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Effects of hypoxia and fatty acids on the distribution of metabolites in rat heart

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The effects of exogenous fatty acids and hypoxia on cardiac energy metabolism were studied by measuring mitochondrial and cytosolic adenine nucleotides as well as CoA and carnitine esters using a tissue fractionation technique in non-aqueous solvents. During normoxia, the administration of 0.5 mM palmitate caused a considerable increase in acyl-CoA and acylcarnitine, particularly in mitochondria. High-energy phosphates, however, were only slightly altered. A 90 min low-flow hypoxia caused a dramatic increase in mitochondrial acyl esters. The mitochondrial ATP content decreased significantly, while the cytosolic concentration was only slightly diminished, suggesting an inhibition of mitochondrial adenine nucleotide translocation by long-chain acyl-CoA. Addition of palmitate during hypoxia amplified hypoxic damage and reduced adenine nucleotides in both compartments considerably, while fatty acid metabolites were only slightly affected. In presence of an inhibitor of fatty acid oxidation (BM 42.304), the fatty-acid-induced acceleration of cardiac injury was prevented. Since BM 42.304 decreased mitochondrial acylcarnitine and increased the cytosolic concentration significantly, BM 42.304 was presumed to inhibit mitochondrial acylcarnitine translocase. However, a causal relationship between lipid metabolites and ischemic damage seemed unlikely.

Introduction

In ischemic myocardium, release of non-esterified fatty acids from endogenous lipid stores is known to be enhanced, while fatty acid degradation is decreased [1,2]. This is associated with an accumulation of acyl-CoA and acylcarnitine within the cell [3,4]. High levels of these metabolites have been shown to induce inhibitory effects in several enzyme systems; fatty acids are potent uncouplers of oxidative phosphorylation and can influence cellular membrane stability [5,6]. Long-chain acyl-CoA has been found to inhibit adenine nucleotide translocase [7,8], acyl-CoA synthetase activity [9] as well as mitochondrial oxidative phosphorylation [10]. Long-chain acylcarnitine has been reported to influence mitochondrial function [11].

Additionally, mechanisms other than accumulation of detrimental metabolites have been discussed as the cause of fatty-acid-induced injury. Considering that fatty acid oxidation by cardiac muscle is associated with a higher expenditure of oxygen compared to carbohydrate oxidation, acceleration of myocardial damage in hypoxia might be due to this additional O_2 consumption [12]. However, there is no conclusive evidence as to what extent and by which mechanism exogenous fatty acid supply contributes to the deleterious effects in oxygen-deficient hearts.

In order to evaluate alterations in metabolic pathways due to exogenous fatty acids and hypoxia, exact knowledge of subcellular distribution of the respective metabolites is necessary. Therefore we used a fractionation of heart tissue in non-aqueous solvents for the determination of mitochondrial and cytosolic contents of high-energy phosphates and acyl esters. This method avoids enzymatic alterations, loss or redistribution of the investigated metabolites during the fractionation procedure [13].

Abbreviations: ANT, adenine nucleotide translocase; BM 42.304, 2-(3-methylcinnamylhydrazono)propionate; Cr, creatine; CP, creatine phosphate; Hepes, *N*-2′-hydroxyethylpiperazineethane sulfonic acid; POCA, sodium 2-(5-(4-chlorophenyl)pentyl)oxirane 2-carboxylate; TDGA, 2-tetradecylglycidic acid.

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It is the purpose of the present study to investigate metabolic alterations caused by hypoxia and the supply of exogenous fatty acids in the mitochondrial and cytosolic compartment. Additionally, the effects of a pharmacologically induced inhibition of fatty acid transport into mitochondria on these changes and on myocardial ischemic tolerance were evaluated. The energy status of the hearts was estimated from the distribution of high-energy phosphates. CoA, carnitine and their long-chain acyl esters were determined as components of fatty acid metabolism.

Methods

Heart perfusion

All tests were performed in hearts from 200–250 g male Wistar rats. After diethyl ether anaesthesia of the animal the thorax was opened and ice-cold NaCl-solution (0.9%) was poured on the heart, which was then rapidly excised and mounted on the cannula of a perfusion apparatus. Hearts were prepared as working hearts. The static pressure afterload was 60 mmHg and the left atrial pressure was maintained at 10 mmHg. All perfusions were performed using a modified Krebs-Henseleit solution containing 135 mM NaCl, 5.9 mM KCl, 1.9 mM $CaCl_2$, 1.2 mM NaH_2PO_4, 1.2 mM Na_2SO_4, 5 mM Hepes, 5 mM glucose, 1 mM sodium lactate and 0.2 mM DL-carnitine. The perfusate was equilibrated with 100% O_2 for normoxic perfusions and 5% O_2/95% N_2 for hypoxic perfusions using a membrane oxygenator. Complete details on the perfusion device have been previously published [14].

For the metabolite determination, five groups of hearts were subjected to different perfusion conditions:
(1) normoxic perfusion without exogenous fatty acids for 15 min;
(2) normoxic perfusion with 0.5 mM exogenous palmitate for 15 min;
(3) hypoxic perfusion without exogenous fatty acids for 90 min;
(4) hypoxic perfusion with 0.5 mM exogenous palmitate;
(5) hypoxic perfusion with palmitate and 10 μM BM 42.304.

For the addition of palmitate a stock solution of fatty acid-albumin complex containing 10 mM palmitate bound to 2 mM fatty-acid-free bovine serum albumin was prepared according to the method of Soltys and Hsia [15]. This solution was continuously added to the perfusate prior to its entry into the left atrium via a small tube by a syringe pump. Hypoxia was performed by perfusing the hearts with reduced pO_2 of 35 mmHg and restricted flow of 2 ml/min in a retrograde manner. The dynamic performance of the heart was monitored by continuous measuring of left ventricular pressure curve, coronary pO_2, coronary flow and cardiac output. All measured data were fed continuously into a PDP11 computer via an AD converter. From the left ventricular pressure curve heart rate, maximum ventricular pressure (P_{max}) and dP/dt_{max} were calculated. O_2 consumption was computed by coronary flow and arteriovenous pO_2 difference. For evaluation of glycolytic energy production, samples from coronary effluent were collected every 15 min for measurement of lactate, which was determined using an enzymatic assay [16].

The perfusions were terminated by freeze-clamping the hearts between aluminium clamps precooled in liquid nitrogen. The frozen tissue was crushed in a mortar containing liquid nitrogen and then freeze-dried at 2 Pa and −50 °C. The lyophilized heart tissue was stored at −70 °C until use.

Fractionation of heart tissue

The freeze-dried tissue of one heart (approx. 0.1 g) was sonicated with highest intensity for 4 min at 5-s intervals (Sonicator W 220 F, Heat Systems Ultrasonics, Plainview, NY, U.S.A.) in 8 ml of a mixture of heptane/carbon tetrachloride ($d = 1.23$ kg/l). This suspension was layered on top of an exponential density gradient ($d = 1.29$–1.35 kg/l) and centrifuged for 3 h at $10\,180 \times g$ (Beckman Centrifuge J-21C with JS-13 swingout rotor, Beckman Instruments, Fullerton, CA, U.S.A.). The preparation of the density gradient is described in more detail by Soboll et al. [13]. The gradient yielded eight fractions, each containing different proportions of mitochondrial and extramitochondrial protein. Each fraction was divided into two aliquots, collected in Eppendorf cups and dried at 14 kPa in a desiccator. In one aliquot the activities of the mitochondrial marker enzyme citrate synthase and of the extramitochondrial marker enzyme phosphoglycerate kinase as well as the protein content were determined [16–18]. In the second aliquot the contents of the high-energy phosphates ATP, ADP, AMP and creatine phosphate (CP), as well as coenzyme A, carnitine, and after alkaline hydrolyses their long-chain acyl derivatives were measured enzymatically [16].

Based on the distribution of the marker enzymes, mitochondrial and cytosolic metabolite contents were calculated by extrapolation to pure mitochondria and cytosol. In order to minimize the statistical errors of enzyme and metabolite distribution for each particular gradient, an average theoretical gradient was calculated from the data of 6–8 single gradients and then extrapolated to pure mitochondria and cytosol [13]. The metabolite contents were converted into concentrations assuming 0.99 μl H_2O/mg mitochondrial protein and 3.5 μl H_2O/mg cytosolic protein, respectively [19,20].

Solubility of palmitoyl-CoA and palmitoyl carnitine

For the fractionation in non-aqueous solvents it is of critical importance that the metabolites to be determined

in the fractions are not dissolved in heptane or CCl_4. Thus, the solubility of long-chain fatty acid esters in non-polar solvents was determined using labelled substances. 2 μl (3.7 kBq) [^{14}C]palmitoyl-CoA and 2 μl (7.4 kBq) [^{14}C]palmitoyl carnitine chloride were sonicated together with 10 mg of lyophilized heart tissue in 1 ml of a mixture of heptane/carbon tetrachloride ($d = 1.23$ kg/l). After centrifugation of the suspension at 15000 $\times g$ the radioactivity of the supernatant and of the pellet, dissolved in H_2O and KOH, was counted (DW 4700 Philips, Kassel, F.R.G.) in 10 ml of Supersolve (Zinsser, Frankfurt, F.R.G.). The results yielded a solubility of 4% for palmitoyl-CoA and of 9% for palmitoylcarnitine in heptane/carbon tetrachloride.

Materials

[^{14}C]Palmitoyl-CoA and [^{14}C]palmitoylcarnitine chloride were obtained from Amersham-Buchler, Braunschweig, F.R.G. and New England Nuclear, Dreieich, F.R.G. Palmitic acid, fatty-acid-free bovine serum albumin, DL-carnitine and Hepes were products of Sigma, Taufkirchen, F.R.G. All other chemicals and solvents of reagent grade were purchased from Merck, Darmstadt, F.R.G.; all enzymes and coenzymes used in the metabolite determinations from Boehringer-Mannheim, F.R.G. For inhibition of fatty acid transport into mitochondria the compound 2-(3-methylcinnamylhydrazono)propionate (BM 42.304) was used [21], which was a gift from Boehringer-Mannheim.

Statistical analysis

All data are given as mean ± S.E. of n independent experiments. Groups of data were compared by use of the Student's t-test. Differences with $P < 0.05$ were regarded as significant.

Results

Haemodynamic parameters

During the aerobic perfusion hearts showed an O_2 consumption of 7.14 ± 0.23 μmol/min per g wet wt., dp/dt_{max} of 3246 ± 287 mmHg/s, maximum ventricular pressure of 95 ± 8 mmHg and mean heart rate of 200 min^{-1}. In the presence of 1 mM lactate a mean lactate uptake of 2.1 ± 0.15 μmol/min per g wet wt. was measured, indicating that about 87% of the total energy production was derived from oxidation of the lactate supplied. At the beginning of the hypoxic period following conversion to a retrograde perfusion, a decrease in all dynamic parameters such as O_2 consumption, dp/dt_{max}, maximum ventricular pressure and heart rate was observed. Neither exogenous fatty acid supply nor BM 42.304 had an influence on these parameters. The arteriovenous difference of lactate indicated a

TABLE I

Tissue content (μmol/g wet wt.) and mitochondrial fraction (%) of high-energy phosphates

Mean ± S.E.

		ATP	ADP	AMP	Σ AxP	CP	CP/Cr
Normoxia without palmitate ($n = 8$)	total content	5.07 ± 0.35	1.10 ± 0.07	0.15 ± 0.03	6.32 ± 0.39	6.20 ± 0.68	0.99 ± 0.001
	mitochondrial	30.9 ± 8.7	23.9 ± 5.7	35.9 ± 8.7	29.9 ± 7.5	–	
Normoxia 0.5 mM palmitate ($n = 6$)	total content	4.79 ± 0.24	0.86 ± 0.08	0.23 ± 0.01 *	5.88 ± 0.31	6.69 ± 0.43	1.02 ± 0.10
	mitochondrial	35.6 ± 11.3	39.1 ± 19.7	28.5 ± 9.0	35.8 ± 14.1	–	
Hypoxia without palmitate	total content	2.52 ± 0.24 **	1.31 ± 0.08	1.04 ± 0.03 **	4.87 ± 0.28 *	2.40 ± 0.10 **	0.27 ± 0.03
	mitochondrial	3.8 ± 2.1 *	56.3 ± 6.4 **	41.9 ± 11.7	27.1 ± 2.5	–	
Hypoxia 0.5 mM palmitate ($n = 6$)	total content	1.43 ± 0.18 ‡‡	0.89 ± 0.12 ‡	1.46 ± 0.21	3.78 ± 0.43 ‡‡	1.99 ± 0.24	0.18 ± 0.02
	mitochondrial	4.6 ± 1.8	26.5 ± 8.4 ‡	12.0 ± 4.6 ‡	13.0 ± 2.6 ‡‡	–	
Hypoxia + palmitate + BM 42.304 ($n = 6$)	total content	2.89 ± 0.08 §§	1.20 ± 0.04 §	0.92 ± 0.11 §	5.00 ± 0.09 §§	3.06 ± 0.14 §§	0.15 ± 0.007
	mitochondrial	4.2 ± 2.6	34.5 ± 6.2	42.7 ± 7.3 §§	19.3 ± 3.6	–	

* $P < 0.05$, ** $P < 0.01$ vs. normoxic control without palmitate.
‡ $P < 0.05$, ‡‡ $P < 0.01$ vs. hypoxia without palmitate.
§ $p < 0.05$, §§ $P < 0.01$ vs. hypoxia with exogenous palmitate.
Σ AxP: ATP + ADP + AMP.

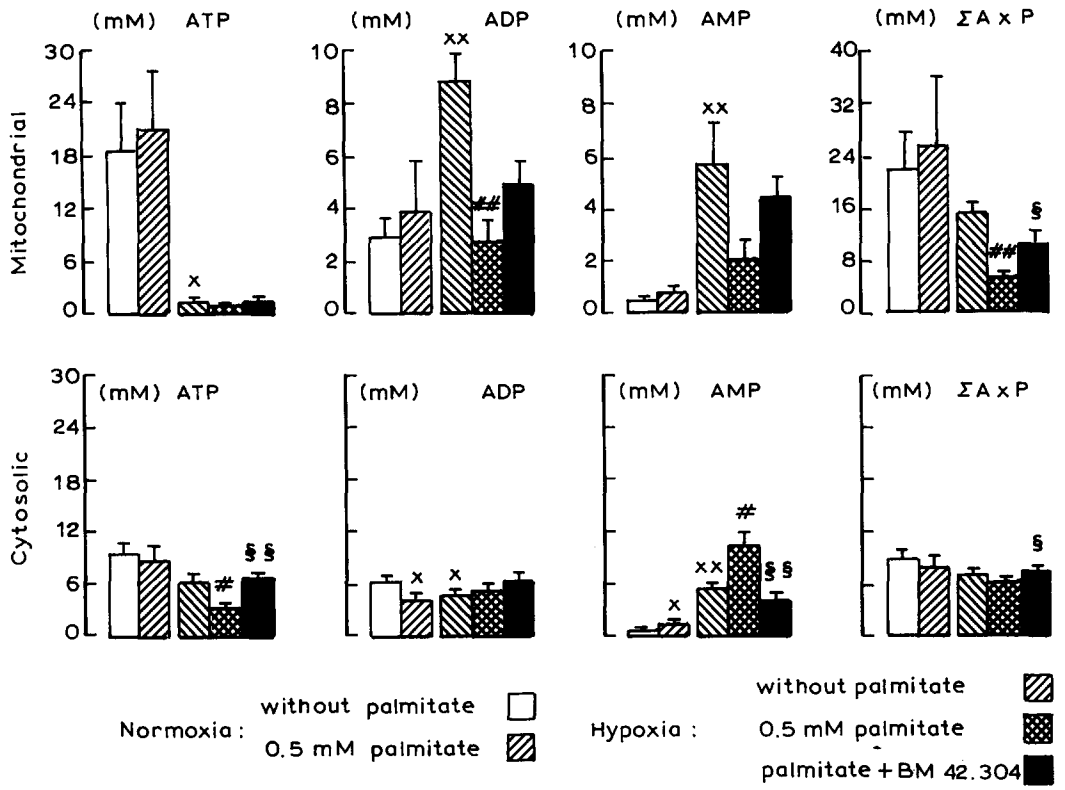

Fig. 1. Effects of palmitate and hypoxia on adenine nucleotide concentrations in the mitochondrial and cytosolic space. Data are mean ± S.E., $n = 8$ for normoxic control; $n = 6$ for the other groups. * $P < 0.05$, ** $P < 0.01$ vs. normoxic control without palmitate. # $P < 0.05$, ## $P < 0.01$ vs. hypoxia without palmitate. § $P < 0.05$, §§ $P < 0.01$ vs. hypoxia with exogenous palmitate. Σ AxP, ATP + ADP + AMP.

lactate release of about 2 μmol/min per g wet wt. under all conditions. Considering that oxygen supply amounted to 0.11 μmol/min per g wet wt. during hypoxia, only about 5% of total ATP was generated aerobically. These results provide evidence that energy metabolism, particularly glycolytic activity, was not affected by exogenous fatty acids or BM 42.304 under these experimental conditions.

High-energy phosphates

Overall tissue contents of adenine nucleotides and CrP/Cr ratios from hearts perfused aerobically with glucose and lactate and from hypoxic hearts in the presence and absence of fatty acids are shown in Table I along with the mitochondrial fraction of all metabolites. The tissue contents of nucleotides are in good agreement with data from other authors [19,22]. Tissue energy state as reflected by adenine nucleotide contents and CrP/Cr ratios appeared to be not significantly changed in the presence of palmitate during normoxia but was considerably decreased on hypoxic perfusion, where palmitate lowered the energy state additionally. This effect was prevented in the presence of BM 42.304 except for CP/Cr. In Fig. 1 mitochondrial and cytosolic concentrations of high-energy phosphates are depicted. In the mitochondria, ATP concentration was twice as high as in cytosol. Similarly, ADP and AMP concentrations were higher in the mitochondrial compartment. Due to the different-sized water spaces of both compartments, only about 30% of total adenine nucleotide contents were located in the mitochondria (Table I). Since CP was found exclusively in the extramitochondrial compartment, which is in accordance with findings of Altschuld et al. [23], only the total CP concentration was determined. Likewise, the total CP/Cr ratio reflects the cytosolic one due to an extramitochondrial localisation of creatine kinase systems. Therefore also only total CP/Cr ratios were determined.

Under hypoxic conditions mitochondrial ATP decreased significantly by 94% compared to normoxic perfused hearts. In cytosol, however, ATP concentration was diminished by only about 34%, probably due to a high glycolytic activity within this compartment. ADP and AMP showed a significant increase in mitochondria. Similarly, cytosolic AMP concentration was considerably enhanced, while ADP was slightly decreased. Particularly in mitochondria, total adenine nucleotide concentration was considerably lowered.

Perfusion with 0.5 mM palmitic acid during hypoxia decreased the overall tissue concentration of adenine nucleotides significantly. In cytosol, predominantly ATP was remarkably diminished while AMP concentration

TABLE II

Tissue content (μmol/g wet wt.) and mitochondrial fraction (%) of CoA and carnitine metabolites

Mean ± S.E.

		Acyl-CoA	CoA	Σ CoA	Acylcarnitine	Carnitine	Σ carnitine
Normoxia without palmitate	total content	0.03 ± 0.01	0.09 ± 0.01	0.12 ± 0.01	0.28 ± 0.03	1.93 ± 0.03	2.21 ± 0.06
	mitochondrial	76.8 ± 7.9	65.5 ± 8.7	68.1 ± 5.5	23.8 ± 5.9	12.4 ± 4.6	13.6 ± 3.1
Normoxia 0.5 mM palmitate ($n = 6$)	total content	0.07 ± 0.01 **	0.04 ± 0.01 **	0.11 ± 0.01	0.29 ± 0.03	1.25 ± 0.07 **	1.54 ± 0.08 **
	mitochondrial	62.4 ± 8.6	49.8 ± 11.5	57.8 ± 9.0	63.8 ± 7.7 **	29.1 ± 9.6	35.4 ± 8.8 *
Hypoxia without palmitate ($n = 6$)	total content	0.15 ± 0.01 **	0.04 ± 0.01 **	0.19 ± 0.01 **	0.48 ± 0.05 **	1.63 ± 0.12 *	2.11 ± 0.16
	mitochondrial	80.5 ± 6.9	78.2 ± 10.0	80.0 ± 7.2	81.0 ± 12.2 **	26.4 ± 7.0	41.5 ± 6.0 **
Hypoxia 0.5 mM palmitate ($n = 6$)	total content	0.16 ± 0.01	0.02 ± 0.01 ##	0.18 ± 0.01	0.61 ± 0.09	1.31 ± 0.15	1.93 ± 0.12
	mitochondrial	86.2 ± 7.7	71.5 ± 16.3	84.4 ± 8.3	82.7 ± 11.9	44.1 ± 7.7	57.6 ± 5.6
Hypoxia + palmitate + BM 42.304 ($n = 6$)	total content	0.17 ± 0.01	0.02 ± 0.01	0.17 ± 0.01	0.38 ± 0.03 §	0.95 ± 0.08	1.32 ± 0.10 §§
	mitochondrial	93.6 ± 14.3	88.9 ± 7.6	93.0 ± 13.1	50.8 ± 13.5	40.7 ± 10.2	43.3 ± 7.6

* $P < 0.05$, ** $P < 0.01$ vs. normoxic control without palmitate.
‡ $P < 0.05$, ## $P < 0.01$ vs. hypoxia without palmitate.
§ $P < 0.05$, §§ $P < 0.01$ vs. hypoxia with exogenous palmitate.
Σ CoA: CoA + acyl-CoA.
Σ carnitine: carnitine + acylcarnitine

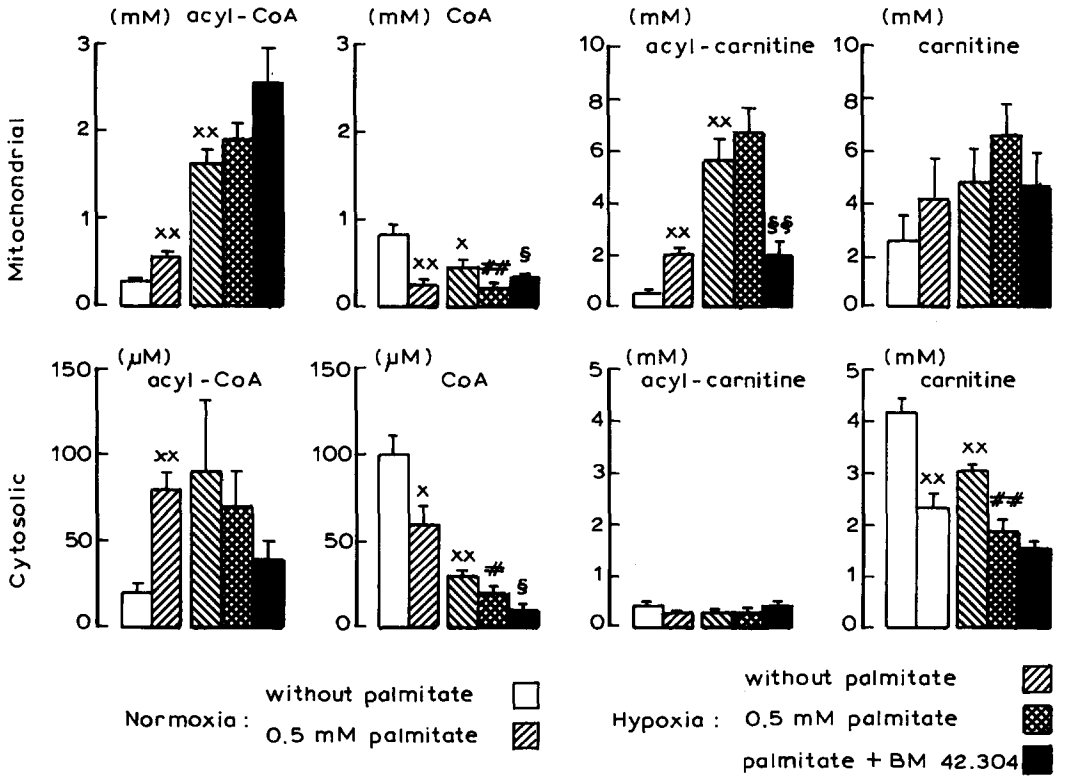

Fig. 2. Subcellular concentrations of CoA, carnitine and their acyl esters from normoxic and hypoxic perfusions with and without fatty acid as well as after addition of 10 μM BM 42.304. For explanation of the symbols see Fig. 1. Data are mean ± S.E. $n = 8$ for normoxic control; $n = 6$ for the other groups.

was significantly increased. Total adenine nucleotide content in the extramitochondrial space, however, was found to be only slightly decreased.

Following addition of BM 42.304, the concentrations of ATP, ADP, AMP and consequently of total adenine nucleotides are enhanced in the mitochondrial compartment. In cytosol, ATP revealed a significant increase while AMP contents were diminished to half. The deterioration of the myocardial energy state caused by hypoxic perfusion and additional exogenous fatty acid supply, was shown to be almost abolished in the presence of the inhibitor BM 42.304.

Lipid metabolites

Table II shows the contents of CoA, carnitine and their long-chain fatty acyl esters in total tissue and their mitochondrial fractions. The results indicate that 23% of CoA and 11% of total carnitine were present as esterified compounds when hearts were perfused aerobically without palmitate. The ratio of acylated and free compounds was considerably higher in the mitochondrial than in the extramitochondrial compartment. CoA was mainly located in the mitochondria, while the major fraction of carnitine was found in the cytosol.

When hearts were perfused with 0.5 mM palmitic acid the concentrations of the acylated compounds – except cytosolic acyl carnitine – increased significantly in both compartments (Fig. 2). The ratio of acylated and free CoA was enhanced about 6-fold in mitochondria as well as in cytosol. The ratio of acylcarnitine and carnitine increased slightly in the mitochondrial space, while the cytosolic ratio was not affected by fatty acid perfusion.

After hypoxic perfusion without exogenous fatty acids, acyl-CoA was found to be enhanced in mitochondrial as well as in cytosolic space, while CoA was significantly reduced compared to normoxia. Acylcarnitine concentration, however, significantly increased only in mitochondria. These results indicated a 10-fold increase in the ratio of acyl-CoA and CoA in mitochondria, while the cytosolic proportion was 15-fold enhanced. These amounts were considerably higher compared to data obtained from normoxic perfused hearts exposed to exogenous fatty acids, while cytosolic acylcarnitine yielded similar results.

The supply of exogenous fatty acids during hypoxia caused a slight increase in acyl-CoA as well as acylcarnitine contents in mitochondria, while free CoA concentration was found to be half of that found in fatty acid-free perfusions. Due to these findings, a considerable enhancement in the mitochondrial ratio of acyl-Coa and CoA and CoA resulted. Cytosolic contents of free carnitine and CoA were found to decrease significantly; the acylated derivates, however, remained nearly constant. In the extramitochondrial compartment a considerable decrease in total carnitine content in favour of mitochondrial contents had occurred. Considering the sum of mitochondrial and cytosolic concentrations of total CoA and carnitine, no significant alterations have been detected. These results indicate that exogenous fatty acid supply as well as hypoxia exert additive effects on the ratios of acylated and free compounds. In hearts perfused with palmitic acid, addition of BM 42.304 caused a significant decrease in mitochondrial acylcarnitine, while free CoA increased. These data indicate that the compound BM 42.304 cannot exert an inhibitory effect on the inner acylcarnitine transferase, as described by Deaciuc et al. [21], since in this case high intramitochondrial acylcarnitine contents have to be expected. In comparison with inhibitor-free perfused hearts, however, elevated cytosolic and low mitochondrial contents of acylcarnitine have been determined, supporting the concept that obviously the preceding transport step, namely the carnitine acylcarnitine translocase, has been affected by BM 42.304. Cytosolic carnitine concentration as well as tissue contents of total carnitines further decreased in presence of BM 42.304, as already shown when solely fatty acids had been supplied during hypoxia. Thereby, the overall carnitine contents were significantly diminished compared to inhibitor-free perfusions.

Discussion

Effect of hypoxia

Since the transfer of adenine nucleotides across the mitochondrial membrane is driven by the membrane potential [2,4], the gradient of ATP/ADP ratios between the mitochondrial and cytosolic compartments reflecting the energy state of the mitochondria should be diminished during hypoxia. However, in our experiments the cytosolic ATP concentration is still nearly as high as the normoxic value. It should be pointed out that with the non-aqueous fractionation technique applied in this study it is not possible to measure accurately the free cytosolic ADP. However, based on calculations of free cytosolic ADP from the creatine kinase equilibrium, it is known that cytosolic ADP in the heart is below 0.1 mM [19] and therefore the cytosolic ATP/ADP ratio is supposed to be much higher than the mitochondrial ratio in normoxia as well as in hypoxia. The cytosolic ATP level could be maintained high during hypoxia due to an increase in glycolysis and to an inhibition of adenine nucleotide transport. From previous reports, an inhibitory effect of long-chain acyl-CoA on the adenine nucleotide translocase (ANT) is well known [8,10,25,26]. However, addition of 0.5 mM palmitate to normoxic hearts caused to changes in subcellular adenine nucleotide distribution, despite increased mitochondrial acyl-CoA. On the other hand, during hypoxia, acyl-CoA levels in the mitochondria are much more increased and may thus explain the inhibition of ANT.

In normoxic perfused hearts, cytosolic AMP contents were shown to be very small, whereas hypoxia caused a considerable increase in this metabolite. AMP can be further degraded by AMP deaminase and 5′-nucleotidase to generate a leak for the overall adenine nucleotide contents. Losses of adenine nucleotides up to 60% during hypoxia have been observed by other authors [27,28]. Our results indicate that cytosolic adenine nucleotide contents remain nearly constant, while mitochondrial contents are distinctly diminished, especially in the presence of exogenous palmitate. These findings might be explained by a compensatory efflux of adenine nucleotides from mitochondria to cytosol by an exchange with cytosolic inorganic phosphate via ATP–P_i exchanger [29] since P_i is considerably enhanced in hypoxia.

Effects of fatty acids during hypoxia

The deleterious effects of exogenous fatty acids on hypoxic myocardium have been the subject of some controversy. In the studies of Gmeiner no effect on myocardial function was observed on reperfusion of hypoxic hearts with palmitate [30]. Ischemic hearts showed uniformly diminished tissue concentrations of ATP and CP in the presence and absence of palmitate [31]. Presumably these results can be attributed to the severity of ischemia as indicated by tissue contents of ATP and CP (0.3 and 0.6 μmol/g (wet wt.), respectively). Therefore, according to these data particular effects caused by fatty acids could not be differentiated. In contradiction to these findings, other groups observed a remarkable depression in myocardial function during reperfusion following ischemia in the presence of fatty acids [12,32,33]. The acceleration of fatty-acid-induced damage to the hypoxic myocardium was suggested to be due to the accumulation of free fatty acids as well as long-chain acyl-CoA and acyl carnitine [3,34]. Tissue concentrations of these metabolites have been correlated with the extent of ischemic injury, but no conclusive evidence for a causal relation has been documented [31]. Our results do not confirm the supposition that deterioration of the myocardial energy state in the presence of fatty acids during hypoxia depends on the accumulation of these fatty acid metabolites. Tissue contents of the acylated derivatives were shown to be enhanced only slightly compared to fatty-acid-free hypoxic perfusions. Possibly the deterioration of the energy state and the increased loss of adenine nucleotides is caused by an accumulation of free fatty acids which cannot be measured in tissue since they are bound to cellular proteins. High concentrations of free fatty acids have been shown to uncouple oxidative phosphorylation in isolated mitochondria [6] as well as in the intact cell [35]. However, this point has been the subject of controversy (for a review see Ref. 36). Thus, others suggested that uncoupling occurs only at unphysiologically high free fatty acid concentrations and that the increase in oxygen consumption is oligomycin-sensitive [37] and is due to an increase in ATP-consuming processes, e.g., Na^+/K^+-ATPase [38]. On the other hand the dramatic decrease in ATP and in energy state observed during hypoxia in the presence of fatty acids (Table I, Fig.1) indicates that under certain extreme physiological conditions free fatty acid may rise to such high levels as to cause an uncoupling like effect on oxidative phosphorylation.

The subcellular distribution of CoA, carnitine and their long-chain acyl derivates in ischemic myocardium was first investigated by the group of Neely [3,34]. They used differential centrifugation of heart homogenates to isolate mitochondrial and postmitochondrial fractions. Following aerobic as well as hypoxic perfusions of the isolated rat heart with 1.2 mM palmitic acid, about 95% of total CoA was located within the mitochondrial space. The amount of total carnitine determined in this compartment rose from 9% in normoxia to 25% in hypoxia. Our results, however, indicate that mitochondrial contents of total CoA amounted to 68% during aerobic perfusions and increased to about 80% in hypoxic perfused hearts. The differences are supposed to be due to different fractionation techniques. Thus, the possibility cannot be excluded that during fractionation of the homogenate in aqueous media, alterations in metabolism as well as in subcellular distribution can occur, whereas this is avoided by the fractionation technique in non-aqueous media. Nevertheless, the alterations induced by hypoxia were found to be comparable. Considering the distribution of total carnitines between mitochondria and cytosol, about 14% were found in the mitochondrial space during normoxia, increasing to a proportion of 42% during the hypoxic perfusions.

After supply of exogenous fatty acids as well as after hypoxia the calculation of total carnitine content, summarized from free carnitine and acylcarnitine, shows an enhancement of these metabolites in the mitochondrial compartment while the cytosolic concentration declined (Table II). This is in accordance with the results of Pande and Parvin, who showed that carnitine-acylcarnitine translocase catalyses not only an exchange mechanism but also an unilateral transport [39].

Effect of a fatty acid metabolism inhibitor

Cinnamoyl derivatives are well known inhibitors of pyruvate transport into the mitochondria. Some of these compounds, such as α-cyano-4-hydroxycinnamate, show inhibitory effects also on the activity of the carnitine-acylcarnitine translocase [40]. These substances are known to react reversibly with thiol groups, forming addition products. In isolated working rat hearts 10 μM BM 42.304 inhibited fatty acid oxidation by more than

80%, this being associated with a reduction in fatty-acid-induced increase in oxygen consumption [14]. This effect is achieved solely with respect to long-chain fatty acids; the oxidation of medium-chain fatty acids, such as octanoate, is not inhibited. As shown by Deaciuc et al. [21], the transport of long-chain fatty acids into mitochondria was inhibited due to the presence of BM 42.304, suggesting that inner acylcarnitine transferase was affected. In this study the carnitine-acylcarnitine translocase was not considered. Our own studies showed a considerable decrease in mitochondrial acylcarnitine contents while cytosolic contents slightly increased. These findings indicate that, in fact, the carnitine-acylcarnitine translocase is inhibited. Similar results have been reported from studies with other inhibitors of fatty acid metabolism. In ischemic rat hearts POCA diminished drastically tissue concentrations of long-chain fatty-acyl-CoA [33]. Acylcarnitine contents, however, were lowered to a much greater extent than those of acyl-CoA. In studies on neonatal rat myocytes hypoxia induced a significant increase in acylcarnitine contents which could be prevented in the presence of POCA [41].

Inhibitors of fatty acid metabolism have also been investigated with respect to prevention of fatty-acid-induced myocardial injury during ischemia or hypoxia, e.g., POCA [33,41], Oxyfencine [42] and TDGA [12]. These compounds were supposed to effect the outer acylcarnitine transferase. Furthermore a blocker of β-oxidation, 4-bromocrotonic acid, has been investigated [42]. In all studies amelioration of cardiac function during reperfusion or improvement of the energy state following hypoxia have been observed. This is consistent with our findings that BM 42.304 practically restored cytosolic contents of adenine nucleotides to normoxic values (Fig. 1). Mitochondrial contents, however, remained as low as without the effector.

The reason for the beneficial effects of fatty acid metabolism inhibitors on energy state of the heart during hypoxia or ischemia in the presence of fatty acids remains unclear. Pearce et al. [12] suggested that the prevention of fatty-acid-induced damage of myocardial function of TDGA was attributed to the reduction in O_2 consumption, due to a shift from fatty acid to carbohydrate oxidation which would result in an enhanced ATP production from the remaining oxygen during ischemia. However this 'oxygen-saving effect' represents no satisfactory explanation for the functional and energetic improvements. Even if fatty acid oxidation is totally inhibited, maximally 30% of oxygen can be spared [12,14], which under hypoxic conditions would amount to about 30 nmol/min per g wet wt.

On the other hand, no conclusions can be drawn concerning the direct relationship of improved hypoxic tolerance and decreased acylcarnitine contents, since fatty acid supply during hypoxia caused no significant changes in mitochondrial acyl-CoA and acylcarnitine contents (Fig. 2).

We assume that addition of fatty acids during hypoxia considerably increases free fatty acid concentration. Addition of BM 42.304 should decrease mitochondrial free fatty acids by preventing the entry of long-chain acyl esters into the mitochondria where they are hydrolyzed to free fatty acids since they cannot be oxidized during hypoxia.

Summarizing these findings, we may conclude that fatty acyl esters such as acyl-CoA and acylcarnitine do not exert harmful effects on heart energy metabolism. The inhibition of adenine nucleotide translocation by acyl-CoA during hypoxia appears to prevent a drastic decrease of cytosolic energy state during hypoxia. The effect of an inhibition of acyl transport into mitochondria, which prevents acceleration of the ischemic injury by exogenous fatty acids, may be explained by a decreased accumulation of free fatty acids in the mitochondrial space.

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