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Effects of *in vivo* treatment of rats with trimethyltin chloride on respiratory properties of rat liver mitochondria

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Abstract

Liver mitochondria isolated from rats treated *in vivo* with trimethyltin chloride show stimulation of respiration using glutamate/malate as substrate, and a transient inhibition on rates of respiration using palmitoyl-L-carnitine as substrate. This phenomenon was observed with both ADP- and FCCP-stimulated respiration. In contrast, rates of respiration by liver mitochondria isolated from rats treated *in vivo* with trimethyltin chloride, following prior treatment with clofibrate, were inhibited when glutamate/malate was respiratory substrates. With palmitoyl-L-carnitine no effect of trimethyltin chloride was observed. *In vitro* treatment of rat liver mitochondria, or of rat liver homogenates, led to the expected, powerful inhibition of respiration. The synthesis of ATP by liver mitochondria isolated from rats treated *in vivo* with trimethyltin chloride was not inhibited compared to mitochondria isolated from control rats. Similarly, ATP synthesis by mitochondria isolated from rats treated with clofibrate, before treatment with trimethyltin chloride, was not inhibited. We, therefore, conclude that the powerful inhibitory effects of trimethyltin found *in vitro*, is not expressed *in vivo* during the first 36 hr following administration. *In vivo* treatment of rats with trimethyltin chloride caused a marked increase in hepatic levels of taurine and glycine, while levels of glutathione and glutamine were diminished. This is consistent with an enhanced oxidative stress in the liver. Our findings lead to the conclusion that increased oxidative stress, rather than inhibition of the mitochondrial ATPase, is a likely major cause of the *in vivo* toxic effects due to trimethyltin chloride.

Keywords: Trimethyltin chloride; Liver mitochondria; Rats; Respiration; Clofibrate; Peroxisomes; ATP synthesis; Amino acids

1. Introduction

The toxicity of trialkyltin compounds is well recognised [1]. The compounds are lipophilic, and are, therefore, readily absorbed *via* the intestine, and can also readily be absorbed across the skin, or *via* lungs [1]. They will also pass readily across the blood–brain barrier [2]. Following injection of trimethyltin into rats, the higher concentration was found in blood, due to the high-affinity binding of trimethyltin to haemoglobin [3,4]. The liver was found to contain the second higher concentration of trimethyltin, followed by kidney and brain [5,6]. In mammals, the major toxic effect appears to involve the central nervous system [7]. The lower molecular weight homologues, i.e. trimethyltin or triethyltin, are most toxic in this respect. Histologically toxicity is characterised by the development of cerebral oedema with triethyltin and neurone degeneration with trimethyltin [7–9]. Recently, trialkyltins have been proposed to bring about oxidative stress and induction of apoptosis [10,11]

Extensive studies attempted to define the toxic effects of these compounds at the biochemical level. In 1955, it was demonstrated that trimethyltin could cause inhibition of oxidative phosphorylation [12]. Subsequent studies using isolated mitochondrial fractions revealed that trialkyltins also cause inhibition of mitochondrial ATP synthesis, while mitochondrial ATP hydrolysis is stimulated [13]. High affinity binding of trialkyltins to other various ATPases has been demonstrated [14–16]. It appears well established that *in vitro* inhibition of oxidative phosphorylation by triethyltin is caused by binding of triethyltin to a high-affinity binding site on the mitochondrial ATPase [14], causing an inhibitory effect similar to that due to

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Abbreviations: FCCP, carbonyl cyanide *p*-trifluoromethoxyphenylhydrazone.

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binding of oligomycin to the F_0-subunit of the mitochondrial ATPase [17–19].

Another *in vitro* effect on mitochondrial function of relatively low concentrations of triethyltin [20] or trimethyltin [21], is the establishment of a rapid Cl^-/OH^- exchange across the inner mitochondrial membrane. This phenomenon also causes mild uncoupling of oxidative phosphorylation, leading to impairment of energy generation. Studies with rat liver lysosomes suggests that trialkyltins may also function as proton carriers in biological membranes [22].

Whereas the previous studies on biochemical effects of trialkyltin were mainly *in vitro* studies, our approach has been to examine properties of mitochondrial function following treatment of rats with a highly toxic, but non-lethal dose, of trimethyltin. We examined respiratory properties using various respiratory substrates as earlier *in vitro* studies suggested that trialkyltins-dependent inhibition was to some extent substrate dependent [23]. The present studies were also designed to ascertain whether the inhibitory effects of trimethyltin found *in vitro* are also observed with mitochondria isolated from rats treated with trimethyltin *in vivo*.

The powerful inhibition of mitochondrial ATPase observed *in vitro* [18,24,25] appears to be reflected as declining levels of intracellular ATP following exposure to cell cultures to trimethyltin [26]. We have, therefore, examined ability of mitochondria isolated from trimethyltin-treated rats to synthesise ATP.

We have also investigated effects of treatment with a single dose of trimethyltin given to rats already subjected to treatment with clofibrate. Clofibrate is known to bring about proliferation of both mitochondria and peroxisomes in rat liver, and in general stimulate the oxidative capacity of the tissue, e.g. activity of respiratory chain, synthesis of ATP [27,28]. We have, therefore, been able to study effects of *in vivo* treatment with trimethyltin on both mitochondrial and peroxisomal metabolism using normal rats as well as rats exhibiting enhanced oxidative capacity due to prior treatment with clofibrate.

Several studies suggest that amino acid metabolism in the brain is severely disturbed following treatment with trialkyltins [29–31]. This may arise due to changes in mitochondrial metabolism and as a result of enhanced oxidative stress. It is not apparent whether treatment with trimethyltin brings about similar changes as regards hepatic levels of amino acids. We, therefore, report data on hepatic levels of amino acids in rats treated *in vivo* with trimethyltin.

2. Materials and methods

2.1. Reagents

ATP, ADP, EGTA, FCCP, L-malic acid, potassium L-glutamate, succinic acid, mannitol, palmitoyl-CoA, NAD^+, CoA and trimethyltin chloride was purchased from Sigma–Aldrich. Clofibrate was purchased from Fluka AG. All other reagents used were of analytical grade.

2.2. Experimental animals

Male Wistar, albino rats (150–200 g body wt.) were used throughout these experiments. During the experimental period the rats were housed in separate cages with *ad lib* access to fodder and water. The cages were kept in a room that had a 12 hr light/dark cycle at a temperature of 21° and a relative humidity of 65%.

Trimethyltin chloride was dissolved in soy oil. Rats were treated with single intraperitoneal (i.p.) injections of trimethyltin chloride in doses up to 16 mg/kg body wt. Although this is in excess of the oral LD_{50}-dose of about 9 mg/kg [32]; this was in our hands a non-lethal dose. The rats were clearly affected by the treatment (diminished mobility), but did access water. None died within the duration of the experiment.

Most rats were killed by decapitation between 2 and 24 hr after injection, although a few were killed 36 hr after injection. The livers were immediately removed, and rat liver mitochondria isolated as described elsewhere [33].

Some rats were also fed on a fodder supplemented with 0.5% (w/w) clofibrate for 14 days before being given a single i.p. injection of trimethyltin chloride. Clofibrate-supplemented fodder was prepared as described previously [34].

2.3. Measurements of rates of mitochondrial respiration

Rates of respiration by isolated rat liver mitochondria were measured polarographically using an YSI Model 52 biological oxygen monitor (Yellow Spring Instruments), fitted with a Clark-type oxygen electrode. The oxygen electrode was calibrated as described elsewhere [35].

In these experiments mitochondria were incubated in a medium containing 130 mM KCl, 20 mM HEPES, 0.1 mM EGTA, 2 mg defatted BSA/mL, and 5 mM $MgCl_2$, pH 7.20. The incubations were started by the addition of 2–4 mg of mitochondrial protein in a final volume of 3 mL. The incubations also contained either 5 mM ADP, or 5 mM ATP or 0.1 μM FCCP. After recording the base line for 1–2 min, respiration was usually started by the addition of a respiratory substrate, e.g. 5 mM glutamate together with 0.5 mM malate. Alternatively, 20 μM palmitoyl-L-carnitine was added, in which case the incubation medium also contained 10 mM malonate to suppress respiratory activity due to citric acid cycle. Respiratory rates were measured as the slope of the linear part of the recorder tracings showing remaining levels of oxygen as a function of time of incubation.

2.4. Assays of ATP

Mitochondrial synthesis of ATP was assayed luminometrically using the 'ATP monitoring kit' (Roche Ltd.).

LKB Wallac 1253 luminometer. Duplicate samples (100 μL) of mitochondrial incubations were quickly transferred into 10 μL of ice-cold 10% (w/v) $HClO_4$ and mixed well. After 4 min of centrifugation at maximum speed at 2° in Eppendorf-centrifuge samples of the supernatants were diluted 100-fold using a Tris–EDTA buffer (0.1 M Tris–acetate, 2 mM EDTA, pH 7.75). Samples (10 μL) of the diluted supernatants were transferred to luminometer cuvettes for assay of content of ATP. With this assay, a linear relationship was observed between the integrated luminescence signal and amounts of ATP of 5–500 pmol per assay sample.

2.5. Analysis of amino acids

Rats were given a single i.p. injection of trimethyltin chloride and decapitated after 2 and 24 hr. The liver was taken out and homogenised in ice-cold water. To the homogenate (0.5 mL) was immediately added 4.5 mL of 3% trichloroacetic acid containing amino adipic acid as internal standard, and left for 20 min to precipitate the proteins. The supernatant was treated with water saturated ether to neutral pH. The sample was analysed on HPLC after derivatisation with *o*-phtalaldehyde in the presence of 2-mercaptoethanol in borate-buffer, pH 12, as described [36].

2.6. Assay of Sn

Rats were injected with trimethyltin chloride (16 mg/kg). After 24 hr the animal the animals were anaesthetised with Hypnorm/Dormicum. The chest was opened and a cannulae inserted into the left ventricle to flush out the blood through the right atrium. The tissue was treated with ultra pure nitric acid for 48 hr at room temperature to dissolve the tissue completely. A sample (10–20 μL) was injected into a graphic furnace and the concentration of Sn was determined by atomic absorption. The concentration of Sn was determined from a standard curve run simultaneously. TMT added to a blood sample showed 86% recovery.

2.7. Assay of protein

Mitochondrial proteins were assayed using the biuret assay [37]. Mitochondrial suspensions were diluted 100-fold with 0.1% (w/v) deoxycholate before being used in assays. The Precimat protein standard solutions (Roche Ltd.) were used as standard protein.

2.8. Statistical analysis

The significance of differences between population means was measured by using Student's unpaired *t*-test with Welch's correction for unequal variance. The analysis was carried out by using the GraphPad Prism program (GraphPad Software).

3. Results

3.1. Effects of treatment with trimethyltin chloride on rates of mitochondrial respiration

3.1.1. Glutamate/malate and succinate as substrates

Data presented in Fig. 1 demonstrate the, expected, powerful *in vitro* inhibition of ADP-stimulated mitochondrial respiration by trimethyltin chloride, 20 μM trimethyltin producing about 85% inhibition of succinate-dependent respiration, and about 75% for glutamate/malate-dependent respiration. An effect of added trimethyltin chloride was also found with FCCP-stimulated respiration, but the extent of inhibition is now markedly less (Fig. 1). This may be due to a direct effect of trimethyltin on the respiratory chain, or due to excessive depolarisation of the inner mitochondrial membrane due to the combined uncoupling effect of FCCP and trimethyltin. These results demonstrate the oligomycin-like effect of trimethyltin added to mitochondrial incubations as described previously [14]. The effects of trimethyltin is essentially substrate independent, and subsequent studies were, therefore, carried out using glutamate/malate alone.

The results presented in Fig. 2a demonstrate that liver mitochondria isolated from rats treated with trimethyltin chloride *in vivo*, in contrast, exhibited increased rates of respiration on glutamate/malate, with either ADP- or FCCP-stimulated respiration. Similarly endogenous rates of respiration were increased (results not shown). Also, this effects was detectable already 2-hr post-injection, and stimulation was further increased 24 hr after injection. In mitochondria isolated 36-hr post-injection, however, respiratory rates were still increased relative to control rates, but less than the rates observed with mitochondria isolated 24-h post-injection (Fig. 2a).

The results presented in Fig 2b show the expected increase in ADP-stimulated respiratory rates commonly observed with mitochondria isolated from rats treated with clofibrate [34]. With mitochondria isolated from rats also treated with trimethyltin chloride, the rates of respiration were, in contrast, consistently decreased following treatment with clofibrate. The extent of inhibition also increased as the time elapsed between injection of trimethyltin and isolation of mitochondria increased. With glutamate/malate as substrates, the rates of respiration with mitochondria isolated 36-hr post-injection was lower than the rates observed with control mitochondria (Fig. 2b).

3.1.2. Palmitoyl-L-carnitine as substrate

Palmitoyl-L-carnitine is an eminent respiratory substrate transported across the inner mitochondrial membrane using the palmitoyl-L-carnitne transferase II (EC. 2.3.1.2.1) and, therefore, entirely different from the transport of dicarboxylic acid substrates. For this reason, we examined effects

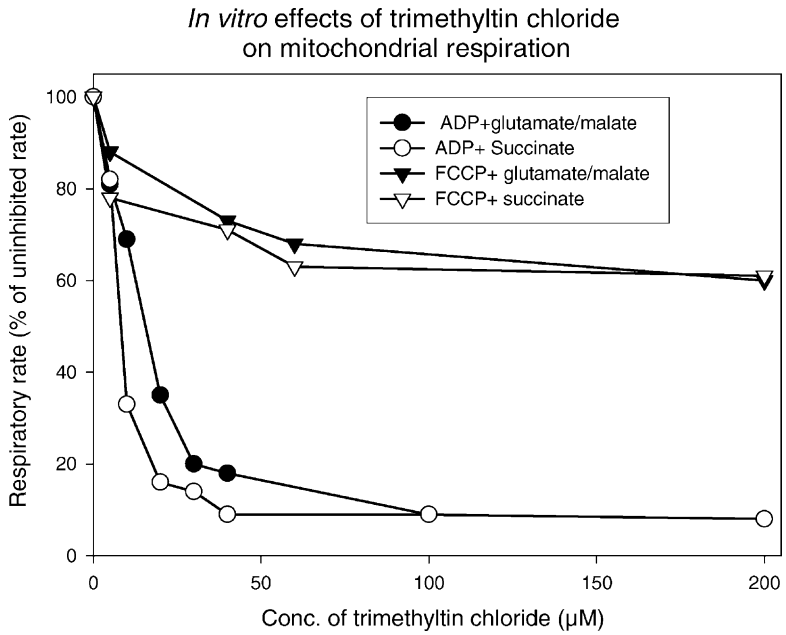

Fig. 1. Effects of *in vitro* trimethyltin chloride on mitochondrial rates of respiration. Rat liver mitochondria (about 2 mg of mitochondrial protein) were incubated in 2 mL of a medium containing 130 mM KCl, 20 mM HEPES, 0.1 mM EGTA, 2 mM KP_i, pH 7.2. In addition the medium contained 2 mM ADP (circle) or 2 M FCCP (inverted triangle). Respiration was started by the addition of 10 mM L-glutamate and 2 mM L-malate (filled symbols), or with 10 mM succinate (open symbols). Where included trimethyltin chloride was added before the addition of the substrates. The concentrations of trimethyltin chloride used are shown in the figure. The consumption of O_2 was monitored polarographically and the respiratory rates were calculated from the linear portion of the recorder-tracing showing O_2 tensions as a function of time of incubation.

of treatment with trimethyltin on palmitoyl-L-carnitine-dependent respiration.

Treatment with trimethyltin chloride now decreased respiratory rates with control mitochondria at 2 and 24 hr after injection, although the effect appeared to vanish 36-hr post-injection of trimethyltin chloride (Fig. 3a). With palmitoyl-L-carnitine as respiratory substrate an expected stimulatory effect on respiratory rates by treatment with clofibrate [38] was observed (e.g. as shown by control rates in Fig. 3a and b). With mitochondria from rats that had been given prior treatment with clofibrate, no marked trend of change was now observed in respiratory rates following injection of trimethyltin chloride (Fig. 3b).

Palmitoyl-L-carnitine-dependent respiration, therefore, responds differently to *in vivo* treatment with trimethyltin as compared to glutamate/malate-dependent respiration. The reason for this is not immediately clear as β-oxidation also makes use of the respiratory chain like glutamate/malate. The measured respiratory rates are, however, much lower as citric acid cycle activity has been suppressed by the malonate included in these incubations. These lower rates of respiration may offer one explanation for this difference, although an effect due to differences in the mechanism of substrate transport into the mitochondrial matrix cannot be excluded. It has previously been reported that β-hydroxybutyrate-dependent respiration and ATP synthesis exhibited lesser sensitivity to *in vitro* trimethyltin as compared to malate, pyruvate or citrate [23].

3.2. Effects of treatment with trimethyltin chloride on peroxisomal β-oxidation

Treatment of rats with clofibrate is known to cause powerful induction of peroxisomal β-oxidation [39]. The results presented in Table 1 show that subsequent treatment with a single injection of trimethyltin chloride had no significant effect on the level of activity of peroxisomal β-oxidation. Also the activity of peroxisomal β-oxidation in control rat liver was unaffected by injected trimethyltin chloride (Table 1). The established peroxisomal activity is, therefore, not affected by subsequent treatment with trimethyltin chloride.

Table 1
Effects of *in vivo* trimethyltin chloride on the activity of hepatic peroxisomal β-oxidation in mitochondria isolated from control- and clofibrate-treated rats

Treatment	Activity (nmol/min/mg protein)
Control	0.43 (0.08)
Trimethyltin chloride treated	0.35 (0.07)
Clofibrate treated	2.25 (0.40)
Clofibrate treated + trimethyltin chloride	2.45 (0.27)

The activity of peroxisomal β-oxidation was assayed in homogenates from both control- and clofibrate-treated rats. Some rats were also given a single peritoneal injection of trimethyltin chloride(16 mg/kg body wt.) 24 hr before experimental use. The values presented represents means, with SD indicated derived from five individual rats in each experimental group. Experimental details are otherwise described in Experimental section.

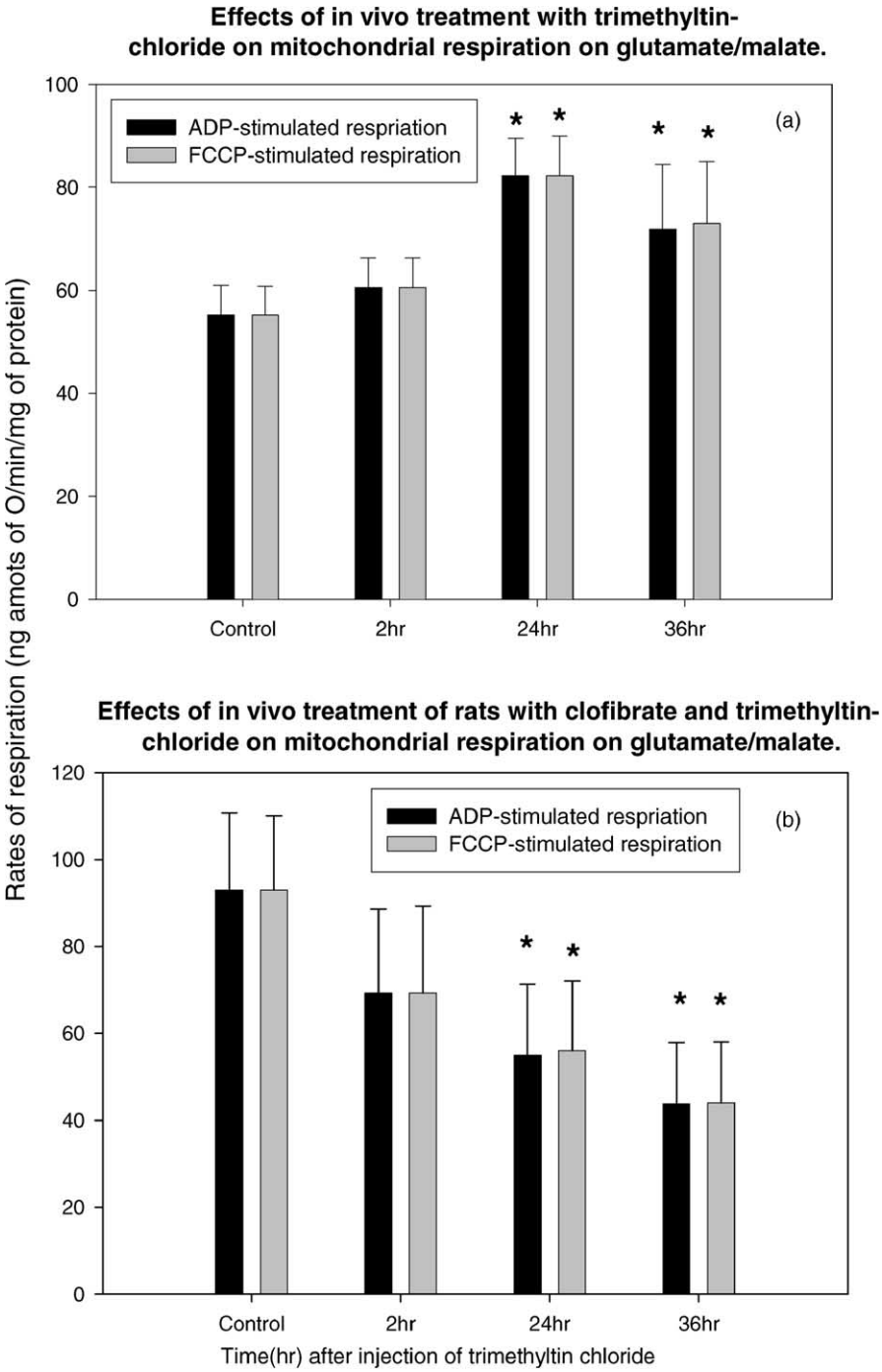

Fig. 2. Effects of *in vivo* trimethyltin chloride on rates of respiration with mitochondria isolated from control- and clofibrate-treated rats using glutamate/malate as substrates. Rat liver mitochondria were isolated from control rats (a) or from rats treated with clofibrate for 10 days before experimental use (b). Some of the rats were given a single i.p. injection of trimethyltin chloride (16 mg/kg body wt.) and mitochondrial were isolated 2, 24 and 36 hr after injection. The respiratory rates were measured polarographically as described in Fig. 1. Mean values labeled (∗) were significantly different from corresponding control mean.

3.3. Effects on treatment with trimethyltin chloride on tissue levels of Sn

The tissue distribution of trimethyltin chloride was studied by measuring the concentration of Sn in various tissues in rats which had been given an i.p. injection of trimethyltin chloride (16 mg/kg body wt.). The rats were killed 24 h after the injection. The results (not shown) demonstrated that the highest tissue levels were found in blood (about 50 μg/mL) while the level in liver and brain was about 1.5 and 0.3 μg/g wet wt. of tissue, in line with earlier findings [3,5,6].

Various concentrations of trimethyltin chloride were also added to rat liver homogenates. After a brief incubation (on ice), mitochondria were isolated from the homogenates

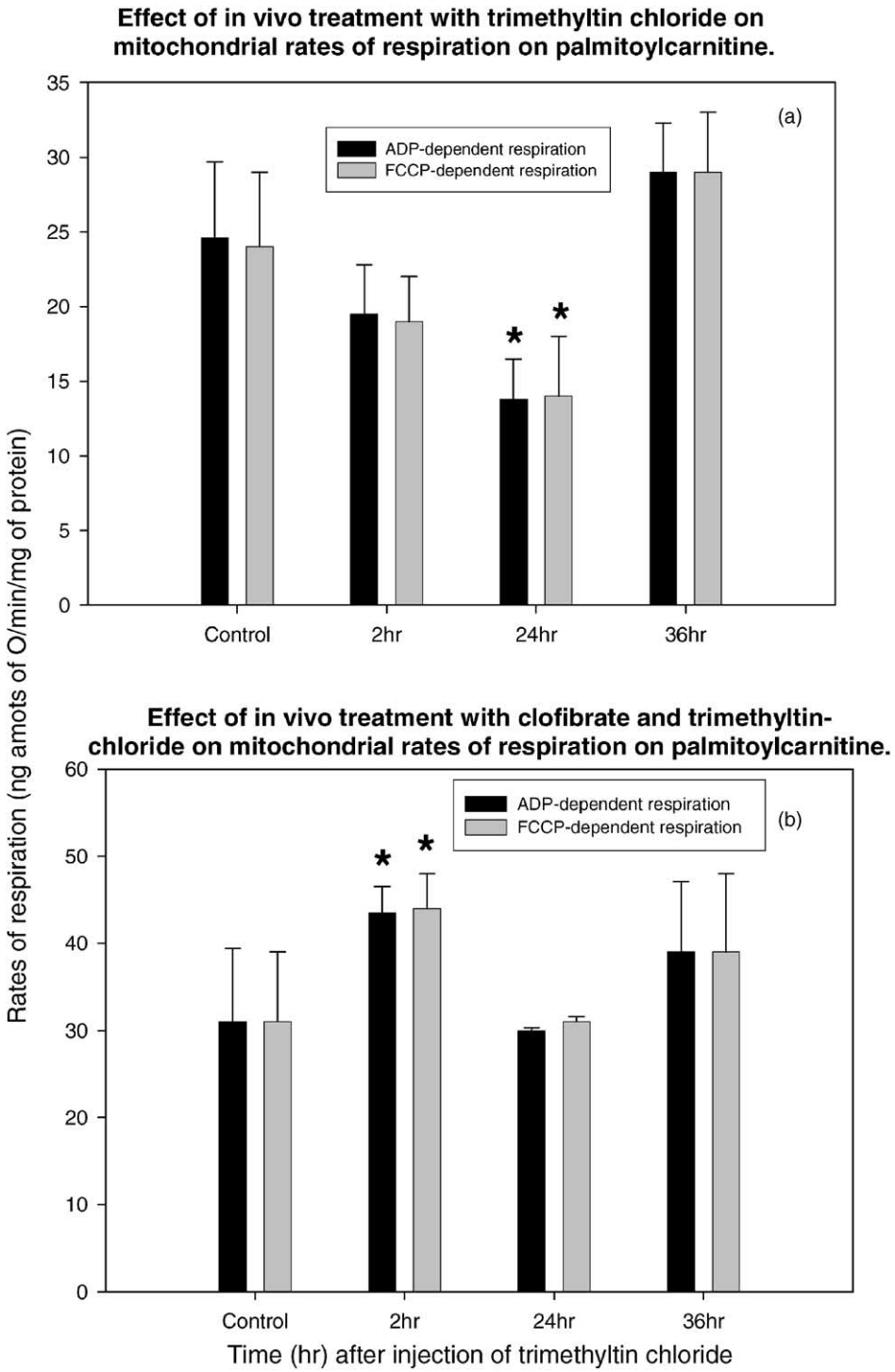

Fig. 3. Effects of *in vivo* trimethyltin chloride on rates of respiration with mitochondria isolated from control- and clofibrate-treated rats using palmitoyl-L-carnitine as substrate. Rat liver mitochondria were isolated from control rats (a) or from rats treated with clofibrate for 10 days before experimental use (b). Some of the rats were given a single peritoneal injection of trimethyltin chloride (16 mg/kg body wt.) and mitochondrial were isolated 2, 24 and 36 hr after injection. The respiratory rates were measured polarographically as described in Fig. 1. The incubation medium now also contained 10 mM malonate to suppress the respiratory activity due the citric acid cycle. Mean values labeled (∗) were significantly different from corresponding control mean.

and their ability to respire on glutamate/malate was measured, and the concentration of Sn in the mitochondrial fraction was assayed. The IC_{50} was about 180 μM (results not shown), which is about twice that reported for triethyltin with brain mitochondrial fractions with respect to inhibition of the mitochondrial ATPase [40].

The results show that respiration was most sensitive to inhibition with concentrations of Sn increasing up to about

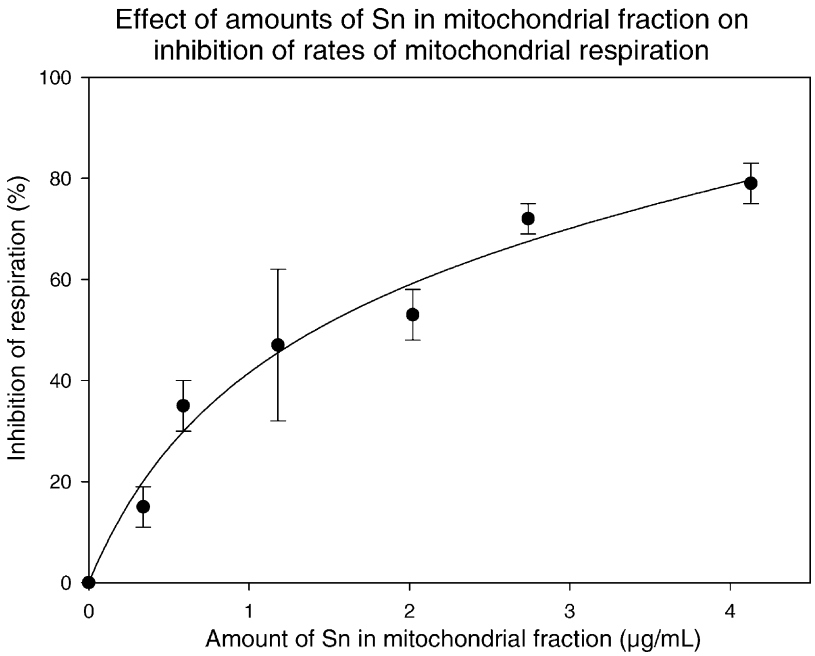

Fig. 4. Effects of trimethyltin chloride added to rat liver homogenates on mitochondrial respiration and on levels of Sn in mitochondrial fractions. Various concentrations of trimethyltin chloride (as shown in the figure) were added to rat liver homogenates (about 18 mg protein/mL). After mixing, the mitochondria were isolated from the homogenates by differential centrifugation. ADP-stimulated mitochondrial rates of respiration on glutamate/malate were subsequently measured as described in Fig. 1. The levels of Sn in the mitochondrial fractions were assayed by atomic absorption as described in Experimental section.

1 µg/mL (Fig. 4). At this concentration about 50% inhibition was observed. Further increase in the concentration of Sn led to progressive inhibition of respiration, but a concentration of 4 µg/mL Sn was required to reach 80% inhibition (Fig. 4).

3.4. Effects of treatment with trimethyltin chloride on hepatic content of amino acids

Rats were given a single i.p. injection of trimethyltin chloride (16 mg/kg body wt.). After 2 or 24 hr, livers were

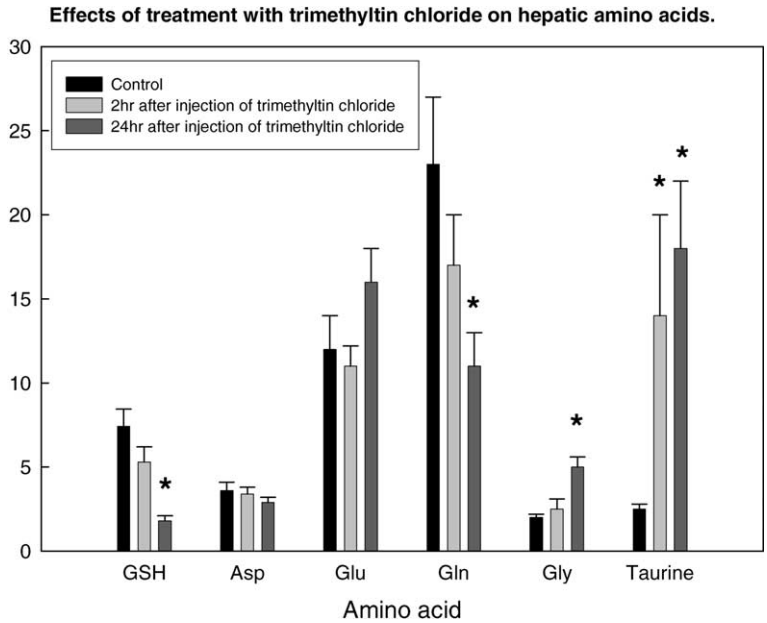

Fig. 5. Effects of *in vivo* trimethyltin chloride on hepatic content of some amino acids. Rats were given a single i.p. injection of trimethyltin chloride (16 mg/kg body wt.). After 2 and 24 hr, livers were removed and hepatic content of amino acids were analysed as described in Experimental section. The data presented represent means and SD derived from measurements from five different animals in each experimental group. Mean values labeled (∗) were significantly different from corresponding control mean.

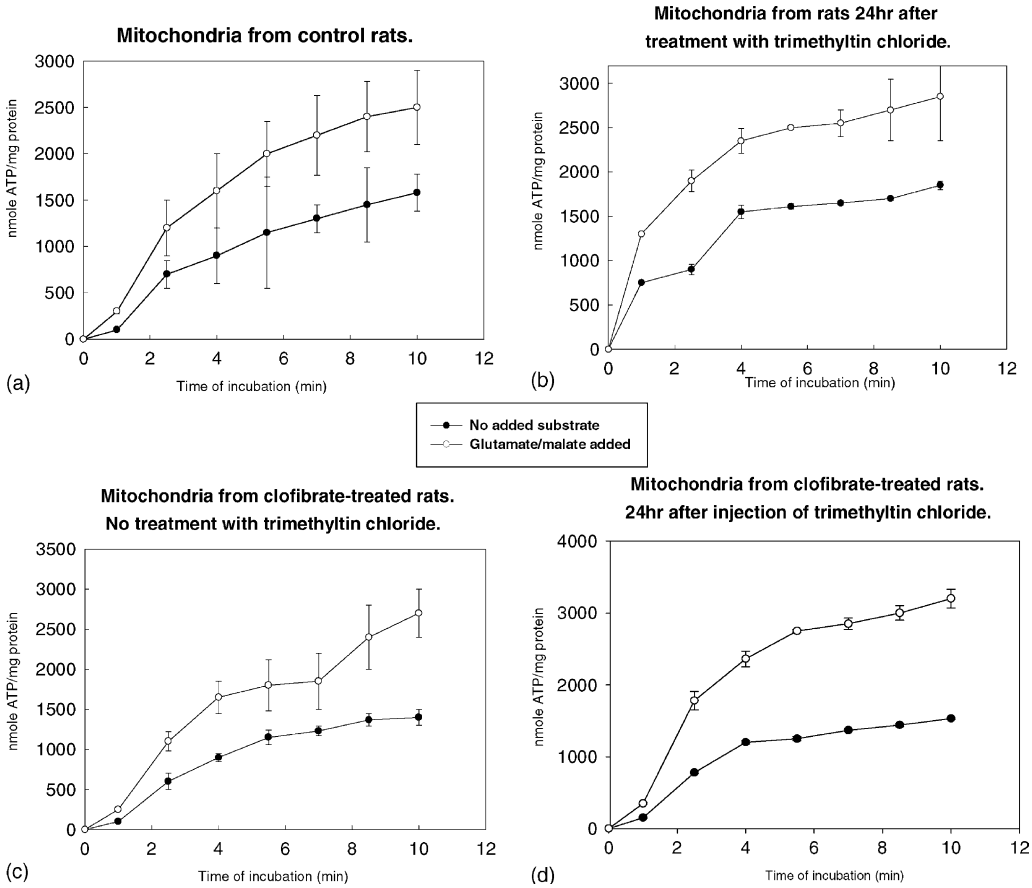

Fig. 6. Effects of *in vivo* trimethyltin chloride on ATP synthesis by isolated rat liver mitochondria with glutamate/malate as respiratory substrates. The ability of mitochondria to synthesise ATP was measured using isolated liver mitochondria from control rats (a), from rats given a single i.p. injection of trimethyltin chloride (16 mg/kg body wt.) (b), from rats treated with clofibrate for 10 days (c) and from rats also given a single injection of trimethyltin chloride (16 mg/kg body wt.) after 10 days of treatment with clofibrate (d). At 24 hr following injection, liver mitochondria were isolated and incubated as described in Fig. 1 (using 2 mM ADP and 2 mM KP_i). At various times samples of the mitochondrial incubation (100 μL) were removed for assay of content of ATP as described in Experimental section. The results presented are the means, with SD indicated, derived from three separate rats in each experimental group.

removed and the hepatic content of free amino acids was assayed. The results showed that the hepatic contents of glutathione (GSH) and glutamine were progressively decreased with increasing time following injection if trimethyltin is used (Fig. 5). Conversely, levels of glycine and taurine were increased.

3.5. *Effects of treatment with trimethyltin chloride on mitochondrial generation of ATP*

Isolated mitochondria incubated as described in the Materials and methods section converted about 80% of added ADP into ATP during 4 min of incubation with glutamate/malate as respiratory substrates (results not shown). In view of the observed oligomycin-like effect of trimethyltin observed *in vitro* [17–19], we decided to investigate if ATP synthesis was impeded in mitochondria isolated from rats that had been treated with trimethyltin chloride. The results presented in Fig. 6 show that ATP synthesis was not inhibited in mitochondria isolated at 24 hr after injection of trimethyltin chloride. Although about 10–20% stimulation apparently was observed, this is not statistically significant. With mitochondrial isolated at 2 or 36 hr after injection of trimethyltin chloride, there was no evidence of progressive inhibition of ATP synthesis (data not shown)—rather ATP synthesis remained apparently unaffected throughout the experimental period.

These results also demonstrate that treatment with clofibrate brought about a measurable increase in amounts of ATP synthesised, particularly as observed after 10 min of incubation (Fig. 6c). With mitochondria isolated 24 hr after treatment also with trimethyltin chloride, no inhibition of ATP synthesis was observed (Fig. 6d). Also at 2 and 36 hr after treatment with trimethyltin, no inhibition was observed—results not shown.

4. Discussion

4.1. *Effect of treatment with trimethyltin chloride on mitochondrial respiration*

It is often assumed that the cause of the extremely severe toxicity of trimethyltin is caused by the well-established,

powerful, irreversible inhibition of the mitochondrial ATPase [17–19]. Our results from *in vitro* treatment of mitochondria with trimethyltin chloride is in agreement with established findings, as shown by the powerful inhibition of ADP-dependent respiration found on addition of 10–20 μM trimethyltin (Fig. 1), and the substantially diminished degree of inhibition observed with FCCP-stimulated respiration (Fig. 1).

When studying mitochondria isolated from rats that had been given prior injections of trimethyltin chloride, however, this inhibition was never observed, at least not in functional terms. In contrast, ADP-stimulated (or FCCP-stimulated) respiration was invariably unchanged, even somewhat stimulated, particularly 24 and 36 hr after injection of trimethyltin chloride when glutamate/malate was used as respiratory substrates (Fig. 2a). Using palmitoyl-L-carnitine as substrate, progressive inhibition was apparent up to 24 hr after injection, but this disappeared at 36-hr post-injection (Fig. 3a). Also ADP- and FCCP-stimulated respiration was similarly unaffected, also suggesting the absence of inhibition of the mitochondrial ATPase. Why palmitoyl-L-carnitine-dependent respiration responds different to treatment with trimethyltin chloride, as compared to glutamate/malate, is not clear. The much lower rate of respiration a with palmitoyl-L-carnitine (because the incubation medium also contained 10 Mm malonate) may be involved. Previously, respiration on β-hydroxybutyrate has been reported to be less sensitive to *in vitro* inhibition by trialkyltins [23].

These findings are further substantiated by the finding of undiminished ability to synthesise ATP from ADP and P_i by mitochondrial respiring on glutamate/malate by mitochondria isolated from rats that had been given prior treatment with trimethyltin chloride. This was apparent both with respect to the synthesis of ATP based on respiration on endogenous substrates, as well as on added glutamate/malate (Fig. 6).

Our results, therefore, suggest that *in vivo* treatment of rats with trimethyltin chloride does not cause severe inhibition of ATP synthesis, at least not in the liver. Conversely, this treatment may even cause some stimulation of respiration and of synthesis of ATP.

4.2. Effects of in vivo treatment with both clofibrate and trimethyltin chloride on mitochondrial respiration and synthesis of ATP

With mitochondria isolated from rats that also had been treated with clofibrate, inhibition of both ATP- and FCCP-dependent respiration on glutamate/malate was observed (Fig. 2b). This suggests that the observed inhibition cannot be due to the established, oligomycin-like inhibition of the ATPase by trimethyltin. This conclusion is supported by the absence of inhibition by injected trimethyltin chloride on the synthesis of ATP by subsequently isolated mitochondria (Fig. 6). With mitochondria from rats treated with both clofibrate and trimethyltin an increase in rate of synthesis of ATP may even be observed (e.g. at 4 min of incubation, see Fig. 6). However, the inhibition of respiration observed with mitochondria from rats given prior treatment with clofibrate, in contrast to mitochondria from rats given trimethyltin alone, remains unexplained. Somehow the clofibrate-induced stimulation of respiration is rendered sensitive to trimethyltin in a manner which clearly cannot involve trimethyltin-dependent inhibition of the mitochondrial ATPase. Also this inhibition of respiration is not manifested as diminished synthesis of ATP (Fig. 6), suggesting that remaining respiratory activity nevertheless can sustain phoshorylation of ADP included in these incubations.

These findings were unexpected, and we have at present no explanation for this phenomenon. More recent data have demonstrated that the established Cl^-/OH^- exchange exhibited by triethyltin [20,21] also operates for trimethyltin [40]. This may lead to uncoupling, and diminished ATP synthesis. The fact that peroxisomal β-oxidation is undiminished 24-hr post-injection of trimethyltin chloride, nevertheless, suggests that there has been no lack of ATP to maintain adequate protein synthesis during this period.

Treatment of rats with trimethyltin is known to cause transient hypothermia in rats [41–43], and it can be speculated that the observed undiminished ATP synthesis found with mitochondria from treated rats is due to a compensatory mechanism attempting to maintain body temperature.

4.3. Effects of treatment with trimethyltin on hepatic amino acids and GSH

Liver amino acid metabolism is, however, also affected by treatment with trimethyltin as demonstrated by the changes in amino acids content of the liver (Fig. 5). It may be significant that the hepatic level of GSH decreased steadily following a single injection of trimethyltin chloride (Fig. 5), suggesting increased oxidative stress. Taurine has also been suggested to function as a hepatic antioxidant [44,45]. The increased hepatic level of taurine (Fig. 5) may, therefore, be regarded as a compensatory mechanism in respect of the diminished hepatic level of GSH.

Studies with mice have also shown that treatment with trimethyltin chloride decreased hepatic content of non-protein sulphydryl groups [46]. This may be in line with the present findings as regards decrease in hepatic content of GSH in the presence of undiminished rates of respiration following treatment with trimethyltin. Whether, and why, the respiratory chain in mitochondria isolated from trimethyltin-treated rats should generate an increased amount of oxygen radicals is not clear. Recent data suggest that tributyltin in cultured thymocytes will increase intracellular Ca^{2+} leading to mitochondrial generation of reactive

oxygen species and cell death [11]. Reactive oxygen species formed by liver mitochondria has been shown to bring about depletion of hepatocyte GSH [47]. Hepatic tributyltin toxicity appears to also involve disruption of hepatocyte Ca^{2+} homeostasis, which will also lead to mitochondrial generation of reactive oxygen species and cell death [48–50]. Similarly, in neuronal cultures [51] and in cerebral granule cells [52] trimethyltin has been show to cause oxidative stress. Therefore, uncontrolled oxidative stress may be the cause of trialkyltin toxicity, while the inhibition of the ATPase remains an *in vitro* phenomenon. This conclusion is in agreement with our observed stimulation of respiration with mitochondria isolated from rats treated with trimethyltin chloride alone.

4.4. Effects of treatment with trimethyltin on hepatic levels of glutamine

Hepatic levels of glutamine decreased progressively with time following treatment with trimethyltin chloride (Fig. 5). In studies of brain, trimethyltin is reported to increase the level of glutamine in all regions examined [53], as well as in hippocampal and caudate dialysates [29]. This is most likely caused by a trimethyltin-dependent increase in astrocytes which is known to be the sole site of glutamine synthetase in the brain [54].

In the liver it is possible, however, that glutamine is steadily converted into glutamate for use in the biosynthesis of GSH. The latter is steadily being consumed, and subsequently hydrolysed. This can explain why the level of glutamate remains relatively steady throughout the experimental period. Alternatively, hepatic glutamine synthetase may be inhibited in treated rats, as was found with rat astrocyte cultures exposed to low concentrations of triethyltin or triphenyltin (although not trimethyltin) [30].

5. Concluding remarks

Our findings strongly suggest that inhibitory phenomena previously observed with *in vitro* studies with trialkyltins are not mirrored with *in vivo* studies, at least not during the first 36 hr following injection. The administered dose of trimethyltin chloride brings about significant levels of Sn in the liver (about 1.5 μg/g wet wt.), although appreciably lower than that found in blood (about 50 μg/mL). It can be argued that this level of trimethyltin in the liver is bound to other sites and, therefore, unable to inhibit the mitochondrial ATPase. Also with mice, *in vivo* treatment with trimethyltin is reported not to inhibit mitochondrial ATP synthesis [46]. A mechanism of toxicity involving cell death due to excessive generation of reactive oxygen species, therefore, remains the more likely mechanism of action *in vivo*. Our finding of undiminished respiratory rates in rats given trimethyltin is in line with this hypothesis.

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