Volume 284, number 1, 103–108 FEBS 09815 June 1991

ADONIS 001457939100524X

Regulation of glutathione *S*-transferase gene expression by phenobarbital in cultured adult rat hepatocytes

Yves Vandenberghe[1], Lisa Tee[2], Fabrice Morel[3], Vera Rogiers[1], André Guillouzo[3] and George Yeoh[2]

[1]*Department of Toxicology, Vrije Universiteit Brussel, Laarbeeklaan 103, 1090 Brussels, Belgium,* [2]*Department of Physiology, University of Western Australia, 6009 Nedlands, Australia* and [3]*INSERM U49, Unité de Recherches Hépatologiques, Hôpital de Pontchaillou, Rennes, France*

Received 27 February 1991

Previous studies, by using Northern blotting analyses, showed that phenobarbital (PB) affects the steady-state mRNA levels of glutathione *S*-transferase (GST) subunits 1/2, 3/4 and 7 in both conventional cultures of adult rat hepatocytes and co-cultures, with rat liver epithelial cells [Vandenberghe et al., 1989, FEBS Lett. 251, 59–64; Morel et al., 1989, FEBS Lett. 258, 99–102]. To determine whether PB acts at the transcriptional level, nuclear 'run-on' experiments using cDNA probes hybridizing to GST subunits 1/2, 3/4 and 7 mRNA were performed on purified nuclei isolated from control and PB treated hepatocytes seeded under conventional and co-culture conditions. Data from this study demonstrate that the increase in steady-state mRNA levels observed in both conventional culture and co-culture after 4 days PB exposure results from an increased transcriptional activity of the GST genes. However, a substantial increase in steady-state mRNA levels in the absence of a commensurate increase in transcriptional activity at 12 days of co-culture, indicates that the barbiturate has also a stabilizing effect in vitro on the GST mRNAs.

Glutathione *S*-transferase; Gene transcription; Rat hepatocyte; Primary culture; Phenobarbital

1. INTRODUCTION

Phenobarbital (PB) has previously been reported to alter the expression, regulation and activity of several liver drug metabolizing enzymes [1–3]. Most attention has been paid to its effect on cytochrome P-450 [4–6], UDP-glucuronyltransferase [7,8] and glutathione *S*-transferase enzymes [9–11]. However, it is yet not clear how this compound affects the regulation of expression of these enzymes. It is not known whether PB induction of transcriptional and translational activities involves one or more receptors [12,13].

It has been shown that PB does not elevate all cytochrome P-450 enzymes to the same extent. Cytochrome P-450 IIB_1 and IIB_2 are the major liver enzymes to be induced [14,15]. The rapid increase in the rates of transcription and the appearance of elevated levels of nuclear pre-mRNA and cytoplasmic mRNAs unequivocally demonstrated that PB elevates levels of cytochrome P-450 IIB_1 and IIB_2 primarily by augmenting the rate of transcription of the respective genes [16–18]. Recent work on cultured hepatocytes [19] showed for the first time that PB elevation of cytochrome P-450 IIB_1 and IIB_2 mRNA levels is the result from an increased transcriptional activity as found for the intact liver.

Liver glutathione *S*-transferase subunits 1 and 3 especially are also induced by PB treatment [11]. Recently, Pickett et al. [20] reported that the increase of GST mRNA in total liver after PB addition results from an elevated GST gene transcription. In cultured hepatocytes, PB treatment induces the GST enzymatic activity [21,22]. It was shown in previous study [21] that, by supplementing the culture medium with 3.2 mM PB, concentrations of individual GST subunits and GST steady-state mRNA levels were changed. However, no information is available on the regulation of GST gene expression by PB in cultured hepatocytes. Therefore the aim of this study was to determine whether the changes observed in mRNA levels are due to altered stability of the mRNAs in the presence of PB or to changes in the rate of gene transcription.

In order to analyze the transcriptional activity of GST 1/2 (alpha family), 3/4 (mu family) and 7 (pi family) genes, a nuclear 'run-on' experiment was performed on nuclei isolated from 4-day conventional cultures and 4- and 12-day co-cultures of adult rat hepatocytes, both with and without 3.2 mM PB treatment.

Abbreviations: GST(s), glutathione *S*-transferase(s); PB, phenobarbital; GAPDH, glyceraldehyde-3-phosphate-dehydrogenase

Correspondence address: Y. Vandenberghe. *Present address:* Dept. Toxicology, Searle European Development Center, Rue Granbompré 11, 1348 Mont-St-Guibert, Belgium. Fax: (32) (10) 450290.

2. METHODS

2.1. *Cell isolation and culture*

Rat hepatocytes were isolated from Sprague-Dawley rats (180–200 g) as described by Guguen et al. [23]. They were seeded in conven-

tional culture and in co-culture with rat liver epithelial cells, as described previously [24]. The culture medium consisted of 75% minimal essential medium and 25% Medium 199, containing 1 mg/ml bovine serum albumin, 10 μg/ml bovine insulin and 10% fetal calf serum (control medium). Four hours after cell seeding, the medium was first renewed and supplemented with 7×10^{-5} M and 7×10^{-6} M hydrocortisone hemisuccinate in conventional cultures and co-cultures respectively. PB (3.2 mM) was added to the culture medium 4 h after cell seeding for conventional cultures and in co-culture when confluency was reached between parenchymal and epithelial cells (approximately after 1 day). Thereafter, PB was added every day when renewing the medium.

2.2. *'Run-on' transcription in isolated nuclei and hybridization*

Nuclei were isolated from freshly isolated, conventional and co-cultured hepatocytes by the method of Becker et al. [26], suspended in 50% glycerol, 5 mM $MnCl_2$, 1 mM $MgCl_2$, 5 mM dithiothreitol, 20 mM Tris, pH 7.4, frozen in liquid nitrogen and stored at −80°C. Concerning the co-cultures, hepatocytes and epithelial cells were not separated for nuclei preparation.

Transcription in isolated nuclei, isolation of ^{32}P-labelled RNA, and hybridization to nitrocellulose filters were performed as described previously [27] with some modifications. Briefly, isolated nuclei (5×10^6) were incubated in a reaction mixture consisting of 50 mM HEPES (pH 8.0), 150 mM NH_4Cl, 1 mg/ml nuclease-free BSA, 1 mM $MnCl_2$, 12.5% glycerol, 0.1 mg/ml heparin, 3.5 mM $MgCl_2$, 1 μl (60 U) RNasin, 0.25 mM dithiothreitol, 0.5 mM ATP, GTP, CTP and UTP, 100 μCi of [α-^{32}P]UTP for 20 min at 25°C. The mixture was adjusted to final concentrations of 5 mM Tris (pH 7.5), 5 mM $MgCl_2$, 5 mM $CaCl_2$, and 1 μl (1 U) RQ1 DNase was added before incubating the mixture for 5 min at 37°C. After treatment with proteinase K (150 μg/ml) at 37°C for 30 min, RNA was isolated by phenol/chloroform extraction followed by trichloroacetic acid and ethanol precipitation in the presence of 0.05 mg/ml transfer RNA. After another treatment with RQ1 DNase as above, RNA was again isolated by phenol/chloroform extraction followed by ethanol precipitation as above, and hybridized at 42°C for 72 h in 55% formamide, 4 × SSC, 0.1 M sodium phosphate (pH 6.8), 5 × Denhardt's solution, 0.1% SDS, 100 μg/ml salmon sperm DNA, and 10% dextran sulphate to 0.25 pmol of each of the following nitrocellulose-bound DNA species: (a) albumin genomic subclones 'B', 'C' and 'D' [28]; (b) GST cDNA pGSTr 155 [29]; (c) GST cDNA JT9L; (d) GST cDNA pGSTr 7 [30]; (e) GAPDH cDNA pRGAPDH-13 [31]; (f) pUC 18; and (g) pBR 322. Filters were washed 3 times in 1 × SSC, 0.1% SDS at 65°C and then twice in 0.1 × SSC, 0.1% SDS and associated radiolabel was visualized by fluorography and assessed by optical densitometry. Densitometric signals were converted to relative transcription rates by subtracting the background signal (pUC 18 or pBR 322) and correcting for the fraction of primary transcripts hybridizable to the recombinant genomic DNA. Corrected signals for the GSTs were normalized relative to the corrected pRGAPDH-13 signal for the respective filter. To enable an indirect comparison of transcription rates of GSTs, the corrected and normalized values were divided by the respective gene length giving the relative proportion of transcripts

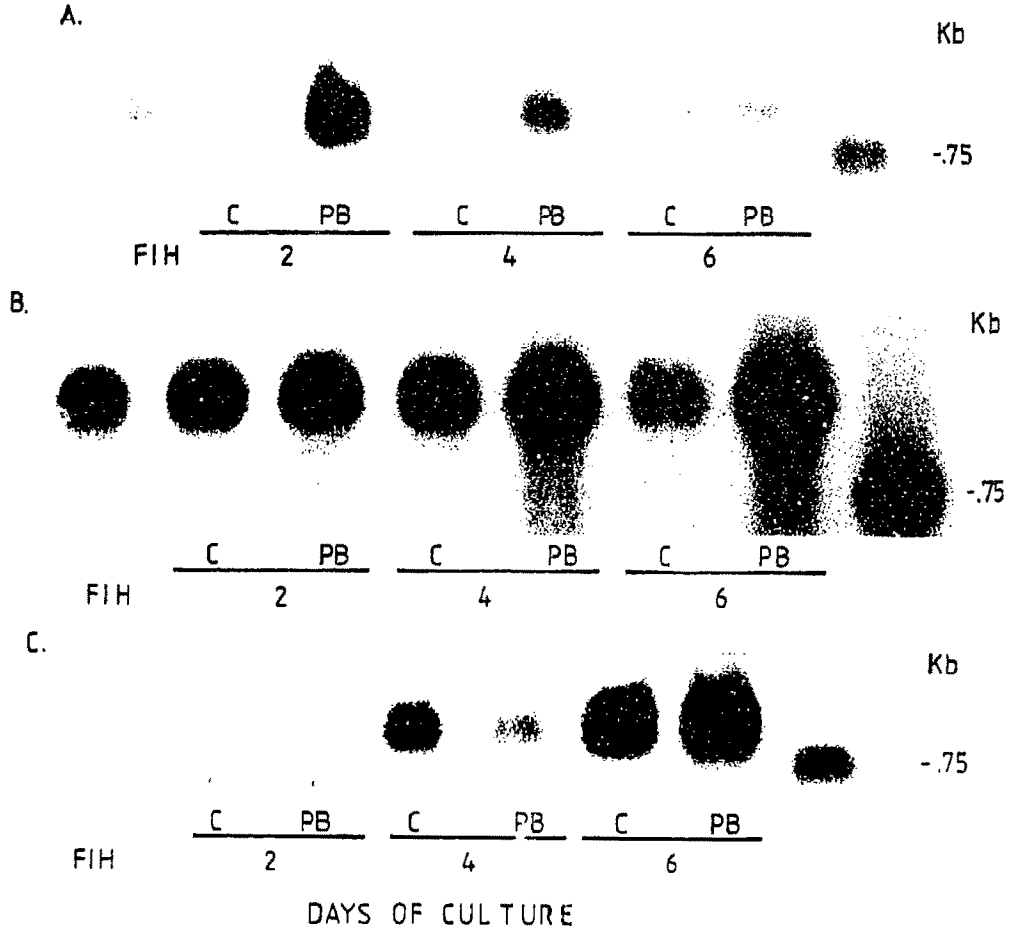

Fig. 1. Effect of phenobarbital on the GST 1/2, 3/4 and 7 mRNA expression in conventionally cultured adult rat hepatocytes. RNA was isolated from control (C) and phenobarbital (PB) treated hepatocytes, maintained in conventional culture for 2, 4 and 6 days and compared to the level of freshly isolated hepatocytes (FIH). ^{32}P-labelled cDNA probes were hybridized to mRNA encoding GST subunits 1/2 (A), 3/4 (B) and 7 (C) as described in section 2.

produced per unit of gene, to the number of GAPDH transcripts produced per unit of time.

3. RESULTS

3.1. *Effect of PB on transcriptional activity of GST 1/2, 3/4 and 7 genes in cultured hepatocytes*

Measurements of GST mRNAs in both conventional cultures and co-cultures with and without PB exposure have already been reported and are summarized in Figs. 1 and 2.

To determine whether changes in steady-state levels of GST 1/2, 3/4 and 7 mRNA from conventional and co-cultured hepatocytes after PB treatment are due to altered stability of the mRNAs in the presence of PB or due to a change in the rate of gene transcription, GST gene transcription was analyzed by a nuclear 'run-on' assay. Nuclei were prepared from freshly isolated, 4-day conventionally cultured and 4- and 12-day co-cultured hepatocytes. Slot blots representative of transcriptional activity are illustrated in Fig. 3. Results, quantified by densitometry, are presented in Table I. The relative rates of transcription of the GST 1/2, 3/4 and 7 genes were calculated relative to transcription of the constitutive gene GAPDH, after subtracting the background signal. Albumin gene transcription rate, included as a control, does not appear to be affected by PB treatment (Fig. 3).

When compared with freshly isolated hepatocytes, transcription of the GST 1/2 genes in 4-day-old conventional cultures (control) was substantially reduced, while a lesser reduction was observed for the GST 3/4 genes. In contrast, transcription of the GST 7 gene was strongly induced (Table I). The pattern in co-culture (control) after 4 days more closely resembled that seen with freshly isolated hepatocytes, although a reduction of GST 1/2 transcription and an induction of GST 7 transcription were evident.

After 4 days of treatment, PB induces transcription of all GST gene families investigated in co-cultures, while in conventional cultures transcription of GST 1/2 and GST 3/4 is increased, but transcription of GST 7 is suppressed. In long term (12-day) co-cultures, transcription of all GST genes is still demonstrable. By this time, the effect of PB on GST gene transcription is minimal. GST 1/2 gene transcription, in the presence of PB, is not detectable anymore. GST 3/4 and 7 genes are transcribed at a level only slightly higher compared to

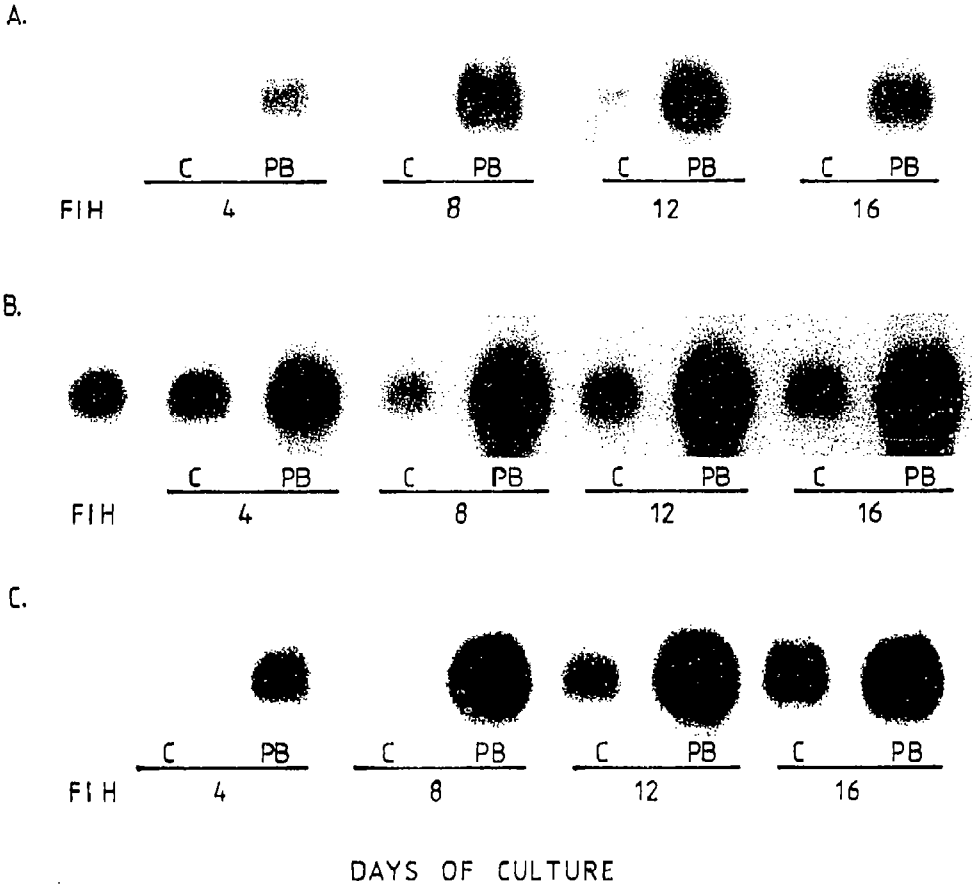

Fig. 2. Effect of phenobarbital on the GST 1/2, 3/4 and 7 mRNA expression in co-cultured adult rat hepatocytes. RNA was isolated from control (C) and phenobarbital (PB) treated hepatocytes, maintained in co-culture for 4, 6, 12 and 16 days and compared to the level of freshly isolated hepatocytes (FIH). ^{32}P-labelled cDNA probes were hybridized to mRNA encoding GST subunits 1/2 (A), 3/4 (B) and 7 (C) as described in section 2.

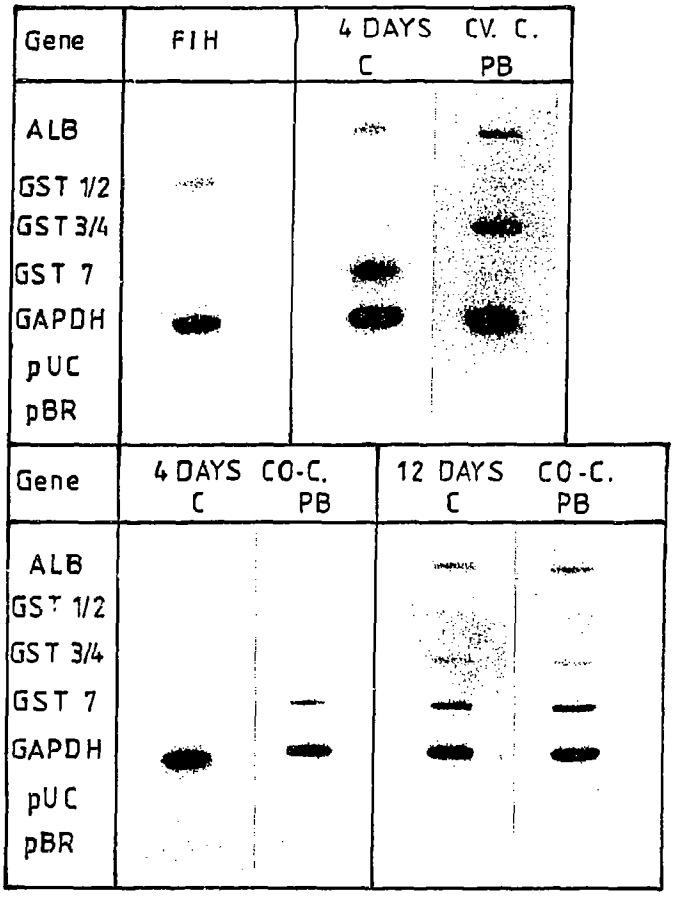

Fig. 3. Effect of phenobarbital on the GST gene transcriptional activity of conventionally and co-cultured adult rat hepatocytes. Transcriptional analysis in nuclei, isolated from freshly isolated hepatocytes (FIH), 4-day conventionally cultured hepatocytes (4 DAYS CV. C.), 4-day (4 DAYS CO-C.) and 12-day co-cultured hepatocytes (12 DAYS CO-C.), comparing control (C) and phenobarbital (PB) treated cells. Nuclei were isolated, and nascent RNA chains were elongated in vitro and hybridized to immobilized DNA from the following genes: albumin (ALB), glutathione *S*-transferase subunits 1 and 2 (GST 1/2), glutathione *S*-transferase subunits 3 and 4 (GST 3/4), glutathione *S*-transferase subunit 7 (GST 7), glyceraldehyde-3-phosphate-dehydrogenase (GAPDH), pUC and pBR.

the controls. These results on GST gene transcription at 12 days of co-culture, when PB treated, do not explain the elevation in GST steady-state mRNA levels obtained under analogous culture conditions (Fig. 2).

Rat liver epithelial cells, used to set up the co-cultures, demonstrate some transcriptional activity of the GST 3/4 and 7 genes, however these levels are not influenced by PB treatment (results not shown).

4. DISCUSSION

Previous studies have demonstrated that treatment with PB, in vivo [11,32] or in vitro [22], increases rat liver GST enzymatic activity. The increase in vivo is caused by an increased amount of GST proteins 1 and 3 especially [11], which is a result of an increased translational activity [13]. The amount of mRNA encoding GST subunits 1, 3 and 4 is increased to max. 8, 6 and 5 times respectively after 24 h of PB treatment [20]. GST 1/2 and 3/4 gene transcriptional activity is increased to max. 5 and 3 times respectively after 8 h of PB treatment [20]. Much less information is available on the effect of PB on GST gene expression and regulation in cultured adult rat hepatocytes.

Using 1-chloro-2,4-dinitrobenzene as a substrate, GST activity increases of up to 300% and 100% in conventional culture and co-culture respectively [21] were observed when 3.2 mM PB was added daily to the hepatocyte cultures. The increase in GST activity in vitro is due to an increase of GST subunits 1, 3 and especially 7, in both culture systems [21]. Northern blot experiments indicate that induction of individual GST subunits by PB treatment in vitro, results from a corresponding increase in the amount of mRNA encoding the GST subunits [21,25]. Nuclear 'run-on' experiments were done to establish whether the elevation in GST 1/2, 3/4 and 7 mRNA levels is due to transcriptional activation or post-transcriptional changes. In conventional culture, correlation between the increase in steady-state mRNA levels, encoding the different GST subunits, and elevation of hybridization signal from transcription assay suggests that PB added to the cultured hepatocytes, activates transcriptional activity of the GST genes.

While a correlation between transcription and steady-state mRNA levels exists in short-term (4-day) co-cultures, the continued increase in steady-state mRNA levels after 12 days in the presence of PB, cannot be accounted for by an increase in transcriptional activity of the corresponding GST gene. In fact, transcriptional activity is decreased (1/2 genes) or stabilized (3/4 and 7 genes) at this stage. In contrast, control co-cultures display an appropriate correlation between mRNA levels and transcription. Therefore these results strongly suggest that PB has increased the stability of the GST mRNAs.

Pickett et al. [20] concluded from in vivo experiments that transcriptional activity of GST genes is sufficient to account for the elevation in mRNA caused by PB administration, suggesting regulation by this xenobiotic at the transcriptional level. However, Francis et al. [12] described that administration in vivo of PB enhanced the half-life of GST 1 and 2 mRNA by nearly two-fold, indicating that the induction of these GSTs may essentially involve stabilization of the mRNAs.

Mechanisms responsible for transcriptional activation of GSTs are still not well described. Transcriptional regulation is primarily determined by specific interactions between *trans*-acting factors and their *cis*-active DNA elements [33]. Telakowski-Hopkins et al. [34] found that the GST Ya (GST 1) subunit structural gene contains at least one *cis*-acting regulatory element

Table I

GST transcription in isolated nuclei prepared from conventionally and co-cultured adult rat hepatocytes

	Vector signal		Hybridization signal* (corrected towards vector)				Relative rate** of transcription		
	pUC	pBR	GST 1/2	GST 3/4	GST 7	GAPDH	GST 1/2	GST 3/4	GST 7
F.I.H.	0.23	0.25	0.20	0.08	N.D.	1.16	0.44	0.21	N.D.
4 Days conventional culture									
Control	0.42	0.43	0.05	0.07	1.05	1.78	0.07	0.12	0.83
PB	0.24	0.28	0.10	0.98	0.11	1.95	0.13	1.60	0.08
4 Days co-culture									
Control	0.10	0.04	0.15	0.07	0.18	1.49	0.25	0.15	0.17
PB	0.13	0.13	0.20	0.18	0.37	0.97	0.53	0.58	0.54
12 Days co-culture									
Control	0.67	0.90	0.14	0.35	1.17	3.29	0.11	0.34	0.50
PB	0.98	1.60	N.D.	0.42	1.46	3.08	N.D.	0.43	0.67

All numerical values refer to optical density units. FIH refers to the values obtained from freshly isolated hepatocytes.

* Data have been corrected for non-specific hybridization by subtraction of vector signal. **GST densitometric signals were corrected for the fraction of primary gene transcripts hybridyzable to the genomic DNA, normalized relative to GAPDH, and converted to relative rates of transcription as described in section 2.

(xenobiotic responsive element, XRE) required for inducible expression by xenobiotics such as 3-methylcholantrene or β-napthaflavone. This finding was recently confirmed by Rushmore et al. [35]. Further investigations did not answer the question of whether the Ah or dioxin receptor directly interacts with specific sequences in the 5′ flanking region of the GST Ya gene [34] as is the case for cytochrome P_1-450 and P-450c genes [36,37].

In co-culture, but not in conventional culture, PB induces the expression of GST subunit 7. The increase in mRNA is a result both of an increased transcriptional activity and an increased stability of the mRNA in the presence of PB. Indeed, the elevation in GST 7 mRNA at 12 days co-culture cannot be explained by elevation in transcriptional activity alone. The reason for the re-expression and inducibility of liver GST 7 in vitro is unknown at present. However, since in cultured hepatocytes, the re-expression of subunit 7 is inhibited by dimethylsulphoxide [38,39], the question arises whether some correlation exists between the expression and inducibility of subunit 7 and its high GSH peroxidase activity. Probably as a result of an increased exposure to free radicals after PB treatment, cultured hepatocytes respond by increasing their GSH peroxidase capacity.

In conclusion, this report is the first to describe the effect of PB on the regulation of GST gene expression in cultured adult rat hepatocytes. Increased levels of GST 1/2, 3/4 and 7 mRNA in cultured hepatocytes, after PB treatment in vitro, are a result of increased transcriptional activity of the GST genes at an early stage of culture. In long-term cultures, there is evidence of increased stability of the GST mRNAs, since the steady-state level is substantially increased without a commensurate increase in transcriptional activity.

Acknowledgements: We would like to thank Prof. B. Ketterer and Dr J. Taylor for the generous gift of the GST cDNA probes pGSTr 155, pGSTr 7 and JTL 9. Y.V.'s stay at the University of Western Australia was supported by the International Union Against Cancer (Yamagiwa-Yoshida Memorial International Cancer Study Grant). We thank Seneca Veerle for technical assistance.

REFERENCES

[1] Pelkonen, R.O. and Lang, M. (1987) in: Enzyme Induction in Man (Sotaniemi, E.A. and Pelkonen, R.O. eds) pp. 317-324, Taylor & Francis, London.
[2] Hietanen, E. (1987) in: Enzyme Induction in Man (Sotaniemi, E.A. and Pelkonen, R.O. eds) pp. 61-68, Taylor and Francis, London.
[3] Caldwell, J. (1988) in: The Liver, Biology and Pathobiology (Arias, I.M., Jakoby, W.B., Popper, H., Schachter, D. and Schafritz, D.P. eds) pp. 355-362, Raven Press, New York.
[4] Atchison, M. and Adesnik, M.C. (1983) J. Biol. Chem. 258, 11285-11295.
[5] Rath, P.C. and Kancingo, M.S. (1988) Biochem. Biophys. Res. Commun. 157, 1403-1409.
[6] Ravishankar, H. and Padmanaban, G. (1985) Biochem. J. 229, 73-79.
[7] Notten, W.R.F., Henderson, P.T. and Kuyper, C.M.A. (1975) Int. J. Biochem. 6, 713-718.
[8] Ullrich, D. and Bock, K.W. (1984) Biochem. Pharmacol. 33, 97-101.
[9] Pickett, C.B., Telakowski-Hopkins, C.A., Ding, G., Argenbright, L. and Lu, A.Y.H. (1984) J. Biol. Chem. 259, 5182-5188.
[10] Ding, V.D.H. and Pickett, C.B. (1985) Arch. Biochem. Biophys. 240, 553-559.
[11] Vos, R.M.E., Snoek, M.C., van Berker, W.J.H., Muller, F. and van Bladeren, P.J. (1988) Biochem. Pharmacol. 37, 1077-1082.
[12] Kocarek, T.A., Schuetz, E.G. and Guzelian, P.S. (1990) Mol. Pharmacol. 38, 440-444.

[13] Pickett, C.B. and Lu, A.Y.H. (1989) Annu. Rev. Biochem. 58, 743-764.
[14] Chianale, J., Dvorak, C., May, M. and Gumucio, J.J. (1986) Hepatology 6, 945-951.
[15] Traber, P.G., Maganto, P., Wojcik, E., Keren, D. and Gumucio, J.J. (1989) J. Biol. Chem. 264, 10292-10298.
[16] Pike, S.F., Shepard, E.A., Rabin, B.R. and Phillips, I.R. (1985) Biochem. Pharmacol. 34, 2489-2494.
[17] Wojcik, E., Dvorak, C., Chianale, J., Traber, P.G., Keren, D. and Gumucio, J.J. (1988) J. Clin. Invest. 82, 658-666.
[18] Giachelli, C.M., Lin-Jones, J. and Omiecinski, C.J. (1989) J. Biol. Chem. 264, 7046-7053.
[19] Schuetz, E.G., Schuetz, J.D., May, B. and Guzelian, P.S. (1990) J. Biol. Chem. 265, 1188-1192.
[20] Pickett, C.B., Telakowski-Hopkins, C.A., Ding, G., Ding, V.D.H. and King, R.G. (1987) in: Glutathione S-Transferases and Carcinogenesis (Mantle, T.J., Pickett, C.B. and Hayes, J.D. eds) pp. 75-85, Taylor and Francis, London.
[21] Vandenberghe, Y., Morel, F., Foriers, A., Ketterer, B., Vercruysse, A., Guillouzo, A. and Rogiers, V. (1989) FEBS Lett. 251, 59-64.
[22] Sasaki, H., Miyaura, S., Horie, K. and Isona, H. (1989) J. Pharmacobio.-Dyn. 12, 775-780.
[23] Guguen, C., Guillouzo, A., Boisnard, M., Le Cam, A. and Bourel, M. (1975) Biol. Gastroenterol. 8, 223-231.
[24] Guguen-Guillouzo, C., Clément, B., Baffet, G., Beaumont, C., Morel-Chany, E., Glaise, D. and Guillouzo, A. (1983) Exp. Cell Res. 143, 47-54.
[25] Morel, F., Vandenberghe, Y., Pemble, S., Taylor, J.B., Ratanasavanh, D., Rogiers, V., Ketterer, B. and Guillouzo, A. (1989) FEBS Lett. 258, 99-102.
[26] Becker, P., Renkawitz, R. and Schütz, G. (1984) EMBO J. 3, 2015-2020.
[27] Shelly, L.L., Tynan, W., Schimd, W., Schütz, G. and Yeoh, G. (1989) J. Cell. Biol. 109, 3403-3410.
[28] Sargent, T.D., Jagodzinski, L.L., Yang, M. and Bonner, J. (1981) Mol. Cell. Biol. 1, 871-883.
[29] Taylor, J.B., Craig, R.K., Beale, D. and Ketterer, B. (1984) Biochem. J. 219, 223-231.
[30] Pemble, S., Taylor, J.B. and Ketterer, B. (1986) Biochem. J. 240, 885-889.
[31] Fort, P., Marty, L., Piechaczyk, M., El Sabrouty, S., Dani, C., Jeanteur, P. and Blanchard, J.M. (1985) Nucleic Acids Res. 13, 1431-1442.
[32] Okuda, H., Potter, B.J., Blades, B., McHugh, T.A., Jacobs, L.N. and Berk, P.D. (1989) Drug Metab. Dispos. 17, 677-682.
[33] Mitchell, P.J. and Tjian, R. (1989) Science 245, 371-378.
[34] Telakowski-Hopkins, C.A., King, R.G. and Pickett, C.B. (1988) Proc. Natl. Acad. Sci. USA 85, 1000-1004.
[35] Rushmore, T.H., King, R.G., Paulson, K.E. and Pickett, C.B. (1990) Proc. Natl. Acad. Sci. USA 87, 3826-3830.
[36] Jones, P.B.C., Durrin, L.K., Fischer, F.M. and Whitlock, J.P. (1986) J. Biol. Chem. 261, 6647-6650.
[37] Sogawa, K., Fujisawa-Sehara, A., Yamane, M. and Fujii-Kuriyama, Y. (1986) Proc. Natl. Acad. Sci. USA 83, 8044-8048.
[38] Vandenberghe, Y., Morel, F., Pemble, S., Taylor, J.B., Rogiers, V., Ratanasavanh, D., Vercruysse, A., Ketterer, B. and Guillouzo, A. (1990) Mol. Pharmacol. 37, 372-376.
[39] Vandenberghe, Y., Foriers, A., Rogiers, V. and Vercruysse, A. (1990) Biochem. Pharmacol. 39, 685-690.