The Modes of Inhibitory Effects of PCBs on Oxidative Phosphorylation of Mitochondria

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The general inhibitory effects on specific enzymes and the toxic effects in humans and animals of PCBs have been widely studied. However, to date, there is no work reported on the differences in inhibitory effects of PCBs according to composition on a cytological level (1).

In order to ascertain the effects of the inhibitory factors, a study was performed on the modes of inhibition of 3 PCBs in oxidative phosphorylation of isolated rat liver mitochondria. The 3 PCBs, locally produced by Kanebuchi Chemical Co., were KC-300, -400 and -500, which comprise different concentration levels respectively of (primarily) trichlorobiphenyl, tetrachlorobiphenyl, pentachlorobiphenyl and their related isomers (2). The modes of antagonism of the 3 different PCBs were followed polarographically as oxygen consumption of respiring mitochondria in their controlled (state 4) and active (state 3) states of respiration.

This report presents the findings of this study.

MATERIALS and METHODS

Rat liver mitochondria was isolated according to the method of Schneider and Hogeboom (3) with the slight modification of 2 extra washings with a chilled mitochondria buffer of 0.25 M sucrose solution consisting of 10 mM Tris-HCl buffer pH 7.2, 10 mM KCl, 5 mM $MgCl_2$, 5 mM potassium phosphate and 0.2 mM EDTA.

Determination of oxygen consumption in respiring mitochondria was performed in a system of mitochondria buffer pH 7.2 and their rates of oxygen consumption at different PCB concentrations in states 3 and 4 were followed with 200 µM ADP and 2 mM succinate at a constant temperature of 20°C. Stock solutions of the 3 different PCBs were prepared in ethanol solutions. Since ethanol is noxious to respiring mitochondria rigid controls were made such that the addition of the PCBs into the system in ethanol solution did not result in an overall ethanol concentration of more than 2 percent. States 3 (active respiration) and 4 (controlled respiration) are definitions of mitochondrial respiratory states in systems containing substrate (succinate), oxygen plus ADP and minus ADP respectively.

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Oxygen consumptions were determined polarographically using a Clark type electrode (4) with a 3.5 ml reaction chamber.

Mitochondria concentrations of the experiments were 0.45 mg protein/ml. The mitochondrial protein content in the system was determined using the Biuret method.

Conversion of absolute oxygen content of the system at 20°C was determined by the addition of excess sodium hydrosulfite to the mitochondrial buffer whereby the evolved O_2 through reduction by sodium hydrosulfite was detected polarographically as intensity in the Clark type electrode.

RESULTS

Respiratory inhibition patterns of the PCBs

Figure 1 shows the respiratory mode pattern tracings of the inhibitory effects of the 3 different PCBs on oxidative phosphorylation in mitochondria at various arbitrarily selected representative concentrations. It can be seen that the respiratory rates of both states (controlled and active) of KC-300 are inhibited at all concentrations in contrast with that of KC-400 and -500, where there is inhibition at low concentrations for both states but rapid stimulation at higher concentrations for state 4 as compared to the controls.

Respiratory inhibition effects of the PCBs

Under various concentrations the respiratory rates of states 3 and 4 for KC-300, -400 and -500 were studied and their rates plotted as a percentage of the respiratory rates of the respective controls taken as 100%.

a) Inhibitory effect of KC-300.

From Figure 2 it can be seen that the respiratory rates of states 3 and 4 are first adversely inhibited to about 54 and 74% respectively below 200 mμM concentrations. A stabilization follows, until low μM orders, where another drastic inhibition of about 18 and 24% respectively occurs.

b) Inhibitory effect of KC-400.

Similarly, in Figure 3, KC-400, states 3 and 4 are first inhibited to around 70 and 82% respectively below 200 mμM concentrations, followed by stabilization until low μM concentrations. At this stage a drastic inhibition in the respiratory rate of state 3 to about 34%, like that of KC-300 occurs. However, in the respiratory rate of state 4 a gradual stimulation to about 140% at mM concentrations, unlike that of KC-300, is observed before stabilization.

c) Inhibitory effect of KC-500.

KC-500, as indicated in Figure 4, shows tendencies of respiratory rate inhibitions similar to those of KC-400 for both states. However, the initial inhibition of the respiratory rates for states 3 and 4 is around 76 and 85% respectively followed by stabilization until

low µM concentrations. Thereafter the second drastic inhibition of state 3 is around 38% and the gradual stimulation of state 4 is around 138% before stabilization is attained at mM concentrations.

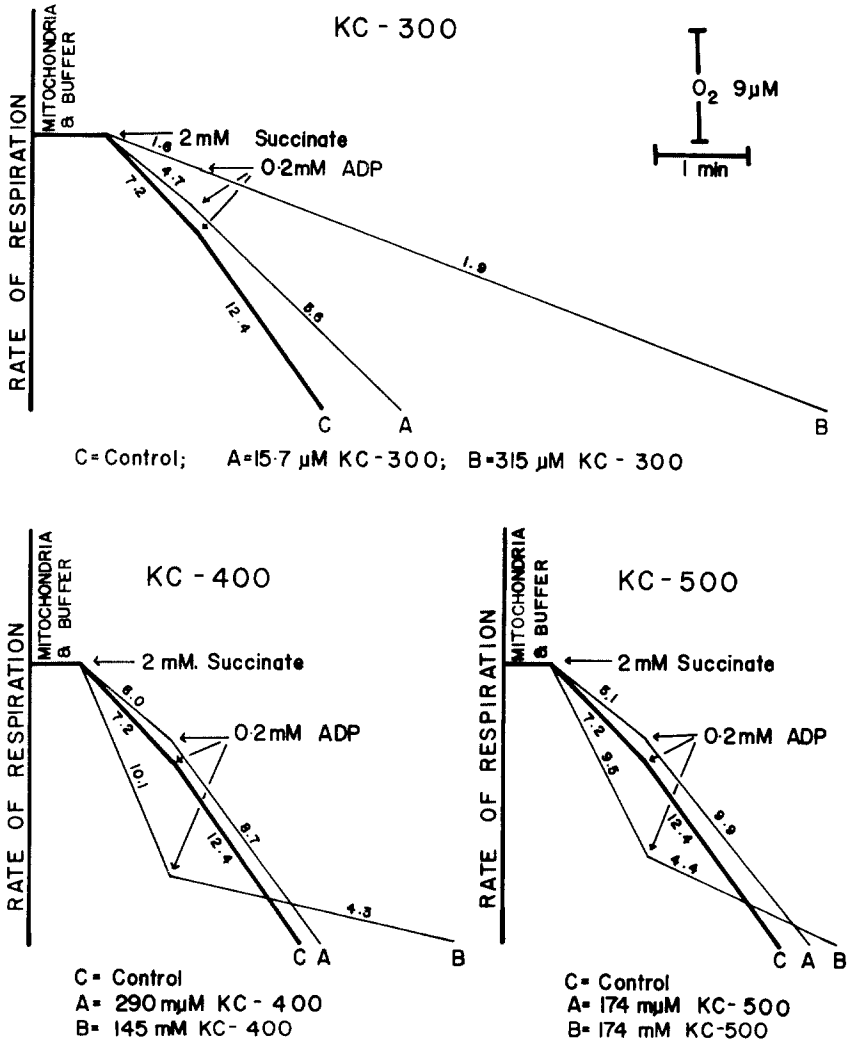

Figure 1. Traces of respiratory patterns of the 3 PCBs. Addition of succinate and ADP were performed as indicated. Rates of respiration of the various representative concentrations of PCBs are as indicated in the respective traces.

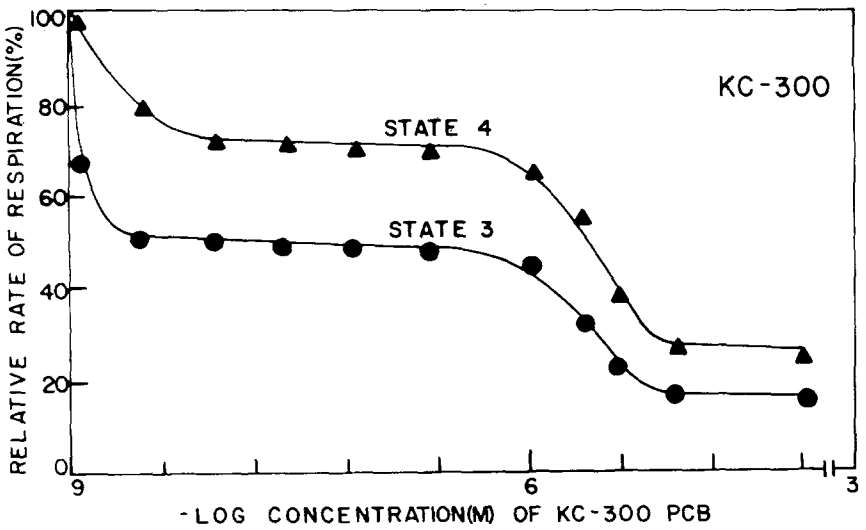

Figure 2. Effect of KC-300 on respiration of states 3 and 4. 100% rate of respiration is equivalent to rate of respiration of the control. Circular and triangular signs in the plots stand for state 3 and 4 rates of respiration respectively.

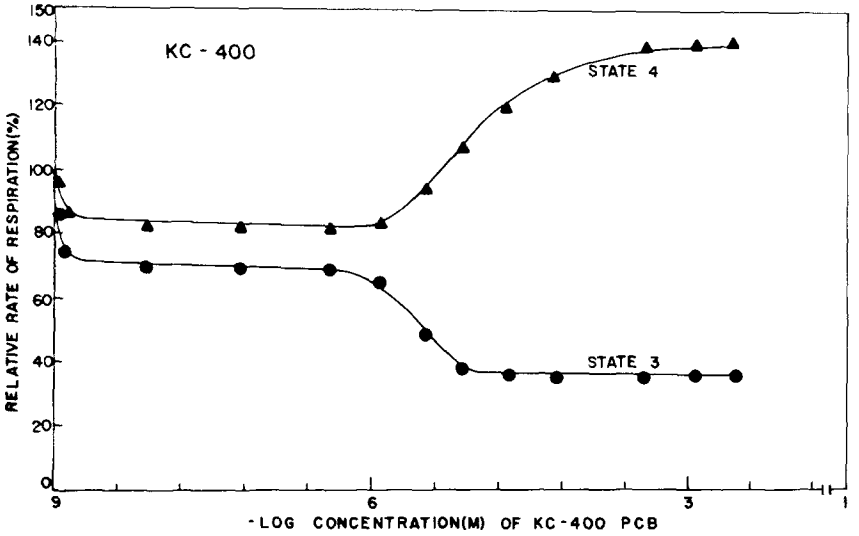

Figure 3. Effect of KC-400 on respiration of states 3 and 4. 100% rate of respiration is equivalent to rate of respiration of the control. Circular and triangular signs in the plots stand for state 3 and 4 rates of respiration respectively.

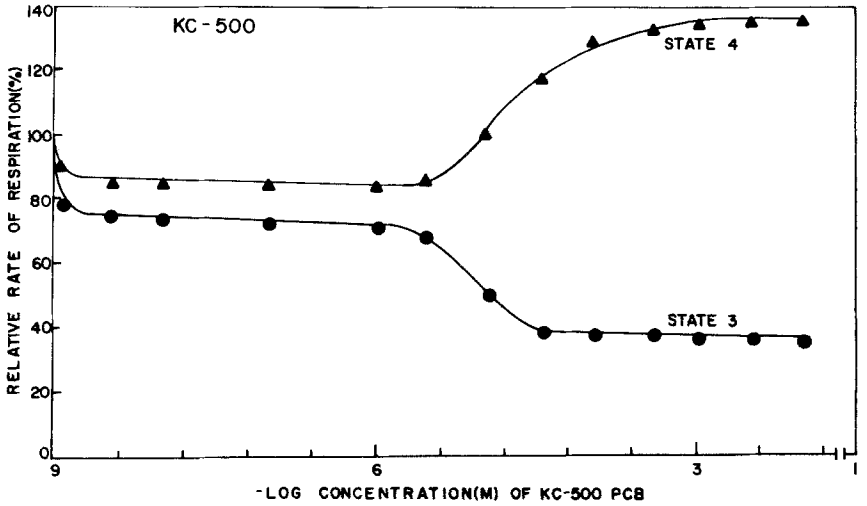

Figure 4. Effect of KC-500 on respiration of states 3 and 4. 100% rate of respiration is equivalent to rate of respiration of the control. Circular and triangular signs in the plots stand for state 3 and 4 rates of respiration respectively.

DISCUSSION

Generally, respiratory inhibition or stimulation in state 3 stands for energy transfer inhibition and uncoupling respectively by the toxic agent (5 - 8). In state 4 it stands for electron transfer system inhibition and uncoupling (5 - 8). Hence, on this basis, Figures 1 and 2 indicate that PCB KC-300 is both an electron transfer system inhibitor and an energy transfer inhibitor at all concentrations. In collaboration, Figures 1, 3 and 4 show that PCBs KC-400 and -500 are electron transfer system inhibitors and energy transfer inhibitors at mμM concentrations with the additional effect of an uncoupler at higher concentrations.

The adverse inhibition at low μM concentrations in the respiratory rate of state 3 of all PCBs examined could be due to either the affinity of the PCBs at this stage or to protein conformational changes of respiring mitochondria as a binding factor. The difference at this stage in the respiratory rate of state 4 for KC-400 and -500 could be attributed to the above reasons along with the additional factor of the influence of the different components of both PCBs as compared to KC-300.

As can be seen from Figures 2, 3 and 4, the toxicity potency of the 3 PCBs varies in the order of KC-300 < KC-400 < KC-500 consistent with the findings of Hoopingarner (9).

Here, it could be concluded safely that the toxicity potency and the modes of antagonism of the 3 PCBs differ, as indicated previously, in accordance with the

overall chlorine concentration and locality of the chlorines in the biphenyls.

SUMMARY

The modes of inhibitory effects of 3 PCBs, KC-300, -400 and -500, on oxidative phosphorylation of respiring mitochondria were studied. It was found that the toxicity potency of the PCBs increases with lower chlorine content. The modes of inhibition of the 3 PCBs with regard to antagonism and toxicity, were found to be different according to their composition. KC-300 was only an energy transfer and electron transfer system inhibitor at all concentration levels in contrast with KC-400 and -500, which were energy transfer and electron transfer system inhibitors at low concentrations but had an uncoupling effect at higher concentrations. A slight difference in inhibitory effects between KC-400 and -500 was also observed.

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