Biochemical Pharmacology, Vol. 32, No. 17, pp. 2612–2615, 1983.
Printed in Great Britain.

0006-2952/83 $03.00 + 0.00

Comparison of the inducibilities of UDP-glucuronosyltransferase and polysubstrate monooxygenase activities in mice exposed to 20 model xenobiotics

(*Received* 12 *July* 1982; *accepted* 5 *April* 1983)

Polysubstrate monooxygenases and UDP-glucuronosyltransferase (EC 2.4.1.17) catalyse successive reactions in the biotransformation of many endogenous and exogenous lipophilic compounds. Owing to the functional proximity, regulation of monooxygenation and glucuronidation reactions may be linked to each other. By removing products of the preceding monooxygenation reactions, conjugating activities may stimulate the activities of monooxygenases [1, 2]. Some monooxygenase and transferase activities are coregulated by the supply of cofactors required by the enzymes [3]. Moreover, these activities are coordinately elevated by common inducers of drug metabolism, such as phenobarbital [4, 5], 3-methylcholanthrene [6], polychlorinated biphenyls (PCBs) [7, 8], 1,1,1-trichloro-2,2-bis(*p*-chlorophenyl)ethane (DDT) [8] and 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) [9].

In recent years it has become evident that polysubstrate monooxygenases and UDP-glucuronosyltransferase exist in multiple molecular forms (e.g. [10]). In light of the present understanding of the multiple monooxygenase and transferase activities, one may ask whether there is any specificity in the coregulation of the activities of these two enzyme systems. Is there a corresponding monooxygenase activity (activities) for each transferase activity? What activities are functionally linked to each other?

In the present study the inducibilities of monooxygenase and transferase activities were compared to each other in order to elucidate the coregulation of the multiple activities of these enzymes. Mice were exposed to 20 different xenobiotics, some of which induced maximally the activities of drug-metabolizing enzymes, others produced either smaller effects or no effects at all. In this experimental approach, those enzyme activities that are coordinately induced would rise together after *in vivo* exposure of various chemicals. As the model compounds, phenobarbital, 3-methylcholanthrene, TCDD and various PCB congeners or isomers were used.

Phenobarbital was purchased from Merck (Darmstadt, F.R.G) and 3-methylcholanthrene from Sigma Chemical Co. (St. Louis, MO). TCDD was a kind gift from Dr. Tuula Thunberg, Karolinska Institutet, Stockholm, Sweden. Mono- and dichlorobiphenyls, as well as 2,4,2′,4′-tetrachlorobiphenyl, were purchased from Ultra-Scientific (Hope, RI). All other PCB congeners and isomers were generously given by Dr. D. J. Ecobichon, McGill University, Montreal, Canada. PCBs were used without further purification. The chemicals were administered i.p. to 4–7-week-old male C57BL/6J mice (22–27 g). Control animals received an equal volume (5 ml/kg) of the vehicle. Phenobarbital was given as an aqueous solution (80 mg/kg × 5), 3-methylcholanthrene (20 mg/kg × 3) and TCDD (20 μg/kg × 1) as their corn oil solutions. The animals were killed and enzyme assays made 1, 2 and 7 days after the last injection of phenobarbital, 3-methylcholanthrene and TCDD, respectively. The chlorobiphenyls, dissolved or suspended in corn oil, were administered as a single dose (0.32 mmole/kg) 7 days before assaying the enzyme activities. Due to the poor solubility, decachlorobiphenyl was given as a suspension in corn oil, and 3,4,3′,4′-tetrachlorobiphenyl as a warm (about 40°) corn oil solution. Solubility problems were not encountered with other PCB isomers.

The enzyme activities were determined from freshly prepared calcium-aggregated hepatic microsomes [11]. As representatives of polysubstrate monooxygenases, arylhydrocarbon hydroxylase (EC 1.14.14.2) activity was measured radiometrically with benzo[*a*]pyrene as the substrate [12], and 7-ethoxycoumarin *O*-deethylase activity with a modification [13] of the fluorometric method of Ullrich and Weber [14]. UDP-Glucuronosyltransferase activity was determined in digitonin-activated microsomes [15] with 4-methylumbelliferone [16, 17] and 2-aminophenol [18] as substrates. Hepatic concentrations of various PCB congeners and isomers were determined modifying the method described by Karppanen *et al.* [19].

The inducibility of polysubstrate monooxygenase activities clearly exceeded that of UDP-glucuronosyltransferases. The former activities elevated maximally 5- to 8-fold (Fig. 1), the latter not more than 2-fold (Fig. 2). The strongest elevation of arylhydrocarbon hydroxylase activity was 4- to 5-fold, caused by 3-methylcholanthrene and TCDD; the activity of 7-ethoxycoumarin *O*-deethylase was enhanced by these compounds to a somewhat smaller extent. Phenobarbital brought about an 8-fold increase in the activity of 7-ethoxycoumarin deethylase, but no change in arylhydrocarbon hydroxylase activity. Hexachlorobiphenyls were generally the most potent PCBs to enhance the activities of polysubstrate monooxygenases. In general, PCBs enhanced more 7-ethoxycoumarin *O*-deethylase than arylhydrocarbon hydroxylase activity. An exception, however, was 3,4,3′,4′-tetrachlorobiphenyl, which enhanced the latter activity more and thus resembled 3-methylcholanthrene and TCDD rather than phenobarbital.

The glucuronidations of both aglycones were increased by phenobarbital and TCDD, whereas only 2-aminophenol conjugation was affected by 3-methylcholanthrene (Fig. 2). The strongest PCBs were about as potent as phenobarbital, 3-methylcholanthrene and TCDD in elevating the activities of UDP-glucuronosyltransferase. As in the case of monooxygenases, hexachlorobiphenyls were generally the most potent PCBs in elevating the glucuronidation reactions. Like 3-methylcholanthrene, 3,4,3′,4′-tetrachlorobiphenyl did not elevate 4-methylumbelliferone glucuronidation. A comparison of the hepatic PCB concentrations and drug-metabolizing enzyme activities showed that those PCBs that accumulated in the liver also caused the strongest enhancement of enzyme activities (Table 1).

Based on the data in Figs. 1 and 2, the inducibilities of the four measured activities were compared to each other by correlation analysis. Accordingly, the monooxygenase activities increased independently from each other, since there was no significant correlation between arylhydrocarbon hydroxylase and 7-ethoxycoumarin *O*-deethylase activities in mice exposed to the model xenobiotics ($r = 0.258$, No. of model compounds 20). Lang and Nebert [20] concluded from their experiments with reconstituted monooxygenase activities that the metabolism of benzo[*a*]pyrene and 7-ethoxycoumarin is catalysed by different forms of cytochrome P-450. The results of the present study, differential induction of monooxygenase activities catalysing the metabolism of benzo[*a*]pyrene and 7-ethoxycoumarin, support their conclusion and indicate separate control of cytochromes P-450 that metabolize the above xenobiotics.

No correlation was seen between the elevations of the two glucuronidation activities ($r = 0.077$, No. of model compounds 14) suggesting that 4-methylumbelliferone and 2-aminophenol transferase activities are under separate

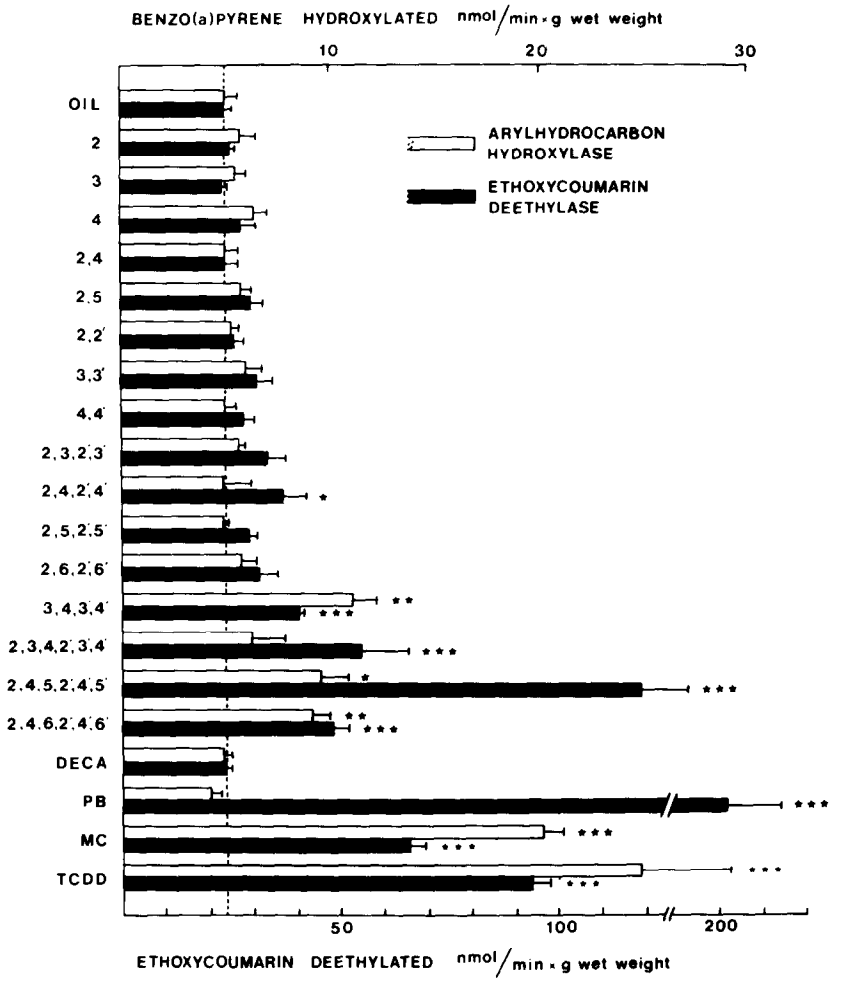

Fig. 1. Hepatic microsomal polysubstrate monooxygenase activities of C57BL/6J mice exposed to model xenobiotics. PCBs were administered as a single i.p. dose (0.32 mmole/kg) 7 days before assaying the enzyme activities. Phenobarbital was given as an aqueous solution (80 mg/kg × 5), 3-methylcholanthrene (20 mg/kg × 3) and TCDD (20 μg/kg × 1) as their corn oil solutions. The animals were killed and enzyme activities determined 1, 2 and 7 days after the last injection of phenobarbital, 3-methylcholanthrene and TCDD, respectively. The values represent mean ± S.E.M. of four to six animals analysed on separate days. Statistical significance (Student's *t*-test) is expressed as follows: * = $2P < 0.05$, ** = $2P < 0.01$, *** = $2P < 0.001$. Numbers on the left refer to positions of chloro groups on the biphenyl molecule. Abbreviations: DECA, decachlorobiphenyl; PB, phenobarbital; MC, 3-methylcholanthrene; TCDD, 2,3,7,8-tetrachlorodibenzo-*p*-dioxin.

Table 1. Hepatic PCB concentrations and drug-metabolizing enzyme activities of mice exposed to selected PCB congeners and isomers*

PCB congener	2,2'	4,4'	2,4,2',4'	2,5,2',5'	2,4,5,2',4',5'	deca
Amount of PCB (nmol/g wet weight)	< 0.76	< 0.30	128 ± 24	2.6 ± 0.5	132 ± 2	16 ± 3
Arylhydrocarbon hydroxylase	104 ± 8	100 ± 10	99 ± 26	99 ± 5	189 ± 28	96 ± 8
7-Ethoxycoumarin O-deethylase	108 ± 9	117 ± 11	156 ± 23	122 ± 8	498 ± 46	99 ± 6
4-Methyl-umbelliferone transferase	126 ± 17	91 ± 7	141 ± 8	106 ± 13	171 ± 7	101 ± 5
2-Aminophenol transferase	106 ± 6		153 ± 16		193 ± 10	

* PCB concentrations and enzyme activities were determined 7 days after a single i.p. administration of the chemical. The values represent mean ± S.E.M. from three to four animals. The data for enzyme activities (given as % of control) are those from Figs. 1 and 2. deca = decachlorobiphenyl.

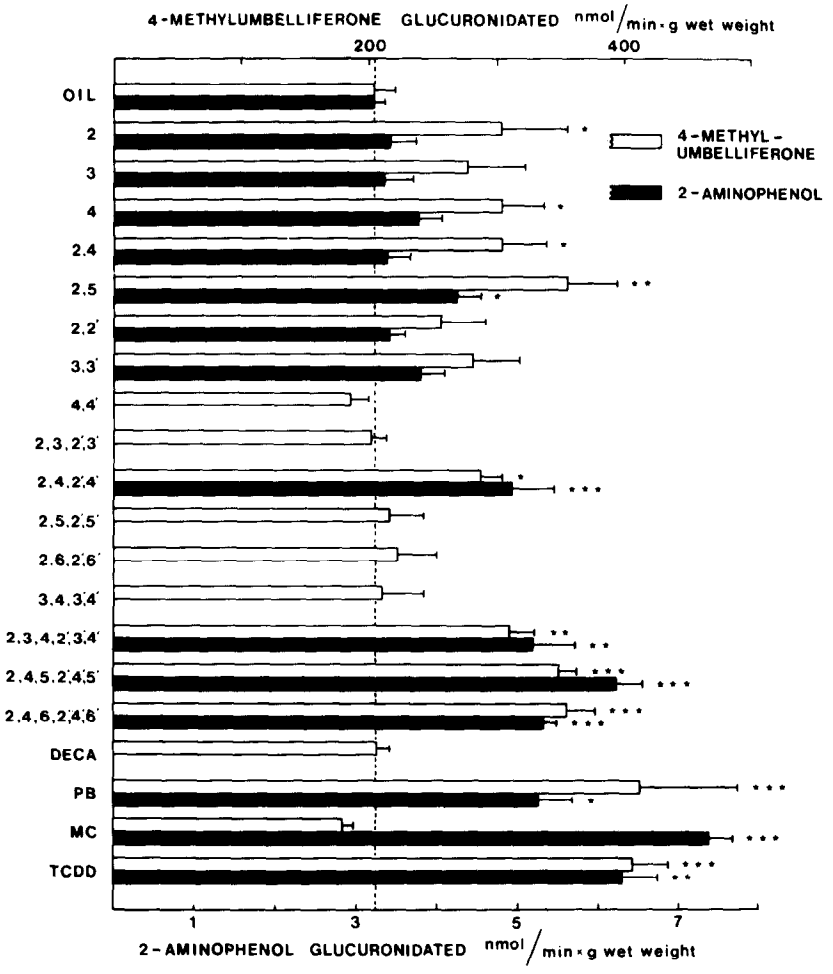

Fig. 2. UDP-Glucuronosyltransferase activity of C57BL/6J mice exposed to model xenobiotics. Enzyme activities were determined in digitonin-activated hepatic microsomes. Mean ± S.E.M. are given. The number of animals, analysed on different days, was four to six. For further details, see Fig. 1.

control. Both these activities have been regarded as 'late-foetal' or 'non-steroidal' transferases [21]. However, differences between 4-methylumbelliferone and 2-aminophenol transferases have been reported with respect to activation of these enzymes, e.g. by digitonin [22], Triton X-100 [22], diethylnitrosamine [23] and metal salts [24]. Moreover, these transferase activities are induced differentially by phenobarbital in the liver of chick embryo [25]. According to the above evidence, it seems that 4-methylumbelliferone and 2-aminophenol transferases represent functionally different forms of UDP-glucuronosyltransferase.

Interestingly, parallel induction of arylhydrocarbon hydroxylase and 2-aminophenol transferase activities ($r = 0.736$, No. of model compounds 14, $2P < 0.01$), as well as of 7-ethoxycoumarin *O*-deethylase and 4-methylumbelliferone transferase activities ($r = 0.585$, No. of model compounds 19, $2P < 0.01$), was found. In the case of the latter monooxygenase-transferase pair, the parallel induction may be regarded as activation of successive reactions in the metabolism of structurally related chemicals. According to a classification by Bock [26], 4-methylumbelliferone transferase belongs to 'GT 1' transferases whose activities are typically induced by 3-methylcholanthrene. In accordance, Owens [27] found induction of hepatic 4-methylumbelliferone transferase activity by 3-methylcholanthrene in responsive mice. Therefore, one would have expected parallel induction of arylhydrocarbon hydroxylase and 4-methylumbelliferone transferase activities. Instead, 3-methylcholanthrene as well as the 3-methylcholanthrene-type PCB 3,4,3',4'-tetrachlorobiphenyl [7], which were potent inducers of arylhydrocarbon hydroxylase, did not significantly elevate 4-methylumbelliferone glucuronidation. On the contrary, phenobarbital was the most potent inducer of 4-methylumbelliferone transferase. Thus, there was no correlation between the inducibilities of arylhydrocarbon hydroxylase and 4-methylumbelliferone transferase activities in the present study. The work of Owens [27] demonstrated that genetic expression of 3-methylcholanthrene inducible 4-methylumbelliferone transferase activity differs from that of arylhydrocarbon hydroxylase activity.

In summary, C57BL/6J mice were exposed to phenobarbital, 3-methylcholanthrene, TCDD and 17 congeners or isomers of PCBs in order to compare the inducibilities of hepatic polysubstrate monooxygenase and UDP-glucuronosyltransferase activities. The aim was to study the coordinated induction of these membrane-bound enzyme systems in light of the known multiplicity of these enzymes. The chemicals were administered to mice as i.p. injections, and the enzyme activities were measured in hepatic calcium-aggregated microsomes. The maximal elevation of arylhydrocarbon hydroxylase activity (5-fold) was caused by TCDD, that of 7-ethoxycoumarin *O*-deethylase (8-fold) by phenobarbital. The highest elevation of 4-methylum-

belliferone glucuronidation (2-fold) was brought about by phenobarbital and TCDD. The conjugation of 2-aminophenol was elevated maximally (2-fold) by 3-methylcholanthrene. Hexachlorobiphenyls were the most potent PCBs to enhance monooxygenase and transferase activities; the effects of most PCBs resembled more those of phenobarbital than those of either 3-methylcholanthrene or TCDD. It was demonstrated that the elevation of drug-metabolizing enzyme activities by PCBs is determined by accumulation of the PCB in the target tissue. The activities of arylhydrocarbon hydroxylase and 7-ethoxycoumarin *O*-deethylase increased independently from each other since there was no correlation between the elevations of these monooxygenases. Similarly, 4-methylumbelliferone and 2-aminophenol transferases were found to be differentially induced. Interestingly, parallel induction of arylhydrocarbon hydroxylase and 2-aminophenol transferase activities, as well as of 7-ethoxycoumarin *O*-deethylase and 4-methylumbelliferone transferase activities, was found. The parallel inductions suggest coordinated regulation of the monooxygenase-transferase pairs.

Acknowledgements—The technical assistance of Ms. Raija Söderholm and Ms. Leena Ruokonen is gratefully acknowledged. This study was financially supported by The Academy of Finland, and by NIH RO1 ES 01684.

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Biochemical Pharmacology, Vol. 32, No. 17, pp. 2615–2618, 1983.
Printed in Great Britain.

0006-2952/83 $03.00 + 0.00

Enhancement of viral growth by the antitumor drug 4′-(9-acridinylamino) methanesulfon-*m*-anisidide (m-AMSA)*

(*Received* 7 *January* 1983; *accepted* 24 *March* 1983)

Patients undergoing extensive drug therapy for treatment of neoplastic disease often become more susceptible to viral infection due to the drug-associated immunosuppression. Patients treated with 4′-(9-acridinylamino) methanesulfon-*m*-anisidide (m-AMSA) are often ambulatory and exposed to viral infection. This report describes the drug augmentation of viral growth *in vitro*, independent of host immune-surveillance. The system consists of HeLa or Vero cells pretreated with m-AMSA and then infected with vaccinia virus or herpes simplex virus type I (HSV). This augmentation of viral growth produced by m-AMSA persists longer than the period of time required for cells to repair detectable DNA damage, suggesting that subtle and persistent alterations in cellular metabolism occur as a consequence of m-AMSA treatment.

m-AMSA is a cytotoxic agent currently undergoing phase III evaluation as a cancer chemotherapeutic agent [1–3]. The exact mechanism for the antitumor action of m-AMSA

* This work was supported in part by Grants CA-24859, CA-28034 and RR-5511-18 from the National Cancer Institute, National Institutes of Health, and Grant G-120 from the Robert A. Welch Foundation.