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脳神経系機能発達への影響と作用メカニズム

Effects on the Brain and Behavioral Development

Brain Development and Behavior as a Toxic Target of Dioxin and Other Environmental Chemicals

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Behavioral teratology describes a discipline whose subject matter is the developing nervous system and its susceptibility to toxic exposures. Japan knows it intimately because much of it had its roots in the Minamata tragedy and the spotlight it shone on methyl mercury. Since then, we have become sensitized to the vulnerability of the developing brain because we've discovered that a remarkable variety of chemicals threaten its integrity, even at low levels of exposure common in the environment. We have learned our lessons from alcohol, lead, PCBs, and other contaminants whose risks to adults convey little information about the risks for the fetus, infant, and child. We are learning new lessons from the chemicals called endocrine disruptors because their modes of action do not always coincide with conventional toxicological principles. Dioxin offers a compelling example of how we need to adjust our perspectives. Views of dioxin's health hazards had been dominated by cancer until evidence began to emerge that it and similar chemicals also interfered with development, particularly of the reproductive system. Only recently have we discovered that aberrant development of the brain and its expression in behavior is one of the most sensitive indications of its harmful effects. The evidence, from my laboratory and others, tells us that certain behaviors are exquisitely responsive to dioxin exposure during gestation, and that one index of susceptibility may be reflected in the differing responses of male and female offspring, a phenomenon termed sexual dimorphism. If we analyze this and related evidence carefully, and with the proper tools, we confront the possibility that current tissue stores of dioxin in the bodies of women may present serious risks to the optimal development of their children's brains and their potential as individuals. This realization should provoke us to examine not just the health implications of current exposures to environmental chemicals, but their ethical implications. Perhaps we need to reconsider our conventional approaches to how we determine prudent exposures, even beyond the Precautionary Principle, and begin asking questions about ethical risks in parallel with our questions about health risks. (My research is funded in part by an Environmental Health Sciences Center grant, ES01247, and a research grant, ES08958, to the University of Rochester School of Medicine and Dentistry from the National Institute of Environmental Health Sciences.)

Thyroid Hormone, Brain Development, and the Environment

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Thyroid hormone (TH) is essential for normal brain development. The syndrome known as cretinism perhaps most often illustrates the validity of this statement. Cretinism is caused by pre- and post-natal hypothyroidism, which is most often attributable to very low dietary iodine. The most prevalent and persistent characteristic of cretinism is severe mental retardation. Postnatal hypothyroidism is also caused sporadically by a syndrome known as congenital hypothyroidism (CH). CH can be cause by thyroid dysgenesis, thyroid agenesis, or defects in the synthetic pathway for TH. CH also can produce severe mental retardation if not identified and treated within a very short window (14 days after birth); as a result, many countries screen all newborns for TH.

Perhaps because of the success of the neonatal screening program, most clinical and experimental research has focused on the developmental effects of postnatal thyroid hormone. However, recent clinical studies have demonstrated that thyroid hormone is important for the fetus as well. One study demonstrates that the offspring of women whose circulating levels of T_4 were within the lowest 10th percentile of the normal range exhibited a significant increase in the incidence of low IQ and attention deficits.

Our laboratory has recently begun to focus on the molecular, cellular and developmental effects of thyroid hormone on fetal brain development. We have identified a number of thyroid hormone-responsive genes in the fetal cerebral cortex prior to the onset of fetal thyroid function. Therefore, these genes are regulated by thyroid hormone of maternal origin. Our studies have led us to the population of early cortical cells that represent the founder population - they are the precursors of all cells in the cerebral cortex. These studies strongly suggest that thyroid hormone from the mother is involved in balancing the production of neurons and glia (helper cells) in specific regions of the early cerebral cortex.

The observation that thyroid hormone of maternal origin can affect fetal brain development also illustrates that any factor that affects maternal thyroid function, or thyroid hormone action, may interfere with fetal brain development in very specific ways. Our laboratory has recently begun to address this issue using polychlorinated biphenyls (PCBs) as a test substance. PCBs are a class of industrial compounds consisting of paired phenyl rings with various degrees of chlorination. Before their production was banned in the United States in the 1970's, over a billion kilograms of PCBs were produced, and they are now ubiquitous, persistent environmental contaminants routinely found in samples of human and animal tissues. PCBs in breast milk has been reported to be in the range of 3 - 40 μM.

Because PCBs are known to reduce circulating levels of thyroid hormone in experimental animals, we examined whether PCB exposure produced effects on brain development consistent with hypothyroidism - using the expression of specific thyroid hormone-responsive genes as a marker of PCB effects. We have found that in both the neonatal and fetal rat, PCB exposure produces thyroid hormone-like effects on gene expression. This includes the expression of genes in the Notch Signaling pathway believed to be responsible for radial gliogenesis.

These studies illustrate several important issues. First, thyroid hormone from the mother can exert specific effects on gene expression in the fetal brain before the onset of fetal thyroid function. There are likely to be many more genes affected that we have yet identified. In addition, the array of genes affected by thyroid

hormone in any cell will likely change as the cell becomes more highly differentiated. Second, the complexity of thyroid hormone action on the developing brain is likely to be more complex that presently appreciated. However, it is surprising, given the importance of thyroid hormone in brain development, that we know so little. Third, environmental factors such as PCBs, can exert effects on gene expression and probably developmental events, that are not predicted based on their effects on circulating levels of gene expression. Considering this, it is essential to development measures of thyroid hormone action that can be used for risk assessment to supplement present studies focused exclusively on measures of thyroid function.

Functional Development of Neuronal Networks in Culture -An *in vitro* Assay System of Developing Brain for Endocrine Disruptors

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Human brain is a chemical machine, which functions by various types of chemical transmitters and hormones including thyroid hormones through their receptors. During its development, neurons are made; synapses are formed through sequential gene expressions followed by defined molecular cascades via many receptor systems, building up the complex structure of the brain. Therefore, such chemicals as endocrine disrupters can easily disrupt development of functional brain, which affects any receptor systems in the fetus and newborn brain. Unfortunately, the blood-brain barrier, protecting the adult brain from toxic chemicals, has not been matured until 2-3 years old. So, toxic chemicals can get in fetus and newborn brain from mother and their food and may affect the developing brain, especially cortical synapse formation that mainly occurs during the perinatal period. So far, we can estimate the total risk of environmental endocrine disrupters is higher for the brain/nervous system than for reproductive and immune system.

However, at present, toxicological and epidemiological data is very limited to answer the serious questions which chemicals from environment can cause which type of deficiencies in the developing brain / behaviors. Obviously, practical screening systems are necessary for testing hundreds of candidate chemicals.

We have developed cell cultures of rat cerebral cortex in which neurons make huge number of synapses each other. The resulting neuronal networks *in vitro* show spontaneous synchronized oscillation of bursting activity. The synchronous oscillation of neuronal excitation can be easily monitored as transient changes of intracellular Ca by video-assisted, multi-sites fluorometry using calcium sensitive dyes (Kuroda et al. Neurosci.Let.,135,235,1992). Various chemicals, which affect any essential functional molecules in this complex system, will change the Ca oscillation. It appeared the frequency of the Ca oscillation increased parallel with the number of synapses formed in the culture. When thyroid hormones are added in the culture, frequency of the Ca oscillation of the neurons increased comparing with the control culture, suggesting thyroid hormones facilitate functional synapse formation. Using this thyroid-dependent increase as an indicator, we can screen added chemicals, which may disrupt thyroid-dependent brain development. Thyroid hormones also stimulate the dendrites extension of cerebellar Purkinje cells in culture.

These data indicate the neuronal cell culture systems will be useful both for practical screening and for molecular and cellular study of toxicity mechanisms of endocrine disruptors.

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Risk Analysis of Endocrine Disrupting Chemicals Using Higher Animals

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It has been studied and reported that endocrine disrupting chemicals (EDCs) disturb estrogenic action on Mollusca, Fish, Amphibian, Reptiles and Avian species and induce a malformation of genital organs. However, it is still controversial of EDCs-effect on higher animals such as mammalians.

Recently, it is postulated that exposure of fetus to the EDCs such as TCDD and PCB may result in a deterioration of neuronal development because these EDCs are able to affect thyroid hormone with their structural similarity rather than estrogen.

In our project, we will try to conduct risk analysis of EDCs using higher animals. The basic analysis on the offspring effect of EDCs taken per orally (PO) starts by the use of pregnant rats. Then pregnant macaque monkeys being more close relatives to human are used. However, it is difficult and gives severe stress to the monkeys administrating EDCs PO everyday, and embedding an osmotic pressure pump for subcutaneous injection (SC) is used. Then, for an extrapolation to human, chimpanzee is used to check a one shot toxicokinetics of the EDCs PO. Thus, extrapolation of the results of long term PO administration in rat, long term SC administration in macaques and one shot chimpanzee by SC and PO is necessary.

The situation is the same for risk analysis of EDCs on neural development measured by behavioral studies. The risk analysis system of EDCs on monkey fetal neurons has not been established as yet. We will propose following check items; 1) apparent malformation, 2) growth and development of neonate, 3) mother-infant interaction (under 6M), 4) first contact behavior (6M- 1Y), 5) finger maze test for cognition and study ability (1.5Y), and 6) drug burden test with monoamines or stimulants.

The risk analysis study of EDCs on neuronal development starts very recently and systematic study using from rodents to primate and great apes comparatively is few. We want try to establish the validation system of EDCs using higher animals.

Evidence for the Role of Environmental Neurotoxicants in Learning and Behavioral Disabilities

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There is increasing evidence that environmental contaminants may affect the development of nervous system function, resulting in cognitive impairment and deficits in attention, impulse control, and social skills. Some of these effects overlap defined cognitive and behavioral disabilities, including learning disabilities, attention deficit hyperactivity disorder (ADHD), and conduct disorders. Learning disabilities, which may affect 15-20% of U.S. children, are defined by deficits in listening, speaking, reading, writing, mathematical abilities, or social skills below that expected by level of intelligence. ADHD is a disability that affects between 3 and 7% of children, with a significant number of individuals continuing to be affected into adolescence and adulthood. ADHD is characterized in part by an inability to organize complex sequences of behavior, to persist in the face of distracting stimuli, and to respond appropriately to the consequences of past behavior. Conduct disorders are characterized by aggression, disruptive behavior, and an unwillingness or inability to respect the rights of others.

Behavioral and cognitive effects that are consistent with these disorders have been documented as a consequence of developmental exposure to lead and PCBs, the environmental neurotoxicants that have received the most attention. Children exposed developmentally to lead are more distractible, impulsive, hyperactive, and disorganized than their peers. Lead exposure results in increased grade retention, need for academic aid, and an increased prevalence of failure to graduate from high school. Children with ADHD, as well as children exposed to lead, exhibit impaired performance on the Wisconsin Card Sort Test, which requires the subject to reverse an already established response strategy without explicit instruction to do so. Lead also produces increased reaction times on both simple and complex vigilance tasks, indicative of impaired attentional processes. Lead exposure also produces increased aggression, delinquency, and other impairments in social behavior. The effects of developmental exposure to polychlorinated biphenyls (PCBs) are in many respects similar to those of lead. *In utero* exposure to PCBs produces small decrements in IQ, deficits in reading ability, increased aggression, and internalizing behavior. Prenatal and/or postnatal exposure results in deficits on a vigilance task, increased activity and inattentiveness, and decreased play behavior.

There are some parallels between the features of ADHD and other behavioral disabilities and the behavior of monkeys exposed developmentally to lead or PCBs, as evidenced by research from our laboratory. Both lead and PCB exposure produce deficits on discrimination reversal and spatial delayed alternation performance; treated monkeys exhibit deficits in their ability to change an already established response strategy and inhibit inappropriate responses. They also perseverate in non-adaptive behavior patterns. Monkeys exposed developmentally to lead or PCBs also perform differently from control monkeys on a fixed interval schedule of reinforcement, which requires the temporal organization of behavior using only internal cues. PCB exposure also produces deficits in DRL performance, a task that requires inhibition of inappropriate responding.

Although the etiology is undoubtedly multifactoral, the possibility that neurotoxic agents in the environment contribute to the prevalence of cognitive and behavioral disabilities warrants further attention.