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Toxicology 216 (2005) 224–231

TOXICOLOGY

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Activation of *CYP1A1* gene expression during primary culture of mouse hepatocytes

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Received 4 June 2005; received in revised form 12 August 2005; accepted 12 August 2005
Available online 15 September 2005

Abstract

Expression of CYP1A1 mRNA in mouse hepatocytes in primary culture was investigated. The expression was obvious on day 3 of culture without addition of any known ligands of the aryl hydrocarbon receptor and increased with culture period. Removal of insulin from and addition of hydrogen peroxide to the medium enhanced and suppressed the expression, respectively. The CYP1A1 mRNA expression was also enhanced in the presence of anti-oxidant, *t*-butylhydroquinone, in the medium. Several kinds of kinase inhibitors markedly increased the CYP1A1 mRNA expression. In contrast, the inhibitory expression was prolonged in the presence of okadaic acid, a potent inhibitor of serine/threonine phosphatase PP1 and PP2. These observations suggest that there might be a repressive pathway in the regulation of CYP1A1 mRNA expression and that the presently observed expression pathway differs at several points from those previously reported, such as ligand-activated aryl hydrocarbon receptor- or omeprazole-mediated expression. Modulation of CYP1A2 mRNA expression after exposing hepatocytes to agents affecting phosphorylation pathways differed from that of CYP1A1 mRNA. This implies that regulatory pathways for CYP1A1 and CYP1A2 expression may differ.
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Keywords: CYP1A1; CYP1A2; Mouse primary culture; Oxidative stress; Anti-oxidant; Protein kinase inhibitor; Okadaic acid

1. Introduction

CYP1A1, a member of the *CYP1* gene family, plays an important role in the metabolism of many xenobiotic compounds (Gonzalez, 1990; Nebert and Gonzalez, 1987). It is highly inducible by TCDD and by planar aromatic hydrocarbons, such as 3-methylcholanthrene or benzo(*a*)pyrene (Gonzalez, 1988; Whitlock et al., 1996). The transcriptional activation of the *CYP1A1* gene has been extensively studied, revealing a mechanism that it is mediated by AhR, a basic helix–loop–helix protein component of the transcription factor complex (Burbach et al., 1992; Nebert et al., 2004). AhR is usually present

Abbreviations: AhR, aryl hydrocarbon receptor; Arnt, aryl hydrocarbon receptor nuclear translocator protein; CYP, cytochrome P450; cDNA, complementary DNA; DCFH-DA, 2,7-dichlorofluorescin diacetate; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; Hsp, heat shock protein; NF-1, nuclear factor 1; SSC, standard saline citrate; SDS-PAGE, sodium dodecyl sulfate-contained polyacrylamide gel electrophoresis; TCDD, 2,3,7,8-tetrachlorodibenzo-*p*-dioxin; XRE, xenobiotic-responsive element

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0300-483X/$ – see front matter © 2005 Published by Elsevier Ireland Ltd.
doi:10.1016/j.tox.2005.08.007

in cytoplasm in association with Hsp90 (Perdew, 1988; Pongratz et al., 1992). Subsequently, upon high-affinity binding to inducing chemicals, the liganded AhR translocates to nuclei, where it switches the partner from Hsp90 to Arnt and binds to the cognate enhancer sequence, XRE, upstream of the targeted *CYP1A1* gene to activate the expression (Mimura et al., 1999).

Recently, there have been reports that CYP1A1 was expressed without the binding of any ligands to AhR. For example, omeprazole, substituted for benzimidazole, has been shown to induce CYP1A1 expression in human hepatocytes and the rat hepatoma cell line H4E, although it is not proved to be a ligand of AhR (Backlund et al., 1997). However, this induction involves activation of the AhR·Arnt complex via intracellular signal transduction systems. Another induction process was reported that *CYP1A1* gene expression might be intimately related with oxidative stress in human lymphoblastoid and HepG2 cells by treatment with carbaryl and thiabendazole, widely used pesticides and non-agonists of AhR (Delescluse et al., 2001). Moreover, exposure to oxygen promptly increased the CYP1A1 mRNA level in cultured lamb lung microvascular endothelial cells (Hazinski et al., 1995), although there are many reports that oxidative stress down-regulated CYP1A1 expression in mammalian and fish cells (Laville et al., 2004; Morel and Barouki, 1998; Shertzer et al., 1999). With regards to the suppressive effects of the oxidative stress, insulin was found to suppress β-naphthoflavone-dependent accumulation of CYP1A1 mRNA in rat hepatocytes (Barker et al., 1994). Therefore, endogenous substances might have a role in the modulation of the CYP1A1 expression. Furthermore, other mechanisms of CYP1A1 expression, not directly explained by mediation of AhR, have been presented, namely those associated with medium change (Nemoto and Sakurai, 1992a; Nolwen et al., 2000). Since CYP1A1 is an important enzyme, which is involved in the activation of several procarcinogens (Gonzalez, 1988, 1990; Nebert and Gonzalez, 1987), further investigations are necessary to elucidate the regulatory mechanisms. Furthermore, since several mouse lines of “humanized” CYPs are now under development for future risk assessment studies of toxicity and carcinogenesis (Nebert et al., 2004), it is an urgent matter to reveal the regulation mechanism of mouse CYPs.

This paper deals with CYP1A1 expression in mouse hepatocytes in primary culture, which mechanism might be different from that mediated by either AhR ligands or omeprazole. The observations suggest that a phosphorylation-regulated suppressive factor might be involved in the CYP1A1 expression.

2. Methods

2.1. Materials

Materials for culturing hepatocytes were purchased from ICN Biomedicals, Inc. (Bedford, MA) and Kyokuto Seiyaku (Tokyo, Japan). Percoll and collagenase (Type I) were products of Pharmacia (Uppsala, Sweden) and Sigma Chemical Co. (St. Louis, MO), respectively. [α-^{32}P] dCTP was obtained from ICN Biochemical (Costa Mesa, CA). Amersham Pharmacia Biotech Co. (Buckingham, England) supplied nylon membrane (Hybond-N). All other laboratory chemicals were of the highest quality and from commercial suppliers.

2.2. Preparation of primary hepatocyte cultures

The liver of C57BL/6NCrj mice was perfused with collagenase, and viable hepatocytes were isolated by Percoll isodensity centrifugation as described (Nemoto and Sakurai, 1992b). Standard culture conditions were as follows, unless otherwise indicated: the cells were dispersed in Waymouth MB 752/1 medium containing bovine serum albumin (2 g/l), transferrin (5 mg/l), selenium (5 μg/l) and dexamethasone (10^{-7} M) and seeded in collagen-uncoated dishes at a density of 2×10^6 cells/(4 ml 60-mm dish). The culture dishes were maintained at 37 °C in a CO_2-humidified incubator. The medium was renewed everyday.

2.3. Hybridization of hepatic RNA with P450 probes

Total RNA was prepared from hepatocytes according to the guanidinium thiocyanate/phenol method (Chomczynski and Sacchi, 1987) and subjected to hybridization as described by Maniatis et al. (1982). Northern transfer experiments were performed after size fractionation of denatured RNA on formaldehyde-containing 1.3% agarose gel. Hybridization was carried out at 42 °C overnight in a mixture containing 50% formamide, 1× Denhardt’s solution, 5× SSC, 50 mM sodium phosphate buffer, pH 6.4, salmon testis DNA at 0.25 mg/ml, and a ^{32}P-labeled cDNA probe. After hybridization, membranes were washed twice for 15 min with 2× SSC and 0.2% SDS at 65 °C. The blots were exposed to Fuji X-ray films at −80 °C with an intensifying screen (DuPont).

2.4. Electrophoretic mobility shift assay

Nuclear extracts were prepared from hepatocytes by the method of Backlund et al. (1997). A double-stranded XRE probe was fill-in-labeled with [α-^{32}P]dCTP using the Klenow fragment (TOYOBO, Osaka, Japan). Unincorporated nucleotides were removed with a NICK column (Pharmacia Biotech, AB). The binding reaction mixture containing 2 μg of crude nuclear extract, 2 μg of poly(dI–dC) (Pharmacia Biotech, AB), about 30,000 cpm of double-stranded probe and 1× binding buffer (16 mM HEPES (pH 7.9), 1% (v/v) glycerol, 0.8 mM dithiothreitol) was incubated at 25 °C for 30 min. For compe-

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