A NUCLEAR FRACTION ANTAGONIZING FATTY ACID EFFECTS ON RAT LIVER MITOCHONDRIA[1]

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Received August 3, 1962

Fatty acids at physiological pH values have several effects on intact mitochondria which depend on concentration and carbon chain length. Long chain fatty acids, at concentrations around 10^{-5} M, produce latent ATP:ase[3] activation (Pressman and Lardy, 1956), decreased P/O ratios, uncoupling of oxidation from ADP dependence, inhibition of ATP-P_i exchange and mitochondrial swelling (Lehninger and Remmert, 1959). At higher concentrations, mitochondrial respiration is inhibited (Björntorp, et al., 1962). The U-factor, which contains long chain fatty acids and is released enzymically from rat liver mitochondria, is considered physiologically significant in regulating mitochondrial oxidation by effecting its uncoupling from ADP dependence (Lehninger and Remmert, 1959).

Serum albumin antagonizes fatty acid effects on mitochondria, probably through binding fatty acids (Björntorp, et al., 1962). Preliminary data demonstrating factors in rat liver nuclei which also antagonize fatty acid effects on mitochondria are presented here and their possible physiological significance as antagonists of the U-factor of Lehninger and Remmert (1959) is considered.

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[1]Supported in part by grants from the National Heart Institute, U.S.P.H.S. (H-2528), the American Heart Association (61 G 14), the Oklahoma State Heart Association and the Ayerst Laboratories, New York.

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[3]Abbreviations: ATP, adenosinetriphosphate; ADP, adenosinediphosphate; P_i, inorganic orthophosphate; P/O ratio, ratio of μMoles P_i esterified to μAtoms of oxygen consumed; αKG, potassium α-ketoglutarate; TRIS, tris(hydroxymethyl) aminomethane hydrochloride buffer; EDTA, ethylenediaminetetraacetic acid.

EXPERIMENTAL

Holtzman male rats, 350-450 g (20 to 24 weeks of age), were sacrificed by decapitation. The liver was perfused through the portal vein with 0.15 M NaCl followed by a mixture of 0.25 M sucrose - 0.001 M EDTA. After homogenization in this mixture (1/5, w/v) in a test tube with glass pestle, cellular fractionation by differential centrifugation was performed (Schneider, 1948). Little if any oxidation was obtained with the nuclear fraction, after five washings with the sucrose - EDTA solution, in a system containing αKG as substrate. This fraction was extracted with 0.15 M NaCl and the remaining pellet suspended in the sucrose - EDTA solution.

Oxygen consumption was determined by Warburg technique as previously described (Björntorp, et al., 1962). Serum albumin (Armour, Fraction V) was extracted by the procedure of Goodman (1957). Protein was determined according to Lowry, et al. (1951).

The mitochondrial swelling reaction was performed in 0.125 M KCl-0.02 M TRIS at 30° C, as described by Lehninger, et al. (1959). No EDTA was used in these experiments.

RESULTS AND DISCUSSION

Table 1 shows the oxidation obtained when cellular fractions were added to a mitochondrial system inhibited by fatty acids. Myristic acid only slightly inhibited homogenate oxidation but almost completely inhibited mitochondrial oxidation. Partial antagonism of the inhibition was obtained by adding the microsomal or soluble fraction, and complete antagonism by adding the nuclei or albumin. The addition of cellular fractions resulted in a slight increase in mitochondrial oxidation of αKG (cf. Schneider, 1948; Stern and Timonen, 1955), but some contamination of the nuclear and microsomal fractions with mitochondria cannot be excluded.

The effect of nuclear subfractions on mitochondrial oxidation is presented in Table 2. The relative increase in oxidation obtained on adding the nuclear fraction to the fatty acid inhibited system was several fold that obtained on adding this fraction to the mitochondrial system alone. The saline extract of

TABLE 1

Antagonism of Rat Liver Cellular Fractions to Fatty Acid Inhibition of Oxidation

	$O_2(\mu l)$
Homogenate	172.2
" + myristic acid	149.8
" + myristic acid + albumin	172.8
Mitochondria	133.5
" + nuclei	152.0
" + microsomes	156.2
" + soluble fraction	150.8
" + myristic acid	12.1
" + myristic acid + nuclei	156.4
" + myristic acid + microsomes	58.9
" + myristic acid + soluble fraction	74.8
" + myristic acid + albumin	142.2

Each Warburg vessel contained ΩKG to a final concentration of 10^{-2} M, 50 μMoles potassium phosphate, 5 μMoles ATP, 6 μMoles $MgCl_2$, 133 μMoles glucose, and 25 units hexokinase (Type III, Sigma). 1 μMole potassium myristate, 0.3 μMoles albumin, mitochondria corresponding to 4.50 mg protein, and homogenate or fractions equivalent to 1/40 original liver specimen were added as indicated. Final volume 3.0 ml, pH 7.5, gas phase air, center well 0.1 ml 20% KOH, temperature 37° C, time 15 minutes.

TABLE 2

Antagonism of Nuclear Subfractions to Fatty Acid Inhibition of Mitochondrial Oxidation

	$O_2(\mu l)$
Mitochondria	96.8
" + nuclei	116.6
" + saline ext. of nuclei	96.0
" + saline-extd. nuclei	81.7
" + boiled nuclei	68.7
" + myristic acid	6.5
" + myristic acid + nuclei	89.2
" + myristic acid + saline ext. of nuclei	47.7
" + myristic acid + saline-extd. nuclei	6.7
" + myristic acid + boiled nuclei	5.7

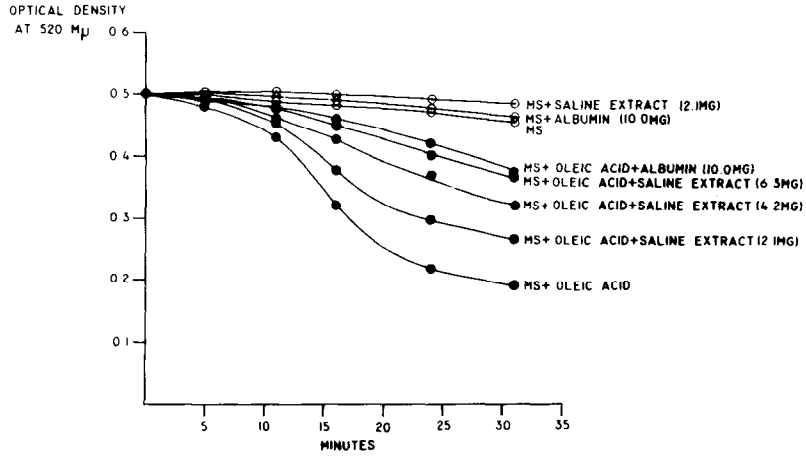
Conditions as in Table 1 except mitochondria corresponding to 3.80 mg protein added to each flask; 2 μMoles potassium myristate, 0.6 μMoles albumin and nuclei or subfractions from 1/40 rat liver were added as indicated.

the nuclear fraction had approximately half the original activity, but no activity was found in the saline-extracted nuclear or boiled nuclear fraction.

The nuclear saline extract or albumin prevented mitochondrial swelling occurring spontaneously or induced by oleic acid, as shown in Fig. 1. Either

FIGURE 1

Effect of Serum Albumin and the Saline Extract of the Nuclear Fraction on Spontaneous or Oleic Acid Induced Mitochondrial Swelling



M S = mitochondrial system. Weights in parentheses are mg protein of saline extract. Original optical density corrected to 0.500. 6×10^{-5} M oleic acid, 2×10^{-5} M albumin.

dialyzing against 0.15 M NaCl or heating for 4 minutes at 100° C reduced the activity of this extract to approximately one-half. Heating the dialyzed fraction destroyed activity. Most of the saline extract activity adsorbed on charcoal.

These results suggest that at least two factors, one dialyzable, the other non-dialyzable and heat-labile, active against mitochondrial swelling are present in the saline extract of the rat liver nuclear fraction. Lehninger, et al. (1959) reported that ATP reverses mitochondrial swelling induced by U-factor. ATP is dialyzable as is one of the saline extract components. It is conceivable that this factor might be effective in the mitochondrial system against swelling but not fatty acid inhibited oxidation, since boiling the nuclear fraction eliminated its effectiveness in the latter system. The heat-labile, non-dialyzable factor differs from C-factor (Lehninger and Schneider, 1959; Neubert, et al., 1962) at least in so far as it does not reverse glutathione swelling of mitochondria. Electrophoresis of the saline extract on

cellulose acetate, pH 8.6, failed to demonstrate a band with the migration rate of albumin.

Barton (1960) demonstrated nuclear proteins of the saline-extractable type in the soluble fraction following centrifugal fractionation and presented other data suggesting their ability to cross the nuclear membrane in vivo. The presence of nuclear proteins may explain the effectiveness of the soluble fraction reported here, although any extra- or intracellular albumin should also be found in this fraction.

Further studies on the identity of the active factors as well as on the mechanism of their action are now under way.

The author gratefully acknowledges the valuable discussions and criticisms provided by Doctors H. Alan Ells, Reagan H. Bradford, and Robert H. Furman and the electrophoretic determinations performed by Doctor Anders Gustafson.

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