

Lead potentiates iron-induced formation of reactive oxygen species

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Abstract

There are reports that lead may promote free-radical initiated events in biological tissue. However, there are also reports on the inability of lead salts to stimulate the production of reactive oxygen species in isolated systems. Furthermore, there is no well understood rationale as to why lead should exhibit pronounced pro-oxidant properties. We are reporting that while lead acetate does not initiate any excess generation of reactive oxygen species in a cerebral synaptosomal suspension, it has a marked ability to enhance the pro-oxidant properties of ferrous iron in the same system. This property was maximal at a lead concentration of 0.5 mM when major precipitation of lead salts occurred. Therefore, it may reside in the ability of iron to form an active chelate on the surface of insoluble lead salts. Such an interaction may account for the discrepancies in the literature concerning the relation between lead toxicity and oxidative stress.

Keywords: Lead; Oxidative stress; Iron; Reactive oxygen

1. Introduction

The basis of the toxicity of lead is undoubtedly multifactorial, involving disruption of a variety of biological processes rather than a single locus of action [1]. The possibility that induction of excess generation of free radicals may in part account for lead toxicity has been repeatedly raised. Evidence for this comes from human exposures which have been associated with elevated levels of superoxide dismutase, presumably induced by oxidative stress [2], and by animal studies [3,4]. Organic lead compounds have also been reported to

elevate levels of lipid peroxidation in tissues of treated animals [5–7]. However, demonstration of the pro-oxidant capacities of lead using isolated preparations has not been unequivocally made; lipid peroxidation can in fact be inhibited under such circumstances [7]. Lead has only a moderate affinity for sulfhydryl groups and does not readily undergo valence changes characteristic of transition metals. Therefore, the mechanism underlying the ability of this metal to promote oxidative stress in lead-exposed tissues, is unclear.

The intent of the current study was to attempt to reconcile the ability of lead to promote lipid

peroxidation in vivo with the failure to demonstrate such a phenomenon in vitro. In a prior study, we have found that aluminum, while it possessed no capacity to stimulate formation of free radicals, was able to potentially enhance the rate at which iron salts stimulated generation of reactive oxygen species [8]. The current report describes how lead is capable of a parallel promotion of pro-oxidant activity when iron is concurrently present.

2. Materials and methods

2.1. Tissue preparation

Male Sprague–Dawley rats (Charles River Laboratories, Wilmington, MA) weighing 150–175 g were utilized. Rats were decapitated, the brains were excised quickly on ice, and the cerebrocortex was dissected out. Tissue was weighed and homogenized in 10 vols. of 0.32 M sucrose and centrifuged at $1800 \times g$ for 10 min. The resulting supernatant fraction was then centrifuged at $31\,500 \times g$ for 10 min to yield the crude cerebral synaptosomal pellet (P2). The P2 pellet was taken up in HEPES buffer to a concentration of 0.1 gequiv./ml. The composition of the HEPES buffer was (mM): NaCl, 120; KCl, 2.5; NaH_2PO_4 , 1.2; MgCl_2 , 0.1; NaHCO_3 , 5.0; glucose, 6.0; CaCl_2 , 1.0; and HEPES, 10; pH 7.4.

2.2. Assay for oxygen reactive species formation

Reactive oxygen species were assayed using 2',7'-dichlorofluorescein diacetate (DCFH-DA), which is de-esterified within cells to the ionized free acid, dichlorofluorescein, DCFH. This is trapped within cells and thus accumulated [9]. DCFH is capable of being oxidized to the fluorescent 2',7'-dichlorofluorescein by reactive oxygen. The utility of this probe in isolated subcellular cerebral systems has been documented [10]. P2 suspensions were diluted in 9 vols. of HEPES buffer. The diluted fractions were then incubated with $5 \mu\text{M}$ DCFH-DA (added from a stock solution of 0.5 mM in 10% ethanol) at 37°C for 15 min. After this loading with DCFH-DA, the frac-

tions were incubated for a further 60 min in the presence of various metallic compounds. At the beginning and at the end of incubation, fluorescence was monitored on a Farrand spectrofluorometer, with excitation wavelength at 488 nm (bandwidth 5 nm), and emission wavelength 525 nm (bandwidth 20 nm). The rate of generation was found to be linear over the incubation period [10]. Autofluorescence of fractions was corrected for, by the inclusion in each experiment of parallel blanks with no DCFH-DA. The correction for autofluorescence was always less than 11% of the total. Oxygen reactive species formation was quantitated from a 2',7'-dichlorofluorescein (DCF) standard curve (0.05–1.0 mM) and results were expressed as nmol DCF formed/15 min/mg protein.

2.3. Materials

2',7'-Dichlorofluorescein diacetate was purchased from Molecular Probes, Inc. (Eugene, OR), while DCF required for calibration was obtained from Polysciences, Inc. (Warrington, PA). Other materials were from Sigma Co., St. Louis, MO.

2.4. Protein determination

Protein concentration was assayed using the method of Bradford [11].

2.5. Statistical analyses

Differences between groups were assessed by one-way Analysis of Variance followed by Fisher's Least Significant Difference Test. The acceptance level of significance was $P < 0.05$ using a two-tailed distribution.

3. Results and discussion

The rate of generation of reactive oxygen species within the cortical P2 fraction was unaltered in the presence of 0.5 mM lead acetate (Fig. 1). In accord with our previous results, there was a major increase in ROS production when $50 \mu\text{M}$

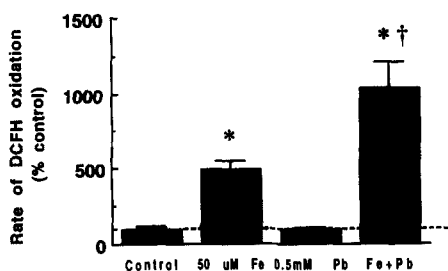


Fig. 1. Effect of ferrous sulfate and lead acetate upon rate of generation of reactive oxygen species within cortical P2 fraction. Each value represents the mean of five or six individual determinations \pm S.E. *Differs from corresponding control value. †Differs from value in presence of iron and absence of lead ($P < 0.05$). Basal level of activity was 3.46 ± 0.27 nmoles DCFH oxidized/mg protein/h.

FeSO₄ was added to the basal reaction mixture. However, when both lead acetate and FeSO₄ were concurrently added to the P2 fraction, the intensity of ROS formation was around twofold greater than that found with FeSO₄ alone (Fig. 1). Thus, lead acetate alone was completely unable to enhance ROS formation but had a pronounced ability to promote iron-initiated pro-oxidant events. A dose-response study of this interaction revealed a maximal promoting effect of lead at a concentration of 0.5 mM (Fig. 2).

In the absence of tissue, Fe-induced oxidation of DCFH was quantitatively minor, but the concurrent presence of 0.25 mM lead acetate signifi-

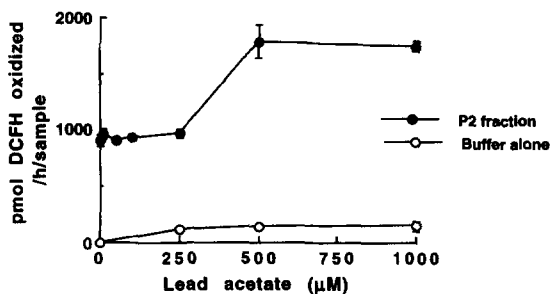


Fig. 2. Rate of generation of reactive oxygen species in presence of 50 μ M FeSO₄ and varying concentrations of lead acetate. Each value represents the mean of four or six individual determinations \pm S.E. When absent, standard errors are smaller than symbols. When P2 fraction was present, each sample contained 75 mg protein. Basal level of activity of P2 fraction in absence of added lead or iron was 34.6 ± 27 pmol DCFH oxidized/mg protein/h.

cantly increased this (Fig. 2). While the total effect was small, the presence of 0.25 mM lead in this case stimulated Fe-induced ROS formation by over 10-fold. However, the effect of lead acetate in the presence of FeSO₄ was much greater when tissue was present than in its absence. Thus potentiation observed was not completely dependent upon the presence of biological tissue but also reflects direct interactions between the metal species. When a parallel study was carried out in the absence of tissue and buffer, substituting distilled water, no ROS formation under any circumstances was detectable (data not shown). In this case, lead acetate formed no precipitate, the final incubation pH being 5.4–6.8, suggesting that the critical interaction is between ferrous ions and an insoluble lead matrix.

This finding may relate to the report that iron-promoted free radical generation may occur at the solid/liquid interface of certain insoluble iron-containing minerals [12]. The mechanism underlying such an interaction between two metals is not obvious. Lead acetate in the presence of HEPES buffer is partially precipitated and the formation of an insoluble complex may be critical. Asbestos fibers are able to potentiate iron-induced hydroxyl radical formation from hydrogen peroxide both in tissue free aqueous media and in isolated macrophages [13,14]. However, iron-related ROS generation was not potentiated by other insoluble materials such as kaolin [8].

A similar enhancement of iron-catalyzed lipid peroxidation has been reported in lead-treated rats, using subcellular fractions of liver and subsequent in vitro addition of 25 μ M Fe³⁺ [15]. However, these authors could find no such interaction in corresponding brain fractions. This difference may be due to the greater pro-oxidant potential of Fe²⁺ over Fe³⁺ (Bondy et al., 1996 unpublished data).

Recently, a synergistic interaction between glutamate and lead has been reported in the promotion of DCFH oxidation by neuroblastoma cells [16]. This was attributed to lead-induced activation of protein kinase C. For this reason, the interaction of lead and iron in the presence of a peptide inhibitor of this enzyme (Thr-Thr-Tyr-Ala-Asp-Phe-Ile-Ala-Ser-Gly-Arg-Thr-Gly-Arg-

Arg-Asn-Ala-Ile-His-Asp . [17]), was examined. However, 0.5 $\mu\text{g/ml}$ of this inhibitor had no effect on the ability of lead to stimulate iron-induced ROS production (data not shown).

The potentiation of oxidative events by a lead salt, evident only in the presence of low levels of Fe^{2+} , may account for the apparent discrepancy between the pro-oxidant properties of lead treatment of intact animals reported on several occasions (described in the introduction) and the paucity of evidence of lead's ability to promote free radical generation in isolated systems. The type of interaction described here also suggests that iron levels may be a significant factor in relating to the severity of lead poisoning.

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