A Study of the Toxic Effects of Six Dibenzofuranes in Mitochondria from Rat Liver

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Abstract

The interactions of five dibenzofurans with the mitochondria from rat liver have been investigated. Results indicate that at low doses all dibenzofurans induce inhibition of ATP synthesis. The efficiency of ATP synthesis is measured from the ratio (R) between the ADP-stimulated respiratory rate (State 3) and basal respiration (State 4). When R = 1, no ATP synthesis occurs. A comparison between the R values for all investigated dibenzofurans (PCDF) shows that the R values are in the same order of magnitude.

Keywords: Dibenzofurans, mitochondria, toxicity.

Introduction

Polychlorinated dibenzo-p-dioxins (PCDD), dibenzofurans (DF) and biphenils (PCB) are industrial compounds or by-products that have been identified as environmental contaminants. Its residues have been detected in fish, wildlife and humans. Studies have been performed in animals to assess the toxic effects in whole organisms, and in tissues, cells and subcellular structures to establish the molecular mechanism or the mechanisms which are responsible for the toxic effects observed in whole animals. At the present time a considerable body of research establishes that in laboratory animals the Ah (aromatic hydrocarbon) receptor (AhR) mediated most toxic effects of halogenated aromatic hydrocarbons [1-4]. The toxicological pattern, however, in whole animals is very complicated and other action mechanisms responsible for acute toxicity, in addition to those alredy proposed, cannot be excluded.

This paper studies the interactions of five DF with the mitochondria from rat liver. The interest of our research on the mitochondria is justified from the fact that mitochondria are a possible target for those toxic compounds which induce acute toxicity since damage to the mitochondria which produce ATP for the cell gives rise to corresponding cell damage. Our results indicate that at low doses all DFs induce inhibition of ATP synthesis, thus explaining the acute toxicity in whole organisms as rats. A comparison with the doses necessary to induce toxic effects in humans is complicated since the effects of DFs on humans are not well characterized [4]. Different degradation rates are postulated to explain the different dose-toxicity relationships observed in whole animals and presumably also in humans.

Experimental Procedures

Mitochondria were prepared from livers of fasted albino winstar rats weighing about 300 g. The livers were isolated and placed in ice-cold sucrose (0.25 M) solution containing Tris-Mops 10 mM pH 7.4 and EGTA 0.1 M (isolation medium). The livers were rinsed three times with ice-cold medium, cut into small pieces with scissors, and homogenized in a glass potter homogeniser equipped with a teflon pestle and kept in an ice-bath, in a volume of 40 ml/liver. Each liver homogenate was diluted with ice-cold medium to 200 ml and intact cells, nuclei and cellular debris were sedimented by 10 min centrifugation at 650xg in a Sorvall RC2B refrigerated centrifuge kept at 2°C (rotor GSA). The supernatant was carefully decanted and centrifuged at 7700xg (rotor SS34) for 10 min. The supernatant was discarded and the mitochondrial pellet was carefully resuspended in ice-cold isolation medium and centrifuged
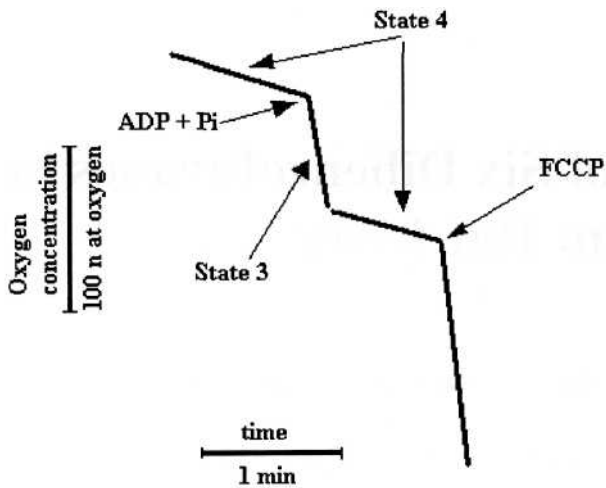
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Fig. 1. A typical experiment of mitochondrial respiration in various conditions (the respiratory rate is the slope of oxygen concentration against time): State 4 is basal respiration. In the presence of 100 μM ADP and Phosphate (Pi), the respiration is stimulated (State 3). When all ADP is consumed and ATP is synthetized, the system returns to State 4. In this condition, the respiratory rate can be stimulated adding an uncoupler (FCCP) is added. Medium composition: sucrose 0.25 M, Hepes pH 7.4 10 mM, succinate 2 mM, acetate 10 mM. Mitochondria 1 mg/ml.

as above. The resulting mitochondrial pellet was resuspended in 0.25 M sucrose containing 10 mM Tris-Mops, pH 7.4 at approximately 200 mg protein/ml and stored in an ice-bath until use. Mitochondrial proteins were measured using Lowry's method [5].

Mitochondrial oxygen consumption was measured with a Clark oxygen electrode (Yellow Springs Instruments, OH, U.S.A.) fitted in a thermostated, closed chamber equipped with magnetic stirring.

All chemicals were of the higest purity commercially available.

Dibenzofurans were a gift from dr. A. Orio.

FCCP (Carbonyl Cyanide p-Trifluoromethoxyphenyl-hydrazone) was furnished by Sigma.

Results

In respiring mitochondria, State 4 is the rate of basal respiration (Fig. 1) (the respiratory rate or respiration is the slope of oxygen concentration against time). State 3 is the respiratory rate when ADP and Pi are added: when ADP and Pi are added, a stimulation in respiratory rate occurs since ATP is synthetized. When all ADP have reacted, the respiratory rate returns in State 4. If in this condition FCCP (a potent uncoupler of oxidative phosphorylation) is added, the respiratory rate is stimulated up to the maximal value.
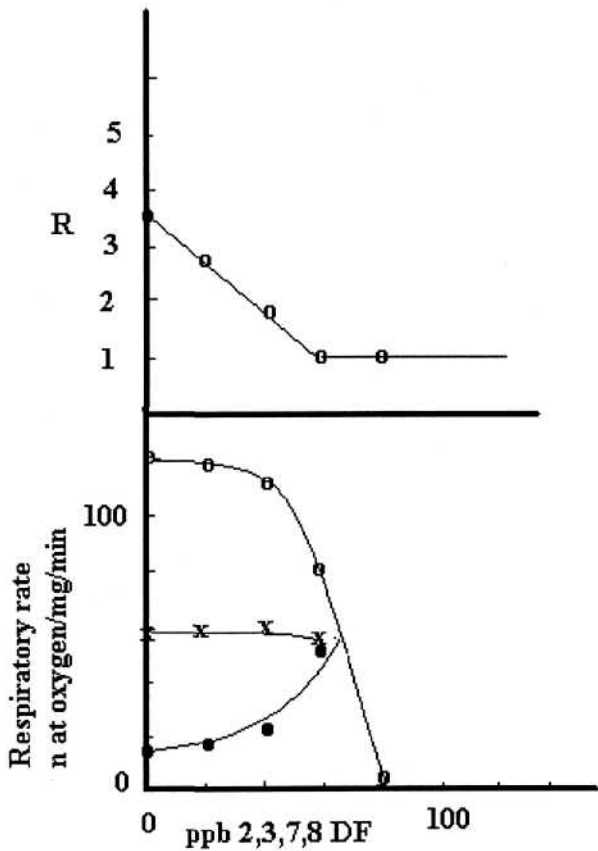

Fig. 2. Respiratory rates of mitochondria in the presence of varying amounts of 2,3,7,8 DF in State 4 (•), State 3 (x) and by FCCP-stimulated respiration (o). In the upper side the R values at any DF concentration are reported. Medium and conditions are those indicated in Fig. 1.
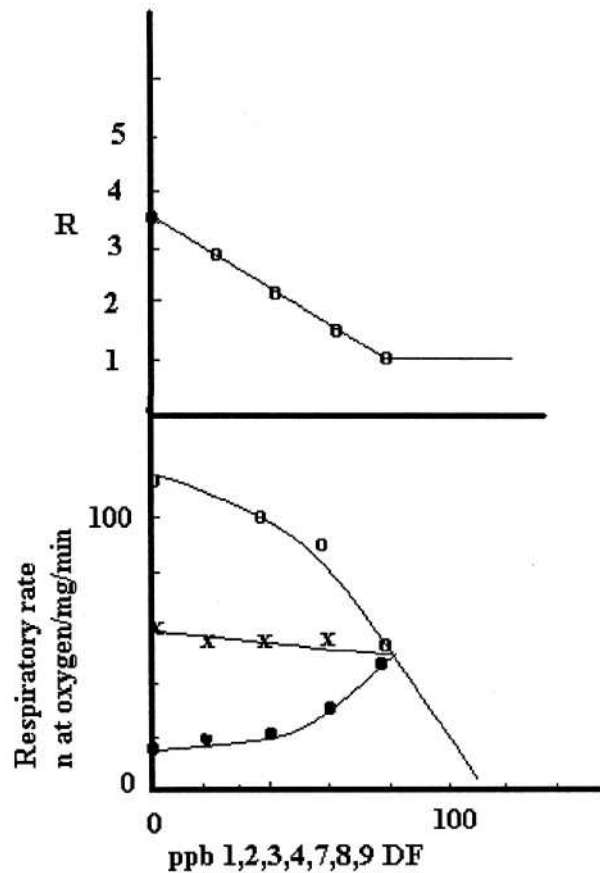

Fig. 3. Respiratory rates of mitochondria in the presence of varying amounts of 1,2,3,4,7,8,9 DF. Medium and conditions as in Figs. 1 and 2.

The literature reports that in well coupled mitochondria, the ratio between State 3 and State 4 respiratory rate (R) is about 5 [6], while when R = 1 no ATP synthesis occurs.

On the basis of these arguments, we have examined the interactions of five DF with the mitochondria. As Fig. 2 shows, when the mitochondria are incubated in the presence of increasing amounts of 2,3,7,8 DF, a significantive increase of State 4 occurs, while State 3 is not substantially modified. As the concentration of 2,3,7,8 DF rises, R value decreases (upper side of Fig. 1). At about 60 ppb DF, R= 1. In this condition, no ATP synthesis occurs. In the figure, the reducing substrate is succinate. The figure also shows that to obtain a complete inhibition of the respiratory chain (FCCP stimulated), the DF dose has to be higher than 60 ppb. This data indicate that the prevailing (more toxic) effect of 2,3,7,8 DF is not an inhibitory effect on the respiratory chain carriers (carriers responsible for the transport of ATP, ADP, Pi or electron carriers as the cytochromes), but an increased State 4 respiratory rate. This effect, as discussed below, has been analyzed in a previous paper [7].

The experiments of Fig. 2 have been performed by succinate as substrate. If glutamate/mahte as reducing substrates are utilized, the same results about the R values are obtained (not shown), but the inhibition of the FCCP-stimulated respiratory rate occurs at higher doses, thus indicating that the first site of the respiratory chain is not the prevailing target of 2,3,7,8 DF. Since the effective toxic effect is the effect with the lowest dose, this aspect has not been further analyzed.

The experiments as in Figs. 1 and 2 have been performed with other four DFs: 1,2,3,4,7,8,9 DF, 1,2,3,4,7,8 DF, 2,3,4,7,8 DF and 2,3,4,6,7,8 DF. Figs. 3-6 report the interactions of the various PCDFs with the respiratory states. The corresponding R values are reported in the upper side of each figure.

Discussion

Results indicate that all DFs inhibit ATP synthesis. This effect occurs at doses lower than that necessary to inhibit the maximal respiratory rate, thus indicating that the prevailing effect on mitochondria is not the inhibition of the respiratory chain. In this regard, the effect on State 4 is similar to an uncoupling effect, but all DF do not possess any acid-base group which is typical of uncouplers. As suggested in a previous paper [7], we suggest therefore that the enhancement in the respiratory rate is a detergent effect. This behaviour could explain many of the different hystopathological behaviours observed in exposed animals such as progressive weight loss and general debilitation and the very extended hystopathological pattern: if low doses of DF cause membrane leaks, this behaviour is not peculiar only to mitochondrial membranes, but a characteristic of all membranes, inducing an effect which is similar to an aging mechanism.

Similar behaviours have also been observed with PCB compounds [8].

Furthermore, data indicate that the doses of DF necessary to inhibit ATP synthesis are all included in the same
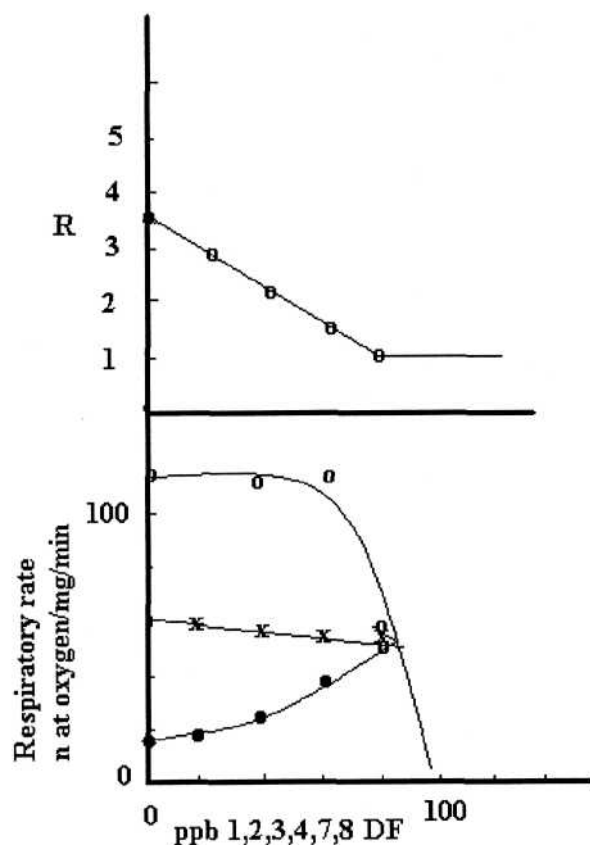

Fig. 4. Respiratory rates of mitochondria in the presence of varying amounts of 1,2,3,4,7,8 DF. Medium and conditions as in Figs. 1 and 2.
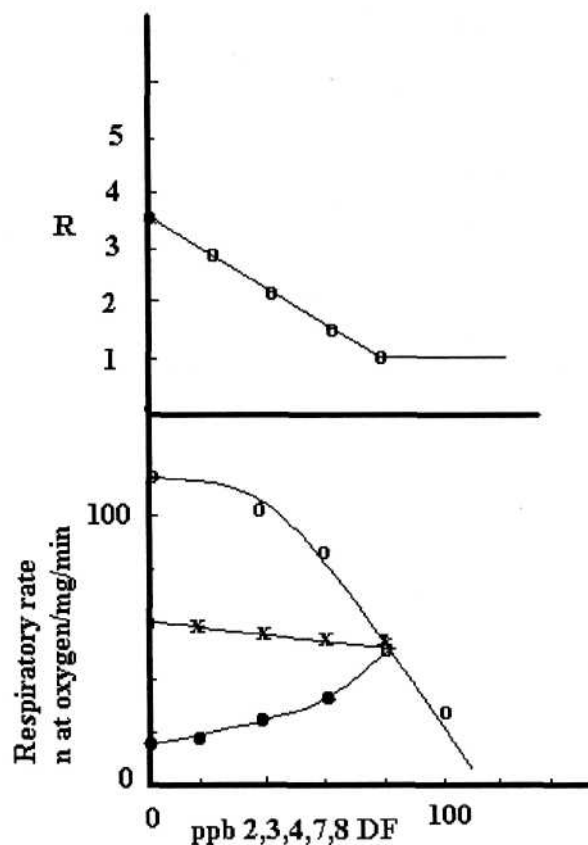

Fig. 5. Respiratory rates of mitochondria in the presence of varying amounts of 2,3,4,7,8 DF. Medium and conditions as in Figs. 1 and 2.
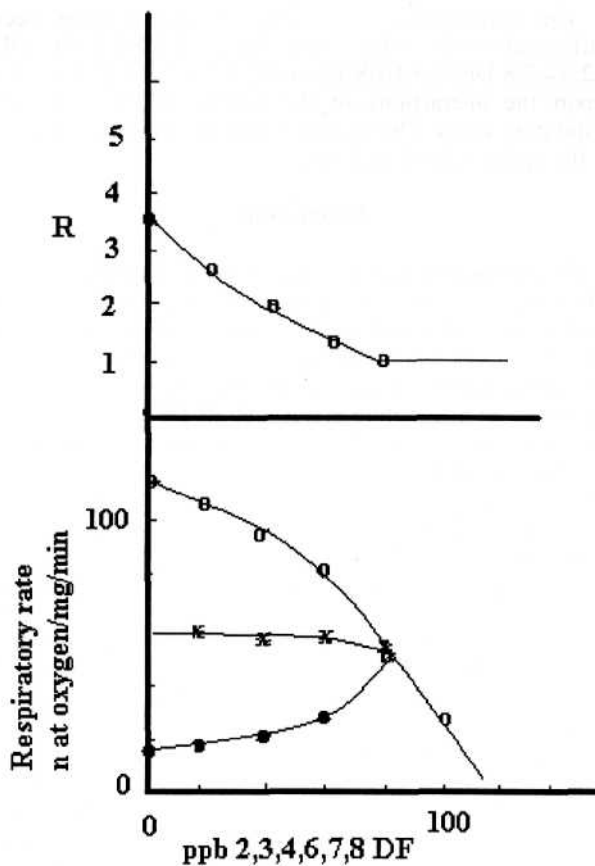
Fig. 6. Respiratory rates of mitochondria in the presence of varying amounts of 2,3,4,6,7,8 DF. Medium and conditions as in Figs. 1 and 2.

order of magnitude. Comparisons with the doses necessary to obtain toxic effects in whole animals, however, are complicated. As suggested by many authors, in humans, the responses dose-effect and the body burdens associated with such effects requires more research [1,3, 4]. Therefore, the proposal which establishes that the toxicity is due to a binding to Ah receptors may not be the only mechanism responsible for toxicity. Therefore, we propose that DFs could contribute to the whole acute toxicity. This hypo-

thesis is supported by the fact that their effect is a membrane permeability enhancement effect [7] since all DFs are membrane-soluble and all membranes show the same characteristics. A possible discrepancy between "in vitro" and "in vivo" experiments could be explained, postulating differents degradation rates for the various DFs. By this model, the major toxicity observed "in vivo" with some DFs is due to their high persistence in the cell, while the low toxicity observed in animals treated with some DFs could be due to a fast degradation of the parent compounds. This different behaviour toward degradation has recently been observed in microorganisms [2].

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