Annu. Rev. Pharmacol. Toxicol. 2004. 44:87–110
doi: 10.1146/annurev.pharmtox.44.101802.121424

First published online as a Review in Advance on August 6, 2003

DEVELOPMENTAL NEUROPATHOLOGY OF ENVIRONMENTAL AGENTS

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Key Words developmental neurotoxicity, methylmercury, lead, antiepileptic drugs, ethanol

■ **Abstract** The developing central nervous system (CNS) is more vulnerable to injury than the adult one. Although a great deal of research has been devoted to subtle effects of developmental exposure, such as neurobehavioral changes, this review instead focuses on a number of chemicals that have been shown, in several experimental models as well as humans, to cause morphological changes in the developing nervous system. Chemicals that are discussed include methylmercury (MeHg), lead (Pb), antiepileptic drugs, and ethanol. Additionally, the issue of silent neurotoxicity, i.e., persistent morphological and/or biochemical injury that remains clinically unapparent until later in life, is discussed.

INTRODUCTION

Neurotoxicity is generally defined as a structural change or a functional alteration of the nervous system, resulting from exposure to a chemical, biological, or physical agent (1). Neurotoxic effects result from a series of events, which includes entry of the agent into the organism and distribution to the site of action, interaction with specific cellular targets, and initiation of biological changes resulting in structural and functional alterations in the nervous system (1). Although neurotoxicity is known to occur in adult individuals, it often involves peripheral nervous system

effects because the central nervous system (CNS) is well protected by the blood-brain barrier, which prevents the passage of many endogenous and exogenous toxic agents into the brain. In contrast, the developing CNS is more vulnerable to injury than the adult CNS owing to several factors that are discussed in the following section. Developmental neurotoxicity is thus a major issue in neurotoxicology, and major neurotoxicants, such as methylmercury (MeHg), lead (Pb), polychlorinated biphenyls (PCBs), or alcohol, are in fact developmental neurotoxicants (2). It has been estimated that up to 12 million children in the United States suffer from learning, neurodevelopmental, or behavioral disabilities, with an estimated cost to society, attributable to environmental exposures, of more than $50 billion (3). Although a great deal of research has been devoted in the past several years to the more subtle effects of developmental chemical exposure, such as neurobehavioral changes, this review instead focuses on a number of chemicals that have been shown, in animal experimental models as well as in humans, to cause clear morphological changes in the developing CNS. The chemicals that are discussed include MeHg, Pb, antiepileptic drugs, and ethanol.

SUSCEPTIBILITY OF THE DEVELOPING BRAIN TO ENVIRONMENTAL INSULT

An understanding of the short- and long-term deleterious effects resulting from any interference with brain development requires not only knowledge of the nature of the interference but also of the nature of the organ at the time of insult (4). In the past several decades, a large number of studies carried out primarily in rodents have provided a mass of information on brain development (4–9). From these studies, one can infer the various stages of brain development in humans, although there are variations in the rates of brain growth among mammals, which depends mostly on the length of gestation (9, 10). Thus, the developmental ages of human and rat embryos or fetuses are comparable when major anatomical features and histological landmarks are similar in appearance in the two species, even though their exact chronological ages are different (9). Different parts of the CNS develop at different stages of development; structures are built by cell proliferation, migration, and a sequence of steps called differentiation (5). Normal function requires a certain number of cells in the correct location, and each cell must have the proper characteristics (5). With autoradiography, the neurogenesis of specific neuronal populations has been determined in rodent brain, and extrapolations were made to the human brain (4, 9). These studies clearly indicate that different brain areas develop at different times during gestation, and within a single brain region, subpopulations of neurons develop at different rates and at different times. For example, in the cerebellum, Purkinje cells develop early (embryonic days 13–15 in the rat, corresponding to weeks 5–7 in humans), whereas granule cells are generated much later (postnatal days 4–19 in the rat, corresponding to gestational weeks 24–40 in humans) (9). Many agents, such as X-ray irradiation, which is

known to cause brain damage, do so by interfering with cell proliferation, and if the insult occurs during the stage of formation of a certain neuronal subpopulation, those cells will not be formed. Cell migration is another important process during brain development by which neurons reach their final location. Such physical contact between the cells is important for the construction of complex circuits, and any interference with cell migration, as seen, for example, with methylmercury, has profound deleterious effects on the developing brain. Migration failure can be caused by either a direct effect on migrating cells; an effect on neighboring cells; or toxic effects on important supporting structures, such as radial glia (5). Like cell proliferation, migration does not proceed at a constant rate throughout development but occurs in waves associated with different cell types (11). To achieve mature function as transmitters of signals, neurons must form connections, and this occurs during the process of synaptogenesis. Though neurons retain the ability to make new synapses throughout life, the developmental period is critical for the formation of the basic circuitry of the nervous system (11). Furthermore, in the developing brain, neurotransmitters may play important roles other than neurotransmission to control body function. Evidence exists that neurotransmitters can modulate proliferation of neural stem cells, neuroblasts, and glioblasts; regulate migration; and induce differentiation (12–15). Consequently, any toxicant that interferes with neurotransmission during development may cause permanent defects in the CNS. The understanding of molecular signals that regulate neuron-glia interactions has increased greatly with the advent of molecular and cellular biological techniques as well as genetically modified mice. Studies in which cell ablations are genetically targeted with ectopic gene expression and gene knockout with single-cell specificity have established the distinct roles played by various cell types during development.

Neurogenesis produces about twice as many neurons in a given structure than the number of neurons that survive in the adult organism. This initial excess of neurons is pruned within a narrow time span, which differs among various brain structures, by a process known as apoptosis or programmed cell death (16). Such physiological processes are regulated by growth factors and cytokines as well as by neurotransmitters, and they are executed by a number of intracellular proteins (17, 18). Any compound that interferes with these processes may trigger apoptotic degeneration of neurons that would not have otherwise been deleted from the developing brain, or may, in contrast, promote survival of unnecessary cells (18). In addition to loss of neurons, pruning, defined as a loss of synapses, also occurs physiologically in the developing brain (19). Such trimming of connections is a longer process than cell death and occurs late in childhood and adolescence (19). Any interference with this process, as has been suggested in the case of Pb, would be expected to affect the number of synaptic connections. Most of the developmental processes discussed so far have focused on neurons. Yet, it is well established that glial cells (astrocytes, oligodendrocytes, and microglia) play a most relevant role in brain function as well as in brain development. An important period of brain development is the so-called brain growth spurt, a transient period of growth when

the brain is growing most rapidly (6). This occurs in the first two postnatal weeks in the rat and in the third trimester of pregnancy and early infancy in humans (7). One of the general features of brain growth throughout mammalian species is that adult neuronal cell number is almost accomplished (with the exception of cerebellar granule cells and few other neurons) before the major phase of glial multiplication begins (7). The brain growth spurt is indeed characterized by rapid proliferation of glial cells, most notably astrocytes and oligodendrocytes, which provide myelination to axons. This period of brain development has been shown to be quite sensitive to the toxic effects of a number of chemicals (e.g., alcohol, certain pesticides, nicotine), which underlines the fact that glial cells represent an important target for neurotoxicity (20). In addition to the sensitive processes described, the developing brain is distinguished by the absence of a blood-brain barrier. The development of this barrier is a gradual process, beginning in utero and complete at approximately six months of age in humans (11). Because the blood-brain barrier restricts the passage of substances from blood to brain, in its absence, toxic agents can freely enter the developing brain.

In summary, the developing brain is extremely vulnerable to toxic insults because a large number of processes occur during an extended period of time. Due to the lack of a protective barrier, chemicals have free access to the developing brain and can exert a number of deleterious effects whose nature and consequences are dependent on the time of exposure.

METHYLMERCURY

Numerous studies have confirmed that methylmercury (MeHg) compounds cause developmental disorders (21). The primary sources of information on such effects in humans have come from accidental exposure incidents in Japan and Iraq (22–24). In both cases, a range of developmental effects was noted in children exposed in utero or at young age. There are considerable differences in the distribution of pathological changes in the young compared to adult brain (25). The brain damage seen after MeHg exposure in adults is primarily localized to specific areas, such as the granule layer of cerebellum and the visual cortex of cerebrum. When exposures to MeHg occur in utero or at early age, the damage is seen at several places throughout the CNS. The earlier the exposure, the more generalized the damage that is observed (26). Such age-related differences are also seen in other mammals, although the specific areas damaged may differ. It has been suggested that these differences are caused by an immature blood-brain barrier, which causes a more generalized distribution of mercury in the developing brain. However, after adult exposure, mercury is found throughout the brain and localization does not correlate with pathological changes. Another possibility is that the more generalized damage in the younger individual compared to older individual is the result of different expressions of toxicity. To examine such differential expression of toxicity, one would need to understand the nature of some possible molecular targets

for MeHg. MeHg readily binds to sulfhydryl groups, and the free concentration of MeHg in a biological system is extremely low (27). Noting the importance of sulfhydryl groups to the proper function of proteins, e.g., enzymes, one can assume that MeHg may cause a range of effects at many locations throughout the body. However, studies in adult animals demonstrate that at moderate dosage of MeHg, the primary target is the CNS, although the organ concentration of MeHg may be considerably higher in other tissues (e.g., liver and kidney). Thus, most cells have a capacity for resisting or repairing the damage inflicted by MeHg. If so, the targets of MeHg would be cells that cannot meet the metabolic challenge of damaged proteins and/or do not have the ability to sequester Hg via the synthesis of metallothionein or other protein and peptides.

In the adult human CNS, characteristic lesions associated with Minamata disease display region-specific distribution; the calcarine cortex was found to be involved with prominent lesions along the anterior portions of the calcarine fissure. In the precentral cortex, severe foci of damage caused the development of secondary bilateral degeneration of the pyramidal tracts. Lesions were also common in deeper layers of the cerebellum, with granular cells seemingly most affected. The peripheral nervous system of Minamata disease patients was also affected, with sensory nerves more affected than motor nerves. In the adult brain, MeHg accumulates in astrocytes, which interferes with glutamate uptake and results in high extracellular glutamate, which neurons cannot tolerate. Such mechanisms may also be in effect in the developing brain, but there are a number of other processes that are unique to the developing brain. Some mechanisms, with possible relevance to MeHg-induced damage, include aberrant neuronal migration from the site of germination to the final destination, the formation of interneuronal contacts, and the apoptotic death of neurons. Animal studies have shown that immature neurons, e.g., cerebellar granule and Purkinje cells, are arrested before or during their migration to their permanent site of function (28). Cell movement is a highly complex process involving major cytoskeletal changes in the neurons as well as the proper function of guiding cells. The interaction of MeHg with the microtubular element of the cytoskeleton has been extensively studied (29). The observed interference of MeHg with neuronal movements may very well be a result of MeHg hindering the repolymerization of microtubules. Indeed, MeHg (0.01 to 10 μM) leads to a concentration- and time-dependent disassembly of microtubules in interphase and mitotic cells in embryonal carcinoma cells in culture (30). As reported by the same authors, spindle microtubules appeared more sensitive than those of interphase cells, corroborating earlier studies on the general sensitivity of microtubules to MeHg and the relative insensitivities of other cytoskeletal components, such as actin and vimentin, to MeHg.

The formation of dendrites and axons depends on the proper function of a growth cone. Studies with primary neurons in culture have shown that one of the earliest effects of MeHg in such cultures is the retraction of growth cones and extensions (31). Apoptosis is a necessary process during the development of the brain. MeHg also induces apoptosis and can thus interfere with cells that are “primed” for

apoptosis so that the process occurs at an inappropriate time (32, 33). Astrocyte-neuron cell-to-cell contact mediated by adhesion molecules is well exemplified by the migration of cerebellar granule cells along Bergmann fibers. Translocation of neuronal cell bodies requires the coordinated temporal and spatial expression of different adhesive molecules, e.g., N-CAM, astrotactin, and L1 (34, 35). Similarly, neurite outgrowth along astrocytic cell surfaces and the extracellular matrix is characterized by a specific spatiotemporal elaboration of a number of adhesive molecules, including L1, N-CAM, N-cadherin, and integrin-class extracellular matrix receptors (36). A prominent feature of prenatal MeHg poisoning is a reduction in CNS mitotic activity (37) and interference with neuron migration (38, 39). Effects of MeHg on neuronal migration in culture reveal cessation of cell movement at 10 μM MeHg. Abnormalities of neuronal migration are also prominent pathological features of MeHg-affected human brains (40). Because neurons migrate along radial glial processes, it is possible that a major effect of MeHg is damage to the astrocytes. Indeed, effects on astrocytes have been shown. The initial site of MeHg injury appears to be the astrocytic plasma membrane, with a marked shift in the distribution of anionic groups and loss of filopodial activity (41). Electron spin resonance studies on cultured astrocyte membranes exposed to MeHg indicate a significant reduction in membrane fluidity following exposure to this organometal (42). There are several mechanisms by which MeHg may cause developmental damage to the CNS, which makes the fetus and the young brain far more vulnerable than the adult brain.

With the devastating and irreversible effects of MeHg on the developing brain, it becomes important to establish a safe dose of exposure. Although animal experiments may provide guidance on this issue, clinical/epidemiological assessments of exposed populations are also required. Two study populations are of prime importance in this regard: one in the Faroe Islands (43) and the other in the Seychelles (44). On both of these locations, there are no industrial sources, and MeHg exposure is entirely due to diet, which consists mainly of pelagic marine animals. In the Faroe Islands, a correlation was found between levels of MeHg in the mother at time of birth (measured in cord blood) and neurological deficits in the offspring. However, such a relation was not found in the Seychelles study. Concomitant exposure to PCBs in the Faroe Island population may be an important confounder, and both MeHg and PCBs may have independently caused neurological effects in this population, as suggested by Rice (45).

LEAD

Pb is a ubiquitous pollutant in the ecosystem. Despite efforts to reduce exposure through cleanup regulations, phasing-out leaded gasoline, and banning Pb-paint, excessive Pb exposure still persists, and Pb poisoning is still considered a primary environmental hazard to children. Pb exposure during pregnancy and in young children is of particular concern because the developing nervous system is especially

susceptible to Pb toxicity (46). Developing fetuses and young children absorb Pb more readily than adults (47, 48), and Pb enters the CNS quite freely, although the exact transport mechanisms have not yet been elucidated. Exposure to Pb during neurodevelopment has significant effects on neurobehavioral and intellectual performance, also resulting in attention, hyperactivity, and learning disorders (49–51). These neurological and intellectual effects have been documented in a variety of cases in which exposure took place either in the intrauterine environment or during early childhood.

Epidemiological studies have associated chronic developmental Pb exposure with impairments in cognitive function and behavioral maturation in young children (52–54). These effects were found despite the absence of any overt signs of toxicity, and the subjects displayed no other apparent alterations in CNS morphology or generalized neurological function. A number of large and well-conducted longitudinal studies lend support to the claim that neurobehavioral and cognitive effects related to environmental Pb exposure during neurodevelopment persist through childhood and adulthood. These reported associations are small when the complexities of identification and selection of confounding factors are considered (55–59).

There is no proven safe lower limit for Pb exposure; effects have been observed in children with blood lead levels (BLLs) of less than 10 μg/dl (0.48 μM) (52, 60). Subtle neurobehavioral dysfunction in children has been associated with an overall median BLL of 5 μg/dl (61). A relatively small study points to an inverse association of low BLL ($<$10 μg/dl) with children's IQ scores at three and five years of age, which suggests that the associated declines in IQ are greater at these low concentrations than at higher Pb concentrations (61a). Animal studies have found observable effects at BLLs of less than 15 μg/dl in primates (62, 63) and less than 20 μg/dl in rats (64). The relationship between Pb exposure and cognitive dysfunctions is similar across species, and cross-species comparisons indicate that chronic low-level exposure to Pb results in similar manifestations of distractibility, neurobehavioral dysfunctions, and learning impairment (50, 61, 65–67).

Pb has been shown to be neurotoxic during neural differentiation (68, 69) and synaptogenesis (70, 71). However, Pb seems to have its greatest effects during the later stages of brain development, perhaps by interfering with the trimming/pruning of synaptic connections (thus affecting the number of synaptic connections; synaptogenesis) and apoptosis (causing neuron death) (71). Pb can also produce significant decreases in the formation of myelin, particularly during late gestational development and during the postnatal period (11, 51). Upon cessation of exposure, some of these effects may be reversible; although there is an apparent accelerated regenerative capacity through two years of age (67), this regeneration may only be partial (56). The blood-brain barrier, which does not fully develop until the middle of the first year of life, is highly sensitive to Pb (72). The endothelial cells that form the main structural component of the blood-brain barrier are the first to be exposed to Pb passage into the brain and have a maturing or differentiating effect on astrocytes (73). Endothelial cells show a marked affinity for Pb and accumulate

it to a higher concentration than other brain structures. However, in vitro cultures of endothelial cells were relatively less sensitive to the cytotoxic effect of Pb at low concentrations (10–50 μM Pb acetate) than astrocytes, as demonstrated by a decrease in cell number and by the presence of intracellular vacuoles and detached cells (73). Toxicological data indicate that cocultures of endothelial cells and astrocytes are less sensitive to toxic agents than their respective monocultures. It is likely, therefore, that Pb ions disrupt the main structural components of the blood-brain barrier by primary injury to astrocytes, with a secondary damage to the endothelial microvasculature (74, 75). The endothelial cells cause a maturing or differentiating effect on the astrocytes, which makes them less susceptible to Pb (73). Acute Pb exposure causes astrocyte inhibition of aerobic energy metabolism that appears to be closely associated with cell damage. The capacity of the astrocyte to sequester Pb in nonmitochondrial intracellular sites may be critical in resistance to Pb toxicity in vitro and in the mature brain (76).

Within the developing brain, Pb-induced damage occurs preferentially in the prefrontal cerebral cortex, hippocampus, and cerebellum (77). Pb toxicity may damage the basal forebrain and the primary visual cortex and cause changes in the permeability of capillaries in the cerebral cortex (78). In rat pups, chronic low-Pb levels caused a delay in biochemical and structural development of the cerebral cortex (70). These changes coincide with delays in the development of exploratory and locomotor activity and impaired learning.

The hippocampus, a critical neural structure for learning and memory, is also affected by low-level inorganic Pb and organolead exposure (79). Developmental Pb exposure causes morphological changes in rat hippocampus; at a BLL of 20 μg/dl, there was an increase in the size and numerical density in the mossy fibers, the granule cell layer, and the commissural-associational area of the dentate molecular layer (80). A number of studies also suggest a dose-dependent bimodal influence on developing hippocampal components, which follows decreased density after higher Pb exposure (BLL of 250 μg/dl) (80, 81). Developmental delay of astrocytes in the rhesus monkey hippocampus has been postulated to occur after both pre- and postnatal chronic low-level Pb exposure (82).

Recent research on the cellular action of Pb has identified target sites where Pb may interfere with the processes underlying the hippocampal synaptic plasticity. These actions include Pb's effect on glutamate release in vivo and on N methyl D aspartate (NMDA) receptor function (83). Pb-induced alterations of ligand binding to NMDA receptors in the hippocampal formation and cortical areas may play a role in Pb-induced neurotoxicity (84, 85). Alternative splicing of the NR1 gene showed selective anatomical and temporal regulation by Pb in the developing rat hippocampus (86). Pb's effect on pre- and postsynaptic glutamatergic function may explain the basis of the Pb-induced changes occurring in long-term potentiation (LTP). Indeed, an effect of Pb on LTP has been observed in many studies from which it is evident that exposure to Pb increases the threshold for induction and diminishes the magnitude of potentiation (87). Exposure to Pb has also been shown to shorten LTP duration by accelerating its rate of decay (88). Perinatal low-level Pb exposure also induced loss of septal cholinergic neurons in neonate rats, which

resulted in a hippocampal cholinergic innervation deficit that persisted into young adulthood (89). This disruption may account for the enduring cognitive impairments associated with early Pb exposure. It has been suggested that Pb-induced cell death in the hippocampus in vivo may partly be due to apoptosis (90).

The cerebellum primarily controls motor movements and may be partially responsible for speech, learning, emotions, and attention. A study of six-year-old children exposed to Pb (BLL 10–14 μg/dl at 1–5 years of age) indicated postural disequilibria (91). Chronic Pb-induced (BLL 20–30 μg/dl) inhibition of postnatal structuring in the rat cerebellum was indicated by an impaired developmental time course of desialylation of the D2-CAM-N-CAM (neural cell adhesion molecules) protein, which may lead to impairment in fine motor skills (92). Histological changes in cerebellum tissue were detected following low-level Pb exposure in rats.

In summary, lead exposure produces neurotoxicity, which results in behavioral, morphological, and electrophysiological effects. Pb affects many different biological activities at the cellular and molecular levels, which may be related to its ability to interfere with the regulatory action of calcium in cell functions. Pb is able to increase intracellular calcium concentrations and serve as a calcium substitute, and some calcium-binding proteins are capable of binding Pb. Subnanomolar concentrations of Pb activate protein kinase C (PKC) in a process that is calcium and phosphorylation dependent (93, 94). The PKC-mediated Pb-induced rise in intracellular free calcium may be the cause of disruption of homeostatic cellular mechanisms (95). This breakdown is expressed in the anatomical site and the neurotransmitter systems, each of which plays a crucial role in modulating emotional response, memory, and learning.

ANTIEPILEPTIC DRUGS

Women with epilepsy need to continue taking medication to control seizures during pregnancy (96); yet, although maternal seizures during pregnancy may pose a risk for the fetus (97), animal and human studies indicate that exposure to anticonvulsant drugs cause developmental toxicity and neurotoxicity. All major antiepileptic drugs (phenobarbital, valproic acid, carbamazepine, and phenytoin) have been found to cause malformations, microcephaly, growth retardation, and impaired behavioral development in infants following prenatal exposure (98–100).

Phenobarbital is the oldest antiepileptic drug and remains one of the most effective agents used to control seizures. Its anticonvulsant effect appears to be due to the ability to potentiate synaptic inhibition through an action at gamma aminobutyric acid ($GABA_A$) receptors. Animal studies have shown that perinatal exposure to phenobarbital can reduce brain weight (101) and cause a reduction of Purkinje and granule cells in the cerebellum (102) and pyramidal and granule cells in the hippocampus (103). Administration of therapeutic doses of phenobarbital to neonatal rats has been shown to cause a wave of apoptotic neurodegeneration, which has been ascribed to the GABA mimetic action of this compound (104, 105). Neurochemical studies have indicated that phenobarbital profoundly

disrupts cholinergic neurotransmission in the hippocampus (106). Results of behavioral studies are consistent with morphological and neurochemical changes, as perinatal exposure of rodents to phenobarbital causes decrements in various spatial learning tasks (106, 107). Long-lasting neurobehavioral effects in humans following in utero exposure to phenobarbital have also been reported and include impaired cognitive development (108) and lower IQ scores (109). Despite the fact that phenobarbital clearly causes developmental neurotoxicity, its embryotoxicity and teratogenic effects appear to be less than other anticonvulsants in animal models (110), although typical malformations associated with in utero exposure to antiepileptic drugs have been reported to occur in humans (111).

Valproic acid is widely used in humans as an anticonvulsant and mood stabilizer. Although its precise mechanism of action has not been elucidated, valproic acid seems to act by increasing the concentration of GABA, primarily by inhibiting its degradation (112). Valproic acid is a clear animal and human teratogen (99). The nervous system appears to be particularly sensitive to the developmental toxicity of valproic acid, as neural tube defects, specifically spina bifida, occur at a high rate upon in utero exposure to this compound (113). Neural tube defects are also seen in mice (114), and strain differences in susceptibility suggest an underlying genetic predisposition (115). Administration of valproic acid to neonatal rats, at doses below the ED_{50} for anticonvulsant action, has been shown to induce apoptotic neurodegeneration in several brain areas, an effect that has been attributed to its GABA mimetic properties (105).

Most mechanistic studies have focused on the major malformations induced by valproic acid (116). At therapeutically relevant concentrations, valproic acid was found to alter the expression of certain homeobox genes, which suggests that teratogenicity may be at least in part mediated by changes in Hox gene expression (115). Concentrations of valproic acid within its therapeutic range have also been found to inhibit histone deacetylase, which is involved in the repression of gene expression and plays an important role in embryonic development (117, 118). Inhibition of histone deacetylase can also prevent cell proliferation and may be responsible for the ability of valproic acid to reduce proliferation of C6 glioma cells (119). This antiproliferative effect of valproic acid may be relevant for its teratogenicity, as alterations of normal proliferation rate of the tissues involved with neuronal tube closure may result in an embryo with a an neural tube defect (116).

Exposure to subteratogenic doses of valproic acid have been shown to cause microencephaly and behavioral changes (deficits in spatial learning tasks and altered locomotor activity) in rodents (110, 120). In humans, in utero exposure to valproic acid has been associated with developmental delays, mental retardation, cognitive impairment, and other behavioral deficits (121, 122). Valproic acid may be more toxic to the developing brain than other anticonvulsants (121), and neural tube defects may only be the tip of the iceberg because valproate carries particular risks to the learning and development of children (123). Also of interest is that in utero exposure of rats to valproic acid causes cerebellar anomalies associated with autism, and an association between exposure to this compound and autistic-type behaviors has been suggested in humans (121, 124).

Similar to valproic acid, in utero exposure to carbamazepine has been associated with an increased risk of spina bifida, with an incidence of about 1% (125). Carbamazepine has indeed been found to be teratogenic in humans, and the pattern of malformations (facial dysmorphic features, microcephaly, growth retardation) resembles that of other anticonvulsants (100, 126). In rodents, incidence and severity of teratogenic effects were less than those observed with other antiepileptic drugs and occurred mostly at high doses (127).

Carbamazepine owes its antiseizure effects to its ability to bind to sodium channels when they are in the inactivated states, thereby slowing the spread of reactivation and reducing high-frequency firing in neurons (128). Such a mechanism has been postulated to be responsible for the widespread degeneration observed in the brains of rats dosed postnatally with carbamazepine (129). Despite this preliminary finding, carbamazepine appears to be the least developmentally neurotoxic compound among the major antiepileptic drugs. Indeed, although mild mental retardation has been reported in children exposed in utero to carbamazepine (130), no neurologic or IQ differences were reported (108, 131).

The last major antiepileptic drug, phenytoin (diphenylhydantoin), is effective against all types of partial and tonic-chronic seizures, and, similar to carbamazepine, its mechanism of action appears to involve an interaction with sodium channels (132). There is ample evidence that phenytoin is a developmental toxicant in animals and humans. The fetal hydantoin syndrome in humans is characterized by facial dysmorphologies, growth retardation, and other anomalies (98, 133), and similar effects have also been seen in rodents (134). Animal studies have also shown that perinatal administration of phenytoin causes a reduction in brain weight (135) and a number of behavioral deficits (seen at subteratogenic doses), which include, in particular, deficits in spatial learning tasks and activity change (hyperactivity) (136–138). Microcephaly, learning disabilities, and decreased IQ scores have also been reported in humans (139–141).

These data suggest that phenytoin can affect the cerebellar system and the hippocampus (110). Indeed, neonatal exposure of mice to phenytoin leads to cerebellar damage, characterized by apoptotic death and delayed migration of granule cells, and altered development of Purkinje cells (142, 143). Early postnatal exposure to phenytoin has also been shown to cause a dose-dependent increase in apoptotic neuronal death in the hippocampus, cortical areas, amygdala, and thalamus (129). In vitro experiments have confirmed that phenytoin induces apoptotic cell death of cultured cerebellar granule cells (144), degeneration of Purkinje cells (145), and toxicity in cerebral cortical cell cultures (146).

ETHANOL

Ethanol is a well-documented developmental toxicant causing a spectrum of physical and mental dysfunctions in children after prenatal exposure. This range of structural and functional abnormalities characterize a syndrome known as fetal alcohol syndrome (FAS) and include pre- and postnatal growth retardation;

craniofacial dysmorphologies; and, in particular, CNS dysfunctions, such as microencephaly, brain malformations, mental retardation, and other behavioral abnormalities (147, 148). In several cases, full-blown FAS may not be present, as evidenced by a lack of characteristic facial features; yet, CNS defects may still be present, and these alcohol-affected children have been classified as alcohol-related birth defects (ARBD) and alcohol-related neurodevelopmental disorders (ARND) (149). The CNS effects of prenatal alcohol exposure are of most concern because they persist into adulthood and are, therefore, irreversible (150, 151).

A great deal of information exists from human and animal investigations on the neuropathological effects of perinatal alcohol exposure. Autopsies of children with FAS have revealed widespread severe damage, including malformations of brain tissue, failure of certain brain regions to develop, and failure of certain cells to migrate to their appropriate locations during development (152, 153). The use of magnetic resonance imaging and computed tomography has allowed a more precise characterization of the developmental effects of alcohol. In addition to a reduced overall brain size (154), certain brain regions appear to be particularly affected by ethanol: These include the corpus callosum, which is reduced in size and sometimes missing altogether (155); the cerebellum, whose size is significantly reduced (156), possibly owing to the loss of specific neuronal cell populations; and the basal ganglia, in particular, the caudate nucleus, whose size is also significantly reduced (156). Other brain areas, such as the cerebral cortex and the limbic system, including the hippocampus, show a much lesser degree of alterations following in utero ethanol exposure in humans.

Detailed information on the neuropathological effects caused by developmental ethanol exposure has been provided by animal studies. A great deal of research has dealt with the issue of microencephaly, as microcephaly has been found to occur in over 80% of FAS cases (147). Studies in rodents have shown that exposure to alcohol, particularly during the third-trimester equivalent (the first two postnatal weeks in the rat) has a profound effect on brain weight. When given during this period, ethanol causes selective microencephaly, which is independent of general growth retardation (157) and is irreversible (158). Further studies have shown that the effect is dose-dependent (159, 160) and exhibits temporal and regional selectivity. For example, early gestational exposure to ethanol (first trimester equivalent) causes a reduction in brainstem growth, whereas postnatal exposure in the rat affects forebrain and cerebellum weight (161). A large number of studies have documented that developmental ethanol exposure causes loss of specific neuronal populations. In addition to temporal and brain-region-specific effects, another important variable is the pattern of exposure. Indeed, binge-like exposure, yielding high blood alcohol levels, has been shown to be much more detrimental than continuous exposure to lower concentrations (162–165). Neuronal cell populations that are reduced by developmental ethanol exposure include neurons in the CA1 region of the hippocampus and granule and Purkinje cells in the cerebellum (163, 166–168). In addition to affecting neurons, developmental exposure to ethanol has also been shown to affect glial cells (169). Evidence exists

of abnormal glial migration in humans with FAS as well as in primates and rats exposed to ethanol during development (152, 170), and a reduction in glial cell number has been reported in rat models of FAS (171, 172). In children affected by FAS, hypoplasia of the corpus callosum and anterior commissure, two areas originally formed by neuroglial cells, has been reported (155).

Investigations on potential mechanisms involved in the developmental neurotoxicity of ethanol abound, as a large number of cellular and intercellular processes are affected by alcohol. Most research in animals and in in vitro systems has focused on the ability of ethanol to cause alterations in cell proliferation and cause cellular death (173). Proliferation of neuronal cell precursors and of glial cells has been shown to be inhibited by ethanol (173, 174). Ethanol has also been shown to cause apoptotic cell death of hippocampal, cerebellar, and cortical neurons in vitro and in vivo (175–177). Such effects may be the result of a direct toxicity of ethanol, of inhibition of neurotrophic properties of glutamate, or of activation of GABA receptors (176).

THE ISSUE OF "SILENT" NEUROTOXICITY

All chemicals discussed in the preceding sections cause severe neurotoxic effects that are manifest shortly after exposure. Yet, evidence is emerging that deleterious effects of toxicants may not become clinically evident for some months, or even several years, after exposure. This period during which the individual may manifest no evidence of toxicity is referred to as a silent period (178). Silent toxicity has been defined as "persistent morphological and/or biochemical injury, which remains clinically unapparent unless unmasked by experimental or natural processes" (178). Silent toxicity has been compared to the process of carcinogenesis, where chemical exposure and cellular damage occurs years, if not decades, before clinical manifestations of the disease are apparent (179). There are several examples of how the concept of silent toxicity may apply to the nervous system. In case of the Parkinsonism-dementia known as Guam's disease, latencies of decades have been reported between alleged exposure to still-undefined substances and clinical signs (180). More recently, bovine spongiform encephalopathy (mad cow disease), a prion disease associated in humans to a variant of Creutzfeld-Jakob disease, has been shown to have an incubation period (latency) of decades (181).

Many possible hypotheses have been proposed to explain the delay between exposure and clinical expression of neurotoxic injury. One possibility is that toxic exposure results in lethal injury to a subpopulation of neuronal cells, but the total number of cells lost is insufficient to immediately compromise functions owing to a reserve provided by surviving cells (178). The deficit would only be unmasked by exogenous influences (stress, disease, additional chemical exposure) or by the natural aging process. A second possibility is that toxic exposure may result in sublethal injury to neural elements, which leads to progressive, incremental loss of function. The organism will initially compensate for the mild deficit, but the

functional reserve and plasticity of the brain would, with time, be overcome, and loss of function would appear (178).

The concept of silent damage and the hypotheses on possible underlying mechanisms may also find a place in developmental toxicity. The concept that adult disease may have a fetal origin has been introduced by David Barker and is known as the Barker hypothesis (182). Exposure to environmental agents may cause damage or alter developmental programming, whose resulting functional deficits become apparent only later in life. A classic example is represented by diethylstilbestrol: In utero exposure to this compound leads to an increase in vaginal adenocarcinoma around the time of puberty (183). With regard to developmental neurotoxicity, evidence is growing that in utero or early postnatal exposure to endocrine disruptors, particularly those affecting thyroid structure and function, can lead later in life to severe behavioral abnormalities (184). A recent study in rats provides evidence that in utero exposure to the Gram(-) bacteriotoxin lipopolysaccharide (LPS) causes an almost 30% loss of dopaminergic neurons in the substantia nigra and the ventral tegmental areas (185). This suggests that in humans, prenatal infections occurring at the appropriate gestational age would result in the birth of an individual with fewer dopaminergic neurons. This, in turn, would be inconsequential early in life, but may predispose an individual to developing Parkinson's disease at a progressive age (185). Though evidence of silent developmental neurotoxicity is still limited, the concept has a solid rationale and a few examples to support it. Clearly, an understanding of its impact would require careful coordination of morphology, biochemistry, and behavior; the use of unmasking paradigms; and, most of all, the implementation of longitudinal (life-long) assessment strategies within developmental neurotoxicity testing.

CONCLUSIONS

Evidence summarized in the previous sections indicates that exposure to certain chemicals during development (prenatally or early postnatally) can cause profound disruptions in the morphology of the CNS. In most cases, evidence for such deleterious effects is provided by animal data and findings in humans. In the case of two widely distributed environmental pollutants, MeHg and Pb, concerns still remain regarding their developmental neurotoxicity. Both are primarily linked to subtle behavioral effects owing to persistent exposure to low levels of these compounds in certain populations owing to consumption of fish (MeHg) or paint (Pb). With regard to ethanol, binge drinking during pregnancy still occurs at an alarming rate, and profound CNS effects are being seen in children diagnosed with FAS. For antipsychotic drugs, the main issue is the balance between benefits for the mother (and indirectly for the fetus) and risk of developmental neurotoxicity for the fetus and newborn. Finally, a more intriguing, as well as most concerning, issue is that of possible silent neurotoxicity. This would occur when exposure to certain chemicals during brain development causes morphological and/biochemical changes that are not apparent until the individual grows, ages, and/or is exposed to additional

environmental challenges. Such silent damage would be difficult to unmask in human clinical/epidemiological studies because of the long interval between alleged exposure and clinical symptoms. This warrants, therefore, careful studies on developmental effects of known and new chemicals in animal models.

ACKNOWLEDGMENTS

Research by the authors has been supported by grants AA08154, ES07033, and ES09601/EPA-R826886 (L.G.C.) and ES10563 and ES07331 (M.A.).

The *Annual Review of Pharmacology and Toxicology* is online at http://pharmtox.annualreviews.org

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