Acta Medica Okayama

Volume 32, *Issue* 6 1978 *Article* 2

DECEMBER 1978

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Masana Ogata[*] Tohoru Hasegawa[†]

Yoshio Yamazaki[‡] Yusaku Nogami[**]

[*]Okayama University,
[†]Okayama University,
[‡]Okayama University,
[**]Okayama University,

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Abstract

The effect of Cd2+ on the respiration of rat liver mitochondria was investigated. The uncoupling effect of Cd2+ was partially restored by the addition of Mg2+. The influence of Cd2+ on adenine nucleotide concentrations in the reaction mixture consisting of mitochondria and ATP was also studied using high performance liquid chromatography. In the presence of added Mg2+, a two-fold increase in AMP concentration was brought about by the addition of Cd2+. There was a concomitant decrease in ATP. In the prence of added ADP, an increase in AMP concentration was also brought about by addition of Cd2+. The results are discussed in relation to ATPase and adenylate kinase activity in mitochondria.

KEYWORDS: cadmium ion, ATPase, adenylate kinase, mitochondria

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*PMID: 154823 [PubMed - indexed for MEDLINE]

Acta Med. Okayama **32**, (6), 387—392 (1978)

EFFECT OF CADMIUM ON CHANGES IN CONCENTRATION OF ADENINE NUCLEOTIDES INDUCED BY MITOCHONDRIA

Masana OGATA, Tohoru HASEGAWA, Yoshio YAMAZAKI and Yusaku NOGAMI

Department of Public Health, Okayama University Medical School, Okayama 700, Japan

Received July 25, 1978

Abstract. The effect of Cd^{2+} on the respiration of rat liver mitochondria was investigated. The uncoupling effect of Cd^{2+} was partially restored by the addition of Mg^{2+}. The influence of Cd^{2+} on adenine nucleotide concentrations in the reaction mixture consisting of mitochondria and ATP was also studied using high performance liquid chromatography. In the presence of added Mg^{2+}, a two-fold increase in AMP concentration was brought about by the addition of Cd^{2+}. There was a concomitant decrease in ATP. In the prence of added ADP, an increase in AMP concentration was also brought about by addition of Cd^{2+}. These results are discussed in relation to ATPase and adenylate kinase activity in mitochondria.

Key words: cadmium ion, ATPase, adenylate kinase, mitochondria

It is well known that Cd^{2+} uncouples oxidative phosphorylation in mitochondria (1), stimulates latent ATPase activity (2, 3), and induces K^+-release from mitochondria (4). This uncoupling action of Cd^{2+} is almost completely prevented by ruthenium red (5), a known inhibitor of Ca^{2+}-transport in mitochondria (6). The release of K^+ induced by Cd^{2+} was prevented by exogenous Mg^{2+} also. It appears, therefore, that the effect of Cd^{2+} on mitochondrial function is antagonistic to Mg^{2+} and Ca^{2+}. On the other hand, the appearance of AMP in the reaction mixture has been reported by Iida, following stimulation of latent mitochondrial ATPase activity by Cd^{2+} (3). Using high performance liquid chromatography (HLC), we investigated the mutual effects of Cd^{2+} and Mg^{2+} on oxidative phosphorylation and also investigated the effect of Cd^{2+} on adenine nucleotide concentrations.

MATERIALS AND METHODS

Animals and preparation of mitochondria. Male Donryu rats weighing approximately 200g and fed on a laboratory stock diet were used. The rats were sacrificed by decapitation: liver mitochondria were isolated according to a modification (7) of the method of Hogeboom *et al* (8). The isolated mitochondria were suspended in ice-cold 0.25 M sucrose and 3 mM Tris-HCl buffer (pH 7.5). Mitochondrial protein was determined by the biuret method.

Measurement of respiratory activity. Rat liver mitochondria (2.5 mg protein/ml) were incubated in 3.5 ml of a reaction mixture containing 0.15 M KCl, 10 mM Tris-HCl buffer (pH 7.5), 2.5 mM phosphate buffer (pH 7.5), 5 mM sodium succinate and 300 μM ADP. Oxygen uptake was measured with a Galvanic oxygen electrode (Kyusui Kagaku Kenkyusho Co., Ltd., Tokyo) connected to an autorecorder.

Quantitative determination of AMP, ADP, and ATP by high performance liquid chromatography. Chromatography was carried out with a Hitachi high performance liquid chromatograph (HLC), model 635, equipped with a UV photometer at 254 nm. The output of the UV detector was recorded on a Shimazu 10 mv recorder at a chart speed of 10 mm/min. The elution phase was 0.5 M KH_2PO_4 solution, and column pressure was 75 Kg/cm^2.

Measurement of adenine nucleotide concentration of mitochondria. Concentrations of adenine nucleotides were measured by determining ATP, ADP and AMP in 2.0 ml of the reaction mixture containing 0.15 M KCl, 10 mM Tris-HCl buffer (pH 7.5), 3 mM ATP and mitochondria (1.5 mg protein/ml) at 25°C. The reaction was started by the addition of mitochondria in the presence of ATP. After 10 min, the reaction was stopped by the addition of ethanol to a final concentration of 50 percent (v/v). The reaction mixture was then centrifuged for 5 min at 3000 rpm, and the resulting supernatant was used for the analysis of adenine nucleotides.

In the presence of ADP, the reaction mixture for the mitochondrial reaction contained, in a final volume of 1.0 ml, 0.25 M sucrose, 4 mM Tris-HCl buffer (pH 7.5), 0.34 mM ADP and mitochondrial suspension (1.25 mg protein/ml). The reaction was started by the addition of ADP and conducted for 15 min at 25°C. The reaction was stopped by the addition of 1.0 ml of ethanol. Adenine nucleotides were analyzed by the same method as for the experiment where ATP was added to the mitochondrial suspension.

RESULTS

The effect of Mg^{2+} on the mitochondrial uncoupling induced by Cd^{2+}. The effect of Mg^{2+} on the uncoupling of respiratory activity induced by Cd^{2+} was investigated. The result is shown in Fig. 1A. The addition of Cd^{2+} alone showed respiratory release indicating the uncoupled state of mitochondria. Respiratory control was observed in the presence of both Cd^{2+} and Mg^{2+}. The results of a quantitative study of Cd^{2+} and the respiratory control index (RCI) are shown in Fig. 1B. RCI decreased with increase in Cd^{2+}, with or without Mg^{2+}. The decrease in RCI was lower in the presence of Mg^{2+} than when Mg^{2+} was absent.

The effect of Cd^{2+} on the concentration of adenine nucleotides in rat liver mitochondria. The effect of Cd^{2+} on the concentration of adenine nucleotides was investigated in the presence of Mg^{2+}. Fig. 2A is a typical HLC pattern obtained after incubation of a reaction mixture containing mitochondria and ATP for 10 min at 25°C. It shows the disappearance of ATP and the appearance of ADP

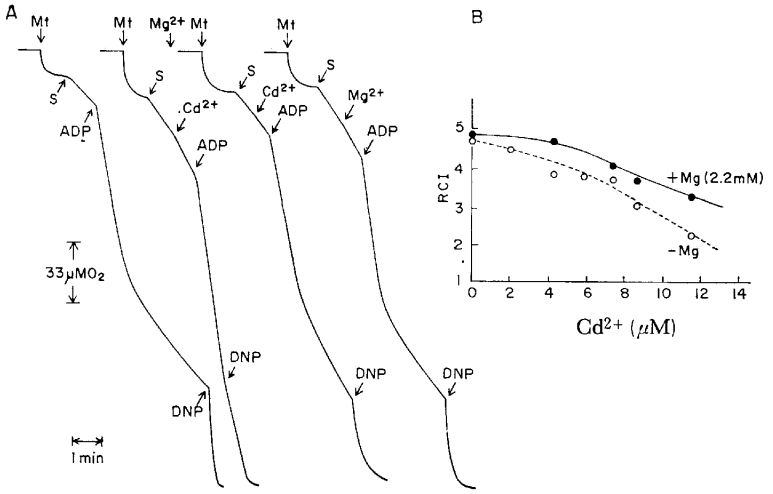

Fig. 1. The effect of Mg^{2+} on uncoupled respiratory activity of rat liver mitochondria induced by Cd^{2+}. A. Actual curves of mitochondrial respiration in the presence of Cd^{2+} and in the presence of Cd^{2+} plus Mg^{2+}. Rat liver mitochondria (2.5 mg protein/ml) were incubated in 3.5 ml of a reaction mixture containing 0.15 M KCl, 10 mM Tris-HCl buffer (pH 7.5) and 2.5 mM phosphate buffer (pH 7.5). This was followed 1 min later by 5 mM sodium succinate as substrate and then 300 μM ADP and 20 μM DNP at certain intervals. The final concentrations of Cd^{2+} and Mg^{2+} were 20 μM and 5 mM, respectively. Incubation was carried out at 25°C with continuos stirring. B. Concentration dependence of the effect of Mg^{2+} on the uncoupled respiratory activity of rat liver mitochondria induced by Cd^{2+}. Experimental conditions were the same as for Fig. 1A. Respiratory control indices (RCI = state 3/state 4) were calculated from the traces of oxygen uptake recorded by the method of Hagihara (17).

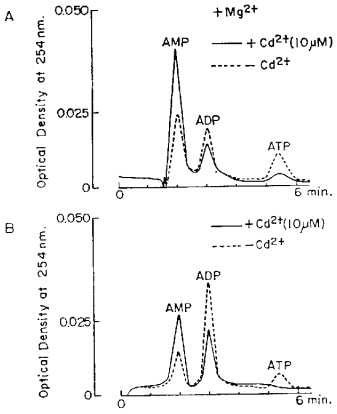

Fig. 2. Typical HLC pattern of the reaction mixture of the mitochondria. Chromatography was carried out with a Hitachi high performance liquid chromatograph. A and B indicate the patterns of the reaction mixture after addition of ATP and ADP to mitochndria.

and AMP. The relationship between the concentration of Cd^{2+} and that of nucleotides after the addition of ATP to mitochondria is shown in Fig. 3. A

decrease in ATP concentration occurred in parallel with increase in the concentration of Cd^{2+} in the presence of 5 mM Mg^{2+}. As shown in Fig. 3B and 3C, ADP decreased and an approximate two-fold increase in AMP concentration was brought about by the addition of Cd^{2+}.

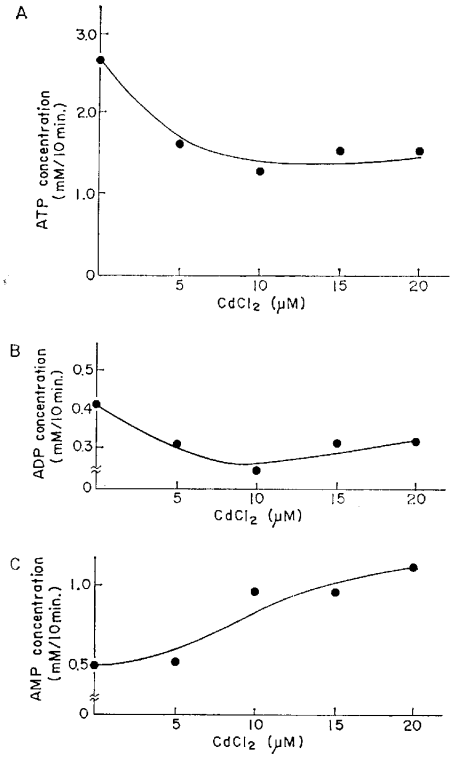

Fig. 3. Effect of Cd^{2+} on the ATP, ADP, and AMP concentrations in mitochondria. Rat liver mitochondria (1.5 mg protein/ml) were incubated in 2.0 ml of a reaction mixture containing 0.15 M KCl, 10 mM Tris-HCl buffer (pH 7.5) and 3 mM ATP as substrate for mitochondria. Ten min later the reaction was stopped by the addition of ethanol to a final concentration of 50 percent (v/v). The supernatant of the reaction mixture was analyzed for ATP, ADP and AMP. A. Change in ATP concentration with increase in Cd^{2+} concentration. B. Change in ADP concentration with increase in Cd^{2+} concentration. C. Change in AMP concentration with increase in Cd^{2+} concentration.

The effect of Cd^{2+} on ADP dismutation with mitochondria. In order to clarify the effect of Cd^{2+} on enzymes other than ATPase, changes in concentration of adenine nucleotides were studied by HLC of the reaction mixture containing 0.34 mM ADP and a variable concentration of Cd^{2+}. As shown in Fig. 2B and Fig. 4, 0.17 mM ADP, 0.08 mM AMP and 0.075 mM ATP occurred in the absence of Cd^{2+}. As shown in Fig. 4, a decrease in ADP concentration and an

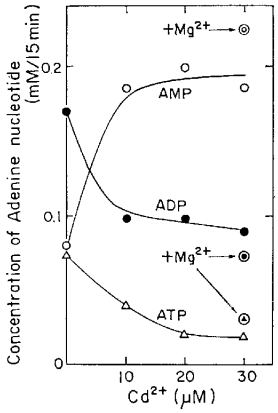

Fig. 4. The effect of Cd^{2+} on ATP, ADP and AMP concentrations in mitochondria with added ADP. Experimental conditions are described in Methods.

increase in AMP concentration occurred in parallel with increase in Cd^{2+} concentration. In the presence of Cd^{2+}, ATP was hydrolyzed by ATPase activated by Cd^{2+} and the decrease in ADP concentration was lower than the increase in AMP concentration. In the presence of 500 μM Mg^{2+}, the decrease in ADP and the increase in AMP concentrations were similar to those in the absence of Mg^{2+}.

DISCUSSION

Diamond *et al* (9) reported that liver mitochondria isolated from chronic cadmium poisoning had a decreased RCI. The results of the present experiment agree with Diamond's report. Our experiments also indicate that the decrease in RCI induced by Cd^{2+} was partially protected by Mg^{2+}, suggesting that Mg^{2+} protects the uncoupling action of Cd^{2+} on mitochondrial oxidative phosphorylation.

The decrease in the ATP concentration of mitochondria in a reaction mixture containing both Cd^{2+} and Mg^{2+} was accompanied by a decrease in ADP and an increase in AMP. This appearance of AMP is not due simply to an ATPase reaction stimulated by Cd^{2+}. It is suggested that besides ATPase, another reaction, such as an adenylate kinase reaction, may be involved. The presence of adenylate kinase in mitochondria has been reported by several authors (10–15).

As shown above, when ADP was incubated with mitochondria in the presence of Cd^{2+}, the decrease in ADP and the increase in AMP occurred within 15 min. Thus, it appears that, in the presence of Cd^{2+}, ADP was produced from ATP by ATPase activated by Cd^{2+}, and AMP was produced from ADP by an adenylate kinase reaction. ATP produced by the adenylate kinase reaction was hydrolyzed again by ATPase.

The production of AMP by mitochondria in the presence of Cd^{2+} may be due to source displacement of the equilibrium of adenylate kinase. Such displacement might be due to replacement, at least in part, of MgATP and/or MgADP by CdATP and/or CdADP (16), or to the binding of Cd^{2+} to the enzyme-protein. Further studies on this problem are needed.

Acknowledgment. The authors express their thanks to Dr. Toshihiko Ubuka, Department of Biochemistry, and to Dr. Kazuhisa Takeda, Department of Internal Medicine, Okayama University for their interest in this work.

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