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MITOCHONDRIAL SUBSTRATE OXIDATION-DEPENDENT PROTECTION AGAINST LIPID PEROXIDATION

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The effect of mitochondrial substrate oxidation on the NADPH-dependent lipid peroxidation of intact mitochondria, microsomes and of homogenate from rat liver was studied. It was found that addition of either succinate or β-OH-butyrate decreased the rate of malondialdehyde production of mitochondria. The effect of succinate was found to be marked (80–90% inhibition). Addition of succinate strongly inhibited the lipid peroxidation of a reconstituted system containing both mitochondria and microsomes. Increasing the amount of mitochondria in this system resulted in enhancement of the inhibition. The same succinate effect on malondialdehyde formation in liver homogenate was also observed. These findings suggest that mitochondria supplied with respiratory substrates may play a role in the protection against the lipid peroxidation in the liver cell.

Lipid peroxidation is a consequence of the free radical production in biological systems. The free radical precursors of the process as well as the lipid hydroperoxides may cause irreversible destruction of living organisms. Thus, it is not surprising that the living organisms have developed mechanisms either for protection against the free radical or for the removal of lipid peroxides formed during the process (see Refs. 1–3). In this paper we present some data suggesting that the mitochondria supplied with respiratory substrates may provide an effective protection against lipid peroxidation in the liver cell.

Preparations used were obtained from male albino rats weighing 120–150 g. Liver mitochondria were isolated according to the generally used procedure [4]. Homogenate of the liver was obtained after precipitation of the nuclear fraction, microsomes were collected from the postmitochondrial supernatant by centrifugation (105 000 × *g*, 60 min). All of the preparations were carried out in 0.15 M Tris-HCl, pH 7.4, except in the case of mitochondria where only the last washing medium was Tris-HCl buffer. Microsomal contamination of mitochondria isolated was assayed by measuring microsomal marker enzymes activity as described in Ref. 5. The glucose-6-phosphatase activity of microsomes and of mitochondria was 77.4 and 10.2 nmol/mg protein per min, respectively. Protein content of the preparation was determined by the method of Lowry et al. [6].

Malondialdehyde formation, as a measure of lipid peroxidation, was assayed by the thiobarbituric acid method of Ottolenghi [7]. Experiments were carried out at 37°C in media consisting of 0.15 M Tris-HCl, pH 7.4 and the additions detailed in the legend of Fig. 1 and the tables. The chemicals used were the purest commercially available.

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Rat liver mitochondria produce malondialdehyde in the presence of NADPH, ADP and ferric ions [8]. Additions of respiratory substrates, such as β-OH-butyrate or succinate, decreased the rate of this malondialdehyde formation as shown in Table I. The strong inhibition caused by succinate was relieved by malonate, and it was hardly influenced by addition of rotenone or antimycin A. These observations are in agreement with the findings of Takayanagi et al. [9], who presented similar effects of succinate on the lipid peroxidation of beef heart submitochondrial particles. The NADH-linked substrate, β-OH-butyrate, also decreased malondialdehyde production, but less effectively (Table I). The effect of this substrate could be abolished by rotenone.

Control experiments indicated that only negligible amount of malondialdehyde was metabolized by mitochondria under the experimental conditions applied (data not shown; cf Ref. 10).

The main source of the free radicals derived from molecular oxygen, which are consumed in the initiation of lipid peroxidation in the liver, is the microsomal NADPH-cytochrome *P*-450 system [11]. Furthermore, the NADPH-dependent lipid peroxidation detected in non-disrupted liver

TABLE I

EFFECT OF RESPIRATORY SUBSTRATES ON THE NADPH-DEPENDENT MALONDIALDEHYDE PRODUCTION OF MITOCHONDRIA

Incubation media contained NADPH (0.3 mM), ADP (3 mM), $FeCl_3$ (15 μM), glucose 6-phosphate (6 mM) and glucose-6-phosphate dehydrogenase (0.4 unit). The concentration of the mitochondrial protein was 3.0 mg/ml. The values are given as means ± S.E. of seven determinations.

Additions	nmol malondialdehyde formed/20 min
None	11.2 ± 3.7
Succinate (6 mM)	1.8 ± 0.5
Succinate plus malonate (15 mM)	10.2 ± 3.3
Succinate plus rotenone (5 μM)	2.2 ± 0.5
Succinate plus antimycin (3 μM)	1.4 ± 0.4
β-OH-butyrate (6 mM)	6.1 ± 3.1
β-OH-butryrate plus rotenone	11.9 ± 3.9
Rotenone	12.0 ± 3.8
Antimycin	8.3 ± 2.8
Malonate	12.2 ± 4.0

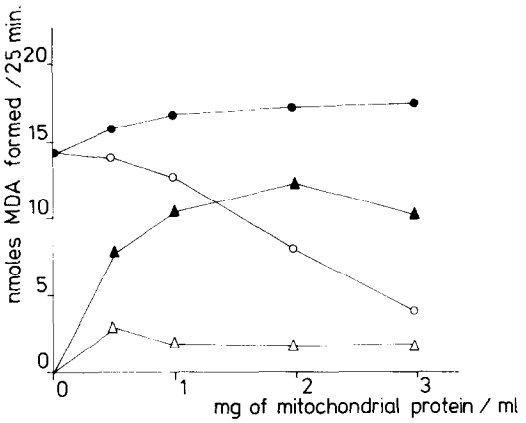

Fig. 1. Effect of succinate on malondialdehyde formation in mitochondria and in mitochondria plus added microsomes. Mitochondrial concentration dependency. Incubations were carried out in the same media as described in Table I: mitochondria alone, ▲; mitochondria supplied with succinate (8 mM), △; mitochondria + added microsomes (2.2 mg protein/ml), ●; mitochondria + added microsomes in the presence of succinate, ○.

mitochondria is due to a microsomal contamination, at least to some extent (as indicated by the glucose-6-phosphatase activity, see above). Thus, the question arose whether the inhibitory effect of mitochondrial succinate oxidation could also effect the lipid peroxidation catalyzed by microsomes.

Fig. 1 shows the rate of malondialdehyde production in a reconstituted system containing both mitochondria and added microsomes in the absence and in the presence of succinate. Malondialdehyde formation in the reconstituted system was decreased as compared to that of the separated particles. Moreover, a further decrease of microsomal lipid peroxidation was observed when succinate was added. This succinate-dependent inhibition was greatly enhanced by increasing the amount of mitochondria in the system. The data presented clearly demonstrate that the inhibitory effect of succinate-oxidizing mitochondria can counterbalance the capacity of microsomes (plus mitochondria) to catalyze lipid peroxidation at a certain ratio of organelles. It is worth mentioning that this inhibition was less effective at higher microsome concentrations (data not shown).

The natural ratio of mitochondria and micro-

TABLE II

EFFECT OF SUCCINATE ON THE MALONDIALDEHYDE PRODUCTION BY RAT LIVER HOMOGENATE

Incubation conditions were the same as in Table I. Protein concentration of the homogenate in the test was 4.0 mg/ml. The values are given as meas ± S.E. of the determinations.

Additions	nmol malondialdehyde formed/40 min
None	7.3 ± 1.1
Succinate (8 mM)	2.9 ± 2.1
Succinate plus malonate (15 mM)	7.2 ± 1.4

somes is preserved in liver homogenate, enabling us to approach the problem of the physiological significance of this type of organelle interaction. Table II indicates that the addition of succinate to liver homogenate also resulted in decreased malondialdehyde production; malonate abolished the succinate effect, as above. The conclusion could be drawn that the succinate-dependent inhibition affects the bulk of the NADPH-dependent lipid peroxidation occurring in the liver homogenate. The results of Takayanagi et al. [9] render probable that it is the reduction of ubiquinone by respiratory substrate which may be responsible for the observed inhibition of lipid peroxidation. Further, the antioxidant property of exogenously added ubiquinols has already been established [12] (and see Ref. 1). Our results with specific electron-transport chain-inhibitors are in accordance with these proposals. The data presented here suggest that mitochondrial substrate oxidation (probably by the reduction of endogenous ubiquinone) provide an effective protection against lipid peroxidation in the liver cell.

References

1 Dianzani, M.U. and Ugazio, G. (1978) in Biochemical Mechanism of Liver Injury (Slater, T.F., ed.), pp. 699–707, Academic Press, New York
2 Slater, T.F. (1979) in Oxygen Free Radicals and Tissue Damage (Ciba Foundation Symposium 65), pp. 143–163, Excerpta Medica, Amsterdam
3 McCay, P.B., Gibson, D.D. and Hornbrook, K.R. (1981) Fed. Proc. 40, 199–205
4 Johnson, D. and Lardy, H. (1967) Methods Enzymol. 10, 94–96
5 Tihanyi, K., Váradi, Gy. and Horváth, I. (1980) Biochim. Biophys. Acta 630, 187–192
6 Lowry, O.H., Rosebrough, N.J., Farr, A.L. and Randall, R.J. (1951) J. Biol. Chem. 193, 265–275
7 Ottolenghi, A. (1959) Arch. Biochem. Biophys. 79, 355–363
8 Pfeifer, P.M. and McCay, P.B. (1972) J. Biol. Chem. 247, 6763–6769
9 Takayanagi, R., Takeshige, K. and Minakami, S. (1980) Biochem. J. 192, 853–860
10 Recknagel, R.O. and Ghoshal, A.K. (1966) Exp. Mol. Pathol. 5, 108–117
11 Pederson, T.C., Buege, I.A. and Aust, S.D. (1973) J. Biol. Chem. 248, 7134–7141
12 Mellors, A. and Tappel, A.L. (1966) J. Biol. Chem. 241, 4353–4356