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Phenobarbital, dexamethasone and benzanthracene induce several cytochrome P450 mRNAs in rat hepatoma cells

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Hepatoma cells derived from the Reuber H35 rat hepatoma express cytochrome P450 enzymes of two major families: polycyclic aromatic hydrocarbon-inducible forms are found in both differentiated and dedifferentiated cells while phenobarbital (PB)-inducible forms are found only in differentiated cells. We report here that (i) benzanthracene and PB induce P450 c mRNA in differentiated and dedifferentiated cells and (ii) dexamethasone and PB induce P450 b/e and/or P450 PB1 mRNAs in differentiated cells but not in dedifferentiated cells.

Cytochrome P-450; Induction; mRNA; (Hepatoma cell, Liver, Rat)

1. INTRODUCTION

Cytochromes P450 belong to a superfamily of enzymes involved in the metabolism of endogenous substrates such as fatty acids and steroids, and in the detoxification as well as in the activation of xenobiotics [1]. Several cytochrome P450 families were initially characterized by their response to inducer compounds such as PB, PAH, glucocorticoids and ethanol [1]. Most cytochrome P450 genes are expressed in the liver of inducer-treated animals. In mice, the results from both in vivo and in vitro studies suggested the existence of a specific receptor, encoded by the Ah locus, and mediating the induction of the P450 P1 gene (equivalent to rat P450 c) by PAH [2]. There is only little information concerning the induction of PB-inducible cytochrome P450 genes by PB [3] due to an acute lack of mutant animal strains and PB-responsive cell lines.

Cells derived from the Reuber H35 rat hepatoma express cytochrome P450 enzyme activities [4]. Aryl hydrocarbon hydroxylase activity has been found in both differentiated and dedifferentiated rat hepatoma lines. Moreover, the differentiated cell lines are exceptional in exhibiting aldrin epoxidase activity, an indicator of PB-inducible cytochromes P450 [4,5]. They thus are likely to constitute a unique in vitro system to carry out genetic and biochemical studies on the regulation of expression of PB-inducible cytochrome P450.

We show here that (i) BA (a typical PAH compound) and PB induce an increase in the amount of P450 c mRNA in both differentiated and dedifferentiated cells and (ii) DEX and PB induce an increase in the amount of P450 b/e and PB1 mRNAs in differentiated cells but not in dedifferentiated cells.

2. MATERIALS AND METHODS

ACI male rats (from which the hepatoma cell lines were initially derived) were injected intraperitoneally once or every 24 h for 3 days; following treatment, they were killed by cer-

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Abbreviations: PB, phenobarbital; DEX, dexamethasone; PAH, polycyclic aromatic hydrocarbon; BA, benzanthracene; 3-MC or MC, 3-methylcholanthrene

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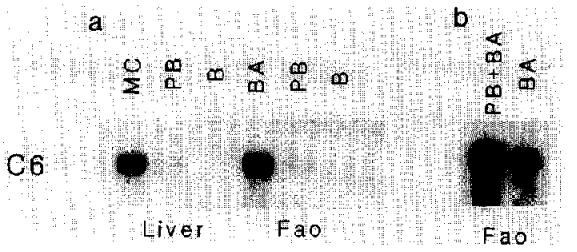

Fig.1. P450 c mRNA levels. (a) Comparison between liver and Fao cells treated by various inducers. (b) For the combination PB + BA, PB for 72 h, BA added for the last 24 h. B stands for basal level.

vical dislocation after overnight starving. 3-MC (100 mg/kg body wt, in oil), PB (100 mg/kg body wt, in 50%, v/v, ethanol/glycerol) and DEX (5 mg/kg body wt, in water) were diluted in normal saline. For cultures, the following conditions of inducer treatments have been determined as optimal, and unless stated otherwise include, BA (for 24 h) in DMSO, PB (72 h) and DEX (24 h) in phosphate buffered saline, added at a final concentration of 10^{-6}, 2×10^{-3} and 10^{-6} M, respectively. As controls, solvents alone were injected into the animals or added to cells. Cell lines and culture conditions have been described [6]. RNA preparation, Northern blotting, hybridization and washes are detailed in [7]. For each RNA gel, 10 or 15 μg of total RNA were loaded per lane. cDNA probes, generously provided by A. Anderson and M. Adesnik, include: C6 [8], which corresponds to P450 c mRNA; R17 [9] to P450 b/e; and pTF2 [10] to P450 PB1. The probes do not cross-hybridize to one another and reveal mRNAs of 3 kb (C6), 2.1 kb (R17) and 1.9 kb (pTF2) long (not shown). The relative intensities of the hybridization signals were estimated by using a BioRad densitometer.

3. RESULTS AND DISCUSSION

Clonal hepatoma cell lines previously characterized for their P450 enzyme activities ([4,5] and Wiebel, F.J., personal communication) were used. The differentiated cells (Fao, C2Rev7 and H4II) express a whole set of hepatic functions including serum proteins (albumin, fibrinogen, α_1-antitrypsin) and enzymes (alcohol dehydrogenase, aminotransferases, fructose diphosphatase, phospho*enol*pyruvate carboxykinase). The dedifferentiated cells (P4, H5-6 and C2) fail to express most or all of these functions [6]. To analyze the profile of induction of cytochromes P450, we compare the results obtained for rat liver and for the widely used differentiated Fao cell line; then, we present the results obtained for the other hepatoma lines.

3.1. *Expression of the cytochrome P450 c gene*

As revealed with the C6 probe, the basal level of P450 c mRNA is very low in both liver and Fao cells (fig.1a). A single injection of 3-MC to the rat, or exposure of Fao cells to BA, lead to comparable increases (20–30-fold) of P450 c mRNA. PB induces a 3–5-fold increase of the mRNA in both rat liver and Fao cells. Since the amounts of P450 c mRNA, following 3-MC (or BA) and PB induction, are comparable in liver and Fao cells, it is likely that the cultured cells use the same mechanisms as hepatocytes for induced expression of the P450 c gene.

Pretreatment of the cells by PB before addition of BA results in a much larger increase (three times the effect of BA alone) of P450 c mRNA than expected for an additive effect (fig.1). This synergism would result if, as has been found for rat liver [8,9], PB induces in Fao cells an increase in the intracellular concentration of receptor molecules for PAH compounds.

As shown in fig.2, the inducibility of P450 c mRNA by BA and/or PB does not depend on the degree of differentiation of the hepatoma cell lines. BA and PB induce this mRNA in Fao and P4 cells; BA but not PB is effective in H4II and H5-6

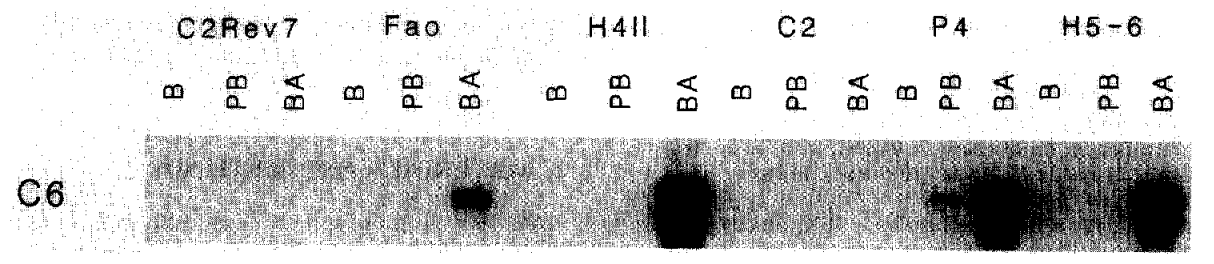

Fig.2. Expression and inducibility of the cytochrome P450 c gene (C6 probe) in three differentiated (left) and three dedifferentiated (right) cell lines. B, basal.

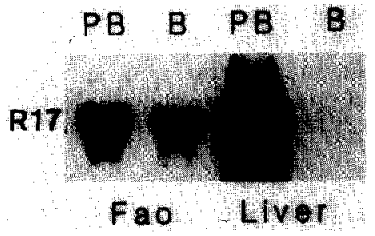

Fig.3. P450 b/e mRNA levels. Comparison between untreated or PB-treated liver and Fao cells. B, basal.

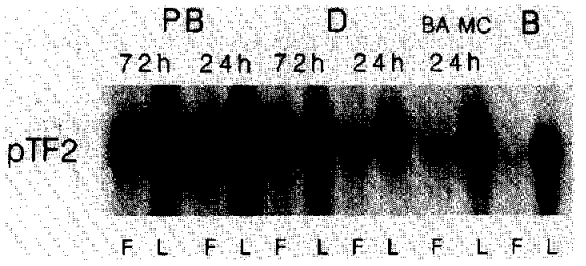

Fig.5. P450 PB1 mRNA levels. Comparison between liver (L) and Fao (F) cells treated by various inducers. B, basal.

cells. Neither BA nor PB is effective in C2Rev7 and C2 cells.

3.2. *Expression of the cytochrome P450 b/e genes*

In liver, as revealed with the R17 probe, the P450 b/e genes are expressed at a very low basal level, but they are highly inducible by PB (50-fold) (fig.3). In contrast, in Fao cells, P450 b/e mRNA is 3–5-fold more abundant than in liver, but it is only slightly (2-fold) inducible by PB.

The results presented in fig.4a show that PB induces P450 b/e mRNA in C2Rev7 cells but not in H4II cells. DEX increases the amount of P450 b/e mRNA in H4II cells but not in the PB-responsive Fao and C2Rev7 cells. Neither PB, nor DEX induces this mRNA in the dedifferentiated cell lines.

3.3. *Expression of the cytochrome P450 PB1 gene*

The level of P450 PB1 mRNA in liver is higher than that of P450 b/e, but it undergoes only a 3-fold increase upon PB induction (fig.5). DEX and 3-MC have no effect on the accumulation of P450 PB1 mRNA in liver. In Fao cells, P450 PB1 mRNA is less abundant than in liver, but it undergoes a 14-fold increase upon PB induction. DEX also induces a large (10-fold) increase in the amount of P450 PB1 mRNA; BA is not effective. Exposure of Fao cells to a mixture of DEX and PB does not result in a further increase in P450 PB1 mRNA (not shown). PB and DEX induce this mRNA in the differentiated C2Rev7 and H4II cells, but not in the dedifferentiated cells (fig.4b).

These results show that the basal and PB and/or DEX-induced expression of P450 b/e and PB1 cytochrome P450 genes is restricted to the differentiated cells. The expression of these genes may thus be considered as a differentiated trait of the rat hepatoma cells, as opposed to the ubiquitous expression of the P450 c gene. The differences in the expression of the P450 b/e and PB1 genes, be-

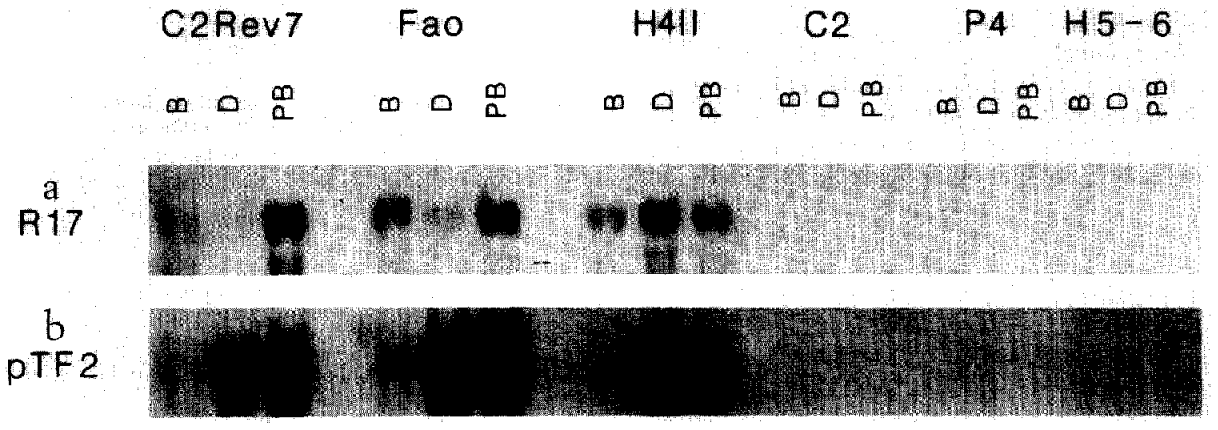

Fig.4. Expression and inducibility, in three differentiated (left) and three dedifferentiated (right) cell lines, of (a) the cytochrome P450 b/e genes (R17 probe) and (b) the cytochrome P450 PB1 gene (pTF2 probe). B, basal.

Table 1

Factor of induction of P450 mRNAs in rat liver and hepatoma cells

		P450 c	P450 b/e	P450 PB1
Liver	MC	27	1[a]	1
	DEX	1[a]	1[a]	1
	PB	3	50	3
Fao	BA	20	1	1
	DEX	1[a]	<1	11
	PB	5	2.2	14
H4II	BA	85	ND[b]	ND
	DEX	ND	1.3	9
	PB	1	1	3
C2Rev7	BA	–[c]	ND	ND
	DEX	–	<1	5.8
	PB	–	1.8	10
H5-6	BA	40	–	–
	DEX	ND	–	–
	PB	1	–	–
P4	BA	60	–	–
	DEX	ND	–	–
	PB	5	–	–
C2	BA	–	–	–
	DEX	ND	–	–
	PB	–	–	–

[a] Data not shown
[b] ND, not determined
[c] –, not detectable

tween rat liver and the differentiated hepatoma cells, might be due to cell heterogeneity either in the cultured cells or in liver. However, since these differences in response to PB are only quantitative, differentiated rat hepatoma cells constitute a valuable in vitro system to investigate the induction pathway of the cytochromes P450 b/e and PB1 by PB.

As summarized in table 1, each of the rat hepatoma cell lines characterized here presents a distinct profile of induced expression of the cytochrome P450 genes. Fao cells are the most responsive; at the other extreme, C2 cells are negative by all criteria. The availability of such a variety of cell lines, either responsive or not to P450 inducers, permits genetic and molecular analyses of factors controlling the expression of rat P450 genes.

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