IN VITRO EFFECTS OF 3,4,3',4'-TETRACHLOROBIPHENYL AND ITS HYDROXYMETABOLITES ON MITOCHONDRIAL FUNCTION

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Abstract
The effects of 3,4,3',4'-tetrachlorobiphenyl and its hydroxymetabolites on mitochondrial function were studied in vitro. The hydroxymetabolites, but not TCB itself, were found to be uncouplers of the oxidative phosphorylation in isolated rat liver mitochondria.

Introduction

3,4,3',4'-Tetrachlorobiphenyl (TCB), one of the most toxic polychlorobiphenyl (PCB) isomers, is known to have marked effects on thyroid hormone status[1,2] and energy metabolism in rats after in vivo exposure. Exposure of rats to TCB induced morphologic changes in mitochondria (megamitochondria) of the rat hepatocyte[3]. Subcellular distribution of labeled TCB in the rat hepatocyte showed a preference for lipid droplets, endoplasmatic reticulum and mitochondria[4]. These findings led to the hypothesis that TCB and/or its hydroxymetabolites might have a direct effect on mitochondrial function.

An in vitro study was performed to investigate the effects of TCB and its hydroxymetabolites on isolated rat liver mitochondria. Oxygen consumption measurements were used as a functional parameter for mitochondrial respiration. The effects of TCB and the hydroxymetabolites on state-4 respiration and oligomycine inhibited state-3 respiration were studied in particular. Dinitrofenol (DNP), an uncoupling agent, was used as a positive control for the uncoupling of state-4 respiration.

Materials and methods

Isolation of mitochondria

Liver mitochondria were isolated from female Wistar rats (200-300 g bodyweight) in 0.25 M sucrose by differential centrifugation according to the method of Hogenboom[5]. The contamination of the isolated mitochondria with other cellular organelles was determined with specific marker enzymes (cytosol: lactate dehydrogenase (LDH); microsomes: ethoxyresorufine-o-deethylase (EROD); lysosomes: acid phosphatase (AP); mitochondria: succinate dehydrogenase (SDH)). The mitochondrial suspension was free of microsomes and cytosol, but did contain 13 % of the lysosomes originally present in the rat liver homogenate.

Mitochondrial oxygen consumption

Mitochondrial oxygen consumption was measured polarigraphically with a Clarke-type oxygen electrode at 25° C. Succinate (FAD-linked respiration) and α-ketoglutarate (NAD-linked respiration) were used as substrates.

Synthesis of hydroxy-3,3',4,4'-tetrachlorobiphenyls

All monomethoxy derivatives of TCB were synthesized according to the *Cadogan variant of the Gomberg-Bachmann coupling reaction*[6,7]. The coupling ractions were performed between the corresponding dichloranilines and dichloranisoles in the presence of iso-amylnitrite. The resulting methoxy-compounds were treated with boron tribromide at room temperature for the cleavage of the etherbond which led to the free phenols of TCB (2-OH-3,3',4,4'-TCB (2-OH-TCB); 4-OH-3,3',4',5-TCB (4-OH-TCB); 5-OH-3,3',4,4'-TCB (5-OH-TCB) and 6-OH-3,3',4,4'-TCB (6-OH-TCB)). They were purified by flush-chromatography and recrystallization. The purity was >99.5% by GC; no dioxins or furans were detected as contaminants. TCB and the hydroxymetabolites were dissolved in methanol and added to the incubation mixture (methanol did not influence the oxygen consumption in volumes < 3% (v/v)).

Results

With α-ketoglutarate as a NAD-linked substrate 3,4,3',4'-TCB was hardly able to stimulate mitochondrial state-4 respiration, not even at high concentrations (0.18-18.0 μM). The hydroxymetabolites 2-OH-TCB, 5-OH-TCB and 6-OH-TCB stimulated state-4 respiration strongly in relatively low doses (0.18-1.8 μM). 4-OH-TCB, however, was only slightly more effective than TCB in stimulating state-4 respiration.

The uncoupling potencies of TCB and the hydroxy metabolites were expressed as the increase in O2 consumption (nmol/min/mg mitochondria) per μM of added TCB or metabolites (table 1). Increase in oxygen consumption was linear when TCB or metabolites were added at

concentrations between 0 - 1.8 μM. The uncoupling potency can be used as a measure for the degree in which TCB or the metabolites are able to uncouple the state-4 respiration.

When succinate was used as an FAD-linked substrate for oxygen consumption, 2-OH-TCB also induced a strong stimulation of state-4 respiration whereas 4-OH-TCB only had minor effects. The 2-OH-TCB, 5-OH-TCB, 6-OH-TCB and to a lesser extent the 4-OH-TCB metabolite were able to release the oligomycine inhibited state-3 respiration with α-ketoglutarate as substrate. None of the hydroxymetabolites of TCB were able to inhibit a DNP-stimulated state-4 respiration, even at high concentrations (3.6 μM).

Table 1 Uncoupling potency of TCB and hydroxy-TCB metabolites on state-4 respiration with α-ketoglutarate as substrate (means ± S.D., n=2)

Compound	Increase of O^2 consumption (nmol/min/mg)	Concentration (μM) of TCB or metabolites	Uncoupling potency (O^2 consumption/μM)
TCB	9.3 ± 2.0	1.8	5.2 ± 1.1
2-OH-TCB	56.9 ± 4.3	1.8	31.6 ± 2.4
4-OH-TCB	23.3 ± 1.5	1.8	12.9 ± 0.8
5-OH-TCB	46.5 ± 1.7	1.8	25.8 ± 0.9
6-OH-TCB	61.6 ± 2.3	1.8	34.2 ± 1.3

Discussion

The results indicate that the hydroxymetabolites of 3,3',4,4'-TCB are able to act in vitro as uncouplers of the oxidative phosphorylation in rat liver mitochondria. The hydroxymetabolites did not only stimulate state-4 respiration, they were also able to release the oligomycine-inhibited state-3 respiration. This implicates that the metabolites act as protonophoric uncouplers of the oxidative phosphorylation. According to their inability to inhibit a DNP-stimulated state-3 respiration the hydroxymetabolites were not able to act as inhibitors of the phosphorylation of ADP (ATP-synthesis) . The strength of uncoupling by the hydroxymetabolites was (in order of potency): 2-OH-TCB = 6-OH-TCB > 5-OH-TCB >> 4-OH-TCB > TCB.

The structural differences between the metabolites may be the cause of

the differences in uncoupling potency. Hydroxylation on the ortho positions of the TCB molecule caused the greatest uncoupling potency (2-OH-TCB and 6-OH-TCB) whereas the 4-OH-TCB metabolite, which can adapt a co-planar conformation like TCB, has the weakest uncoupling potency. Because of its co-planarity 3,4,3',4'-TCB caused relatively little disruption in the inner mitochondrial membrane when compared to non-planar types of TCBs[8]. Ortho-hydroxylated metabolites, which cannot adapt a co-planar conformation, may cause more disruption of the inner mitochondrial membrane. This can lead to loss of the proton gradient across the mitochondrial membrane and uncoupling may take place. Uncoupling of the oxidative phosphorylation by the hydroxymetabolites may result in lower ATP production and the release of energy by heat production, so less energy-dependent processes in the cell may take place. These changes in mitochondrial energy metabolism may also be of relevance to the PCB-induced wasting syndrome in rodents.

References

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