POSSIBLE RELATIONSHIP BETWEEN TISSUE LEVEL OF LONG CHAIN ACYL-CoA AND THE ABILITY OF THE OVERLOADED MYOCARDIUM TO OXIDIZE AN EXCESS OF REDUCED PYRIDINE NUCLEOTIDE

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Received 27 February 1980

1. Introduction

The inhibitory effect of acyl-CoA derivatives on adenyl nucleotide translocase and respiration of isolated mitochondria is now rather well documented [9,14,16,20,25,41]. Comparing the respective K_i determined in vitro, it appears that long chain fatty acyl esters of coenzyme A are 10 times more effective than the bongkrekic acid [4] and only 5 times less active than actually the most potent inhibitor of AdNT, actractyloside [28]. On the basis of the above findings it has been suggested that acyl-CoA esters might be involved in the control of adenyl nucleotide translocation and thus of energy production of intact tissues [16,29,31]. In fact, under conditions of increased energy requirements, such as increasing heart work, the intracellular content of acyl-CoA decreases [21,23]. On the other hand, there is an accumulation of long chain acyl-CoA in ischemic myocardium [11, 31,36] and during the experimental diabetes [8] which both lead to impaired mitochondrial function [8,13]. Direct evidence of in situ regulatory function of long chain acyl-CoA derivatives is still lacking, however.

In our previous report on optical monitoring of respiratory activity of isolated perfused hearts from animals with compensated mechanical overload of the left ventricle, we noticed an increased ability of hypertrophied myocardium to reoxidize an excess of reduced pyridine nucleotide accumulated during short period of anoxia [18,19]. In this work we tested the effect of short anoxia on two of the parameters able to explain the apparent increase of maximal rate of pyridine nucleotide reoxidation: (1) instantaneous phosphate potential (ATP/ADP P_i) of total adenine nucleotide [3] and (2) tissue levels of acyl-CoA esters, considered as prospective modulator of adenine nucleotide translocase activity both in vitro [6,7,29, 33,39] and in vivo [12,22,30,37,38].

2. Material and methods

Adult female Wistar rats were used throughout this study. A communication between abdominal aorta and inferior vena cava was done under nembutal anesthesia, according to the technique developed by Hatt [10]. The animals were sacrificed one month later and their hearts perfused at 37°C with Krebs Henseleit bicarbonate buffer (KHB) containing 2.5 mM pyruvate. Two 95 cm columns (O_2 and N_2 equilibrated) were connected to a three-way miniature solenoid valve (General Valve Corporation) permitting to switch rapidly from oxygenated to anoxic solution and vice versa.

All hearts were perfused for 15 min with oxygenated KHB (95% O_2 and 5% CO_2 equilibrated). Spontaneously beating "normoxic" hearts (controls) were frozen at the end of this period, those of the second, "anoxic" group were perfused for a further 3 min by N_2-equilibrated KHB. They were then frozen by a Wollenberger clamp precooled in liquid nitrogen. Reduction degree of pyridine nucleotides was monitored by the method described previously [18,19]. No systematic control of hemodynamic parameters,

except for heart rate and coronary flow rate, was done in this series of experiments.

The frozen samples were then deproteinized in 5 ml of perchloric acid (4°C) containing 50 μl of 0.1 M dithiothreitol per ml. The supernatant was neutralized to pH 5.8 and assayed for ATP and creatine phosphate, ADP and AMP and CP and P_i [5]. The tissue content of free and esterified coenzyme A was determined fluorometrically (Eppendorf 1101 M) according to Williamson [5]. An aliquot of frozen tissue was dried overnight and metabolite concentrations expressed in μmol (nmol)/g dry weight.

3. Results

In fig.1 and in table 1 we resume our data concerning the in situ optical recording of maximal respiratory activity of volume-overloaded hearts presented in detail in another paper [19]. It can be seen that one month after the surgery, once the overloading has been compensated by heart hypertrophy, the ability of the myocardium to reoxidize the anoxia-induced increment of reduced pyridine nucleotides was significantly improved. Modifications of both maximal rate of NADH reoxidation (dF/dt) and time necessary to reestablish the initial redox conditions (T) suggest a significantly higher rate of electron transfer in overloaded hearts during the early phase of reoxygenation. The increased respiratory activity of hypertrophied heart was apparently unrelated to improved oxygen supply to tissue, since neither the maximal coronary flow rate (Table 1), nor the speed of myoglobin reoxygenation [19] were changed under the same conditions.

Another explanation of the increased rate of NADH reoxidation might be the shift in cytosolic ATP/ADP

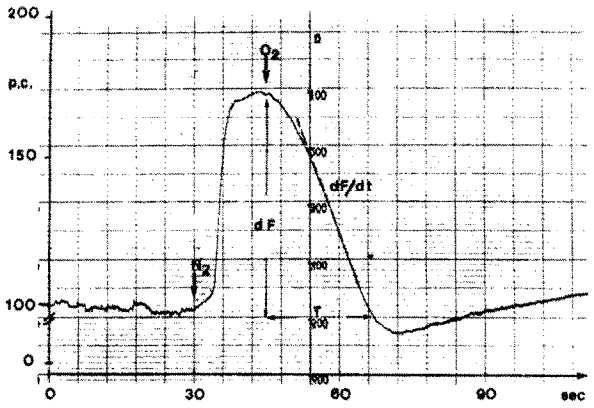

Fig.1. Effect of short period of anoxia on fluorescence emission of reduced pyridine nucleotide of perfused rat heart (KHB containing 2.5 mM pyruvate, pH 7.4, respective pO_2 640 and 60 Torr); dF: amplitude of the anoxic signal at 365–470 nm; dF/dt: maximal rate of reduced PdN reoxidation; T: time necessary to reach normoxic conditions.

× P_i ratio [39] or adenylate charge [2]. We tried to assess the above possibility by determinations of tissue adenine nucleotide content. It can be seen from Table 2 that energy states of control and overloaded hearts were rather comparable, both under normoxic and anoxic conditions. It follows that the increased respiratory activity of overloaded hearts is not simply related to altered phosphate potential resulting from increased ADP or P_i accumulation in the myocardium.

In contrast, a significant difference between the two groups of hearts was found at the level of CoA esters governing the AdN distribution between cytosol and mitochondria. While under normoxic conditions, the CoA distribution is rather comparable, the anoxic patterns differ considerably (Table 3). The most significant differences is the absence of anoxia induced

Table 1
Effect of volume overload on heart weight, coronary flow rate and kinetics of PdN reoxidation

	Heart weight[a] (g dw/100 g bw)	Coronary flow (ml/min/g dw)	Pyridine nucleotide		
			F_2 (%)	T (s)	dF/dt
Controls (n = 20)	0.050 ± 0.001	75.80 ± 3.10	185.45 ± 5.99	38.66 ± 2.61	3.71 ± 0.01
1-month fistulas (n = 10)	0.080** ± 0.004	76.84 ± 6.25	172.00 ± 15.0	28.10* ± 4.0	7.91** ± 0.80

[a] bw, body weight; dw, dry weight

$\bar{m}$ ± SEM; *$P < 0.05$; **$P < 0.01$

Table 2
Effect of short anoxia (3 min) on tissue content of adenine nucleotides

	CP	ATP	ADP	AMP	P_i	ATP / ADP × P_i
Controls						
Normoxic	42.2 ± 0.7	22.0 ± 0.4	4.33 ± 0.40	0.52 ± 0.03	12.3 ± 1.0	0.409 ± 0.050
Anoxic	6.7* ± 0.2	17.4* ± 0.3	7.26* ± 0.13	1.13* ± 0.04	47.7* ± 1.0	0.050* ± 0.001
1-month fistulas						
Normoxic	46.5 ± 2.1	25.7 ± 1.3	4.18 ± 0.20	0.59 ± 0.04	12.6 ± 0.7	0.530 ± 0.090
Anoxic	8.8* ± 1.1	17.41* ± 1.5	7.37* ± 0.74	1.79* ± 0.20	51.9* ± 2.4	0.049* ± 0.005

n = 10; $\bar{x}$ ± SEM; *$P < 0.001$; μmol/g dw

accumulation of long chain acyl-CoA. Both acetyl-CoA and short chain acyl-CoA levels were also less affected in volume overloaded hearts, as compared with controls. Only non-significant modifications of CoA and of total CoA levels could be seen during anoxia in any of two groups tested.

4. Discussion

Recently, it has been suggested that principal determinants of instantaneous respiratory activity of intact tissues are (1) cytosolic [ATP]/[ADP].[P_i], (2) intramitochondrial [NAD^+]/[NADH] and (3) oxygen availability [39]. For phosphorylation state of adenine nucleotides could modulate the rate of electron transport, at least two conditions have to be satisfied: (1) near equilibrium must exist between respiratory chain and mitochondrial pool of adenine nucleotides, (2) rate of adenine nucleotide translocation must be high enough to avoid that adenine nucleotide translocase became the rate-limiting step of cell respiration [37]. The first condition seems to be satisfied over a range of experimental conditions from isolated mitochondria [7,39], through cell suspensions [38] to intact tissues, such as isolated perfused heart [22]. The second point, i.e. the prospective control of cell respiration of the level of adenine nucleotide translocation is still under discussion [35].

As concerns the heart muscle, it has been suggested that under pathological conditions, such as ischemia [30] and experimental diabetes [8], mitochondrial adenine nucleotide translocation could be affected by long chain fatty acyl-CoA accumulation in the myocardium. In fact, it has been shown in vitro (isolated mitochondria) that long chain fatty acid inhibit both, $^{32}P_i$-ATP exchange and ^{14}C-ADP translocation through the mitochondrial membrane [9,20, 40]. These inhibitory effects were abolished by uncouplers and they could be reversed by compounds preventing CoA esterification with fatty acids or by external carnitine, facilitating the oxidation of acyl-CoA esters to short chain metabolites. These experiments suggested that in vitro in presence of exogenous fatty acids adenine nucleotide translocase can become

Table 3
Effect of short anoxia (3 min) on the redistribution of coenzyme A

		CoA	Acetyl-CoA	Short chain acyl-CoA	Long chain acyl-CoA
Controls	Normoxic	300 ± 16	37 ± 4	204 ± 17	74 ± 6
	Anoxic	297 ± 15	105* ± 12	59* ± 10	144* ± 14
1-month fistulas	Normoxic	281 ± 20	38 ± 3	155 ± 17	68 ± 10
	Anoxic	303 ± 30	64 ± 12	130 ± 13	68 ± 8

n = 9–10; $\bar{x}$ ± SEM; *$P < 0.001$, nmol/g dw

rate limiting and that its inhibition is mediated by long chain fatty acid esters of CoA, rather than by fatty acids themselves [25,41].

Whether a similar situation could also appear in situ is still under discussion. A number of indirect evidence, such as extremely high tissue levels of long chain acyl-CoA, low K_i values of adenyl translocase for fatty acid-CoA, etc may argue in the sense of that possibility [4,28]. However, it has been objected [35] that the majority of intracellular fatty acyl-CoA is bound to proteins and thus ineffective at the level of mitochondrial membrane.

In this respect, the results described in the present paper should be considered as direct proof of the thesis [6,15,37] according to which adenine nucleotide translocation is involved in the control of cell respiration. The improved ability of overloaded hearts to reoxidize an excess of reduced pyridine nucleotide we monitored optically may be related to the absence of anoxia-induced long chain acyl-CoA accumulation. In absence of any difference in adenine nucleotide concentrations, the above possibility seems to be one of a few plausible explanations of increased respiratory activity of overloaded myocardium. Another factor should, however, be kept in mind: modifications of cytochrome aa_3 interaction with oxygen [1,17,26]. Experiments are in progress in order to discriminate between these two possibilities. Whatever the exact mechanism of improved respiratory activity of overloaded myocardium is our results suggest that biochemical adaptations of energy production can appear during the stage of "compensated" heart hypertrophy.

Acknowledgement

Research Supported by D.G.R.S.T., Grant No. 74.7.0790.

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