Bull. Environ. Contam. Toxicol. (1984) 32:410–416

Environmental Contamination and Toxicology

Neonatal Aspartate Treatment: Effects on Arcuate Nucleus Morphology, Organ Weights, and Reproductive Function

James N. Pasley,[1] Ervin W. Powell,[2] and Nancy Lamoreux[2]

Departments of [1]Physiology and [2]Anatomy, University of Arkansas for Medical Sciences, Little Rock, AR 72205

Aspartate (ASP) and glutamate (GLU), excitotoxic compounds, are known to induce specific morphological alterations in the arcuate nucleus of the hypothalamus when administered to neonatal rats, mice or monkeys (OKANIWA et al., 1979; OLNEY 1969; PIZZI et al., 1978; POWELL & PASLEY, 1982; REYNOLDS et al., 1976). The potential toxicity of aspartic acid, a naturally occurring amino acid used by the food processing industry as an artificial sweetener has recently been extensively reviewed by OLNEY (1980). Adult rodents which have been injected with ASP during the neonatal period manifest several endocrinological and metabolic abnormalities as a consequence of this selective destruction of cell bodies located within the arcuate nucleus (OKANIWA et al)., with reduced pituitary, gonadal, uterine, and thyroid organ weights have been noted in rodents treated with excitotoxic amino acids (LAMPERTI & BLAHA, 1976; NEMEROFF et al., 1981; OKANIWA et al., 1979; PLNEY & HO, 1970; PIZZI et al., 1978). In addition, adult ASP treated rats are both stunted and obese (SHAINKER & OLNEY, 1974, 1980).

Since most reports concerning the effects of these compounds involve large subcutaneous doses (4 or 8 mg/gm), we examined the effects of single small subcutaneous doses (0.5 mg/gm or 2 mg/gm) of ASP on specific morphologic changes in the arcuate nucleus, reproductive organ development and fecundity in brown house mice.

MATERIALS AND METHODS

Eight day old, male and female brown house mice from a wild-derived colony maintained in our laboratory for 12 years received a single injection s.c. of either 0.5 mg/gm or 2 mg/gm aspartate or saline. The animals were maintained in a controlled environment (24±1° C, 12/12 light-dark cycle) in the animal facility and fed laboratory chow (Purina mouse chow) and water ad libitum. The mice were weaned at 21-23 days, separated by sex and kept 4-6 per cage. At 60 days of age the mice were euthanized by cervical dislocation. Brains were removed and fixed in 10% sucrose formalin. Serial frozen sections were made and examined under light microscopy. Cells within the arcuate nucleus were

[1]To whom correspondence should be addressed

counted blindly three times in the serial sections from the three groups, i.e., saline injected, 0.5 mg/gm, and 2 mg/gm aspartic acid treated mice. Reproductive organs and adrenal glands were also removed and fixed in 10% formalin. The organs were later weighed wet to the nearest 0.001 gm, embedded in paraffin and sectioned at 5 μ for examination by light microscopy.

Three other groups of mice were neonatally treated with 2 mg/gm aspartate or saline and were reared to 60 days of age and their reproductive function assessed by mating tests. The mice were caged in pairs and tested by pairing one ASP male with a normal female, or one normal male with one ASP female, or one normal male with one normal female. Female mice were examined daily for a vaginal plug. Day one of pregnancy was designated the day after a plug was found. After parturition litter size and weight was recorded. The animals were also weighed at weaning and at 60 days of age when they were killed.

Statistical comparisons between saline-treated controls and aspartic acid treated mice were performed by Student's "t" test or analysis of variance where appropriate.

RESULTS AND DISCUSSION

The aspartic acid treated mice exhibited a dose dependent decrease in the number of cells within the arcuate nucleus (Table 1).

Table 1. Arcuate nuclear cell counts per $100\mu^3$ from adult house mice treated neonatally with saline or aspartate (mean±SEM). All slides were mixed and counted blindly three times.

Treatment	N	AV Cells/$100\mu^3$
Control (Saline)	6	0.999±.02
Aspartate - 0.5 mg/gm	7	0.746±0.2**
Aspartate - 2.0 mg/gm	5	0.310±.02**

**=P<0.01 vs. control, Student's "t" test.

Arcuate nuclei from mice treated with 0.5 mg/gm and 2.0 mg/gm are depicted in Figure 1. The arcuate nuclei from mice treated with 2.0 mg/gm aspartate were nearly devoid of cells. Although the general appearance of the arcuate nuclei from 0.5 mg/gm aspartate treated mice differed little from saline controls, careful counting revealed a lesser number of neurons per cubic micron significantly different that controls of Table 1. Animals neonatally treated with excitotoxic compounds are characteristically obese (OLNEY, 1980). Obesity was absent in our animals.

Table 2. Mean (± S.E.) body and organ weights from house mice receiving a single dose of saline or aspartate (0.5 or 2.0 mg/gm) at 8-10 days of age. Final body and organ weights were taken at 60 days of age.

Male	N	Initial Body Wt. (g)	Final Body Wt. (g)	Adrenal (wt) (mg)	Testis/Ovary Wt. (mg)	Seminal Vesicle/ Uterus Wt. (mg)
Control (Saline)	10	3.4±0.4	15.6±1.0	4.4±0.4	148.2±8.2	103.3±8.7
Aspartate 0.5mg/gm	10	3.5±0.1	17.4±1.0	4.5±0.4	129.1±9.0	69.8±9.2**
Aspartate 2.0mg/gm	5	3.7±0.3	14.4±0.7	3.8±0.8	116.8±4.4**	52.5±12.6**
Females						
Control (Saline)	5	3.7±0.4	16.9±1.1	7.7±0.7	3.9±0.2	53.0±6.3
Aspartate 0.5mg/gm	5	4.1±0.1	15.8±0.4	7.7±1.1	1.8±0.4*	28.1±3.8
Aspartate 2.0mg/gm	5	3.6±0.2	12.6±1.1	7.9±1.5**	2.2±0.4*	18.9±1.6**

*P<0.05 vs. control
**P<0.01 vs. control.

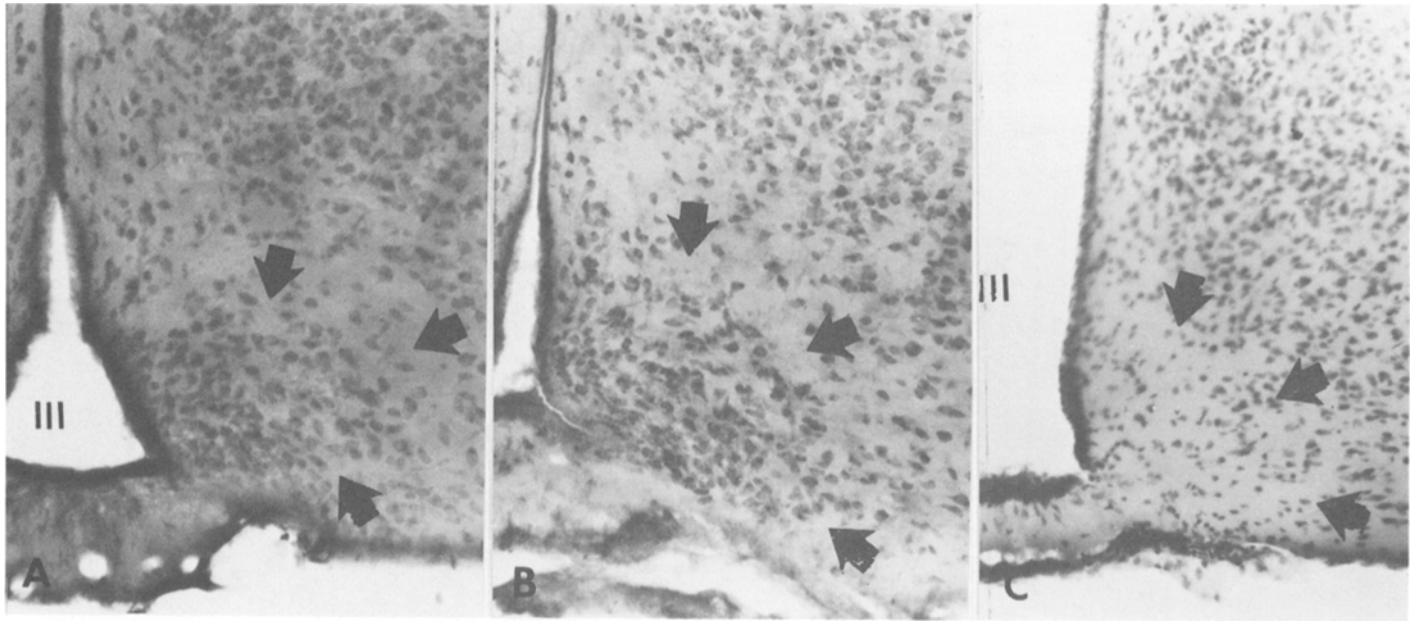

Figure 1. The arcuate nucleus from a neonatally treated saline control adult house mouse (A). Typical appearance of the arcuate nucleus in the adult mouse following neonatal administration of 0.5 mg/gm aspartate (B). Adult mouse hypothalami that demonstrate an obvious loss of neurons following a single 2.0 mg/gm dose of aspartate at 8 days of age (C). The large arrows indicate the area of the arcuate nucleus, III, third ventricle. Cresyl/Violet strain X 450.

This may have been due to the fact that obesity often takes months to develop and our animals were approximately 60 days old when terminated. OLNEY (1969), however, found that body weight of GLU treated mice surpassed littermate controls at 45 days of age. Male aspartate treated mice receiving 0.5 and 2.0 mg/gm aspartate had smaller seminal vesicles than their respective sex matched controls (Table 2). Testicular weight was also reduced in the 2.0 mg/gm group compared to controls. Decreased testicular weights have also been reported in mice receiving multiple doses of ASP from 2.2 mg/g to 4.4 mg/g body weight from days 2-11 of life (PIZZI et al., 1978). Wet weights of ovaries and uteri were less in both 0.5 mg/gm and 2.0 mg/gm aspartate treated mice. No differences were found in adrenal weight in male mice but 2.0 mg/gm ASP reduced adrenal weights in female mice. This contrasts with unpublished work by MASSEY et al. (1978) cited in a report by OLNEY & PRICE (1978) who found increased corticosterone levels in mice following GLU treatment. Moreover, the data are inconsistent with the findings of PIZZI et al. (1978) who reported no differences in adrenal weight in male and female mice after ASP treatment. LAMPERTI AND BLAHA (1976), however, reported a reduction in adrenal size in female hamsters after repeated large doses of monosodium glutamate (MSG).

Results of reproductive function testing of mice treated with 2.0 mg/gm ASP are seen in Table 3.

Table 3. Effects of 2.0 mg/gm body weight sodium aspartate on reproductive function. Data are means and their standard errors.

Pairs	Pregnancy rate	Litter size	Pup Birth wt. (g)	Pup Wean wt. (g)	B.W. 60 days(g)
Control Male & control female	7/6	6.9±0.5	1.6±0.3	7.0±0.6	15.9±0.7
Control male & ASP female	5/4	3.6±0.8*	1.5±0.1	7.6±0.8	14.8±0.7
ASP male & control female	8/8	4.4±0.7*	1.4±0.3	6.5±0.5	15.0±0.6

*$P<0.05$

Although litter size was significantly less in mice paired with either an ASP treated female or ASP treated male, mean birth weight, weaning weight or body weight at 60 days did not differ among the pups from the different treatment pairings. PIZZI et al. (1978) found that offspring of ASP-treated males had lower body weights at birth and at 30 days of age after multiple doses of sodium ASP ranging from 2.2 mg/gm to 4.4 mg/gm.

Thus, the depression of reproductive weights and the smaller litter size among sodium aspartate treated mice suggest inhibition of reproductive function by a single subcutaneous dose of ASP during the neonatal period.

The present study extends previous observations on the description of the arcuate nucleus and the hypothalmic-pituitary-gonadal axis in adult rodents treated in the neonatal period with relatively high doses of aspartate (PIZZI et al., 1978; OLNEY, 1980). This report using lower dosages of aspartate in the neonate demonstrates detectable dose dependent damage to the arcuate nucleus in the adult which could account for altered reproductive function in mice. Because of the chemical similarity between ASP and GLU, aspartate may be acting like MSG in that it also produces destruction of neuronal perikarya in the arcuate nucleus (OLNEY, 1980). The destruction of certain arcuate perikarya in MSG-treated rats which are believed to accumulate ^{3}H-estradiol would likely impair feedback regulation of the hypothalamic-pituitary-gonadal axis (GRANT et al., 1978). Destruction of arcuate neurons would thus reduce feedback receptor sites and lead to a decrease in luteinizing hormone (LH) release, a gonadotropin vital for normal reproductive function. Neonatally MSG treated adult rodents have been found to exhibit a marked reduction in gonadotropin and gonadal steroid hormone levels (NEMEROFF et al., 1981). This might then be expected following treatment with aspartate since OLNEY has shown that MSG and ASP produce similar hypothalamic lesions. Our finding of arcuate nucleus damage with small doses of aspartate also indicates that neuroendocrine dysfunction may still be occurring. The dose required to induce hypothalamic lesions with potassium aspartate apparently varies considerably with the age of animals and route of administration (OKANIWA et al., 1979).

REFERENCES

Conte-Devolx B, Giraud P, Castanos E, Boudouresque F, Orlando M, Gillioz P, Oliver C (1981) Effect of neonatal treatment with monosodium glutamate on the secretion of α-MSH, β-endorphin and ACTH in the rat. Neuroendocrinology 33:207-211

Grant LD, Stumpf WE, Sar M, Nemeroff CB, Kizer JS (1978) Monosodium glutamate (MSG) destruction of estrogen-feedback neurons in hypothalamic arcuate nucleus-median eminence. Fed. Proc. 37:297

Lamperti A, Blaha G (1976) The effects of neonatally administered monosodium glutamate on the reproductive system of adult hamster. Biol. Reprod. 14:362-369

Nemeroff CB, Lamartiniere CA, Mason GA, Squibb RE, Hong JS, Bondy SC (1981) Marked reduction in gonadal steroid hormone levels in rats treated neonatally with Monosodium-L-Glutamate: Further evidence for disruption of hypothalamic-pituitary-gonadal axis regulation. Neuroendocrinology 33:265-267

Okaniwa A, Hori M, Masuda M, Takeshita M, Hayaski N, Wada I, (1979) Histopathological study on effects of potassium aspartate on the hypothalamus of rats. J. Toxicol. Sci. 4:31-46

Olney JW (1969) Brain lesions, obesity and other disturbances in mice treated with monosodium glutamate Science 164: 719-721

Olney JW (1980) Excitatory neurotoxins as food additives: An evaluation of risk. Neurotoxicology 2: 163-192

Olney JW, Ho OL (1970) Brain damage in infant mice following oral intake of glutamate, aspartate or cysteine. Nature 227: 609-10

Olney JW, Price MT (1978) Excitotoxic Amino Acids as Neuroendocrine probes In: McGeer EG, Olney JW, MCgeer PL, (eds) Kainic Acid as a tool in Neurobiology. Raven Press, New York, pp. 248-249.

Pizzi WJ, Tabor JM, Barnhart JE (1978) Somatic behavioral and reproductive disturbances in mice following neonatal administration of sodium L-aspartate. Pharmac. Biochem. Behav. 9:481-485

Powell EW, Pasley JN (1982) The effects of neonatally administered aspartic acid on the reproductive system of adult house mice. Endocrine Soc. Abst. 64:431

Reynolds WA, Butler V, Lemlay-Johnston N (1976) Hypothalamic morphology following ingestion of aspartate of MSG in the neonatal rodent and primate; A preliminary report. J. Toxicol. Environ. Health 2:471-480

Schainker B, Olney JW (1974) Glutamate-type hypothalamic-pituitary syndrome in mice treated with aspartate or cysteate in infancy. J. Neural Transm. 35: 207-15

Received August 9, 1983, Accepted September 16, 1983