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Effects of trialkyllead compounds on mitochondrial energy conservation

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The effects of triethyllead acetate and tri-n-butyllead acetate on rat liver mitochondrial ATPase, succinate-driven ATP synthase and mitochondrial membrane potential have been compared with those of the equivalent organotin compounds. ATP synthase I_{50} values were approximately four times the ATPase I_{50} values for organotin compounds but the reverse pattern of activity is observed with trialkyllead compounds, which are 5–10 times more effective inhibitors of ATP synthase than of ATPase activity. The primary effects of trialkyltins are as inhibitors of the ATPase complex with relatively minor effects on mitochondrial membrane potential ($\Delta\Psi$). In contrast, trialkylleads are potent uncoupling agents, which accounts for their potent inhibition of ATP synthesis. The uncoupling action of trialkylleads and trialkyltins is independent of chloride concentration and is unlikely to be due to Cl^-/OH^- exchange.

Keywords: Mitochondria, membrane potential, trialkyllead, trialkyltin

INTRODUCTION

Triethyllead is a toxic metabolic product of tetraethyllead and its effect on mitochondrial energy conservation reaction, together with other alkylleads and alkyltins, has been extensively studied by Aldridge and co-workers.[1] As described for trialkyltins, the trialkylleads act on mitochondria by three basic mechanisms: (a) direct inhibition of the mitochondrial ATPase complex;[1] (b) stimulation of chloride/hydroxyl exchange;[1–3] (c) gross swelling of the mitochondral membrane.[1] Similarly to the trialkyltins, the trialkylleads show increased inhibition of the ATPase complex with increasing alkyl chain length, with maximal activity being exhibited by tri-n-butyl derivatives and by triaryl derivatives such as triphenyllead.[1] The modes of action of triorganotin and triorganolead compounds are thus dependent on the type of compound used and the composition of the incubation medium, particularly the presence or absence of chloride ion.[1]

This paper examines the mode of action of triorganolead and triorganotin compounds on mitochondrial ATPase, ATP synthase and mitochondrial membrane potential ($\Delta\Psi$), all in minimal chloride media. It is shown that triorganotins and triorganoleads have differential effects on mitochondrial ATPase and ATP synthase. Also, triorganoleads are shown to be potent uncoupling agents in minimal chloride media under conditions where Cl^-/OH^- exchange is unlikely to be of significance.

EXPERIMENTAL

Materials

Triethyltin sulphate was made as previously described.[4] Tri-n-butyltin acetate was a gift from Schering Industrie Chemikalien, West Germany. All trialkyltins were added as ethanolic solutions. Triethyllead acetate and tri-n-butyllead acetate were obtained from K & K laboratories (Plainview, New York) and were added as diethylformamide solutions. 2-(4-Dimethylaminostyryl-1-methylpyridinium iodide (DSMP-I) was obtained from Aldrich Chemical Co. [^{3}H]-DSMP-I was a gift from Professor J. Rafael (University of Heidelberg).

Rat liver mitochondria were prepared as described by Selwyn *et al.*[5] and were suspended finally in 250 mmol dm^{-3} sucrose, 10 mmol dm^{-3} Hepes buffer (pH 7.5) at protein concentrations of 50 mg cm^{-3}. Other materials and methods, including ATPase and succinate-driven ATP synthase assays, have been described previously.[4,6,7] The media used contained no

added chloride and were minimal chloride media containing less than 5 μmol dm^{-3} chloride ion.

Methods

Determination of mitochondrial $\Delta\Psi$ and ΔpH by ion distribution

Membrane potential ($\Delta\Psi$) and ΔpH were determined by the ion distribution methods of Chappell and Crompton[8] using [^{3}H]methyltriphenylphosphonium ($TPMP^+$) ion for $\Delta\Psi$ estimation and [^{14}C]lactate for ΔpH estimation. Mitochondrial matrix volumes were determined using [^{14}C]sucrose and [^{3}H]water.

Fluorimetric determination of $\Delta\Psi$ using $DSMP^+$

The method closely follows that described by Mewes and Rafael[9] using a Perkin–Elmer MPF44 spectrofluorimeter (excitation, 479 nm; emission, 589 nm) at 25°C. Routinely, mitochondria (1 mg cm^{-3}) were incubated with $DSMP^+$ (2 nmol mg^{-1} protein) in 250 mmol dm^{-3} sucrose, 10 mmol dm^{-3} Hepes, pH 7.5; 5 mmol dm^{-3} succinate and 8 μmol dm^{-3} rotenone. Uptake of $DSMP^+$ was followed by an increase in fluorescence until maximum fluorescence increase was attained, which is equivalent to a $\Delta\Psi$ of 180–190 mV.[9] Organotins and organoleads were then added and the decrease in fluorescence was monitored.

Fluorescence changes were calibrated utilising [3H]$DSMP^+$ ion distribution for determination of $\Delta\Psi$, or fluorescence changes were directly utilized to determine $\Delta\Psi$ as described by Mewes and Rafael.[9] Uncouplers or any reagent which affected the mitochondrial membrane potential led to a decrease in fluorescence and an equivalent decrease in $\Delta\psi$.

RESULTS

Inhibition of ATPase and ATP synthase activities

The effect of trialkyltins and trialkylleads on liver mitochondrial ATPase and ATP synthase is shown in Fig. 1 and the appropriate I_{50} values (50% inhibition values) are listed in Table 1. The tributyl derivatives are more effective inhibitors than triethyl derivatives but are less effective than the triphenyl derivatives (results not shown). These findings are essentially similar to those

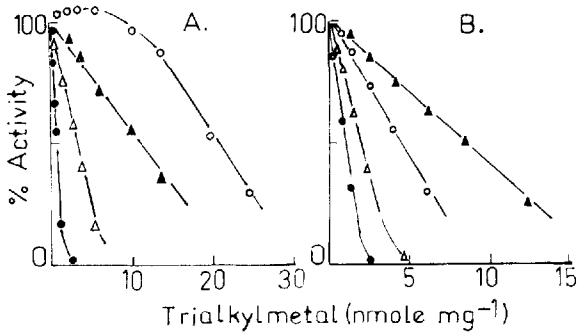

Figure 1 Inhibition of ATP synthesis and hydrolysis in rat liver mitochondria by trialkyl-leads and -tins. (A) Titration profiles for triethyllead acetate (○, ●) and triethyltin sulphate (△, ▲). (B) Titration profiles for tributyllead acetate (○, ●) and tributyltin acetate (△, ▲). These estimates were performed as described under Experimental. The open and filled symbols are ATPase and ATP synthase respectively, where activities are expressed as percentiles of control values (ATPase 37–40 nmol mg^{-1} min^{-1}, ATP synthesis 73–80 nmol mg^{-1} min^{-1}).

reported by Aldridge *et al.*[1] for isethionate media. The trialkyltin derivatives are approximately four times more effective inhibitors of ATPase activity than of succinate-driven ATP synthase activity. The differential inhibition of ATPase activity has been described previously[7] and has been ascribed to changes in substrate affinity in non-energized and energized states.[10]

The reverse pattern of sensitivity is shown by trialkylleads, which are potent inhibitors of succinate-driven ATP synthase and relatively ineffective inhibitors of mitochondrial ATPase. Triethyllead completely onhibits succinate-driven ATP synthase at concentrations where ATPase is unaffected. Examination of the effects of trialkytins and trialkylleads on mitochondrial membrane potential provides an explanation for these different sensitivity patterns.

Effects on mitochondrial membrane potential ($\Delta\Psi$)

The effects of trialkyltins and trialkylleads were examined in the $DSMP^+$ fluorescence assay of Mewes and Rafael[9] using minimal chloride media. Trialkyllead compounds are potent uncouplers which reduce $\Delta\Psi$ by +100 mV at values of 4.0 and 2.25 nmol per mg protein for triethyllead and tributyllead, respectively. These values are approximately five times less than those obtained with the equivalent trialkyltin

Table 1 The sensitivities of mitochondrial ATPase and ATP synthase and mitochondrial $\Delta\Psi$ to triorganoleads and triorganotins

Trialkylmetal		I_{50} (n mol mg protein^{-1}) ± S.D. ATPase	ATP synthase	$\Delta\Psi = +100$ mV ± S.D.[a]
Triethyllead acetate	(3)	18 ± 1.6	1.6 ± 0.4	4.0 ± 0.5
Triethyltin sulphate	(5)	2.8 ± 0.4	9.8 ± 0.5	19.0 ± 2.3
Tributyllead acetate	(3)	4.8 ± 0.5	1.0 ± 0.2	2.25 ± 0.3
Tributyltin acetate	(3)	2.0 ± 0.2	7.8 + 0.2	13.0 ± 2.6

[a]$\Delta\Psi$ values are given as the amount of trialkylmetal required to change $\Delta\Psi$ by +100 mV and are approximately twice the levels required to reduce $\Delta\Psi$ to −120 mV.

Notes. ATPase and succinate-driven ATP synthase activities were estimated as described in the Experimental section. The numbers in parentheses indicate the number of duplicate experiments performed. Control rates of ATPase and ATP synthase were in the ranges 35–40 and 80–100 nmol mg^{-1} min^{-1} respectively. $\Delta\Psi$ estimations were made by the $DSMP^+$ fluorescence method of Mewes and Rafael.[9] Fluorescence changes were calibrated using the distribution of [^{3}H]DSMP-I or were used directly for $\Delta\Psi$ estimation as described by Mewes and Rafael.[9]

compounds. Trialkyllead compounds are clearly potent uncouplers and the concentrations required to reduce the membrane potential to −120 mV are equivalent to the I_{50} values for ATP synthase inhibition by trialkylleads. The uncoupling activity of trialkylleads is thus directly correlated with their capacity to inhibit ATP synthesis.

These experiments were carried out in media containing no added chloride, where the maximal chloride concentration was less than 5 μmol dm^{-3}. Addition of potassium chloride up to 1 mmol dm^{-3} levels did not enhance the uncoupling activity of the trialkyltin or trialkyllead compounds that were used.

DISCUSSION

The fluorimetric method of Mewes and Rafael[9] has been shown to be a simple and reliable technique for the estimation of mitochondrial $\Delta\Psi$ and for studies of the effects of organotins and organoleads on mitochondrial $\Delta\Psi$. Current studies have shown the method can provide the basis for a simple *in vitro* screening system for compounds which affect membrane function (D. E. Griffiths, unpublished studies).

Here it is shown that triorganolead compounds are relatively poor inhibitors of the ATP synthase complex and that their main mode of action is as agents which dissipate the membrane potential, $\Delta\Psi$. They also dissipate the ΔpH component of the mitochondrial protonmotive force and their mode of action is assumed to be mediation of a chloride/hydroxyl exchange reaction, as has been proposed for triorganotin compounds.[1,2,5] How-

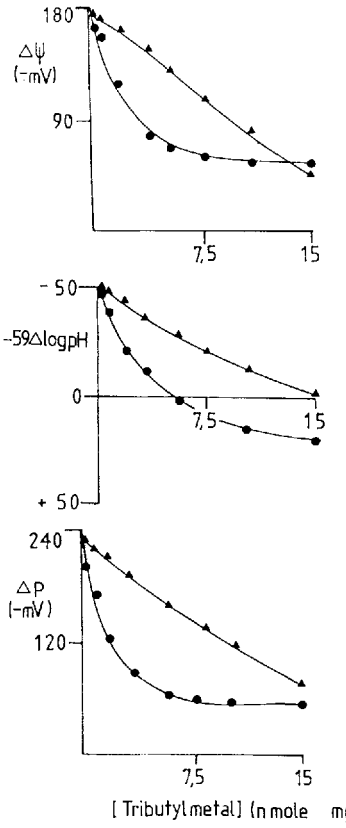

Figure 2 The effects of tributyl-leads and -tins on mitochondrial $\Delta\Psi$, ΔpH and Δp in minimal chloride media. $\Delta\Psi$ and Δp estimates were based on those obtained with [^{3}H]TPMP$^+$. The distributions of 2 μmol dm^{-3} (1 μCi) [^{3}H]TPMP$^+$ and 25 μmol dm^{-3} (0.5 μCi) [^{14}C]lactate were measured by the method of Chappell and Crompton[8] as described in the Experimental section. The abcissa units are nmol organometal per mg protein. ▲, Tributyltin acetate; ●, Tributyllead acetate.

ever, the rapid dissipation of $\Delta\Psi$ by both the triorganotins and the triorganolead compounds tested in the absence of added chloride raises doubts as to the role of the chloride/hydroxyl exchange reaction. The mechanism of action of both the triorganotins and the triorganoleads in dissipation of the mitochondrial membrane remains to be established.

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