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DIRECT *IN VITRO* EFFECTS OF BIS(TRI-*N*-BUTYLTIN)OXIDE ON HEPATIC CYTOCHROME P-450

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Abstract—Bis(tri-*n*-butyltin)oxide, an agriculturally important biocidal agent, when added *in vitro* to liver microsomes containing the phenobarbital-induced form of cytochrome P-450, produced a typical type I binding spectrum (an absorption maximum at 390 nm; an absorption minimum at 420 nm). Studies with microsomal preparations containing cytochrome P-448, induced by 3-methylcholanthrene or β-naphthoflavone, revealed that this hemeprotein was more susceptible to direct degradation by bis(tri-*n*-butyltin)oxide than was the uninduced or phenobarbital-induced forms of cytochrome P-450. The disappearance of spectrally detectable cytochrome P-450 was accompanied by an increase in cytochrome P-420. The formation of cytochrome P-420 was both time and temperature dependent, and it also occurred to a greater extent in microsomal preparations containing cytochrome P-448 than in microsomes containing the phenobarbital-induced form of cytochrome P-450. In all cases, the decreases in spectrally detectable cytochrome P-450 produced by the organotin were not accompanied by decreases in microsomal heme or cytochrome b_5 content. The findings provide evidence for the direct interaction followed by conversion of cytochrome P-450 to cytochrome P-420 produced by a trialkyltin compound *in vitro*, and indicate that different susceptibilities to degradation exist within the various subspecies of this hemeprotein.

The liver endoplasmic reticulum is highly susceptible to damage resulting from exposure to certain environmental chemicals. Disruption of liver function is often manifested by changes in the functional activity of the cytochrome P-450-dependent monooxygenase system localized within these membranes. The particular susceptibility of cytochrome P-450 to chemically-mediated damage relates directly to the centrol role of this hemeprotein in the chemical activation/detoxification of a wide variety of lipophilic drug substrates.

Although a vast majority of exogenous substrates of the monooxygenase system are efficiently metabolized and cleared from the cell without affecting the functioning of cytochrome P-450, a small number of chemicals have been recognized to produce decreases in the cellular content and functional activity of this hemeprotein [1–9]. Attempts at correlating the structure or physical properties of these compounds with their toxic effects have been largely unsuccessful. Several distinct chemical mechanisms by which cytochrome P-450 is degraded on interaction with foreign chemicals have been proposed (see review by De Matteis [10]). These include the conversion of cytochrome P-450 to cytochrome P-420, covalent modification of the heme moiety, direct damage to the apoprotein, and lipid peroxidation of microsomal membrane lipids [10].

Previous work from this laboratory has demonstrated the potent ability of metal ions [11–13] as well as metalloporphyrins [14, 15] and organotin compounds [16, 17] to produce marked decreases in the content of cytochrome P-450 *in vivo*. In this report, we present evidence for the direct conversion of hepatic cytochrome P-450 to cytochrome P-420 by the agriculturally important organotin biocidal agent, bis(tri-*n*-butyltin)oxide. In addition, evidence is provided for the highly specific interaction of this trialkyltin compound *in vitro* with specific forms of cytochrome P-450.

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MATERIALS AND METHODS

Materials

Male Sprague–Dawley rats (175–225 g), purchased from Taconic Farms (Germantown, NY), were used throughout the present study. Bis(tri-*n*-butyltin)oxide was obtained from the Ventron Corp. (Danvers, MA). Sodium phenobarbital was purchased from Mallinckrodt (St. Louis, MO), 3-methylcholanthrene from the Eastman Kodak Co. (Rochester, NY), and β-naphthoflavone from the Aldrich Chemical Co. (Milwaukee, WI). All other reagents were of the highest grade commercially available and were purchased from the Sigma Chemical Co. (St. Louis, MO).

Methods

Treatment of animals and preparation of microsomes. Phenobarbital and β-naphthoflavone were each injected i.p. in a single daily dose for 3 days. Phenobarbital (80 mg/kg body wt) was dissolved in saline. β-Naphthoflavone (40 mg/kg) was suspended in corn oil. 3-Methylcholanthrene (25 mg/kg) was suspended in corn oil and injected i.p. 72 and 48 hr prior to killing the animals. Microsomes prepared from control animals receiving either corn oil or saline (1.0 ml/kg, i.p.) responded to *in vitro* organotin treatment identically, and such microsomes are

referred to as "untreated" in the figures and tables. Animals were allowed free access to water but were deprived of food for the 16 hr prior to sacrifice.

Livers were exhaustively perfused *in situ* with ice-cold 0.9% NaCl and homogenized in 3 vol. of potassium phosphate buffer (0.1 M, pH 7.4) containing sucrose (0.25 M). The homogenates were centrifuged at 9,000 *g* for 20 min, and the resultant supernatant fraction was centrifuged at 105,000 *g* for 60 min to prepare a microsomal pellet which was suspended in potassium phosphate buffer (0.1 M, pH 7.4), to a final protein concentration of approximately 15–20 mg/ml. The microsomal suspension was then either used immediately or stored in liquid N_2 (−70°). Storage of microsomes in liquid N_2 for several months did not result in a detectable loss in spectrally measurable cytochrome P-450 concentration.

Substrate-induced spectral studies. Microsomes, which were prepared as described above, were diluted with potassium phosphate buffer (0.1 M, pH 7.4) to obtain a final protein concentration of approximately 2 mg/ml. Binding studies were performed at room temperature and all spectra were recorded between 350 and 500 nm using an Aminco DW2A spectrophotometer in the split beam mode. Bis(tri-*n*-butyltin)oxide was dissolved in ethanol (95%) in a stock solution of 1 mM. An equal volume of ethanol (95%) was added to the reference cuvette after each addition of the organotin to the sample cuvette. The total volume of added solvent never exceeded 4% (v/v) of the total microsomal suspension. The magnitude of spectral change after each addition of substrate was determined as the difference in absorbance at 390 and 420 nm [18, 19]. Spectral dissociation constants were then calculated from the double reciprocal (Lineweaver–Burk) plots in which substrate binding $(\Delta Abs)^{-1}$ was plotted against substrate concentration $(\mu M)^{-1}$. The data were examined by linear regression analysis. The K_s determination was repeated on five freshly prepared microsomal samples. No difference was noted in the K_s determined from fresh and liquid N_2-stored microsomes.

Incubation in vitro. Microsomes obtained from the livers of rats pretreated with the various inducers and prepared by the above procedure were then diluted to a protein concentration of 1.5 mg/ml with potassium phosphate buffer (0.1 M, pH 7.4) containing 1 mM EDTA. The samples were placed in 25-ml Erlenmeyer flasks. The microsomes (15 ml) were incubated aerobically with constant shaking for various times (indicated in the legends of figures and tables) up to 60 min in the presence of 0.2 mM bis(tri-*n*-butyltin)oxide. The organotin (dissolved in 95% ethanol) was added directly to the microsomal suspension in a volume of 50 μl (0.3%, v/v). After incubation for an appropriate time interval, aliquots were removed and placed in an ice water bath prior to recording the spectra.

For studies involving the rate of formation of cytochrome P-420 as a function of temperature, a single cuvette containing the dithionite-reduced microsomal preparation was scanned in the dual-wavelength mode at two fixed wavelengths (420 and 490 nm) following CO addition to the sample cuvette. The scan speed was adjusted to 100 sec/inch. Cuvette temperature was regulated by continuous water flow through an FEN temperature regulator (Haake Instruments, Inc., Saddle Brook, NJ).

The concentrations of cytochrome P-450 and cytochrome P-420 were estimated by the dithionite-reduced minus carbon monoxide (CO) difference spectrum [20, 21]. Cytochrome b_5 content was quantitated by the NADH-reduced minus oxidized difference spectrum, using an extinction coefficient of 185 mM^{-1} cm^{-1} between 412 and 425 nm [20]. The total concentration of microsomal heme was determined by the pyridine-hemochromagen method as described by Porra and Jones [22], using the dithionite-reduced minus oxidized difference spectrum between 541 and 557 nm and an extinction coefficient of 20.7 mM^{-1} cm^{-1}. Protein concentration was determined by the method of Lowry *et al.* [23], using crystalline bovine serum albumin as standard.

Statistical analysis. The data were analyzed by the standard *t*-test, and the indicated *p* value was regarded as denoting significance.

RESULTS

The sequential addition of increasing amounts of bis(tri-*n*-butyltin)oxide to liver microsomes obtained from phenobarbital-pretreated animals resulted in the formation of typical type I substrate-induced difference spectra, with an absorbance peak at 390 nm and a trough at 420 nm (Fig. 1). The double-reciprocal (Lineweaver–Burk) plot for bis(tri-*n*-butyltin)oxide is also shown in the figure (insert). Ethanol, which can bind with low affinity

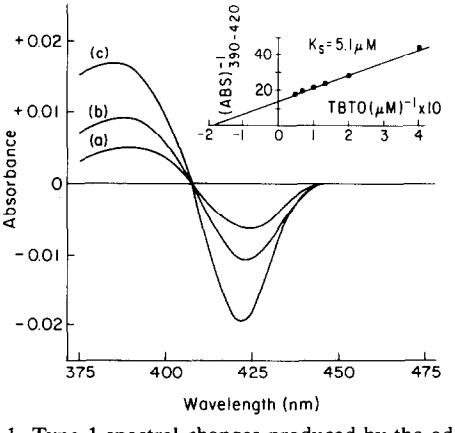

Fig. 1. Type 1 spectral changes produced by the addition of bis(tri-*n*-butyltin)oxide (TBTO) to rat liver microsomes. Microsomes prepared from phenobarbital-pretreated rats were adjusted to a protein concentration of 2 mg/ml in 0.1 M potassium phosphate buffer (pH 7.4) and divided equally between two cuvettes. The baseline was corrected for equal absorbance prior to the addition of substrate, bis(tri-*n*-butyltin)oxide, dissolved in 95% ethanol. Increasing concentrations of bis(tri-*n*-butyltin)oxide were added to the sample cuvette, while an equal volume of ethanol alone was added to the reference cuvette, and the spectra recorded after each addition. Key: (a) 1 μM, (b) 2.5 μM, and (c) 10 μM. Inset: Double-reciprocal Lineweaver–Burk plot of the change in absorbance vs increasing substrate concentrations ($r = 0.99$).

Table 1. Binding of bis(tri-*n*-butyltin)oxide to microsomes*

Pretreatment	Cytochrome P-450 (nmoles/mg)	Abs_{max} (nm)	Abs_{min} (nm)	$\Delta(Abs_{max} - Abs_{min})$/nmole cytochrome P-450
Untreated	0.74	395	424	0.0108
Phenobarbital	2.18	390	420	0.0101
3-Methylcholanthrene	1.86	392	422	0.0036
β-Naphthoflavone	1.34	390	422	0.0055

* Hepatic microsomal suspensions (2 mg protein/ml) were prepared from rats, and difference spectra were obtained as described in Materials and Methods. Values represent the average of two separate experiments.

to the heme iron of cytochrome P-450 [24], but which does not interfere at these concentrations with the development of a type I spectrum, was used in these experiments because the organotin compound is readily soluble in this solvent at concentrations up to 50 mM.

The binding of bis(tri-*n*-butyltin)oxide to liver microsomes was further examined in microsomes obtained from animals pretreated with inducers of specific forms of cytochrome P-450 (Table 1). Similar type I binding spectra were obtained, although there were differences in the extent to which the organotin was capable of eliciting a spectral response. The relative degree of substrate binding was expressed as the $\Delta(Abs_{max} - Abs_{min})$ per nmole of cytochrome P-450 present in the microsomal suspension (Table 1), and by this criteria was similar both in microsomes obtained from untreated (0.0108) and phenobarbital-pretreated (0.0101) animals. The two inducers of cytochrome P-448 that were examined, 3-methylcholanthrene and β-naphthoflavone, while resulting in substantial elevation in the concentration of cytochrome P-448, actually diminished the maximum spectral change elicited by bis(tri-*n*-butyltin)oxide. Pretreatment with 3-methylcholanthrene resulted in a 65–70% decrease in the $\Delta(Abs_{max} - Abs_{min})$ per nmole of cytochrome P-448 produced by addition of bis(tri-*n*-butyltin)oxide, while β-naphthoflavone lowered this relative binding parameter by ~50%. The positions of the absorption maxima (390–395 nm) and minima (420–424 nm), however, did not vary significantly among the four different microsomal preparations (Table 1), indicating that a similar type I spectral response was produced by the organotin upon interaction with both cytochrome P-450 and cytochrome P-448.

Liver microsomal suspensions obtained from untreated animals, or animals pretreated with either phenobarbital, 3-methylcholanthrene or β-naphthoflavone, were incubated at 37° for up to 60 min in the presence of 0.2 mM bis(tri-*n*-butyltin)oxide. This concentration of organotin is approximately 40-fold in excess of the spectral dissociation constant (K_s) of 5.1 μM calculated by linear regression (Fig. 1). As shown in Fig. 2, bis(tri-*n*-butyltin)oxide produced a time-dependent decrease in the spectrally detectable content of cytochrome P-450 (448) in each microsomal preparation examined. Furthermore, the addition of NADPH was not required for such changes to occur, suggesting a direct effect of the organotin on the cytochrome. This loss in cytochrome P-450 content was considerably more pronounced in microsomes obtained from 3-methylcholanthrene- or β-naphthoflavone-pretreated animals in which cytochrome P-448 is the predominant form of the hemeprotein. Cytochrome P-448 content was lowered to approximately 50% of its initial concentration within 5 min. This is in comparison with only a 25–30% decrease in cytochrome P-450 content at this time point, 5 min, in microsomes obtained from either untreated or phenobarbital-pretreated animals. At 30 and 60 min, when 35–40% of cytochrome P-450 was lost in the untreated and phenobarbital microsomes, as much as 70–75% of the spectrally detectable cytochrome P-448 had disappeared in the 3-methylcholanthrene and β-naphthoflavone groups (Fig. 2).

These observed decreases in cytochrome P-450 (448) were accompanied by the formation of cytochrome P-420 in the untreated and phenobarbital treatment groups. This conversion to cytochrome P-420, however, was much more pronounced in microsomes obtained from rats pretreated with 3-methylcholanthrene and β-naphthoflavone. Following a 60-min incubation with the organotin, as much as 20–30% of the initial CO-binding cytochrome was present in the form of cytochrome P-420 in these latter two treatment groups. The greatest dissimilarity between preparations, however, was apparent upon examination of the relative proportion of cytochrome P-420 to cytochrome P-450 following incubation with the organotin. Cytochrome P-420 represented approximately 40% (β-naphthoflavone) and 50% (3-methylcholanthrene) of the remaining CO-binding cytochrome (Fig. 2). Furthermore, the spectrally measurable total CO-binding cytochrome was found to decrease in microsomes as a function of incubation time. Again, the decreases in total CO-binding cytochrome were greater in the 3-methylcholanthrene (45%) and β-naphthoflavone (50%) treatment groups than in the untreated (25%) and phenobarbital (15%) groups (Fig. 2).

The effect of bis(tri-*n*-butyltin)oxide (0.2 mM) on the CO/dithionite-reduced difference spectrum of cytochrome P-448 (β-naphthoflavone-induced) is shown in Fig. 3. The organotin compound was added to the microsomal suspension immediately after reduction with dithionite, and the development of the CO-induced difference spectrum was recorded prior to and following a 15-min incubation at 37°. Consistent with the data shown above (Fig. 2) for microsomes incubated aerobically without prior

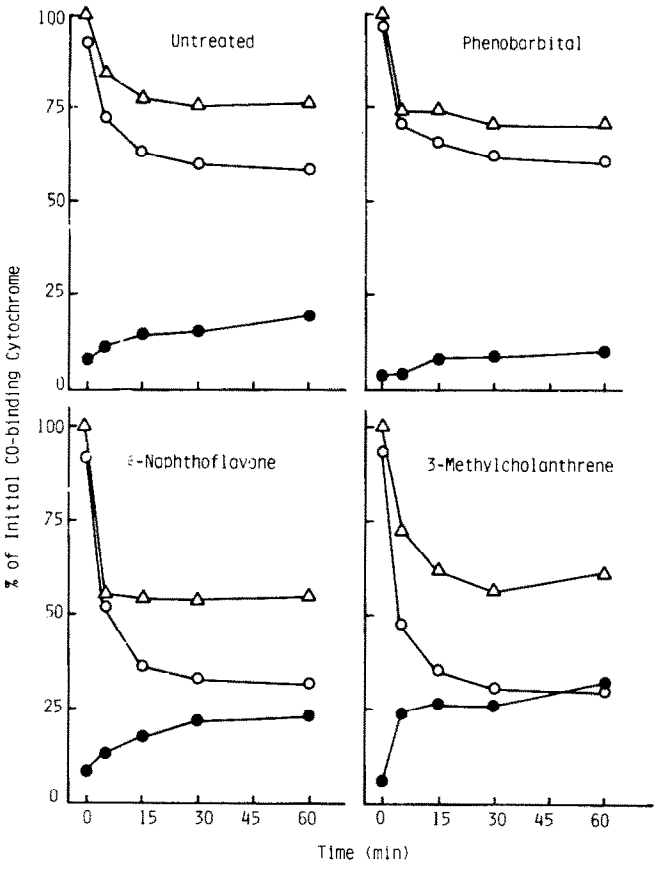

Fig. 2. *In vitro* time-dependent loss of cytochrome P-450 in the presence of bis(tri-*n*-butyltin)oxide. Microsomes were prepared from the livers of rats as described in Materials and Methods. The microsomal suspensions (1.5 mg protein/ml) in a total volume of 15 ml were incubated at 37° in the presence of 0.2 mM bis(tri-*n*-butyltin)oxide. At the times indicated, 1.5-ml aliquots were removed, and the content of cytochrome P-450 (○—○) and P-420 (●—●) was determined [20, 21]. The values are reported as a percentage of initial total CO-binding cytochrome (△—△) with solvent (ethanol) alone. Values represent the average of three separate determinations.

reduction, there was a significant decrease in peak height at 448 nm, accompanied by a shift in the absorption maximum from 448 to 450 nm. This spectral shift to a longer wavelength is probably due to a negative contribution of cytochrome P-420 to the absorption maxima at 448 nm [20].

These decreases in spectrally measurable cytochrome P-450 content were not accompanied by a lowering of the levels of total microsomal heme nor of cytochrome b_5 concentration (Table 2). Microsomal suspensions were incubated at 37° with or without bis(tri-*n*-butyltin)oxide for 30 min. Although the concentration of cytochrome P-450 was decreased to approximately 65% (untreated and phenobarbital) and 35% (3-methylcholanthrene and β-naphthoflavone) of initial levels, total microsomal heme content was in no case diminished in any of the treatment groups examined. Furthermore, there were no differences observed in the spectral appearance of the pyridine hemochromagen difference spectrum in the presence or absence of the organotin, suggesting that the heme moiety itself may not be a direct target of the organotin effects. The other major heme protein in the microsomal membrane, cytochrome b_5, was also unaffected by incubation with bis(tri-*n*-butyltin)oxide in all four preparations (Table 2), nor were any spectral perturbations apparent in the NADH-reduced difference spectrum.

The rate of formation of cytochrome P-420 was also temperature dependent (Fig. 4). The appearance of cytochrome P-420 was monitored with time at two fixed wavelengths (420 and 490 nm), using a dithionite-reduced microsomal preparation obtained from 3-methylcholanthrene-treated animals. At the two lower temperatures examined (26° and 32°), the formation of cytochrome P-420 was linear with time. However at 38°, the highest temperature examined, the initial rate of formation was stimulated markedly, resulting in a hyperbolic curve with a greater absorption at 420 nm.

DISCUSSION

These studies demonstrate the potent ability of bis(tri-*n*-butyltin)oxide, a commercially important organotin biocidal agent, to interact with and to degrade hepatic cytochrome P-450 *in vitro*. These

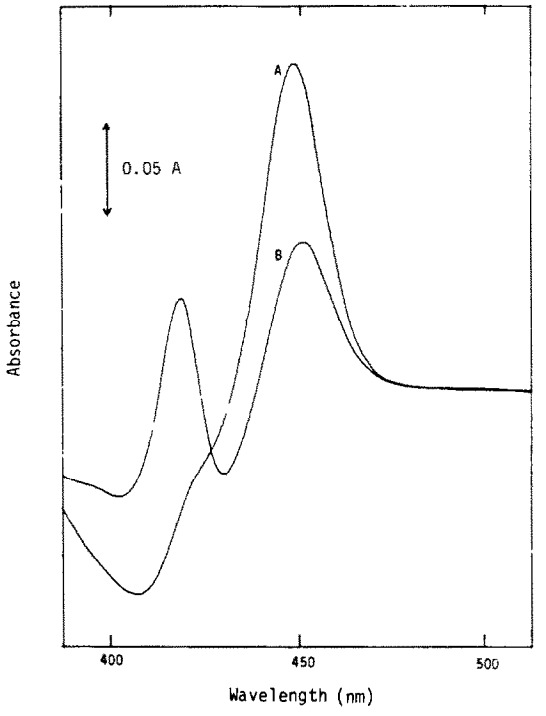

Fig. 3. Conversion of cytochrome P-448 to cytochrome P-420. Microsomal suspensions (1.5 mg protein/ml) were prepared from β-naphthoflavone-pretreated animals. The microsomes were reduced with dithionite prior to the addition of 0.2 mM bis(tri-*n*-butyltin)oxide. CO was then bubbled into the sample cuvette, and the difference spectrum was recorded prior to (A) and immediately following (B) a 15-min incubation at 37°.

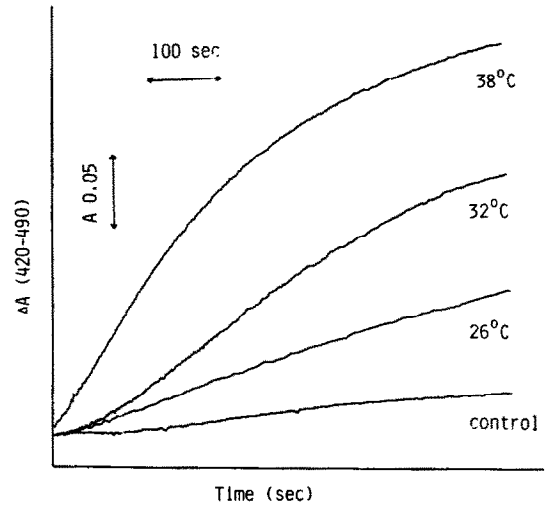

Fig. 4. Tempurature-dependent formation of cytochrome P-420 in the presence of 0.2 mM bis(tri-*n*-butyltin)oxide. Microsomal suspensions of 1 ml (1.5 mg protein/ml) prepared from 3-methylcholanthrene-treated animals were reduced with sodium dithionite and saturated with CO. After establishing a stable baseline, bis(tri-*n*-butyltin)oxide (0.2 mM) was added to the cuvette, and the rate of formation of cytochrome P-420 was monitored in the dual-wavelength mode (420 nm–490 nm) at the temperatures indicated for 10 min. The control represents an identical microsomal suspension containing vehicle (ethanol) alone incubated at 38°.

Table 2. *In vitro* disappearance of spectrally detectable cytochrome P-450 in the presence of bis(tri-*n*-butyltin)oxide without concurrent decreases in cytochrome b_5 and microsomal heme content*

Pretreatment	Organotin (−/+)	Cytochrome P-450	Cytochrome P-420 (nmoles/mg protein)	Cytochrome b_5	Heme
Untreated	—	0.83 ± 0.05	0.08 ± 0.02	0.42 ± 0.01	1.28 ± 0.01
	+	0.54 ± 0.03†	0.18 ± 0.04‡	0.42 ± 0.04	1.49 ± 0.13
Phenobarbital	−	2.10 ± 0.18	0.09 ± 0.03	0.48 ± 0.02	2.82 ± 0.07
	+	1.36 ± 0.12§	0.18 ± 0.04	0.50 ± 0.04	2.92 ± 0.04
3-Methylcholanthrene	−	1.46 ± 0.06	0.07 ± 0.02	0.47 ± 0.02	1.89 ± 0.26
	+	0.51 ± 0.02†	0.39 ± 0.03†	0.46 ± 0.02	1.74 ± 0.30
β-Naphthoflavone	−	1.40 ± 0.04	0.14 ± 0.03	0.44 ± 0.02	1.71 ± 0.12
	+	0.50 ± 0.03†	0.34 ± 0.03†	0.42 ± 0.01	1.85 ± 0.14

* Microsomal suspensions (1.5 mg protein/ml) were prepared from rats as described in Materials and Methods. Samples were incubated at 37° with constant shaking for 30 min in the presence of 0.2 mM bis(tri-*n*-butyltin)oxide. The concentrations of cytochromes P-450 and P-420 [20, 21], cytochrome b_5 [20] and microsomal heme [22] were determined immediately. Values are reported as the means ± S.E.M. of at least three separate experiments.

† $P < 0.01$ as compared with organotin (−).

‡ $P < 0.05$.

§ $P < 0.02$.

effects appear to be direct in that the addition of NADPH was not required for metabolic activation of the organotin. Furthermore, the decreases in cytochrome P-450 content were accompanied to a certain extent by the formation of cytochrome P-420, although concurrent losses in microsomal heme and cytochrome b_5 did not occur. In addition, microsomes obtained from rats pretreated with inducers of cytochrome P-448 (3-methylcholanthrene and β-naphthoflavone) exhibited greater susceptibility to these effects than microsomes in which the phenobarbital-induced form of cytochrome P-450 is predominant.

The type I spectral binding characteristics exhibited by bis(tri-*n*-butyltin)oxide are those typical of a lipophilic hydrocarbon interaction with cytochrome P-450 [18, 19] and occurred, although to a differing extent, in microsomes containing a predominance of cytochrome P-450 or cytochrome P-448 (Table 1). Binding affinity has been shown to increase proportionately with substrate hydrophobicity [25–28]. Therefore, one might anticipate that cytochrome P-450 would have a lower binding affinity (higher K_s) for polar dialkyltin compounds. Indeed, diethyltin dichloride, a dialkytin compound previously shown to be marginally effective in degrading hepatic cytochrome P-450 *in vitro* [17], exhibited only a weak spectral interaction ($K_s \sim 1.4$ mM) with induced hepatic microsomes from phenobarbital-pretreated animals (results not shown). This is in marked contrast to the highly specific spectral interaction displayed by the more hydrophobic organotin compound, bis(tri-*n*-butyltin)oxide ($K_s \sim 5$ μM). The difference in degree of spectral binding displayed by diethyltin dichloride might also partially explain the relative lack of potency of this organotin in degrading cytochrome P-450 *in vitro* [17], if, in fact, access to the active site of the hemeprotein is prerequisite to enzyme degradation.

The *in vitro* dose- and time-dependent effects produced by various organotin compounds on the phenobarbital-induced form of cytochrome P-450 have been described previously by this laboratory [17]. In the present investigation, the direct effects (i.e. independently of exogenous NADPH and therefore presumably metabolic activation) of bis(tri-*n*-butyltin)oxide were examined in microsomal preparations containing a predominance of cytochrome P-448; the time-dependent decrease in spectrally detectable cytochrome P-448 was found to be enhanced considerably (Figs. 2 and 3). This greater susceptibility of the β-naphthoflavone-induced form of the hemeprotein to organotin degradation confirms an earlier report using triethyltin bromide to produce such an effect [29]. There are other examples of isozyme selectivity in which specific subspecies of cytochrome P-450 are preferentially inactivated as a result of chemically-mediated damage. Allylisopropylacetamide [1, 9, 10, 30], ethynyl substituted steroids, such as norethindrone and ethynyl estradiol [6], and various olefin derivatives [8, 30] preferentially inactivate the phenobarbital-induced form of cytochrome P-450, while a purified preparation of the β-naphthoflavone isozyme has been shown to be more susceptible to 2,2,2-trifluorethyl vinyl ether than the phenobarbital isozyme [31]. In the case of these unsaturated chemicals, however, there is an absolute requirement for metabolic activation of the substrate, with activity dependent on the addition of NADPH. Furthermore, the destruction of cytochrome P-450 is accompanied by a direct covalent modification of the heme moiety. There are, however, relatively few examples of chemically-mediated inactivation of cytochrome P-450 occurring *in vitro* in the absence of metabolic activation with NADPH as occurs with bis(tri-*n*-butyltin)oxide.

In the present studies, the greater lability of cytochrome P-448 to the *in vitro* effects of the organotin compound is explained in part by the more facile conversion of this subspecies to the spectrally altered form of the hemeprotein, cytochrome P-420. While less than 10% of the total CO-binding cytochrome (cytochromes P-450 and P-420) in microsomes obtained from phenobarbital-pretreated animals was present as cytochrome P-420 following a 30-min incubation with the organotin, as much as 25% of this spectrally detectable CO-binding cytochrome was present as cytochrome P-420 in microsomes obtained from β-naphthoflavone- and 3-methylcholanthrene-pretreated animals.

Direct damage to the heme moiety of the cytochrome may be eliminated as a factor contributing to the ultimate degradation of the hemeprotein upon interaction with bis(tri-*n*-butyltin)oxide since the specific content of microsomal heme was in no case diminished as a result of organotin treatment (Table 2). Furthermore, there were no modifications in the pyridine-hemochromagen difference spectrum in the presence of the organotin. Of a number of organotin compounds that we have tested *in vitro* with hepatic microsomes, only the trialkyltins have produced substantial decreases in cytochrome P-450 levels (unpublished observations). Since the trialkyltin compounds, with their high degree of lipid solubility, are more likely to penetrate the hydrophobic membrane environment in which the cytochrome P-450 molecule is embedded [32], and thereby gain access to the active site of the hemeprotein [as evidenced by the low K_m exhibited by, for example, bis(tri-*n*-butyltin)oxide $\simeq 5$ μM], there is thus a far greater potential for a disruptive interaction to occur. The active site of cytochrome P-450 consists of a heme moiety embedded within a hydrophobic pocket of the apoprotein [28]. A thiol ligand contributed by a cysteine residue has been proposed to constitute the fifth ligand to the penta- or hexacoordinate iron [33], forming a bond which is critical to the unique spectral properties displayed by cytochrome P-450 [20]. Disruption of this coordinate covalent bond by various sulfhydryl reagents causes a conversion of cytochrome P-450 to cytochrome P-420 (see Ref. 34 for a review). It is thus possible that bis(tri-*n*-butyltin)oxide, after gaining access to the substrate binding center, may cause some perturbation in the sulfur–iron bond. Tetravalent tin, with varying numbers of alkyl side chains, is known to readily form coordination complexes with various hetero atoms such as sulfur and nitrogen [35–37]. Therefore, the possibility of complex formation between the tin atom and either a pyrollic nitrogen, or perhaps a

nitrogen or sulfur atom of an amino group located in proximity to the heme moiety cannot be ruled out.

In conclusion, this study demonstrates the ability of bis(tri-*n*-butyltin)oxide to interact with hepatic microsomes, resulting in the formation of a type I spectral interaction that is characteristic of a multitude of hydrophobic substrates of the cytochrome P-450-dependent monooxygenase system. One of the direct consequences of this interaction within the microsomal membrane is an apparent degradation of cytochrome P-450, at least by the spectral criteria we have examined, accompanied by a partial conversion to cytochrome P-420. These effects are considerably more pronounced in microsomes containing the 3-methylcholanthrene- and β-naphthoflavone-induced forms of the cytochrome. These findings are important to our understanding of the underlying mechanisms by which organotin compounds can directly affect the levels of the various subspecies of cytochrome P-450, resulting in a potentially altered metabolic response to other environmental chemicals, drugs and carcinogens.

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