EFFECT OF CHRYSENE AND CARBON TETRACHLORIDE ADMINISTRATION ON RAT HEPATIC MICROSOMAL MONOOXYGENASE AND UDPGLUCURONOSYLTRANSFERASE ACTIVITY

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1. Introduction

Induction of hepatic microsomal mixed function oxidase and UDPglucuronosyltransferase follow conspicuously different time scales after 3-methylcholanthrene, benzpyrene, phenobarbital and DDT administration [1–3], but see also [4]. The extent of induction of mixed function oxidase is several fold over that of UDPglucuronosyltransferase [1]. Chlorpromazine is a potent inducer of mixed function oxidase [5], but failed to induce UDPglucuronosyltransferase [6]. On the other hand, human placental aryl hydrocarbon hydroxylase is induced by cigarette smoking [7,8], but UDPglucuronosyltransferase in the human placenta is evidently independent of the smoking of the mother [9]. To study further the interdependence of the induction of mixed function oxidase and UDPglucuronosyltransferase, rats were given chrysene, a potent inducer of aryl hydrocarbon hydroxylase [10], solely and in combination with carbon tetrachloride, a depressor of mixed function oxidase [11,12] and the activity of aryl hydrocarbon hydroxylase and UDPglucuronosyltransferase determined.

Chrysene enhanced aryl hydrocarbon hydroxylase activity 15-fold, but failed to affect UDPglucuronosyltransferase. Carbon tetrachloride depressed aryl hydrocarbon hydroxylase, both induced and noninduced, by about 60 per cent. Carbon tetrachloride caused an about 2-fold in vivo activation of UDPglucuronosyltransferase.

2. Material and methods

Microsomes were prepared with the calcium aggregation method [13] from the livers of adult male Wistar rats (32 in all) as described earlier [14]. Activation of UDPglucuronosyltransferase was achieved with the method of Hänninen [3,15] by treating microsomes with digitonin. The activity of aryl hydrocarbon hydroxylase (EC 1. 14. 14. 2) was determined using 3,4-benzpyrene as the substrate [16]. The amount of the cytochrome P-450 (448) was estimated with a Unicam SP-800 spectrophotometer with 91 $mmole^{-1}$ cm^{-2} as the molar extinction coefficient [17]. To determine the activity of UDPglucuronosyltransferase (EC 2. 4. 1. 17) *p*-nitrophenol and 4-methylumbelliferone were used as the aglycones [18]. The microsomal protein content was determined with the biuret method [19] with bovine serum albumin as the reference protein.

Chrysene (1,2-benzophenanthrene) (Koch-Light Laboratories, Ltd., Colnbrook, England) was administered intraperitoneally (20 mg/kg) as a solution (4 mg/ml) in corn oil, carbon tetrachloride (1 ml/kg) intragastrically in corn oil (0.2 ml/ml) 24 hr before killing the rats. The control rats were given the same amount (0.5 ml/kg) corn oil intragastrically.

3. Results and discussion

Carbon tetrachloride caused a marked decrease in

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Table 1
The effect of carbon tetrachloride (1 ml/kg i.g.) and chrysene (20 mg/kg i.p.) on the protein and cytochrome P450 content and arylhydrocarbon hydroxylase activity of rat liver microsomes harvested with the calcium aggregation method. The means and standard errors of the means (SEM) are indicated.
The number of experiments in parentheses

Treatment	Microsomal protein mg/g liver	Cytochrome P450 nmole/g liver	3,4-Benzpyrene hydroxylated nmole/min/g liver
Control [8]	27.8 ± 1.8	8.15 ± 0.8	0.143 ± 0.021
Carbon tetrachloride [8]	34.1 ± 4.04	2.28 ± 0.18	0.059 ± 0.011
Chrysene [8]	29.1 ± 2.0	9.07 ± 0.53	1.92 ± 0.12
Carbon tetrachloride + Chrysene [7]	32.2 ± 2.4	2.05 ± 0.14	0.611 ± 0.118

the carbon monoxide binding pigment content and aryl hydrocarbon hydroxylase activity in rat liver microsomes (table 1). Simultaneously the cytochrome was changed to a great extent to the inactive P-420 form, as has also earlier been found [20], but see also [21]. A decrease in the cytochrome P-450 content and mixed function oxidase after a small CCl_4 dose has been found to coincide with a protection of the animal against the toxic effect of a lethal CCl_4 intoxication [22,23]. The dose of CCl_4 in the present study, 1 ml/kg, was somewhat bigger than that needed for the protection effect. The effect of CCl_4 on the amount of the carbon monoxide binding pigment and on the aryl hydrocarbon hydroxylase was about similar both in the control rats and rats treated simultaneously with chrysene, a decrease to one third. Thus it seems probable that the sensitivity of both cytochrome P-450 and P-448 is similar to carbon tetrachloride (see also [20]).

Carbon tetrachloride administration to rats produced a two fold activation of UDPglucuronosyltransferase towards both 4-methylumbelliferone and *p*-nitrophenol (table 2). Evidently the increase of activity was not due to induction, but to activitation because in the digitonin treated microsomes a depression of activity was seen instead of activation. The effect of carbon tetrachloride thus evidently resembles that of digitonin. Carbon tetrachloride did not affect UDPglucuronosyltransferase, when added to the incubation mixture in concentrations up to 8 mmole/l (added in 2 M ethanol) or up to 170 mmole/l (added in 2.4 M dimethylsulfoxide, which however itself caused a decrease of activity to half the control value). Carbon tetrachloride causes peroxidation of lipids [24,25]. This lipid peroxidation might cause an alteration in the permeability of the microsomal membranes thus explaining the activation seen in the measurable UDP glucuronosyl-transferase activity. On the other hand,

Table 2
The effect of carbon tetrachloride (CCl_4) and chrysene on the UDPglucuronosyltransferase (4-methylumbelliferone and *p*-nitrophenol) activity of rat liver nonactivated and digitonin activated calcium harvested microsomes as well as on the yield of protein in digitonin activated microsomal fraction. Means and standard errors of the means are indicated.
Number of experiments in parentheses

Treatment	4-Methylumbelliferone conjugated nmole/min/g		*p*-Nitrophenol conjugated nmole/min/g		Protein in digitonin treated microsomes mg/g liver
	Nonactivated	Digitonin activated	Nonactivated	Digitonin activated	
Control [8]	20.7 ± 1.4	198 ± 10	9.3 ± 1.0	95 ± 7	18.6 ± 1.6
CCl_4 [8]	38.4 ± 2.0	173 ± 17	24.2 + 2.1	99 ± 9	19.0 ± 1.9
Chrysene [8]	23.3 ± 1.5	238 ± 15	11.1 ± 1.0	111 ± 7	19.8 ± 1.4
Chrysene + CCl_4 [7]	37.9 ± 2.4	187 ± 11	22.6 ± 1.7	105 ± 10	19.6 ± 1.7

high concentrations of chloroform activate UDPglucuronosyltransferase [26], and chloroform is one of the metabolites of carbon tetrachloride [27,28]. Attack on membrane lipids by carbon tetrachloride also produces fatty acids which are capable of activating UDPglucuronosyltransferase in vitro [29]. UDPglucoronosyltransferase has been postulated to lie deep in the microsomal membrane [30], a view supported by the findings of in vitro activation of the enzyme by many membrane perturbating agents (sonication, surfactants, organic solvents, dialysis against alkaline EDTA, ageing, different enzyme treatments (for refs. see [31] many of which simultaneously cause a decrease of the activity of the more superficial mixed function oxidase. The present data on an in vivo activation of UDPglucuronosyltransferase with CCl_4 support the concept.

Chrysene was a potent inducer of the cytochrome P-450 and aryl hydrocarbon hydroxylase, in accord with an earlier study [10]. Simultaneously the absorption maximum of the reduced CO-binding pigment shifted from 450 nm to 448 nm, a phenomenon peculiar to the induction of monooxygenase by polycyclic aromatic hydrocarbons [32,33]. The effect of chrysene on UDPglucuronosyltransferase was slight, only a tendence to rise was seen (table 2). Even after treating the microsomes with digitonin, which reveals latent induction of UDPglucuronosyltransferase after administration of polycyclic hydrocarbons [14] no induction was seen. It is, however, possible that later an induction could have been seen, because induction of UDPglucuronosyltransferase is slower to appear than that of monooxygenase after treating the animal with polycyclic hydrocarbons [1]. Another plausible explanation of the failure of chrysene to induce UDPglucuronosyltransferase is that induction of UDPglucuronosyltransferase is caused by metabolic intermediates, not by the parent hydrocarbon [1–3,34], and that chrysene does not give rise to such intermediates.

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