Divergent Modes of Induction of Rat Hepatic and Pulmonary CYP3A1 by Dexamethasone and Pregnenolone 16α-Carbonitrile

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Both the glucocorticoid receptor (GR) agonist dexamethasone (DEX) and GR antagonist pregnenolone 16α-carbonitrile (PCN) enhanced the transcription of CYP3A1 in rats though in a different fashion. Seven-week old male Wister rats were intraperitoneally administered one to three times with corn oil (vehicle) or DEX and/or PCN (80 mg/kg each) at 24-hr intervals according to various schedules. The animals were sacrificed twenty-four hours after the last treatment, and the dissected livers and lungs were examined for mRNA contents of CYP3A1 and pregnane X receptor (PXR) by real time PCR. DEX appeared to act mainly as an inducer of PXR, that in turn transactivated the *CYP3A1* gene, by activating the GR, while PCN was deduced to have a direct effect on the expression of the *CYP3A1* gene *via* activation of PXR from the rapid increase in CYP3A1 mRNA in comparison with DEX. Sequential treatment with DEX and PCN in this order induced CYP3A1 mRNA expression more effectively than treatment in the opposite order. When DEX and PCN were administered simultaneously, the induction of PXR by DEX was reversed by PCN, resulting from their competitive effects on the GR. In the lung, an increase in the CYP3A1 mRNA level was observed in the presence of DEX but not PCN, independently of the GR-activation.

Key words —— CYP3A1, dexamethasone, pregnenolone 16α-carbonitrile, pregnane X receptor, glucocorticoid receptor, Wistar rat

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INTRODUCTION

Mammalian *CYP3A* genes coding for predominant drug-metabolizing enzymes were initially recognized to harbor elements responsive to both classical glucocorticoids and antiglucocorticoids.[1] Studies on the mechanism of induction of CYP3A by structurally diverse compounds including both glucocorticoids and antiglucocorticoids have culminated in the discovery of pregnane X receptor (PXR). Since the tissue distribution and relative abundance of PXR mRNA resembled those of CYP3As, PXR was considered to be important not only for the induction but also for the constitutive expression of these enzymes.[2] A dual effect of dexamethasone (DEX) was reported on the expression of human CYP3A4 and rat CYP3A23,[3,4] which may be an allelic variant of CYP3A1,[5] in various cell culture systems including rat primary hepatocytes. Under physiological conditions (submicromolar concentrations), DEX induces PXR, which might be responsible for the ligand-independent activation of CYP3A,[2] through the classical glucocorticoid receptor (GR) pathway.[6] At micromolar to supramicromolar concentrations (under stress), DEX plays the role of an PXR agonist, causing the transactivation of CYP3A4 and CYP3A23.[3] A synthetic steroid, pregnenolone-16α-carbonitrile (PCN), appears to stimulate the hepatic metabolism and detoxification of lithocholic acids and xenobiotics by binding to the rodent type of PXR which then interacts with a distinct DNA-response element upstream of the *CYP3A* genes.[7] The fact that the agonist and antagonist for one receptor (in the case of GR, DEX and PCN, respectively) share the nature of an agonist for another receptor (PXR) is considered to be a part of an adaptive system against endogenous and exogenous toxic compounds. Although the functions of DEX and PCN have been well characterized using cultured cells including primary hepatocytes, the details of their behavior *in vivo*, especially when administered in various combinations, remain to be elucidated.

MATERIALS AND METHODS

Animals and Treatments —— Seven-week-old male Wistar rats (Clea Japan, Inc., Tokyo, Japan) were housed under a 12-hr light-dark cycle and allowed free access to food and water. The rats were injected i.p. with DEX (WAKO Pure Chemical

Table 1. Time Schedule of the Treatments

treatments	0 hr	24 hr	48 hr
C	corn oil	*a)*	
P1	PCN	*a)*	
D1	DX	*a)*	
P2	PCN	—	*a)*
D2	DX	—	*a)*
PP	PCN	PCN	*a)*
DD	DX	DX	*a)*
PD	PCN	DX	*a)*
DP	DX	PCN	*a)*
X1	PCN + DX	*a)*	
X2	PCN + DX	—	*a)*
X3	PCN + DX	PCN + DX	*a)*

a) Removal of the organs, +: and, —: no treatment.

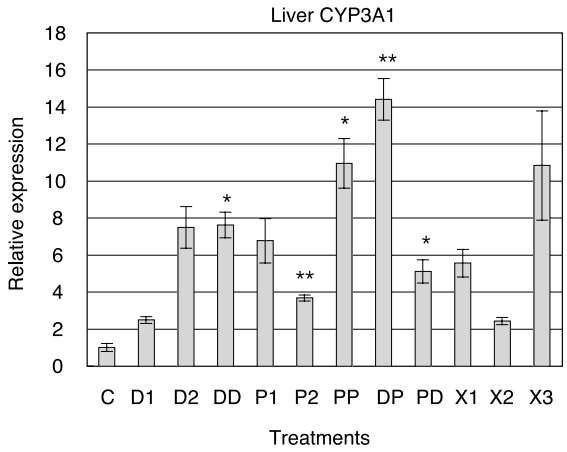

Fig. 1. Expression of CYP3A1 mRNA in Livers of Wistar Rats after Treatment with Dexamethasone and/or Pregnenolone 16α-Carbonitrile

Rats were injected i.p. with DEX and/or PCN (80 mg/kg body weight) or with corn oil as a control according to the schedules shown in Table 1. Livers were removed from the animals 24 hr after the last treatment for the isolation of total RNA. CYP3A1 mRNA was measured by real-time PCR method using individual total RNA (2 μg/ml). The expression of mRNA is presented relative to that for the control group. The data are shown as the mean ± S.E. for 3 animals with significant differences compared to the control at $*p < 0.05$, $**p < 0.01$.

Industries, Osaka, Japan, 80 mg/kg body weight) and/or PCN (WAKO Pure Chemical Industries, 80 mg/kg body weight) or with corn oil as a control according to the manner described in Table 1. Livers and lungs were removed from the animals 24 hr after the last treatment for the isolation of total RNA.

Quantitation of CYP3A-, PXR- and GR-mRNAs by Real-Time PCR —— Total RNA was extracted from the homogenate of 25 mg of rat liver or lung using an RNeasy Kit (QIAGEN, Hilden, Germany). After incubation at 65°C for 10 min, the extracts were quickly placed in an ice-cold water bath. Total RNA and oligo-dT primer were added to RTG You-Prime First-Strand Beads (Amersham Biosciences, NJ, U.S.A.), and left at room temperature for 1 min. Reverse transcription was then performed at 37°C for 1 hr to obtain cDNA. Real-time PCR was carried out for the quantitation of each transcript in a reaction mixture consisting of 2 μl of the cDNA, 1 μl each of a pair of primers, 21 μl of water and 25 μl of iQ SYBER™ Green Supermix (BIO-RAD, CA, U.S.A.). The PCR was performed with an initial enzyme activation step at 95°C for 5 min followed by 50 cycles of denaturation at 95°C for 30 sec, annealing at 56°C for 30 sec and extension at 72°C for 45 sec in a real-time DNA thermal cycler (iCycler™, BIO-RAD). The following oligonucleotides were used as forward and reverse primers, respectively: 5′-GTTCACCAGTGGAAGACTCA-3′ and 5′-CTGTAGGCACCAAACACTTC-3′ for CYP3A1, 5′-AACAGGAACCTGGGAGTG-3′ and 5′-ACGC AGCTGTAGCTTCTTC-3′ for PXR, 5′-TCTACC CTGCATGTATGACG-3′ and 5′-GGCTCTTCAG ACCTTCCTT-3′ for GR, and 5′-ACCACAGTCC ATGCCATCAC-3′ and 5′-TCCACCACCCTGTT GCTGTA-3′ for glyceraldehyde-3-phosphate dehydrogenase (GAPDH). The amplified cDNA was quantitated by the number of cycles (or cross point) at which the fluorescence signal is greater than a defined threshold in the logarithmic phase of amplification. The results were normalized using those for GAPDH.

Statistics —— Statistical significance was determined using Student's *t*-test. A 0.05 level of probability was adopted as the criterion of significance.

RESULTS AND DISCUSSION

Effect of DEX and PCN on the Expression of Rat Liver CYP3A1, PXR and GR mRNAs

The time schedules by which the rats were treated with DEX and/or PCN in various combinations are summarized in Table 1.

The level of CYP3A1 mRNA went up marginally 1 day after the DEX-treatment (80 mg/kg body weight) (Fig. 1, D1). A further rise was seen after another day (Fig. 1, D2). On the other hand, the same dose of PCN increased the level of CYP3A1 mRNA promptly 1 day after the PCN-treatment (Fig. 1, P1), though the level decreased 2 days after the treatment (Fig. 1, P2). Therefore, both agents are confirmed

to be active inducers of CYP3A1 mRNA expression in rats under the adopted experimental conditions. The proximal and distal PXR-responsive modules were discovered in the promoter region of CYP3A1/23.[8] A GR-responsive element of the *CYP3A1*/*IGC2* gene was identified in transfection experiments with HepG2 cells,[9] with the maximum induction achieved at 50–100 nmol/l DEX. Although DEX and PCN were reported to be a GR agonist and antagonist, respectively, at submicromolar concentrations using *in vitro* assays, both share the nature of a PXR agonist at micromolar to supramicromolar concentrations. The increase in the CYP3A1 mRNA level in the presence of PCN could be attributed to the role of PXR. The time course for the induction of CYP3A1 mRNA shown by DEX is distinct from the PCN-dependent one, suggesting a PXR-independent mechanism in the case of DEX. Based on the results shown in Fig. 1 and the findings made *in vitro*, DEX appeared to play the role of a GR agonist, promoting the transcription of PXR, which in turn transactivated the *CYP3A1* gene *via* a PXR-response element upstream in an PXR agonist-independent manner. The differential bioavailabilities of DEX and PCN might underlie the divergent mechanisms for the induction of CYP3A1 mRNA.

Supporting this hypothesis, a temporary accumulation of PXR mRNA was observed 1 day after the DEX-treatment (Fig. 2, D1 and D2). During the 1-day lag time between the peaks of PXR and CYP3A1 mRNA expression in the livers from the rats treated with a single intraperitoneal injection of DEX (Fig. 1, D2; Fig. 2, D1), newly transcribed PXR becomes ready for the transactivation of the *CYP3A1* gene. Since the level of PXR mRNA is rather constant 1 day after the final administration of DEX irrespective of the preceding DEX-treatment (Fig. 2, D1 and DD), an excess amount of GR over that of PCN is expected in the rat liver before the DEX-administration. Likewise, the amount of PXR is surplus to that of PCN because the CYP3A1 mRNA level in the case of two consecutive administrations of PCN is equivalent to the sum of those 1 and 2 days after the single PCN administration (Fig. 1, P1, P2 and PP).

When DEX and PCN were individually administered on the two consecutive days in this order (Fig. 1, DP) or the inverse order (Fig. 1, PD), the CYP3A1 mRNA level was higher than the simple addition of the two separate components attained only in the rats treated with DEX on day 1 and PCN on day 2. The PXR induced by DEX with the help

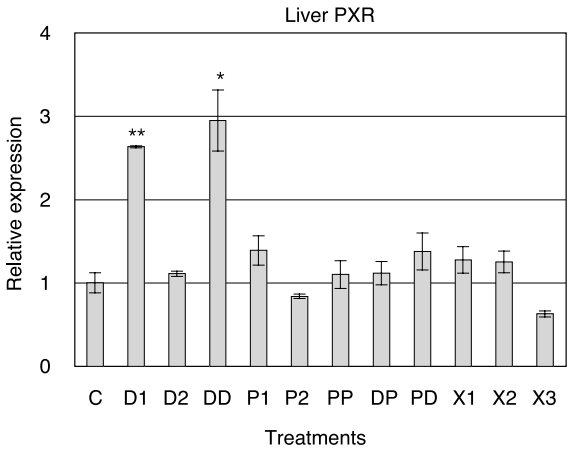

Fig. 2. Expression of PXR mRNA in Livers of Wistar Rats after Treatment with Dexamethasone and/or Pregnenolone 16α-Carbonitrile

Rats were treated as described in the legend to Fig. 1. PXR mRNA was measured by the real-time PCR method using individual total RNA (2 μg/ml). The expression of mRNA is presented relative to that for the control group. The data are shown as the mean ± S.E. for 3 animals with significant differences compared to the control at $*p < 0.05$, $**p < 0.01$.

of GR is activated by PCN to start the transcription of the *CYP3A1* gene in the DEX-treated rats, while the PCN-activated PXR and DEX-activated GR-dependent PXR induction have no connection. The simultaneous administration of DEX and PCN (80 mg/kg body weight, each) resulted in the competition between them, especially for the GR, because a marked induction of hepatic CYP3A1 mRNA 2 days after the DEX-administration was not observed in the rats treated simultaneously with DEX and PCN (Fig. 1, D2 and X2). The PXR-response element may be upstream of the *GR* gene, as shown in Fig. 3 (P2), though this remains to be confirmed.

Effect of DEX and PCN on the Expression of Rat Lung CYP3A1 and PXR mRNAs

In mice, six isoforms of the Cyp3a subfamily have been reported, *i.e.*, Cyp3a11, Cyp3a13, Cyp3a16, Cyp3a25, Cyp3a41 and Cyp3a44. Among them, the inducibility of Cyp3a11 and Cyp3a13 by DEX and PCN has been reported.[10] Lung levels of Cyp3a mRNA were enhanced in mice treated for 4 consecutive days with either DEX or a mixture of DEX and PCN, but not with PCN alone.[11] PXR mRNA levels were below the detectable limit in the lungs from control or PCN-treated mice but the mRNA was detected by RT-PCR at significant levels in the lungs from mice treated with DEX or DEX + PCN. These authors conclude that PXR induced by DEX was activated by PCN, resulting in the co-

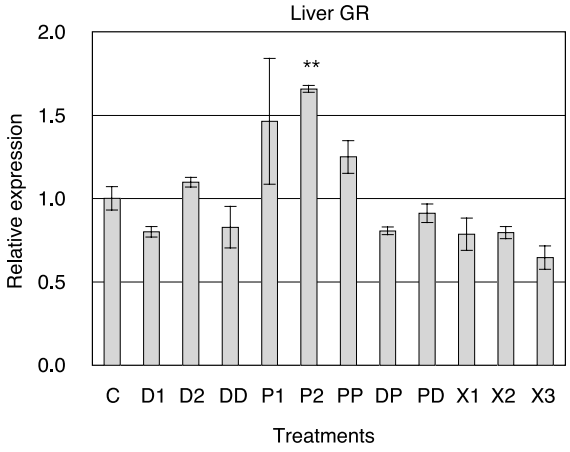

Fig. 3. Expression of GR mRNA in Livers of Wistar Rats after Treatment with Dexamethasone and/or Pregnenolone 16α-Carbonitrile

Rats were treated as described in the legend to Fig. 1. GR mRNA was measured by the real-time PCR method using individual total RNA (2 μg/ml). The expression of mRNA is presented relative to that for the control group. The data are shown as the mean ± S.E. for 3 animals with significant differences compared to the control at $^{**}p < 0.01$.

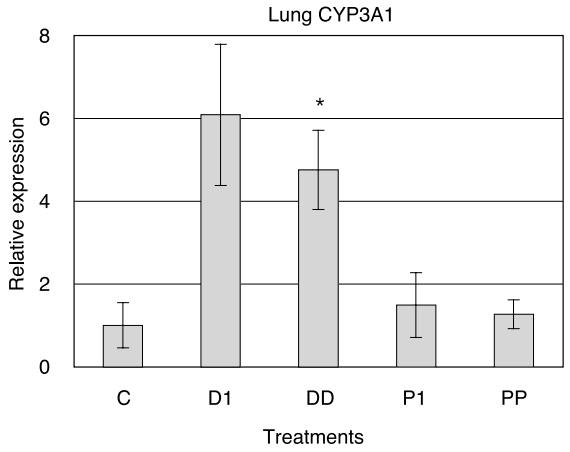

Fig. 4. Expression of CYP3A1 mRNA in Lungs of Wistar Rats after Treatment with Dexamethasone and/or Pregnenolone 16α-Carbonitrile

Rats were treated as described in the legend to Fig. 1. Lungs were removed from the animals 24 hr after the last treatment for the isolation of total RNA. CYP3A1 mRNA was measured by the real-time PCR method using individual total RNA (2 μg/ml). The expression of mRNA is presented relative to that for the control group. The data are shown as the mean ± S.E. for 3 animals with significant differences compared to the control at $^{*}p < 0.05$.

operative effect on the expression of Cyp3a mRNA. However, we could not observe the expression of PXR mRNA in the lungs from rats even after DEX-treatment (data not shown). Coincidently, the increased expression of pulmonary CYP3A1 mRNA was not observed in the presence of PCN as shown in Fig. 4. The increased expression of CYP3A1 mRNA was observed after the DEX-treatment in a different manner from that of its hepatic counterpart (Fig. 4), excluding the GR-dependent mechanism. In the lung where most nuclear receptors such as CAR and PXR are not found, other transcriptional activators determine the responsiveness to DEX as Rodrigues, *et al.*[12] demonstrated the accumulation of CCAAT/enhancer binding protein α (C/EBPα) in the rat liver nuclei in response to DEX and binding to the CYP3A1 promoter elements. The discrepancy between the observations of the present study and those presented by Yanagimoto *et al.*[10] would be attributable to the species difference or the experimental conditions.

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