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Mini-Review

Developmental Neurotoxicology

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The developing brain has a distinctive set of characteristics that make it unusually sensitive to damage by toxic agents. Mechanistic understanding of the vulnerability of the immature nervous system to various chemicals is important from a preventive perspective but has also frequently given us new insights into maturation of neural circuitry. This review examines some of the developmental consequences of contact with various exogenous agents, including metals, solvents, pharmaceuticals, and natural products. This review emphasizes how subtle suboptimal brain function rather than acute toxicity can be a consequence of chemical exposures occurring during ontogenesis. The rate of brain aging may be influenced by events taking place in embryogenesis, following a prolonged asymptomatic period. The potential for appearance of adverse effects after prolonged latent periods is underscored.

Key words: neurotoxicity; metals; solvents; development; xenobiotics; maturation

The maturation of the human brain is an extended process. The consequences of early exposure to chemicals that are specifically harmful to the brain (neurotoxic agents), can be very subtle and may be evident only after a prolonged latency. This makes the establishment of causal relationships between a chemical exposure and an adverse neurological effect difficult to pinpoint. Recent studies are emerging showing how exposure to a toxic stimulus can cause ''imprinting,'' a process by which early environmental factors may permanently alter the gene expression profile of an organism. The consequences of such imprinting might not manifest themselves until adulthood. Exactly how aging triggers activation of these silent genetic changes is at present unknown. Any agent that interrupts or alters the normal development of a fetus with postnatal adverse consequences is known as a *teratogen*.

Another important consequence of early exposure to toxins is alteration of neuronal morphogenesis, which can permanently modify behavior. Such changes may not be dramatic but are important. Even modest deficits, resulting from suboptimal neural ontogenesis, when taking place across a broad spectrum of the population, can have a significant societal impact. Minor developmental shortfalls may be more readily detected in animal models compared with a more heterogeneous human population. The first part of this review discusses the features of brain maturation that make it vulnerable to chemical interference. The second section surveys some representative chemicals that can adversely and permanently influence this process. The focus is on a limited number of agents drawn from a few major chemical classes. This overview is intended to exemplify general features of damage to the immature nervous system rather than to provide a comprehensive cataloging. A noteworthy laboratory use of neurotoxic agents, which is not discussed in this review, is that, by selectively interfering with specific developmental steps, they can have potential value as research tools.

SPECIAL ATTRIBUTES OF THE DEVELOPING BRAIN WITH RESPECT TO NEUROTOXIC EXPOSURE

CNS Plasticity

The immature brain is a much more dynamically active tissue than the mature brain. It has a high degree of plasticity and a broad range of potential developmental directions. An initial high mitotic rate is followed by very precise movement of cells to defined loci. Appropriate guidance of such migrations is critical. Further maturation involves increasingly irreversible establishment of neuronal pathways and selective apoptotic destruction of a host of extraneous cells. Neurons originate in the germinal matrix and then migrate outward along the radial glia to a predestined terminus in one of the several layers of the brain. Finally, features characteristic of a mature brain emerge. These include the deposition of myelin, establishment of the blood–brain bar-

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rier, and biochemical differentiation characterizing adult neurons and glia.

Developmental Stages

Disruption of the complex sequence of events culminating in a fully functioning nervous system can lead to a less than optimal variant of a maximally effective brain. The precise time of a neurotoxic exposure can greatly affect the outcome. Maturation of the fetus can be described as taking place in three major stages. 1) The embryo during the first period of pregnancy (early in the first trimester) has a high proliferation rate, which makes it susceptible to antimitotic agents, such as those used in cancer therapy. However, at such an early period, major commitment to cell differentiation has not been irrevocably made. If the very early embryo is transiently exposed to a toxic chemical, the result is either death of the fetus and fetal abortion or an effective reorganization of the damaged embryo with compensatory replacement of lost cells. 2) The second phase of pregnancy is when organogenesis is initiated. In the case of the nervous system, this involves irrevocable establishment of neural pathways. Because the blood–brain barrier has not yet appeared, harmful agents have good access to the immature brain. This is true for metals, such as lead and mercury, and also for drugs of abuse, such as phencyclidine (PCP), levels of which can be reached in excess of those in the corresponding maternal brain (see below). Furthermore, the fetus has no functioning kidney and very limited metabolic detoxifying capacity, because mixed-function oxidases are present only at very low levels. This means that excretion is a relatively slow process, relying largely on diffusion. Major organ damage can ensue following exposure to harmful agents at this developmental stage. Examples include fetal alcohol syndrome (FAS; see below) and the skeletal abnormalities attributed to thalidomide. Interruption of mitosis associated with this stage of cerebral development can lead to permanent reduction of cell number in the affected area. Each brain region is characterized by a critical proliferative period. Thus, application of an antimitotic agent early in neurogenesis can lead to reduced cortical size, whereas other areas, which differentiate later, such as the cerebellum, remain essentially unaffected. Conversely, a similar exposure at a later stage of fetal development can result in almost total ablation of the cerebellum, although cortical changes are minimal (Matsutani et al., 1983). 3) All organ systems are largely developed at the final stage of pregnancy, but the blood–brain barrier is still not well developed. Toxic insult to the brain at this stage can no longer lead to gross anatomical abnormality but can lead to potentially permanent behavioral deficits, presumably as a consequence of more subtle deficits in the laying down of neuronal circuitry.

Unique Biochemistry of the Immature Brain

The biochemical milieu of the immature brain is very different from that found in the adult tissue. The high glycolytic rate and lower velocity of oxidative metabolism of the embryo are evolutionarily driven by the problem of obtaining an adequate oxygen supply from maternal tissue. Despite the existence of a distinctive form of hemoglobin designed to compensate in part for this, in comparison with the adult, the fetus lives in an environment with a low partial pressure of oxygen. As a consequence, newborn animals born at a relatively early stage of maturation, such as the rat, can live in an anoxic environment for much longer periods than the corresponding adult. Because inhibitory circuitry appears prior to the differentiation of excitatory neurons, the immature nervous system is less susceptible to excitotoxicity. Thus convulsants can be more damaging to the adult than to the fetus. All these apparent advantages of the fetus to certain types of toxic stimulus are balanced by a greater likelihood of permanently imprinting an irrevocable change within the younger brain.

Pre- and Postnatal Maturation

The brain of the fetus is in a milieu that differs significantly from that of the adult. The absence of a mature blood–brain barrier allows access of ions and hydrophilic compounds to a much greater degree than in the mature brain. This can increase exposure, for example, to any toxic metals, such as lead or mercury, found within the blood stream. Furthermore, the lack of a developed mixed-function oxidase system in fetal brain or liver impedes the metabolic transformation of lipophilic organic compounds first to polar materials and then to conjugates. The absence of cytochrome P450 systems in combination with lack of a functioning kidney means that levels of xenobiotic polar compounds within fetal brain can be reduced only by simple diffusion gradients into the placenta. Thus because of the readiness of access combined with the tardiness of metabolic excretion, the fetus can be especially vulnerable to both toxic polar and lipophilic chemicals. Levels of toxic chemicals within fetal brain may then be much greater than those present in the corresponding maternal brain. For instance, treatment of pregnant rats with the drug of abuse PCP can result in over a 100-fold greater concentration in the brain of the embryo compared with that of the treated mother (Ahmad et al., 1987). Analysis of corresponding blood levels shows that this difference is due to the blood–brain barrier of the adult rats largely excluding this drug from the central nervous system (CNS). Despite this enormous difference, the relatively small amount of PCP that did reach the maternal brain was sufficient to cause marked behavioral changes. Thus the amount of the drug that the fetal brain was exposed to was much greater than the pharmacologically active dose in the mother.

The brain has a prolonged period of postnatal maturation, and myelination is not complete until adolescence. Significant plasticity persists in the young adult brain, which is more adaptable than in the more mature adult CNS. The biological basis of this gradual loss is unclear, but it illustrates how the progression from ontogenesis to senescence is part of a single continuum.

CLASSES OF NEUROTOXIC AGENTS

The potential of several agents for adversely affecting normal brain development is briefly described. This listing is by no means a comprehensive survey of toxic agents but is intended to exemplify some general features by which the developing nervous system becomes an especially vulnerable target.

Metals

Lead. The prevalence of acute lead poisoning among the population has decreased greatly in the last 50 years. This is due to the implementation of more rigorous occupational health standards and reduction or elimination of lead from sources where the general population could be exposed. Thus lead has been removed from household paints, gasoline, toys, and soldering of food-containing cans. Its use in plumbing has also been reduced. However, lead is indispensable in many industrial processes and is still present in homes as a battery component and in soldering materials. Levels of lead originally accepted as safe have been repeatedly reduced, and the current ''no effect'' level is likely to be further lowered. Although lead toxicity was originally regarded as an overt event involving clear encephalopathy of relatively few individuals, the current view is that low levels of lead can exert an insidious adverse effect over a much broader population. The consequences of lead exposure for the immature brain can be difficult to detect but may have broad societal sequelae. A distinct deficit in behavioral parameters has been found in young children exposed gestationally to relatively minor levels of lead. This has been unequivocally demonstrated by following neonatal progression and relating this to umbilical lead levels at birth (Bellinger, 1994). The credibility of these studies has been strengthened by the fact that a wide range of potential confounders has been taken into account, including birth order, parental smoking and drinking habits, and family income levels. When all these are factored in, a clear dose–response relationship emerges at levels of blood lead previously considered insignificant (0–15 μg/100 ml blood).

The different susceptibility of the fetal and adult brain to lead is in part attributable to the blood–brain barrier, which can protect the adult brain to a significant extent. This is illustrated by the difference in acute toxicity of inorganic lead salts and the amphiphilic organic lead compound triethyl lead, which can readily bypass the blood–brain barrier to the mature brain. Less than 1 g of the latter compound can rapidly prove lethal to humans.

Many distinct mechanisms have been proposed to account for lead toxicity. These include elevated levels of free radicals, competition with calcium and zinc in key biological processes, modulation of neurotransmitter systems, energy deficits, and membrane damage. The issue of the mechanism of lead toxicity becomes simpler when extremely low levels of lead, similar to those currently found in the general population, are considered. At such concentrations, at which no effects are evident on those parameters described above, lead has been found to interfere with the function of zinc finger proteins (Basha et al., 2003). These transcription factors of the SP family of proteins contain repeating cysteine and histidine domains folded around zinc and bind to guanosine cytidine boxes. They are much higher in cells undergoing differentiation. SP-1 can mediate the expression of a variety of genes, including that for amyloid precursor protein (Basha et al., 2005). The substitution of lead for zinc initially reduces the strength of binding of these proteins to DNA but may later lead to intensification of normal binding (Zawia, 2003). This may account for the finding that the developmental profile of mRNAs is shifted in the brains of lead-exposed young animals so that their premature expression is followed by untimely depression. For example, lead-exposed fetal brain exhibits abnormally early neurite outgrowth, myelination, and then hypomyelination (Zawia et al., 1998). The ability of lead either to promote or to inhibit neurite outgrowth has been related to interference with signaling pathways involving specific kinases (Crumpton et al., 2001; Schneider et al., 2003), and this may also underlie changes in gene expression.

After neonatal exposure to lead, enhanced expression of amyloid precursor protein and accelerated formation of toxic amyloid peptides in aged rats can be delayed and be manifested as long as 20 months after the original exposure (Basha et al., 2005). However, the formation of amyloid peptides could be initiated only after early exposure to lead, and treatment of adult or aged animals produced no effect on amyloidogenesis. This illustrates how the consequences of genetic ''imprinting'' by lead may be reflected in overt phenotypic changes only after a prolonged interval and how the aging process might be a necessary cofactor in this delayed expression.

Mercury. The effect of mercury on development of the CNS was made dramatically apparent following inadvertent extended exposures during gestation, such as those seen in the Minamata Bay disaster. This followed the discharge of industrially produced, relatively nontoxic inorganic mercury salts into a bay with restricted access to the sea. These salts were converted to organic methylmercury by marine microorganisms. In this manner, mercury entered the food chain, reaching progressively higher levels with each predator species. This biotransformation followed by bioconcentration allowed low concentrations of inorganic mercury to be converted to toxic concentrations of methylmercury within tissues of larger fish. A further exacerbating factor was the prolonged residence time of mercury within the body (found also with lead). Neurotoxicity was found in humans after extended periods of fish consumption by populations involved in fishing as a major occupation. The slow onset of symptoms resulted in a high incidence of severe, largely irreversible damage to the CNS, which was especially marked in gestationally exposed infants. This again illustrates how interference with cerebral

metabolism at a plastic stage of development can often be much more harmful than a parallel assault on the mature brain, because interference with complex cell migration, as well as differentiation, can take place. In addition, fetal inability to excrete materials can further contribute to the teratogenicity of mercury. There have been other major outbreaks of mercury poisoning, such as that following consumption of grain treated with methylmercury as a fungicide and intended for planting, in Iraq (Myers and Davidson, 2000).

The major current issue of concern is the presence of significant amounts of mercury in fish and how such dietary exposure could affect the immature brain in a less dramatic yet deleterious manner. It is unclear what constitutes safe levels of mercury ingestion. As with lead, there is a significant likelihood of insidious adverse effects occurring with gestational exposure. Such relationships have been sought in populations consuming large amounts of fish, such as the inhabitants of the Faeroe and Seychelle Islands. However, the beneficial effects of maternal fish consumption may outweigh the harmful effects of low amounts of accompanying mercury (Clarkson and Strain, 2003), and this can confound human studies. Contamination of several rivers in the Amazonian basin has occurred as a consequence of gold mining in which mercury is used to amalgamate gold particles, and this also has led to an excessive accumulation of mercury in fish (Lebel et al., 1988). As yet there is no clear consensus on what the adverse effects are in low-level gestational exposures.

Treatment of cell cultures, containing both neuronal and glial cells derived from fetal rat brain during early development, with low concentrations of either organic or inorganic mercury compounds leads to cell death. In cell cultures derived from a more mature fetal stage, the organic form of mercury was more toxic and showed specific neuronal toxicity. Below the cytotoxic concentration of mercury (>1 μM), pronounced gliosis was observed (Monnet-Tschudi et al., 1996). This further illustrates the selective vulnerability of the fetal brain at different stages of maturation. The mechanism of mercury neurotoxicity is unclear, and several major events resembling those described for lead have been suggested. Mercury salts have a strong affinity for sulfhydryl groups, and this is also likely to play a role in accounting for their neurotoxicity. The disruption of the active sites of a broad class of enzymes by metals with an affinity for sulfhydryl groups is likely to affect many processes and thus produce multiple defects. Oxidative stress leading to lipid peroxidation and DNA damage may also underlie the toxicity of mercurials. In a human fetal hepatic cell line, exposure to low concentrations of inorganic mercury lead to lipid peroxidation and single-strand breaks in the DNA (Bucio et al., 1999). Furthermore, treatment of pregnant mice with N-acetyl-L-cysteine, an antioxidant and precursor of glutathione, reduces the teratogenicity of organic mercury (Ornaghi et al., 1993).

Vaccines containing ethylmercury, in the form of the preservative Thimerosal, have been suggested to play a role in autism (Bernard et al., 2001). Repetitive and abnormal movements as well as an inability to relate socially with others characterize this neurological disorder. It is estimated that children treated with these mercury-containing vaccines are exposed to levels of the metal far beyond those considered safe by FDA standards. This has led to a class action lawsuit against the manufacturer of Thimerosal by parents with autistic children or children who have not yet developed any symptoms but were exposed to high concentrations of mercury contained in the preservative. However, more recently it has been reported that the association between mercury-containing vaccines and autism might have no firm basis (Ip et al., 2004).

Ethanol and Other Solvents

One of the more dramatic and prevalent effects of fetal neurotoxic damage consequent to solvent exposure is that seen following maternal alcohol abuse. Jones and Smith (1973) first described FAS. However, a relation between alcohol consumption and this severe birth defect has been suspected, proposed, and then forgotten over a considerable period. The Old Testament (Judges, chapter 13, verse 7) contains the text; ''behold thou shall conceive a child. Now drink no wine or strong liquor,'' suggesting that this correlation was known over 2,500 years ago. Excess consumption of alcohol in adults causes irreversible damage to the brain only following many years of abuse. However, even transient gestational exposure to this solvent can lead to offspring with permanent and severe mental retardation as well as other physical abnormalities (Olney, 2004). The mechanism underlying this likely relates to the ability of this solvent to inhibit neuritic outgrowth by interfering with glial guidance of this process (Bingham et al., 2004) and with regulatory peptides, such as the neural cell adhesion molecule L1, which is also necessary for neuritic outgrowth (Bearer, 2001). Because this molecule is down-regulated after developmental synaptogenesis, such interference can occur only in the fetus. The characteristic facial and CNS abnormalities of FAS seen in human offspring has also been closely paralleled in many experimental animal models, including dog, mouse, rat, pig, and monkey (Slikker, 1994). Several other organic solvents, both aliphatic and aromatic, are capable of producing teratogenic effects closely resembling FAS. These include methanol and toluene (Wilkins-Haug, 1997; Costa et al., 2002).

Pharmacological Agents

Anticonvulsants. Craniofacial anomalies, similar but not identical to those seen with FAS, have been related to exposure to a variety of anticonvulsants, including phenytoin, phenobarbital, and trimethadione (Orup et al., 2003). Valproic acid use has been associated with neural tube defects such as spina bifida (Yerby, 2003), and this has been reproduced in an animal model (Padmanabhan and Shafiullah, 2003). Deficiency of folic

acid, while not causing severe symptoms in mothers, can cause failure of neural tube closure in the fetus (Geisel, 2003), and animal studies have shown that the administration of several antiepileptic drugs can result in folate depletion and teratogenic effects (Lewis et al., 2002; Burgoon et al., 2002). A common feature of all of these exposures seems to be deficits of neural migration patterns. The locus of action of most anticonvulsants is at least partially understood. However, it is generally not known whether their adverse effects on neurogenesis are related to untoward consequences of actions at such primary loci or are due to events provoked at undefined secondary foci. Phenytoin-induced deficits have been found to involve altered gene expression at crucial times of neural development (Gelineau-van Waes et al., 1999). Neural tube disorders, resulting from failure of the neural tube to close during the fourth week of embryogenesis, are among the most common severely disabling birth defects in the United States, with a frequency of approximately 1 in every 2,000 births (Northrup and Vlocik, 2000).

Associations have also been made with autism and gestational exposure to valproic acid and also to thalidomide (Arndt et al., 2005). These agents elevate serotonin levels in immature animals and cause behavioral disturbances reminiscent of autism (Narita et al., 2002). This association is strengthened by the finding that autism is also associated with elevated serotonergic activity (Whitaker-Azmitia, 2005). During embryogenesis, serotonin acts as an important neurotrophic factor (van Kesteren and Spencer, 2003).

Chemotherapeutic agents. Many chemotherapeutic agents used in cancer treatment to inhibit mitosis, disrupt the normal strict sequential pattern of cell division, emergence of distinct cell types in germinal layers, and cell migration to precise target areas. By this means, their utilization may lead to a range of craniofacial abnormalities and neural deficits. The mechanism of action of methotrexate is known to involve inhibition of dihydrofolate reductase, leading to deficiency of folate (Juchau, 1993), and this underlies its teratogenic properties. Another chemical known to cause craniofacial abnormalities in humans and experimental animals is vitamin A (Yu et al., 2003; McCaffery et al., 2003). Both the cis and trans isomers of this vitamin have teratogenic activity.

Anesthetics and tranquilizers. Even transient exposure of the fetus to anesthetics, sedatives, or tranquilizers during the later period of gestation and the early neonatal period has the potential to trigger apoptotic events during neurogenesis (Olney et al., 2000). The molecular basis of this may involve transient blockade of the responsivity of N-methyl-D-aspartate (NMDA) receptor to excitatory amino acids. During development, glutamate appears to play a critical role in inhibiting apoptosis, thus ensuring cell survival (Ikonomidou et al., 1999; de Lima et al., 2004).

Drugs of abuse. Another class of compounds whose potential effects on the immature nervous system has received much attention is drugs of abuse other than ethanol. These include cocaine, PCP, mescaline, nicotine, and various amphetamine derivatives. Use of each of these drugs during pregnancy has been associated with behavioral abnormality of neonates. The mechanism of action of these chemicals is likely to involve rather selective modulation of the formation and migration of specific neurotransmitter pathways. Thus, although such changes may not be overtly teratogenic, they may involve more subtle, long-lasting behavioral changes. The difficulty of establishing a causal relationship between drug use and relatively minor behavioral abnormality in humans is compounded by a large number of potentially confounding factors, such as simultaneous abuse of several drugs, nutritional status, and maternal behavior. Mother–infant bonding, critical for human maturation, can be disrupted when either the mother or the child exhibits abnormal behavior. Even in experimental animal models, a range of possible confounders has to be considered, such as the effect of chemicals being tested on the overall systemic well being of animals. Variability of maternal behavior following drug treatment can be taken into account by cross-fostering. However, the use of mixed litters containing control pups and somewhat abnormal offspring from treated mothers can also complicate analysis of results.

Thus, a large group of unrelated pharmaceuticals is able to disturb the process of deposition of the different anatomical components of the mature brain. This illustrates the complexity of the process and its great susceptibility to several kinds of disruption. A common feature, which may underlie such events, might involve reduction of DNA synthesis and depression of mitosis. The resulting absence of a critical cell population during ontogenesis can upset the normal pattern of recognition among cells and impair critical cell migrations. In addition, modulation of the normal expression of elements by which cells communicate, such as growth factors or cytokines, is likely to play a role in teratogenesis.

Natural Products

Several organic materials present in the diet of some populations are known to be neuroteratogenic. Neural tube deficits have been associated with the cycad plant genotoxin cycasin, which is the β-D-glucoside of methylazoxymethanol (MAM). MAM, an alkylating agent, has been shown to damage neuronal DNA and thus compromise DNA repair and perturb neuronal gene expression (Kisby et al., 1999), and it has been found to be a potent neuroteratogen in experimental animals (Lu et al., 2000). This agent is suspected to be a causal factor in ALS/parkinsonian dementia complex, a progressive adult disorder confined to islands of the western Pacific. Thus, the origins of some adult neurodegenerative disease may lie in the precipitation of a slowly evolving pathology originating very early in development (Fiore et al., 2000). Although ALS/parkinsonian dementia is a rare disorder attributed to a disappearing environmental factor confined to the western Pacific, the toxicological

profile of cycasin might have a much broader relevance to the effects of unknown environmental factors on wider populations.

While, many plant teratogens are recognized in the field of veterinary medicine, relatively few involving humans are well documented. High incidences of neural tube defects (NTD) occur in some regions of the world, including parts of China and South Africa, where there is substantial consumption of fumonisins, a class of mycotoxin produced when moulds grow on food crops in the field or in storage. Fumosins cause neural tube and craniofacial defects in mouse embryos in culture, and many of these effects are prevented by supplemental folic acid (Marasas et al., 2004). An association has also been made between spina bifida and the steroidal alkaloid glycosides solanine and chaconine, which can be induced by light exposure in potatoes. This class of compounds is a neuroteratogen in experimental animals, causing craniofacial malformations (Gaffield and Keeler, 1996), but a clear association with spina bifida in humans has not been made. Once again, supplementation with a folate analogue can be protective. Overall, interference with folate-requiring processes seems to be a common basis for the action of many neuroteratogens.

Persistent Xenobiotic Environmental Contaminants

Transient chemical exposures, focused on a distinct metabolic target, may ensue as a consequence of pharmacological therapies intended to be of limited duration. However, many environmentally prevalent contaminants have no inherent pharmaceutical design. Such materials may have more than a single locus of action and may also be retained for extended periods within tissues.

A range of organic agents of anthropomorphic origin and possessing great stability is broadly present in the environment. These agents include polychlorinated biphenyls (PCBs), polybrominated biphenyls (PBBs), o′p′-DDT, and dioxins, all of which are planar bicyclic compounds. These are known to be toxic in adults, and it is very likely that they have significant effects on the embryonic brain. Gestational and lactational exposure to PCBs has been reported to impair cognitive development of infants (Aoki, 2001). Many of these long-lived compounds have hormonal properties and thus have the potential to act as endocrine disruptors. Dioxins and PCBs appear to affect both thyroid and estrogen function (Schantz and Widholm, 2001; Ohsako et al., 2002). Such agents can interfere with both the maturation of the neuroendocrine axis and the elaboration of neuronal networks (Kakeyama and Tohyama, 2003). Treatment of embryos with dioxins can effect permanent changes in gene expression. This imprinting may involve altered methylation of DNA within specific genetic loci (Wu et al., 2004). The biological activity of these organohalogens is likely to have multiple bases. Their stability suggests allosteric interactions with specific receptors or genetic loci rather than covalent reactions.

INTERACTION OF NEUROTOXIC EXPOSURES WITH OTHER FACTORS

Several extraneous factors govern the extent to which a chemical may manifest itself as toxic in the immature nervous system. These include genetic factors that can affect susceptibility and nutritional status. Malnutrition can under certain circumstances enhance the potential for gestational insult of a material that otherwise would be less toxic. For example, a protein-deficient diet can lead to an inadequate intake of sulfur-containing amino acids, which are used in the conversion of cyanide to thiocyanate by rhodanase. This can lead to reduced detoxification of cyanide-producing glycosides found in cassava. This is a widely consumed plant known to cause spastic paraplegia in adults (Tor-Agbidye et al., 1999) and also likely to be a teratogen (Frakes et al., 1986).

Vitamin deficiency can also potentiate the adverse effects of compounds and can be incurred by selective depletion by or competition with a toxic agent. Thus, the microcephaly induced in experimental animals by X-ray irradiation can be protected against by pretreatment with α-tocopherol (Tanaka et al., 1986). About 65% of radiation-induced damage is due to formation of reactive oxygen species (ROS) (Han et al., 1976), and this strongly implicates free radicals as a disruptor of fetal brain maturation. Many neurotoxic agents promote excess formation of ROS. This may occur in a direct manner, as is the case with redox-active metals such as copper or redox-active organic compounds such as quinones. Elevated ROS generation is also associated with cell injury, reflecting impaired oxidative metabolism consequent to energy depletion. Thus abnormal rates of ROS production may represent a final common path of many toxic agents whose impact may be at a variety of unrelated sites (Campbell and Bondy, 2004). Therefore, quenching of the oxidant properties of metabolites by coexposure to antioxidants, can generally be neuroprotective.

CONCLUSIONS

Gross morphologic abnormalities or disruption of processes such as neurogenesis or myelination induced by exposure to a neurotoxic agent during fetal or early postnatal life are readily detectable. However, important behavioral deficits that are not readily associated with morphological changes can also incur following such exposures. In such circumstances, which are likely to be more prevalent than major toxic lesions, the detection of damage to the immature human brain and establishment of a causal relationship with a prenatal exposure is problematic. It has been calculated that, were newborn infants to experience a loss of 30 IQ points resulting from a transient prenatal exposure to a toxic agent, one would be very unlikely to uncover the cause of this deficit. In the absence of spectacular and obvious physical changes, such as those incurred with prenatal exposure to thalidomide, minor behavioral impairments are very

difficult to detect and attribute to a gestational origin. It is only because FAS presents with a distinctive physical appearance as well as mental retardation that an association with maternal drinking was made. It should also be borne in mind that compounds known to cause gross teratogenic changes, when applied at lower levels, have generally been found to cause behavioral impairment in the absence of marked neuropathological changes.

An important distinction is between the effect of a minor insult to an individual and that on society as a whole. Thus, if an exposure to a chemical agent were to cause a drop in IQ of 5 points compared with the IQ achievable under optimal conditions, this would probably not affect an individual greatly. However, a widespread exposure (for example, such as that existing for lead) could spread such a deficit over the whole population. This would markedly affect the lower and upper ends of a bell-shaped intelligence distribution curve and result in a significantly greater percentage of the population who are not able to care for themselves as well as a decrease in the number of highly gifted individuals.

Early exposures to toxic agents may be expressed much later in the life of an organism. After maternal exposure to a neuroteratogen, undesirable effects may appear after a prolonged refractory period, leading to cognitive defects that appear in childhood or adolescence (Slotkin, 1998). The time of an insult may thus be considerably removed from the expression of damage. For example, prenatal exposure to low levels of carbon monoxide may not affect the initial postnatal spurt in myelination but only affect later myelin deposition (Carratu et al., 2000). The study of Basha et al. (2005) involving neonatal administration of lead with ensuing acceleration of aging as judged by earlier appearance of amyloid deposition, illustrates how gestational events can profoundly affect the aging process after a very long latency interval. The establishment of firm causal relationships over such extended periods necessitates the design of a convincing animal model. The only way to consolidate suggestive but inconclusive epidemiological results is by demonstration of parallel events in an animal model with well-controlled conditions. This situation can never be achieved in human studies.

In summary, the human brain has a prolonged period of postnatal maturation, and many developmental events such as myelination are not fully complete until adolescence. This gives an extended opportunity for neurotoxic agents to subtly but permanently affect the integral functioning of the adult nervous system.

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