LOSS OF OXIDATIVE PHOSPHORYLATION IN MITOCHONDRIA ISOLATED FROM KIDNEYS OF MERCURY POISONED RATS

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SUMMARY: Mercurials at low concentrations suppress oxidative phosphorylation in isolated rat kidney mitochondria. The kidneys of rats administered toxic doses of $HgCl_2$ have been found to accumulate mercury selectively compared e.g. to liver. Mitochondria isolated from such kidneys are defective in coupled ATP synthesis. Providing the time subsequent to the injection of a toxic dose of $HgCl_2$ does not exceed a critical time, the defect in the mitochondria isolated from the kidneys of such poisoned rats is correctable by addition of Mg^{++} to the assay medium. Acute mercury toxicity in rats appears to be referable to the impairment of kidney function in turn caused by impairment of mitochondrial function.

The ready interaction of mercurials with thiol groups of proteins, and the alterations in protein conformation and enzymic function which such interactions induce are very well documented phenomena (1, 2). The concentration levels of mercury at which different proteins react with the heavy metal is highly variable, and this variability together with the concentration gradients introduced by the circulation system underlies the selective action of mercurials when administered to a whole animal. Kidneys selectively accumulate relatively large amounts of mercury compared to other organs (3-6); and within the kidney, the cortex accumulates more mercury than the medulla (7,8). Even within individual cells there is a gradation in the distribution of mercury (9, 10). Mitochondria are among the subcellular organelles with a relatively high capability for accumulating significant levels of mercury (11). In view of the critical role of the mitochondrion in generating ATP in all aerobic cells, it is a reasonable presumption that mercurial toxicity would necessarily involve mitochondrial dysfunction in the target organs where accumulation of mercury is greatest. But this correlation has

TABLE I. Effect of $HgCl_2$ on the P/O ratios of isolated rat kidney mitochondria.*

$HgCl_2$ added to the medium nmoles/mg protein	P/O
0	1.50 ± 0.10
6	0.62 ± 0.10
12	0.20 ± 0.10
18	0.05 ± 0.05

*The procedures for the isolation of rat kidney mitochondria and for the determination of P/O ratios are given in Materials and Methods. Mitochondria were incubated in the presence of $HgCl_2$ for 1-2 minutes.

never been examined experimentally.

Our laboratory has been concerned for some time with the effects of mercurials on mitochondrial structure, function, and control and we have been aware that the capability of rat kidney mitochondria for oxidative phosphorylation is suppressed at relatively low levels of mercury (Table I). Since kidney is known to be the principal and critical target organ in mercury poisoning in most experimental animals, we have attempted to determine in rats administered toxic doses of $HgCl_2$ whether the failure in kidney function could be a consequence of mitochondrial dysfunction. The present communication is addressed to this objection.

MATERIALS AND METHODS

Rats (Holtzman strain, 250-300g) were lightly anesthetized with ether and injected with a $HgCl_2$ solution (in 0.15 m NaCl) in the tail vein. After 4 or 24 hours, two rats were sacrificed and the kidneys pooled for the preparation of mitochondria. Results reported were carried out in three separate experiments. Mitochondria from kidneys of untreated and treated rats were isolated by the procedure of Johnson and Lardy (12) in a medium 0.25 M in sucrose and 10 mM in Tris-Cl, pH 7.4. The amount of ATP synthesized (nmoles)

per amount of oxygen consumed (natoms) (P/O ratio) was determined at 30° by allowing the mitochondria to consume a known amount of O_2 in a closed reaction cell in a medium 80 mM in KCl, 50 mM in sucrose, 10 mM in Tris-Cl, pH 7.4, 10 mM in potassium succinate, 10 mM in potassium phosphate (^{32}P), 1 mM in ADP and 5 mM in glucose. The medium also contained 5 units of hexokinase per ml, rotenone (10 μM) and 1 mg of mitochondrial protein. When $MgCl_2$ was present in the medium, the final concentration was 5 mM. The reaction was stopped by the addition of trichloroacetic acid (final concentration 2.0%). Esterified phosphate was separated from nonesterified phosphate by the procedure of Lindberg and Ernster (13) and the amount of esterified phosphate (^{32}P) determined as described previously by Lee *et al.* (14). Oxygen concentration was monitored with a Beckman oxygen analyzer and oxygen sensitive electrode. The concentration of merucry was determined by atomic absorption spectroscopy (15). Protein was determined by the Biuret procedure described by Gornall *et al.* (16).

RESULTS AND DISCUSSION

The toxic dose administered to the experimental animals (3 mg Hg^{++} in the form of $HgCl_2$ per kg rat) was fatal to 90% of the animals in 2 to 4 days. The poisoned animals were sacrificed after 4 hours, and a macroscopic examination of various organs revealed extensive alteration in the appearance of the kidneys (extensive hemmorhage) but normal appearance of other organs (heart, liver, spleen). Mitochondria were isolated from the kidneys of poisoned animals at 4 and 24 hours post-injection and these were compared with the mitochondria isolated from the kidneys of untreated animals. The results are summarized in Table II. Kidney mitochondria from untreated animals show almost theoretical efficiency in coupling succinate-dependent electron transfer to synthesis of ATP (P/O ratio of about 1.5). It is to be noted that the presence of Mg^{++} in the assay medium was not required for oxidative phosphorylation and did not significantly affect the values of the P/O ratios when the mitochondria were isolated from untreated animals. Kidney mitochondria

TABLE II. Effect of intravenous injection of $HgCl_2$ on the P/O ratios of mitochondria isolated from rat kidney.*

$HgCl_2$ injected mg Hg^{++}/Kg body wt.	Injection of mercurial (Hrs.)	P/O $-Mg^{++}$	P/O $+Mg^{++}$
0	-	1.4 ± 0.2 (3)	1.5 ± 0.2 (5)
3.0	4	0.05 ± 0.03 (3)	1.4 ± 0.2 (3)
3.0	24	<0.01 (3)	<0.01 (3)

*Methods for the intravenous injection of $HgCl_2$, isolation of kidney mitochondria and determination of P/O ratios are described in Materials and Methods. Number in parenthesis indicates the number of times the particular experiment was repeated.

TABLE III. Mercury concentration in kidney mitochondria isolated from rats exposed to a lethal dose of $MgCl_2$.*

Treatment of the animal	Mercury in kidney mitochondria nmoles/mg
None	<0.05 (2)
$HgCl_2$ (3 mg Hg^{++}/kg)	3.70 - 5.40 (2)

*Mercury content of isolated rat kidney mitochondria was determined by the method described in Materials and Methods.

from the treated animals showed a quite different pattern. The P/O ratio in absence of added Mg^{++} in the assay medium was about 0.05 and the addition of Mg^{++} to the assay medium restored the value of the P/O ratio to the level of the control mitochondria. This result establishes that at 4 hours, the mitochondria from the kidneys of animals exposed to a toxic dose of mercury were incompetent in respect to oxidative phosphorylation but not irreversibly. Normal function could be restored by addition of 5 mM $MgCl_2$ to the assay medium. At 24 hours post-injection, the P/O ratio was essentially zero with

or without added Mg^{++} in the assay medium, and succinate or pyruvate plus malate respiration was over 90% inhibited. The stage of irreversible damage to the mitochondria had apparently been reached. Presumably, the same would apply to kidney function. At 4 hours, kidney function could be saved by appropriate correctional means. After 24 hours, these same means would be ineffectual. Elsewhere, we shall report that BAL can effectively neutralize the toxic effects of a dose of mercury equal to the dose administered in the present series of experiments providing it is injected no later than 4 hours after administration of the mercury.

We have determined the level of mercury in the mitochondria isolated from the kidney of animals administered a toxic dose of mercury (Table III). The levels are clearly in the range shown to impair oxidative phosphorylation of isolated kidney mitochondria. However, slightly higher levels of mercury are required *in vitro* to induce the same degree of uncoupling *in vivo*. The reason for this apparent discrepancy is revealed when the level of calcium in normal mitochondria after isolation is compared to the level in kidney mitochondria isolated from mercury treated rats (Table IV). The latter have three times more calcium than the former. It is this combination of calcium and mercury that effectively uncouples kidney mitochondria isolated from mercury poisoned rats. This is substantiated by the results of Table V which shows that in isolated mitochondria the combination of mercury and calcium is much more effective in uncoupling than either agent alone.

Two important conclusions may be drawn from these data. First, rat kidney can rapidly concentrate mercury injected intravenously to the point where within four hours a concentration level is reached sufficient to diminish or abolish the capacity for oxidative phosphorylation significantly. Second, the damage sustained by the mitochondria at this concentration level is not irreversible and therefore the defect is still capable of being corrected.

Two observations we have made in this study are highly relevant to the correlation between the toxic effects of heavy metals and the suppression of

TABLE IV. Calcium and magnesium in kidney mitochondria isolated from rats exposed to a lethal dose of $HgCl_2$.*

Treatment of animal	Mg^{++} natoms/mg protein	Ca^{++}
None	22 ± 12	38 ± 8
$HgCl_2$ (3 mg Hg^{++}/kg)	17 ± 2	112 ± 10

*Calcium and magnesium content of isolated rat kidney mitochondria was determined by atomic absorption spectrometry as described by (18).

TABLE V. Effect of calcium and mercury on the P/O ratio of isolated rat kidney mitochondria.*

Addition	P/O
None	1.26 ± 0.10
$CaCl_2$ (0.1-0.3 mM)	0.49 ± 0.05
$HgCl_2$ (5 nmoles/mg)	0.95 ± 0.10
$HgCl_2$ + $CaCl_2$	0.05 ± 0.02

*The method for the isolation of kidney mitochondria and the determination of P/O ratios are described in Materials and Methods.

mitochondrial function in the target organ. First, in the rat, the liver accumulates far less mercury per unit dry weight of protein than does the kidney under conditions of acute toxicity. Moreover, mitochondria isolated from the liver of rats administered a dose of mercury sufficient to damage the kidney and impair the function of kidney mitochondria show no demonstrable loss of capacity for oxidative phosphorylation. Second, when toxic levels of cadmium are administered to the rat, it is the liver and liver mitochondria which sustain damage; the kidney and kidney mitochondria are essentially normal four hours after injection (17). These collateral observations reinforce

our confidence that the correlation we have established between mercurial toxicity and mitochondrial dysfunction in rat kidney is highly significant. On the basis of this close correlation, we are proposing that the dysfunction of kidney mitochondria is indeed the primary cause of mercury toxicity in rats.

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