Ah receptor-mediated CYP1A induction without ligand binding

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Introduction

Hazard and risk assessment of complex halogenated aromatic hydrocarbons (HAHs) such as polychlorodibenzodioxins (PCDDs), polychlorodibenzofurans (PCDFs) and polychlorobiphenyls (PCBs) have utilized a toxic equivalency factor (TEF) approach (1).
Biological responses to these HAHs are mediated by initial binding to cytosolic aryl hydrocarbon receptor (AhR) protein which then activates the transcription of CYP1A gene and other genes (2,3). As AhR ligands, the known CYP1A inducers meet relatively strict structural requirements. They are hydrophobic, polycyclic and planar molecules of particular size (4). Recently, several non-HAH molecules have been found to activate CYP1A gene. Even though these chemicals are inefficient in competing with tetraCDD (TCDD) for binding to AhR (5-11). In other cases, the inducer may be a stable metabolite and not the parent compound .
Number of drugs are designed by associating an active hydrophilic function and a fat soluble carrier (i.e. aromatic cycles). Carbaryl (CAR) is a model compound with a bulky part (carbamate function) and a planar part (naphtalene NAPn). Naphtol (NAPol) is the main stable metabolite from carbamate E1CB hydrolysis. The aim of the present work is to examine the potential for CAR and NAPn, to induce changes in the levels of rat hepatic CYP1A1 and 1A2 proteins and the corresponding enzymatic activities (ethoxyresorufin O-deethylase and methoxyresorufin O-demethylase). Moreover the affinity for AhR can also be related to species variation. Receptors from primate are known to exhibit lower affinity than those purified from rodents (3). The *in vitro* relative affinities of CAR, NAPol, and NAPn for the AhR in rat, mice, dog, monkey and human are determined.

Material and methods

Treatment of animals : Groups of 4 SD rats were received a single intraperitoneal injection of CAR or NAPn in corn oil (2 ml/ kg) at the following doses (0, 20, 80, 150 µmol/kg). 24h after injection, animals were killed by decapitation. Livers were immediately excised and microsomal fractions were prepared as described previously (12) and stored at -80°C until used.

Enzyme assays : Protein concentrations were determined as described previously (13). Ethoxyresorufin O-deethylase (EROD), marker of CYP1A1 and methoxyresorufin O-demethylase (MROD), marker of CYP1A2 were determined according to the method of (14).
Western blot analysis was conduct as described previously (15).
In vitro studies : 2 male Sprague-Dawley (SD) rats (180-200g) were injected i.p with phenobarbital (PB) dissolved in corn oil (80 mg/kg bw, 3 consecutive days), the last injection 24h before sacrifice. C57/BL6 mice (7 weeks old) were pretreated with 1,4-bis[2-(3,5-dichloropyridyloxy)]-benzene (TCPOBOP) dissolved in sunflower oil (3mg/kg (ip)) and were euthanized 72h following injection. Samples of frozen livers (nearly 10g) of dogs (Beagle) and monkeys (Cynomolgous monkey) aged 4 months were purchased from IMT-Pharmatox society (St Pierre des Corps, France); Fresh hepatic liver of human was kindly provided by M. Creppy (University Bordeaux II).
Preparation of hepatic cytosols of PB-pretreated rat, TCPOBOP-pretreated mice, dog, monkey and human were prepared as described by (15). Molybdate was added to buffer for samples of dog, monkey and human. Cytosolic protein concentrations were determined using bicinchoninic acid (16). ***Preparation of enriched 9S*** was obtained by sucrose density gradients (17) and ***binding experiments*** were determined as described (15).
Statistical analysis : Enzyme activities were submitted to a one-way ANOVA followed by Tukey test. The data are expressed as means ± standard deviations.

Results

in vivo studies

The effects of CAR and NAPn treatement on EROD and MROD activities in rat liver, are presented in figure 1. CAR induced a significant increase in EROD and MROD activities only at the higher dose tested. NAPn showed no significant change on these both activities at all doses tested.

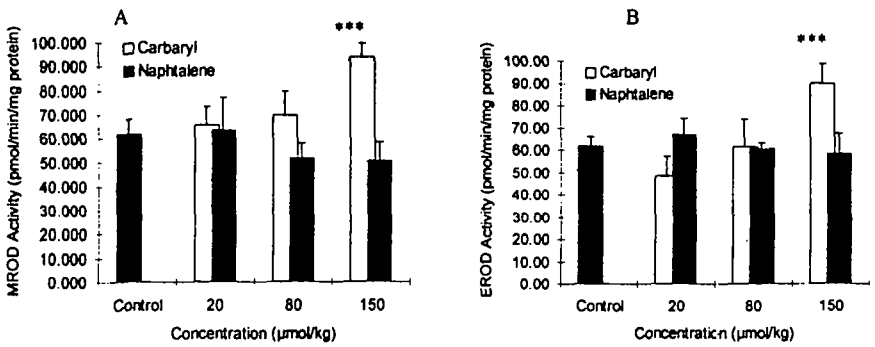

Figure 1: EROD (A) and MROD (A) activities of liver microsomes from SD rats treated with CAR or NAPn at the following doses : 0, 20, 80, 150 µmol/kg. *** significantly different from control ($p<0.01$).

Western blot analysis of microsomal proteins (Fig. 2) showed that the observed increases in EROD and MROD activities by CAR at the highest dose were accompanied by appearence of immunodetected CYP1A1 isoform and a weak rise in immunodetected CYP1A2.

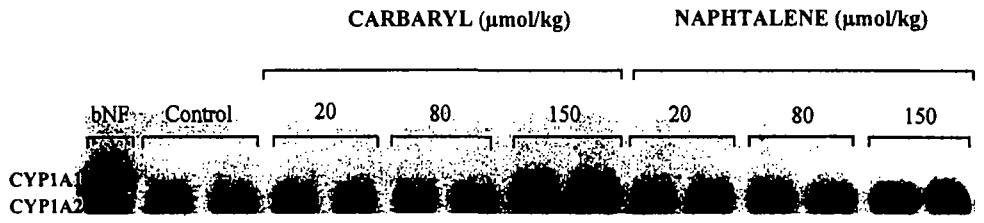

Figure 2 : Immunodetection of CYP1A1 and 1A2 from rats pretreated with CAR or NAPn. Rat pretreated with β-naphtoflavone (βNF) represents a positive control of CYP1A1 induction.

In vitro studies

When rat liver 9S was incubated with [3H]TCDD and analysed on sucrose density gradient, a peak of radioactivity reflecting the TCDD-AhR complex was obtained in the 9S region (Fig. 3). As expected, the peak almost totally disappeared when the incubation was carried out in the presence of a 1000-fold excess of unlabeled TCDF. In contrast, when CAR, NAPn or NAPol were used as competitors, even in 8000-fold excess with respect to the radiolabeled ligand, the peak was not affected, showing that these compounds did not compete with [3H]TCDD for the specific binding site of SD rat AhR. Moreover, no competition was observed in mice, dog, monkey or human (Table 1).

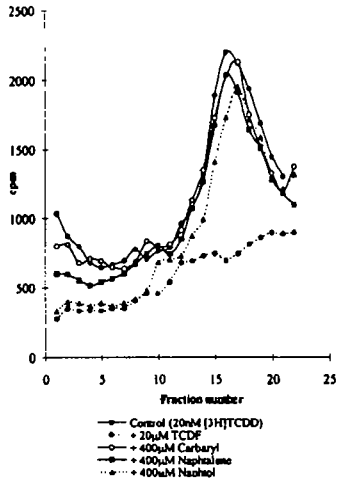

Figure 3 : 5-20% sucrose gradient sedimentation of SD rat AhR (9S enriched fraction) incubated with [3H]TCDD in absence or presence of 20µM TCDF or 8000-fold excess of CAR, NAPn or NAPol.

Animal species	Specific ligand [3H]TCDD	% of specific ligand binding after addtion of 8000-fold excess of		
		CAR	NAPn	NAPol
PB-treated SD rat	20nM	93.2	97.2	92.7
TCPOBOB-treated C57/BL6 mice	25nM	69.5	93.9	87.3
Beagle dog	50nM	98.5	82.6	95.7
Cynomolgous monkey	50nM	106	88.8	100
Human	50nM	98.6	80.9	97.9

Table 1: Lack of competitive effect of CAR, NAPn and NAPol with [3H]TCDD for AhR in all animal species.

Discussion

This study shows that CAR induces CYP1A1 and 1A2 proteins at 150µmol/kg. This induction was characterized by an increase of EROD and MROD activities, which are correlated with a rise of CYP1A1 and CY1A2 proteins levels. NAPn, which not possess the hydrophilic carbamate function is not able to induce these responses. The absence of competitive binding of

the insecticide to the AhR suggests that native carbaryl is probably not a ligand of this receptor. This result could have been expected, since carbaryl chemical structure is completely different from classical HAHs, as it is neither a planar nor a polycyclic aromatic chemical. NAPn, the planar structure of CAR, is unable to displace [3H]TCDD specific binding. This effect was also found for AhR purified from other species including human. The results shows the relative importance of the carbamate hydrophilic function of CAR in CY1A induction and suggests alternative mechanisms. Several recent studies described some ligand-independent activation mechanisms, mediated by intracellular signal transduction systems, possibly involving tyrosine kinase (11). It must also be considered that CAR may be bound to a second binding site, distinct from the TCDD binding site. In summary, the data presented demonstrate that a non planar compound with a hydrophylic part, can also modulate the AhR-mediated responses. These mechanism, have to be considered for the TEF approach.

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