Environmental Health Perspectives
Vol. 89, pp. 91-94, 1990

Lead Poisoning and Brain Cell Function

By Gary W. Goldstein*

Exposure to excessive amounts of inorganic lead during the toddler years may produce lasting adverse effects upon brain function. Maximal ingestion of lead occurs at an age when major changes are occurring in the density of brain synaptic connections. The developmental reorganization of synapses is, in part, mediated by protein kinases, and these enzymes are particularly sensitive to stimulation by lead. By inappropriately activating specific protein kinases, lead poisoning may disrupt the development of neural networks without producing overt pathological alterations. The blood-brain barrier is another potential vulnerable site for the neurotoxic action of lead. Protein kinases appear to regulate the development of brain capillaries and the expression of the blood-brain barrier properties. Stimulation of protein kinase by lead may disrupt barrier development and alter the precise regulation of the neuronal environment that is required for normal brain function. Together, these findings suggest that the sensitivity of protein kinases to lead may in part underlie the brain dysfunction observed in children poisoned by this toxicant.

Introduction

Subtle but convincing defects in cognitive function and behavior occur in children with blood levels of lead frequently encountered in the general population (*1*). If the adverse effects described in the prospective studies of children reported at this conference persist into adult life, we face considerable loss from exposure to this environmental toxicant. The mechanisms by which lead disrupts brain function and the reasons for the apparent heightened sensitivity of the immature nervous system are not well understood. The goal of this review is to describe two neural systems, injury to either of which may underlie the developmental vulnerability of the brain. A potential biochemical site for the toxic action of lead is also described.

At a cellular level of organization, the brain can broadly be divided into two components. The neurons with their processes and insulating myelin are the information system, while the endothelial cells and astrocytes that regulate the fluid environment within brain are the homeostatic system.

Synaptogenesis

Neural networks are formed by interconnecting neurons with extensive processes consisting of dendrites that receive and axons and nerve endings that transmit information (*2*). In these networks, axons signal their targets (dendrites and neuronal cell bodies) by release of neurotransmitters in response to an excitatory depolarization of the axonal membrane. The oligodendroglial cells surround the axons to form the myelin sheath, which acts to speed the wave of depolarization from the neuronal cell body to the nerve terminal. Receptors on the dendrites and cell body respond to the neurotransmitters and summate excitatory and inhibitory signals to produce episodic depolarization of the target neuron. The combination of a nerve terminal and its receptor site on an adjacent neuron is termed a synapse. Since a large number of nerve terminals converge on each target neuron, the activity in the neural network can be exceedingly complex, especially considering the number of neurons (10^{11}) and synapses (10^{15}) in the human brain. It is the pattern of synaptic activity in these circuits that determines all aspects of brain function: control of mobility, perceptions, behavior, and ideas. If low-level exposure to lead alters aspects of behavior, there must be a direct or indirect effect of the toxin upon the function of certain neural networks. Damage at low doses of lead, however, is not overt since there is no pathologic indication of brain cell injury or death.

One clue to the toxic mechanism may be the developmental vulnerability. Blood levels of lead peak between 18 and 36 months of age, and it is the magnitude of these elevations that corresponds best with future neurobehavioral deficits (*3*). Examination of the child's brain at this stage of development reveals an ongoing reorganization of dendrite-nerve terminal connections. Almost all of cerebral neurons form during the second trimester of gestation and migrate to their adult location well before birth. Neuronal connections at birth, however, are sparse compared to the adult (*4*). This is reflected in a relatively low metabolic rate measured by positive emission tomography scan (*5*). During the first 24 months of life, a progressive increase occurs in synaptic density and complexity paralleled by an increase in cerebral metabolic rate. By the third year of life, both measures exceed the normal adult pattern by almost 2-fold.

During the next stage of brain development, an apparent pruning of these exuberant connections takes place so that dendritic arborization and brain metabolic rate decline to adult values. From infancy to 8 months, production of synapses exceeds their removal, leading to a density of connections

*Departments of Neurology and Pediatrics, The Johns Hopkins School of Medicine and The Kennedy Institute, 707 N. Broadway, Baltimore, MD 21205.

greater than that present at maturity. During subsequent years, the rate of removal exceeds the rate of formation, and the density of connection falls. Thereafter, formation and disappearance reach an equilibrium that leaves the density of synapses at a nearly constant value.

This developmental pattern occurs in a number of species and appears to be genetically determined. However, given the huge number of synapses (greater than 10^{15}), individual genes that number about 100,000 cannot control which synapses are formed, retained, or lost. Current theories suggest that survival of synaptic connections and dendrites occurs by a Darwinian selection process resulting from competition for a limited amount of trophic factors and influenced by the amount and complexity of activity in a given neuronal circuit (*6*).

To consider how lead might disrupt this selection process, it is necessary to understand the biochemical events that modulate activity in the nerve terminal (*7*). Transmission of information (excitation or inhibition) from one neuron to another is mediated by the release of neurotransmitters at specific nerve terminals. The sequence of biochemical events that control neurotransmitter release appears to be similar in most neurons. When the threshold for excitation at the cell body is exceeded, the portion of the axon nearest the cell body is depolarized by an opening of sodium channels. The resulting reversal of electrical potential produces a wave of depolarization that travels down the axon to the nerve terminal. When the nerve terminal itself is depolarized, calcium channels in the plasma membrane open, and calcium enters the terminal. This increase in calcium concentration causes a fusion of neurotransmitter-containing membrane vesicles with the plasma membrane and release of their contents into the synaptic junction. Neurotransmitter molecules are thereby made available for the receptors on the target neuron, and activity is passed on to the next cell in the network.

There are a number of influences that modulate the sensitivity of this sequence of events, and it is at this level of control that the biochemical basis for synaptic remodeling and learning appear to reside. With repeated depolarization sequenced in an appropriate pattern, nerve terminals and their associated target cell receptor zones become more efficient and may expand their surface area. The apparent biochemical mediators of this enhanced efficiency are a class of enzymes called protein kinases (*8*). These enzymes facilitate the transfer of the terminal phosphate group from ATP to specific amino acids on regulatory proteins. Because phosphate groups are negatively charged, phosphorylation alters the configuration and function of the target protein. Thus, a protein that acts as a gate in a membrane channel may open and allow more calcium to enter the nerve terminal after depolarization. Another protein that acts as a receptor for neurotransmitters may become less responsive to neurotransmitters after phosphorylation. Enzymes involved in neurotransmitter synthesis or breakdown are also subject to phosphorylation. Protein kinases are important to the control of neural transmission and play a central role in regulating the activity and development of neural networks.

Protein kinases are classified by the type of intracellular messenger by which they are activated. The major classes of intracellular messengers include cyclic AMP, cyclic GMP, calmodulin, diacyglycerol, inositol triphosphate, and calcium. In each case, the cascade of events initiating the activation of a protein kinase begins when a neurotransmitter (the first messenger) occupies a receptor site on the cell surface. The signal produced by receptor occupancy is transmitted through the plasma membrane by a transduction molecule (G-protein), which in turns stimulates an enzyme to produce a soluble molecule (the intracellular second messenger) that signals activation of its protein kinase. One such pathway involves the breakdown of a plasma membrane lipid, phosphoinositide bisphosphate, to two second messengers: diacylglycerol and inositol triphosphate (*9*). Diacylglycerol, in the presence of calcium, activates protein kinase C. Protein kinase C in turn phosphorylates proteins in the nerve terminal that enhance neurotransmitter release after depolarization, possibly by increasing calcium entry (*10*). In this way, protein kinase C may influence synapse formation and persistence.

We found that very low concentrations of lead appear capable of replacing calcium in this reaction sequence (*11*). Calcium inside a cell acts as a second messenger to activate a wide variety of biochemical events in addition to protein kinase C. Protein kinase C is, however, particularly sensitive to the calciumlike effects of lead. By acting as a calcium substitute, lead may disrupt the normal regulation of protein kinase C. For example, prolonged activation of this enzyme could produce sustained release of neurotransmitters from nerve terminals. Such unregulated activity would have a number of disruptive effects. Depending upon the type of neurotransmitter released, target neurons may have their threshold for excitation raised or lowered. In addition, the ability to modulate the neurotransmitter release mechanism would be decreased if an important regulator like protein kinase C has a diminished range of response because of sustained stimulation by lead. This loss could limit the complexity of activity in a neural circuit and place those synapses at a disadvantage during the developmental selection process. In humans, the result may be diminished cognitive function and altered behavior patterns. Such a pathologic mechanism for the developmental toxicity of lead is consistent with normal brain anatomy: the problem would not be in number of neurons or synaptic connections but rather with the inefficient manner in which synapses are formed and retained (*12*).

Two other protein kinases in the nerve terminal may be disrupted by the presence of lead. Surges in calcium concentration within the nerve terminal alter the configuration of calmodulin, a calcium-binding regulatory protein (*13*). Calmodulin serves as a sensor for the free concentration of calcium inside the nerve terminal. When its binding sites are occupied by calcium, calmodulin changes shape, binds to, and activates regulatory proteins including enzymes and transporters. Lead can replace calcium in this reaction and activate several biochemical events normally regulated by the concentration of calcium. Calmodulin stimulates a specific protein kinase in the nerve terminal that phosphorylates synapsin. Synapsin is a protein present in high concentra-

tions around synaptic vesicles, the storage packets for neurotransmitters in the nerve ending (*8*). When synapsin is phosphorylated by calmodulin-protein kinase, the synaptic vesicles are more sensitive to depolarization and release more neurotransmitter into the synaptic cleft between the nerve terminal and the dendrite. In this way, calmodulin-protein kinase modulates the release of neurotransmitters. Normally, the concentration of calcium in the nerve terminal is lower than that required to activate calmodulin. One mechanism to elevate the calcium level is through the phosphoinositide second messenger system described above. When phosphoinositide bisphosphate is hydrolyzed in response to occupancy of a neurotransmitter receptor, two second messengers are produced (*9*). One is the diacylglycerol that activates protein kinase C, and the second is inositol triphosphate, which causes release of calcium from the endoplasmic reticulum into the cytosol of the nerve terminal. Elevations in the concentration of calcium then activate calmodulin and its protein kinase to phosphorylate synapsin and enhance neurotransmitter release. In a test tube, lead activates calmodulin in the absence of calcium (*14,15*). If such activation occurs in the intact nervous system, control of neurotransmitter release would be disturbed and adjustments of neural net performance blunted.

Yet another type of protein kinase exists in the nerve terminal. This one is activated by cyclic AMP, the second messenger produced from ATP by adenylate cyclase after external exposure of the neuron to β-adrenergic neurotransmitters (*8*). Cyclic AMP activates a protein kinase that also phosphorylates synapsin. In this case, lead may inhibit the phosphorylation of synapsin because the toxin inhibits the formation of cyclic AMP by adenylate cyclase (*16*). Cyclic AMP levels may be even further depressed since the enzyme that degrades cyclic AMP (cyclic AMP-phosphodiesterase) is activated by calmodulin in the presence of lead (*15*). Thus, cyclic AMP levels may be diminished both by lead inhibition of adenylate cyclase and lead stimulation of phosphodiesterase.

All three protein kinases, protein kinase C, calmodulin-protein kinase, and cyclic AMP-protein kinase, are present in the nerve terminal and serve to modulate the release of neurotransmitters. Although the exact steps are unclear, modulation of neurotransmitter release appears to be of central importance to both short-term and long-term cognitive and behavioral function. Regional selectivity in vulnerability to lead may be explained by the differential location of the various protein kinases (*17*). *In vitro* studies of lead toxicity suggest the following pattern of sensitivity to lead: protein kinase C $>>$ calmodulin-protein kinase C $>$ cyclic AMP protein kinase (*11,16,18*).

In addition to effects upon neurotransmitter release, the protein kinases, by means of their phosphorylated products, signal the nucleus of neurons to alter gene expression. Thus, long-term alterations in neural net function may be regulated not only by biochemical events in the nerve terminal but also by changes in nuclear function. Given the information available, it would appear important to explore the effects of lead upon gene function. Protein kinase C is of special interest because it appears particularly sensitive to lead and is known to be involved in regulating gene expression (*19*).

In nerve endings isolated from brain, lead stimulates the basal release of neurotransmitters (*20*). Although there are multiple potential sites at which lead might produce these changes, one unifying hypothesis is that the enhanced release of neurotransmitters is related to the stimulation of protein kinase C by low doses of lead.

Blood-Brain Barrier

By altering protein kinase-mediated control of cell function, lead could also disrupt the function of the homeostatic support system of the brain: the endothelial cells and the astrocytes. The fluid environment of the nervous system is maintained under strict control by the blood-brain barrier (*21*). In contrast to other organs, the passage of water-soluble molecules between the blood and the brain fluid spaces does not occur by simple diffusion. Instead, selected molecules including the essential amino acids, glucose, calcium, sodium and potassium are transported across the capillary by means of carriers located in the plasma membrane of brain endothelial cells. These transport systems are highly specific and exclude most nonessential water-soluble molecules. Elsewhere in the body, there are gaps between capillary endothelial cells that allow for the free passage of most dissolved substances in the blood stream. This difference is important for normal brain function because the concentrations of many hormones and ions in the blood vary with diet and stress and would interfere with brain function if they had ready access to neurons. Thus, the small blood vessels in brain have many special features resembling those found in epithelial transport tissues such as the renal tubule. This added complexity may underlie their vulnerability to injury by toxicants.

Lead appears to be preferentially accumulated by endothelial cells in brain (*22*). High-level exposure to lead results in a loss of normal barrier function and a movement of plasma into the interstitial fluid spaces of brain (*23*). Edema ensues with increased intracranial pressure and diminished perfusion of the brain with blood and its essential substrates (oxygen and glucose). This cascade of events can and often does produce irreversible brain damage. Exposure to low levels of lead may change the microenvironment of the brain in ways less overt than associated with the acute encephalopathy that nevertheless interfere with normal developmental processes.

Our laboratory investigates the effects of lead upon blood-brain barrier development and function. For the most part, studies are carried out in small blood vessels freshly isolated from brain or in endothelial cells maintained in tissue culture (*24*). Although the endothelial cells and their tight junctional contacts create the blood-brain barrier, a large body of information suggests that the signals important for the expression of the barrier result from interactions between endothelial cells and astrocytes (*25*). Astrocytes actually outnumber neurons in the brain. Their processes surround the endothelial cells and interdigitate among the axons and dendrites in areas of synaptic connections (*2*). We found that the interaction between endothelial cells and astrocytes influence the toxic action of lead (26).

Control of cell growth and differentiation is a particularly well-established function of protein kinase C (*19*). Thus, the sensitivity of this enzyme to lead may be relevant not only to synaptogenesis, as discussed in the preceding section, but also to the regulation of permeability at the blood-brain barrier. To pursue this possibility, we investigated the activation of protein kinase C in isolated brain microvessels. We found that low concentrations of lead cause a translocation of protein kinase C from the cytosol to the membrane fraction of microvessels prepared from newborn rat brain (*27*). Such a translocation is usually produced as a response to growth factors and appears linked to the maturation of brain endothelial cells. In normal rats, protein kinase C activity in brain microvessels is in the cytosol until 10 to 15 days of age, after which it associates with cellular membranes (*28*). Our *in vitro* studies suggest that exposure to lead causes a premature translocation of protein kinase C and disrupts the normal developmental sequence. In this way, lead could alter the microenvironment of the brain and disrupt normal development without producing pathologic signs of cell death or edema.

Summary

The neurobehavioral changes found in lead-poisoned children may result from damage to the blood-brain barrier or direct effects of lead upon neuronal activity and synaptogenesis. A promising direction for future biochemical investigation is suggested by the sensitivity to lead of cell messenger systems. Definition of the interaction of lead with the binding of a neurotransmitter to cell surface receptors, the transduction process producing intracellular second messengers, the activation of specific protein kinases, and the alterations of cellular function and gene expression produced by phosphorylation of regulatory proteins should be pursued. A comprehensive investigation of these regulators should provide new insights into the biologic basis for the deficits that occur in children with low level exposure to lead.

This work was supported in part by grant ES02380 from the NIH (EHS).

REFERENCES

1. McMichael, A. J., Baghurst, P. A., Wigg, N. R., Vimpani, G. V., Robertson, E. E., and Roberts, R. J. Port Pirie Cohort Study: environmental exposure to lead and children's abilities at the age of four years. N. Engl. J. Med. 319: 468–475 (1988).
2. Raine, C. S. Neurocellular anatomy. In: Basic Neurochemistry, 4th ed. (G. Siegel, B. Agranoff, R. W. Albers, and P. Molinoff, Eds.), Raven Press, New York, 1988, pp. 3–33.
3. Bellinger, D., Leviton, A., Waternaux, C., Needleman, H., and Rabinowitz, M. Longitudinal analyses of prenatal and postnatal lead exposure and early cognitive development. N. Engl. J. Med. 316: 1037–1043 (1987).
4. Huttenlocher, P. R., and deCourten, C. The development of synapses in striate cortex of man. Hum. Neurobiol. 6: 1–9 (1987).
5. Chugani, H. T., Phelps, M. E., and Mazziotta, J. C. Positron emission tomography study of human brain functional development. Ann. Neurol. 22: 487–497 (1987).
6. Purves, D. Body and Brain: A Trophic Theory of Neural Connections. Harvard University Press, Boston, MA, 1988.
7. Erulkar, S. D. Chemically mediated synaptic transmission: an overview. In: Basic Neurochemistry, 4th ed. (G. Siegel, B. Agranoff, R. W. Albers, and P. Molinoff, Eds.), Raven Press, New York, 1988, pp. 151–182.
8. Nestler, E. J., and Greengard, P. Protein phosphorylation and the regulation of neuronal function. In: Basic Neurochemistry, 4th ed. (G. Siegel, B. Agranoff, R. W. Albers, and P. Molinoff, Eds.), Raven Press, New York, 1988, pp. 373–398.
9. Agranoff, B. W. Phosphoinositides. In: Basic Neurochemistry, 4th ed. (G. Siegel, B. Agranoff, R. W. Albers, and P. Molinoff, Eds.), Raven Press, New York, 1988, pp. 333–347.
10. Malhotra, R. K., Bhave, S. V., Wakade, R. D., and Wakade, A. R. Protein kinase C of sympathetic neuronal membrane is activated by phorbol ester—correlation between transmitter release, $^{45}Ca^{2+}$ uptake, and the enzyme activity. J. Neurochem. 51: 967–974 (1988).
11. Markovac, J., and Goldstein, G. W. Picomolar concentrations of lead stimulate brain protein kinase C. Nature 334: 71–73 (1988).
12. Petit, T. L. Developmental effects of lead: its mechanism in intellectual functioning and neural plasticity. Neurotoxicology 7: 483–496 (1986).
13. Stoclet, J. -C., Gerard, D., Kilhoffer, M. C., Lugnier, C., Miller, R., and Schaeffer, P. Calmodulin and its role in intracellular calcium regulation. Prog. Neurobiol. 23: 321–364 (1987).
14. Habermann, E., Crowell, K., and Janicki, P. Lead and other metals can substitute for Ca^{2+} in calmodulin. Arch. Toxicol. 54: 61–70 (1983).
15. Goldstein, G. W., and Ar, D. Lead activates calmodulin sensitive processes. Life Sci. 33: 1001–1006 (1983).
16. Nathanson, J. A., and Bloom, F. E. Lead-induced inhibition of brain adenylate cyclase. Nature 255: 419–420 (1975).
17. Worley, P. F., Baraban, J. M., DeSouza, E. B., and Snyder, S. H. Mapping second messenger systems in the brain: differential localizations of adenylate cyclase and protein kinase C. Proc. Natl. Acad. Sci. USA 83: 4053–4057 (1986).
18. Simons, T. J. B. Cellular interactions between lead and calcium. Br. Med. Bull. 42: 432–434 (1986).
19. Nishizuka, Y. The molecular heterogeneity of protein kinase C and its implications for cellular regulation. Nature 334: 661–665 (1988).
20. Minnema, D. J., Michaelson, I. A., and Cooper, G. P. Calcium efflux and neurotransmitter release from rat hippocampal synatposome exposed to lead. Toxicol. Appl. Pharmacol. 92: 351–357 (1988).
21. Goldstein, G. W., and Betz, A. L. The blood-brain barrier. Sci. Am. 254: 74–83 (1986).
22. Toews, A. D., Kolber, A., Hayward, J., Krigman, M. R., and Morell, P. Experimental lead encephalopathy in the suckling rat: concentration of lead in cellular fractions enriched in brain capillaries. Brain Res. 147: 131–138 (1978).
23. Goldstein, G. W. Brain capillaries: a target for inorganic lead poisoning. Neurotoxicology 5: 167–176 (1984).
24. Bowman, P. D., Ennis, S. R., Rarey, R. W., Betz, A. L., and Goldstein, G. W. Brain microvessel endothelial cells in tissue culture: a model for study of blood-brain barrier permeability. Ann. Neurol. 14: 396–402 (1983).
25. Goldstein, G. W. Endothelial cell-astrocyte interactions: A cellular model of the blood-brain barrier. Ann. N.Y. Acad. Sci. 529: 31–39 (1988).
26. Gebhart, A. M., and Goldstein, G. W. Use of an in vitro system to study the effects of lead on astrocyte-endothelial cell interactions: a model for studying toxic injury to the blood-brain barrier. Toxicol. Appl. Pharmacol. 94: 191–206 (1988).
27. Markovac, J., and Goldstein, G. W. Lead activates protein kinase C in immature rat brain microvessels. Toxicol. Appl. Pharmacol. 96: 14–23 (1988).
28. Markovac, J., and Goldstein, G. W. Transforming growth factor beta activates protein kinase C in microvessels isolated from immature rat brain. Biochem. Biophys. Res. Commun. 150: 575–582 (1988).