Identification of an enhancer element of class Pi glutathione S-transferase gene required for expression by a co-planar polychlorinated biphenyl

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3,3′,4,4′,5-Pentachlorobiphenyl (PenCB), one of the most toxic co-planar polychlorinated biphenyl congeners, specifically induces class Pi glutathione S-transferase (GSTP1) as well as cytochrome *P*-450 1A1 in primary cultured rat liver parenchymal cells [Aoki, Matsumoto and Suzuki (1993) FEBS Lett. **333**, 114–118]. However, the 5′-flanking sequence of the GSTP1 gene does not contain a xenobiotic responsive element, to which arylhydrocarbon receptor binds. Using a chloramphenicol acetyltransferase assay we demonstrate here that the enhancer termed GSTP1 enhancer I (GPEI) is necessary for the stimulation by PenCB of GSTP1 gene expression in primary cultured rat liver parenchymal cells. GPEI is already known to contain a dyad of PMA responsive element-like elements oriented palindromically. It is suggested that a novel signal transduction pathway activated by PenCB contributes to the stimulation of GSTP1 expression.

Key words: antioxidant responsive element, lipofection, primary culture, rat liver parenchymal cells, transfection.

INTRODUCTION

Co-planar polychlorinated biphenyl (PCB) congeners and related compounds are known to be potent hepatocarcinogens on the basis of experimental results and epidemiological studies [1–3]. Co-planar PCB congeners are chlorinated at the *meta* and *para* positions of the aromatic rings and are stereo-structurally similar to 2,3,7,8-tetrachlorodibenzo-*p*-dioxin and 2,3,7,8-tetrachlorodibenzofuran. These compounds are teratogenic [4,5] and have anti-oestrogenic and endocrine-disrupting activities [6,7]. Co-planar PCB congeners are not mutagens and are categorized as tumour promoters [8]. However, the manner in which co-planar PCB congeners regulate gene expression is unclear except for their effect on the genes for cytochrome *P*-450 1A1 and related drug-metabolizing enzymes [9]. The transcriptional activation of these genes is mediated by a ligand-dependent transcription factor termed arylhydrocarbon receptor. We systematically surveyed the genes induced by co-planar PCB congeners. Studies on mechanisms for inducing the expression of these genes might be expected to reveal a novel signal transduction pathway involved in the adverse effects caused by co-planar PCB.

Previously we showed that class Pi glutathione S-transferase (GSTP1) was specifically induced in primary cultured rat liver parenchymal cells by 3,3′,4,4′,5-pentachlorobiphenyl (PenCB) [10], which is one of the most toxic co-planar PCB congeners [1,3]. GSTP1 is a marker enzyme for preneoplastic hepatic foci and hepatocarcinomas but is latent in normal rat liver [11,12]. The GSTP1 gene structure has been intensively investigated [13–16]. The 5′ upstream region of the GSTP1 gene contains a PMA responsive element (TRE) and a related enhancer element termed GSTP1 enhancer I (GPEI), but not a xenobiotic responsive element [17,18], to which the ligand-bound arylhydrocarbon receptor and auxiliary factors (e.g. arylhydrocarbon receptor nuclear translocator protein) bind. GPEI contains a dyad of TRE-like elements [13,14] identified as *cis*-elements required for GSTP1 expression in tumour cells [14,15].

We have showed previously that protein kinase C or a related protein kinase has a role in the PenCB-induced expression of GSTP1 mRNA in rat liver parenchymal cells [19] and that the phosphorylation of c-Jun was stimulated by PenCB [20]. To understand the mechanism for the induction of GSTP1, we have now identified a 5′ upstream region required for the induction of GSTP1 by PenCB. Plasmids containing a 3.0 kb fragment of the 5′-flanking region of the GSTP1 gene and its deletion mutants fused with the chloramphenicol acetyltransferase (CAT) gene were transfected into primary cultured rat liver parenchymal cells by lipofection; CAT activity was determined after treatment of the cells with PenCB. Here we report that GPEI is required for the stimulation of GSTP1 gene expression by PenCB.

MATERIALS AND METHODS

Chemicals

PenCB was kindly provided by Dr. M. Morita. ECAT, 1CAT, 2CAT and GPEI/Δ-56kCAT plasmids, and their synthetic fragments and mutants, and pGP5 were prepared as described previously [13,14]. Vector pmiwZ was obtained from the Japanese Cancer Resources Bank (Gene) (deposit number VE052). pRSVLUC was prepared by the method of de Wet [21]. pSVLUC and pSVβGAL were purchased from Promega (Madison, WI, U.S.A.); mouse epidermal growth factor (EGF) and acetyl coenzyme A were from Wako Pure Chemicals (Tokyo, Japan); cell culture media and dexamethasone were from Sigma Chemical Co. (St. Louis, MO, U.S.A.); fetal bovine serum was from Bioproducts (Walkersville, MD, U.S.A.); Lipofectin was from Life Technologies (Gaithersburg, MD, U.S.A.); 1-deoxy-[*dichloroacetyl*-1-^{14}C]chloramphenicol was from Amersham (Little Chalfont, Bucks., U.K.); β-galactosidase reporter gene

Abbreviations used: ARE, antioxidant responsive element; CAT, chloramphenicol acetyltransferase; EGF, epidermal growth factor; β-Gal, *Escherichia coli* β-galactosidase; GPEI, class Pi glutathione S-transferase enhancer I; GPEIs, 38 bp core sequence of GPEI; GSTP1, rat class Pi glutathione S-transferase; PCB, polychlorinated biphenyl; PenCB, 3,3′,4,4′,5-pentachlorobiphenyl; TRE, PMA responsive element.

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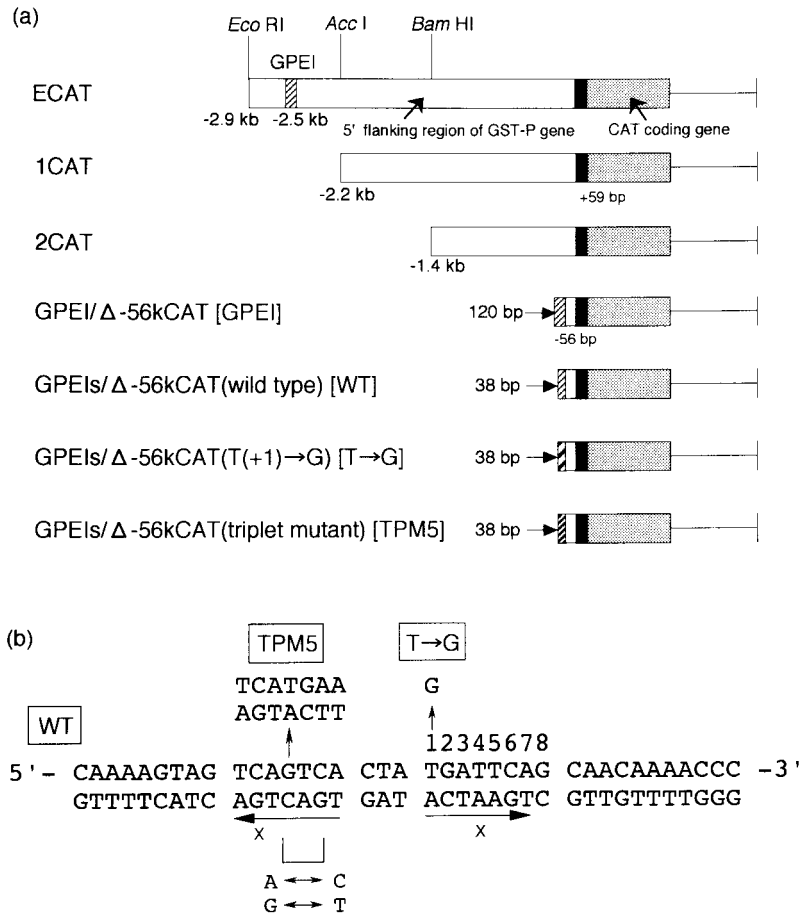

Figure 1 Structures of GSTP1 promoter–CAT fusion plasmid constructs

(**a**) Structures of the constructs used for transfections. The abbreviations in the square brackets show the plasmid DNA species used for Figure 5. (**b**) Structure of GPEIs and its synthetic mutants [14]. The TRE-like sequence is indicated by long arrows. The positions of nucleotides in the TRE-like sequence are numbered in accordance with the rule described by Angel et al. [41]. The single point-mutated nucleotide is indicated by a short vertical arrow. TPM5 indicates the mutant in which the nucleotides as a consecutive triplet are changed to non-complementary ones (A ↔ C, G ↔ T). The TPM5 sequence is shown above the wild-type sequence. The positions of nucleotides different from those of the consensus TRE are indicated by x.

assay kit and luciferase reporter gene assay kit were from Boehringer Mannheim (Mannheim, Germany).

Preparation of monolayer cultures of rat liver parenchymal cells

Monolayer culturing of the cells was performed essentially as described previously [19]. Liver parenchymal cells were isolated from a male Wistar rat liver that had been digested with collagenase. Cells (2.5×10^6) in 3 ml of modified Williams medium E, pH 7.4, containing 10 mM sodium pyruvate, 100 i.u./ml penicillin, 100 μg/ml streptomycin, 0.25 μg/ml amphotericin B, 25 mM Hepes/NaOH buffer, 1 nM insulin, 100 nM dexamethasone and 10% (v/v) fetal bovine serum were plated in collagen-coated 60 mm plates. After the cells had been cultured for 4 h at 37 °C in air/CO_2 (19:1), the medium was changed to serum-free Williams medium E containing 1 nM insulin, 100 nM dexamethasone and 0.1 μg/ml aprotinin; the cells were then incubated for 20 h.

Construction of Reporter gene plasmids

CAT fusion plasmids were constructed by the method of Sakai et al. [13]. A 3.0 kb fragment between −2.9 kb and +59 bp of the gene for GSTP1 [22,23] was inserted into the *Hin*dIII site of pSV0CAT [24] and designated ECAT. A series of 5′ deletion mutants were constructed from the ECAT by using appropriate restriction enzymes [13]. GPEI fragment, a synthetic 38 bp fragment of the GPEI core (GPEIs) and its mutants were connected to Δ-56kCAT (an enhancer-negative plasmid that contains a GC box and a TATA box) by the method of Okuda et al. [14]. The structures of ECAT, 1CAT, 2CAT, GPEI/Δ-56kCAT, GPEIs/Δ-56kCAT and its mutants are illustrated in Figure 1.

Vector pmiwZ [25] includes the Rous sarcoma virus 5′ long terminal repeat [26] and a chicken β-actin promoter in its 5′-flanking region. pRSVLUC includes the Rous sarcoma virus 5′ long terminal repeat. pSVβGAL and pSVLUC include the SV40 promoter and enhancer [27]. As reporter genes, pmiwZ and pSVβGAL contain a *lac*Z [*Escherichia coli* β-galactosidase (β-Gal)] gene, and pRSVLUC and pSVLUC contain a firefly luciferase gene [21].

DNA transfection and treatment with chemicals

Plasmid transfection of monolayer cultured rat liver parenchymal cells was performed by the lipofection method [28–30]. The

Table 1 Luciferase or β-galactosidase activity in reporter-gene-transfected primary cultured rat hepatocytes

Rat liver parenchymal cells were transfected with the indicated amounts of the reporter gene construct. Luciferase or β-galactosidase activity in the cells was determined with a chemiluminescence assay. Values represent the enzyme activities as cpm per μg of protein.

			Luciferase activity	
Plasmid	Cell type	Amount of DNA (μg)	(c.p.m./μg of protein)	(fold)
pRSVLUC	Control	0	3.70 ± 0.93	1.0
	Transfected	5	11.23 ± 0.46	3.0
		10	12.45 ± 3.07	3.4
		20	12.71 ± 2.75	3.4
pSVLUC	Control	0	3.04 ± 0.14	1.0
	Transfected	5	7.65 ± 0.20	2.5
		10	7.96 ± 0.00	2.6
		20	8.94 ± 0.00	2.9
			β-galactosidase activity	
Plasmid	Cell type	Amount of DNA (μg)	(c.p.m./μg of protein)	(fold)
pmiwZ	Control	0	4980 ± 1280	1.0
	Transfected	5	218000 ± 9470	438
		10	239000 ± 58400	480
		20	416000 ± 83800	835
pSVβGAL	Control	0	11700 ± 5980	1.0
	Transfected	5	21500 ± 5650	1.8
		10	31600 ± 6040	2.7
		20	58800 ± 31700	5.0

culture medium was changed to 3 ml of Williams medium E without fetal bovine serum before lipofection; the plasmids were then transfected into the cells with 30 μl of Lipofectin reagent in each 60 mm plate. CAT plasmid (12 μg) was transfected into the cells with 4 μg of pmiwZ unless stated otherwise. These amounts of plasmids were optimal for obtaining maximum CAT activity. After the cells were incubated for 12 h at 37 °C in air/CO_2 (19:1), the medium was replaced with serum-free Williams medium E containing 10 mM sodium pyruvate, 100 i.u./ml penicillin, 100 μg/ml streptomycin, 0.25 μg/ml amphotericin B, 25 mM Hepes/NaOH buffer, 1 nM insulin, 0.1 μg/ml aprotinin and 6 mM nicotinamide. If the cells were treated with PenCB and/or dexamethasone, or with EGF, the reagent was added to this medium at the indicated concentrations as described previously [19]. The cells were further incubated for the indicated times.

Cells were harvested by scraping in PBS. Cell extracts were prepared by four cycles of freezing and thawing in 0.25 M Tris/HCl, pH 7.5, and the protein concentration of the extracts was determined by the procedure of Bradford [31].

Reporter gene assay

β-Gal or luciferase activity in the cell extracts was measured by using chemiluminescent assays [32,33] with reporter gene assay kits from Boehringer Mannheim; chemiluminescence was determined with a liquid-scintillation analyser (model 2500TR; Tri-carb, Meriden, CT, U.S.A.). For CAT assays [34] the extracts were heated at 65 °C for 10 min and the precipitates were removed by centrifugation at 15000 ***g*** for 5 min. The reaction mixtures (final volume 125 μl) containing cell extract, 210 mM Tris/HCl, pH 7.5, 11 kBq of 1-deoxy[^{14}C]chloramphenicol [35] and 80 nmol of acetyl-CoA were incubated at 37 °C for 2 h. Reactions were terminated by the addition of 1 ml of cold ethyl acetate. The product (3-acetyl-1-deoxychloramphenicol) and unreacted substrate were extracted with ethyl acetate. After ethyl acetate had been evaporated, the residue was dissolved in 20 μl of ethyl acetate and chromatographed on a thin-layer plate (LK6D; Whatman, Maidstone, Kent, U.K.) with chloroform/methanol (94:6, v/v). The radioactivity of product and substrate was analysed by using the Fuji BAS 2000 system (Fuji Photo, Tokyo, Japan). CAT activity was determined as the ratio of radioactivity of the product to the total radioactivity and was standardized with β-Gal.

RNA preparation and Northern blot analysis

When the level of GSTP1 mRNA induced in primary cultured rat liver parenchymal cells by PenCB was determined, untransfected cells were cultured in the presence of PenCB in the same manner as transfected cells. After the cells had been treated with 100 nM PenCB for 24, 36 or 48 h, total RNA was extracted from the cells as described by Chomczynski and Sacchi [36]. RNA (10 μg) was separated on a formaldehyde/1.2 % (w/v) agarose gel containing 0.02 % ethidium bromide. After RNA had been separated on the gel, the positions of rRNA species were revealed by UV illumination. The separated RNA was transferred to a nylon membrane (Amersham). The membrane was incubated with GSTP1 cDNA probe, which is an *Eco*RI–*Sal*I fragment of pGP5 [22] as described previously [19,37]. Radioactive bands on the membrane were detected by autoradiography.

RESULTS

Before we performed the CAT assays we looked for an expression vector with high sensitivity as a control plasmid. We intended to standardize CAT activity with reporter gene activity of a control

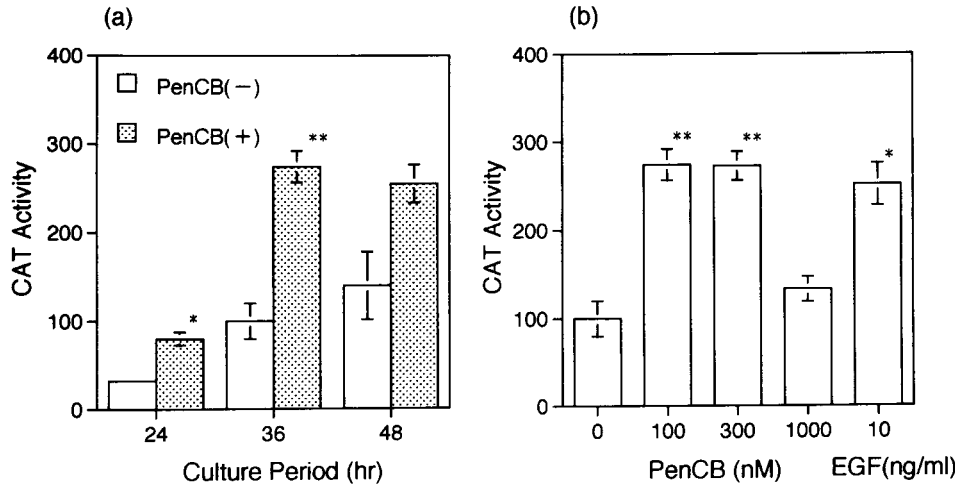

Figure 2 Effect of PenCB or EGF on the expression of the gene for CAT with GSTP1 promoter (ECAT) in primary cultured rat liver parenchymal cells

Rat liver parenchymal cells were transfected with 12 μg of ECAT and 4 μg of pmiwZ on each 60 mm plate, then treated with PenCB. The CAT activity in the cells was assayed; it was standardized with β-galactosidase activity. Each value is the mean ± S.E.M. for three separate experiments. (**a**) Effect of culture period after transfection. Open bars show the CAT activity of the cells without PenCB treatment [PenCB(−)]; stippled bars show that of the cells treated with 100 nM of PenCB [PenCB(+)]. The activity in the cells cultured for 36 h without PenCB treatment is represented as 100. $^{*}P < 0.05$, $^{**}P < 0.01$, compared with PenCB(−). (**b**) Effects of PenCB and EGF. The cells were cultured for 36 h after transfection. The activity in the cells cultured without PenCB and EGF treatment is represented as 100. $^{*}P < 0.05$, $^{**}P < 0.01$, compared with control.

plasmid because the transfection of primary cultures of rat liver parenchymal cells is generally inefficient and variable between cultures. A vector expressing β-Gal or luciferase (pmiwZ, pSVβGAL, pRSVLUC or pSVLUC) was transfected into primary cultured rat liver parenchymal cells and enzyme activities in the cell lysates were measured. As shown in Table 1, β-Gal activity was higher than luciferase activity in the cell lysates. Of the two β-Gal-expressing vectors, pmiwZ showed higher activity than pSVβGAL. pmiwZ was the most appropriate of the four vectors for co-transfection with CAT fusion plasmid to standardize the CAT activity, because pmiwZ was expected to show the highest activity of the four even when a small amount (less than 5 μg) of plasmid was transfected. When 4 μg of pmiwZ per 60 mm dish was co-transfected with 12 μg of CAT plasmid (ECAT), sufficient β-Gal activity and optimum CAT activity were obtained (results not shown).

The optimal condition for the stimulation of CAT activity by PenCB was determined. ECAT-transfected primary cultured rat liver parenchymal cells were exposed to 100 nM PenCB for various durations (24–48 h). PenCB stimulated the expression of CAT, the activity of which increased with the duration of incubation in both the presence and the absence of PenCB. Optimum stimulation by PenCB was observed after incubation for 36 h (Figure 2a). GSTP1 mRNA in the cells was determined after treatment with 100 nM PenCB for 24, 36 and 48 h (Figure 3). Although GSTP1 mRNA became detectable even in untreated cells after incubation for 36 and 48 h, expression of GSTP1 mRNA was stimulated in PenCB-treated cells after exposure for 24–48 h. This result shows that the increase in CAT activity by PenCB was correlated with the increase in the amount of GSTP1 mRNA. The expression of GSTP1 became leaky in primary cultures of liver parenchymal cells after a long period of culture, as reported previously [38,39]. The expression of CAT in the absence of PenCB could be explained by this leakage of GSTP1 gene expression in primary cultured cells. Some factor required for the PenCB-independent expression of GSTP1 might be induced during the culturing of the cells.

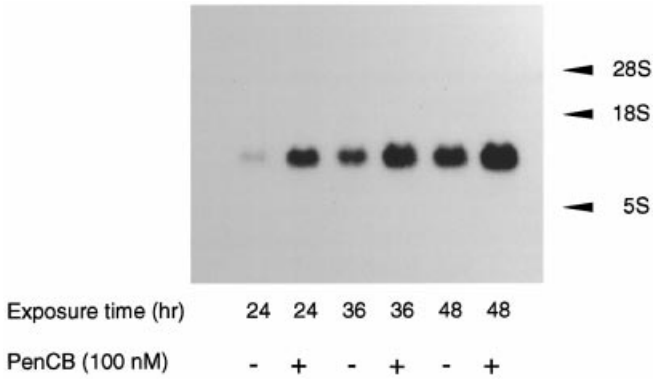

Figure 3 Induction of GSTP1 mRNA by PenCB

Rat liver parenchymal cells were treated with 100 nM PenCB for 24, 36 or 48 h after preculturing for 36 h. GSTP1 mRNA levels in PenCB-treated cells and in the control cells were determined by Northern blot analysis. The positions of 28 S, 18 S and 5 S ribosomal RNA are indicated at the right.

ECAT-transfected cells were treated with PenCB (100 nM to 1 μM) for 36 h (Figure 2b). PenCB stimulated the expression of CAT at concentrations of 100 and 300 nM but not at 1 μM PenCB. This suppression of CAT activity might be caused by an inhibitory effect of PenCB on CAT activity, because cytotoxicity was not observed at 1 μM PenCB (results not shown). CAT activity was also stimulated in ECAT-transfected cells by treatment with 10 ng/ml EGF, an inducer of GSTP1 [19]. This indicates that the 2.9 kb upstream flanking region of GSTP1 gene was required for stimulation by EGF as well as by PenCB.

To determine the region required for the stimulation of GSTP1 expression by PenCB, rat liver cells were transfected with ECAT or two types of 5′-deletion mutants (1CAT and 2CAT). As shown in Figure 1, 1CAT is a fragment between −2.2 kb and

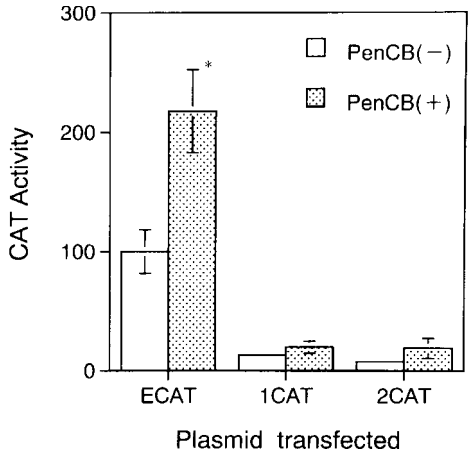

Figure 4 Effect of PenCB on the expression of the gene for CAT in primary cultured rat liver parenchymal cells transfected with ECAT, 1CAT or 2CAT

Rat liver parenchymal cells were transfected with 12 μg of CAT construct and 4 μg of pmiwZ on each 60 mm plate, then treated with 100 nM PenCB for 36 h. Each value is the mean ± S.E.M. for three separate experiments. Open bars show the CAT activity of the cells without PenCB treatment [PenCB(−)]; stippled bars show that of the cells treated with PenCB [PenCB(+)]. $^{*}P < 0.05$, compared with PenCB(−).

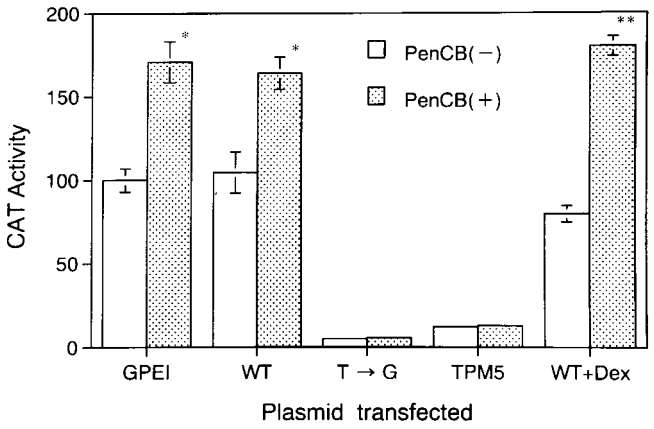

Figure 5 Effect of PenCB on the expression of the gene for CAT in primary cultured rat liver parenchymal cells transfected with wild GPEI, GPEIs and mutant GPEIs/CAT constructs

Culture and transfection were performed as described in the Materials and methods section and in the legend to Figure 4. The structures and abbreviated names of the plasmid constructs are shown in Figure 1. 'WT + Dex' indicates that the cells transfected with 'wild-type' plasmid (GPEIs) were treated with 100 nM dexamethasone. Each value is the mean ± S.E.M. for four separate experiments. Open bars show the CAT activity of the cells without PenCB treatment [PenCB(−)]; stippled bars show that of the cells treated with PenCB [PenCB(+)]. $^{*}P < 0.05$, $^{**}P < 0.01$, compared with PenCB(−).

+59 bp of the GSTP1 gene fused with the CAT-coding region, and 2CAT is a fragment between −1.4 kb and +59 bp. After the transfected cells had been treated with 100 nM PenCB for 36 h (Figure 4), CAT activity was stimulated approx. 2-fold by PenCB in ECAT-transfected cells. However, in cells transfected with 1CAT or 2CAT, CAT activity was much lower than in the ECAT-transfected cells in the absence of PenCB, and PenCB did not stimulate CAT activity. This suggests that the region between 2.9 and 2.2 kb upstream of the transcription initiation site of the GSTP1 gene is involved in the stimulation of GSTP1 expression by PenCB and in its basal level of expression.

An enhancer termed GPEI, which is required for the expression of GSTP1 in tumour cells, is located 2.5 kb upstream [13,14]. GPEI is 120 bp long and its core 38 bp region, GPEIs (Figure 1b), contains a dyad of TRE-like elements oriented palindromically with a 3 bp interval. We determined whether GPEI is required for the stimulation of GSTP1 expression by PenCB (Figure 5). GPEI fragment, GPEIs and its mutant fragments fused with CAT plasmid were transfected into the cells, which were treated with 100 nM PenCB. The expression of GPEIs/Δ-56kCAT (wild-type) was stimulated by PenCB, as was that of GPEI/Δ-56kCAT. However, CAT activity in the cells transfected with the plasmid containing mutant GPEIs [a single base alteration (T → G) and triplet alteration (TPM5)] [14] was slight, and was not stimulated by PenCB. Dexamethasone (100 nM) suppressed the induction of GSTP1 mRNA by PenCB in our previous study [19] but did not inhibit the PenCB-dependent stimulation of CAT activity in GPEIs/Δ-56kCAT-transfected rat liver cells at 100 nM (Figure 5). This result shows that glucocorticoid does not inhibit GPEI-mediated transcription.

DISCUSSION

In this report we have shown that the region between 2.9 and 2.2 kb upstream of the GSTP1 gene contains a *cis*-element required for the stimulation of GSTP1 expression by PenCB. In this region the GPEI enhancer element required for GSTP1 expression in hepatocarcinoma is located 2.5 kb upstream of the initiation site. CAT activity was stimulated by PenCB in cells transfected with GPEI or GPEIs but not in cells transfected with mutant GPEIs. The level of stimulation by PenCB was the same in GPEIs-transfected cells and in GPEI-transfected cells. These results indicate that the stimulation of GSTP1 expression by PenCB is regulated at the transcriptional level and that the GPEIs region is a major *cis*-element involved in the stimulation.

In cells transfected with GPEI or GPEIs, CAT activity was expressed constitutively in the absence of PenCB; a small amount of GSTP1 mRNA was detected in the cells after incubation for 24 h (Figures 3 and 5). The CAT activity was stimulated approx. 2-fold by PenCB, but the magnitude of stimulation was smaller than that expected from PenCB-dependent GSTP1 mRNA expression. This suggests that some transcription factor binds to the GPEI core in the absence of PenCB and that one or more other factors required for the stimulation are potentiated to bind to the GPEI core by treatment with PenCB. The low efficiency of stimulation is probably caused by a difference in the accessibility of transcription factor to the GPEI core between the GSTP1 gene in genomic DNA and in the transiently transfected CAT gene [40]. Because the genomic GSTP1 gene is integrated into the chromatin, a transcription factor for the constitutive expression could be excluded from the gene. This organized structure of the genomic GSTP1 gene probably accelerated the attachment to the GPEI core of a factor required for PenCB-dependent stimulation. In contrast, because the transfected plasmids were disorganized in the nucleus, constitutive expression could not be suppressed by the chromatin structure, and the factor for stimulation could not be attached to the GPEI core efficiently.

It has been difficult to perform CAT assays with primary cultured liver parenchymal cells because transfection of these cells is inefficient. In spite of this difficulty we used primary cultured cells because GSTP1 is expressed constitutively in carcinoma cell lines [13,14]. We observed that the gene for

GSTP1 is also expressed in rat hepatocytes immortalized with simian virus 40 as well as in hepatomas, and that this expression is slightly stimulated by PenCB (results not shown).

Our observation indicates that arylhydrocarbon receptor might not act on GPEI as a transactivating factor [19], and the sequence of GPEI is different from that of xenobiotic responsive element [consensus sequence 5′-(T/G)NGCGTG(A/C)(G/C)A-3′] [17,18]. It is suggested that PenCB activates a novel signal transduction pathway that potentiates some factor required for expressing a GPEI-containing gene (GSTP1 gene). The core region of GPEI, GPEIs, contains a dyad of TRE-like elements palindromically oriented with a 3 bp interval [14,15]. The mutant GPEIs/Δ-56kCAT plasmids that were transfected into the cells (TG and TPM5) have a single point mutation in the downstream half-site TRE-like sequence of GPEI and three consecutive point mutations in the upstream half-site TRE-like sequence (Figure 1). PenCB did not stimulate CAT expression in the cells transfected with the mutant GPEIs/Δ-56kCAT plasmids. These mutant plasmids also did not show CAT activity in hepatocarcinoma cell lines [14]. These results suggest that transcription factors for GSTP1 expression in hepatocarcinomas are identical with those for the stimulation of GSTP1 expression by PenCB in cultured normal liver cells and that both of the two TRE-like elements are required for the stimulation of GSTP1 expression by PenCB.

TRE is an element to which AP-1 (a heterodimer of c-Jun and c-Fos) binds [41–43]. The binding of AP-1 to TRE is known to be regulated by phosphorylation/dephosphorylation of c-Jun and/or expression of the gene for c-Jun. The phosphorylation of c-Jun is stimulated by a protein kinase cascade containing protein kinase C and related kinases. We have already shown that a protein kinase C-like kinase is required for the PenCB-dependent expression of GSTP1 [19] and that PenCB stimulates c-Jun phosphorylation [20]. However, a transcription factor for the stimulation of GSTP1 expression by PenCB has yet to be identified. c-Jun, c-Fos, Maf and related factors are candidates that act on GPEI for expressing GSTP1 in carcinoma [16], and one or more novel factors other than AP-1 have been found to bind to GPEI in F9 cells and HeLa cells [44]. These factors might also contribute to the GPEI-dependent stimulation of GSTP1 expression by PenCB. In this report we have shown that dexamethasone, an antagonist of c-Jun [45–47], did not inhibit GPEIs-mediated transcription, which indicates that c-Jun is not involved in this stimulation. It was shown that the GPEI sequence is similar to those of the antioxidant responsive element (ARE) and the element recognized by nuclear factor erythroid 2, which is a heterodimer of p45 and a member of the small Maf family proteins [48,49]. Favreau and Pickett [50] reported that t-butylhydroquinone and PMA stimulate CAT expression in HepG2 cells transfected with CAT constructs containing ARE or GPEI, and that GPEI might recognize the same nucleoprotein complex as ARE in HepG2 cells. These observations suggest that GPEI is responsive to antioxidants and xenobiotics other than PenCB. Recently, Nrf2 (nuclear factor erythroid 2-related factor 2), one of the p45-related molecules, was identified as a transcription factor for the expression of genes containing an ARE by using Nrf2 knock-out mice [49]. Nrf2 is a possible factor required for the stimulation of GSTP1 expression by PenCB.

We have shown that a GPEI-binding protein is induced in the nucleus of PenCB-treated primary cultures of liver parenchymal cells by a gel-shift assay (Y. Ohban and Y. Aoki, unpublished work). Further studies to identify one or more factors binding to GPEI by treatment with PenCB are required to understand the signal transduction pathway activated by PenCB and its relation to the toxicity of PenCB.

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