ARACHIDONATE RELEASE FROM RAT LIVER MITOCHONDRIA IN ENDOTOXIN SHOCK

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1. Introduction

A series of biochemical and haemodynamic changes occur in endotoxic shock [1–3] including changes in both mitochondrial membranes from different origins and in the cation content [4], (Na-K) ATPase activity [5], respiratory control [6–9] and other functional parameters have been described [10–12]; some discrepancies have been observed, however, on the variation of mitochondrial substrate oxidation [10,11]. Effectively, an intact mitochondrial membrane is neccesary for oxidative phosphorylation and active cation transport [13,14] this integrity depending largely upon phospholipids [15] but also upon ionic, hydrogen, and hydrophobic bonding [16]. Nevertheless, changes in mitochondria may be secondary to other endotoxemic effects [17]. The alterations of cellular pH, cation levels and water shifts across membranes activate some lysosomal enzymes which may contribute to the damage of the mitochondria [18]. Prostaglandins and their synthetase system are considered of great importance in endotoxic shock [19]; however, prostaglandin A_2, E_1 and F_2 did not enhance the survival of the treated animals whereas arachidonic acid significantly prevented the accumulation of lactic acid dehydrogenase and glutamine–pyruvic transaminase activities in the plasma of shocked animals [20].

Extensive subcellular variations in the hepatic parenchymal cell are related to the decrease in blood glucose levels and disordered energy metabolism [21–23]. However, there is now general agreement that, in the liver, hemorrhage and endotoxemia cause mitochondrial alterations that can be characterized by both morphologic and enzymatic alterations.

This paper aims to relate the change in the fatty acid patterns of the major mitochondrial lipid classes with some functional parameters of this subcellular fraction from rat liver under endotoxic shock.

2. Experimental

In all experiments male Wistar rats (200–250 g) were used. They were fasted overnight and allowed water until the experiments.

Endotoxin shock was induced in unanesthesized rats by an intravenous injection of *Escherichia coli* 0127:B8 (Difco Labs.) lipopolysaccharide B at 5 mg/kg body wt in 5 mg/ml saline solution. Control animals received a dose of saline solution. Animals were sacrificed after reaching circulatory collapse.

Livers were quickly excised from the rats and mitochondrial preparations were obtained according to [24].

Total lipids [25] were fractionated into lipid classes by thin-layer chromatography as in [26]. Fatty acid methyl esters [27] were quantitated by the internal patron method [28].

Mitochondrial respiratory activity (RCR and ADP/O) was assayed in a Clark oxygen electrode using either glutamate–malate or succinate as substrates according to [29]. Succinate–cytochrome *c* reductase activity was assayed as in [30].

Interdependency between the two variables, arachidonic and linoleic acid release and mitochondrial parameters (RCR), was determined by the correlation coefficient (r) using a computer program. The population coefficient was also determined.

3. Results and discussion

The content of the major fatty acids acylating

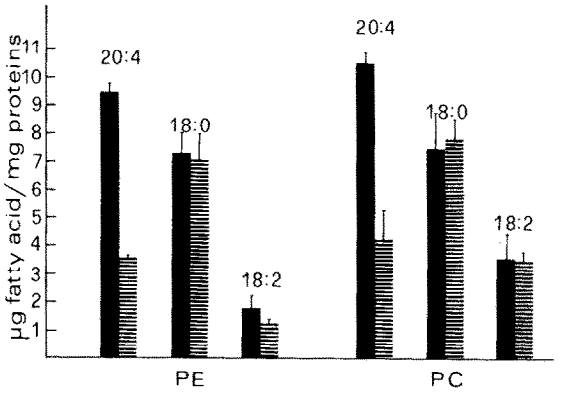

Fig.1. Levels of arachidonic acid (20:4), stearic acid (18:0) and linoleic acid (18:2) in phosphatidylethanolamine (PE) and phosphatidylcholine (PC) from normal (black bars) and shocked (striped bars) mitochondria. SD are given in the bars.

phosphatidylcholine and phosphatidylethanolamine of mitochondrial preparations, from both control and under endotoxin shock animals, is given in fig.1. Results are given as the absolute amounts of fatty acids/mitochondrial protein wt; thus, the fatty acid content only expresses the variations of the individual fatty acids. From these data it is clear that the content in arachidonic acid is highly diminished in both phosphoglyceride classes from shocked mitochondria; the content of the other major fatty acids, 18:0 and 18:2, does not exhibit significant variations. On the other hand, the released arachidonic acid contributed to increase its content in the pool of mitochondrial free fatty acids [31].

This removal of linked fatty acids from phosphatidylcholine and phosphatidylethanolamine by the phospholipid-hydrolysing mechanisms must be the result of the pre-activation of a lipolytic system; nevertheless, the nature of the initial attack remains unresolved.

In concomitant studies, changes elicited by lipopolysaccharide in the respiratory control (RCR and ADP/O ratio) and in succinate–cytochrome *c* reductase were determined in the liver mitochondria. Values are given in table 1. These parameters changed significantly as compared to the untreated mitochondrial values. These results are in agreement with in vitro work [7–11] that correlates the onset of endotoxin shock with the mitochondrial ability lose to maintain the membrane-bound Mg^{2+}.

We have carried out a correlation analysis between

Table 1
Respiratory control ratio ($RCR_{3/2}$) using glutamate–malate as substrate, ADP/O and succinate–cytochrome *c* reductase activity (mol reduced cytochrome *c* . min^{-1} . mg $protein^{-1}$) in liver mitochondria of control and shocked rats

Mitochondrial activities	Control animals	Shocked animals
$RCR_{3/2}$	9.2 ± 1.3	2.5 ± 0.5
ADP/O	2.5 ± 0.1	1.4 ± 0.1
Succinate–cyt. *c* reductase	45.8 ± 1.9	22.0 ± 2.2

the levels of fatty acids in phosphatidylcholine and phosphatidylethanolamine/mg mitochondrial proteins and the mitochondrial parameters; correlation coefficients (*r*) were calculated for the $RCR_{3/2}$ values using succinate as substrate and the contents of linoleic and arachidonic acids in either phosphatidylcholine or phosphatidylethanolamine (fig.2). These data show the existence of a significant difference between the correlation coefficients due to the linoleic acid levels and those obtained with the arachidonic acid contents. Taking into account the population coefficient, it is possible to attribute the high correlation between both variables to the onset of endotoxic shock.

The study of the mechanisms of stimulation of mitochondrial lipolytic activities carried out by the lipopolysaccharide and the fate of arachidonic acid are in due course.

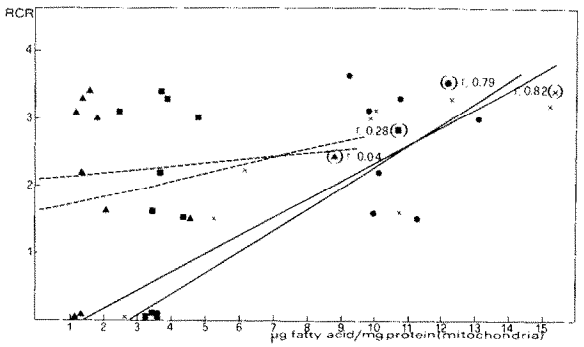

Fig.2. Correlation coefficients (*r*) between $RCR_{3/2}$ using succinate as substrate and the amounts of fatty acids for a series of liver mitochondrial preparations of shocked rats. Linoleic acid (– – –) from phosphatidylethanolamine (▲) and phosphatidylcholine (■). Arachidonic acid (——) from phosphatidylethanolamine (●) and phosphatidylcholine (×).

References

[1] Carr, M. H. and Scholerb, P. R. (1959) J. Lab. Clin. Med. 53, 646–652.
[2] Lavenson, S. M., Nagler, A. N. and Enheber, A. (1964) in: Shock (Hershey, S. G. ed) Churchill, London.
[3] Berman, I. R., Moseley, R. V., Lamborn, P. B. and Seeman, H. K. (1969) Ann. Surg. 169, 202–209.
[4] Baue, A. E., Wurth, M. A. and Sayeed, M. M. (1970) Surg. Forum 21, 8.
[5] Wurth, M. A., Sayeed, M. M. and Baue, A. E. (1972) Proc. Soc. Exp. Biol. Med. 139, 1238–1241.
[6] Greer, G. G. and Melazzo, F. H. (1975) Can. J. Microbiol. 21, 877–883.
[7] Mela, L., Bacalzo, L. V. and Miller, L. D. (1969) Am. J. Physiol. 220, 571–577.
[8] Greer, G. G., Epps, N. A. and Vail, W. J. (1973) J. Infect. Dis. 128, 724–729.
[9] Kato, M. (1972) Jap. J. Med. Sci. Biol. 25, 225–230.
[10] Moss, G. S., Erve, P. O. and Schumer, W. (1969) Surg. Forum 20, 24–25.
[11] Harris, R. A., Harris, D. L. and Green, D. E. (1960) Arch. Biochem. Biophys. 128, 219–230.
[12] Berry, L. J. Moore, R. N., Goodrum, K. J. and Couch, R. E., in: Microbiology, (Schlessinger, D. ed) pp. 286–292, Am. Soc. Microbiol.
[13] Hagberg, S., Haljamae, H. and Rockert, H. (1968) Ann. Surg. 168, 243–248.
[14] Haljamae, M. (1970) Acta Physiol. Scand. 78, 189–200.
[15] Green, D. E. and Perdue, J. F. (1966) Proc. Natl. Acad. Sci. USA 55, 1295–1302.
[16] Fleischer, S., Fleischer, B. and Stoeckenius, W. (1967) 32, 193–208.
[17] Berry, L. J. (1977) CRC Crit. Rev. Toxicol. 5, 239–318.
[18] Mela, L. M., Miller, L. D. and Nicholas, G. G. (1972) Surgery 72, 102–110.
[19] Priano, L. L., Miller, T. H. and Traber, D. L. (1974) Circ. Shock 1, 221–230.
[20] Flynn, J. T. (1978) J. Pharm. Exp. Ther. 206, 555–566.
[21] DePalma, R. G., Coil, J., Davis, J. H. and Holden, W. D. (1967) Surgery 62, 505–515.
[22] DePalma, R. G., Harano, Y., Robinson, A. and Holden, W. D. (1970) Surg. Forum 21, 3–6.
[23] Rhodes, R. S., DePalma, R. G. and Robinson, A. V. (1975) Am. J. Surg. 130, 560–564.
[24] Schneider, W. C. (1948) J. Biol. Chem. 176, 259–266.
[25] Folch-Pi, J., Lees, M. and Sloane-Stanley, G. H. (1957) J. Biol. Chem. 226, 497–509.
[26] Rouser, G., Fleischer, S. and Yamamoto, A. (1970) Lipids 5, 494–496.
[27] Metcalfe, L. D. and Schmitz, A. A. (1961) Anal. Chem. 33, 363–364.
[28] Christie, W. W., Noble, R. C. and Moore, J. H. (1970) Analyst 95, 940–944.
[29] Estabrook, R. W. (1967) Methods Enzymol. 10, 41–47.
[30] Tisdale, H. D. (1967) Methods Enzymol. 10, 213–215.
[31] Conde, G. (1980) PhD Thesis, Complutensis University, Madrid.