Acetyl-CoA inhibition of adenine nucleotide translocation in ischemic myocardium

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SHUG, A. L., E. SHRAGO, N. BITTAR, J. D. FOLTS, AND J. R. KOKE. Acetyl-CoA inhibition of adenine nucleotide translocation in ischemic myocardium. Am. J. Physiol. 228(3): 689-692. 1975.—The translocation of adenine nucleotides across the inner mitochondrial membrane and the tissue concentration of long-chain acyl-CoA esters were studied in dog heart after experimental myocardial ischemia. Ligation of the anterior coronary artery initiated events leading to an early decrease in adenine nucleotide translocase activity. A reciprocal increase in the concentration of heart tissue long-chain acyl-CoA esters was also observed. Adjacent nonischemic tissue showed changes intermediate between that of ischemic and normal heart tissue. It is postulated that a decrease in fatty acid oxidation after myocardial ischemia would lead to an accumulation of long-chain acyl-CoA esters, which in turn would inhibit adenine nucleotide translocation. The net result would be a lowering of the energy charge of the cell, adversely affecting muscle contraction and electrical conduction.

in vitro experiments showed that long-chain acyl-CoA esters did indeed inhibit adenine nucleotide translocation when added directly to heart mitochondria (21). Because of the possible significance of these findings in heart disease, we have conducted a series of experiments whose purpose was to show that long-chain fatty acyl-CoA esters accumulate in the ischemic myocardium, resulting in an inhibition of adenine nucleotide translocase, and in this manner alter the cell’s metabolism as just described.

MATERIALS AND METHODS

Healthy mongrel dogs weighing between 20 and 30 kg were used. Anesthesia was produced with subcutaneous morphine sulfate (3 mg/kg) followed in 1 h by intravenous pentobarbital (30 mg/kg). Ventilation was maintained by means of a Harvard animal respirator with room air. In all cases, short segments of the anterior descending branch of the left coronary artery were dissected free, allowing blood flow to be measured with a Biotronix flowmeter and flow transducer. Epicardial electrodes were sutured to the anterior and lateral walls of the left ventricle. Normal dogs were those in which no intervention was carried out. Ischemia was produced by ligating either 1) only the anterior descending coronary artery or 2) in addition distal segments of the diagonal branches and circumflex artery.

At designated times, cardiac inflow and outflow were stopped by clamps and areas of ischemia and nonischemia were monitored visibly by gross appearance and by changes in the electrocardiogram. Tissues representing the ischemic and nonischemic areas of the left ventricle were then rapidly excised. Confirmation of the state of the tissue was obtained by electron microscopy. Samples of left ventricle were homogenized in a modified medium of Pande and Blanchaer (15) containing 250 mM sucrose, 50 mM Tris-HCl (pH 7.4), and 1.0 mM EGTA. In early experiments bovine serum albumin was included, but later was omitted when it was found to have little effect on adenine nucleotide translocase activity and oxygen consumption. Mitochondria were obtained by differential centrifugation according to the method of Henderson and Lardy (3). Oxygen consumption and respiratory control ratios in mitochondria were measured polarographically as described by Estabrook (2) by use of a Clark oxygen electrode supplied with the Gilson oxigraph. Translocation of adenine nucleotides in mitochondria was determined by the method of Wojtczak and...
Zaluska (26), with a slight modification previously described (8, 20).

Determination of tissue levels of long-chain fatty acyl-CoA esters was carried out on excised ischemic and nonischemic heart tissue quickly frozen in liquid nitrogen. For the most part the analysis was completed on the day of the experiment, but it was found feasible to store the frozen tissue at 70°C for several weeks without loss of activity. Extraction and determination of long-chain acyl-CoA esters were essentially by the method of Williamson and Corkey (25). For accuracy and reproducibility it seemed necessary to use between 1.5 and 2.5 g frozen tissue. Finely powdered tissue was homogenized in 3.5 vol ice-cold 6% perchloric acid. After centrifugation of the homogenate at 25,000 X g for 10 min in a Sorval RC 2B refrigerated centrifuge, the pellet was washed twice in 6% perchloric acid, once with water, and finally suspended in 0.4 vol 100 mM dithiothreitol and 1.6 vol water. Saponification of the acyl-CoA to liberate free CoA was carried out by alkaline hydrolysis at 50-55°C for 10 min. The mixture was then chilled in an ice bath for 10 min, acidified to pH 4.6-5.0 with 6% perchloric acid and 500 mM trichloroacetic and centrifuged as above. The supernatant was assayed for free CoA by the phosphotransacetylase system (23).

Protein was determined by the biuret method (3) after prior lysis of the mitochondria with deoxycholate. Dry weight was obtained by heating small pieces of tissue to a constant dry weight in a drying oven.

The [14C]ADP (sp act 450 mCi/mmol) was purchased from Amersham-Searle Company. Acetyl phosphate, phosphotransacetylase, and dithiothreitol were purchased from Sigma Chemical Company. Carbonyl cyanide m-chlorophenylhydrazone was a gift from Dr. H. A. Lardy, Enzyme Institute, University of Wisconsin. All other reagents were of the highest quality commercially obtainable.

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EFFECT OF FATTY ACIDS ON HEART

Adenine nucleotide translocase, now recognized as one of the more important enzymes in the regulation of cell metabolism, has been characterized primarily by its extreme sensitivity to the inhibitors atracyloside (1) and bongkrekic acid (5). More recently it has been shown that long-chain acyl-CoA esters can act as natural effectors of adenine nucleotide translocation in the regulation of cell metabolism. A correlation between actual levels of long-chain acyl-CoA esters and inhibited adenine nucleotide translocation in liver, during altered metabolic and nutritional states, has been pointed out and used as an explanation for the resulting effects on gluconeogenesis, ketogenesis, and energy-linked respiration (9, 20, 24).

In heart tissue fatty acids are the preferred substrate for energy purposes (10, 11, 18). During myocardial ischemia it has been observed that the oxidation of fatty acids is decreased and their conversion to triglyceride increased (17). It was postulated that impaired oxidation of fatty acids during ischemia might lead to accumulation of their long-chain acyl-CoA esters, with resulting inhibition of adenine nucleotide translocation (21). In vitro experiments with isolated mitochondria were consistent with this postulate, and the possibility that an inhibition of adenine nucleotide translocation was one of the early events after myocardial infarction was considered (21). The present experiments support this proposal. Mitochondria isolated from the ischemic area of the left ventricle within 15 min after occlusion of the anterior coronary artery show inhibition of adenine nucleotide translocation and progressive inhibition of this enzyme up to 60 min of ischemia. During this time interval, an increase in the level of heart tissue long-chain acyl-CoA esters accompanies the inhibition of translocase. Changes in adenine nucleotide translocation and long-chain CoA esters in control heart tissue obtained adjacent to the ischemic area are found consistently. The morphology both by light and electron microscopy was completely normal in this control area. Sufficient evidence is not yet available on the reversibility of the biochemical lesions observed in either the ischemic or nonischemic control tissue. This investigation and a previous in vitro study (21), however, show that addition of L-carnitine to the mitochondrial reaction mixture at least partially reverses the decrease in adenine nucleotide translocation.

In the aerobic rat heart perfused by the Langendorff technique with high concentrations of palmitate, tissue levels of long-chain acyl-CoA esters increased about 50% (14). Rapid oxidation of the long-chain acyl-CoA esters prevented their excessive accumulation. In myocardial ischemia, reduction in fatty acid oxidation was observed to be accompanied by lower tissue levels of acetyl-CoA and acetylcarnitine and by increased levels of fatty acyl-CoA and fatty acylcarnitine (14). The carnitine acyltransferase enzyme (or enzymes) that translocates long-chain CoA esters across the inner mitochondrial membrane as the acylcarnitine derivative, and could thereby play a part in lowering long-chain acyl-CoA levels, has been found to be reduced after 30 min of ischemia (27).

Deleterious effects of fatty acids on myocardial function have been noted under both experimental and clinical conditions. Studies on isolated dog heart preparations demonstrated a depressant effect of fatty acids on muscular mechanics (6). Free fatty acids were found to play a significant role in increasing myocardial oxygen requirement and

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**FIG. 2.** Adenine nucleotide translocase activity and long-chain acyl-CoA levels in heart during early intervals after experimental myocardial ischemia. Methods and results are as given in Table 1 and in MATERIALS AND METHODS.

exchange of about 100 nmol ADP/min per mg at 0°C. Ligation of one artery (anterior descending coronary) for 60 min produced severe inhibition of adenine nucleotide translocase, reducing the activity in the ischemic tissue to about 34% of normal. Additional ligations of the circumflex and distal diagonal branches decreased the activation even further. As in experiments measuring oxygen consumption, the control or nonischemic area shows inhibited adenine nucleotide translocase activity that also becomes more marked as the ischemia is intensified.

Subsequent studies were directed toward detecting changes in adenine nucleotide translocase at earlier time intervals than 60 min of ischemia as well as correlating the carrier activity with tissue levels of long-chain acyl-CoA esters. In studies shown in Fig. 2, cardiac tissue was examined at 15, 30, and 60 min after coronary ligation (anterior descending) for both adenine nucleotide translocase activity and long-chain acyl-CoA ester concentration. In comparison with the normal heart, a decrease in adenine nucleotide translocase can be detected as early as 15 min in the ischemic area, with a further drop up to 60 min. Of significance is the fact that the decrease in adenine nucleotide translocase is accompanied by an increase in the concentration of long-chain acyl-CoA esters for each time interval, with the greatest increment occurring at 15 min. More important may be the consistent observation that the adjacent nonischemic area showed similar though lesser changes in adenine nucleotide translocase activity and long-chain acyl-CoA levels that under proper conditions might be reversed toward normal.

**DISCUSSION**

Adenine nucleotide translocase, now recognized as one of the more important enzymes in the regulation of cell metabolism, has been characterized primarily by its extreme sensitivity to the inhibitors atracyloside (1) and bongkrekic acid (5). More recently it has been shown that long-chain acyl esters can, at low concentrations, reversibly inhibit translocation of adenine nucleotides across the inner mitochon-
promoting depression of contractility of the hypoxic heart in experimental animals (6). Free fatty acid-albumin infusions administered to dogs with myocardial infarction produced serious ventricular arrhythmias in a significant number of animals (13). Such experimental studies have been used to corroborate the clinical observations of Oliver (12), who found increased levels of serum free fatty acids during acute myocardial infarction that were associated with an increase in severe complications. This correlation was not observed by Regan et al. (16), who suggested that many of the complications, particularly arrhythmias, could be interpreted as a reflection of increased catecholamine secretion rather than free fatty acids acting independently.

Inhibition of adenine nucleotide translocation by long-chain fatty acyl-CoA esters offers a unifying concept to explain a variety of clinical as well as experimental observations resulting from the effects of fatty acids on myocardial function. Most of the pathophysiological and clinical responses described could be secondary to an inhibition of translocase resulting in an altered energy charge and redox state of the heart cell. Additional experiments are necessary, however, before any definitive conclusions can be reached. Long-chain CoA esters were measured in whole-heart tissue and not analyzed specifically in mitochondria, the site of effective translocase inhibition. If technically feasible, the actual separate determination of long-chain acyl-CoA esters as well as adenine nucleotide levels in mitochondria and cytosol would be extremely important. The adenine nucleotide translocation of rat liver mitochondria has a Km for ATP of less than 0.5 μM and Kt for palmitoyl-CoA of 0.3 μM (19). Although these values would appear to be consistent with the range of their expected mitochondrial concentrations, information on the relative concentration of each at a particular time would be of value.

In conclusion, we acknowledge that the present methodology only suggests a causal relationship between the increase in long-chain fatty acyl-CoA esters and inhibition of adenine nucleotide translocation in heart tissue after myocardial infarction. However, if it can be ascertained that the accumulation of long-chain fatty acyl-CoA esters is an initiating factor in decreasing the energy charge of the cell after myocardial ischemia, it may constitute a reversible biochemical lesion and prevent destruction of the myocardium.

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REFERENCES


