Neurotransmitters in Rats Fed Fumonisin B1

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Abstract. **Fumonisin B1, a toxin produced by *Fusarium moniliforme*, has been associated with a neurotoxic syndrome in horses known as equine leukoencephlomalacia. Previous investigations showed that *F. moniliforme* cultured on corn and incorporated into rat chow increased brain 5-hydroxyindoleacetic acid (5HIAA) and 5HIAA: serotonin (5HT) ratios in these animals. Therefore, this study was undertaken to determine whether fumonisin B1 would produce related neurochemical effects in the brain and pineal gland of male and female rats. Rats were fed fumonisin B1 at 15, 50, and 150 ppm for 4 weeks. No differences occurred in brain concentrations of norepinephrine, dopamine, 3,4-dihydroxyphenylacetic acid, 3-methoxytyramine, homovanillic acid, 5HT, 5HIAA, and the 5HIAA to 5HT ratios in either male or female rats, nor where there differences between the sexes. When compared across sexes, the norepinephrine to dopamine ratios were decreased ($P < 0.05$) in the 150-ppm-treated animals. This may suggest a fumonisin B1-induced imbalance in brain norepinephrine and/or dopamine. No differences were observed in pineal norepinephrine, 5HT, 5HIAA, and the 5HIAA to 5HT ratios. Since fumonisin B1 failed to duplicate the effects of the *F. moniliforme*-induced imbalances in 5HT and 5HIAA metabolism in the brains of rats, other mycotoxins from *F. moniliforme* may be responsible for these effects.** [P.S.E.B.M. 1993, Vol 202]

Fusarium moniliforme J. Sheld. is a common fungal infection in corn and is directly related to equine leukoencephalomalacia (1). The gross manifestation of this disease is liquefactive necrosis and cystic cavitation of the brains in horses (1). The lesions are caused by fumonisin B1, a toxin produced by *F. moniliforme* (2, 3). Fumonisin B1 also acts as a cancer promoter and initiator in laboratory rats (4, 5). Several fumonisins have been isolated from commercial corn and corn-based human foodstuffs worldwide (6). The production of these toxins by most isolates of *F. moniliforme* from various agricultural commodities and geographic areas strongly suggests that they are ubiquitous (7–9).

Hepatoxicities and/or nephrotoxicities in domestic and laboratory animals have been associated with naturally infected *F. moniliforme* corn, *F. moniliforme* cultured on corn in the laboratory, and the fumonisins (5, 9–13). There is a significant correlation between *F. moniliforme*-contaminated corn, the fumonisins, and esophageal cancer in humans whose diets are based on corn and corn products (i.e., South Africa) (14). The clinical manifestations and target organs of the fumonisins may be dose dependent and species specific. To date, only horses have demonstrated neurotoxicities. However, Porter *et al.* (15) have reported that corn naturally infected with *F. moniliforme*, as well as *F. moniliforme* cultured on corn, altered brain serotonin (5HT) and 5-hydroxyindoleacetic acid (5HIAA) in rats. Fumonisin B1 in the diets of these animals was 139 ppm (15).

The objectives of this study were to compare the effects of fumonisin B1 on neurotransmitters and metabolites in male and female rats, and to determine whether this mycotoxin is responsible for the *F. moniliforme*-induced changes in metabolism in the brains of male rats. Since the pineal gland has the highest concentration of 5HT and 5HIAA (i.e., quantity of compound/gland), an additional objective was to compare the effects of fumonisin B1 on neurotransmitters in the pineal gland of these animals.

Materials and Methods

Animals. Male and female Sprague-Dawley rats (Harlan Industries Inc., Indianapolis, IN; $n = 3$/treat-

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ment), 4–5 weeks of age, were acclimated for 2 weeks on a rodent chow diet (Agway, Inc., Waverley, NY). The rats were housed individually in wire mesh steel cages equipped with an automatic waste water flush system in a room maintained with 12:12-hr light:dark photoperiod at a temperature of 22°C. Water and feed were provided *ad libitum* for 4 weeks. Animals were weighed initially and at weekly intervals. Food consumption was measured weekly and animals were observed for toxic signs throughout the study.

Isolation of Fumonisin B1 and Diet Preparation. *F. moniliforme*, MRC 826, was cultured on corn as described (15). Fumonisin B1 (>99%) was isolated from the culture material and purified using high pressure liquid chromatography. The complete isolation procedure will be reported elsewhere. Briefly, the culture material (approximately 750 g/flask) was extracted with 2 liters of acetonitrile:water (1:1 v/v) and filtered (Whatman No. 1 filter paper), and the acetonitrile was evaporated *in vacuo* (≤40°C). The aqueous concentrate (pH = 5.7) was then extracted with chloroform until the chloroform layer was clear (approximately 4 equivalent vol). The aqueous solubles were then placed on an XAD2 column (3000 g: Aldrich Chemical Co., Inc., Milwaukee, WI) and the column was eluted with water (6 liters), methanol (4 liters), and chloroform (4 liters), respectively. The methanol fraction was evaporated to dryness *in vacuo* (≤40°C), the residue was dissolved in a minimum amount of water, and the solution was placed on a 2.5- × 25-cm, RP-C_{18} column (Waters Associates, Milford, MA). The column was eluted with 200 ml each of water, acetonitrile:water (15:85 v/v), acetonitrile:water (70:30 v/v), and acetonitrile. Fumonisin B1 eluted in the 70:30 acetonitrile:water fraction, which was concentrated to dryness as above. The residue was subjected to a preparative clean-up procedure using high pressure liquid chromatography on a Waters 500A preparative liquid chromatography system (Waters Associates) with a mobile phase consisting of methanol:water:0.1% acetic acid (65:35:0.1 v/v/v). The residue was dissolved in a minimum amount of mobile phase, placed on a PrePak-500/C_{18} column, and monitored with a refractive index detector. The fractions containing fumonisin B1 were collected, recombined, and subjected to repeated preparative-liquid chromatography until 99% purity was obtained. The isolation and purification of fumonisin B1 were compared with an authentic standard (OPA-derivative, 10; GC/MS, 16) obtained from R. Vleggaarr, Medical Research Council, Tygerburg, Republic of South Africa.

The purified mycotoxin was added to basal rat chow powder at 15, 50, and 150 ppm using a Patterson-Kelly LB-16 mixer equipped with an intensifier bar (Patterson Kelly, East Stroudsburg, PA) and the diets were stored at −18°C in plastic-lined containers. Feed was provided daily and controls were fed powdered basal rat chow.

Tissue Preparation, Neurochemical-Metabolite, and Statistical Analyses. Animals were sacrificed (CO_2; decapitation) and brains and pineals were collected and processed (13, 15–18). Norepinephrine, dopamine, dihydroxyphenylacetic acid, homovanillic acid, 5HT, and 5HIAA were assayed in the brains using high-performance liquid chromatography with electrochemical detection (Bioanalytical Systems, West Lafayette, IN). Mobile phase and instrument conditions were as reported (17, 18), with minor modifications in work-up procedures. Dihydroxybenzylamine was used as an internal standard. Briefly, brains were collected over ice, placed in 0.05 *M* $HClO_4$/0.1% cysteine solution (150 mg brain/ml solution), minced with scissors, and stored at −80°C until analyzed. Tissues were thawed over ice, homogenized, and centrifuged (15–18), and 10 μl of dihydroxybenzylamine solution (0.01 μg/μl) were added to 1,990 μl of the supernatant. This solution was filtered through a 0.2-μm Acrodics (Waters Associates) and analyzed using a 20-μl injection loop. The peak height ratios of dihydroxybenzylamine to the individual compounds at 700 mV and 10 nA (external and internal standards) were used to quantitate the individual compounds. The pineal glands were placed in individual 1.5-ml polypropylene tubes with 200 μl of a 0.05 *M* $HClO_4$/0.1% cysteine solution, sonicated (Microson ultrasonic cell disruptor; Heat Systems, Atlanta, GA) for 10 sec (ice-salt bath), and centrifuged at 20,000 rpm (Beckman J2–21 centrifuge; Beckman, Palo Alto, CA) and 2°C for 30 min. The supernatant was decanted into fresh tubes and stored at −80°C until analyzed for norepinephrine, 5HT, and 5HIAA as above. Standards were obtained from Sigma Chemical Co. (St. Louis, MO) and the results are reported as the free compounds (i.e., brain, ng compound/g tissue; pineal, ng compound/gland). Body weight changes, food consumption, serum chemistry, and total histopathology are reported elsewhere (13). Neurotransmitters, metabolites, and ratios in the brain and pineal glands were analyzed by analysis of variance (19). Duncan's multiple range test was used to locate mean differences ($P < 0.05$).

Results and Discussion

Fumonisin B1 had no apparent effects on brain neurotransmitters and metabolites in either male or female rats (Tables I and II). Also, there were no differences in the brain neurotransmitters and metabolites between the sexes. When the data were compared without respects for sex (i.e., $n = 6$/treatment), brain norepinephrine to dopamine ratios were significantly decreased in the 150-ppm-treated animals (150 ppm, 0.636 vs controls, 0.705; $P < 0.05$). Such decreases were also observed in a previous study (15), when the rat

Table I. Brain Neurotransmitters and Metabolites in Male Rats Fed Fumonisin B1[a]

Compounds	Controls[b] (0 ppm)	Treatment 1[c] (15 ppm)	Treatment 2[c] (50 ppm)	Treatment 3[c] (150 ppm)
Norepinephrine	531 ± 20	498 ± 19	521 ± 27	518 ± 28
Dopamine	728 ± 46	699 ± 50	752 ± 38	773 ± 17
3,4-Dihydroxyphenylacetic acid	143 ± 14	135 ± 17	133 ± 26	136 ± 22
Homovanillic acid	69 ± 5	72 ± 4	67 ± 6	80 ± 22
3-Methoxytyramine	69 ± 3	73 ± 7	68 ± 5	75 ± 12
5HIAA	370 ± 20	375 ± 24	363 ± 25	400 ± 52
5HT	376 ± 20	360 ± 21	369 ± 15	385 ± 18
5HIAA/5HT	0.98 ± 0.02	1.04 ± 0.01	0.98 ± 0.03	1.04 ± 0.09
Brain wt (mg)	1663 ± 21	1717 ± 38	1687 ± 12	1740 ± 89
Body wt (g)	291 ± 20	303 ± 6	287 ± 24	277 ± 25
Brain wt:body wt	5.74 ± 0.36	5.66 ± 0.19	5.91 ± 0.44	6.30 ± 0.44

[a] Data are expressed as mean ng/g ± SD of brain; $n = 3$ animals/treatment.
[b] Certified rat chow (Agway).
[c] Certified rodent chow plus purified fumonisin B1.

Table II. Brain Neurotransmitters and Metabolites in Female Rats Fed Fumonisin B1[a]

Compounds	Controls[b] (0 ppm)	Treatment 1[c] (15 ppm)	Treatment 2[c] (50 ppm)	Treatment 3[c] (150 ppm)
Norepinephrine	492 ± 4	473 ± 15	466 ± 72	455 ± 30
Dopamine	727 ± 40	670 ± 36	693 ± 129	763 ± 117
3,4-Dihydroxyphenylacetic acid	144 ± 21	139 ± 15	149 ± 27	151 ± 16
Homovanillic acid	89 ± 10	81 ± 3	83 ± 5	89 ± 5
3-Methoxytyramine	89 ± 11	80 ± 8	89 ± 5	98 ± 9
5HIAA	378 ± 13	349 ± 52	338 ± 35	415 ± 30
5HT	367 ± 24	346 ± 7	330 ± 57	344 ± 49
5HIAA/5HT	1.03 ± 0.04	1.01 ± 52	1.03 ± 0.09	1.25 ± 0.50
Brain wt (mg)	1527 ± 21	1540 ± 46	1597 ± 168	1490 ± 156
Body wt (g)	173 ± 2	168 ± 6	173 ± 22	159 ± 17
Brain wt:body wt	8.82 ± 0.07	9.16 ± 0.49	9.28 ± 0.38	9.38 ± 0.86

[a] Data are expressed as mean ng/g ± SD of brain; $n = 3$ animals/treatment.
[b] Certified rodent chow (Agway).
[c] Certified rodent chow plus fumonisin B1.

diets contained 16% *F. moniliforme* cultured on corn. The decrease in the norepinephrine to dopamine ratio may reflect either a fumonisin B1-induced imbalance in brain norepinephrine or dopamine concentrations or possibly secondary effects relative to hepatic or renal toxicities. Nevertheless, the possible fumonisin B1-induced imbalances in norepinephrine and dopamine warrant further investigations.

There were significant differences ($P < 0.02$) in brain weights between the male and female rats for the 15 ppm and the 150 ppm treatments (males > females), but there were no differences between the sexes in the controls or the 50 ppm treatments (Tables I and II). As expected, body weight for the males was greater than that for the females ($P < 0.01$) and the brain weight to body weight ratio for the females was greater ($P < 0.01$) than that for the males (Tables I and II). However, no significant differences were observed in the brain weight, body weight, or brain weight to body weight ratios among treatments within the sexes (Tables I and II).

Porter *et al.* (15) reported that an isolate of *F. moniliforme*, MRC 826, cultured on corn and incorporated into rat chow resulted in elevated brain 5HIAA and 5HIAA to 5HT ratios. In the current investigations, although not statistically different, numerically elevated brain 5HIAA and 5HIAA to 5HT ratios were observed in the 150 ppm treatments (Tables I and II) of both sexes as compared with their controls.

There were no differences in pineal norepinephrine, 5HT, 5HIAA, and the 5HIAA to 5HT ratios in either the males or females with regard to the effects of fumonisin B1. There were no differences between the sexes, nor were there any differences when the sexes were combined (i.e., $n = 6$/treatment) among treatments (Tables III and IV). In the control animals, numerically reduced values were observed for the pineal 5HT and 5HIAA of the females as compared with the males. These findings are consistent with the results of Wakabayashi and Shimada (20). Although fumonisin B1 did not appear to affect brain neurotransmitters,

Table III. Effects of Fumonisin B1 on Pineal Norepinephrine, 5HT, and 5HIAA in Male Rats[a]

Compounds	Controls[b] (0 ppm)	Treatment 1[c] (15 ppm)	Treatment 2[c] (50 ppm)	Treatment 3[c] (150 ppm)
Norepinephrine	0.67 ± 0.09	0.51 ± 0.09	0.61 ± 0.28	0.52 ± 0.20
5HT	84 ± 52	64 ± 15	69 ± 9	73 ± 16
5HIAA	32 ± 5	29 ± 6	25 ± 2	30 ± 6
5HIAA:5HT	0.46 ± 0.23	0.46 ± 0.11	0.37 ± 0.09	0.41 ± 0.02

[a] Data are expressed as mean ng/pineal ± SD; $n = 3$ animals/treatment.
[b] Certified rodent chow (Agway).
[c] Certified rodent chow plus fumonisin B1.

Table IV. Effects of Fumonisin B1 on Pineal Norepinephrine, 5HT, and 5HIAA in Female Rats[a]

Compounds	Controls[b] (0 ppm)	Treatment 1[c] (15 ppm)	Treatment 2[c] (50 ppm)	Treatment 3[c,d] (150 ppm)
Norepinephrine	0.60 ± 0.12	0.43 ± 0.15	0.40 ± 0.09	0.69 ± 0.01
5HT	55 ± 15	47 ± 2	51 ± 12	44 ± 40
5HIAA	19 ± 1	21 ± 7	22 ± 1	19 ± 1
5HIAA:5HT	0.36 ± 0.08	0.45 ± 0.15	0.46 ± 0.09	0.73 ± 0.65

[a] Data are expressed as mean ng/pineal ± SD; $n = 3$ animals/treatment.
[b] Certified rodent chow (Agway).
[c] Certified rodent chow plus fumonisin B1.
[d] One pineal gland was lost; therefore, $n = 2$/treatment.

metabolites, or pineal norepinephrine, 5HT, and 5HIAA in this study, serum chemistry, hepatic, and renal effects were consistent with *F. moniliforme* toxicity in rats (13).

These results suggest that fumonisin B1 is not responsible for the *F. moniliforme*-induced changes in 5HIAA and the 5HIAA to 5HT ratios in the brains of rats (15). Fusaric acid (5-butylpicolinic acid), a phytotoxin, is produced by most isolates of *F. moniliforme* (21; C. W. Bacon, unpublished results) and has been shown to induce similar imbalances in 5HIAA and 5HIAA to 5HT ratios in the brains of rats (22). Smith and MacDonald (23) reported that fusaric acid induced increases in hypothalamic tryptophan, 5HT, 5HIAA, and 5HIAA to 5HT ratios of pigs and suggested that fusaric acid may act synergistically with other Fusarium mycotoxins (i.e., *trichothecenes*) in causing feed refusal in swine. Fusaric acid has been reported to inhibit dopamine β-hydroxylase activity (24–26) and subsequently affects dopamine and norepinephrine concentrations and metabolism (25;27). Therefore, the combined effects of the fumonisins, fusaric acid, and other *F. moniliforme* toxins warrant further investigations.

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1. Marasas WFO, Kellerman TS, Pienaar TS, Naude TW. Leukoencephalomalacia: A mycotoxicosis of equidae caused by *Fusarium moniliforme* Sheldon. Onderstepoort J Vet Res **43**:113–122, 1976.
2. Kellerman TS, Marasas WFO, Theil PG, Gelderblom WCA, Cawood M, Coetzer JAW. Leukoencephalomalacia in two horses induced by oral dosing of fumonisin B1. Onderstepoort J Vet Res **57**:269–275, 1990.
3. Marasas WFO, Kellerman TS, Gelderblom WCA, Coetzer JAW, Theil PG, Van der Lugt JJ. Leukoencephalomalacia in a horse induced by fumonisin B1, isolated from *Fusarium moniliforme*. Onderstepoort J Vet Res **55**:197–203, 1988.
4. Gelderblom WCA, Kriek NPJ, Marasas WFO, Thiel PG. Toxicity and carcinogenicity of *Fusarium moniliforme* metabolite, fumonisin B1, in rats. Carcinogenesis **12**:1247–1251, 1991.
5. Marasas WFO, Kriek NPJ, Fincham JE, van Rensburg SJ. Primary liver cancer and oesophageal basal cell hyperplasia in rats caused by *Fusarium moniliforme*. Int J Cancer **34**:383–387, 1984.
6. Sydenham EW, Shephard GS, Thiel PG, Marasas WFO, Stockenstrom S. Fumonisin contamination of commercial corn-based human foodstuffs. J Agric Food Chem **39**:2014–2018, 1991.
7. Leslie JF, Plattner RD, Desjardins AE, Klittich CJR. Fumonisin B1 production by strains from different mating populations of *Gibberella fujikuroi* (*Fusarium* section *Liseola*). Phytopathology **82**:341–345, 1992.
8. Nelson PE, Plattner RD, Shackelford DD, Desjardins AE. Production of fumonisins by *Fusarium moniliforme* strains from various substrates and geographic areas. Appl Environ Microbiol **57**:2410–2412, 1991.
9. Thiel PG, Marasas WFO, Sydenham EW, Shephard GS, Gelderblom WCA, Nieuwenhuis JJ. Survey of fumonisin production by *Fusarium* species. Appl Environ Microbiol **57**:1089–1093, 1991.
10. Ross PF, Rice LG, Plattner RD, Osweiler GD, Wilson TM, Owens DL, Nelson HA, Richards JL. Concentrations of fumonisin B1 in feeds associated with animal health problems. Mycopathologia **114**:129–135, 1991.
11. Voss KA, Plattner RD, Bacon CW, Norred WP. Comparative studies of hepatotoxicity and fumonisin B1 and B2 content of

water and chloroform/methanol extracts of *Fusarium moniliforme* strain MRC 826 culture material. Mycopathologia **112**:81–92, 1990.
12. Voss KA, Norred WP, Plattner RD, Bacon CW. Hepatotoxicity and renal toxicity in rats on corn samples associated with field cases of equine leukoencephalomalacia. Food Chem Toxicol **27**:89–96, 1989.
13. Voss KA, Chamberlain WJ, Bacon CW, Norred WP. Renal toxicity and hepatotoxicity in rats fed fumonisin B1. J Nat Toxins (in press).
14. Sydenham EW, Thiel PG, Marasas WFO, Shephard GS, Van Schalkwyk DJ, Koch KR. Natural occurrence of some *Fusarium* mycotoxins in corn from low and high esophageal cancer prevalence areas of the Transkei, Southern Africa. J Agric Food Chem **38**:1900–1903, 1990.
15. Porter JK, Voss KA, Bacon CW, Norred WP. Effects of *Fusarium moniliforme* and corn associated with equine leukoencephalomalacia on rat neurotransmitters and metabolites. Proc Soc Exp Biol Med **194**:265–269, 1990.
16. Plattner RD, Norred WP, Bacon CW, Voss KA, Peterson R, Shackleford DD, Weisleder D. A method of detection of fumonisins in corn samples associated with field cases of equine leukoencephalomalacia. Mycologia **82**:698–702, 1990.
17. Nishie K, Porter JK, Cole RJ, Dorner JW. Neurochemical and pharmacological effects of cyclopiazonic acid, chlorpromazine, and reserpine. Res Commun Psychol Psychiatry Behav **10**:291–302, 1985.
18. Lipham LB, Porter JK, Norred WP, Booth NH, Robbins JD. Quipazine metaclopramide inhibition of CB-154 induced prolactin suppression in rats: Neurotransmitter-metabolite correlation. Proc Soc Exp Biol Med **184**:250–255, 1987.
19. SAS Institute, Inc. SAS user's guide: Statistics: Cary, NC: SAS Institute, Inc., 1982.
20. Wakabayashi H, Shimada K. Variations of melatonin and serotonin content in rat pineal gland with sex and oestrous phase difference determined by high-performance liquid chromatography with fluorimetric detection. J Chromatogr **381**:21–28, 1986.
21. Burmister HR, Grove MD, Peterson RE, Weisleder D, Plattner RD. Isolation and characterization of two new fusaric acid analogs from *Fusarium moniliforme* NRRL 13,163. Appl Environ Microbiol **50**:311–314, 1985.
22. Chaouloff F, Laude D, Merino D, Serrurrier B, Elghozi JL. Peripheral and central short-term effects of fusaric acid, a DBH inhibitor, on tryptophan and serotonin metabolism in the rat. J Neural Transm **65**:219–232, 1986.
23. Smith TK, MacDonald EJ. Effect of fusaric acid on brain regional neurochemistry and vomiting behavior in swine. J Anim Sci **69**:2044–2049, 1991.
24. Hidaka H, Nagatsu T, Takeya K, Tatsuhisa T, Suda H, Kojiri K, Matsuzaki M, Umezawa H. Fusaric acid, a hypotensive agent produced by fungi. J Antibiot **22**:228–230, 1969.
25. Nagatsu T, Hidaka H, Kuzuya H, Takeya K, Umezawa H, Takeuchi T, Suda H. Inhibition of dopamine *beta*-hydroxylase by fusaric acid (5-butylpicolinic acid) in vitro and in vivo. Biochem Pharmacol **19**:35–44, 1970.
26. Suda H, Takeuchi T, Nagatsu T, Matuszaki M, Matsumoto I, Umezawa H. Inhibition of dopamine *beta*-hydroxylase by 5-alkylpicolinic acid and their hypotensive effects. Chem Pharm Bull **17**:2377–2380, 1969.
27. Racz K, Kuchel O, Buu NT. *In vivo* release of adrenal catecholamines in rats by fusaric acid. Eur J Pharmacol **109**:1–7, 1985.