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RESEARCH COMMUNICATION

Induction of peroxisomal β-oxidation genes by retinoic acid in cultured rat hepatocytes

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Retinoic acid is reported here to induce peroxisomal β-oxidation activities in cultured rat hepatocytes, with a concomitant increase in respective peroxisomal mRNAs. The concentrations of retinoic acid required for inducing liver peroxisomal acyl-CoA oxidase were similar to those required for inducing liver transglutaminase. A putative 5′-flanking response element for retinoic acid may be found within the enhancer region involved in the induction of peroxisomal genes by xenobiotic amphipathic carboxylates.

INTRODUCTION

Liver peroxisomal genes may be induced by a variety of xenobiotics, such as fibrate drugs [1], phthalate plasticizers [2], substituted long-chain dicarboxylic acids [3], and others [4]. The induction of liver peroxisomal genes by 'peroxisomal proliferators' is accompanied by induction of specific non-peroxisomal activities (i.e. microsomal cytochrome *P*-452 [5]) and is mediated by transcriptional activation of the respective genes [6]. A common structural denominator for the above-mentioned xenobiotics has been previously proposed to consist of a carboxylic function carried on a hydrophobic backbone to yield an amphipathic carboxylate [3,7]. The amphipathic-carboxylic nature of xenobiotic inducers of peroxisomal genes has initiated our search for endogenous amphipathic carboxylates acting as 'peroxisomal proliferators' [7]. Retinoic acid (RA), having a carboxyl function carried on a hydrophobic polyisoprenoid backbone, may indeed be considered an example of an endogenous amphipathic carboxylate, thus initiating this study to evaluate its capacity as an inducer of peroxisomal genes in cultured rat hepatocytes.

MATERIALS AND METHODS

Cultured cells

Primary cultured rat hepatocytes were prepared as described in [8] with modifications as previously described [7]. Cells were plated on rat tail collagen gel prepared as described in [9] and grown in RPMI 1640 medium containing 10% fetal-calf serum, 100 μunits of insulin/ml, 10 μg of cortisol/ml, 50 μg of streptomycin sulphate/ml and 50 μg of penicillin G/ml. Peroxisomal proliferators and RA (200 × conc. stock solutions in dimethyl sulphoxide) were added to the culture medium at the final concentrations indicated.

Enzyme activities

Cultured cells were harvested by collagenase treatment and washed with modified Hanks' buffered saline. The cell pellet was suspended in 10 mM-sodium phosphate-buffered saline (pH 7.0) and sonicated. Peroxisomal acyl-CoA oxidase (AOX) activity was determined in the culture homogenate as previously described [7]. Transglutaminase activity was determined in the culture homogenate as described in [10].

RNA preparation and analysis

Total RNA was prepared by using guanidinium thiocyanate and centrifugation through a CsCl cushion as described in [11]. The RNA was analysed by Northern-blot analysis and quantified by dot-blot hybridization. Peroxisomal AOX mRNA levels were determined by using the (1600 + 1000 + 750) bp pSTI restriction fragments excised from the recombinant DNA pMJ125 [12]. Peroxisomal enoyl-CoA hydratase mRNA levels were determined by using the 2324 bp *Eco*RI restriction fragment excised from the recombinant DNA pMJ26 [13]. Peroxisomal thiolase mRNA levels were determined by using the 730 bp *Pst*I restriction fragment excised from the recombinant DNA pMJ203 [14]. Catalase mRNA levels were determined by using the 500 bp *Pst*I restriction fragment excised from the recombinant DNA pMJ504 [15]. Peroxisomal cDNA probes were kindly provided by Dr. T. Hashimoto (Shinsha University, Matsumato, Nagano, Japan). cDNA probes were ^{32}P-labelled by nick-translation [16].

RESULTS AND DISCUSSION

The induction of peroxisomal genes by RA was studied here by incubating cultured rat hepatocytes in the presence of increasing concentrations of RA added to the culture medium. As shown in Fig. 1(*a*), AOX activity was induced dose-dependently by RA, being accompanied by a dose-dependent increase in peroxisomal AOX mRNA (Figs. 1*b* and 1*c*). Similarly to xenobiotic peroxisomal proliferators, RA was found to induce the peroxisomal activities in a selective pattern (Table 1). It is worth noting, however, that the selectivity of RA as an inducer of peroxisomal genes differed from that of nafenopin, serving here as a xenobiotic peroxisomal proliferator of the fibrate family. Thus, although the induction of the hydratase mRNA by nafenopin was significantly more pronounced than that of the oxidase, both mRNAs were similarly induced by RA.

Since the inducing concentrations of RA were in the range of 1–30 μM, being substantially higher than those required for initiating differentiation by RA in epithelial [17] or HL-60 [18] cells, the induction of liver peroxisomal genes by RA was further evaluated in comparison with liver transglutaminase, classically considered as a reference marker for RA action in cell lines [19,20] and liver [10]. As shown in Fig. 2(*a*), induction of liver transglutaminase by RA was biphasic, being characterized by a

Abbreviations used: AOX, acyl-CoA oxidase (EC 1.3.3.6); RA, retinoic acid.

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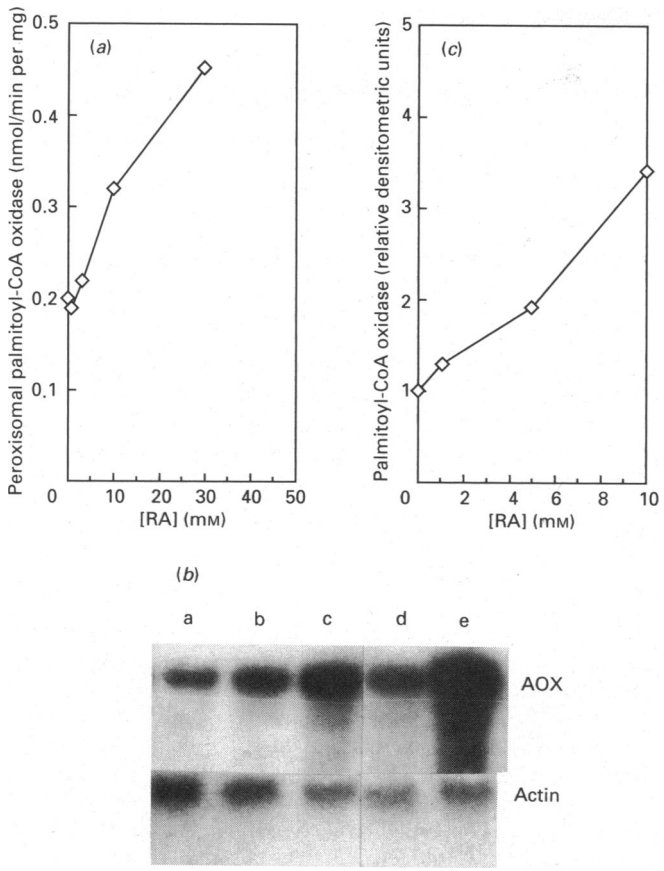

Fig. 1. Induction of AOX by RA in cultured rat hepatocytes

(*a*) AOX activity as a function of RA in medium after incubation for 48 h. The culture medium was replaced every 24 h. (*b*) AOX mRNA Northern blot after incubation for 24 h: a, no additions; b, 5 μM-RA; c, 10 μM-RA; *d*, 5 μM-nafenopin; *e*, 50 M-nafenopin. (*c*) AOX mRNA dot-blot after incubation for 24 h.

Table 1. Induction of peroxisomal genes by RA and nafenopin

Cultured rat hepatocytes were incubated for 48 h as indicated. Total RNA was isolated and peroxisomal β-oxidation mRNAs were determined by dot-blot hybridization as described in the Materials and methods section. mRNA results are presented in densitometric units relative to non-treated cultures.

Addition to culture	AOX mRNA	Enoyl-CoA hydratase mRNA	Thiolase mRNA	Catalase mRNA
No addition	1	1	1	1
10 μM-RA	3	3	2	1
50 μM-Nafenopin	10	26	8	1.5

3-fold increase in activity at RA concentrations in the range 0.1–1 μM, with most of the transglutaminase activity being induced at RA concentrations in the micromolar range. The biphasic nature of liver transglutaminase induction by RA was further analysed by studying the respective Eadie–Scatchard plot. As shown in Fig. 2(*b*), the K_d for RA induction of most of the liver transglutaminase activity was 6.4 μM, compared with a K_d of 0.1 μM for RA acting as an inducer of liver transglutaminase in the sub-micromolar range. In contrast with transglutaminase,

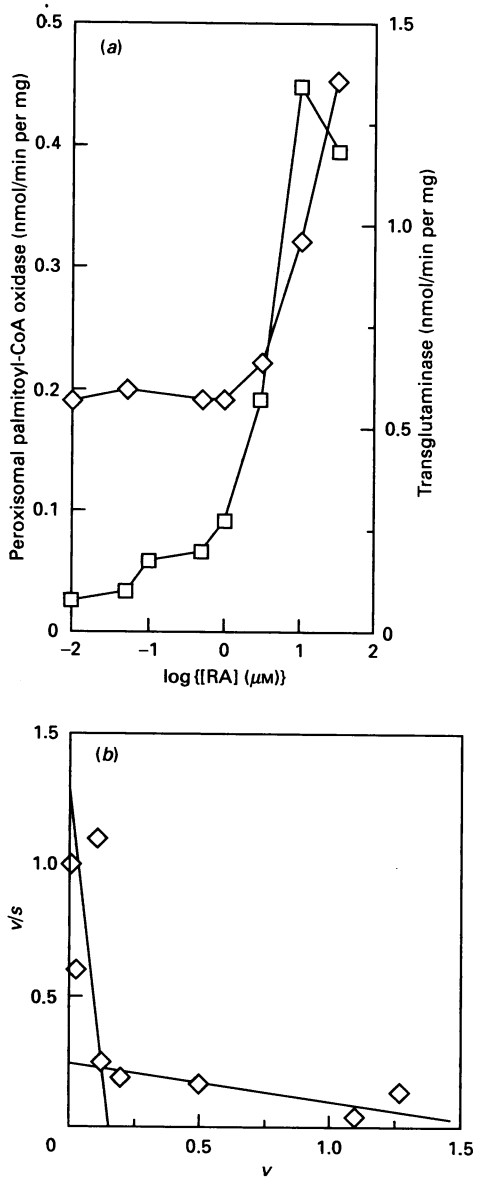

Fig. 2. Induction of AOX and transglutaminase as a function of RA

(*a*) AOX (◇) and transglutaminase (□) activities after incubation for 48 h. (*b*) Eadie–Scatchard plot for transglutaminase activity.

no AOX activity was induced within the lower concentration range of RA (Fig. 2*a*), whereas the extent of AOX induction by micromolar concentrations of RA correlated well with that of liver transglutaminase. Co-induction of liver transglutaminase and peroxisomal AOX by RA may indicate that RA may act as an inducer of peroxisomal genes within its physiological concentration range.

A putative 5′-flanking response element for RA may be found within the (−597/−472) enhancer region recently reported to be involved in the induction of peroxisomal AOX by ciprofibrate or di-(2-ethylhexyl) phthalate [21]. Indeed, the (−570/−559) sequence TGACCTTTGTCC appears to be similar to the (−451/−440) sequence TGACCTTTGGCC recently reported to mediate the effect of RA on phosphoenolpyruvate carboxykinase [22].

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