Lead (Pb) exposure and its effect on APP proteolysis and Aβ aggregation

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SPECIFIC AIMS

Alzheimer's disease (AD) is characterized by excessive deposition of aggregated β-amyloid (Aβ) peptide of 40-42 residue, which is derived from the amyloid precursor protein (APP) following processing by β-secretase and γ-secretase. We have reported that developmental exposure to Pb up-regulates the expression levels of APP and its amyloidogenic Aβ products late in life. This provided the first evidence for the developmental and environmental link for disturbances in AD-associated proteins. Our aim was to examine whether latent up-regulation in APP expression and Aβ levels are exacerbated by concurrent disturbances in APP processing or Aβ aggregation.

PRINCIPAL FINDINGS

1. Nanomolar levels of Pb promote Aβ aggregation

Increased APP expression and/or processing are believed to accelerate AD pathogenesis in humans and animal models of AD due to the increased production of the amyloidogenic peptides $A\beta_{1\text{-}40}$ and $A\beta_{1\text{-}42}$. Environmental agents could exacerbate such a situation by directly promoting Aβ aggregation; therefore, we decided to examine the effects of xenobiotic metals on human Aβ aggregation in vitro.

The fluorescent dye 1,1-bis(anilino)napthaline-5,5-disulfonic acid (bis-ANS) was used to probe the conformational properties of Aβ and Aβ aggregates in the absence and presence of metals at various concentrations. Bis-ANS is known to bind aggregated but not monomeric Aβ. The non-amyloidogenic Aβ 40-1 was used as a control in these experiments. Among the environmental metals tested, only Aβ solutions containing Pb were observed to bind tightly to bis-ANS, consistent with the presence of Aβ aggregates. The aggrega-

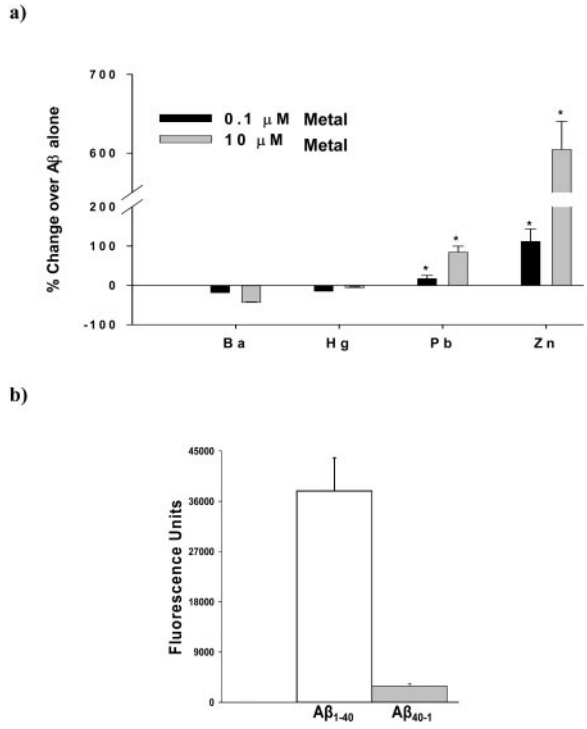

Figure 1. Fluorimetric detection of $A\beta_{1\text{-}40}$ aggregation and its reverse sequence peptide $A\beta_{40\text{-}1}$ (control) using bis-ANS. *a)* $A\beta_{1\text{-}40}$ samples were prepared for the fluorescence studies and the emission spectra was recorded at 455–550 nm. The intensity of fluorescence was used as a measure of aggregation. Values shown are the mean ± SE (n=4). *Significantly different from control (P<0.05) as determined by ANOVA and Duncan's post hoc test. *b)* Affinity of bis-ANS to bind $A\beta_{1\text{-}40}$ and $A\beta_{40\text{-}1}$ (control peptide).

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tion patterns observed using this method were dose dependent.

2. Developmental exposure to Pb has no latent effect on activity of secretase enzymes

We have previously shown that exposure to Pb during development up-regulates APP expression. Here we wanted to determine whether APP processing by secretases was also affected by developmental events. We profiled the cortical activity levels of α-, β-, and γ-secretases across the life span of rodents. We found that the activities of α-, β-, and γ-secretases peaked during early brain development, decreased to basal levels in adulthood, and remained constant for the rest of the animals' lives. We examined the effects of Pb exposure on the activity of each secretase using cortical tissue from three different groups: control, Pb-E (exposed to 200 ppm Pb in drinking water from birth to weaning), and Pb-L (exposed to 200 ppm Pb in drinking water from 18 to 20 months). We found that there were no significant changes in the activity levels of any of the three enzymes among these groups at 20 months of age (**Fig. 2**).

Pb-Levels

Sample	Blood µg/dL	Cortex µg/g wet wt. of tissue
Control (PND 20)	<2.0	<0.2
Pb (PND 20)	46.43 ± 1.95*	0.41 ± 0.04*
Control (20 month)	<2.0	<0.2
Pb-E	<2.0	<0.2
Pb-L	60.1 ± 15.01*	0.32 ± 0.03*

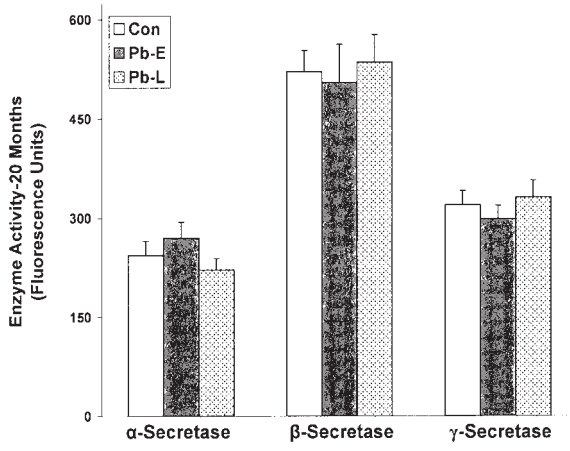

Figure 2. Effect of developmental and aging exposure to Pb on the activity levels of α-, β-, and γ-secretases in the cortex: Animals were exposed to 200 ppm Pb-acetate (Pb-E: birth through weaning; Pb-L: 18–20 months of age). Brain cortices were obtained from 20-month-old control, Pb-E, and Pb-L animals. Cell lysates were prepared from these tissues and analyzed for the activity levels of α-, β-, and γ-secretases using the R&D Systems kit. The intensity of fluorescence was used as a measure of enzyme activity. Each data point represents the mean ± SE (n=4–6). Blood and tissue levels of Pb are shown in the insert. *Significantly different from their corresponding controls (P<0.05).

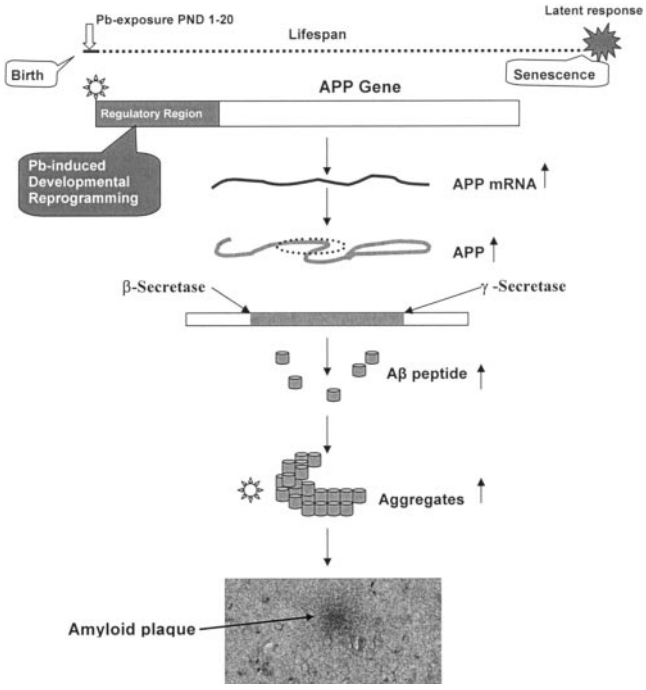

Figure 3. Schematic diagram showing the possible sites of action for Pb (☼) in the amyloidogenesis pathway: Rodent studies show that developmental exposure to Pb results in a latent effect on the expression of APP via the transcriptional mechanisms. This overexpression of the APP gene leads to an over production of the APP and its amyloidogenic product Aβ, thereby causing neurodegeneration and plaques formation in the brain. In vitro studies using a synthetic Aβ peptide suggest that Pb can promote the aggregation of Aβ. Items marked with an arrow (↑) show an elevation in intermediates in the pathway due to Pb exposure.

CONCLUSIONS

AD is a progressive neurodegenerative disorder whose clinical manifestations appear in old age. Most theories and mechanisms proposed to explain the pathogenesis of sporadic AD focus on events or disturbances that occur during old age. The long latency of AD suggests that this neurodegenerative disease remains asymptomatic for decades before a progressive accumulation of damage becomes clinically detectable. Our findings suggest that the initial events which trigger this disease begin very early in life and may be worsened by re-exposure to environmental agents late in life. Therefore, we propose that amyloidogenesis is promoted by a latent response to developmental reprogramming of the expression of the APP gene by early exposure to Pb as well as enhancement of Aβ aggregation in old age. In rodents, these events occur without Pb-induced disturbances to the enzymatic processing of APP. The findings described above provide further evidence for the developmental basis of amyloidogenesis and late life disturbances in AD-associated proteins by environmental agents. FJ