BIOLOGIJA. 2004. Nr. 1. P. 30–32

Identification of targets of the noxious action of 2,2',5,5'-tetrachlorbiphenyl in the respiratory chain of rat liver mitochondria

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The effect of 2,2',5,5'-tetrachlorinated biphenil (TCB) on the activity of the respiratory chain modules of rat liver mitochondria oxidizing succinate (+ rotenone) in state 3 was studied using a modular kinetic approach. The kinetic dependencies of CoQ and cytochrome c reducing and oxidizing modules on the reduction state of common intermediates (CoQ and cytochrome c, respectively) were determined in the control and in the presence of 20 μM TCB. Analysis around cytochrome c revealed that TCB inhibited components of both modules. The same type of analysis around CoQ revealed that TCB had no effect on the kinetics of the CoQ-reducing module, however, it inhibited the CoQ-oxidizing module. In conclusion, our results showed that TCB inhibited cytochrome bc_1 but did not affect succinate dehydrogenase. However, this study did not provide evidence whether or not TCB has a direct effect on cytochrome oxidase.

Key words: 2,2',5,5'- tetrachlorbiphenyl, liver mitochondria, respiratory chain, kinetic analysis

INTRODUCTION

Organochlorines actually dominate over all lists of global contaminants and environmental health hazards. Many of these compounds resist natural degradation, even very dilute discharges tend to build up in the enviroment over time. Organochlorines are fat-soluble and therefore bioaccumulate in the fatty tissues of living organisms [1]. Biological and toxic effects of polychlorinated biphenyls (PCB) have been extensively studied in animal models. The liver is especially susceptible to deleterious effects of PCBs. An impairment of mitochondrial functions by different PCBs has been extensively studied, and the results indicated that 2,2',5,5'-tetrachlorobiphenyl (TCB) is one of the most potent inhibitors of mitochondrial functions [2–5]. TCB uncouples oxidative phosphorylation [2], inhibits several components of the respiratory chain (namely, succinate dehydrogenase and cytochrome bc_1 [2], Complex I [6]), and ATP synthase [6].

The aim of this study was to lokalise in more detail the inhibitory sites of TCB within the respiratory chain of the rat liver mitochondria. For this purpose, we applied modular kinetic analysis around two mobile respiratory electron carriers: ubiquinone and cytochrome c. We determined which modules of the respiratory chain around those carriers were affected by TCB.

MATERIALS AND METHODS

Mitochondria were isolated by differential centrifugation from the liver of male Wistar rats weighing 275–300 g [6]. The mitochondrial pellet was resuspended to an approximate protein concentration of 50 mg/ml. The protein concentration was determined by the Pierce BCA method [7].

The experiments were performed at 25 °C using 5 mM succinate (+ 2 μM rotenone) as a substrate. Mitochondrial concentration in experiments was 1.0 mg/ml. The rate of mitochondrial respiration was measured using the Clark electrode after preincubation of mitochondria for 3 min in the absence of TCB or in the presence of 20 μM TCB in the incubation medium containing 110 mM KCl, 20 mM Tris-HCl, 5 mM KH_2PO_4, 50 mM creatine, excess of creatine kinase, 1 mM $MgCl_2$, pH 7.2. The reaction was started by addition of 1 mM of ATP. We

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used TCB (99.8 % purity) manufactured by Chem Service, USA.

RESULTS

Modular kinetic analysis was performed with the aim of localizing the effects of TCB on the respiratory chain of the rat liver mitochondria respiring with succinate (+rotenone) in state 3. The respiratory chain is conceptually divided into two modules around two different intermediates – coenzyme Q (CoQ) (CoQ-reducing and CoQ-oxidizing modules) and cytochrome c (cytochrome c-reducing and cytochrome c-oxidizing modules). The steady-state rate of oxygen consumption and steady-state reduction level of an intermediate were measured at the same time. The kinetic dependencies of cytochrome c reducing and oxidizing modules on cytochrome c reduction state were determined by measuring a number of steady states in which the rate of cytochrome c oxidation and reduction was varied by titration with KCN and malonate, respectively. The kinetics of the CoQ reduction was determined by modulating the activity of the CoQ-oxidizing module by myxothiazole titration and the kinetics of CoQ oxidation – by malonate titration of the CoQ reducing module as described earlier [8].

The kinetic dependencies of fluxes through each module (with succinate as a respiratory substrate) were determined in the absence and in the presence of 20 μM (or 20 nmol/mg) TCB. The results showed that TCB decreased the rate of oxygen consumption from 132 ± 12 to 101 ± 8 nmol O/min per mg (n = 6). The kinetic curves of cytochrome c reduction and cytochrome c oxidation were obviously shifted by TCB towards the lower rates at the same level of cytochrome c reduction (Figure, A) implying that TCB inhibited the components in both modules. The same type of analysis around CoQ revealed that TCB had no effect on the kinetics of CoQ-reducing module (dicarboxylate carrier and succinate dehydrogenase), however, it inhibited the CoQ-oxidizing module (Figure, B). From these two sets of results (Figure, A and B) a conclusion was derived that the inhibition of the respiratory chain by TCB is explainable by inhibition of cytochrome bc_1 (since dicarboxylate carrier + succinate dehydrogenase, the other components of cytochrome c reducing module, are not affected) and inhibition of the process catalysed by cytochrome oxidase.

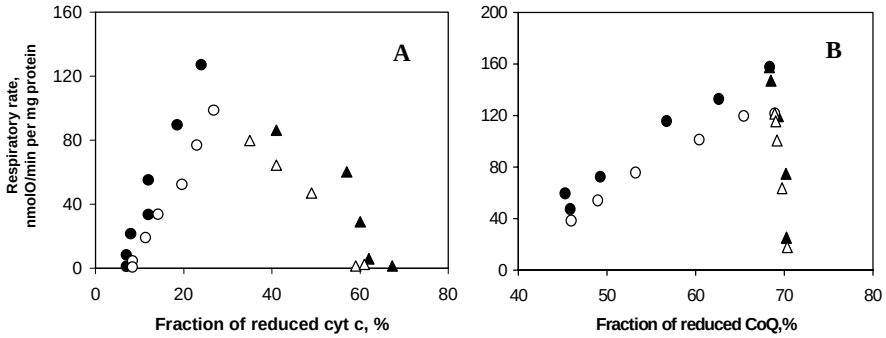

Figure. The effect of TCB on the kinetics of cytochrome c and CoQ reducing and oxidizing modules
A – ●,○ – kinetics of cytochrome c-oxidizing module, ▲,△ – kinetics of cytochrome c reducing module; ●,▲ – in the absence of TCB, ○,△ – + 20 μM TCB; B – ●,○– kinetics of CoQ-oxidizing module; ▲,△ – kinetics of CoQ-reducing module; ●,▲ – in the absence of TCB, ○,△ – + 20 μM TCB

From the kinetic dependencies (Figure) the elasticity of each module towards the common intermediate was determined and the flux control coefficients were calculated (Table). One can see that the contribution of the CoQ-reducing module (dicarboxylate carrier and succinate dehydrogenase) to the control of respiration was negligible, most of the flux control resided in the CoQ-oxidizing module (cytochrome bc_1 + cytochrome oxidizing module). The flux control between the two modules around cytochrome c was distributed to a larger degree, however, the dominating role belonged to the cytochrome c-reducing module (dicarboxylate carrier + succinate dehydrogenase + cytochrome bc_1). The flux control of cytochrome bc_1 calculated by subtracting the value of either $C_{CoQ\text{-}red}$ from $C_{cyt\ c\text{-}red}$, or $C_{cyt\ c\text{-}ox}$ from $C_{CoQ\text{-}ox}$ equalled to 0.59. This value increased to 0.69 upon exposure to TCB. The shift of the control from the cytochrome c oxidizing to the reducing module was not statistically significant, possibly because in this case TCB inhibited one component in each of the modules.

Table. **The flux control coefficients of the CoQ-reducing ($C_{CoQ\text{-}red}$), CoQ-oxidizing ($C_{CoQ\text{-}ox}$), cytochrome c-reducing ($C_{cyt\ c\text{-}red}$) and cytochrome c-oxidizing ($C_{cyt\ c\text{-}ox}$) modules over the rate of respiration under state 3 conditions in liver mitochondria in the absence of TCB and in the presence of 20 μM TCB**

C_i	Without TCB	20 μM TCB
$C_{CoQ\text{-}red}$	0.08 ± 0.01	0.04 ± 0.01*
$C_{CoQ\text{-}ox}$	0.92 ± 0.01	0.96 ± 0.01*
$C_{cyt\ c\text{-}red}$	0.67 ± 0.07	0.73 ± 0.06
$C_{cyt\ c\text{-}ox}$	0.33 ± 0.07	0.27 ± 0.06

Substrate–succinate (+rotenone). * indicates a statistically significant difference ($p < 0.05$), n = 3.

DISCUSSION

In this study, we demonstrate the usefulness of modular kinetic analysis in decoding the individual molecular targets of toxic multi-site effects in a multienzyme system. The results obtained indicate that TCB inhibited cytochrome bc_1 but did not affect succinate dehydrogenase in the respiratory chain of liver mitochondria in state 3. Since our experiments are performed in state 3, the module-oxidizing cytochrome c is comprised of cytochrome oxidase operating together with two subsystems consuming the membrane potential – the phosphorylation module and the proton leak module. We have shown in the previous study [6] that the proton leak is stimulated, whereas the phosphorylation subsystem is inhibited by TCB, and the resulting small increase in the membrane potential was observed in mitochondria respiring with succinate in state 3. These two effects, through changes in the membrane potential [6], may contribute to the inhibition on the overall activity of the module oxidizing cytochrome c. Therefore the results obtained in this study do not provide evidence whether TCB has a direct effect on cytochrome oxidase.

Interaction of TCB with the myxothiazole-sensitive site in cytochrome bc_1 was reported by other authors [4], and our study confirms their finding. However, in contrast to their suggestions, our results indicate that succinate dehydrogenase is not affected, but instead, the process catalysed by cytochrome oxidase is inhibited by TCB. This contradiction may be explained by the fact that TCB effect on the activity of succinate dehydrogenase cytochrome oxidase was directly estimated by Nishihara's group under the conditions when mitochondria could not maintain the transmembrane proton gradient [4]. Possibly, the interaction of lypophylic toxin with membrane protein complexes might depend on their conformational changes induced by membrane energization.

Received 14 November 2003
Accepted 4 November 2003

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2,2',5,5'-TETRACHLORBIFENILO POVEIKIO VIETØ NUSTATYMAS ÞIURKËS KEPENØ MITOCHONDRIJØ KVËPAVIMO GRANDINËJE

S a n t r a u k a

Toksinis 2,2',5,5'-tetrachlorbifenilo (TCB) poveikis oksidacinio fosforilinimo sistemos kvëpavimo posistemei buvo tiriamas kinetinës moduliø analizës metodu þiurkës kepenø mitochondrijose, oksiduojanèiose sukcinatà (+ rotenonà) treèios metabolinës bûsenos metu. Tiriamoji sistema buvo suskirstyta á modulius, sujungtus dviejø tarpiniø metabolitø – kofermento Q ir citochromo c. Citochromà c redukuojanèio ir oksiduojanèio modulio kinetikos pokyèiai parodë, jog TCB inhibavo abu modulius. TCB inhibavo kofermentà Q oksiduojantá modulá, taèiau neveikë redukuojanèio modulio. Apibendrinus gautus duomenis, galima daryti iðvadà, kad 20 μM TCB neveikia sukcinato dehidrogenazës ir inhibuoja citochromà bc_1. Taèiau lieka neaišku, ar TCB tiesiogiai veikia citochromo oksidazæ.