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STUDIES ON THE UPTAKE OF PORPHYRIN BY ISOLATED RAT LIVER MITOCHONDRIA

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Summary

1. The uptake of deuteroporphyrin by isolated rat liver mitochondria proceeds by two different mechanisms, a passive binding, and a mechanism sensitive to CCCP plus valinomycin, with different pH, temperature and time dependencies.

2. The CCCP plus valinomycin-sensitive uptake of deuteroporphyrin parallels the transmembrane potassium gradient ($[K^+_{in}]/[K^+_{out}]$).

3. Only that deuteroporphyrin taken up in parallel to the transmembrane potassium gradient is accessible to ferrochelatase.

4. The uptake of deuteroporphyrin at high concentrations is followed by a series of damaging effcects on the mitochondria: uncoupling, dissipation of the mitochondrial energy potential, increased ion permeability and leakage of endogenous potassium.

5. The detrimental effects of porphyrins at high concentrations on mitochondrial structure might explain the apparently unrelated metabolic aberrations characteristic of certain porphyric diseases.

Introduction

The ferrochelatase reaction of intact mitochondria differs from that of ultrasonically treated mitochondria [1].

The uncoupler CCCP which has no effect on the ferrochelatase activity of sonicated mitochondria [1] inhibits the ferrochelatase activity of iron-loaded whole mitochondria by approx. 70% [1]. Moreover, the ferrochelatase of intact mitochondria has significantly higher K'_m values for iron and porphyrin, a V approx. two-thirds that of sonicated mitochondria, and a tempera-

Abbreviations: CCCP, carbonyl cyanide *m*-chlorophenylhydrazone; EPPS, 4-(2-hydroxyethyl)-1-piperazine propane sulphonic acid; HEPES, *N*,-2-hydroxyethylpiperazine-*N'*-2-ethanesulphonic acid; PIPES, piperazine-*N*,*N'*-2-bis-(2-ethanesulphonic acid).

ture optimum 10°C higher than that of sonicated mitochondria. These results suggest that the mitochondrial inner membrane may represent a permeability barrier to both iron ions [2,3] and porphyrins.

The present paper deals with uptake of deuteroporphyrin by intact mitochondria and the dependence of at least part of this uptake on metabolic energy.

It was found that rat liver mitochondria took up deuteroporphyrin by two mechanisms: (1) a passive, energy-independent binding to structures of the outer compartment; (2) an uptake depending on the transmembrane potassium gradient. In agreement with previous studies [1,4], only deuteroporphyrin taken up by the latter process was accessible to the ferrochelatase.

A preliminary account of certain aspects of this work has already appeared [5].

Materials and Methods

Preparation of mitochondria and deuteroporphyrin. Rat liver mitochondria were prepared as previously described [6]. For the experiments with potassium-free media, the buffer was adjusted to the appropriate pH with Tris/base. The functional integrity of the preparations was tested by measuring the respiratory control ratio, using succinate as substrate [7]. Only preparations with respiratory control ratios greater than 4 were used.

Deuteroporphyrin IX was prepared as previously described [1]. The purity of the preparation was determined by thin-layer chromatography on silica gel after esterification [8]. The chromatograms were scanned on a Shimadzu dual wavelength thin-layer chromatography-scanner, model CS-900 equipped with a fluorescence accessory. Approx. 90% of the fluorescent material was recovered as a single spot (R_F-value 0.74).

Uptake of deuteroporphyrin. Mitochondria, approx. 2 mg of protein were preincubated in a final volume of 1 ml for 5 min at 25°C with 0.25 mol/l sucrose, 10 mmol/l HEPES buffer, pH 7.40 (adjusted with KOH). Further additions or omissions were as described in legends to figures and tables. The reaction was initiated by adding deuteroporphyrin, 15 μmol/l unless stated otherwise. At 30 s aliquots of 0.5 ml were withdrawn and the accumulation of deuteroporphyrin was terminated by adding 2 volumes of ice-cooled buffer, followed immediately by centrifugation in an Eppendorf microcentrifuge (Type 3200).

The amount of deuteroporphyrin accumulated was determined either from the decrease in the fluorescence of the supernatant following centrifugation, or from the increase in the fluorescence of the mitochondrial pellet extracted according to Chisolm and Brown [9]. Fluorescence was measured in a Jasco FP-4 fluorescence spectrophotometer on aliquots of the supernatant or of the mitochondrial extract diluted in 0.1 mol/l HCl. Exitation and emission wavelengths were at 393 and 593 nm, respectively. The spectral bandwidths of the exitation and the emission monochromators were 10 nm.

Other analytical methods. The spectral shift of safranine was determined by incubating mitochondria, approx. 1 mg/ml in the medium of the accumulation experiments (see above), but supplemented with 40 μmol/l safranine.

Further additions and experimental conditions were as described (see Figs. 1 and 7). The spectra were recorded on an Aminco DW-2 UV/VIS spectrophotometer using the wavelength pair 524—554 nm [10].

Mitochondrial swelling was determined by incubating the mitochondria approx. 1 mg protein/ml in the medium of the accumulation experiments (see above). Further additions were as described in legend to Fig. 2. The changes in absorbance at 520 nm were recorded.

Potassium was determined by flame photometry. The endogenous potassium concentration of the mitochondria was calculated by subtracting the potassium content of extramitochondrial pellet water determined according to the amount of [^{14}C]sucrose recovered in the pellet.

Deuteroheme was determined as pyridine deuterohemochrome [11].

Protein was determined by the Folin-Ciocalteau reagent [12]. All experiments were performed in subdued light.

Chemicals. ADP, ATP, oligomycin, 4-(2-hydroxyethyl)-1-piperazine propane sulphonic acid (EPPS), *N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulphonic acid (HEPES) (A grade), piperazine-*N*,*N'*-2-bis-(2-ethanesulphonic acid) (PIPES), carbonyl cyanide *m*-chlorophenylhydrazone (CCCP) and valinomycin were obtained from the Sigma Chemical Co. (St. Louis, Mo., U.S.A.). Deuteroporphyrin IX was purchased from the Porphyrin Products (Logan, Utah, U.S.A.). Safranine was purchased from Merck, AG (Darmstadt, G.F.R.), and [^{14}C]-sucrose was a product from The Radiochemical Center (Amersham, England). Nigericin was a gift from Professor K. van Dam (B.C.P. Jansen Institute, Amsterdam, The Netherlands). Other chemicals were of the highest purity commercially available. Double quartz distilled water was used throughout.

Results

Porphyrins are known to cause photodynamic damage to erythrocyte cell membranes as shown by damaged ultrastructure and colloid hemolysis [13—16]. These effects have been ascribed to lipid peoxidation and/or cross-linking of membrane proteins by singlet exited oxygen, generated by porphyrins exposed to long-wavelength ultraviolet light under aerobic conditions [14,15,17,18]. Furthermore, in aquous solution deuteroporphyrin as well as a number of uncouplers of oxidative phosphorylation behave as weak acids with amphophilic and lipophilic properties [19]. Thus it is not unexpected that deuteroporphyrin might have detrimental effects on the integrity of the mitochondria.

Deuteroporphyrin-lecithin micelles, equivalent to 15 nmol deuteroporphyrin/mg protein have no effect on the respiratory rate of rat liver mitochondria [4]. However, deuteroporphyrin added to respiring mitochondria in the absence of lecithin, induced a marked increase in the State 4 respiration rate at concentrations as low as 10 nmol deuteroporphyrin/mg protein (Fig. 1). In contrast to the rapid transient respiratory bursts obtained in the course of cation accumulation [20], the response with deuteroporphyrin was slow, reaching steady-state respiratory rate after a period of 4—6 min, whereafter it proceeded linearly untill anaerobiosis (figure not shown).

In rat liver mitochondria the energy-dependent stacking of safranine corre-

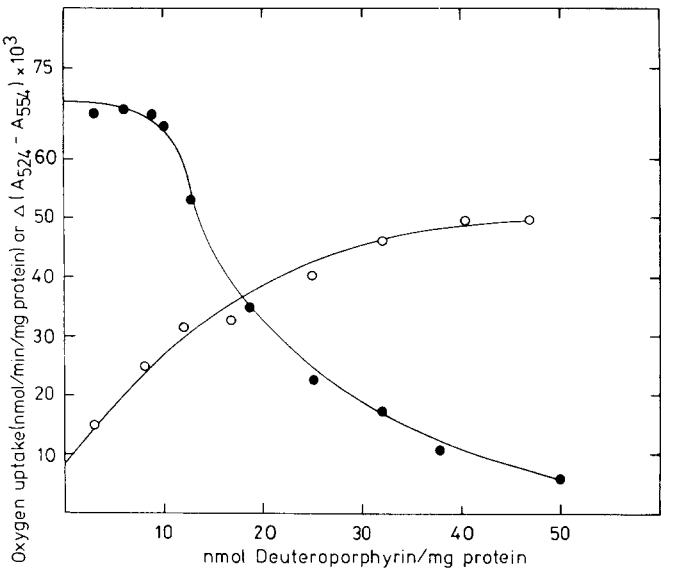

Fig. 1. Effect of deuteroporphyrin on the State 4 respiration rate (○) and on the CCCP-sensitive stacking of safranine in rat liver mitochondria (●). Mitochondria, approx. 2 mg of protein were incubated in a total volume of 3.0 ml: 50 mmol/l glucose, 175 mmol/l sucrose, 5 mmol/l Mg^{2+}, 5 mmol/l P_i and 10 mmol/l HEPES buffer, pH 7.40. The reaction was initiated by adding 10 mmol/l succinate. At steady-state respiration rate of deuteroporphyrin was added, and the new steady-state respiration rate (obtained within 4—6 min) was recorded. Temperature 25°C. In parallel experiments mitochondria, approx. 1 mg protein/ml were incubated as described (see Materials and Methods) in the presence of 40 μmol/l safranine. Temperature 25°C. Deuteroporphyrin was added, followed at 2 min by 5 μmol/l CCCP. The change in absorbancy $\Delta(A_{524nm} - A_{554nm})$ after adding CCCP was recorded.

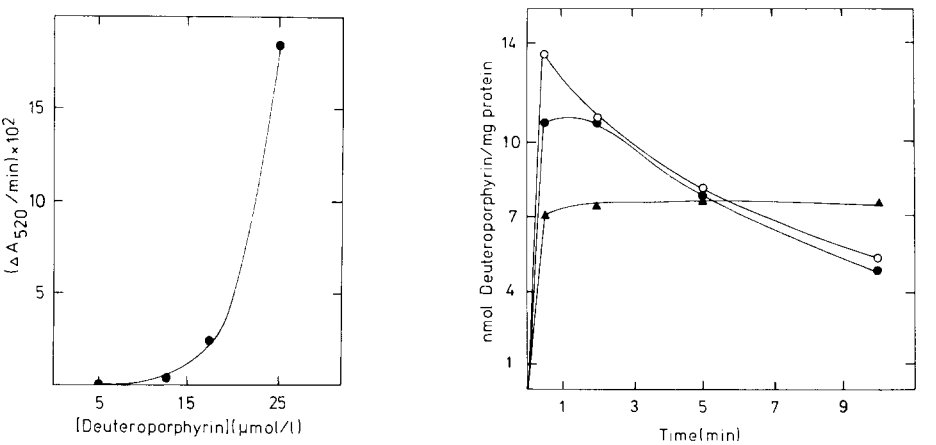

Fig. 2. Effect of deuteroporphyrin on the swelling of rat liver mitochondria. Mitochondria, approx. 2 mg protein/ml were incubated as described (see Materials and Methods). Deuteroporphyrin was added, and the swelling was determined from the steady-state rate of changes in turbidity, measured at 520 nm. The steady-state rate was obtain within 3—4 min.

Fig. 3. Time progress curve for the accumulation of deuteroporphyrin. Mitochondria, approx. 2 mg of protein were incubated as described (see Materials and Methods). Deuteroporphyrin, 8 nmol/mg protein (▲), 12 nmol/mg protein (●) and 16 nmol/mg protein (○), was added. At the time indicated, aliquots were withdrawn, and the uptake of deuteroporphyrin was determined as described (see Materials and Methods).

lates to the membrane potential under conditions of small variations in the transmembrane pH gradient [21,22]. As shown in Fig. 1 deuteroporphyrin at concentrations below 10 nmol/mg protein had no effect on the stacking of safranine in the time interval indicated.

The effect of deuteroporphyrin on the integrity of the mitochondria is evident also from the experiments reported in Fig. 2. At concentrations of deuteroporphyrin above 10—15 nmol/mg protein swelling of the mitochondria occurred concomittantly with collapse of the transmembrane pH gradient and release of matrix proteins (figure not shown).

The relevance of these experiments to this study is evident from results presented in Fig. 3. When mitochondria were incubated with increasing concentrations of deuteroporphyrin, the time-progress curves differed depending on the concentration of deuteroporphyrin. At 8 nmol/mg protein the uptake reached saturation level at 30 s, whereafter the uptake remained constant during the following 10 min. By increasing the concentration of deuteroporphyrin to 12 or 16 nmol/mg protein the uptake still reached a maximum at 30 s, but was followed by a discharge of the deuteroporphyrin which was quite marked after 3—4 min. This may be due to a time-dependent disruption of mitochondria at concentrations of deuteroporphyrin above 8—10 nmol/mg protein, in agreement with the results reported in Figs. 1 and 2. Therefore in the following experiments the incubation period has been limited to 30 s, and unless stated otherwise, the amount of deuteroporphyrin has been kept at 8—10 nmol/mg protein.

When the accumulation of deuteroporphyrin was determined from the increased fluorescence of the mitochondrial pellet, the uptake was approx. 95% of that determined from the decreased fluorescence of the supernatant (see Materials and Methods). Therefore being the least elaborate assay we have in most experiments determined the uptake of deuteroporphyrin from the decrease in the fluorescence of the supernatant.

Effect of respiratory substrates, inhibitors of metabolic activities and uncoupler on the uptake of deuteroporphyrin

The ferrochelatase activity of intact mitochondria was markedly influenced by changes in the energy state of the inner membrane and these phenomena could not be explained by an effect on the enzyme [1].

As seen from Fig. 4 the mitochondria revealed a CCCP-sensitive as well as a CCCP-insensitive uptake of deuteroporphyrin. In the absence of CCCP the uptake reached a saturation level of 15 nmol deuteroporphyrin/mg protein at concentrations of deuteroporphyrin between 50 and 100 μmol/l. In the presence of CCCP, the accumulation was largely reduced. The CCCP-sensitive uptake, i.e. the uptake in the absence, minus uptake in the presence of CCCP, reached a saturation level of approx. 9 nmol/mg protein at approx. 75 μmol/l of deuteroporphyrin.

The experiments reported in Fig. 4 were performed on mitochondria respiring on endogenous substrates. By supplementing the mitochondria with exogenous energy source (succinate, ATP), or inhibiting the energy-yielding reactions (CN^-, oligomycin), there were only negligible changes in the uptake of deuteroporphyrin (Table I). However, by adding valinomycin plus CCCP,

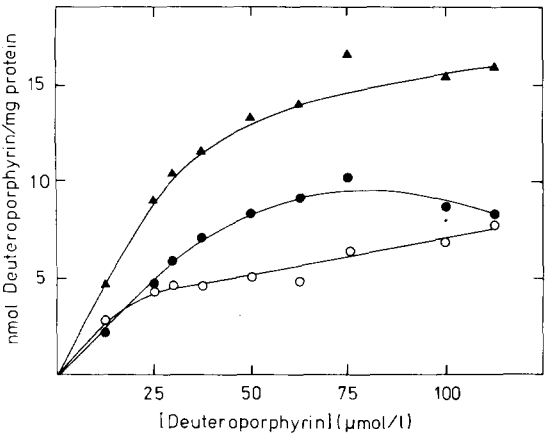

Fig. 4. Effect of increasing concentrations of deuteroporphyrin on the uptake of deuteroporphyrin. Mitochondria, approx. 2 mg of protein were incubated as described (see Materials and Methods) in the absence and presence of 5 μmol/l CCCP. Deuteroporphyrin was added, and the reaction was terminated at 30 s (for further details, see Materials and Methods). ▲, uptake of deuteroporphyrin in the absence of CCCP; ○, uptake in the presence of CCCP; ●, uptake of deuteroporphyrin in the absence of CCCP minus the uptake in the presence of CCCP.

there was a marked reduction in the amount of deuteroporphyrin taken up (Table I). These results suggested that the energy requirement for deuteroporphyrin uptake was low, or that the CCCP effect was indirect, mediated through the discharge of the transmembrane potassium gradient (see below). Valino-

TABLE I

EFFECT OF RESPIRATORY SUBSTRATES, INHIBITORS AND IONOPHORES ON THE ACCUMULATION OF DEUTEROPORPHYRIN

Mitochondria, approx. 2 mg protein/ml were incubated as described (see Materials and Methods). Further additions were (final concentration, added 4 min before deuteroporphyrin): 10 mmol/l succinate, 2 μg oligomycin/mg protein, 3 mmol/l KCN, 50 μmol/l ATP, 5 μmol/l CCCP and 0.5 μg valinomycin/mg protein. The results which are the means and ranges (in parentheses) from six separate experiments are given as the per cent values relative to that obtained in the absence of exogenous substrates or inhibitors.

	Deuteroporphyrin accumulated (%)	
Control	100 *	
+ succinate	103	(91—111)
+ oligomycin	101	(93—104)
+ valinomycin	101	(98—106)
+ ATP	99	(94—106)
+ KCN	98	(91—102)
+ valinomycin	99	(93—102)
+ oligomycin	97	(91—99)
+ KCN	87	(79—93)
+ valinomycin	100	(99—103)
+ CCCP	45	(33—48)
+ valinomycin	19	(9—34)
+ oligomycin	20	(10—39)

* The mean 100% value was 4.3 nmol deuteroporphyrin/mg protein.

mycin, alone or in combination with succinate or ATP, had no effect on the uptake of deuteroporphyrin.

Release of deuteroporphyrin taken up

From the localisation of the ferrochelatase [3], the effect of CCCP on ferrochelatase activity of iron-loaded mitochondria [1] and the effect of CCCP on the uptake of deuteroporphyrin by intact mitochondria (Fig. 4), it is tempting to suggest that the CCCP-sensitive uptake represents an influx of deuteroporphyrin possibly to the M-side of the inner membrane. This uptake obviously creates a considerable transmembrane concentration gradient of deuteroporphyrin. Thus if the deuteroporphyrin taken up distributes in an inner membrane and matrix space of approx. 1 μl/mg protein [23], deuteroporphyrin concentrations up to 9 mmol/l (Fig. 4) would be expected.

Preservation of concentration gradients created by mitochondrial accumulation of ions, mostly depends on available metabolic energy [20]. With iron, however, the matrix loading is essentially an irreversible unidirectional flux of iron ions [24].

The relationship between deuteroporphyrin release and available metabolic energy is outlined in Fig. 5. By rapidly cooling deuteroporphyrin-loaded mitochondria to +4°C, the mitochondria retained the deuteroporphyrin taken up by the CCCP-sensitive mechanism for approx. 3 min after which there was a significant release of deuteroporphyrin. The CCCP-insensitive deuteroporphyrin uptake remained essentially unchanged for at least 10 min at +4°C (figure not shown). Deuteroporphyrin release was proceeded by discharge

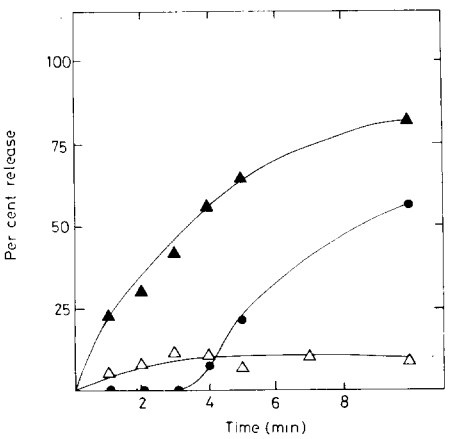

Fig. 5. Release of deuteroporphyrin and potassium from mitochondria at +4°C. Mitochondria, approx. 2 mg of protein were incubated with 15 μmol porphyrin/l as described (see Materials and Methods) in the absence and presence of 5 μmol/l CCCP. Deuteroporphyrin was added, and the reaction was terminated by adding two volumes of ice-cooled buffer. The deuteroporphyrin-loaded mitochondria, brought to +4°C were pelleted at the time intervals indicated. The results are given as the per cent release of deuteroporphyrin or potassium relative to the values found in mitochondria pelleted by the standard procedure. ●, release of CCCP-sensitive accumulated deuteroporphyrin; ▲, release of endogenous potassium in the presence of deuteroporphyrin. In parallel experiments release of endogenous potassium in the absence of deuteroporphyrin was measured (△).

of endogenous potassium, essentially as reported for photohemolysis induced by protoporphyrin in normal erythrocytes [13]. Note that in the absence of deuteroporphyrin there was minimal release of endogenous potassium.

Effect of pH and temperature on the uptake of deuteroporphyrin

The uptake of deuteroporphyrin was strongly dependent on pH. Thus, whereas, the CCCP-insensitive uptake increased with decreasing pH, approaching a level at pH approx. 6.5, the CCCP-dependent uptake, revealed a pH optimum at 7.4.

The uptake of deuteroporphyrin was also markedly influenced by changes in the temperature. A temperature optimum was found at 25—30°C for the CCCP-dependent uptake. This differs significantly from the temperature optimum found for the ferrochelatase reaction of intact mitochondria [1], but it is in agreement with temperature optima for other energy-dependent ion transport systems of mitochondria [2,20]. The CCCP-insensitive uptake of deuteroporphyrin increased in parallel to increasing temperature in the range tested (10—55°C).

Effect of potassium and the relationship between the uptake of deuteroporphyrin and the energy-dependent stacking of safranine

Valinomycin in the presence of CCCP markedly depressed the uptake of deuteroporphyrin (Table I) indicating that the intra-mitochondrial potassium concentration is important in controlling the uptake of deuteroporphyrin.

Table II shows the effect of CCCP with and without valinomycin and nigericin on the uptake of deuteroporphyrin and on the mitochondrial potassium content in potassium-free as well as in potassium-containing media. The inhibitors lowered, in parallel, both the potassium content and the uptake of deuteroporphyrin. The effect of nigericin was essentially as that obtained with CCCP plus valinomycin.

TABLE II

THE RELATIONSHIP BETWEEN THE ENDOGENOUS CONCENTRATION OF POTASSIUM AND THE ACCUMULATION OF DEUTEROPORPHYRIN

Mitochondria prepared in potassium-free media, were incubated as described (see Fig. 1) in a potassium-free medium (A) or in a medium containing 10 mmol/l potassium (B). The uptake of deuteroporphyrin and the potassium concentration in the pellet were determined as described (see Materials and Methods). The results, which are the means and the ranges (in parentheses) from six separate experiments, are given as the per cent values relative to that obtained in the absence of ionophores.

	A		B	
	Potassium of the pellet	Deuteroporphyrin accumulated	Potassium of the pellet	Deuteroporphyrin accumulated
5 μmol/l CCCP	60 (50—70) *	67 (64—72) **	63 (53—71) ***	63 (50—80) †
0.5 μg valinomycin/mg protein	53 (50—56)	62 (60—64)	90 (70—100)	85 (70—100)
0.5 μg valinomycin/mg protein + 5 μmol/l CCCP	25 (20—31)	40 (34—47)	41 (30—52)	40 (10—15)
0.5 μg nigericin/mg protein	28 (21—45)	35 (32—51)	40 (30—61)	44 (21—55)

The mean 100% values were: * 150 nmol/mg protein, ** 5.0 nmol/mg protein, *** 220 nmol/mg protein, † 5.2 nmol/mg protein.

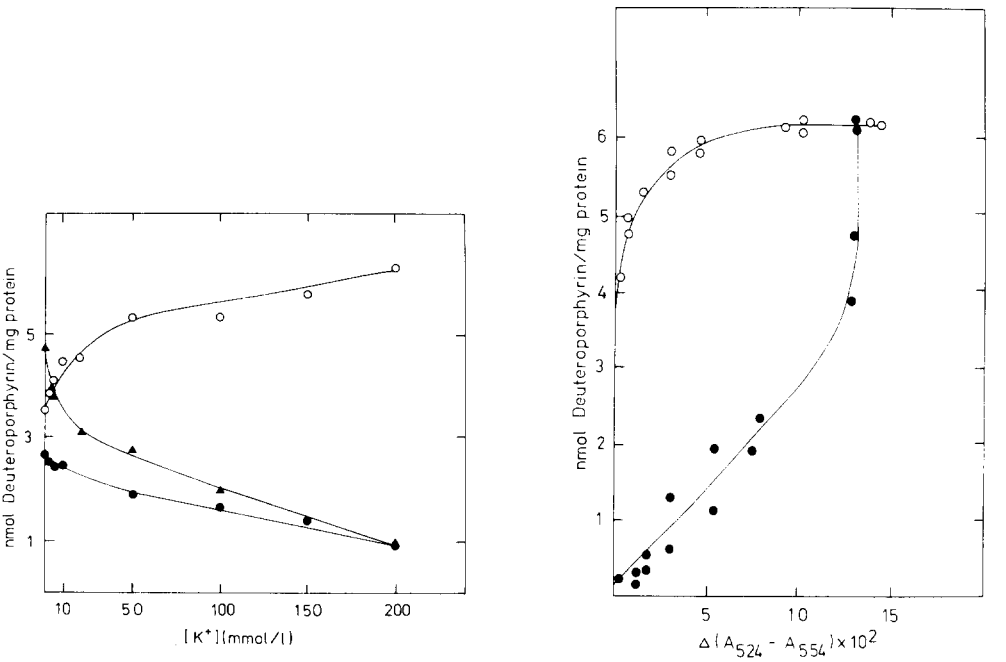

Fig. 6. Effect of increasing concentrations of potassium on the CCCP-insensitive (○), CCCP-sensitive (●) and CCCP plus valinomycin-sensitive uptake of deuteroporphyrin (▲). Mitochondria, approx. 2 mg of protein were incubated as described (see Materials and Methods) at increasing concentrations of KCl. The CCCP-sensitive uptake was calculated as described (see Fig. 4). The CCCP plus valinomycin-sensitive uptake was calculated from the difference between the uptake in the absence of ionophores minus the uptake in the presence of 5 μmol/l CCCP plus 1 μg valinomycin. The osmolarity of the incubation medium was kept constant by varying the concentration of sucrose.

Fig. 7. Relationship between CCCP-sensitive stacking of safranine and uptake of deuteroporphyrin in the absence and presence of valinomycin. Mitochondria, approx. 2 mg protein/ml were suspended in incubation buffer at increasing concentrations (0—5 μmol/l) of CCCP (○). After a preincubation period of 5 min, aliquots were removed for determination of the safranine stacking (see Fig. 1) or the uptake of deuteroporphyrin. In a parallel set of experiments the mitochondria were preincubated with 1 μg valinomycin plus increasing concentrations of CCCP (●).

By increasing exogenous potassium concentration, the effect of CCCP plus valinomycin leveled off (Fig. 6), indicating that rather than the intracellular concentration of potassium, it is the transmembrane potassium gradient ($[K^+_{in}]/[K^+_{out}]$) which is the driving force in the uptake of deuteroporphyrin.

We have studied the relationship between energy-dependent stacking of safranine [20,21] and uptake of deuteroporphyrin by titrating mitochondria with CCCP in the absence and presence of valinomycin (Fig. 7). By increasing the concentration of CCCP in the absence of valinomycin, the uptake of deuteroporphyrin was relatively unaffected within a wide range of changes in stacking of safranine, in agreement with the findings reported in Table I. By supplementing the mitochondria with valinomycin, a reduction in stacking of safranine upon addition of CCCP was associated with a decrease in the uptake of deuteroporphyrin. These findings were supported also by measuring the formation of deuteroheme in whole mitochondria. Thus in whole mitochondria, valinomycin had only marginal effects on deuteroheme synthesis

as determined by the pyridine deuterohemochrome method [11]. However, in the presence of CCCP, or CCCP plus valinomycin, the ferrochelatase reaction was inhibited approx. 70 and 90%, respectively.

Discussion

The partition of the enzymes concerned in the biosynthesis of heme both in mitochondria and cytosol has been suggested to be an important regulating factor in the overall heme synthesis [25]. However, although the fluxes of δ-aminolevulinic acid [26], iron [2] and heme [27] across the mitochondrial membranes are well documented and appearently strictly controlled, the significance of these transport processes to the regulation of the in vivo heme synthesis is as yet not known.

Recently we reported that CCCP had a marked inhibitory effect on ferrochelatase activity of intact mitochondria but had virtually no effect on the ferrochelatase of sonicated mitochondria [1]. Also the CCCP-sensitive ferrochelatase activity had a temperature optimum at 25—30° C [1]. These observations are in agreement with the results presented in this study, i.e. isolated rat liver mitochondria have a CCCP-sensitive mechanism for the uptake of porphyrin necessary for heme synthesis.

Porphyrins are known to bind to normal cells [28], neoplastic cells [29], lipids, lipoproteins and proteins [30—32]. According to Kosaki et al. [28,30, 31] porphyrins with carboxyl groups of the III-isomer series, but not those of the I-isomer series, bind to mitochondria with high affinity. Deuteroporphyrin IX, structurally very similar to protoporphyrin IX and slightly negatively charged at pH 7.40, possesses hydrophobic as well as hydrophilic groups [19], which might bind to ligands as reported for other porphyrins.

The transfer of deuteroporphyrin to the ferrochelatase within the mitochondria takes place by mechanism(s) quantitatively very similar to that reported for the uptake of iron [2]. An interesting feature is the time delay from the uptake in intact mitochondria, ending at 30 s, to the initiation of deuteroheme synthesis 2—3 min later (Figs. 3 and 4, and Fig. 2 of ref. 1). Apparently in whole mitochondria rather than in sonicated mitochondria there is some rate-limiting reaction(s) between the CCCP-sensitive step(s) and the ferrochelatase reaction. This applies to the uptake of deuteroporphyrin as well as the uptake of iron [1].

The uptake of iron depends on reducing equivalents supplied by the respiratory chain and it operates at a relatively high energy pressure [6]. For the uptake of deuteroporphyrin, the requirement of metabolic energy might be an indirect one through the maintenance of a transmembrane potassium gradient.

The importance of the transmembrane potassium gradient to the uptake and retention of deuteroporphyrin is supported by several findings: the inhibition of the uptake by CCCP plus valinomycin (or nigericin) (Table II), the inhibition of the uptake by increasing the exogenous concentrations of potassium (Fig. 6), the disproportion between the energy-dependent stacking of safranine and the uptake of deuteroporphyrin in the presence of increasing concentrations of CCCP plus valinomycin (Fig. 7), and the release of deuteroporphyrin

subsequent to the discharge of intra-mitochondrial potassium (Fig. 5). These results all indicate that the accumulation and the retention of deuteroporphyrin in a compartement and in a form available to deuteroheme synthesis depends on a potassium gradient across the inner membrane. Considering the anionic nature of the deuteroporphyrin molecule these results all agree with the findings that the uptake of metabolite anions parallels the amount of endogenous potassium [33—36]. However, whereas the uptake of metabolite anions depends on the transmembrane pH gradient rather than the transmembrane potassium gradient, to the uptake of deuteroporphyrin the potassium gradient appears the more important (Fig. 7). This assumption is supported also by the observation that the rate of potassium efflux in the presence of uncoupler is far below that obtained in the presence of uncoupler plus valinomycin [37] (see Table II and Fig. 5). Our results (Table II) agree with the observations that in the absence of respiratory substrates, the influx of potassium in valinomycin-treated mitochondria depends on the exogenous potassium concentration [33—37]. No increase in deuteroporphyrin uptake was seen in media supplemented with an energy source (succinate or ATP) plus valinomycin (Table I), thus differing from results obtained with metabolite anions [23,38]. This could be explained by a considerable energy reserve of the mitochondria (respiratory control ratios mostly above 5), a relatively low capacity for deuteroporphyrin accumulation, and the operation of the uptake at zero-order rate with respect to the transmembrane potassium gradient above certain limits.

An important aspect of this study concerns the detrimental effect on the mitochondrial integrity observed at increasing concentrations of deuteroporphyrin (Figs. 1—3). Thus, at >10 nmol deuterophorphyrin/mg protein and incubation times beyond 2—3 min, the mitochondria became uncoupled, dissipated the energy potential, underwent marked swelling and discharged their potassium content. These effects, although mostly determined by the exogenous deuteroporphyrin concentration, were accentuated in mitochondria accumulating deuteroporphyrin.

Ten years ago, Labbe [39] forwarded the hypothesis that impaired mitochondrial energy generation might be responsible for some of the aberrations characteristic of human porphyrias. Evidence supporting this hypothesis are the inverse correlation between ATP and porphyrin concentration in rat liver after administration of the porphyrinogenic drug apronal [40], an enhanced State 4 respiration rate in mitochondria isolated from allylisopropylacetamid-treated rats [44], a 3-fold increase in State 4 respiration rate in fibroblasts cultured from patients with acute intermittent porphyria [42], an inverse correlation between the effect of porphyrinogenic drugs on the inhibition of terminal NADH oxidase activity and induction of porphyrin synthesis [43], and a marked inhibition of the ferrochelatase of bone marrow cells at protoporphyrin concentrations above 10^{-4} mol/l [44]. These observations all support the assumption that porphyrins at appropriate concentrations might affect the mitochondrial energy generation, as shown in the present study. Quantitatively, however, it is difficult to extrapolate our results to the in situ situation, mainly because porphyrins are not free in the cytosol [45].

In erythrocytes from patients with erythropoietic protoporphyria photo-

oxidation and colloid-osmotic hemolysis were proceeded by potassium loss [13,14]. Similar results were found when erythrocytes from healthy persons were incubated with high concentrations of porphyrin and exposed to long-wavelength ultraviolet light [13,14]. Our findings in mitochondria are in accordance with these observations. It should be noted that the mutilating effects of deuteroporphyrin are not dependent on light exposure, and to some extent they can be counterbalanced by providing the mitochondria with an energy source (Fig. 3 compared to Fig. 5).

In conclusion, it is tempting to suggest that mechanisms similar to those here described, i.e. the uptake of porphyrin by the mitochondria to noxious levels, energy dissipation, potassium loss and discharge of non-committed porphyrin to the cytosol might be operative in porphyria.

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