ON A SLOW INHIBITORY EFFECT OF FREE FATTY ACIDS ON THE RESPIRATORY CHAIN OF NON-PHOSPHORYLATING SUBMITOCHONDRIAL PARTICLES FROM BEEF HEART

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1. Introduction

Free fatty acids (FFA) exert a variety of inhibitory effects on the energy metabolism of mitochondria. They uncouple the oxidative phosphorylation [1–4], inhibit the ATP–P_i exchange [2,3], stimulate the Mg-dependent ATPase [1,2] and act as endogenous ionophores for potassium ions [5]. Moreover, the CoA-esters of fatty acids are strong inhibitors of the adenine nucleotide translocase [6] and the pyridine nucleotide transhydrogenase system [7]. FFA exhibit an instantaneous multi-site inhibition of the electron transport system of non-phosphorylating submitochondrial particles (ETP) which is relieved or prevented by serum albumin [8,9]. In this report a new inhibitory effect of FFA is described which appears slowly.

2. Materials and methods

ETP were obtained by milk alkaline treatment of beef heart mitochondria with the aid of a modified procedure according to Crane et al. [10].

Before use the ETP were stored below −30°C; freezing and thawing were carried out rapidly (solvent–dry ice mixture and water bath of 37°C resp.) and were without influence on the results.

The FFA used were from commercial sources. The unsaturated acids were extracted from a suspension of their urea adducts in water after acidification.

The incubation mixture was as follows: 200 μl 0.1 M potassium phosphate buffer, pH 7.4; 5 μl fatty acid in methanol (freshly destilled over KOH) and 50 μl ETP (38–145 μg protein–biuret method). After the incubation at 37°C for a given time 0.8 ml 0.05 M Tris–H_2SO_4 buffer, pH 8.0, containing 1 mg human serum albumin (Forschungsinstitut für Impfstoffe Dessau) per ml and 10 μl NADH (VEB Arzneimittelwerk Dresden), 20 mg/ml were added and the NADH oxidase activity was recorded by means of the spectrophotometer Unicam SP 800 at 340 nm. The content of serum albumin in the sample was high enough to overcome the known instant inhibition by FFA as determined by parallel experiments without preincubation.

3. Results

Fig. 1 shows the time-dependence of that part of FFA inhibition which cannot be reversed by serum albumin. The slowness of the effect is shown by the curve for myristic acid; maximal inhibition appears only after 8 hr preincubation. The rate of inhibition increases with higher concentrations of the FFA. From the titration curve of myristic acid after 8 hr preincubation (fig. 2) it is evident, that a certain amount of the FFA can be added without any effect on the NADH oxidase system; beyond a threshold value a steep increase of the inhibition occurs:

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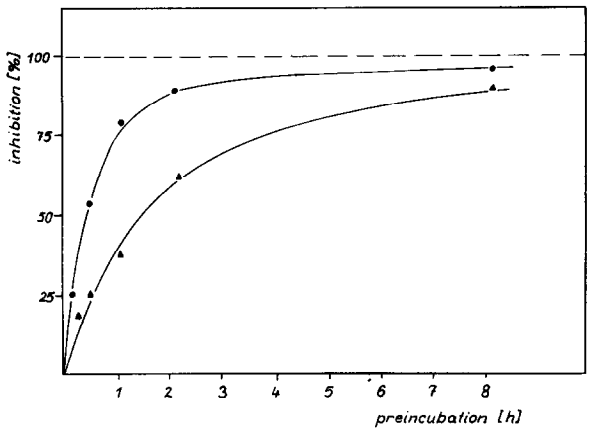

Fig. 1. Time-dependence of the slow inhibition of the NADH oxidase system by FFA: (●——●) oleic acid (222 nmoles/mg ETP protein); (▲——▲) myristic acid (125 nmoles/mg ETP protein).

obviously a certain amount of FFA may be bound to sites other than those sensitive to the inhibition. In another experiment we found that an equivalent amount of a methanol extract of ETP added to the test system caused a nearly total inhibition after long-time preincubation; this was due to the endogenous FFA content of the ETP. Again it would appear that the FFA of ETP are masked. An analogous situation was observed for the uncoupling activity of FFA on brown adipose tissue mitochondria [11].

Fig. 3 illustrates the inhibitory activity of FFA, expressed as the reciprocal amount necessary for half-

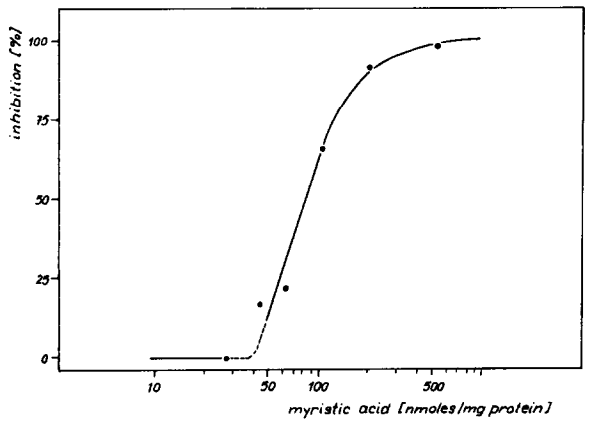

Fig. 2. Titration curve of the slow inhibition by myristic acid; 8 hr preincubation.

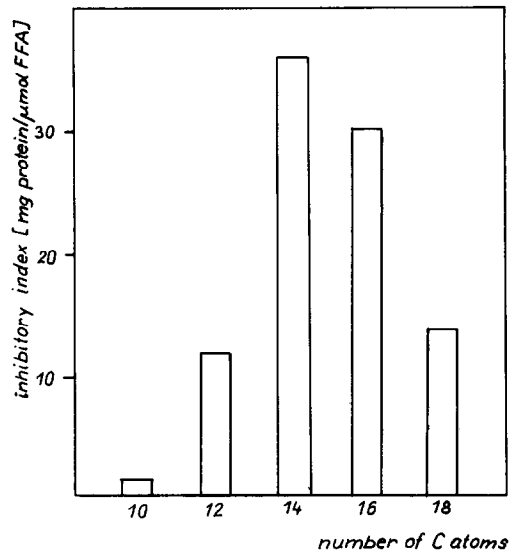

Fig. 3. Dependence of the slow inhibition on the chain length of FFA. Mean values of 5 experiments with two different ETP preparations. The inhibitory index represents the amount of ETP protein which is half-inhibited by 1 µmol FFA after 8 hr preincubation.

inhibition, as a function of the chain length of the even-membered saturated acids. A maximum at C_{14} (myristic acid) is observed, the same that was found for the instant inhibition [9]. However, the cis-unsaturated C_{18}-acids (oleic, linoleic and linolenic acid) show about the same effect as myristic acid and do not differ significantly among themselves. The approximately equal efficiency of FFA, their methyl-esters and their monoglycerides is in great contrast to the instant actions of these compounds; here the monoglycerides show a higher efficiency than FFA, whereas the methylesters are ineffective [9].

The action site in the respiratory chain for the effect here described appears to be located in the NADH-ubiquinone segment (unpublished results). On the other hand, the instant action of FFA is exerted both on the NADH oxidase and the succinate oxidase system, in either case primarily at a site on the substrate side of cytochrome *b* [8].

Electronmicroscopic pictures of the ETP (negative staining) did not reveal any structural damages accompanying the long-time inhibition of FFA on the NADH oxidase system which makes an unspecific effect on the membrane structure of the ETP unlikely.

The slow inhibition by FFA seems to be irreversible, since serum albumin did not reverse this effect.

4. Discussion

The effect here described appears to have escaped the attention so far. From the structure–activity relationship it is evident that the active moiety of the fatty acid molecule is the unpolar chain; therefore, hydrophobic interactions may be primarily involved. The slowness of the effect requires additional explanations such as compartmentation of the bound fatty acids at different sites of the mitochondrial inner membrane with a limited transport between the compartments. Furthermore, it is conceivable that the FFA do not react directly on the respiratory chain but activate a masked endogenous phospholipase in the sense of a feed-forward stimulation.

The amount of FFA which is required for the slow inhibition is in the same range as the endogenous FFA content of isolated mitochondria from various sources. Therefore, the effect may be of biological importance in vivo. For example an accumulation of FFA may contribute to the irreversible damage of the respiratory chain during prolonged ischemia. In vitro the endogenous FFA may be responsible for the storage lability.

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