INDUCTION OF CYTOCHROME P-450 BY PERFLUORODECALIN IN MOUSE LIVER MICROSOMES

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One of the most important properties of cytochrome P-450 is its inducibility. This describes an increase in the concentration of cytochrome P-450 by enzymes coupled with it in the membranes of the endoplasmic reticulum and also intensification of enzyme activity relative to oxidation substrates in response to administration of an inducing agent [11]. More than 300 chemical compounds capable of inducing cytochrome P-450 are known. Recently most attention has been paid to the group of organic perfluoro compounds, some of which, notably perfluorodecalin, have a strong inducing action on cytochrome P-450 in the liver [5]. Since these substances are utilized as plasma expanders, the study of their action on the microsomal system is particularly important. Meanwhile the study of the molecular structure of these compounds would help us to understand better the mechanism of induction of cytochrome P-450. Organic perfluoro compounds form a type 1 enzyme-substrate complex with cytochrome P-450 [17], accelerate oxidation of NADPH, and increase oxygen consumption, but they themselves are not hydroxylated in the process [5]. This is because of particular features of the molecular structure of these compounds. The limiting electronegativity of the fluorine atom is the reason for their complete biochemical inertness [13]. Although the direct addition of organic perfluoro compounds to the suspension of microsomes [8] or perfusion of the liver with them [9] does not cause any appreciable change in the metabolism of substrates of cytochrome P-450, they do increase the concentration of the latter by several times and also accelerate hydroxylation of the substrates 24 h or more after their administration [5].

The aim of this investigation was to study the inducing action of perfluorodecalin on cytochrome P-450 in mouse liver. Despite the similarity in their mechanism of induction, perfluorodecalin has a much stronger inducing action than phenobarbital.

EXPERIMENTAL METHOD

CBA mice weighing 25-35 g were used in the experiments. The animals were kept at 24°C and were given food and water ad libitum. All injections were given intraperitoneally. Phenobarbital sodium (100 mg/kg) was injected in 0.9% NaCl solution, whereas perfluorodecalin (from 0.03 to 1 ml/kg) was given in the composition of phosphatidylcholine liposomes. A known quantity of the phospholipids was dissolved in chloroform, dried in vacuo on a rotary evaporator at 35°C, then treated twice with diethyl ether to remove all the solvent. The thin layer of lipids formed on the walls of the flask was removed with water with vigorous shaking after the addition of a certain quantity of perfluorodecalin. The liposomes were then sonicated at 20,000 Hz at 10°C for 30 min, with intervals of 30 steps every 2 min. The resulting suspension, containing 60 μl of perfluorodecalin in 1 ml, was used immediately before preparation. Control animals received an equal volume of 0.9% NaCL solution or of the suspension of liposomes. Microsomes were isolated by differential centrifugation [3], with reprecipitation in a solution of 100 mM sodium pyrophosphate and 100 mM sodium citrate to remove any adsorbed proteins. The concentration of cytochrome P-450 in the suspension of microsomes and in the liver tissue homogenate was determined spectrophotometrically [12, 15]. Microsomal proteins were separated by electrophoresis in the presence of sodium dodecylsulfate [10]. After staining with

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TABLE 1. Time Dependence of Cytochrome P-450 Induction by Perfluorodecalin and Phenobarbital in CBA Mouse Liver (M ± m)

Time of induction, h	Cytochrome P-450 concentration (in nmoles) on induction by			
	perfluorodecalin		phenobarbital	
	homogenate, nmoles/g liver	microsomes, nmoles/mg microsomal proteins	homogenate, nmoles/g liver	microsomes, nmoles/mg microsomal proteins
0	28,5±4,3	0,9±0,2	28,5±4,3	0,9±0,2
3	35,6±5,1	0,8±0,2	35,9±1,8	0,9±0,1
6	38,9±3,0	1,0±0,1	38,0±2,4	1,0±0,1
12	59,1±3,9	1,3±0,1	41,1±2,8	1,3±0,2
24	66,0±6,2	1,7±0,1	61,3±3,1	1,6±0,1
48	124,3±4,8	5,3±0,2	51,6±4,7	1,4±0,1
72	61,8±4,7	1,5±0,1	—	—

TABLE 2. Dose Dependence of Cytochrome P-450 Induction by Perfluorodecalin in CBA Mouse Liver (M ± m)

Dose, ml/kg	Cytochrome P-450 concentration (in nmoles) on induction by perfluorodecalin*	
	homogenate, nmoles/g liver	microsomes, nmoles/mg microsomal proteins
0	28,5±4,3	0,9±0,2
0,03	21,7±4,1	0,8±0,1
0,1	62,2±3,2	3,8±0,2
0,3	90,4±3,0	3,9±0,2
0,4	90,7±4,1	5,3±0,3
0,6	124,3±6,5	3,8±0,5
1,0	121,0±5,5	4,2±0,4

Legend. *Perfluorodecalin was injected into the animals 48 h before sacrifice.

Coomassie brilliant blue R-250 the gel disks were scanned on a densitometer (Helena, France) equipped with an integrator. The reaction velocity of N-demethylation of aminopyrine was determined by accumulation of formaldehyde [14], and of *p*-hydroxylation of aniline by the accumulation of *p*-aminophenol [7]. The protein concentration was determined by Lowry's method [11]. The results were subjected to statistical analysis by the usual methods [6].

EXPERIMENTAL RESULTS

The aim of the investigation was to study induction of cytochrome P-450 in the liver by perfluorodecalin. For this purpose we determined how induction of cytochrome P-450 depended on the dose of perfluorodecalin and the time after its injection. The well known inducer phenobarbital was used for comparison. The cytochrome P-450 concentration in this case reached a maximum 24 h after intraperitoneal injection of phenobarbital in a dose of 100 mg/kg (Table 1), in good agreement with data in the literature [4]. In the case of perfluorodecalin, the peak concentration of cytochrome P-450 was reached 48 h after injection of the optimal dose of the inducer (0.6 ml/kg for homogenate and 0.4 ml/kg for microsomes). The concentration of cytochrome P-450 both in the homogenate and in the microsomes 24 h after injection of perfluorodecalin was twice as high as the control level, and the same result was observed during induction by phenobarbital. However, between 24 and 48 h after injection of perfluorodecalin the cytochrome P-450 concentration in the liver continued to rise (fourfold and sixfold for homogenates and microsomes respectively). The dose dependence of cytochrome P-450 induction in the mouse liver for perfluorodecalin was determined using an optimal induction time of 48 h (Table 1). As Table 2 shows, the cytochrome P-450 concentration in the liver rose gradually with an increase in the dose of perfluorodecalin and reached a maximum in response to injection of optimal doses of the inducer (see above). Increasing the dose of perfluorodecalin to 1 ml/kg did not cause any increase in the cytochrome P-450 concentration in the liver. The relative concentration of the hemoprotein,

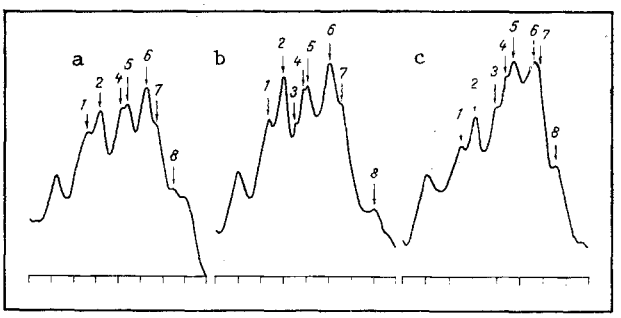

Fig. 1. Densitograms of electrophoretic fractionation of liver microsomal proteins of control animals (a), 24 h after injection of phenobarbital (b), and 48 h after injection of perfluorodecalin (c) into CBA mice. Mol. wt. of isoforms: 1) 59.5 kilodaltons (kD), 2) 58 kD, 3) 56 kD, 4) 55 kD, 5) 54 kD, 6) 51 kD, 7) 50 kD, 8) 48 kD.

TABLE 3. Effect of Induction of Cytochrome P-450 by Perfluorodecalin and Phenobarbital in Liver Microsomes of CBA Mice on Velocity of NADPH-Dependent Hydroxylation of Aniline and N-Demethylation of Aminopyrine (M ± m)

Inducer	Dose (kg body weight)	Velocity of hydroxylation of aniline, nmoles p-aminophenol/min		Per cent of control, relative to		Velocity of N-demethylation of aminopyrine, nmoles formaldehyde/min		Per cent of control, relative to	
		per milligram protein	per nanomole cytochrome P-450	protein	cytochrome P-450	per milligram protein	per nanomole cytochrome P-450	protein	cytochrome P-450
PFD	0,03 ml	2,9±0,06	3,63±0,06	72	81	8,4±0,9	10,5±0,9	94	106
PFD	0,1 ml	4,89±0,19	1,28±0,04	121	29	36,3±1,6	9,6±0,4	408	97
PFD	0,4 ml	6,64±0,27	1,20±0,05	165	27	57,1±4,5	11,7±0,9	642	108
PFD	0,6 ml	3,93±0,31	1,03±0,07	98	23	45,4±4,0	11,9±1,3	510	122
PFD	1,0 ml	4,42±0,29	1,05±0,05	110	23	68,4±5,9	16,3±1,2	769	165
PB	100 mg	2,54±0,21	1,59±0,09	63	35	25,9±2,3	16,2±1,3	291	163
Control		4,03±0,09	4,48±0,06	100	100	8,8±0,7	9,9±0,4	100	100

Legend. PFD) perfluorodecalin, induction 48 h; PB) phenobarbital, induction 24 h.

calculated per milligram of microsomal protein, actually was reduced a little, evidently due to induction of other microsomal proteins [5], for which the doses giving maximal induction may be higher.

On fractionation of the microsomal proteins by gradient polyacrylamide gel electrophoresis, 7 protein bands were found in intact animals and 8 bands in mice receiving phenobarbital and perfluorodecalin, in the region from 48 to 60 kilodaltons, corresponding to cytochrome P-450 isoforms (Fig. 1). The isoform with mol. wt. 56 kilodaltons appeared during induction by both phenobarbital and perfluorodecalin, but was not found in microsomes of the control mice. These data are in agreement with our own results obtained for intact and phenobarbital-induced C58BL/6 mice [2]. During induction by perfluorodecalin, besides the appearance of the new isoform, there was also an increase in the relative content of isoforms with mol. wt. of 50, 54, and 55 kilodaltons, and in the case of induction by phenobarbital, of isoforms with mol. wt. of 50, 51, and 58 kilodaltons.

The next stage of the work was to study the effect of induction by perfluorodecalin on the rate of oxidation of substrates of cytochrome P-450 (Table 3). The type 1 substrate aminopyrine and the type 2 substrate aniline were used. For comparison, oxidation of these same substrates after induction with phenobarbital was investigated. The velocity of *p*-hydroxylation of aniline fell gradually to 25% of the control level when calculated per nanomole cytochrome P-450 with an increase in the dose of the inducer to ml/kg. If calculated per milligram of microsomal protein, the velocity of aniline hydroxylation first rose to reach a maximum after injection of perfluorodecalin in a dose of 0.4 ml/kg (165% of the control). With higher doses the velocity fell to control values (Table 3). The velocity of oxidation of aniline also fell after induction by phenobarbital. The directly opposite picture was observed when N-demethylation of aminopyrine was studied. An increase in the dose of perfluorodecalin from 0.03 to 1 ml/kg led to a gradual increase in the velocity of demethylation of animopyrine, just as after induction with phenobarbital (Table 3).

Similarity was thus found in the induction of cytochrome P-450 by perfluorodecalin than phenobarbital. These compounds give rise to similar changes in the spectrum of isoforms induced by them, and isoforms of cytochrome P-450 hydroxylate the type 1 substrate aminopyrine better than the type 2 substrate aniline. Meanwhile perfluorodecalin is a more powerful inducer of the mono-oxygenase system than phenobarbital, for mice at least.

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Ca^{++}-DEPENDENT SEROTONIN SECRETION FROM THE RAT HYPOTHALAMUS DURING ONTOGENY

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One of the most important functions of the serotoninergic system of the brain is its role in regulation of the secretion of pituitary trophic hormones. At the hypothalamic level serotonin (5-hydroxytryptamine; 5-HT) is involved as a neurotransmitter or neuromodulator in the regulation of secretion of adenohypophysiotropic neurohormones, whereas at the pituitary level, as a neurohormone it influences the secretion of pituitary trophic hormones [2].

In adult animals the main source of the 5-HT innervation of the hypothalamus is the dorsal and medial nuclei raphe in the midbrain. Differentiation of 5-HT-containing neurons in these nuclei takes place after the 13th day of embryonic life [11]. After the 16th day the fetal hypothalamus begins to be innervated by 5-HT fibers, and on the 18th day, serotonin-like neurons appear in it [10]. It is considered that in the early stages of ontogeny 5-HT plays the role of inducer of neurogenesis [5], but later it becomes involved in the regulation of function of the anterior lobe of the pituitary.

Data in the literature relate mainly to structural aspects of development of the serotonin system of the hypothalamus in ontogeny but give no idea about functional maturation of the serotoninergic fibers forming it.

The aim of this investigation was to study the formation of one of the most important properties of the hypothalamic serotoninergic fibers, namely Ca^{++}-dependent serotonin secretion in response to potassium depolarization, during ontogeny of the rat.

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