EFFECTS OF CHLORINATED MONO AROMATIC HYDROCARBONS ON MITOCHONDRIAL OXIDATIVE PHOSPHORYLATION IN RATS LIVER

Masana OGATA, Tohoru HASEGAWA, Takaaki MORI and Tadamichi MEGURO

Department of Public Health, Okayama University Medical School
2-5-1 Shikata-cho, Okayama-shi, 700 Japan

(Received October 31, 1980)

Abstract: The effects of chlorinated mono aromatic hydrocarbons on mitochondrial oxidative phosphorylation in rats were investigated. Of the chlorinated mono aromatic hydrocarbons examined, the tetra- and trichlorinated compounds strongly inhibited respiratory control with increasing state 4 respiration and decreasing state 3 respiration. The effect of the chlorinated mono aromatic hydrocarbons on the respiratory control of mitochondria was in the order of tetra > tri > di > mono chlorinated mono aromatic hydrocarbons, and the effect of isomers of the dichlorinated compounds was in the order of o > m > p-dichlorinated hydrocarbons. Chlorinated mono aromatic hydrocarbons also induced K+ release from mitochondria in the same order as the inhibitory effect on respiratory control, suggesting its interaction with the mitochondrial membrane and induction of a change in permeability to ions.

It is concluded that chlorinated mono aromatic hydrocarbons act primarily as an uncoupler of the mitochondrial oxidative phosphorylation rather than as an electron transport or an energy transfer inhibitor. The destructive effect of the chlorinated mono aromatic hydrocarbons on the mitochondrial membrane was also demonstrated by the K+ release.

Key words: Mitochondria—Oxidative phosphorylation—K+ release—Chlorinated hydrocarbons—Rat

INTRODUCTION

Aromatic hydrocarbons are widely employed as solvents, fumigants and chemical intermediates. For example, chlorobenzene is used as a solvent and chemical intermediate, and o-dichlorobenzene is used as a solvent, fumigant, insecticide and chemical intermediate. Also, m- and p-chlorobenzenes have been found in mud.

Inhalation of monochlorobenzene causes histological changes in the lungs, liver and kidneys. Such substances may affect the function of biological membranes. This communication describes the results of an investigation undertaken to elucidate the effects
of chlorinated aromatic hydrocarbons at the level of subcellular particles on the energy transfer reaction of isolated rat liver mitochondria.

METHODS

Animals and preparation of mitochondria: Male Donryu rats weighing approximately 200 g and fed on a laboratory stock diet were used. The rats were fasted overnight and were sacrificed by decapitation. Liver mitochondria were isolated according to a modification of the method of Hogeboom et al. in a medium containing 0.25 M sucrose, 0.2 mM EDTA, and 3 mM Tris- HCl buffer (pH 7.4). The isolated mitochondria were resuspended and washed twice in the EDTA-free isolation medium described above.

Mitochondrial protein was determined by the Biuret reaction using bovine serum albumin as a standard.

Measurement of oxygen uptake and oxidative phosphorylation: Oxygen uptake was measured with a Galvanic oxygen electrode (Kyusui Kagaku Kenkyusho Co., Ltd., Tokyo) connected to an autorecorder. The ADP : O ratios and respiratory control indexes (RCI=state 3/state 4) were calculated from traces of the oxygen uptake recorded according to the method of Hagihara.

Reagents: The chlorinated aromatic hydrocarbons (CAHC) employed, viz. monochlorobenzene (MoCB), o-, m-, and p-dichlorobenzene (DiCB), 1,2,3-trichlorobenzene (TriCB), and 1,2,3,4-tetrachlorobenzene (TetCB), were obtained commercially. These were dissolved in absolute ethanol when used and only small volumes were added to the reaction mixtures; the controls were given an equal volume of solvent alone. ADP and ATP were purchased from Sigma Chemical Co., and the other chemicals were commercial products of reagent grade.

RESULTS

Relationship between chlorinated aromatic hydrocarbons and the respiratory control index.

Typical changes in the rate of oxygen uptake by o-DiCB are shown in Fig. 1. Such changes were, in the case of relatively low concentrations of o-DiCB, due to an increase in state 4 respiration and a decrease in state 3 respiration.

The effects of the chlorinated aromatic hydrocarbons on the respiration and oxidative phosphorylation of mitochondria isolated from rat livers are summarized in Table 1. The effect of ethanol as a solvent on the respiratory control index was negligible at a final concentration of 1% or less. In the presence of chlorinated aromatic hydrocarbons such as MoCB, DiCB, TriCB, and TetCB, at a final concentration of 0.24 mM or less, a remarkable decrease in the respiratory control index was observed. The decrease in respiratory control caused by these chlorinated aromatic hydrocarbons derived from inhibition of state 3 respiration and/or acceleration of state 4 respiration as in the case
EFFECTS OF CHLORINATED AROMATIC HYDROCARBONS ON MITOCHONDRIA

Fig 1. Effect of o-dichlorinated benzene on the ADP stimulated respiration of rat liver mitochondria. The experimental conditions were as shown in Table 1.

Table 1. Effect of chlorinated hydrocarbons on the oxidative phosphorylation of rat liver mitochondria.

<table>
<thead>
<tr>
<th></th>
<th>RCI±SEM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (complete)</td>
<td>4.93±0.113</td>
</tr>
<tr>
<td>MoCB</td>
<td>4.31±0.095</td>
</tr>
<tr>
<td>o-DiCB</td>
<td>3.42±0.098</td>
</tr>
<tr>
<td>m-DiCB</td>
<td>3.60±0.053</td>
</tr>
<tr>
<td>p-DiCB</td>
<td>4.01±0.117</td>
</tr>
<tr>
<td>TriCB (1,2,3-)</td>
<td>uncoupling</td>
</tr>
<tr>
<td>TetCB (1,2,3,4-)</td>
<td>uncoupling</td>
</tr>
</tbody>
</table>

Mitochondria (2.7 mg protein/ml) were incubated with 0.24 mM chlorinated mono aromatic hydrocarbon for 1 min before the addition of 5 mM succinate.
of o-DiCB (Fig. 1).

Among the chlorinated aromatic hydrocarbons examined, the action of TetCB and TriCB was the most significant on the mitochondrial oxidative phosphorylation. The respiratory control index was in the order: control, MoCB, DiCB, TriCB>TetCB, and o-, m- and p-DiCB among DiCB at 0.24 mM final concentration (Table 1).

| Table 2. One way ANOVA between the control, monochlorobenzene and dichlorobenzenes |
|---------------------------------|----------------|----------------|
|                                | DF  | Sum of squares | F-value |
| A                               | 9.385 | 2.3463         | 36.634* |
| E                               | 1.601 | 0.0640         |         |
| T                               | 10.986 |                |         |

* Rejected at the 5% level.

One way ANOVA of the RCI between five groups is shown in Table 2. Significant differences are apparent among the control, MoCB, o-, m- and p-DiCB. Therefore, significant tests on the difference between any two sample means were performed. Significant differences were detected at the 5% rejection limit except in two test, i.e. between the control and MoCB, and between o-DiCB and m-DiCB, among all groups. The percent of RCI for 1,2,3-TriCB against 1,2,3,4-TetCB at 0.21 mM was 94%, suggesting that the action of TetCB on the mitochondrial energy transfer reaction was the most effective.

Effect of chlorinated aromatic hydrocarbons on the potassium compartmentation of mitochondria.

The intramitochondrial K+ content is considered to be closely related to the mitochondrial energy transfer reaction\(^5\).\(^6\).\(^7\). It probably reflects the mitochondrial energy state due to the ion gradient between the outside and the inside of the mitochondrial membrane. In the presence of a heavy metal ion such as Cd\(^{2+}\),\(^8\), K+ release from the mitochondria is induced and is accompanied by a change in the mitochondrial energy state. It is necessary therefore to examine the effect of chlorinated aromatic hydrocarbons on the permeability to ions such as K+.

Fig. 2 shows the change in K+ concentration in the reaction medium during incubation of mitochondria at 25°C. A low K+ efflux was observed in untreated mitochondria, and 1% ethanol as solvent exerted little effect on it. When chlorinated aromatic hydrocarbons were introduced into the medium, remarkable K+ release from the mitochondria was induced depending on the concentration of chlorinated aromatic hydrocarbon added. The effect was in the order of TetCB>TriCB>DiCB>MoCB and in the case of DiCB, was in the order of o-DiCB>m-DiCB>p-DiCB.
DISCUSSION

The present data indicate that a decrease in the RCI of chlorinated aromatic hydrocarbons is paralleled by K⁺ release. This suggests that chlorinated aromatic hydrocarbons may exert a destructive effect on the mitochondrial membrane.

The effects of chlorinated aliphatic hydrocarbons on the oxidative phosphorylation of rat liver mitochondria have been described previously by Ogata and Hasegawa⁹. However, no reports have so far considered chlorinated aromatic hydrocarbons.

In the present experiments, it was shown that aromatic hydrocarbons with three or four chloro atoms exerted stronger effects. The data indicated that in the case of mitochondrial oxidative phosphorylation, chloro atoms accelerated the inhibitory reactions.
M. OGATA, T. HASEGAWA, T. MORI AND T. MEGURO

Isomers also showed different grades of inhibition. The precise mechanism of the effects of these chlorinated aromatic hydrocarbons on liver mitochondrial oxidative phosphorylation will be investigated in the near future.

ACKNOWLEDGEMENT

This work was supported in part by a grant from the Ministry of Education, Japan, Agency No. 503077.

REFERENCES