	78.4	83.0

Only adenosine and adenosinesulfate are able to inhibit oxygen consumption and CO_2 production clearly when palmitate is oxidized by heart or liver mitochondria. Details are given in the next paragraph.

Effects of adenosine and Nagarse on mitochondrial palmitate oxidation

In previous experiments conducted with the Clark oxygen electrode with rat

heart mitochondria it was often noted that ADP inhibited palmitate oxidation. A typical tracing is shown in Fig. 4. We expected to find a stimulation by ADP, like we observed with substrates like palmitoylcarnitine and pyruvate (see Fig. 4), because of the transition of the mitochondria from State 4 to State 3. WARSHAW AND TERRY[20] showed (in their Fig. 5) that palmitate oxidation in calf heart mitochondria was impaired by ADP addition, without discussing the effect.

Addition of AMP or adenosine, breakdown products of ADP, also inhibited palmitate oxidation. This led us to think that adenosine as it could be generated from ADP or AMP, might be responsible for the inhibition. The effects, however, were quite variable in the short-lasting experiments with the oxygen electrode. Therefore, we

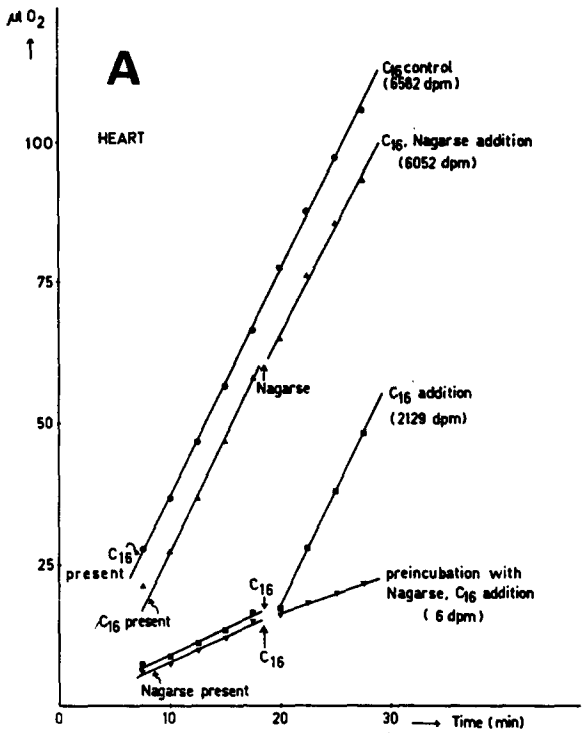
TABLE IV

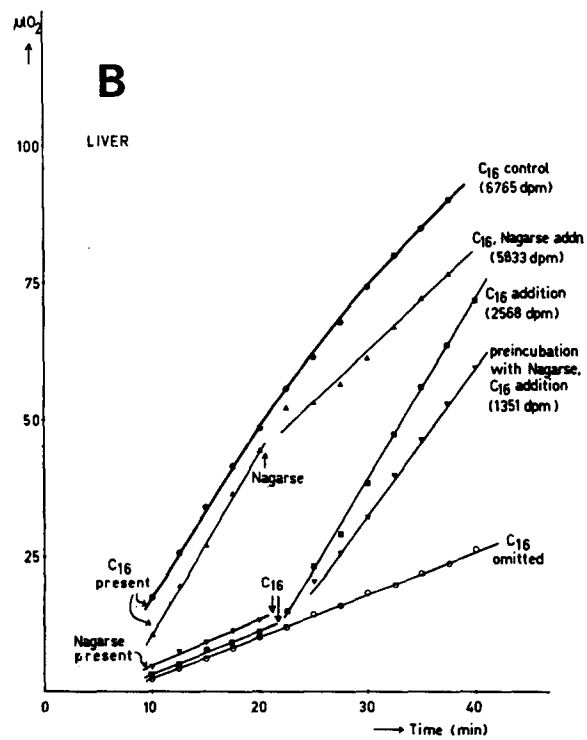
INFLUENCE OF ADENOSINE AND SOME ANALOGS ON PALMITATE OXIDATION IN HEART MITOCHONDRIA

Heart mitochondria were isolated as described under *Preparations*. Oxygen uptake was measured with a differential respirometer at 25.0°. Reaction vessels contained 0.25 mM [1-^{14}C]palmitate (0.040 μC/μmole)/0.04 mM albumin, 1.0 mM L-malate, 2.5 mM ATP, 2.5 mM L-carnitine, 0.1 mM CoASH, 10 mM potassium phosphate (pH 7.4), 5.0 mM $MgCl_2$, 30 mM KCl, 1.1 mM EDTA, 75 mM Tris–HCl buffer (pH 7.4), about 2.8 mg mitochondrial protein and 25 mM sucrose. Adenosine and analogs were tested in a final concentration of 2.0 mM. The reaction volume was 1.0 ml. All constituents were added from stock solutions, if necessary neutralized with KOH. The centre well of the vessels was provided with 0.05 ml 2.5 N KOH and a filter paper. The side arm of the flasks contained 0.05 ml 70% $HClO_4$. The reaction was started with the mitochondria. Readings were taken at 2.5 min intervals. After 30 min the reaction was terminated by addition of $HClO_4$. Shaking was continued for 30–60 min. The KOH-soaked paper in the centre well was transferred with 10 ml scintillation mixture to a counting vial. For details of the measurement of $^{14}CO_2$ see *Methods*. Total O_2 consumption was calculated by extrapolation.

Condition	*Total O_2 uptake (Σ μl/mg protein)*		*$^{14}CO_2$ production (disint./min per mg protein)*	
	Expt. I	*Expt. II*	*Expt. I*	*Expt. II*
Palmitate/albumin omitted	10	10	—	—
Palmitate albumin	46	49	2438	2548
+ adenosine	20	23	523	721
+ deoxyadenosine	—	46	—	2535
+ inosine	42	49	2362	2466
+ guanosine	41	—	2150	—
+ xanthosine	44	—	2328	—
+ 5′-AMP	47	—	2871	—
+ 5′-IMP	42	—	2195	—
+ adenosinesulfate	18	21	441	601
+ adenine	42	—	2160	—
+ hypoxanthine	—	46	—	2475

Fig. 5. The effect of Nagarse on [1-^{14}C]palmitate oxidation in heart mitochondria (A) and liver mitochondria (B). (A) 2.7 mg heart mitochondrial protein was present; further conditions were as indicated in the legend to Table IV. In experiment (B) 3.3 mg liver mitochondrial protein was used. The palmitate oxidation was stimulated in this case by the addition of 25 mM D-glucose and 3 I.U. dialyzed hexokinase (EC 2.7.1.1) (*cf.* ref. 1). 25 mM potassium phosphate (pH 7.4), 20 mM KCl, 60 mM Tris–HCl buffer (pH 7.4) were included in the medium. The reaction was started by the addition of mitochondria. Other conditions for liver were identical to those given in the legend to Table IV. Where indicated in (A) and (B) Nagarse (0.5 mg) or [1-^{14}C]palmitate were present from the start or added after preincubation. The reactions were stopped after 30 min (heart) or 40 min (liver) and $^{14}CO_2$ was counted (see Table IV). The amounts of $^{14}CO_2$ present at the end of the experiment are indicated in (A) and (B). dpm = disint./min.


μl O2
A
HEART
100
75
50
25
C16 control (6582 dpm)
C16, Nagarse addition (6052 dpm)
Nagarse
C16 addition (2129 dpm)
C16 present
C16 present
C16
preincubation with Nagarse, C16 addition (6 dpm)
C16
Nagarse present
0
10
20
30
Time (min)


μO2
B
LIVER
100
75
50
25
C16 control (6765 dpm)
C16, Nagarse addn (5833 dpm)
C16 addition (2568 dpm)
preincubation with Nagarse, C16 addition (1351 dpm)
Nagarse
C16 omitted
C16 present
C16
Nagarse present
0
10
20
30
40
Time (min)

turned to manometry, which enabled us to use radioactive labelled palmitate to differentiate between endogenous and added substrate. Table IV shows the oxidation of [1-^{14}C]palmitate by heart mitochondria. In the medium 0.1 mM coenzyme A is included, because it is found that coenzyme A is easily washed out from the mitochondria. Leakage of coenzyme A from rat liver mitochondria is demonstrated by SKREDE AND BREMER[5]. Several compounds are tried for their ability to inhibit palmitate oxidation. When tested in a concentration of 2.0 mM only adenosine or adenosinesulfate are inhibitory to a significant extent. The same compounds are powerful inhibitors of palmitoyl-CoA synthetase (Tables II and III). The discrepancy of oxygen uptake and $^{14}CO_2$ production as far as the degree of inhibition is concerned, is probably due to the oxidation of endogenous substrates (mainly fatty acids). For instance lauroyl-CoA synthetase (personal communication of H. R. SCHOLTE) and octanoyl-CoA synthetase (unpublished observation) are relatively insensitive to the inhibitors. When instead of heart mitochondria liver mitochondria were tested the inhibition of palmitate oxidation was found to be less. In those experiments (not shown) adenosine and adenosinesulfate inhibited oxygen uptake and $^{14}CO_2$ production only half as strong. In those experiments the conditions used were not the same. In the experiments with heart mitochondria phosphate acceptor was generated, from the 2.5 mM ATP present, by Mg^{2+}-stimulated ATPase. In the experiments with liver mitochondria phosphate acceptor was generated by the addition of hexokinase and glucose. The conditions used for palmitate oxidation by liver mitochondria are given in the legend to Fig. 5. It should be emphasized that in this case the ATP concentration in the vessels is very low, contrary to the experiments conducted with heart mitochondria. When it is kept in mind that the inhibition by adenosine of palmitoyl-CoA synthetase is competitive with respect to ATP (Figs. 1 and 2), one could argue that cardiac palmitate oxidation is even more sensitive to adenosine than hepatic palmitate oxidation. It is quite difficult to oxidize palmitate under identical conditions as was shown before[1].

In an earlier publication it was demonstrated that in mitochondria isolated from homogenates of liver and heart treated with Nagarse palmitoyl-CoA synthetase activity was almost completely lost[2]. Palmitate oxidation was severely impaired in the case of Nagarse-treated heart mitochondria as was shown by DE JONG AND HÜLSMANN[1]. The effect of Nagarse on isolated mitochondria was studied in greater detail. Addition of quite large amounts of Nagarse to heart mitochondria oxidizing palmitate, failed to produce the expected inhibition. If, however, the fatty acid is added after Nagarse no palmitate can be oxidized (Fig. 5). Apparently Nagarse is not able to destroy the palmitoyl-CoA synthetase in heart mitochondria when conditions are favorable for fatty acid activation, suggesting a change in conformation. This was confirmed by experiments (not shown) in which sonicates were preincubated with Nagarse in the presence of palmitate, ATP and coenzyme A. Palmitoyl-CoA synthetase was found to be quite insensitive to the Nagarse action under this condition. Preincubation with either palmitate, ATP or coenzyme A was much less effective to protect the enzyme. Also palmitoyl-CoA (0.2 μM) was not able to protect the palmitoyl-CoA synthetase from Nagarse digestion. Under the conditions specified in Fig. 5, palmitate oxidation by liver mitochondria is much less sensitive to preincubation with Nagarse. The palmitoyl-CoA synthetase present in the outer membrane, is destroyed, but the inner membrane enzyme (insensitive to Nagarse treatment[4]), is able to provide adequate amounts of palmitoyl-CoA[1].

CONCLUSIONS

The effects of Nagarse, hexokinase, adenosine and carnitine on palmitate activation and oxidation in liver and heart mitochondria are summarized in Table V. We conclude that in the oxidation experiments with liver mitochondria, as shown in this communication and in the preliminary note[1], palmitate is activated mainly in the inner membrane matrix compartment. BEATTIE[21] found that 95 % of the total palmitate oxidation activity (measured as formazan formed from tetrazolium salt) of rat liver mitochondria was recovered in the inner membrane matrix fraction, as obtained by digitonin treatment. LIPPEL AND BEATTIE[7] concluded from this experiment that palmitoyl-CoA synthetase was localized in the inner membrane fraction. This conclusion does not seem justified since the preparation contained approx. 11 % outer membrane[21], which has a very high palmitoyl-CoA synthetase activity[1,4-6,8,22]. VAN TOL AND HÜLSMANN[4] showed that during hepatic fatty acid oxidation at low concentrations of palmitate (*i.e.* below 40 μM), with carnitine present, the outer membrane acyl-CoA synthetase is operative.

TABLE V

SUMMARY OF THE EFFECTS OF NAGARSE, HEXOKINASE, ADENOSINE AND CARNITINE ON LIVER AND HEART MITOCHONDRIAL PALMITATE ACTIVATION AND OXIDATION

The results were compiled from experiments shown in Figs. 1, 2 and 5, Table IV and the preliminary note[1] (Table I).

	Palmitate oxidation		*Palmitate activation*		
	Liver	*Heart*	*Liver*		*Heart*
			Inner membrane matrix	*Outer membrane*	
Nagarse	No effect	Inhibition	No effect	Inhibition	Inhibition
Hexokinase	Stimulation	Inhibition	No effect	Inhibition	Inhibition
Adenosine	Small inhibition	Inhibition	Small inhibition	Inhibition	Inhibition
Carnitine	No effect	Obligate	—	—	—

In heart mitochondria we find only evidence for a palmitoyl-CoA synthetase located on the outside of the outer membrane (see also the preliminary note[1]).

Adenosine is a potent vasodilator as has been shown a long time ago by DRURY AND SZENT-GYÖRGYI[23] (*cf.* also ref. 24). Many workers reported the formation of adenosine in the heart by breakdown of adenine nucleotides under hypoxic conditions (see, for instance, refs. 25 and 26). KATORI AND BERNE[27] emphasized the importance of adenosine as the possible metabolite responsible for autoregulation of coronary blood flow. It is possible that adenosine reduces mitochondrial palmitoyl-CoA formation, when the cardiac oxygen level is low, so that adenosine not only functions as a regulator of blood flow contributing to oxygenation and lactate removal, but also as a metabolic inhibitor.

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