Prolonged induction of hepatic haem oxygenase and decreases in cytochrome *P*-450 content by organotin compounds

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The administration of organotin compounds to rats in single doses causes a significant and prolonged induction of haem oxygenase and a sustained decrease in haemoprotein content in the liver. The extent of induction of hepatic haem oxygenase varied between 3 and 5-fold at 72 h after a single injection of water-insoluble organotins of differing structure. The alterations in haem metabolism produced by tricyclohexyltin hydroxide were studied in detail. The effects were dose-dependent, with doses as low as 3.75 mg/kg body wt. resulting in significant induction of haem oxygenase and a decrease in cytochrome *P*-450 and cytochrome *b*₅ contents at 72 h in the liver. The effects with time of a single dose of tricyclohexyltin on various parameters of liver haem metabolism were also examined. The organotin produced a substantial and very prolonged induction of haem oxygenase accompanied by a steady decline in cytochrome *P*-450 content for periods up to 8 days. The long duration of action of these organotins with respect to induction of haem oxygenase and depletion of cellular haemoprotein content provides a highly sensitive metabolic system with which to define further the toxic potential of organometals as well as to study the adaptive responses in liver to long-term perturbations of haem metabolism by foreign chemicals.

A number of metals have the ability to induce, in liver, kidney and other tissues (Maines & Kappas, 1974, 1977a), the synthesis *de novo* of haem oxygenase (Tenhunen *et al.*, 1969), the rate-limiting enzyme of haem degradation. Haem oxygenase-inducing metals include cobalt, cadmium, platinum, chromium, nickel and tin, among others (Maines & Kappas, 1975, 1976, 1977b). Tin has an especially potent ability to induce the enzyme in kidney (Kappas & Maines, 1976; Drummond & Kappas, 1979). DeMatteis (1978) reviewed the potential mechanism(s) by which metals, as well as other agents, induce haem oxygenase.

Tin is widely used industrially in a modified chemical state; that is, covalently bound to organic substituents. Since organometals might be expected to have different biological effects than their inorganic counterparts, we have tested a number of organotin compounds to determine whether they have the ability to induce haem oxygenase and to decrease cellular cytochrome *P*-450 content in a manner comparable with that of 'inorganic' tin.

The results of these studies are described in the present paper; they indicate that the administration of certain organotin compounds in single doses can produce a significant and prolonged induction of haem oxygenase and a sustained decrease in cytochrome *P*-450 content in the liver. The term induction is used to describe new enzyme synthesis, since this response to metals has been shown in previous reports to be inhibited by agents that block nucleic acid and protein synthesis (Maines & Kappas, 1975). Since there is a good deal of evidence suggesting that organotins are metabolized by a cytochrome *P*-450-dependent system (Aldridge *et al.*, 1977; Kimmel *et al.*, 1977), an interesting relationship between detoxification and regulation of haemoprotein metabolism becomes evident. The toxicological importance of these organotin effects on haem metabolism may also be considerable because of the widespread use and distribution of such organometals in the environment.

Experimental

Male Sprague–Dawley rats (180–225 g) purchased from Holtzman (Madison, WI, U.S.A.), were used throughout the present study. Tricyclo-

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hexyltin bromide, di-n-butyltin dichloride, bis(tri-n-butyltin) oxide and diethyltin dichloride were obtained from the Ventron Corp., Danvers, MA, U.S.A. Tricyclohexyltin hydroxide and triphenyltin acetate were a generous gift of the M & T Chemical Co., Rahway, NJ, U.S.A. The organometals were of at least 95% purity as determined by the manufacturer. All other reagents were obtained from Sigma, St. Louis, MO, U.S.A.

Rats were injected subcutaneously with the organometal compounds in 95% ethanol in a single dose of amounts up to 15 mg/kg body wt., unless otherwise specified. Control rats received an equal volume (1 ml/kg body wt.) of ethanol. The ethanol-treated control rats were identical with saline-treated animals in all parameters studied. 'Inorganic' tin (SnCl_2) was given as a saline suspension in a single subcutaneous injection. Animals were starved for 16 h before being killed by decapitation at the time points indicated.

Haem oxygenase activity was measured in the 105 000 g microsomal fraction, as described previously (Maines & Kappas, 1976). Cytochrome P-450 and cytochrome b_5 contents were measured by the method of Omura & Sato (1964). The data were analysed by the standard *t* test and the value of $P < 0.02$ was regarded as denoting significance.

Results and discussion

Various organotin compounds of differing structure were screened for their ability to induce haem oxygenase in liver and kidney, organs known to be responsive to metal-induced changes in this enzyme. The effects of the administration of a single dose of each of six organotin compounds on haem oxygenase activity and haemoprotein (cytochrome P-450 and cytochrome b_5) content in the liver at 72 h

are shown in Table 1. Five of the compounds tested induced haem oxygenase significantly with the induction response varying between 3 and 5-fold.

The highly water-soluble compound diethyltin dichloride did not produce significant induction at 72 h, although the effect of this organotin on haem oxygenase induction in both liver and kidney at 16–24 h after its administration was substantial (activity was increased four and ten times in the liver and kidney respectively). The least water-soluble organotins produced the greatest induction of hepatic haem oxygenase at 72 h and had little or no effect on the enzyme in the kidney. The decrease in hepatic microsomal haemoprotein content observed at 72 h after administration of the non-polar compounds was marked in comparison with that produced by the water-soluble compound diethyltin, suggesting that the basis for the prolonged induction of the enzyme might reside, in part, in the lipid solubility of the organometal.

Since the least-polar organotins did not significantly induce renal haem oxygenase, in contrast with the marked kidney response elicited by 'inorganic' tin (Drummond & Kappas, 1979), it is clear that the organification of the metal somehow results in a shifting of its site of action, either through a change in its tissue distribution and/or a qualitative change in the nature of its interaction with the regulatory mechanism for this enzyme in various organs.

Included in Table 1 is a comparison of the effects of tricyclohexyltin hydroxide and tricyclohexyltin bromide. The parameters studied were essentially identical at 72 h, suggesting that the anionic moiety was not a major determinant of the enzymic and other responses to the organometals.

The effects of administering increasing doses of tricyclohexyltin hydroxide, a widely used miticide,

Table 1. *The effects of organotin compounds on hepatic microsomal haemoproteins and haem oxygenase at 72 h*
Rats were injected subcutaneously with 15 mg of the organotin compounds/kg body wt. (25–55 $\mu\text{mol/kg}$ body wt.) in 95% ethanol 72 h before killing. The animals were starved 16 h before killing. Hepatic microsomal fractions were prepared and assays were performed as described in the Experimental section. Values are reported as means $\pm$ s.e.m. for at least two separate experiments involving a minimum of six animals.

Treatment	Haem oxygenase (nmol of bilirubin formed/h per mg of microsomal protein)	Cytochrome P-450 (nmol/mg of microsomal protein)	Cytochrome b_5 (nmol/mg of microsomal protein)
Control	1.79 $\pm$ 0.18	0.75 $\pm$ 0.08	0.33 $\pm$ 0.01
Diethyltin dichloride	3.00 $\pm$ 0.10*	0.52 $\pm$ 0.01*	0.24 $\pm$ 0.03*
Di-n-butyltin dichloride	7.93 $\pm$ 0.60*	0.41 $\pm$ 0.02*	0.23 $\pm$ 0.01*
Triphenyltin acetate	5.42 $\pm$ 0.06*	0.49 $\pm$ 0.05*	0.23 $\pm$ 0.01*
Tricyclohexyltin bromide	6.60 $\pm$ 0.25*	0.33 $\pm$ 0.03*	0.20 $\pm$ 0.02*
Tricyclohexyltin hydroxide	6.94 $\pm$ 0.46*	0.27 $\pm$ 0.02*	0.20 $\pm$ 0.01*
Bis(tri-n-butyltin) oxide	6.22 $\pm$ 0.21*	0.26 $\pm$ 0.01*	0.16 $\pm$ 0.01*

* $P < 0.02$

on various parameters of haem metabolism were examined at 72 h in the liver, as shown in Table 2. Tricyclohexyltin produced, in doses up to 7.5 mg/kg body wt., a dose-dependent alteration of hepatic microsomal haem metabolism, with the lowest dose (3.75 mg/kg body wt.) resulting in significant losses in the content of cytochrome *P*-450 (−44%) and cytochrome *b*₅ (−31%) at 72 h compared with solvent-treated controls. These dose-dependent changes paralleled the rise in activity of hepatic haem oxygenase. At doses of 15 mg/kg body wt. the extent of induction of haem oxygenase reached a plateau at activities approx. 5-fold greater than the control. The decreases observed in cytochrome *P*-450 and cytochrome *b*₅ varied inversely with the increases in haem oxygenase activity. As with haem oxygenase, the maximum changes in haemoprotein content were reached at doses of the organotin in the range of 15 mg/kg body wt., suggesting a saturation effect in response to the compound. Tricyclohexyltin did not induce splenic or renal haem oxygenase at any of the doses studied. The addition *in vitro* of tricyclohexyltin to the haem oxygenase assay system derived from control microsomal fraction was without effect at concentrations up to 500 μ mol/litre.

We extended our observations to examine the effect of tricyclohexyltin on various parameters of liver haem metabolism with time (Fig. 1). Tricyclohexyltin was administered subcutaneously to rats at a single dose of 15 mg/kg body wt. (40 μ mol/kg body wt.), the molar tin equivalent being approx. 10 μ mol/kg body wt. On an equivalent molar basis, this is approx. $\frac{1}{3}$ of the dose of cobalt that significantly induces hepatic haem oxygenase (Maines & Kappas, 1974). The organotin produced a substantial and prolonged induction of haem oxygenase, the peak activity (four times the control activity) being reached by 48 h (Fig. 1) and remaining still highly increased at 96 h; in fact

enzyme activity remained above control values in this and other experiments for as long as 8 days after treatment (2.5 times the control value). This sustained enzyme induction contrasts with the effects of 'inorganic' metal inducers of haem oxygenase, which generally result in maximum induction at 16–24 h with a return to normal values by approx. 48 h (Maines & Kappas, 1977a). The tricyclohexyltin induction of haem oxygenase was accompanied by a steady decline in the content of cytochrome *P*-450

Fig. 1. Time course of tricyclohexyltin hydroxide effects on hepatic microsomal haemoprotein content and haem oxygenase activity

Tricyclohexyltin hydroxide (15 mg/kg body wt.) or SnCl_2 (8.78 mg/kg body wt.) was administered subcutaneously in a single injection to 18 rats, divided into three rats per time point. Hepatic microsomal fractions were prepared and assays were performed as described in the Experimental section. The hatched and unfilled bars show the effects respectively of SnCl_2 and tricyclohexyltin hydroxide on haem oxygenase activity (nmol of bilirubin formed/h per mg of microsomal protein). ●, Cytochrome *P*-450 content (nmol/mg of microsomal protein). Values are expressed as means $\pm$ S.D. for at least two separate experiments.

Table 2. The effects of tricyclohexyltin hydroxide on hepatic microsomal haemoproteins and haem oxygenase at 72 h. Tricyclohexyltin hydroxide was administered subcutaneously in 95% ethanol in a single injection at the doses indicated to 15 rats, divided into three rats per dose concentration. The animals were starved for 16 h before death and killed 72 h after the single injection. Hepatic microsomal fractions were prepared and assays were performed as described in the Experimental section. Values are reported as means $\pm$ S.E.M. for at least two separate experiments involving a minimum of six animals.

Dose (mg/kg body wt.)	Haem oxygenase (nmol of bilirubin formed/h per mg of microsomal protein)	Cytochrome <i>P</i> -450 (nmol/mg of microsomal protein)	Cytochrome <i>b</i> ₅ (nmol/mg of microsomal protein)
0	1.35 $\pm$ 0.28	0.75 $\pm$ 0.02	0.36 $\pm$ 0.01
3.75	3.84 $\pm$ 0.27*	0.42 $\pm$ 0.02*	0.25 $\pm$ 0.01*
7.5	6.05 $\pm$ 0.32*	0.36 $\pm$ 0.03*	0.22 $\pm$ 0.01*
15	6.57 $\pm$ 0.77*	0.27 $\pm$ 0.03*	0.22 $\pm$ 0.01*
30	6.54 $\pm$ 0.24*	0.28 $\pm$ 0.02*	0.23 $\pm$ 0.003

* $P < 0.02$.

up to 72 h (Fig. 1) and this content remained low thereafter until haem oxygenase activity returned to normal. Cytochrome b_5 content did not change until 48 h (results not shown), consistent with its longer half-life (Bock & Siekevitz, 1970), after which it reached a value approx. 30% below normal before gradually returning towards control values during the succeeding week.

To determine whether or not an equivalent amount of 'inorganic' tin would produce a quantitatively similar alteration of hepatic haem metabolism, a dose of SnCl_2 calculated to contain the molar equivalent of tin found in the organometal was administered subcutaneously in a single injection. The effects on hepatic haem oxygenase activity are shown in Fig. 1 (hatched bars). The extent of induction of this enzyme and the concomitant perturbations in microsomal haem metabolism produced by the 'inorganic' metal were negligible throughout the period of study, indicating the important role of the organic substituent in determining the ability of the organotin to induce prolonged perturbations of haem metabolism.

The long duration of action of the organotins with respect to induction of microsomal haem oxygenase and depletion of cytochrome $P-450$ and cytochrome b_5 contents is probably due to their highly water-insoluble nature [e.g. water solubility of tricyclohexyltin hydroxide ($< 1 \text{ mg/litre}$)], as well as their prolonged biological half-life in the body (McCollister & Schober, 1975).

These organometal-induced perturbations in haem metabolism merit further investigation for toxicological as well as biochemical reasons. Although numerous studies concerning various aspects of organotin toxicity have appeared in the literature in recent years (Barnes & Stoner, 1959; Rose & Aldridge, 1968; Kimbrough, 1976; Aldridge *et al.*, 1977), the alterations described in the present paper report a novel and highly sensitive metabolic system with which to further define the toxic potential of organometal compounds. Moreover, prolonged induction of hepatic haem oxygenase, accompanied by the significant depletion of microsomal haemoproteins for periods up to 8 days or longer provides

another valuable experimental system with which to study the adaptive responses (Sassa *et al.*, 1979) of haem and haemoprotein metabolism in liver to perturbations by foreign chemicals.

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