CYTOCHROME P-450 CONCENTRATIONS IN ADRENAL AND LIVER MICROSOMES FROM GUINEA PIGS WITH GENETIC DIFFERENCES IN CORTISOL HYDROXYLATION

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Considerable evidence has now been accumulated which implicates the hemoprotein from microsomes which binds carbon monoxide (cytochrome P-450) as the terminal oxidase in many NADPH dependent enzymatic hydroxylation processes of steroids and drugs (Estabrook et al., 1963; Omura et al., 1965; Remmer and Merker, 1965). The concentration in liver of cytochrome P-450 increases following administration of drugs such as phenobarbital which also increase severalfold the NADPH dependent drug-metabolizing and steroid hydroxylation activities (Remmer and Merker, 1965; Conney et al., 1965). There seems to exist in general a parallelism between the concentration of cytochrome P-450 and these enzymatic activities (Merker and Remmer, 1965; Kato, 1966).

It seemed therefore of interest to study the concentration of cytochrome P-450 in 2 strains of guinea pigs (high and low producers) which have been shown to differ considerably in their adrenal cortisol hydroxylation activity at position C-2α and C-6β (Burstein et al., 1965a, 1967; Bhavnani and Burstein, 1966). This was especially interesting because in the guinea pig the products of these hydroxylations--2α- and 6β-hydroxycortisol--occur naturally as a result of the hydroxylation of the endogenously produced cortisol and have been isolated from guinea pig urine (Burstein et al., 1955; Burstein, 1956; Burstein et al., 1965b).

The results of this report indicate a higher concentration of cytochrome P-450 in adrenal microsomes from the guinea pig strain with the higher adrenal

cortisol hydroxylation activity. No significant strain differences were found in hepatic microsomes.

METHODS. The guinea pigs used were male strain 13 (high producers) and Hartley (low producers). The strain 13 were descendants of highly inbred strain 13 guinea pigs originally purchased from the Animal Production Section of the National Institutes of Health and randomly bred in this laboratory. The strain 13 animals were homozygous for high 2α-hydroxycortisol production. The Hartley guinea pigs were descendants of selected Hartley animals with a low production of 2α-hydroxycortisol and were homozygous for this trait (Burstein et al., 1965b).

The animals were kept in one room which was maintained at 71 ± 2 F and were fed Purina Guinea Pig Chow (Ralston Purina Co.) supplemented with fresh vegetables.

After decapitation the excised adrenals and livers were immediately placed in ice-cold isotonic (1.15%) KCl. The adrenals were homogenized in 10 ml of the KCl solution without prior cutting of the tissue. The livers were cut to small pieces with scissors and 1 g representative portions homogenized with 10 ml of isotonic KCl. The microsomal fraction was prepared by centrifugation for 1 hour at 330,000 x g (International Equipment Co. Ultracentrifuge T60) of the supernatant resulting from centrifugation at 15,000 x g for 10 min (Sorvall SS-1 Superspeed angle centrifuge). The microsomal pellets were resuspended in 3.0 ml isotonic KCl and 1.0-2.0 ml aliquots taken for cytochrome P-450 and protein determinations. Protein was determined according to Lowry et al. (1951). Cytochrome P-450 was determined essentially according to Omura and Sato (1964) from the difference spectrum of carbon monoxide treated (1.0 min CO bubbling) dithionite reduced microsomes in tris pH 7.2 buffer. The spectra were determined in a Cary model 14 recording spectrophotometer with a 0-0.1 slidewire. The difference in absorbance between 450 and 500 mμ ($\Delta OD_{450-500}$ light path 1 cm) was taken as an arbitrary

measure of cytochrome P-450. The results are expressed as $\Delta OD_{450-500}$ per mg protein per ml of solution in which the measurement was made. When care is taken to avoid excessive foaming a fair linear relationship between $\Delta OD_{450-500}$ and protein concentration obtained with both strains. (Thanks are due to Dr. R. W. Estabrook for valuable suggestions regarding the methodology of cytochrome P-450 determination.)

TABLE 1. Cytochrome P-450 in high (strain 13) and low (Hartley) producer guinea pig adrenal microsomes

Strain 13					Hartley				
No.	Age, Months	Body Weight g	Adrenal Wt mg	Cytochrome P-450[a]	No.	Age, Months	Body Wt g	Adrenal Wt mg	Cytochrome P-450[a]
1	3	600	259	0.122	1	2	650	370	0.054
2	3	570	260	0.160	2	2	650	251	0.072
3	3	670	292	0.161	3	3	800	263	0.059
4	3	570	240	0.168	4	3	820	249	0.089
5	3	550	240	0.153	5	3	850	256	0.088
6	3	660	290	0.161	6	3	800	600	0.100
7	4	690	295	0.177	7	3	830	427	0.117
8	4	710	272	0.184	8	3	860	465	0.088
9	4	760	289	0.151	9	3	860	435	0.073
10	4	670	307	0.163	10	3	800	402	0.086
11	4	620	293	0.152	11	4	820	279	0.046
12	4	670	287	0.170	12	4	900	343	0.063
13	4	600	261	0.157	13	4	760	244	0.053
14	5	720	367	0.165	14	4	720	278	0.069
15	5	750	378	0.145	15	5	760	310	0.078
16	5	760	322	0.082	16	5	750	370	0.091
17	5	770	303	0.113	17	5	730	356	0.046
18	5	760	369	0.110					
19	5	770	424	0.120					
20	5	710	331	0.089					
21	5	730	391	0.125					
			Mean ± SD	0.144 ± 0.029			Mean ± SD		0.075 ± 0.020

$t = 8.46$ ($p < 0.001$)

[a] Expressed as $\Delta OD_{450-500}$ per mg protein per ml.

EXPERIMENTAL AND RESULTS. The difference spectra of carbon monoxide-treated adrenal microsomes from the low producers exhibited in most cases in addition to the 450 mμ maximum also a peak (of reduced intensity) at 420 mμ. With the high producer adrenal microsomes the peak at 420 mμ was in most cases either absent, greatly reduced in intensity as compared to the low producers, or present only in the form of a shoulder. A peak at 420 mμ (considerably smaller than the peak at 450 mμ) was present in the spectra from the hepatic microsomes of both strains. The intensity of the 420 mμ peak varied considerably from experiment to experiment and it was assumed that it represented contamination by hemoglobin. Care to avoid contamination with blood during the removal of the adrenals and livers and repeated washings with isotonic KCl of the microsomes reduced the intensity of the 420 mμ peak but did not eliminate it completely. The possibility that contamination with hemoglobin might affect the determination of cytochrome P-450 was checked by adding hemoglobin to microsomal preparations. The addition of guinea pig hemoglobin

TABLE 2. Cytochrome P-450 in high (strain 13) and low (Hartley) producer guinea pig hepatic microsomes

Strain 13					Hartley				
No.	Age, Months	Body Weight g	Liver Weight g	Cytochrome P-450[a]	No.	Age, Months	Body Weight g	Liver Weight g	Cytochrome P-450[a]
7	4	690	20.5	0.042	11	4	820	30.1	0.054
8	4	710	24.6	0.038	12	4	900	35.7	0.033
9	4	760	25.1	0.051	13	4	760	26.9	0.047
10	4	670	19.0	0.048	14	4	720	27.7	0.057
11	4	620	21.3	0.041	1	2	650	23.8	0.046
12	4	670	22.3	0.056	2	2	650	19.9	0.032
13	4	600	21.4	0.050	18	2	570	18.5	0.053
		Mean ± SD		0.047 ± 0.006			Mean ± SD		0.046 ± 0.010

Strain 13 animals Nos. 7-13 and Hartley Nos. 1, 2, 11-14 are the same animals listed in Table 1. Their ages and body weights were duplicated in this Table for convenience.

[a]Expressed as $\Delta OD_{450-500}$ per mg protein per ml.

to give a final concentration of 40-60 μg/ml gave an immeasurable absorbance (considerably larger than 0.2) at 420 mμ but had no significant effect on $\Delta OD_{450-500}$.

The cytochrome P-450 concentrations in adrenal and liver microsomes from high and low producers are given in Tables 1 and 2. The high producers exhibited a significantly larger concentration of cytochrome P-450 in adrenal microsomes than the low producers. No significant strain difference was found with the hepatic microsomes.

DISCUSSION. The results of this study indicate a 1.9-fold higher concentration of cytochrome P-450 in terms of protein concentration in adrenal microsomes from the high producer guinea pigs in comparison with the low producers, which parallels their differences in adrenal cortisol hydroxylation activities. Relatively little is known about the kinetic aspects of hydroxylation in respect to cytochrome P-450 concentration and the fact that the strain differences in hydroxylation activity were considerably higher (approximately 7-fold, Bhavnani and Burstein, 1966; Burstein et al., 1967) than the cytochrome differences cannot be easily interpreted. One possibility may be that the differences between the strains in their total hydroxylation activity in which cytochrome P-450 plays a role is only a narrow one. For instance, the 2 strains do not differ significantly in their ability to produce cortisol (Burstein et al., 1965b) and one would not expect significant differences in respect to the microsomal hydroxylation activity of steroid precursors at positions C-17 and C-21. In addition, there may be no differences between the hydroxylation activities of certain cells of the adrenal (such as the medulla). If each hydroxylase is a separate macromolecular assembly containing its own cytochrome P-450 only a small difference in its overall concentration may obtain.

There was no significant strain difference in respect to the hepatic cytochrome P-450 concentration which was lower than that in adrenal. It is noteworthy that guinea pig liver microsomes possess considerably less enzymatic

cortisol-hydroxylation activity than adrenals and that the strain difference in hepatic enzymatic activity is also much smaller as compared to adrenal preparations (Burstein, S., to be published).

The existence of a peak at 420 mμ in addition to the peak at 450 mμ in adrenal microsomes primarily from the animals with low hydroxylation activity remains unexplained. The presence of hemoglobin seems likely but definitive proof was not obtained. Hemoglobin even in relatively high concentrations did not interfere with the determination of $\Delta OD_{450-500}$.

Recent studies indicate the possibility of existence of multiple P-450 hemoproteins or forms (Imai and Sato, 1966; Sladek and Mannering, 1966). Genetic variants of hemoproteins such as hemoglobin and catalase have been extensively studied in man and animals (cf Stanbury et al., 1966). Genetic mutants of cytochrome systems have been reported in Neurospora and Saccharomyces cerevisiae (cf De Busk, 1956).

Differences in hepatic drug metabolism before and after phenobarbital treatment have been described in various rabbit strains (Cram et al., 1965). It would be interesting to determine the cytochrome P-450 concentrations in these animals.

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