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MITOCHONDRIAL METABOLISM IN PORPHYRIC RAT LIVER

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SUMMARY

The metabolism of the mitochondria (porphyric mitochondria) isolated from the porphyric livers of rats whose porphyrin synthesis has been stimulated by administration of allylisopropylacetamide was studied and compared with the metabolism of normal mitochondria. Polarographic analysis of the porphyric mitochondria showed a lowered respiratory control with an enhanced rate of the State 4 respiration and no changes in ADP/O ratios. The disturbance of respiration was specifically located in the NAD-linked substrate oxidation; succinate oxidation was far less affected. The porphyric mitochondria showed slightly enhanced Mg^{2+}- and dinitrophenol-dependent latent ATPase activities and also higher ratios of cytochrome $c+c_1$ relative to mitochondrial cytochromes, *a* and *b*. Similar respiratory disturbances could be induced *in vitro* in normal mitochondria by adding δ-aminolevulinic acid.

INTRODUCTION

The initial step in the biosynthesis of heme in the mammalian liver cells involves the mitochondrial enzyme δ-aminolevulinic acid synthetase[1,2]. The enzyme can be readily induced *in vivo* by administering a porphyrinogenic chemical and experimental porphyria results[1–4]. Beattie and Stuchell[5] have reported that induction of mitochondrial δ-aminolevulinic acid synthetase in rat liver by allylisopropylacetamide is followed by increased synthesis of mitochondrial cytochromes $a+a_3$, *b* and $c+c_1$. They have suggested a close relationship between mitochondrial biogenesis and porphyrin biosynthesis in which the rate-limiting step is δ-aminolevulinic acid synthetase[5]. It remains unclear how these metabolic processes are related to each other especially as Porra and his associates[6], on the other hand, reported that the level of δ-aminolevulinic acid synthetase is about 10-fold higher in the semianaerobic than in the aerobic yeast. The present paper describes that the liver mitochondria having a high δ-aminolevulinic acid synthetase activity show some marked changes in their metabolism.

MATERIALS AND METHODS

To stimulate δ-aminolevulinic acid synthetase activity in mitochondria allylisopropylacetamide was subcutaneously injected three times every 12 h to female

Wister strain albino rats (100–180 g body wt) which have been fasted for 24 h. The dose of the agent was 20–25 mg per 100 g body wt at each administration and the animals were fasted throughout the treatment[4]. For each experiment porphyric liver mitochondria were prepared by the method of Hogeboom[7] from 4 to 6 rats, 12 h after the last injection.

Assay of mitochondrial activities

Oxygen uptake was estimated polarographically using a Clark oxygen electrode at 25 °C in a medium composed as follows, with a final volume of 2 ml: 100 mM sucrose, 20 mM KCl, 3 mM $MgCl_2$ and 5 mM Tris–HCl buffer (pH 7.4)[8]. ATPase activity was determined by the method of Takahashi[9] by measuring the P_i liberated from the added ATP at 25 °C in the medium containing 100 mM KCl, 5 mM $MgCl_2$ and 10 mM Tris–HCl buffer (pH 7.4) in a final volume of 2 ml. Cytochrome concentrations were determined by difference spectroscopy with the method of Chance[10] and Iwata *et al.*[11]. Protein concentrations were measured by the biuret method after solubilizing the mitochondria with 0.5% cholate and 0.9% deoxycholate[12]. Allylisopropylacetamide was a generous gift of Hoffman La Roche Co., Ltd. ATP, ADP and δ-aminolevulinic acid were obtained from Sigma Chemical Co., Ltd. The other chemicals were commercial products of the highest purity.

RESULTS

Mitochondria isolated from livers of rats into which allylisopropylacetamide has been injected show about 10-fold higher activity of δ-aminolevulinic acid synthetase than in normal mitochondria[4]. A typical experiment for comparing the respiratory activities of porphyric and normal mitochondria is shown in Fig. 1. State 4 respiration (respiration limited by lack of ADP) in the presence of β-hydroxybutyrate is significantly higher in porphyric mitochondria. Similar enhanced rates of State 4 respiration are also observed with NAD-linked substrates, such as pyruvate–malate, α-ketoglutarate or glutamate (not indicated in the data). The enhancement of respiration in the State 3 respiration, however, is much lower than in State 4 respiration. Hence the respiratory control index is significantly lowered in the NAD-linked respiration of porphyric mitochondria. On the other hand, the ADP/O ratio and the dinitrophenol-dependent respiration remain unchanged in porphyric mitochondria. However, State 4 and State 3 respirations in the succinate- or ascorbate-linked respiration are enhanced slightly so that the respiratory control values for both substrates are similar to those in the normal mitochondria. These results suggest one or more lesions between the respiratory chain and the energy transfer reactions in porphyric mitochondria. The data also suggest that the lesion is located in the respiratory chain between NAD and coenzyme Q; the succinate oxidation is far less affected.

Cowger and his coworkers[14,15] have reported that porphyrinogenic compounds are potent inhibitors of NADH oxidase. 5 mM allylisopropylacetamide produced 30% inhibition and 10 mM produced 60% inhibition without any effects on succinate oxidase in beef heart submitochondrial particles[14]. It is possible that the presence of allylisopropylacetamide may itself cause such disturbances. Similar results, however, were not observed on addition of allylisopropylacetamide to intact mitochondria isolated from livers of normal rats. As indicated in Table I, 8 mM allylisopropyl-

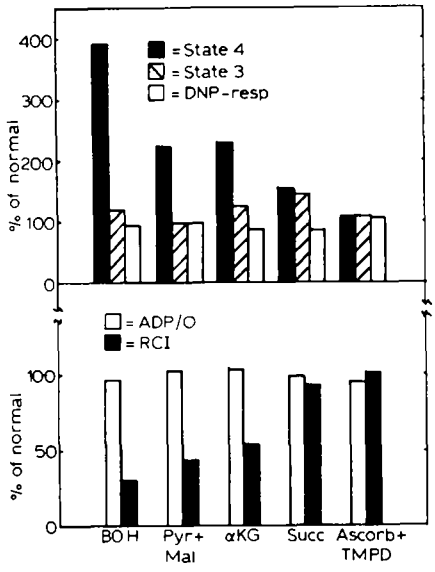

Fig. 1. The properties in the respiration and oxidative phosphorylation in mitochondria isolated from porphyric livers of rats treated with allylisopropylacetamide. To determine each parameter the experiment was carried out in the reaction medium containing mitochondria (4.0–5.46 mg protein), 2 mM P_i and a respiratory substrate. The final concentration of the substrate was 2 mM in β-hydroxybutyrate (BOH), α-ketoglutarate (αKG) and succinate (Succ), respectively, except 2 mM pyruvate (Pyr)+0.1 mM malate (Mal) and 2 mM ascorbate (Ascorb)+0.1 mM TMPD. The concentration of ADP and dinitrophenol (DNP) was 200 μM and 50 μM, respectively. The mean values of porphyric mitochondria were calculated from 3 different experiments and they were expressed as the percent activities or values of those in normal mitochondria. ADP/O ratio and the respiratory control index (RCI) were determined by the method of Hagihara[13]. The absolute values of each parameter in normal mitochondria was as follows: with BOH State 4, 1.95 natoms/min per mg protein; State 3, 27.0 natoms/min per mg protein; DNP-resp, 17.7 natoms/min per mg protein; ADP/O ratio, 3.13 and RCI, 13.84; with Pyr+Mal State 4, 2.67; State 3, 27.4; DNP-resp, 29.3; ADP/O, 2.99 and RCI, 10.30; with αKG State 4, 3.08; State 3, 39.4; DNP-resp, 37.0, ADP/O, 3.15 and RCI, 12.78; with Succ State 4, 11.91; State 3, 66.9; DNP-resp, 57.8, ADP/O, 2.15 and RCI, 5.62; with Ascorb+TMPD State 4, 40.4; State 3, 73.8; DNP-resp, 54.0; ADP/O, 1.89 and RCI, 1.83. DNP-resp, DNP-induced respiration. State 4 and 3, the respiration defined by Chance and Williams[21].

acetamide did not cause any disturbances in the succinate- or NAD-linked respiration. The results suggest that the intact rat liver mitochondria may be impermeable to allylisopropylacetamide.

Recently Matteis and Gibbs[16] reported that allylisopropylacetamide induced an excessive *de novo* synthesis of hepatic δ-aminolevulinic acid synthetase leading to an accumulation of δ-aminolevulinic acid in mitochondria. Thus, it is possible that δ-aminolevulinic acid might interfere with mitochondrial oxidative activities. The possibility was examined by the addition of δ-aminolevulinic acid to mitochondria isolated from livers of normal rats. Succinate- and β-hydroxybutyrate-linked respiration were measured, and showed characteristic features of porphyric mitochondria. As shown in Figs 2 and 3, δ-aminolevulinic acid stimulates State 4 respiration without any effects on the State 3 respiration in the presence of β-hydroxybutyrate as a respi-

TABLE I

NO EFFECTS OF ALLYLISOPROPYLACETAMIDE ON THE RESPIRATION AND OXIDATIVE PHOSPHORYLATION IN MITOCHONDRIA ISOLATED FROM LIVERS OF NORMAL RATS

Mitochondria (3.92 mg protein) were added to the reaction medium containing a respiratory substrate and 4 mM P_i and allylisopropylacetamide. The signs − and + show the absence and the presence of allylisopropylacetamide, respectively. For details refer to the legend in Fig. 1.

Substrate	*Allylisopropyl-acetamide* (8×10^{-3} *M*)	*Respiratory activity* (*natoms/mg protein per min*)			*Respiratory control index*	*ADP/O*
		State 4	*State 3*	*Dinitro-phenol*		
4 mM succinate	−	34.8	194.8	153.2	5.60	2.03
	+	34.2	198.4	150.7	5.80	2.01
4 mM α-ketoglutarate	−	10.9	80.6	98.8	7.42	3.08
	+	13.0	93.6	96.8	7.20	3.05
4 mM β-hydroxybutyrate	−	14.1	80.6	62.5	5.71	3.03
	+	12.9	74.6	50.1	5.78	2.94
2 mM ascorbate	−	194.8	305.5	265.8	1.58	1.18
+ 0.1 mM TMPD	+	191.4	300.5	251.0	1.57	1.20

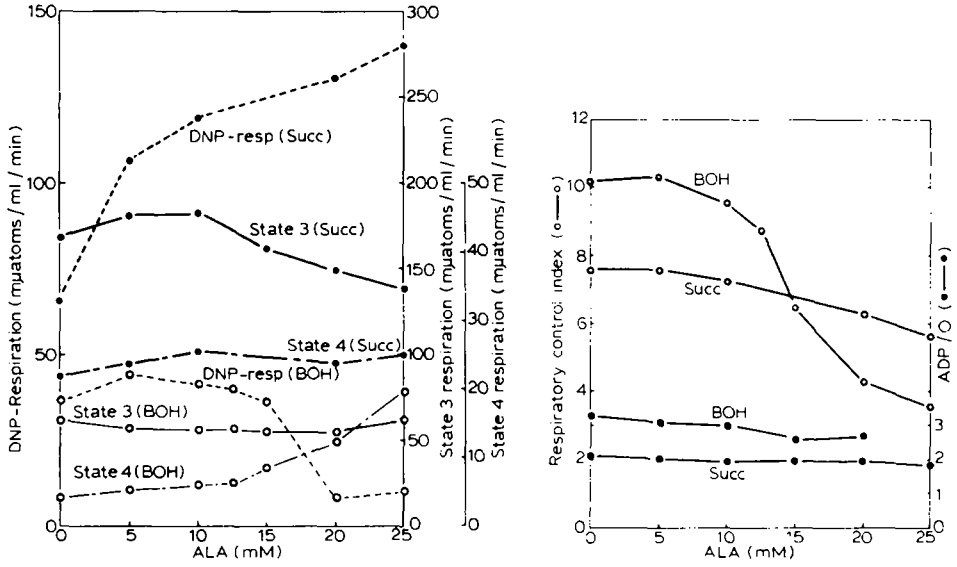

Fig. 2. The effects of δ-aminolevulinic acid (ALA) on the succinate- and β-hydroxybutyrate-linked respirations in mitochondria isolated from livers of normal rats. The reaction was carried out by adding mitochondria (4.8 mg protein) in the presence of 4 mM P_i, 4 mM succinate or 4 mM β-hydroxybutyrate and ALA in the concentration as indicated in the figure. The concentration of added ADP and dinitrophenol (DNP) was 250 μM and 10 μM, respectively. For details refer to the legend in Fig. 1.

Fig. 3. The effects of δ-aminolevulinic acid (ALA) on the oxidative phosphorylation in mitochondria isolated from livers of normal rats in the presence of succinate or β-hydroxybutyrate as a respiratory substrate. The data were deduced from the experiment presented in Fig. 2. For meaning of symbols see data in Fig. 1.

ratory substrate. As shown in Fig. 3, the respiratory control index decreases markedly along with an increase in the δ-aminolevulinic acid concentration. In succinate oxidation, 10 mM δ-aminolevulinic acid causes hardly significant effects in State 4 and State 3 respirations, but at a concentration higher than 10 mM the State 3 respiration is depressed so that a small but gradual decrease in the respiratory control index occurs with increasing δ-aminolevulinic acid concentration (Fig. 3). The ADP/O ratio in the presence of succinate remained normal and the ratio with β-hydroxybutyrate was only slightly affected (Fig. 3). δ-Aminolevulinic acid, however, stimulated dinitrophenol-dependent respiration in the presence of succinate and inhibited it in the presence of β-hydroxybutyrate.

The cytochrome contents of the porphyric mitochondria were compared with that of normal mitochondria (Table II). The concentration of cytochromes *a*, *b* and $c+c_1$ in normal mitochondria was approximately 0.19, 0.12 and 0.17 nmole per mg mitochondrial protein, respectively, that is in a ratio of 1:0.6:0.9. This is close to the ratio reported by Williams[17] and Iwata *et al.*[11]. However, the cytochromes *a* and *b* in porphyric mitochondria decreased as shown in Table II, but cytochrome $c+c_1$ concentrations were in an almost normal range. Thus in porphyric mitochondria there is a higher concentration of cytochrome $c+c_1$ relative to the other cytochromes.

TABLE II

COMPARISON OF THE CYTOCHROME CONCENTRATIONS IN THE MITOCHONDRIA ISOLATED FROM NORMAL AND PORPHYRIC RAT LIVERS

For porphyric livers rats were treated with allylisopropylacetamide (25 mg/100 g body wt) 3 times as described in the text. The concentration of each cytochrome was determined by the method of Chance[10] and Iwata *et al.*[11]. Concentrations are expressed as nmoles/mg protein.

Expt No.	*Mitochondria*	*Cytochrome a*	*Cytochrome b*	*Cytochrome* $c+c_1$	*Ratio of cytochrome contents (Cytochrome a:cytochrome b:cytochrome* $c+c_1$*)*
1	Normal	0.221	0.127	0.191	1:0.61:0.90
2		0.172	0.108	0.156	1:0.64:0.91
1	Porphyric	0.100	0.064	0.119	1:0.64:1.19
2		0.106	0.091	0.172	1:0.86:1.68
3		0.133	0.103	0.178	1:0.78:1.34
4		0.164	0.110	0.178	1:0.67:1.09
5		0.148	0.096		1:0.65
6		0.121	0.105	0.174	1:0.87:1.44
7		0.112	0.106	0.171	1:0.95:1.55

The lowered respiratory control in porphyric mitochondria suggests a changed activity in partial reactions of the energy transfer system. As shown in Fig. 4, dinitrophenol-dependent latent ATPase activity is about 6 times higher than that of Mg^{2+}-dependent ATPase in normal mitochondria. On the other hand, in porphyric mitochondria Mg^{2+}-dependent ATPase increased 2-fold and the activity of dinitrophenol-dependent latent ATPase also increased approximately 1.3-fold. Hence the ratio of dinitrophenol-ATPase to Mg^{2+}-ATPase is about 3.7 in porphyric mitochondria.

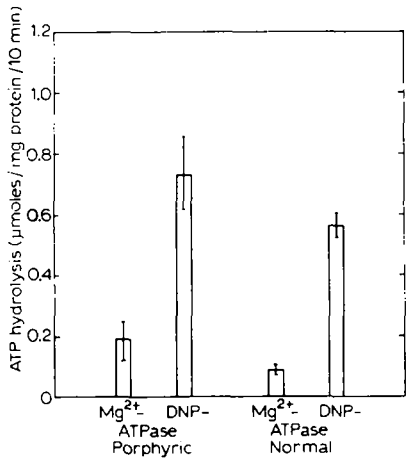

Fig. 4. Comparison of the ATPase activities in mitochondria isolated from normal and porphyric livers of rats. The reaction medium contained 150 mM KCl, 5 mM $MgCl_2$, 10 mM Tris-HCl buffer (pH 7.4) and 3 mM ATP with or without 10 μM dinitrophenol (DNP). The reaction was started by adding mitochondria (3.1 to 5.6 mg protein) and it was allowed to proceed for 10 min. The reaction was stopped by adding cold 1 M $HClO_4$ and the supernatant fluid was collected by a centrifugation after left standing 60 min in an ice-water bath. P_i released from the added ATP in the supernatant was determined by the method of Takahashi[9]. The bars in the columns indicate standard errors from 4 experiments.

DISCUSSION

The changes in mitochondrial metabolism, as reported here, occurring in porphyric mitochondria do not appear to be directly caused by allylisopropylacetamide as reported by Cowger and his co-workers[14,15]. But the present results cannot yet completely exclude a direct effect of allylisopropylacetamide to the metabolism in liver mitochondria when the agent is administered *in vivo*. It is possible that normal mitochondria, *in vitro* are impermeable to the porphyrinogenic agent.

The present data show that δ-aminolevulinic acid which may be produced in large amounts by allylisopropylacetamide injection causes disturbances in the NAD-linked substrate oxidation in the normal mitochondria similar to those seen in porphyric mitochondria except the action on the dinitrophenol-dependent respiration. The concentration of δ-aminolevulinic acid used in the present study may be much higher than is encountered in porphyric mitochondria so that the results may not be directly transposed to the metabolism of porphyric mitochondria. Jones and Jones[18], however, reported that δ-aminolevulinic acid passes through the mitochondrial membrane with difficulty so that a high concentration of δ-aminolevulinic acid may be required in the medium to permit a δ-aminolevulinic acid concentration in the mitochondrion equivalent to that which occurs in porphyric mitochondria.

It has been generally accepted that ADP/O ratio and the respiratory control index are correlated in respiratory chain oxidative phosphorylation system[19,20]. The present paper, however, indicated that the porphyric mitochondria showed a lowered respiratory control without any changes in ADP/O ratios in the NAD-linked respi-

ration and that the similar disturbances could also be induced *in vitro* in normal mitochondria by δ-aminolevulinic acid.

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