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ACTION ON MITOCHONDRIA AND TOXICITY OF METABOLITES OF TRI-*n*-BUTYLTIN DERIVATIVES

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Abstract—Tri-*n*-butyltin derivatives are metabolized by a cytochrome P450-dependent rat liver microsomal monooxygenase system and by mice to yield carbon-hydroxylated metabolites, i.e. the α-, β-, γ- and δ-hydroxybutyl-dibutyltin derivatives, as well as the corresponding γ-ketone. The latter three metabolites are sufficiently stable at physiological pH for comparisons with tributyltin chloride with regard to their action on mitochondrial functions and intraperitoneal toxicity to mice. The δ-hydroxy compound differs most greatly from the other metabolites in potency for altering mitochondrial functions possibly because of its greater polarity or its lower propensity for intramolecular coordination of the introduced oxygen with the tin atom. The γ-hydroxy, δ-hydroxy and γ-keto compounds are less toxic to mice than tributyltin derivatives and do not increase the water content of the brain under conditions where triethyltin bromide does.

Tri-*n*-butyltin derivatives are important biocides [1] and biochemical probes in studies on mitochondrial functions [2]. The *in vitro* metabolism of tributyltin derivatives by the cytochrome P450-dependent monooxygenase system of rat liver microsomes yields carbon-hydroxylated metabolites identified as the α-, β-, γ- and δ-hydroxybutyldibutyltin derivatives [3–7]. An additional metabolite is the γ-keto compound from further oxidation of the γ-hydroxy metabolite [3–7]. Structures of these metabolites are as follows:

Designation	Structure (Bu = $CH_3CH_2CH_2CH_2$–)
Parent compound	$Bu_2Sn(X)CH_2CH_2CH_2CH_3$
α-Hydroxy	$Bu_2Sn(X)CH(OH)CH_2CH_2CH_3$
β-Hydroxy	$Bu_2Sn(X)CH_2CH(OH)CH_2CH_3$
γ-Hydroxy	$Bu_2Sn(X)CH_2CH_2CH(OH)CH_3$
δ-Hydroxy	$Bu_2Sn(X)CH_2CH_2CH_2CH_2OH$
γ-Keto	$Bu_2Sn(X)CH_2CH_2C(O)CH_3$

Several of these metabolites are also detected in the liver and feces of orally-treated mice [7]. The α- and β-hydroxy organotin metabolites rapidly destannylate (carbon–tin cleavage) under physiological or mild acid conditions, respectively, but the γ- and δ-hydroxy and γ-keto metabolites are sufficiently stable for toxicological evaluations [3, 4, 6].

The various homologs of the trialkyltin series differ considerably in their physical properties such as water and lipid solubilities, in their toxicity and clinical manifestations [8, 9], and in their potency for disrupting three aspects of mitochondrial function [2]. The following effects are seen upon increasing the concentration of triorganotin derivatives: (1) increased ATP hydrolysis due to energy consumption in maintaining the mitochondrial hydroxyl ion concentration while it is being continuously exchanged for chloride in the presence of the trialkyltin derivative [2, 10–12]; (2) decreased ATP hydrolysis attributable to disruption of the energy conservation system, resembling the action of oligomycin in this respect [2, 11–14]; (3) increased ATP hydrolysis at the highest concentrations of trialkyltin derivatives due to gross swelling [2, 15].

The hydroxy and keto metabolites of tributyltin derivatives differ from the parent compounds in solubility properties and in their ability to undergo intramolecular coordination of the oxygen-containing substituents with the tin atom [16]. It is therefore of interest to determine the action on mitochondria and the toxicity of metabolites of tributyltin derivatives.

MATERIALS AND METHODS

Chemicals. Tributyltin chloride, γ-hydroxybutyldibutyltin acetate, δ-hydroxybutyldibutyltin chloride, and γ-ketobutyldibutyltin bromide were synthesized and purified as described by Fish *et al.* [3]. Di-*n*-butyltin dichloride and triethyltin bromide were obtained from commercial sources.

Mitochondrial studies. The preparation of the mitochondrial fraction from rat liver and the determination of ATP synthesis, ATP hydrolysis, P_i and protein were as previously described [17]. Swelling in sodium chloride solution was measured by a reported procedure [18]. The chloride and isethionate (hydroxyethane sulphonate) media contained 0.1 M of the respective potassium salts.

The organotin compounds were dissolved in dimethylformamide and stored at 5° in the dark. The final concentration of dimethylformamide in the mitochondrial medium was 1%. The concentrations of triorganotin derivatives necessary for 50 per cent inhibition were determined from the straight lines relating log [inhibitor] against log (per cent activity/per cent inhibition). Each mitochondrial preparation was used

to compare three compounds. The results are presented with both the concentrations of triorganotin derivatives to which the mitochondria were added and the concentration of mitochondrial protein to permit calculation of the amount of triorganotin derivative added per unit of protein.

Toxicity studies. Male albino mice (20 ± 0.5 g, Swiss Webster strain, Simonsen Laboratories, Inc., Gilroy, CA) were treated by the intraperitoneal route on two consecutive days with a solution (25 μl) of the test compound dissolved in 10% (w/v) Tween 80 in distilled water. On the third day (72 hr) the mortality was recorded and the surviving animals were sacrificed for determination of water content of the brain by weighing the brain immediately after removal and after drying to constant weight (110° for 24 hr).

RESULTS

ATP hydrolysis in a chloride medium. The concentrations of trialkyltin derivatives used in the present study do not cause an increase in ATP hydrolysis due to gross swelling. There are three ways of expressing the stimulation of ATP hydrolysis in a chloride medium within the organotin derivative concentration range examined: (a) the lowest concentration of triorganotin derivative causing an increase in ATP hydrolysis; (b) the concentration causing maximum ATP hydrolysis; (c) the maximal rate of ATP hydrolysis attained.

It is difficult to achieve any precision for (a), but visual inspection of the experimental results (Fig. 1B and similar data for the other compounds) indicates that the order from the most to the least active is the δ-hydroxy compound, the γ-hydroxy compound, tributyltin chloride, and the γ-keto compound. Measures (b) and (c) are given in Table 1. The maximal rate of ATP hydrolysis achieved by a particular compound depends on the degree of separation of the concentrations bringing about the two actions leading to an increase and then a decrease in ATP hydrolysis (effects 1 and 2 discussed above in the introduction). The rate of ATP hydrolysis found with 0.5–1.8 μM of the δ-hydroxy compound approaches that found for trimethyl and triethyltin derivatives as well as the lower members of the trialkyllead series [19]; it is probable that this is the maximal chloride dependent rate which can be achieved.

ATP synthesis linked to the oxidation of succinate. Inhibition of ATP synthesis linked to the oxidation of succinate in chloride or isethionate medium results from mechanisms 1 and 2 previously discussed. Mechanism 1 is chloride dependent while mechanism 2 is

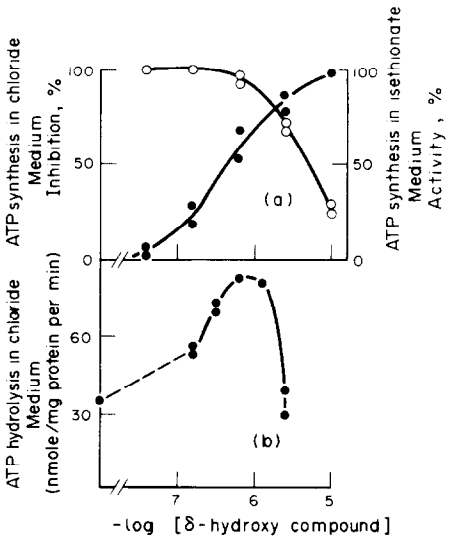

Fig. 1. Effect of δ-hydroxy-*n*-butyl dibutyltin chloride on ATP synthesis linked to the oxidation of succinate and ATP hydrolysis. ATP synthesis (a) was measured over 2 min and the control rates were 277 and 327 in a chloride medium (● = per cent inhibition) and 297 and 274 nmoles/mg protein/min in an isethionate medium (○ = per cent activity). ATP hydrolysis (b) was measured over 10 min. In the presence of 20 μM 2:4-dinitrophenol for 2 min ATP hydrolysis was 319 nmoles/mg protein/min for each experiment.

independent of the main anion and is due to an action on the system bringing about the synthesis of ATP from ADP and P_i. The correspondence of the concentrations bringing about changes in ATP hydrolysis and in ATP synthesis are shown in Figs 1(a) and (b). The difference between the concentrations inhibiting ATP synthesis in isethionate and in chloride medium is greatest for the δ-hydroxy-organotin metabolite, less for the γ-hydroxy metabolite and within experimental error nonexistent for tributyltin chloride and the γ-keto compound (Table 2).

Swelling in a sodium chloride medium. Since rat liver mitochondria are permeable to sodium ions, gross swelling will occur when trialkyltin derivatives facilitate the exchange of chloride for intramitochondrial hydroxyl ion [18, 20]. Thus swelling in 0.15 M sodium chloride is a measure of the ability of the trialkyltin derivative to bring about this chloride-hydroxyl exchange. The decreasing order of activity is δ-hydroxy > γ-hydroxy > parent compound > γ-keto (Table 2).

Table 1. ATP hydrolysis in a chloride medium stimulated by tri-*n*-butyltin chloride and various metabolites

Compound	Mitochondrial protein, mg/ml	[R_3SnX]*	ATP hydrolysis (nmoles/mg protein/min) Control (A)	Exp. (B)	(B-A)
Tri-*n*-butyltin chloride	1.20	0.60 (6)	35.0	43.3	8.3
γ-Keto compound	0.92	0.31 (1)	34.8	41.9	7.1
γ-Hydroxy compound	1.24	0.57 (6)	36.0	69.0	33.0
δ-Hydroxy compound	1.05	0.56–1.8 (2)	34.9	82.8	47.9

* [R_3SnX] is the concentration of trialkyltin derivative causing maximal stimulation of ATP hydrolysis. Figures in parentheses are number of experiments.

Table 2. Inhibition of ATP synthesis by tri-*n*-butyltin chloride and various metabolites

Compound	ATP synthesis in a chloride medium $I_{50}(\mu M)$*	ATP synthesis in an isethionate medium $I_{50}(\mu M)$*	Swelling in 0.15 M NaCl $I_{50}(\mu M)$*
Tri-*n*-butyltin chloride	0.66–0.90 (4)	0.50–0.70 (4)	0.56–0.70 (2)
γ-Keto compound	0.66–0.79 (2)	0.89 (2)	0.80 (1)
γ-Hydroxy compound	0.40–0.63 (3)	0.71–0.89 (3)	0.18–0.30 (2)
δ-Hydroxy compound	0.45–0.63 (2)	4.5–5.0 (2)	0.10 (1)

* I_{50} is the concentration of the trialkyltin derivative causing 50 per cent effect and the figures in parentheses are the number of experiments. The mean mitochondrial protein concentrations for each set of experiments varied only between 1.04 and 1.14 mg/ml.

Table 3. Mortality and brain edema in mice following intraperitoneal administration of tri-*n*-butyltin chloride and various metabolites or comparison compounds for 2 consecutive days with determinations on the third day

Compound	Effect at indicated daily dose, μmoles/kg: Mortality (%) 12.5	25	50	Water content of brain (% ± S.D.)* 12.5	25	50
			Tri-*n*-butyltin chloride and metabolites			
Tri-*n*-butyltin chloride	10 (20)†	36 (25)		79.2 ± 0.4 (17)	79.0 ± 0.3 (16)	
γ-Keto compound		10 (10)	53 (15)		79.2 ± 0.2 (9)	79.0 ± 0.4 (7)
γ-Hydroxy compound		0 (10)	35 (20)		79.3 ± 0.2 (10)	79.1 ± 0.3 (12)
δ-Hydroxy compound		20 (20)	47 (30)		79.1 ± 0.2 (16)	79.0 ± 0.3 (16)
			Comparison compounds			
Triethyltin bromide	0 (20)	45 (25)		79.4 ± 0.2 (18)	79.9 ± 0.6 (23)	
Di-*n*-butyltin dichloride	0 (10)	90 (30)		79.1 ± 0.2 (10)	78.9 ± 0.1 (3)	

* Water content of brain in control mice was 79.1 ± 0.4%.
† The number of animals examined is given in parenthesis.

Toxicity and cerebral water content. Tributyltin chloride is about 2-fold more toxic than three of its metabolites (γ-hydroxy, δ-hydroxy and γ-keto compounds) to mice treated intraperitoneally with two consecutive daily doses (Table 3). Another metabolite, the dibutyltin derivative, is more toxic than any of the previously-mentioned trialkyltin derivatives.

Triethyltin bromide produces a significant increase in cerebral water content following two consecutive daily intraperitoneal doses (Table 3). Tributyltin derivatives and those metabolites of it tested are inactive under the same test conditions. However, it is known that after repeated dosing or after the animals are held for some time, it is possible to produce an increase in brain weight or the water content of brain and spinal cord on administering tributyltin derivatives orally to rats [9] and intraperitoneally to mice [21, 22].

DISCUSSION

The δ-hydroxy and to a lesser extent the γ-hydroxy metabolites of tributyltin derivatives differ from the parent compound and the γ-keto metabolite in their actions on mitochondria as follows: increased potency for effects brought about by chloride-hydroxide exchange (mitochondrial swelling in chloride medium); decreased potency for chloride-independent binding to a component in the energy conservation system (inhibition of ATP synthesis in isethionate medium); the resulting potency differential for chloride-dependent and -independent effects (inhibition of ATP synthesis in chloride versus isethionate media).

In their actions on mitochondrial functions the more polar metabolites of tributyltin derivatives, the γ- and δ-hydroxy metabolites, somewhat resemble the lower trialkyltin compounds [12, 17] suggesting a possible relationship between potency and polarity. The action of the metabolites might also be influenced by the propensity of the hydroxyl or keto function to coordinate intramolecularly with the tin atom to form a trigonal bipyramidal inner coordination complex, which probably retards chloride-hydroxide exchange at the tin atom. Although intramolecular tin–oxygen coordination possibly contributes to the differences in action of the γ- and δ-hydroxy compounds, the expected order for ease of coordination (γ-keto > γ-hydroxy > δ-hydroxy) does not parallel the overall findings when the parent compound is also considered since tributyltin derivatives and the γ-keto metabolite have very similar actions on mitochondrial functions. The hydroxybutyldibutyltin derivatives and other hydroxyalkyldialkyltin compounds may be useful probes in further differentiating the various actions of triorganotin derivatives on mitochondrial functions.

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