ENHANCEMENT BY CHELATING AGENTS OF LEAD TOXICITY TO MITOCHONDRIA IN THE PRESENCE OF INORGANIC PHOSPHATE

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1. Introduction

Inorganic lead is harmful to plants and animals. A major subcellular result of Pb^{2+} administration to rats is altered mitochondrial structure, including loss of cristae, and impaired phosphorylation performance when these organelles are subsequently isolated [1–3]. In vitro mitochondria bind and accumulate Pb^{2+} in the absence of P_i [4,5], and this alters respiration and substrate uptake [4–8]. It has thus been suggested that mitochondria may be loci for Pb^{2+} toxicity in vivo [4,9]. However, despite the ubiquitous presence of phosphate in living organisms, many workers have stressed that in vitro the presence of this anion reduces or abolishes the effects of Pb^{2+} on mitochondria [4,6–9], because of the very low solubility product of lead phosphate (7.9×10^{-43}) [10], and the lack of interaction of the precipitate with mitochondria [9]. Thus, although the formation in the mitochondrial matrix of granules, probably containing lead, has been reported after Pb^{2+} administration in vivo [11] and in vitro [12] and ^{210}Pb accumulates in mitochondria [13], no studies have been made of the effects of this cation on mitochondrial phosphorylation in vitro, because this process requires added P_i. In this paper we report that Pb^{2+} does inhibit phosphorylation, despite the presence of P_i, and show that certain chelating agents, notably ATP, exascerbate the deterioration in phosphorylation performance.

2. Methods

We prepared rat heart mitochondria as previously described [14], taking care to remove the 'fluffy layer' which often covers the mitochondrial pellet. The final mitochondrial suspensions were supplemented with 0.5 mg purified bovine serum albumin/mg protein. We determined the effects of Pb^{2+} on the phosphorylation rates of mitochondria respiring on pyruvate and malate, although rates, P/O ratios and respiratory control ratios usually varied in concert, as illustrated in fig.2.

3. Results

Even in the pressence of 4.7 mM P_i, without added adenine nucleotide, phosphorylation rates decline when Pb^{2+} is added in excess of about 100 nmol/mg protein (fig.1). The deterioration also increases with the time elapsing between Pb^{2+} administration and the addition of ADP. In these respects, Pb^{2+} resembles Ca^{2+}, but the two differ very significantly in the effect of ATP on the damage which they cause. Phosphorylation of exogenous ADP before addition of Ca^{2+} lessens the decrease in phosphorylation rate caused by this cation in the presence of P_i [15], but the decrease caused by Pb^{2+} is much greater when it is added after an ADP cycle, and the extent of the deterioration is directly related to the amount of ADP phosphorylated before the administration of Pb^{2+} (fig.2). We observed a similar effect on adding ATP as such, and found that when Pb^{2+} was added without the prior phosphorylation of ADP, of two subsequent phosphorylation cycles, the second was much worse than the first, due, we propose, to the ATP produced by the first cycle. ADP enters mitochondria in exchange for internal ATP, which is also in dynamic equilibrium with external ATP [16]. Since

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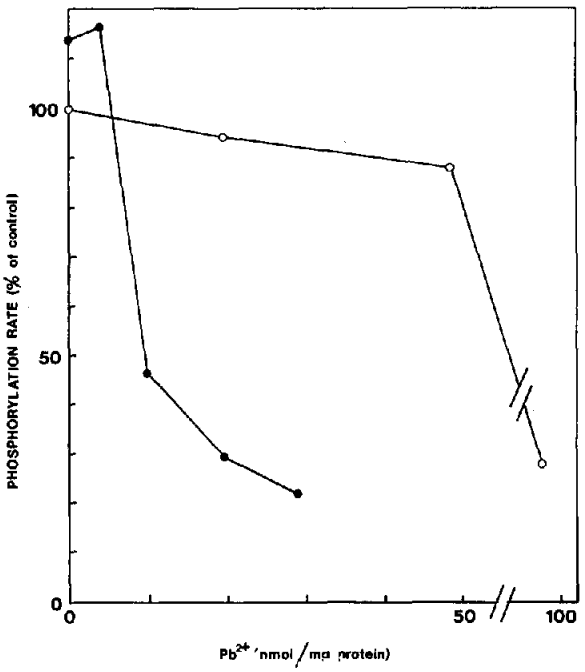

Fig.1. Effect of Pb^{2+} on the phosphorylation rate of rat heart mitochondria before and after the phosphorylation of added ADP. 2.6 mg of mitochondrial protein was incubated in 150 mM KCl, 10 mM Tris HEPES buffer at pH 7.0, and the Tris salts of pyruvate, malate and phosphate, each at 4.7 mM, in a total volume of 3.25 ml at 27°C in a Clarke-type oxygen electrode. Various amounts of lead were added as 5 mM $Pb(NO_3)_2$ either with (•) or without (○) the prior phosphorylation of 0.6 μmol of ADP. ADP was always added exactly 2 min after the Pb^{2+}. Phosphorylation rates are expressed as percentages of the control rate obtained before the addition of Pb^{2+}. When a prior phosphorylation cycle was included, this was used as the control; when it was not included the control was achieved by a separate run. The mean phosphorylation rate in the absence of Pb^{2+} was 505 nmol ADP/mg protein/min.

ATP also has a much higher affinity for Pb^{2+} than ADP [10], we believe that it is principally ATP which causes this increase in lead toxicity to mitochondria, through the solubilising effect of the formation of a Pb–ATP complex. Such complexes are known to increase the effective solubility of $Pb_2(PO_4)_3$, or the more likely species $PbHPO_4$ [17], and we confirmed this by atomic adsorption spectroscopy. Fig.1 shows that the prior phosphorylation of 0.6 μmol ADP reduces the amount of Pb^{2+} which causes a decrease in phosphorylation rate in the presence of P_i by about an order of magnitude. We do not believe that ATP is acting as an additional energy source, thus increasing Pb^{2+} uptake, because of the similar effect achieved with other complexing agents, such as sucrose and dextran [18].

Citrate, another natural chelating agent which protects heart mitochondria against Ca^{2+} damage in the presence of P_i, by reducing both the rate and extent of its uptake [15], also increases the damage done by Pb^{2+} in the absence of added adenine nucleotide, although less so than does ATP (table 1). The presence of citrate does not result in additional

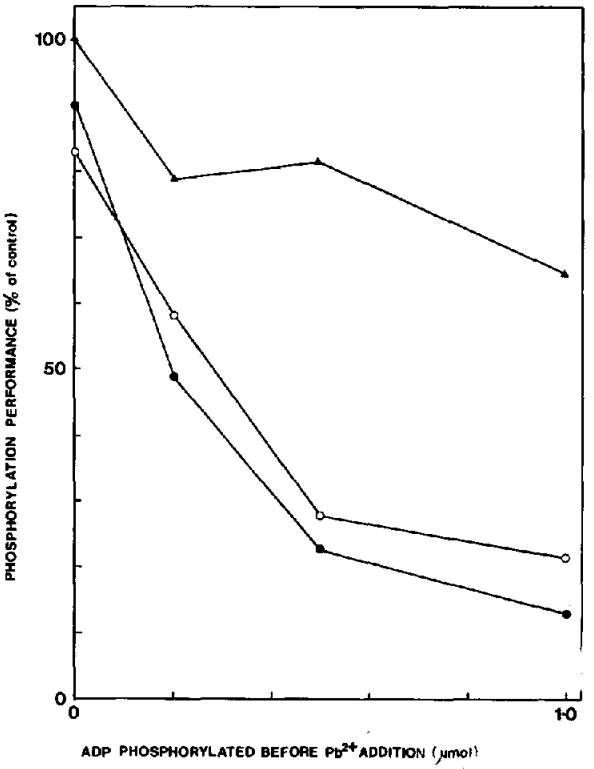

Fig.2. Effect of the length of a prior phosphorylation cycle on the Pb^{2+}-induced deterioration in phosphorylation performance of rat heart mitochondria 1.6 mg of mitochondrial protein was incubated in the conditions described in the legend to fig.1. Various amounts of ADP were phosphorylated, then 100 nmol $Pb(NO_3)_2$ was added, and after exactly 2 min, a further 0.5 μmol ADP was added. The phosphorylation rates (•), P/O ratios (▲) and respiratory control ratios (○) of the second ADP cycles are expressed as percentages of the mean values achieved in five experiments in which 0.5 μmol of ADP was added to mitochondria in the absence of Pb^{2+}.

Table 1
Effect of citrate on Pb^{2+} inhibition of mitochondrial phosphorylation rate in the presence of P_i, with and without the phosphorylation of previously added ADP

Citrate concn. (mM)	Phosphorylation rate after Pb^{2+} as % of rate before Pb^{2+}	
	Rates ± Pb^{2+} determined from different runs, i.e. effect of Pb in the absence of added adenine nucleotide	Rates ± Pb^{2+} determined from the same run, i.e. effect of Pb in the presence of added adenine nucleotide
0.0	52	16
2.3	30	25
4.5	27	23
9.0	–	17

2.0 mg of mitochondrial protein was incubated in the conditions described in the legend to fig.1. ADP was added 0.6 μmol at a time, before and/or after 250 nmol of Pb^{2+}; the ADP was added exactly 2 min after the Pb^{2+}. When a prior phosphorylation cycle was included, this was used as the control; when it was not included, the control was achieved by a separate run. The mean phosphorylation rate in the absence of Pb^{2+} was 588 nmol ADP/mg protein/min.

damage when Pb^{2+} is administered after ADP, and small amounts of citrate even have a small protective effect against the increased damage due to ATP (table 1). Heart mitochondria, in the presence of malate, take up citrate very slowly [19] which is why the increased Pb^{2+} damage caused in the presence of this anion is less than with ATP. The fact that whilst chelating some Pb^{2+} in the medium, citrate largely remains there explains why, in the presence of preformed ATP, it has a limited protective effect. With rat liver mitochondria, into which citrate enters more freely [19], preliminary experiments indicate that it causes a greater increase in Pb^{2+} damage.

Unlike ATP and citrate, EDTA, which forms a very stable chelate with Pb^{2+} [10], prevents the effect of Pb^{2+} on phosphorylation performance. Since EDTA is unable to cross the inner mitochondrial membrane, this suggest that Pb^{2+} must not only be in a soluble form to inhibit phosphorylation, but must also be able to cross the inner membrane. The protection afforded by EDTA to mitochondria contrasts with the increased transfer across the plasma membrane which this agent is reported to cause [20], perhaps by pinocytosis [21], and suggests that the use of similar compounds in the treatment of Pb^{2+} poisoning will not damage mitochondria.

4. Discussion

We have shown that despite the presence of P_i, low concentrations of Pb^{2+} do damage mitochondria, much more than might be expected from a simple consideration of the solubility of its phosphate, due to the solubilising action of natural chelating agents, especially ATP. Such an effect must be particularly great in cells and conditions in which the cytoplasmic ATP levels are high. One of the first symptoms of plumbism in man is anaemia, and Pb^{2+} inhibits several of the mitochondrial steps in haem biosynthesis. Enzymes affected by low levels of Pb^{2+} in vivo are located in the blood or the plasma membrane [22–24]. This, coupled with the ability of mitochondria to accumulate Pb^{2+} is consistent with our suggestion that after passage through the plasma membrane, which may occur with ease [13,25], intracellular Pb^{2+} will preferentially affect mitochondrial enzymes and function, although non-mitochondrial enzymes may be inhibited in vitro [26]. This contrasts with the suggestion [21] that nuclear protein–Pb^{2+} inclusion bodies may be depots which effectively remove Pb^{2+} from the cytosol.

In a KCl medium with 2.5% dextran, and after the phosphorylation of 0.5 μmol ADP, we have shown a deterioration in phosphorylation rate in the presence of Pi after the administration of as little as 1 nmol Pb^{2+}/mg protein. Thus, assuming 35 mg mitochondrial protein/g cardiac tissue [27], about 7 ppm of Pb^{2+} in heart tissue could affect phosphorylation, if it was all located in mitochondria, as evidence suggests it may be [13,25]. Although such concentrations were not found in heart muscle of normal or exposed individuals by Barry [28], they were found in other soft tissues, notably the aorta, and recalculation of data for occupationally exposed men recorded by Petkou et al. [29] gives values for Pb^{2+} in heart tissue of about 3.5 ppm. It is quite possible that conditions are such in vivo that lower levels of Pb^{2+} have effect, and that in our experiments some Pb^{2+} was precipitated even in the presence of ATP. We therefore sug-

gest that the etiology of Pb^{2+} toxicity, including possible neurological and behavioural disorders at what are regarded as normal levels, may have their origins in effects on mitochondria.

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