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NEUROTRANSMITTER AND RECEPTOR CHANGES IN THE BRAINS OF FETUSES FROM AREAS OF ENDEMIC FLUOROSIS

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SUMMARY: The levels of neurotransmitters and receptors in brain tissue of aborted fetuses from areas of endemic fluorosis were tested. The results showed that in 10 fetuses from a high fluoride area ranging in age from 5 to 7 months, the levels of norepinephrine, 5-hydroxytryptamine, and α_1 -receptor were lower, and the level of epinephrine was higher as compared with levels seen in the control fetuses from a non-endemic fluorosis area; each of these differences was statistically significant ($p < 0.05$). Other monoamine neurotransmitters and metabolic products, such as dopamine, 5-hydroxyindoleacetic acid and 3,4-dihydroxybenzoic acid, showed nonsignificant differences ($p > 0.05$). The results suggest that the accumulation of fluoride in the brain tissue can disrupt the synthesis of certain neurotransmitters and receptors in nerve cells, leading to neural dysplasia or other damage.

Keywords: Aborted fetuses; Brain receptors; Fetal fluoride; Nerve cell receptors; Neurotransmitters; Placenta.

INTRODUCTION

There have already been several reports in the literature¹⁻⁵ demonstrating that, in cases of chronic fluoride poisoning, the pregnant mother's body load of fluoride may pass through the placenta and collect in the body of the fetus, and even continue through the blood-brain barrier and into brain tissue, thereby inflicting damage that slows brain development, manifesting as abnormalities to such features as the brain tissue morphology, ultrastructure, and neurotransmitters. In this work we took the next step and measured the density and function of α_1 -receptors as well as levels of the monoamine neurotransmitters in aborted fetuses from an endemic fluorosis area in order to investigate the effects of fluoride poisoning on receptors and neurotransmitters in brain tissue.

MATERIALS AND METHODS

Research material:

(i). *Fetuses from the endemic fluorosis zone:* Ten specimens were drawn from an endemic fluorosis region of Zhijin County in Guizhou Province. In each case abortion was induced by water bag. The age of each fetus was calculated with reference to suspension of the mother's menstrual cycle and the CR length; all fetuses were found to be between five and seven months of age. The mothers all lived permanently in an endemic fluorosis area and ate diets rich in high fluoride grains, and each showed some degree of dental fluorosis. However, no other symptoms or manifestations of fluorosis and no other diseases that would affect bone metabolism were present.

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(ii). *Fetuses from the nonendemic zone:* Ten specimens were drawn from a nonfluoride endemic section of Guiyang city in Guizhou Province. The fetuses were otherwise the same as the fluoride endemic fetuses, i.e. calculated to be five to seven months of age. The mothers showed no signs of dental fluorosis.

Tests:

(i). *Fluoride content of the pregnant women's urine and blood serum:* Intravenous blood samples were taken in the morning on an empty stomach, and the serum was separated out. The first morning urination was collected, and both urine and serum were analyzed for fluoride content using the fluoride ion selective electrode method.

(ii). *Fluoride content of the fetal brain and bone tissue samples:* A weighed sample of fresh tissue was removed from the brain specimen of each fetus, dried, carbonized, and incinerated. The right femur of each fetus was extracted, dried, carbonized, and incinerated. The remains of each sample were subjected to a fluoride ion selective electrode test to determine fluoride content.

(iii). *Preservation of the brain specimens:* After each fetus was aborted using the water bag method, the tissue from the upper cerebrum was extracted and stored at a temperature of -20°C .

(iv). *Test of monoamine neurotransmitters in fetal brain tissue:* The levels of the monoamine neurotransmitters were determined using a high performance liquid chromatograph manufactured in the United States by the Waters Corporation. The norepinephrine, epinephrine, 5-hydroxytryptamine, dopamine, 5-hydroxyindoleacetic acid, and 3,4-dihydroxybenzoic acid were purchased from Sigma Chemical Co.

(v). *Test of α_1 -receptor in the fetal brains:* Membrane suspension was prepared using methods described in the literature.⁶ A small sample of the membrane suspension was subjected to Lowry's method to determine the membrane protein count. The remaining liquid was subjected to a radioligand binding assay of receptor saturation; the receptor density (Bmax) and the disassociation constant (Kd) were determined by a 2550 TR/LL liquid scintillation counter (manufactured by US-based Packard Instrument Co.). The radioligand used was Canadian-produced ^3H -Prazosin (specific activity 42 Ci/mmol).

RESULTS

The urine and serum fluoride levels of the pregnant women and the bone and brain fluoride content of the fetuses are shown in Table 1.

Table 1. Urine and serum fluoride of the pregnant women, and bone and brain fluoride of the fetuses^a (mean \pm SD)

Group	n	Pregnant women		Fetuses	
		Urine F ⁻ ($\mu\text{g}/\text{mL}$)	Serum F ⁻ ($\mu\text{g}/\text{mL}$)	Bone F ⁻ ($\mu\text{g}/\text{g}$)	Brain F ⁻ ($\mu\text{g}/\text{g}$)
Non-fluorosis area	10	1.67 \pm 0.82 [*]	0.41 \pm 0.15	2.50 \pm 0.11 [*]	1.23 \pm 0.24 [*]
Fluorosis area	10	4.32 \pm 2.94	0.55 \pm 0.21	2.87 \pm 0.25	1.65 \pm 0.33

^aThe bone and brain fetal fluoride levels are dry tissue analyses; ^{*}p<0.05 comparing the non-fluorosis and the fluorosis are a groups.

As seen in Table 1, the women from the endemic fluorosis area had higher urine fluoride than the women from the non-endemic area, and the difference is statistically significant. Both brain and bone tissue of the fetuses from the fluorosis endemic area showed higher fluoride content than the control fetuses, and these differences were also statistically significant.

The levels of monoamine neurotransmitters in the fetal brain tissue are given in Table 2.

Table 2. Levels of monoamine neurotransmitters in fetal brain tissue (mean±SD)

Group	n	Nor-epinephrine (mg/g)	Epi-nephrine (mg/g)	Dopamine (ng/g)	3,4-dihydroxybenzoic acid (ng/g)	5-hydroxytryptamine (ng/g)	5-hydroxyindoleacetic acid (ng/g)
Non-fluorosis area	10	92.92±41.12*	17.44±2.82*	5.28±2.79	8.01±4.36	450.60±319.93*	37.53±20.14
Fluorosis area	10	32.81±18.30	21.84±2.92	7.34±3.92	10.34±8.66	123.34±31.91	31.28±18.95

*p<0.05 comparing the non-fluorosis and the fluorosis area groups.

As can be seen in Table 2, the levels of norepinephrine and 5-hydroxytryptamine in brain tissue taken from the fetuses of the fluoride-exposed mothers are lower than the levels of the control fetuses, and the difference is statistically significant (p<0.05). Moreover, the level of epinephrine in brain tissue from the subject group is higher than in the control, and the difference is statistically significant (p<0.05).

The receptor density and disassociation constant of the α_1 -receptors are recorded in Table 3.

Table 3. Receptor density and disassociation constant of α_1 -receptors (mean±SD)

Group	n	α_1 -receptor density (fmol/mg pr)	Receptor-ligand disassociation constant (nmo/L)
Non-fluorosis area	6	15.83±3.96*	73.67±34.35*
Fluorosis area	4	0.043±0.02	8.84±5.92

*p<0.05 comparing the non-fluorosis and the fluorosis area groups.

As is evident from Table 3, the brain tissue of the fetuses from the endemic fluorosis area showed a marked decrease in α_1 -receptor density and receptor-ligand disassociation constant as compared to the non-endemic area fetuses; the differences were statistically significant.

DISCUSSION

The mothers of the ten fetuses that formed the subject group for this study all had dental fluorosis, with a corresponding increase in urinary fluoride, indicating that these pregnant women were suffering from chronic fluoride poisoning. The excess fluoride of the mother was passed through the placental barrier into the fetus, and from there through the blood-brain barrier to accumulate in the fetal brain, leading to a significant rise in bone and brain fluoride levels. Our results are consistent with earlier reports.⁸

Previous experiments have shown that the brains of fetuses from endemic fluorosis areas as well as fluoride poisoned rats manifest morphological changes.¹⁻⁴ Following experimental testing of the monoamine neurotransmitters in fetuses from fluorosis endemic areas, the present study found lowered levels of

norepinephrine, and elevated levels of epinephrine. The presence of norepinephrine in the brain allows the organism to become alert, and guards against the intensification of reflex reactions and other behavior. Norepinephrine also plays a role in the regulation of complex response mechanisms, emotions, cerebrocardiovascular function, etc. When norepinephrine levels drop, the ability to maintain an appropriate state of activation in the central nervous system is weakened. The elevated levels of epinephrine could be due to a blockage of the pathway that transforms epinephrine into norepinephrine or possibly due to suppression of the relevant metabolic enzymes, causing the brain levels of epinephrine to increase, and the levels of norepinephrine to decrease.

There have been reports that in cases of chronic fluoride poisoning, the hypothalamic 5-hydroxytryptamine system shows diminished activity, and the sufferers develop hyperalgesia.⁵ The present study found that the levels of 5-hydroxytryptamine in the brains of fetuses from fluoride endemic areas was low; this is likely a causal factor in the abnormal 5-hydroxytryptamine system activity of the fluoride poisoning cases.

The fact that a comparison of the levels of dopamine and its metabolic product, 3,4-dihydroxybenzoic acid, in the fluoride-exposed and non-fluoride-exposed fetal brains showed no significant difference may be due to the fact that there are relevantly few locations with dopamine neurons in that part of the brain, so major differences are not revealed by the data.

The physiological function of neurotransmission is accomplished by means of specialized receptors. Norepinephrine released from nerve endings serves its function primarily via activated epinephrine receptors.⁹ While testing the fluoride endemic fetuses for brain levels of norepinephrine, we also tested the nearby α_1 -receptors, discovering that the fetuses from the endemic fluorosis area showed a significant lack of α_1 -receptors, with a drop in the disassociation constant between the α_1 -receptor and the ligand, indicating a significant increase in the α_1 -receptor's affinity with the ligand.

In the central nervous system, 5-hydroxytryptamine and norepinephrine are interdependent, with the presence of one strengthening the effect of the other.⁹ Chronic fluoride poisoning or excess fluoride causes a drop in the levels of transmitters, such as 5-hydroxytryptamine and norepinephrine, and also the density of norepinephrine-specialized α_1 -receptors, leading to abnormalities in nerve function. Integrating the results of previous experiments, we theorize that a reduced synthesis of neurotransmitters and a decrease in the density and function of their receptors are the physical basis for the various functional neurological deficits seen in fluoride poisoning cases, thereby further aggravating general neural dysplasia and other damage.

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REFERENCES

- 1 Guan Zhizhong, Yu Yanni, Liu Jialiu. Changes in brain morphology in the offspring of chronically fluoride poisoned rats. *Chinese Journal of Pathology* 1986;15(4):297-9.
- 2 Guan Zhizhong, Yu Yanni, Liu Jialiu. Effects of chronic fluoride poisoning on levels of DNA and RNA in the brains of rats. *Journal of Guiyang Medical College* 1985;10(4):250-3.
- 3 Liu Wanxing, Dong Zhong, Liu Jialiu. Experimental research of the behavior and brain tissue morphology of the offspring of chronically fluoride poisoned rats. *Chinese Journal of Pathology* 1989;48(4):290-1.
- 4 Du Li, Wan Changwu, Cao Xumao, et al. The effects of chronic fluoride poisoning on fetal development. *Chinese Journal of Pathology* 1982;21(4):218-20.
- 5 Yuan Sude, Xie Qiwen, Lu Fuying. The neurological symptoms and brain neurotransmitters of chronic fluoride poisoned patients. *Fluoride Research Newsletter* 1989;3:19.
- 6 Feng Yipu, Zhang Liying, Ceng Guiyun. Methods for measuring epinephrine receptors and some of our experiences. *Journal of Nuclear Medicine* 1983;13(3):8-10.
- 7 Lowry OH, Rosebrough N., Farr AL, Randall RJ. Protein measurements with folin phenol reagent. *Journal of Biological Chemistry* 1951;193:2550-4.
- 8 Chen Zaishe, Liu Weiqun, Su Guifang. Research into the distribution of fluoride in fetal tissue. *Chinese Journal of Endemic Diseases* 1990;9(6):345-6.
- 9 Han Jisheng, editor. *Essentials of Neuroscience*. Beijing: Beijing Medical University and Peking Union Medical College Press; 1993. p. 363-783.