The Effect of Lead on Astroglial Glutamate Uptake

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Low-level lead has been shown to cause cognitive and attention deficits in children. Research has focused on elucidating the underlying molecular and biochemical mechanisms of these abnormalities. In recent years, the importance of astroglia in neurological diseases has been realized and they are no longer merely considered passive cells. Because astroglia have previously been shown to sequester lead, this study examined the effects of lead on an important astroglial function: glutamate uptake. In immature astroglia treated with lead for 1 and 7 days, glutamate uptake at 10 minutes was increased beyond control levels. However, mature astroglia treated with lead for 14 days showed an initial increase in glutamate uptake at 5 minutes, then a decrease in uptake at 10 minutes. Overall, the change in glutamate uptake varied with age in culture. Further studies are warranted in order to determine areas of Pb insult and possible recovery mechanisms.

Introduction

Exposure to low-level lead is detrimental to the developing nervous system. Research has shown blood lead levels as low as 10 μg/100 ml to cause reduction in IQ scores, nerve conduction velocity, and attentiveness (Bellinger et al., 1989; Philip and Gerson, 1994). Although the brain is not the major lead depot, it does accumulate lead and has no known mechanism for lead removal (Tiffany-Castiglioni et al., 1996). Research has focused on the morphological, physiological and biochemical responses of neurons and glia to lead. In general, these studies have shown neurons to be more sensitive to lead than glial cells (Tiffany-Castiglioni et al., 1986). For example, astroglia take up and sequester lead from the extracellular medium (Tiffany-Castiglioni et al., 1986; 1987) but show little viability change with lead exposure (Holtzman et al., 1987). Holtzman et al. (1984) proposed the "lead sink" theory hypothesizing that astroglia sequester lead in order to protect more sensitive cells in the central nervous system (CNS). Astroglia are now believed to be key cells in neuronal survival after lead exposure and, consequently, have become the focus of our lead toxicity studies.

Astroglia have many functions in the CNS. Physically, astroglial foot processes surround 100% of vascular surfaces in the mammalian brain (Vaquera-Orte et al., 1981) and are necessary for inducing tight junctions between these endothelial cells (Janzer and Raff, 1987) thus forming the blood-brain barrier. Biochemically, astroglia regulate ion concentrations in the extracellular fluid (Dermietzel et al., 1991) and metabolize some neurotransmitters (Kimelberg, 1986). For example, glutamine synthetase (GS) is localized to astroglia in the brain (Norrenberg and Martinez-Hernandez, 1979) and is responsible for converting the excitatory neurotransmitter glutamate to the amino acid glutamine (Benjamin, 1983). As neurons release glutamate at synapses, it is taken up by astroglia, converted to

glutamine through GS, released, and can then be used as a glutamate precursor by neurons. This glutamate/glutamine cycle is important for neuronal survival; if glutamate is not removed from the extracellular space, it is excitotoxic to neurons causing impairment and death (Rosenberg, 1991; Rothstein et al., 1993).

Glutamate metabolism is a potential site for lead-induced injury in astroglia. GS activity decreases in lead treated astroglia (Sierra and Tiffany-Castiglioni, 1991; Engle and Volpe, 1990; Ronnback and Hansson, 1992) although the exact mechanism is unknown. If GS activity decreases, there could be an increased concentration of glutamate in astroglia or in the extracellular space. In their review, Ronnback and Hansson (1992) reported that glutamate uptake also decreases in lead-treated astroglia. This could exacerbate an extracellular buildup of glutamate levels. The focus of this study was to determine if there is an age-dependent effect of lead on astroglial glutamate uptake.

Materials and Methods

Materials - [^{3}H]-glutamate was obtained from Amersham Laboratories (Elk Grove, IL). Waymouth's 705/1 and fetal bovine serum (FBS) were obtained from Gibco BRL (Grand Island, NY). Six-well plates were obtained from Baxter (The Woodlands, TX). Sucrose was obtained from VWR (Gibbstown, NJ). Tris nitrate was obtained from USB (Cleveland, OH). All other chemical reagents were purchased from Sigma (St. Louis, MO).

Cell Culture - Primary cultures of astroglia were prepared from the cerebral hemispheres of 0 to 1 day old Sprague Dawley rat pups (Charles River, Raleigh, NC) as described by McCarthy (1983) and modified by Tiffany-Castiglioni et al. (1987; 1989). Cells were cultured in Waymouth's 705/1 MD with 10% FBS and were plated into six-well plates. The

growth medium was replaced four days after plating and three times per week thereafter.

Lead Treatment - A 1 mM stock solution of lead acetate (Pb) was prepared in sterile water. Lead treatment was initiated at 14 days (immature cells) or 21 days (mature cells) of culture. On the appropriate day, Pb was added to the growth medium at a final concentration of 1 μM and the cells were fed as usual. Cells were treated for up to 14 days with six samples per dose group per time point. In some experiments, glutathione or N-acetylcysteine was added to the medium to test the potential protective effects of these compounds. These results were inconclusive and are not reported.

[^{3}H]-Glutamate Uptake - Glutamate uptake was measured according to the radioisotope method of Aschner et al. (1990; personal communication). Cell cultures were analyzed for glutamate uptake on days 1, 7, and 14 of Pb exposure. Growth medium was decanted and the cell monolayers were washed three times with HEPES-buffered medium consisting of the following components: 122 mM NaCl, 3.3 mM KCl, 0.4 mM $MgSO_4$, 1.3 mM $CaCl_2$, 1.2 mM KH_2PO_4, 10 mM D(+)glucose and 25 mM HEPES. HEPES-buffered solutions were maintained at pH 7.4 by addition of 1N NaOH. The cultures were incubated in HEPES medium for 30 minutes in an incubator at 5% CO_2/95% air atmosphere. The medium was then aspirated and 0.5 ml of the same prewarmed medium containing radiolabeled glutamate was added to each well at a concentration of 0.2 μCi/well. Uptake was measured at room temperature for 0, 5, and 10 minutes. Uptake was terminated by removing the buffer and washing five times with approximately 1 ml of ice-cold stop solution consisting of : 0.29 M sucrose, 10 mM tris nitrate and 0.5 mM $Ca(NO_3)_2$, pH 7.4. The cells were solubilized in the wells in 1 ml of 1 N NaOH at room temperature for at least one hour.

Aliquots were taken for scintillation counting and protein quantification by the BCA method (Smith et al., 1985).

Statistical Analysis - All values are expressed as means ± standard error of the mean. Differences between treatments were analyzed statistically by Student's t-test (GraphPAD InStat, 1990).

Results

Uptake of glutamate was determined for both immature and mature astroglia. In immature astroglia, no significant change in glutamate uptake was seen at 5 minutes on days 1 and 7 of Pb treatment (Fig. 1 and 2). However, at 10 minutes, glutamate uptake was higher in Pb-exposed cells than in controls for both 1 ($p<0.05$) and 7 days of treatment. On day 14 of lead exposure, no change from control values was seen at 5 or 10 minutes (Fig. 3). In mature astroglia, glutamate uptake was decreased at both 5 and 10 minutes in cultures treated with Pb for one day, though it did not reach statistical significance (Fig. 4). At 7 days of treatment no change from control values in glutamate uptake was seen at either time point (Fig. 5). In contrast, exposure of mature astroglia to Pb for 14 days resulted in an initial increase at 5 minutes ($p<0.001$) and then a decrease at 10 minutes ($p<0.001$) of glutamate uptake (Fig. 6).

Discussion

Glutamate uptake was examined because of its possible role in neuronal damage. Lead exposure has been shown to decrease GS activity (Sierra and Tiffany-Castiglioni, 1991; Engle and Volpe, 1990; Ronnback and Hansson, 1992). If the glutamate/glutamine cycle

is disrupted and if glutamate remains in the extracellular space, it could be toxic to neurons. Our results show that the effects of Pb on glutamate uptake varied with the age of the culture and the time period over which glutamate uptake was monitored.

This variation can best be explained when the results are viewed with respect to the age of the cells in culture. In immature astroglia, Pb exposure caused an increase in glutamate uptake on days 1 and 7 of treatment (i.e. the cells were 15 and 21 days in culture respectively). Likewise, on day 1 of Pb exposure in mature astroglia (i.e. the cells were 22 days in culture), glutamate uptake was increased when compared to control levels at 10 minutes. However, in immature cells exposed to Pb for 14 days and mature astroglia exposed to Pb for 7 days (i.e. the cells were 28 days in culture) no change from control levels of glutamate uptake was seen. These data suggest a consistency in cell maturation in culture both in the presence and absence of Pb. Uptake for both sets of cultures varies presumably because many factors can affect astroglial maturation rates. Although astroglial cells isolated by the method described have been previously shown to be nearly pure astroglia (Tiffany-Castiglioni et al., 1987), any contamination from neurons, fibroblasts, or endothelial cells could change maturation rates of astroglial cultures (Hatten, 1985; 1987; Legare, 1995; Lerea and McCarthy, 1990). Therefore, another possible source of variation in glutamate uptake could also correlate with astroglial development.

Several studies have indicated that the brain is vulnerable to lead exposure at various stages of development. Immature astroglia (less than 21 days) may not yet have the capacity to protect other cells in the CNS from injury. In fact, Tiffany-Castiglioni et al. (1996) showed that 7 day-old cultures were unable to sequester lead as efficiently as 21 day old cultures. Tiffany-Castiglioni (1993) also postulated that astroglia may become tolerant to Pb toxicity in view of their ability to sequester Pb with little viability change. One possible

lead tolerance mechanism in astroglia is increased glutathione (GSH) levels in the cytoplasm after Pb exposure (Legare et al., 1993). During our study, we also looked at the possibility of GSH acting as a defense mechanism to lead toxicity; however, the results were inconsistent and therefore have not been reported.

Our results were in contrast to those of Ronnback and Hansson (1992) who found that glutamate uptake decreased after a low level Pb exposure of 60 minutes. The possible explanations of this difference are numerous, with the most obvious of those being Pb exposure time. Other possible variations in the procedure include differences in: purity or age of astroglial cultures, growth medium, and time or concentration of [^{3}H]-glutamate exposure. Purity of astroglial cultures is important because, as mentioned earlier, the presence of other CNS cells in astroglial cultures affects maturity. The choice of growth medium is also important in that some media (such as MEM) contain little or no glutamate while Waymouth's 705/1 contains high glutamate levels (150 mg/L) which could affect glutamate transport. Finally, [^{3}H]-glutamate exposure time and concentration is important. For example, Ronnback and Hansson (1992) studied glutamate uptake using various concentrations of glutamate while we chose to use the method of Aschner et al. (1990) which studies glutamate uptake over time. Our assay also used only [^{3}H]-glutamate while that of Ronnback and Hansson (1992) used both [^{3}H]-glutamate and cold glutamate for a higher final concentration of glutamate in the uptake assay.

We have focused our analysis on our most robust findings. Problems with other time points (day 3 of treatment at each age, not shown) may reflect uneven rates of differentiation in the cell population at very young and intermediate ages. Another problem may be the rate at which glutamate is metabolized; our glutamate uptake times may have been too long if Pb alters efflux of glutamate metabolites. The 5 and 10 minute time points

were selected based on the work of Aschner et al. (1990) who reported that mercury reduces glutamate uptake in astroglia. In the future, possible variations of this study should be considered. Perhaps the time points of the glutamate uptake assay should be revised to include 1, 2.5 and 5 minutes. This revision would allow a closer study of initial glutamate uptake by the cell. Also, other treatment groups should be pursued, such as GSH, in order to elucidate areas of lead insult and possible mechanisms of recovery.

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Figure 1. ^{3}H-glutamate uptake after 1 day of Pb exposure in immature astroglia. The 10-minute uptake was significantly increased ($p<0.05$) in the Pb-exposed cells when compared to that of controls.

Figure 2. ^{3}H-glutamate uptake after 7 days of Pb exposure in immature astroglia. The 10-minute uptake was significantly increased ($p<0.05$) in the Pb-exposed cells when compared to that of controls.

Figure 3. ^{3}H-glutamate uptake after 14 days of Pb exposure in immature astroglia. Uptake was the same at all time points for both control and Pb-treated cells.

Figure 4. ^{3}H-glutamate uptake after 1 day of Pb exposure in mature astroglia. Uptake was reduced in the Pb-treated cells at both time points, though not significantly.

Figure 5. ^{3}H-glutamate uptake after 7 days of Pb exposure in mature astroglia. Uptake was the same at all time points for both control and Pb-treated cells. Compare this figure with Figure 3. See the discussion section for an explanation.

Figure 6. ^{3}H-glutamate uptake after 14 days of Pb exposure in mature astroglia. Uptake was increased ($p<0.001$) at 5 minutes and decreased ($p<0.001$) at 10 minutes in the Pb-exposed cells.

Figure 1

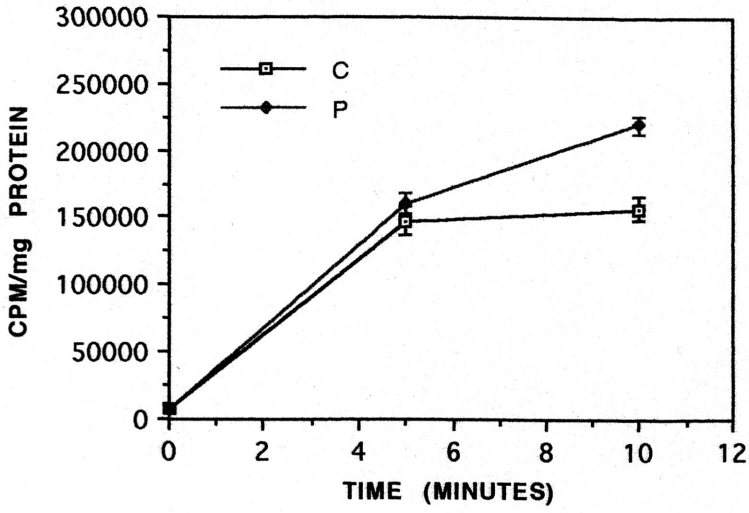

Figure 2

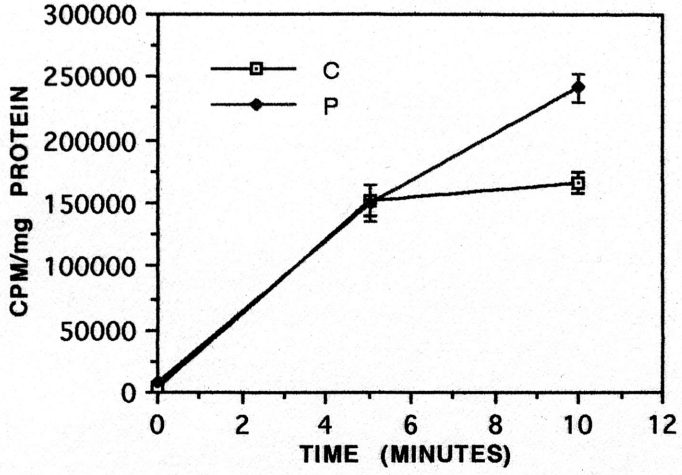

Figure 3

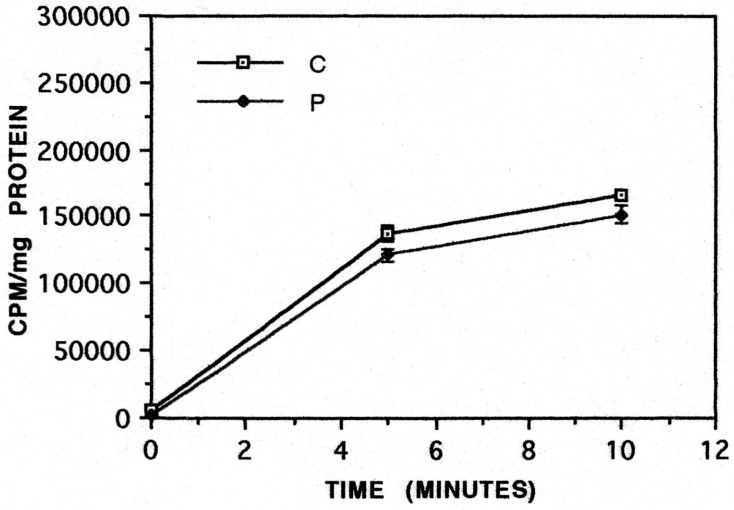

Figure 4

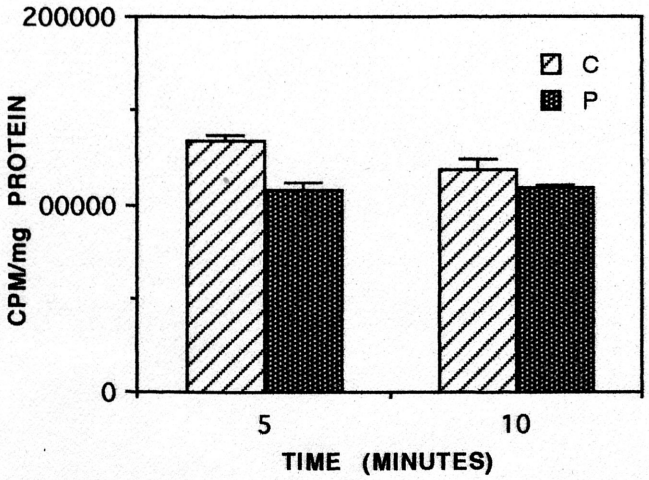

Figure 5

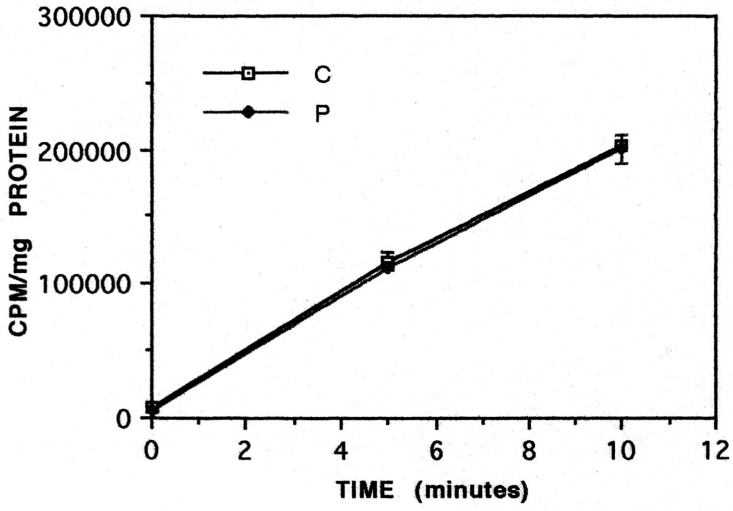

Figure 6