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Polychlorinated biphenyls modulate protooncogene expression in Chang liver cells

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Abstract

In Chang liver cells we studied the influence of polychlorinated biphenyls (PCB) on the expression of different protooncogenes. In cells incubated with medium supplemented with PCB we observed an early effect after 3 h on c-*erb*A and c-*erb*B RNA level. This reduction of RNA was due to a delayed transcriptional activation of the genes. The PCB congener 3,3',4,4',5-pentachlorobiphenyl (5-CB) in a 1,000-fold lower concentration influenced protooncogene expression in the same manner. In contrast c-*raf* RNA level increased transiently after 24 h. The fact that persistent chemicals like PCB interfere with protooncogene expression is particularly interesting in view of their tumor promoting activity.

Key words: PCB; Protooncogene expression; c-*erb*A; c-*erb*B; c-*raf*; Tumor promoter

1. Introduction

Accumulation and storage of PCB in human and animal tissues have been recognized as an environmental problem [1]. Like many other cyclic hydrocarbons, PCB are known to increase the incidence of hepatocellular carcinomas in rodents by their tumor promoting activity [2]. For other tumor promoters it is well documented that they interact with important pathways of growth control and differentiation in the cell [3].

We investigated the influence of PCB on protooncogene expression in a human liver cell line. Various oncogenes, chosen to represent several recognized stages in signal transduction cascades [4] were selected for studying mRNA levels and transcription rates.

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Abbreviations: PCB, polychlorinated biphenyls; 5-CB, 3,3',4,4',5-pentachlorobiphenyl; DMEM, Dulbecco's modified Eagle's medium; DMSO, dimethylsulfoxide; EGF, epidermal growth factor; PDGF, platelet derived growth factor; TCDD, 2,3,7,8-tetrachlorodibenzo-*p*-dioxin.

2. Materials and methods

2.1. Cell culture

Human Chang liver cells (American Type Culture Collection No. CCL 13) were grown in DMEM supplemented with glutamine (2 mM), $NaHCO_3$ (45 mM) and 8% fetal calf serum in petri dishes to approximately 70% confluence, then incubated in PCB medium containing 50 μM Arochlor 1254 (a mixture of PCB congeners), 5-CB medium containing 3 nM, 15 nM or 300 nM 5-CB or control medium containing the solvent DMSO.

2.2. RNA preparation

Total RNA was isolated according to [5], RNA from 5-CB treated cells according to [6].

2.3. RNA blotting and hybridization

For slot blot hybridization, denatured total RNA was applied onto nitrocellulose filters with a minifold II apparatus (Schleicher & Schüll, Germany). Northern blot hybridization was carried out as described in [7]. The autoradiographs of the filters were evaluated by densitometric scanning (Elscript 400, Hirschmann, Germany).

2.4. Run on transcription assay

Nuclei of Chang liver cells were isolated and frozen according to [8]. The assay with the thawed nuclei ($1 \times 10^7/100$ μl) was performed as described in [9].

2.5. DNA-probes

v-*erb*A, a *Pst*I–*Pst*I fragment (500 bp) of pAE-*Pst* [10]; v-*erb*B, a *Eco*RI–*Eco*RI fragment (5.1 kb) of pAE11 [10]; c-*myc* (mouse), a *Bam*HI–*Xba*I fragment (4.8 kb) of pSVc-*myc*1 [11]; v-*raf*, a *Xho*I–*Bgl*II fragment (1.38 kb) of 3611MSV [12]; actin (human) λHAc-69A [13].

2.6. Chemicals

Reagents and enzymes were purchased from the following sources: Arochlor 1254 from Dr. Wrabetz, Bayer AG; 5-CB from Promochem; radioactive substances, enzyme kits from Amersham; enzymes from Boehringer; all other chemicals from Sigma.

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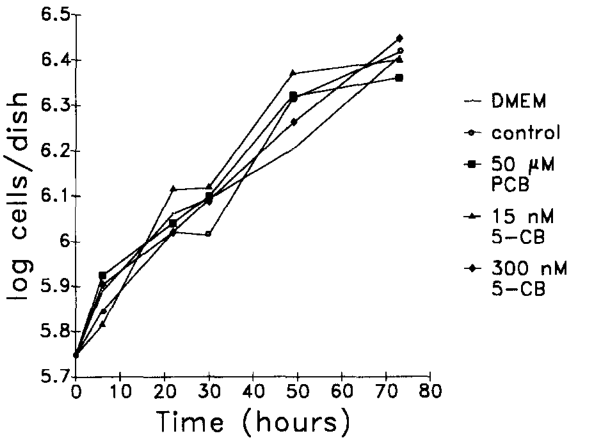

Fig. 1. Growth of Chang liver cells in PCB and 5-CB supplemented media.

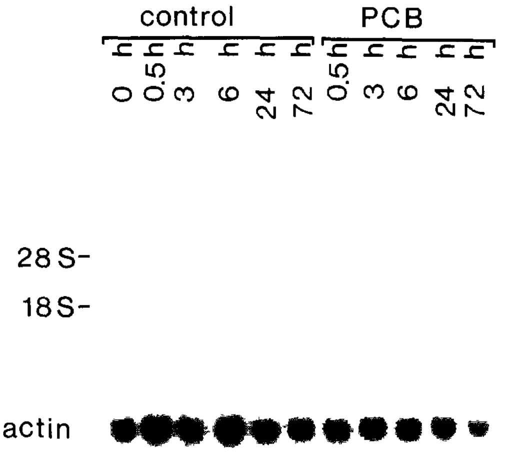

Fig. 3. Northern blot analysis of c-*raf* RNA. Total RNA (20 µg/lane) isolated from cells incubated with PCB medium or control medium. The rehybridization of the stripped filter with an actin probe is a control for equal amounts of RNA.

3. Results

At the concentrations used, neither PCB (50 µM) nor the congener 5-CB (5 nM, 15 nM and 300 nM) had an influence on viability, growth rate (Fig. 1) or morphology of the cells. After incubation of the cells with PCB medium or control medium for different times transcript levels of 9 protooncogenes were determined by slot blot analysis of the isolated RNA. No expression of c-*fms* was detectable and the RNA levels of c-*fos*, c-*mos*, c-*myb*, c-Ha-*ras* (data not shown) and c-*myc* were not affected. However, the transcripts of both c-*erb*A and c-*erb*B transiently decreased in PCB treated cells after 3–6 h to 30–60% of the level in control cells (Fig. 2). After 24 h the normal level was restored or even exceeded. For c-*raf* RNA an increase upto two-fold of the normal level was observed 24 h after PCB treatment. These data were confirmed by Northern blot analysis of isolated RNA, a specific c-*raf* RNA of approximately 3.5 kb size [14] was detectable (Fig. 3). Further investigations on c-*erb*A and c-*erb*B expression were carried out with the congener 5-CB to exclude effects due to contaminants in the PCB mixture. 5-CB is one of the most toxic PCB congeners [15]. We observed the same effect on c-*erb*A and c-*erb*B RNA levels as described for the PCB mixture. Different 5-CB concentrations were tested (Fig. 4). 3 nM 5-CB corresponded to the amount of this congener in the PCB mixture Arochlor 1254. The 5-fold concentration had a more pronounced effect.The highest concentration, 300 nM 5-CB, caused a dramatic decrease of c-*erbA* and c-*erbB* RNA, followed by a strong increase of the specific RNA. Northern blot analysis of samples taken after 3 and 24 h of incubation confirmed these data (Fig. 5). Changes of mRNA levels can be caused by changes in transcription rate or alternatively by altered mRNA half life. Therefore we performed nuclear run on transcription assays. Nuclei were isolated after PCB incubation of the cells and incubated in vitro in the presence of [^{32}P]UTP, to allow elongation of in vivo-initiated nascent RNA transcripts. In nuclei of cells treated only with control medium, transcription of c-*erb*A and c-*erb*B was induced (Fig. 6). In nuclei isolated after incubation with PCB medium, no transcriptional induction was observed after 3 h, after 6 h the transcription rate increased. The effect on c-*erb*B transcription was not as strong as on c-*erb*A transcription. PCB treatment did not affect transcription of actin and c-*myc*.

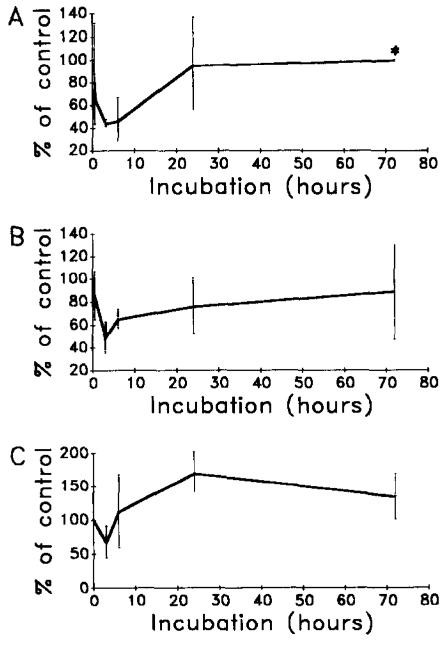

Fig. 2. Slot blot analysis quantitated by densitometric evaluation, mean of five independent experiments shown. RNA (20 µg/slot) isolated from Chang liver cells treated with PCB medium (50 µM PCB) plotted relative to RNA from cells treated with control medium. (A) c-*erb*A RNA (*only one experiment), (B) c-*erb*B RNA, (C) c-*raf* RNA.

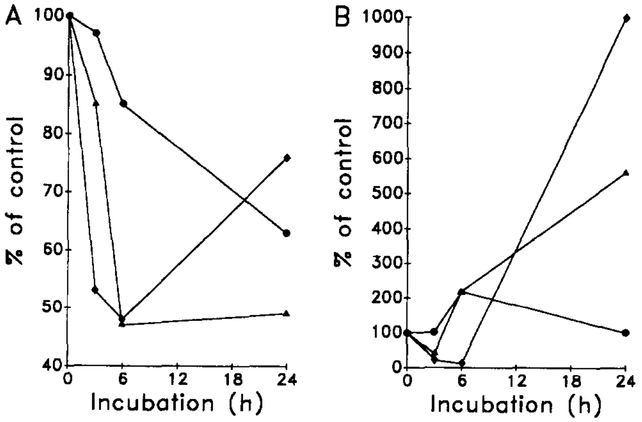

Fig. 4. Concentration dependent effect of 5-CB on (A) c-*erb*A and (B) c-*erb*B RNA level. The slot blot analysis was evaluated by densitometric scanning. RNA isolated from 5-CB treated cells was plotted relative to RNA of control cells. (●) 3 nM 5-CB, (▲) 15 nM 5-CB, (◆) 300 nM 5-CB.

4. Discussion

The nuclear run on transcription data indicate that the reduced amount of c-*erb*A and c-*erb*B RNA in cells treated with PCB is the consequence of a transcript depletion due to a delayed transcriptional induction of these genes in the presence of PCB. A decrease of c-*erb*A specific RNA coding for the thyroid hormone receptor [16] to 50% of the control and down-regulation of thyroid hormone receptor is also reported for rat GH3 cells when the thyroid hormone T3 was added to the cells after preincubation in a T3/T4 depleted medium [17]. The rapid decrease/increase of c-*erbA* RNA after PCB treatment of the cells might be mediated by a similar, specific mechanism. There is evidence that aromatic hydrocarbons can interact with thyroid hormone pathways [18]. TCDD and related compounds like PCB are structually related to the thyroid hormones and may function as potent agonists or antagonists [19]. The thyroid status has been shown to be important for the manifestation of neoplastic transformation [20]. Borek et al. demonstrated that a preincubation of primary hamster embryo cells and mouse C3H/10T1/2 cells with thyroid hormone, which did not affect cell growth, increased the transformation rate by benzo[a]pyrene and *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine, a direct acting mutagen. In rodents treated with PCB [9] or TCDD [21] also c-*erb*A expression in the liver is affected. The interference of PCB with thyroid hormone action might be important with regard to the tumor promoting activity of PCB.

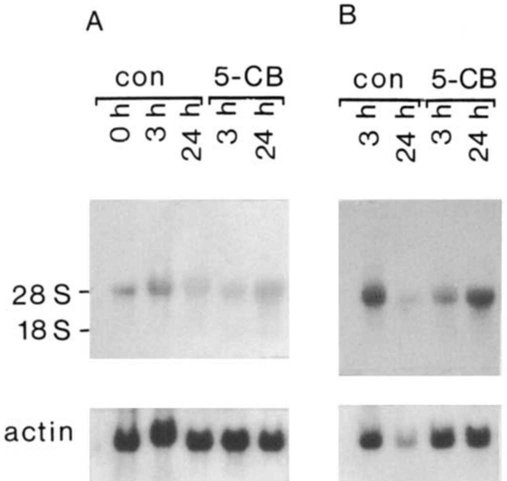

Fig. 5. Northern blot analysis of (A) c-*erb*A and (B) c-*erb*B RNA after incubation of Chang liver cells with medium containing 15 nM 5-CB. The rehybridization of the stripped filter with actin is a control for equal amounts of RNA (20 μg/lane).

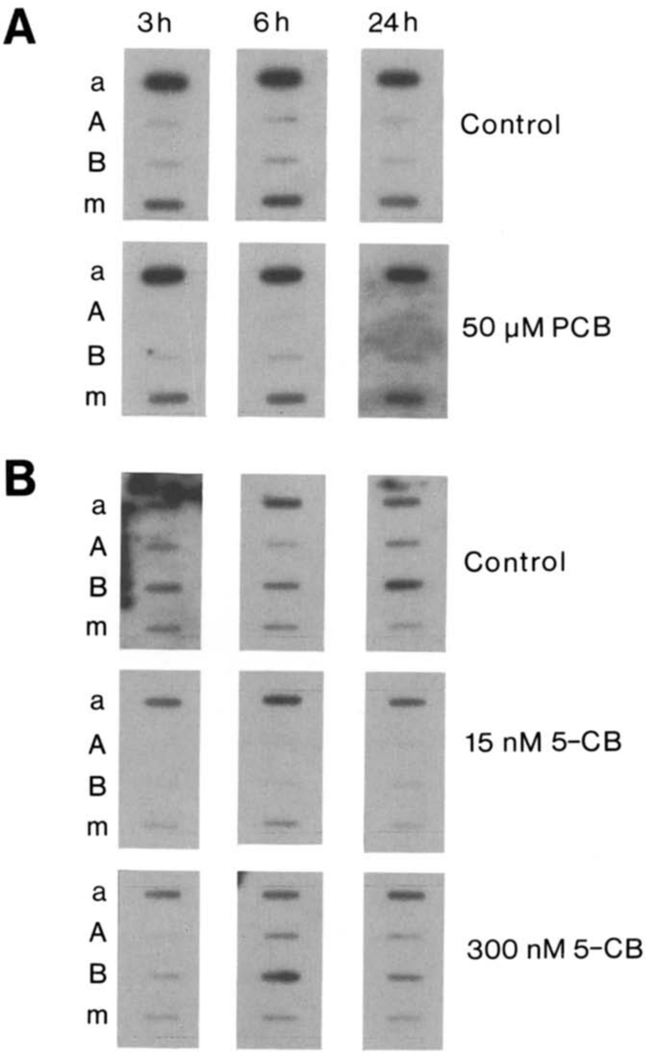

Fig. 6. Run on transcription analysis of protooncogenes after PCB treatment. DNA representing actin (a), c-*erb*A (A), c-*erb*B (B), and c-*myc* (m) sequences fixed onto nitrocellulose were hybridized with ^{32}P-labelled run on transcripts. (A) Transcription after incubation of the cells with medium containing 50 μM PCB. (B) Transcription after incubation of the cells with medium containing 15 nM or 300 nM 5-CB.

The expression of the EGF-receptor gene (c-*erb*B) was affected in a comparable time course by PCB treatment as the c-*erb*A gene. It has been described, that agents causing a reduction in the number of EGF receptors in vivo elicit cellular responses that are similar to those caused by exposure to excess doses of growth factors [22]. This correlates the effect of PCB on the c-*erb*B gene with its tumor promoting potential.

C-raf encodes a cytoplasmic serine/threonine kinase. The RAF-protein has been implicated in the mitogenic EGF and PDGF signal transduction cascade following receptor autophosphorylation and c-*raf* activation [23]. *C-raf* expression might play an important role in the development of preneoplastic foci in the liver. A 7-fold elevation of c-*raf* expression was also found in preneoplastic foci of rats fed a PCB supplemented diet (H.-S. Jenke, manuscript in preparation).

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