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Neurotoxicity of Sodium Fluoride in Rats

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MULLENIX, P. J., P. K. DENBESTEN, A. SCHUNIOR AND W. J. KERNAN. *Neurotoxicity of sodium fluoride in rats.* NEUROTOXICOL TERATOL 17(2) 169–177, 1995.—Fluoride (F) is known to affect mineralizing tissues, but effects upon the developing brain have not been previously considered. This study in Sprague–Dawley rats compares behavior, body weight, plasma and brain F levels after sodium fluoride (NaF) exposures during late gestation, at weaning or in adults. For prenatal exposures, dams received injections (SC) of 0.13 mg/kg NaF or saline on gestational days 14–18 or 17–19. Weanlings received drinking water containing 0, 75, 100, or 125 ppm F for 6 or 20 weeks, and 3 month-old adults received water containing 100 ppm F for 6 weeks. Behavior was tested in a computer pattern recognition system that classified acts in a novel environment and quantified act initiations, total times and time structures. Fluoride exposures caused sex- and dose-specific behavioral deficits with a common pattern. Males were most sensitive to prenatal day 17–19 exposure, whereas females were more sensitive to weanling and adult exposures. After fluoride ingestion, the severity of the effect on behavior increased directly with plasma F levels and F concentrations in specific brain regions. Such association is important considering that plasma levels in this rat model (0.059 to 0.640 ppm F) are similar to those reported in humans exposed to high levels of fluoride.

Fluoride Neurotoxicity Central nervous system

DENTAL fluorosis has been on the rise since the 1950s, indicating that our total fluoride exposure is increasing (9). Fluoride, including sodium fluoride (NaF), has been added to public water supplies for over 40 years in the United States as a preventative measure against dental caries. Other sources of fluoride exposure include processed beverages, toothpastes, mouth rinses, dietary supplements, and food. Although dental fluorosis causes discoloration of teeth, it is not considered a public health concern because it does not hinder tooth function or oral health. In addition, no clear link has been established between fluoride and cancer risk, bone fractures, birth defects, or problems of the gastrointestinal, genito-urinary, or respiratory systems (1). Therefore, the impetus to limit total fluoride exposure in the United States is currently based on cosmetic concerns and a general desire not to expose the public to any more fluoride than the amount necessary to prevent dental caries.

One concern that has not been fully investigated is the link between fluoride and effects on the central nervous system (CNS). In vitro studies have shown that intracellular fluoride can alter the kinetic properties of calcium currents in hippocampal neurons (22). Fluoride is a normal component of cerebrospinal fluid (21), but it has not been found to accumulate there during endemic fluorosis or nervous system disease (21,41). Yet, there have been reports from Chinese investigators that high levels of fluoride in drinking water (i.e., 3–11 ppm) affect the nervous system directly without first causing physical deformations from skeletal fluorosis (13,20,40). One study of adult humans found attention affected by sublingual drops containing 100 ppm of sodium fluoride (39), an exposure level potentially relevant to humans because toothpastes contain 1000 to 1500 ppm fluoride (8,48) and mouthrinses contain 230–900 ppm fluoride (48).

Many years of ubiquitous fluoride exposure have not resulted in obvious CNS problems such as seizures, lethargy, salivation, tremors, paralysis, or sensory deficits. Still unexplored, however, is the possibility that fluoride exposure is linked with subtle brain dysfunction. The present study evaluates the neurotoxic potential of sodium fluoride in an animal model. It uses behavioral methodology that focuses on behavioral repertoire, responses to novelty and the temporal or sequential organization of spontaneous behavior, all important

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DISCUSSION

This study demonstrates a link between certain fluoride exposures and behavioral disruption in the rat. The effect on behavior varied with the timing of exposure during CNS development. Behavioral changes common to weanling and adult exposures were different from those after prenatal exposures. Prenatal exposure on GDs 17–19 dispersed many behaviors as seen in drug-induced hyperactivity (34), while weanling and adult exposures led to behavior-specific changes more related to cognitive deficits (35,36). Prenatally induced behavioral effects were unaccompanied by changes in body weight or elevated plasma fluoride levels. Rather, the most obvious hypothesis is that the effects relied on transient peaks in maternal plasma fluoride levels, fluoride passing the placenta, and fluoride penetrating the blood–brain barrier of the fetus. Fluoride has been reported to pass the placenta in rats (45), and on GD 17–19 the blood–brain barrier is immature and readily penetrable (52). In contrast, the behavioral effects induced by weanling and adult exposures were accompanied often by weight reduction and always by elevated plasma fluoride levels. In fact, effects on behavior related directly to plasma fluoride levels and the fluoride accumulation in the brain. This contradicts findings from short-term fluoride kinetic studies, which found that the adult blood–brain barrier was relatively impermeable to fluoride when whole brain fluoride levels were measured within 1 h following IV injection (49,50). Considering the brain fluoride accumulations found in this study, such impermeability does not apply to chronic exposure situations.

Hyperactivity and cognitive deficits are generally linked with hippocampal damage (3), and in fact, the hippocampus is considered to be the central processor which integrates inputs from the environment, memory, and motivational stimuli to produce behavioral decisions and modify memory (12). GDs 17–19 in the rat is a period when pyramidal cells of the hippocampus are forming (6), and granule cells of the dentate gyrus of the hippocampus form at the ages when weanling and adult exposures were administered (7). Involvement of different cell types would explain variation in behavioral outcomes between prenatal, weanling, and adult exposures. The hypothalamus and the hippocampus in normal female rat brains have the lowest concentrations of fluorine, the element which was found to be the most regionally distributed by instrumental neutron activation analysis (10). The method used for ionic fluoride analysis in the present study also revealed that the brain region containing the lowest fluoride concentrations was the hippocampus of controls but only in females. This hippocampal selectivity was disrupted when adult females were exposed for 6 weeks to 100 ppm fluoride; hippocampal fluoride levels increased and behavior was affected. Adult males receiving the same fluoride exposure did not have significantly elevated fluoride levels in the hippocampus, nor did they have significant behavioral disturbances. Sex differences in hippocampal function have been described recently in other studies (2,47). Overall, the behavioral changes from fluoride exposure are consistent with interrupted hippocampal development. Whether the hippocamus is indeed the brain region most susceptible to fluoride is a possibility deserving consideration in future studies.

Interruption of normal brain development often results in responses that are sex-dependent. The brain responds differently to drugs depending on which hormones are present at the time and whether the brain is male or female (30). In male primates the orbital cortex matures earlier than in females, and such developmental differences are thought responsible for the consequences of perinatal injuries appearing more frequently in males (18). This type of developmental difference might explain why transient peaks of fluoride on prenatal days 17–19 affected males and not females. The effects of chronic fluoride exposures at weanling and adult stages may have involved still other sexual dimorphisms. There are developmentally regulated sexual dimorphisms in hypothalamic somatostatin and growth-hormone-releasing factor signaling, growth hormone secretion and even hepatic metabolism (5,29,38). The sexually dimorphic control of growth would be especially important to fluoride distribution. The rate of fluoride uptake by bone depends on age or the stage of skeletal development; fluoride is deposited in mineralizing new bone more readily than in existing bone (49). As males experience greater and more prolonged growth spurts than females, their plasma fluoride might be directed more to bone than to brain, perhaps explaining why longer exposures and higher plasma fluoride levels were needed in males to affect behavior. Fluoride's tendency to seek developing bone may also explain why adult female rats had behavioral effects at a lower plasma fluoride concentration than did weanling female rats. Levels of fluoride in plasma and bone must be correlated with those in specific brain regions of both sexes to fully understand behavioral consequences.

Rats ingested 75–125 ppm fluoride for weeks to attain plasma fluoride levels of 0.059–0.640 ppm. Six weeks of consuming 75 and 100 ppm fluoride produced higher plasma fluoride levels than did 125 ppm. Perhaps a taste aversion limited water consumption at the 125 ppm level, prolonging the period needed to attain plasma levels that were achieved in 6 weeks by the two lower exposure levels. Regardless, it was fluoride levels in plasma, not fluoride levels of exposure, which best predicted effects on behavior. Similar plasma fluoride levels of 0.076–0.25 ppm have been found in humans ingesting 5–10 ppm fluoride in drinking water (19,37,42), and plasma levels as high as 0.28 to 0.43 ppm have been measured in children drinking water containing 16 ppm fluoride (44). This plasma fluoride range also occurs in certain therapies. Fasting serum fluoride levels of 0.2 to 0.3 ppm are used in the treatment of osteoporosis (31), and plasma fluoride levels as high as 1.44 ppm are found in children 1 h after receiving topical applications of an acidulated phosphate fluoride (1.23%) gel (14,15).

Because humans occasionally are exposed to high amounts of fluoride and plasma levels as high as those found in this rat study, neurotoxic risks deserve further evaluation. This is the first laboratory study to demonstrate that CNS functional output is vulnerable to fluoride, that the effects on behavior depend on the age at exposure and that fluoride accumulates in brain tissues. Experience with other developmental neurotoxicants prompts expectations that changes in behavioral function will be comparable across species, especially humans and rats (16,43). Of course behaviors per se do not extrapolate, but a generic behavioral pattern disruption as found in this rat study can be indicative of a potential for motor dysfunction, IQ deficits and/or learning disabilities in humans. Substances that accumulate in brain tissue potentiate concerns about neurotoxic risks, but the conditions leading to fluoride deposits in any species are still not clear such that quantitative extrapolations are not possible at this time. Thus, conclusions concerning the neurotoxic potential of fluoride require further rat and human studies, both focused on the relationship of plasma fluoride levels with the brain, behavior, and skeletal growth.

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