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Effect of unbalanced diets on incorporation of δ-aminolevulinic acid into cytochrome P-450

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The in vivo syntheses of two liver microsomal cytochromes P-450 PB_{3a}, P-450 UT_{50} [(1987) Eur. J. Biochem., submitted] (M_r 50 000, 52 000) have been estimated by measuring the specific activity 2 h after i.p. administration of δ-[^{3}H]aminolevulinic acid to male Sprague Dawley rats. The animals were fed either a standard rat chow (5% lard, 22% casein) or unbalanced diets (high lipid, 30% lard or low protein, 6% casein) with or without 50 ppm Phenoclor DP6. The high-lipid diet supported a more rapid body weight gain but had little impact on cytochrome P-450 content, expressed either per whole liver or per mg microsomal protein, and on the incorporation of the precursor into cytochrome P-450. The latter was determined by measuring the radioactivity incorporated into the cytochrome P-450 fraction, partially purified by afffinity chromatography, as well as into two cytochrome P-450 isozymes (M_r 50 000 or 52 000) purified by DEAE-52 cellulose ion-exchange chromatography. The low-protein diet, on the other hand, severely depressed body weight gain and cytochrome P-450 content as well as incorporation of radioactivity, the lower-M_r cytochrome (M_r 50 000) being particularly affected. However, when a potent inducer, Phenoclor DP6, was added to the low-protein diet, cytochrome synthesis was restored indicating that the effect was reversible.

Cytochrome P-450 synthesis; Dietary protein; Polychlorobiphenyl; Nutritional effect; δ-Aminolevulinic acid

1. INTRODUCTION

In rat liver several diverse cytochromes (cyt.) P-450 exist, many of which can be increased by treatment with different xenobiotics [1-3]. Moreover, nutritional disorders may cause changes in the levels of total cytochrome P-450 [4-7] and their characteristics [8-10]. Knowledge of the rate of synthesis of cyt. P-450 is needed for the interpretation of regulatory mechanisms in concentration changes.

Here, we have evaluated the synthesis rate of two cyt. P-450 isoenzymes from liver of rats fed either standard or unbalanced diets with or without treatment with the inducer Phenoclor DP6. From N-terminal sequence analysis, these proteins cyt. P-450 PB_{3a} and cyt. P-450 UT_{50} can be compared with cyt. P-450b and $P\text{-}450_1$ described by Botelho et al. [2] and Levin et al. [11], respectively.

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2. MATERIALS AND METHODS

Weanling Sprague Dawley male rats were fed a standard diet (St) containing 22% casein and 5% lard ad libitum for 1 week and then divided into 3 groups. Group 1 was fed the standard diet, group 2 a high-lipid diet (HL 30% lard), and group 3 a low-protein diet (L_P 6% casein). Experimental diets were fed for 6 weeks. Each group was further divided, half of the animals being fed the ex-

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perimental diet containing 50 ppm Phenoclor DP6 [a French polychlorobiphenyl (PCB) from Prodelec] for the last 4 weeks. δ-[^{3}H]Aminolevulinic acid (δALA, 510 mCi/mmol) from New England Nuclear dissolved in 0.9% NaCl solution was administered to rats by intraperitoneal injection (50 μCi/100 g body wt) 2 h before killing. Microsomes were isolated from liver as in [12]. Cyt. P-450 was solubilized with sodium cholate and purified by affinity chromatography on ω-*n*-diaminooctyl-Sepharose 4B (AOS), prepared as described [13]. Purified cyt. P-450 was eluted with 0.08% non-ionic detergent (Emulgen 911) at a specific content of 17 nmol per mg protein with a recovery of approx. 45% of total microsomal cyt. P-450. Cyt. P-450 eluted from the affinity column was immediately pooled and dialyzed overnight against 10 mM phosphate buffer and applied on a Whatman DEAE-52 cellulose ion-exchange column previously equilibrated with 600 ml of 10 mM potassium phosphate buffer (pH 7.25) containing 20% glycerol, 0.1 mM EDTA and 0.2% sodium cholate. After sample application the column was washed with equilibration buffer followed by a linear gradient of NaCl (0–300 mM) in 500 ml of the same buffer during which cytochrome isoenzymes were sequentially eluted. Each showed a single protein staining band when analyzed by SDS-polyacrylamide gel electrophoresis and molecular masses of 50 and 52 kDa, respectively. The identification of cytochrome isoenzymes by means of antibody cross-reaction, amino acid analysis, catalytic activity and N-terminal analysis has been reported separately [14]. The specific contents were 14–19 nmol cyt. P-450/mg protein. Samples of total cyt. P-450 eluted from the aminooctyl-Sepharose column (AOS P-450) and the purified isoenzymes were dissolved in soluene 350 and toluene PPO-POPOP mixture. Radioactivity was determined using a Searle 300 liquid scintillation counter. Protein was assayed as described by Lowry et al. [15]. Total microsomal cyt. P-450 was measured as reported by Omura and Sato [16].

3. RESULTS AND DISCUSSION

The rate of biosynthesis of total cyt. P-450 and those of two purified cyt. P-450 isozymes were estimated in the liver of rats treated with standard and unbalanced diets with and without Phenoclor DP6, a potent cyt. P-450 inducer.

Levels of translatable mRNA coding for cytochromes P-450 are increased by various inducers [17] indicating an increased rate of cyt. P-450 synthesis. Previous estimations of the turnover rate of cyt. P-450 have been based on the rate of degradation of the heme moiety of cyt. P-450 after removal of cyt. b_5 by protease digestion [18]. In 1981 we showed [19] by using simultaneously [^{3}H]δALA and [^{14}C]guanidinoarginine (low reutilizable precursor) that the turnover of the two moieties of cyt. P-450 was synchronous. This observation was confirmed in 1982 in purified cyt. P-450 [20]. Thus, the level of incorporation of [^{3}H]δALA into the cyt. P-450 heme can be related to the synthesis rate of the two cytochrome moieties.

Prolonged dietary and inducer treatments were chosen to ensure steade-state conditions for cyt. P-450 isozyme biosynthesis. Therefore, when the precursor was injected the precursor pool/cyt. P-450 pool ratio was quite different in the experimental groups. Thus, the specific values calculated in dpm/nmol cyt. P-450 must be corrected by a factor to account for these differences as first suggested by Kuriyama et al. [21], these factors being reported in table 1. For example, control rats were injected with 50 μCi/100 g body wt, namely 148 μCi. The total content of liver cytochrome was 253 nmol. For the low-protein group 63 μCi δALA were injected, the cyt. P-450 liver content being 68 nmol. The precursor/cytochrome ratio was calculated as follows: body wt of the standard diet group × total liver cyt. P-450 of the treated group/body wt of treated group × total liver cyt. P-450 in the standard diet group (treated groups refer to unbalanced diet fed groups and DP6-treated groups). Table 2 lists the effects of unbalanced diets and DP6 treatment on the incorporation of [^{3}H]δALA into cyt. P-450. The interpretation must be carried out on the basis of the corrected values.

In all experimental groups the incorporation of precursor was higher for the higher-M_r cyt. P-450 isozyme, P-450 PB_{3a}. Based on our characterizations of ligand binding, catalytic activity, amino acid composition and N-terminal sequences ([11] and unpublished), this protein can be compared with the cytochromes P-450 purified by Botelho et

Table 1

Body weight and hepatic cytochrome P-450 content of liver microsomes from rats fed standard or unbalanced diets with or without DP6

	St[a]	HL	Lp	St + P	HL + P	Lp + P
Body weight (g)	296 ± 7[b]	329 ± 8	127 ± 6	297 ± 9	320 ± 11	129 ± 7
Cytochrome P-450						
(nmol/whole liver)	253 ± 25	287 ± 18	68 ± 6	747 ± 88	1328 ± 97	233 ± 11
(nmol/mg microsomal protein)	0.925 ± 0.084	1.027 ± 0.061	0.720 ± 0.104	1.684 ± 0.061	1.959 ± 0.079	1.450 ± 0.146
Correction factor[c]	1	1.02	0.63	2.94	4.86	2.13

[a] Rats were fed standard diet (St) or unbalanced diets (HL, high-lipid; Lp, low-protein) with (+ P) or without Phenoclor DP6

[b] Means ± SD from 8 animals are shown

[c] Correction factor: (body wt St × total liver P-450 treated)/(body wt treated × total liver P-450 St)

Table 2

Effect of unbalanced diet and DP6 treatment on the incorporation of [^{3}H]δALA into cytochrome P-450

	Cytochrome P-450 (dpm/nmol)					
	Uncorrected values			Corrected values		
Group	AOS P-450[a]	P-450 UT_{50}	P-450 PB_{3a}	AOS P-450	P-450 UT_{50}	P-450 PB_{3a}
St[b]	42492[c] ± 3806	33846 ± 2309	56258 ± 4308	42000	34000	56000
HL	43068 ± 2968	33420 ± 2030	55348 ± 3785	43000	34000	56000
Lp	1118 ± 103	288 ± 90	2141 ± 129	700	200	14000
St + P	29006 ± 2202	15208 ± 1304	36155 ± 2628	85000	45000	106000
HL + P	15856 ± 1185	8415 ± 597	20079 ± 1504	77000	41000	98000
Lp + P	20591 ± 1692	19025 ± 1008	33036 ± 2466	44000	40000	70000

[a] AOS P-450: total cytochrome P-450 eluted from ω-aminooctyl-Sepharose by Emulgen 911 as indicated in section 2

[b] See footnotes to table 1

[c] Means ± SD of 4 determinations are shown

al. [2], cyt. P-450 UT_{50} being identical or very similar to cytochrome $P\text{-}450_1$ [11]. From the decay of radioactivity in the heme and the protein moieties, Parkinson and co-workers [22], studying cyt. P-450 turnover in Arochlor 1254-treated immature male long Evans rats, assumed that two kinetically distinct populations of cyt. P-450a may exist. The half-life calculated for cyt. P-450a from the slow kinetics of radioactivity decay was 40% longer than that calculated from the decay of radioactivity in cyt. P-450b. In the present experiments the incorporation of [^{3}H]δALA was 40% lower in cyt. P-450 UT_{50} than in cyt. P-450 PB_{3a} in untreated rats. From this it may be concluded that cyt. P-450 UT_{50} isolated from untreated rats corresponds to the slow kinetic population of cyt. P-450a described by Parkinson et al. [23]. The results also suggest that a new population of cyt. P-450 UT_{50} with a fast kinetic incorporation of radioactivity appears after treatment by inducers. With high-lipid diet incorporation into the enzymic forms of cyt. P-450 remained unchanged compared with the standard diet group. However, the low-protein diet strongly decreased incorporation of the precursor in both isoenzymes and AOS P-450. Compared with the standard diet group incorporation was only 1.6% in AOS P-450, 0.5% in P-450 UT_{50} and 25% in P-450 PB_{3a}. This decrease in synthesis may explain the decrease in total liver cyt. P-450 to one quarter of that present during the standard diet (table 1). The treatment with Phenoclor DP6 increases the incorporation of [^{3}H]δALA into AOS cyt. P-450 up to 2-fold. The synthesis of cyt. P-450 PB_{3a} shows a larger increase (90%) than that of cyt. P-450 UT_{50} (32%). Parkinson et al. [23] showed that cyt. P-450b was highly induced by 2,2′,4,4′,5,5′-hexachlorobiphenyl and that the induction reached a maximum 5 days after PCB treatment and remained maximally induced for 2 weeks after treatment. 2,2′,4,4′,5,5′-Hexachlorobiphenyl is a major constituent of Phenoclor DP6 and the induction of cyt. P-450b can be related to the high induction in demethylase activity by this PCB [23].

The increased incorporation of precursor in cyt. P-450 UT_{50} may be due either to an increase in the rate of synthesis of the population of cyt. P-450 UT_{50} found in untreated rats or to the appearance of a new population of cyt. P-450 UT_{50} with fast kinetic parameters. We have previously shown [14] that the catalytic activities were quite different between cyt. P-450 UT_{50} isolated from untreated animals and cyt. P-450 UT_{50} from DP6-treated rats whereas the catalytic activities of cyt. P-450 PB_{3a} remained largely unchanged on DP6 treatment. When Ryan and co-workers [24] isolated P-450a from immature male Long Evans rats pretreated with Aroclor 1254, phenobarbital or 3-methylcholanthrene, they found considerable variations in the specific activities of benzo(*a*)-pyrene hydroxylase, ethoxycoumarin-*O*-deethylase and zoxazolamine hydroxylase [24]. Although these authors did not purify cyt. P-450a from the livers of untreated rats, one explanation for the

variation in activity they observed in induced rats may be the different extents to which individual members of a cyt. P-450a family are increased following inducer treatment. Our results support the existence of a new population of cyt. P-450 UT_{50}. The increases in precursor incorporation into cyt. P-450 isoenzymes by Phenoclor DP6 were not modified by high-lipid diet. In the group fed a low protein + DP6 diet the incorporation of [^{3}H]-δALA is slightly increased (20%) compared with animals on the standard diet and not treated with inducers. However, when the influence of DP6 in the animals fed the low-protein diet was studied the increase caused by DP6 was 200-fold for cyt. P-450 UT_{50} and 5-fold for cyt. P-450 PB_{3a}. Thus it can be concluded that low-protein diet strongly decreases the synthesis of cyt. P-450 UT_{50} and P-450 PB_{3a}, but treatment with DP6 restores a normal synthesis rate to both enzymic forms.

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