INHIBITION OF BOVINE HEART Na^+,K^+-ATPase BY PALMITYLCARNITINE AND PALMITYL-CoA

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Summary

The activity of a partially purified bovine heart Na^+,K^+-ATPase is inhibited by DL- and L- palmitylcarnitine (I_{50}=44-48μM). Palmitylcarnitine with a I_{50} of 25μM also markedly inhibits K^+-phosphatase activity. Palmityl-CoA decreases Na^+,K^+-ATPase activity, but to a lesser extent (I_{50}=80μM). Both palmitic acid and hexanoic acid produce 10 to 15% inhibition of activity at concentrations of 70μM and 3-5mM, respectively. These free fatty acids protect the enzyme against inhibition by 40μM palmitylcarnitine. However, at 50μM palmitylcarnitine, the protective effect by hexanoic acid is no longer apparent. Addition of 40μM palmitylcarnitine to the Na^+,K^+-ATPase in the presence of varying concentrations of palmityl-CoA produces an additive inhibition of enzyme activity, suggesting two different sites on the enzyme susceptible to inhibition by the two ester forms of the fatty acid.

Introduction

Reduced coronary flow to the myocardium produces an abrupt increase in levels of potassium in the coronary sinus (1). Beller et al (2) reported a significant reduction in microsomal Na^+,K^+-ATPase after two hours of coronary ligation. We found, in contrast, decreased Na^+,K^+-ATPase only after chronic (several days) myocardial ischemia (3). Acute potassium loss during brief periods of coronary occlusion is followed by rapid reuptake of potassium when flow is restored (1). Thus, the cation pump mechanism for restitution of myocardial cellular potassium after interruption of coronary flow appears to be resistant and can operate, even after prolonged (one hour) periods of oxygen deficit On the other hand, the pump is probably affected immediately

after an occlusion but may be reversibly inhibited by accumulation of some intracellular metabolite. During the isolation process, it is possible that the metabolite is removed from the enzyme, particularly during the deoxycholate extraction procedure (3). Ahmed and Thomas (4) found that free fatty acids inhibit brain microsomal Na^+,K^+-ATPase. The intracellular concentrations of long chain free fatty acids are small, with the majority of the lipid esterified to coenzyme A or l-carnitine for oxidation via the carnitine palmityltransferase system in mitochondria (5). During myocardial ischemia, concentrations of long chain acyl-CoA and acylcarnitine increase by two and three-fold probably due to limited oxidative metabolism of fatty acids by the β-oxidation pathway in mitochondria (6). Although the intracellular distribution of these esters is unknown in the cell *in vivo*, their membrane-active properties make it likely that both acyl-CoA and acylcarnitine are bound to membrane structures. Since acylcarnitine readily penetrates the inner mitochondrial membrane, intercalation of the long chain fatty acyl groups into the membrane bilayer may be a consequence of ester accumulation. The present report demonstrates a potent inhibitory effect of the carnitine and coenzyme A esters of palmitic acid on a partially purified Na^+,K^+-ATPase from bovine heart.

Material and Methods

Bovine heart Na^+,K^+-ATPase was partially purified by a procedure developed in this laboratory (7). The specific activity of the enzyme used in these experiments was 160-200μmoles phosphate liberated per hour per mg. Enzyme activity was assayed at 37°C by a coupled enzyme system, pyruvate kinase-lactic dehydrogenase, as previously described (8). The Na^+,K^+-ATPase (approximately 15μg/ml) was incubated in the assay medium in the presence and absence of fatty acid and fatty acyl esters as indicated in the figure legends. The reaction was initiated by addition of Mg ATP to a final concentration of 3mM. This procedure eliminated precipitation of palmityl-CoA and the free fatty acids as Mg^{++} salts (Ref. 4 and personal communication, Dr. K. Ahmed). No effect of

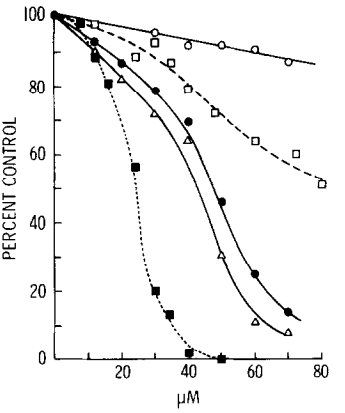

Fig. 1: The effect of palmitic acid and palmityl esters on bovine heart Na^+,K^+-ATPase and K^+ phosphatase

1) % control = % activity, Na^+,K^+-ATPase (171μmoles P_i/mg/hour)

o——o + palmitic acid

□----□ + palmityl-CoA

●——● + DL- palmitylcarnitine

△——△ + L- palmitylcarnitine

2) % control = % activity, K^+ phosphatase (41μmoles PNPP/mg/hour)

■----■ + DL- palmitylcarnitine

The data in this and subsequent figures are typical of numerous replicate experiments on two separate enzyme preparations.

200μM palmitylcarnitine or 0.4mM l-carnitine was observed on the coupled enzyme rates (measured in the presence of 0.3mM ADP at 340nm, enzymes diluted 1:800). The possibility that palmityl-CoA may inhibit pyruvate kinase (9) was tested. After an 800-fold dilution of the coupled enzymes (2.5μg enzymes) 60μM palmityl-CoA inhibited NAD production by only 17%. Since the pyruvate kinase/lactic dehydrogenase rate is normally in 50-fold excess (50μg enzymes) over the ATPase rate under our assay conditions, the inhibition of the coupled enzymes by palmityl-CoA was not rate-limiting to the assay.

Potassium phosphatase activity associated with the cardiac Na^+,K^+-ATPase enzyme (8) was assayed as previously described (10) with p-nitrophenol phosphate as substrate, except that 25mM Imidazole -HCl, pH 7.8, was used instead of Tris buffer. The increase in absorbance at 410nm at 37°C was measured. The reaction was initiated by addition of a final concentration of 20mM K^+. Since the assay medium for preincubation contains 4mM $MgCl_2$, only the effect of palmitylcarnitine on K^+- phosphatase activity was examined.

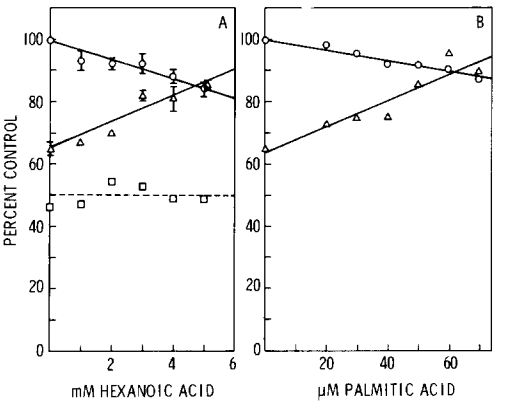

Fig. 2: The effect of free fatty acid on bovine heart Na^+,K^+-ATPase in the presence and absence of palmitylcarnitine

A. Hexanoic acid

o——o hexanoic acid only

Δ——Δ hexanoic acid + 40μM palmitylcarnitine

□----□ hexanoic acid + 50μM palmitylcarnitine

B. Palmitic acid

o——o palmitic acid only

Δ——Δ palmitic acid + 40μM palmitylcarnitine

Ordinate = % activity of Na^+,K^+-ATPase as measured in the absence of fatty acids.

Both palmitic and hexanoic acids were added as ethanolic solutions, with no more than 0.02ml added to the experimental cuvettes. Control cuvettes received an identical volume of ethanol. This amount of ethanol had no effect on the control enzyme rate. Palmityl-CoA (Sigma Biochemicals) and palmitylcarnitine (P/L Biochemicals) were added as aqueous solutions, at pH 7.4. Palmitylcarnitine was maintained at 37°C to avoid precipitation from solution which may occur at room temperature. The pH of the reaction medium was measured before and after the assays and found not to change.

Results

Inhibition of bovine heart Na^+,K^+-ATPase by palmitic acid, palmityl-CoA and palmitylcarnitine is concentration-dependent

and varies according to the esterfied group (Fig. 1). Palmitic acid alone produces only a slight effect on enzyme activity with 13% inhibition at 80μM acid. Palmityl-CoA produces a 50% inhibition of Na^+,K^+-ATPase activity at 80μM added, whereas both the DL- and L- isomers of palmitylcarnitine are approximately twice as potent as palmityl-CoA with I_{50} values of 48 and 44μM, respectively (Fig. 1). Addition of DL- palmitylcarnitine to the K^+-phosphatase assay results in a marked depression of activity with 50% inhibition observed at 25μM.

Although palmitic acid has only a slight effect on Na^+,K^+-ATPase activity, evidence suggests that the free acid is associated with enzyme protein. When palmitylcarnitine (40μM) is added to the enzyme reaction in the presence of palmitic acid, increases in the concentration of free fatty acid decrease the inhibition of Na^+,K^+-ATPase elicited by palmitylcarnitine (Fig. 2). Similar results are obtained in the presence of 1-5mM hexanoic acid (Fig. 2). Inhibition of Na^+,K^+-ATPase by 5mM hexanoic acid proceeds to only 15%. However, in the presence of 40μM palmitylcarnitine, increases in hexanoic acid concentration produce a reversal of the inhibition by palmitylcarnitine (Fig. 2). When the concentration of palmitylcarnitine is increased above its I_{50} for Na^+,K^+-ATPase, hexanoic acid can no longer prevent the inhibitory effects of this ester (Fig. 2). The inhibition of Na^+,K^+-ATPase by palmitylcarnitine does not appear to be freely reversible, since the enzyme remains inhibited after incubation with palmitylcarnitine, centrifugation, and resuspension (data not shown). When palmitylcarnitine (40μM) is added to the assay medium for Na^+,K^+-ATPase in the presence of varying concentrations of palmityl-CoA, inhibition is increased (Fig. 3). No protective effect of palmitylcarnitine inhibition by palmityl-CoA is observed and enzyme activity appears to be additively decreased.

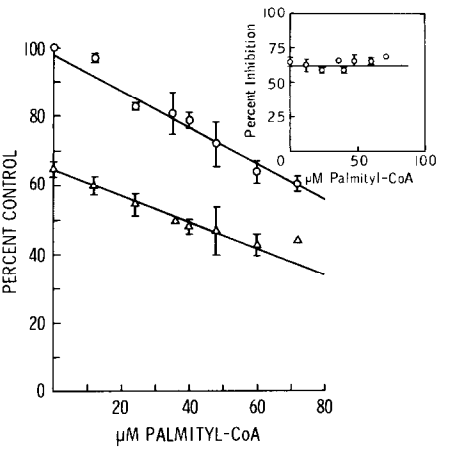

Fig. 3: The effect of palmityl-CoA on bovine heart Na^+,K^+-ATPase in the presence and absence of palmitylcarnitine

o——o palmityl-CoA only

Δ——Δ palmityl-CoA + 40μM palmitylcarnitine

Ordinate = % activity of Na^+,K^+-ATPase as measured in the absence of palmityl-CoA and palmitylcarnitine.

Inset: Ordinate = percent inhibition of the Na^+,K^+-ATPase in the presence of palmityl-CoA after addition of 40μM palmitylcarnitine.

Discussion

Palmityl-CoA inhibits a variety of metabolic processes, including mitochondrial di- and tricarboxylic acid transport and adenine nucleotide transport (11-13). The physiological significance of these effects of palmityl-CoA in metabolic regulation is unclear because of the relatively high concentrations employed (14). An exception to these observations is the adenine nucleotide translocase that is inhibited by palmityl-CoA at a concentration well below the critical micelle level (K_I=.1-.2μM) (13). The inhibition of Na^+,K^+-ATPase by palmityl-CoA is observed at concentrations above 20μM, and the concentrations employed are in

the range of palmityl-CoA levels found in the myocardial cell under both normal and pathological conditions (5,6). However, the binding of palmityl-CoA to protein constituents in the cell most likely alters the amount of this fatty acyl ester that is available to interact with the plasma membrane. On the other hand, palmitylcarnitine within physiological range of concentrations has not previously been reported to inhibit enzymatic processes in the cell. In fact, palmitylcarnitine inhibition of mitochondrial metabolism has been attributed to the conversion of this metabolite to palmityl-CoA by the mitochondrial lipid transport enzyme, carnitine palmityltransferase (15). Pande and Blanchaer (15) reported that, unlike palmityl-CoA, (-) palmitylcarnitine did not inhibit dinitrophenol-stimulated ATPase activity in mitochondria, even at concentrations approaching 400μM.

The inhibition of heart Na^+,K^+-ATPase by palmitylcarnitine appears to take place at site (s) different from the sites inhibited by palmityl-CoA. Since Ahmed and Thomas (4) have previously reported that long chain fatty acids are competitive inhibitors of brain microsomal Na^+,K^+-ATPase with respect to K^+, the possibility that inhibition occurs at a cation binding site on the enzyme is under investigation. These data provide a possible explanation for altered ion fluxes during early periods of myocardial ischemia as a physiological consequence of palmitylcarnitine interaction with Na^+,K^+-ATPase.

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