

**DAMAGE IN THE NEONATAL
MOUSE BRAIN FOLLOWING
INGESTION OF ASPARTAME**

Searle Laboratories
Division of G.D.S.
P.O. Box 5111
Chicago, Illinois 60680

DAMAGE IN THE NEONATAL MOUSE BRAIN FOLLOWING INGESTION OF ASPARTAME

Naomi Lemkey-Johnston, Ph.D.
W. Ann Reynolds, Ph.D.
Henri Kulikowski, M.S.

Illinois State Institute for Developmental Disabilities
and
Dept. of Anatomy, University of Illinois College of Medicine

Introduction

Aspartame (APM), under consideration as a sweetening agent, is a dipeptide consisting of aspartic acid and phenylalanine. Aspartic acid given orally at levels of 1 gm/kg to neonatal mice is capable of eliciting both hypothalamic and retinal lesions (1). Inouye and Murakami (2) have reported damage to the hypothalamus of the mouse following ingestion of monosodium L-Aspartate while phenylalanine was found to be non-toxic at equivalent levels. The present report is concerned with the effects of the oral consumption of APM upon the hypothalamus of the neonatal mouse. Of special interest is whether dose levels of APM yield damage similar to that found with equivalent dosages of other acidic amino acids in that very sensitive model, the neonatal mouse.

Materials and Methods

Animal Dosing

All mice used in this study were A/JAX-ICR hybrids between 6 and 10 days of age of both sexes. Because of its low solubility, Aspartame was administered as a slurry via stomach tube. After treatment, mice were isolated from their dams but kept warm on a warming table until the end of the treatment interval of 3 hours. Aspartame was administered as a 10% w/v solution at dosages of 2, 1.5, 1, 0.5 and 0.25 mg/gm (see Table 1).

Preparations of Brains for Light Microscopy

Heads were cut off, the cranium dissected to expose the dorsal aspects of the cerebrum and cerebellum and immersed in full strength formalin. Following routine dehydration and embedding, sections were cut at 6 μ and stained with Cresyl Echt Violet solution. All brains were cut and analyzed serially in the sagittal plane which included anteriorly, the olfactory bulb to posteriorly, the cervical spinal cord.

Results

In analyzing paraffin sections the criteria were the same as used for lesions following monosodium glutamate (MSG), sodium chloride and sucrose ingestion by the neonatal mouse (3). The word "lesion" refers to specific brain damage involving the neurons and neuropil which will be reported as being distinct from changes in vasculature and hemorrhages.

Major Areas of Damage

Major lesions were largely confined to midline structures, namely, the hypothalamic arcuate nucleus, the subfornical organ and the area postrema. The locations and incidence of damage are summarized in Table II.

2mg/g: At this dosage 25-100 cells of midsagittal sections of the arcuate nucleus exhibited shrunken neurons with pyknotic nuclei, enlarged dendrites but little or no edema in the neuropil. Most susceptible was the median portion of the nucleus and its ventral portions near the median eminence. At this dosage the lesioned cells were distributed throughout the entire nucleus. The subfornical organ had pyknotic cells of which the medial and ventral portions, which are closest to the ventricle, were most involved. The area postrema showed extensive damage throughout with pyknotic nuclei.

1.5 mg/g: In the arcuate nucleus, the number of lesioned cells and extent of the lesion was quite similar to that encountered with the 2.0 mg/g dose. Between 30 and 100 neurons appeared to be damaged in midsagittal sections. The area postrema and SFO appeared damaged to the same extent as at 2.0 mg/g.

1.0 mg/g: The arcuate nucleus definitely exhibited lesser damage than at 2.0 or 1.5 mg/g. In midline sections, about 8-20 cells were pyknotic and the lesion as a whole had a far less lateral extent. The area postrema also contained far less damage, with about 35 pyknotic cells involved per section, which is about 1/3 the number encountered with the higher dosages. This area contrasted with the arcuate nucleus in that pyknotic cells were found in both medial and lateral regions.

At 0.5 and 0.25 mg/g, no neuronal lesions were observed.

Less Frequently Observed Regions of Damage

Pyknosis and edema were observed in other areas of the brain, usually in close proximity of the CSF.

At 2 mg/g, 4 of 18 animals were observed to have the following lesions: pyknotic cells in the dorsal dentate gyrus, and considerable damage to the dorsal anterior region of the habenula.

At 1.5 mg/g, the dentate gyrus, and most frequently the habenular nucleus were pyknotic. The marginal ventral aspects of the hippocampus showed pyknotic cells. Five of 17 brains contained lesions involving the above structures.

At 1.0 mg/g, a few animals contained lesions. The most prevalent site was again the habenula wherein the lesions were very slight but slight lesions could also be seen in the marginal ventral hippocampus and in the dorsal dentate gyrus. Only two animals showed distinct lesions. Those with lesions in the habenula were slight and could be enumerated only to a few (3-5) definite instances.

Vascular Changes

a. Internal to CNS Structures

Vascular changes were observed at higher dosages. The cerebellar folia, as well as the molecular and granular layer, contained enlarged blood vessels. Enlarged blood vessels were noted in animals receiving the 2.0, 1.5 and 1.0 mg/g dosages in the following regions: Ventral cerebral cortex; marginal lenticular nucleus, internal thalamus, the external aspects of the ventral and dorsal hippocampus, internal and external aspects of the tectum, the internal mesencephalon, the external ventral pons and ventral medulla. No major vascular alterations were encountered at the 0.5 and 0.25 mg/g levels.

b. External to CNS Structures

At the 2.0 and 1.5 mg/g dosages, the choroid plexus was almost always dramatically dilated. In several brains, there were enlarged arachnoid spaces over the cerebrum, the tectum, the cerebellum, the ventral pons, and medulla.

In almost every brain containing damage to the arcuate nucleus, regardless of dosage, portal vessels underneath the median eminence were enlarged.

In addition, at 2.0, 1.5 and 1.0 mg/g dosages, the following structures exhibited dilated blood vessels in the subarachnoid space proximal to them: the olfactory bulb, the tectum, the dentate hippocampus, the dorsal and ventral aspects of the cerebellum, the dorsal surface of the thalamus, the pons, the mammillary bodies, and the subcommissural organ. Again, the lower dosage levels (0.5 and 0.25 mg/g) did not seem to cause major blood vessel alterations in these structures.

Enlarged Ventricles

Brains from mice receiving the 2.0, 1.5, 1.0, and 0.5 mg/g loads often had dilated lateral cerebral ventricles and third ventricles. This phenomenon was noted in a large number of brains, whether or not lesions were present. Dilatation of the ventricles was not encountered in animals receiving 0.25 mg/g.

Discussion

It is noteworthy that the three main structures in the brain showing lesions were in a medial location and were close to the ventricles. Was the access to the structures via CSF in the ventricles, as we have well demonstrated with MSG (3,4) or could access have been gained via blood vessels which are specialized with respect to the blood-brain barrier? Both the subfornical organ and area postrema are known to have fenestrated blood vessels. The portal vessels underlying the arcuate nucleus are likewise known to be fenestrated. But many other structures showing pyknosis and edema are located in close proximity to the CSF.

At equivalent dosages (say 2 mg/g) far fewer brain regions are damaged, and the lesions are less extensive in size following ingestion of APM than is observed with MSG (4). Of course, only roughly one-half the molecule is aspartic acid, capable of causing neuronal death at high dosages in the rodent. Further, the limited solubility of APM in water forces its use as a slurry for dosing. It is likely that the gastric absorption time for a slurry is longer than for dissolved amino acids which probably lowers the maximum blood levels of aspartic acid attained following dosing.

The lowest dose level causing any neuronal pyknosis was 1.0 mg/g. This would require an enormous acute intake for a human being; roughly 50-100 g for an adult in a single dose. We have demonstrated that one of the sequelae of such big acute dosages is a major increase in the osmotic load of the blood (3). Indeed, many of the vascular effects observed, including dilatation of the ventricles, enlarged choroid plexus and blood vessels, probably

result from hyperosmolality. It is significant that these alterations, likely due to acute osmotic loading, are seen even at the lower dosages of APM (0.25 and 0.50 mg/g) where no neuronal death was observed. This leads us again to the working hypothesis that hyperosmolality alters the integrity of the blood-brain barrier, a phenomenon well-known to neuroscientists (5). Then, altered capillary permeability permits egress of substances in large concentrations from the blood into the neuropile. In this instance, the substance would likely be aspartic acid, normally barred from free exchange with brain tissue by the blood-brain barrier.

TABLE I

NEONATAL BRAINS ANALYZED

<u>Dosage (mg/g)</u>	<u>Number of Animals</u>	<u>Number of Litters</u>	<u>Age at Dosing</u>
2.0	18	5	6,7,9 10 days
1.5	17	4	9,10 days
1.0	21	3	6,7,9 days
0.5	19	5	6,8,9 days
0.25	12	3	7,8,9 days
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TABLE II

INCIDENCE OF LESIONS IN NEONATAL MICE AFTER ASPARTAME INGESTION

Dosage (mg/g)	Structure	Number of Animals with lesions*	Percentage of Total Sampled
2.0	arcuate nucleus	7	39%
	area postrema	7	39%
	subfornical organ (SFO)	7	39%
1.5	arcuate nucleus	7	41%
	area postrema	7	41%
	SFO	6	35%
1.0	arcuate nucleus	2	10%
	area postrema	3	14%
	SFO	1	5%
0.5	--	0	0
0.25	--	0	0

*Lesions were not always encountered in different locations within the same animal.

References

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