Serum Prolactin and Brain Catecholamine Metabolite Depression in Rats Administered Extracts of Endophyte-Infected Fescue

James K. Porter,* Joe D. Robbins, William P. Norred, George B. Garner, and Charles W. Bacon

Male Sprague-Dawley rats were dosed with extracts from *Epichloë typhina* (*Acremonium coenophialum*) infected fescue seed and their serum prolactin, brain catecholamine, and metabolites were compared to rats dosed with extracts from noninfected seeds. Prolactin, dihydroxyphenylacetic acid and homovanillic acid levels were significantly lowered in the rats dosed with extracts from fungus-infected seed. The results support using prolactin and possibly brain catecholamine and metabolites in rats as a bioassay for toxic fungus-infected fescue.

Tall fescue (*Festuca arundinaceae*, Screb.) is the dominant and most important cultivated forage grass on more than 35 million acres in the United States (Hoveland, 1983). Bacon et al. (1977) have reported a high occurrence of the fungus endophyte *Epichloë typhina* (*Acremonium coenophialum*; Morgan-Jones and Gams, 1982) in pastures where cattle exhibited fescue toxicity symptoms, and Porter et al. (1979, 1981) found this fungus to produce the ergot peptide alkaloid ergovaline in vitro. More recently Plattner and Yates (1984) identified ergovaline as the major ergot peptide alkaloid in highly toxic, endophyte-infected fescue. Investigations have continued to implicate endophyte-infected fescue with poor weight gains (Bacon et al., 1977; Hoveland et al., 1980), elevated temperatures and respiration rates (Bacon et al., 1977; Hemken et al., 1981) and reproduction problems in cattle and mares (Daniels et al., 1983; Henton et al., 1983) and sheep (Bond et al., 1981).

Hemken et al. (1981) using temperature-controlled rooms have shown a significant correlation between heat-stressed animals ("summer syndrome") and reduced weight gains, lowered prolactin levels, and lowered internal temperatures in cattle maintained on a variety of fescue highly infected with the endophyte. In addition, Hurley et al. (1981) and Stidham et al. (1982) have demonstrated decreased serum prolactin levels in calves fed (toxic) fescue, and Daniels et al. (1981, 1983) have shown that extracts of toxic fescue produced abortions in rats.

The purpose of this study was to develop bioassays in laboratory animals and study possible enzymatic mechanisms by which endophyte-infected fescue affects the serum prolactin and brain catecholamine and metabolites (norepinephrine, dopamine, dihydroxyphenylacetic acid, homovanillic acid, 5-hydroxy-3-indoleacetic acid, and serotonin) in rats dosed with extracts of fungus-free vs. fungus-infected fescue seed.

MATERIALS AND METHODS

Male Sprague-Dawley rats (Harlan Sprague-Dawley, Indianapolis, IN) were housed in individual cages (12-h light/12-h darkness), and fed certified feed (Agway RMH 3000, Ithaca, NY) and water (ad libitum) for 2 weeks prior to and during the experiment. Eight animals per group (300–330 g, 2 months of age) were dosed orally via intragastric cannula on a daily basis for 3 days. Control animals (group 1) received 2 mL of distilled water; CB-154 animals (group 2) received ip 0.25 mg of bromoergocriptine mesylate (Sandoz Pharmaceuticals, East Hanover, NJ) dissolved in 1:9 (v/v) 95% ethanol–water; experimental control animals (group 3) received 2 mL orally of fungus-free seed extracts, and the experimental animals (group 4) received 2 mL orally of the fungus-infected seed extracts. All animals received 2 mg of reserpine (Sigma Chemical Co., St. Louis, MO) ip as a single dose (aqueous suspension) 24 h prior to exsanguination on day 3. Animals were anesthetized (diethyl ether) and exsanguinated by cardiac puncture.

Prolactin Radioimmunoassay. Blood serum prolactin was determined by a double-antibody radioimmunoassay (RIA). RIA materials were supplied by Dr. Raiti Salvatore through the National Hormone and Pituitary Program, NIAMDD, NIH. The procedure followed was essentially that outlined for NIAMDD Rat Prolactin PP-2, except the labeling reaction with Chloramine T was run with a pH 6.5 phosphate buffer. The ^{125}I-PRL was purified on a 0.7 × 1.8 cm anion-exchange column (AG 1-X8, Cl form; BioRad Laboratories, CA). The antiserum produced in goats was purchased from Antibodies, Inc., Davis, CA.

Liquid Chromatography/Electrochemical Analyses. For catecholamine and catecholamine metabolite analyses, whole brains were collected immediately over ice and stored at −80 °C in 30% glycerol/0.25 M K_3PO_4, pH 7.25, containing 1.0 mM EDTA. Whole brains were thawed, weighed, and sonicated for 3 min (ice–salt bath) in 0.05 M $HClO_4$ (containing 0.1% cysteine) by using a Model W140D sonifier cell disruptor (Ultrasonics, Inc., Plainview, Long Island, NY). Homogenates were equivalent to 150 mg of brain tissue/mL of solution. The suspensions were centrifuged (2 °C) for 10 min at 15000 rpm by using a Beckman Model J-21 centrifuge. The supernatant was decanted and analyzed (100-μL volume injected routinely throughout the experiment) directly for norepinephrine (NE), dopamine (DA), dihydroxyphenylacetic acid (DOPAC), homovanillic acid (HVA), 5-hydroxy-3-indoleacetic acid (5HIAA), and serotonin (5HT). Standards were either Sigma Chemical Co. (St. Louis, MO) or Bioanalytical Systems (West Lafayette, IN) dissolved in the $HClO_4$/cysteine solution at the equivalent of 0.2 ng/μL.

Liquid chromatography/electrochemical detection was conducted on a Bioanalytical Systems Catecholamine Analyzer No. 3 using a 20-μL injection loop, a glassy carbon dual parallel electrode flow cell, and a dual-pen recorder (Omniscribe D-5000, Bausch & Lomb, Houston Instruments). The column stationary phase was Biophase ODS 5 μm [250 × 4.6 mm (l × i.d.)], the column temperature was 30 °C with a flow rate of 1.0 mL/min, and the chart speed was 0.5 cm/min. The W_1 and W_2 electrodes were 800 and 700 mV, respectively (vs. Ag/AgCl). The range

Toxicology and Biological Constituents Research Unit, R. B. Russell Agricultural Research Center, U.S. Department of Agriculture, Agricultural Research Service, Athens, Georgia 30613 (J.K.P., J.D.R., W.P.N., and C.W.B.), and Department of Animal Science, University of Missouri, Columbia, Missouri 65211 (G.B.G.).

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Table I. Prolactin Concentrations, ng/mL (±SD)[a]

group	
1, control	43 (17)[b]
2, CB-154	17 (3)[c]
3, noninfected extracts	52 (28)[b]
4, infected extracts	21 (3)[c]

[a] Eight animals per treatment. Each determination was made in triplicate. [b,c] Values with different superscripts differ significantly ($P < 0.05$).

setting was 5 nA, and the mobile phase consisted of 7 parts of acetonitrile and 93 parts (v/v) of 0.15 M monochloracetic acid (pH 3.0) buffer containing 200 mg/L sodium octylsulfate and 250 mg/L Na_2EDTA (Mayer and Shoup, 1982). Compound identification was based on authentic standards' chromatographic retention times and by comparison of the ratios for the parallel outputs at 800 mV (W_1) and 700 mV (W_2), respectively, according to reported procedures (Mayer and Shoup, 1982, 1983).

Analyses of variance procedures were used for statistical analysis (SAS Institute, Inc., 1982). When significant F values were obtained, Duncan's multiple range test was used for determining mean separation.

Extraction of Endophyte-Infected and -Noninfected Fescue Seeds. Endophyte-infected seed was obtained from a commercial source that gave the positive enzyme-linked immunosorbent assay (ELISA) test (Johnson et al., 1982), and showed infection by **E. typhina** on microscopic examination. Intrarumen administration of extracts of these seeds produced "fescue foot" in cattle at the University of Missouri (Garner, 1983). The seeds (200 g) were placed in a 4-L percolator and allowed to stand for 12 h in 50% aqueous ethanol solution (1450 mL). The extract was drained and the pH adjusted to 5.6 with acetic acid. The residual seed was treated as above, and the combined extracts (~3 L) were filtered by suction (Büchner funnel). The filtrate was then concentrated in vacuo (≤40 °C) to 180 mL. The pH was adjusted to 7.0 with NaOH (0.2 N), and 100 mL was concentrated to the equivalent of 1.5 g of seed/mL of solution.

Noninfected fescue seed (also from a commercial source) that gave a negative ELISA test and were negative for *E. typhina* on microscopic examination were treated as above such that the concentrate was equivalent to 1.5 g of seed/mL of solution.

RESULTS AND DISCUSSION

Extracts of endophyte-infected fescue seed significantly lowered serum prolactin levels ($P < 0.01$), and the levels of the brain catecholamine metabolite DOPAC ($P < 0.01$) (Tables I and II) as compared to both control (group 1) and endophyte-free fescue seed extracts (group 3). HVA levels of the infected seed extracts (group 4) were depressed significantly ($P < 0.01$) from the noninfected seed extracts (group 3) but not significantly from the controls (group 1, Table II). Although brain levels of DA appeared higher, the levels of this neurotransmitter along with NE, 5HIAA, and 5HT were not significantly different among the treatment groups. DOPAC was significantly higher in the brains of rats dosed with extracts of fungus-free seed (group 3, Table II) as compared to control animals (Group 1) although levels of serum prolactin were unaffected (Table I). The prolactin concentrations in rats dosed with CB-154 (2-bromo-α-ergocryptine) were lowered as expected (Berde and Schild, 1978) (cf. Table I). Typical chromatograms for analyses of brain homogenates are shown in Figure 1.

Prolactin plays a vital biochemical role in reproduction processes, mammary gland development, and milk secretion (Wallner et al., 1983). It has been suggested that increased average daily weight gains observed in heifers were related to the increased prolactin secretions induced in these animals by exposure to light (Tucker and Ringer, 1982). Prolatin secretions from the anterior pituitary gland is controlled primarily by serotonin, which stimulates prolactin secretions via the prolactin-releasing hormone from the hypothalamus. Dopamine (and other brain catecholamines) inhibits secretions of prolactin from the pituitary gland. Thus, prolactin secretions may be affected by either interfering with serotonin, mimicking dopamine processes (e.g., general mechanisms attributed to ergot alkaloids) or by interfering with enzymatic mechanisms regulating the concentrations of these biogenic amines.

Table II. Levels of Catecholamines in Brain Tissue (±SD)[a,b,h]

	group			
	(1) control	(2) CB-154	(3) noninf. extracts	(4) inf. extracts
NE	646 (93)	609 (52)	656 (73)	604 (52)
DA	799 (125)	742 (57)	723 (138)	815 (116)
DOPAC	119 (24)[c]	114 (14)[c]	138 (14)[d]	94 (12)[e]
HVA	101 (25)[f,g]	81 (14)[f]	118 (23)[g]	87 (12)[f]
5HIAA	287 (19)	299 (30)	306 (44)	285 (32)
5HT	372 (35)	343 (39)	357 (55)	373 (57)

[a] Nanograms per gram of brain tissue. [b] Eight animals per treatment. [c-g] Values with different superscripts differ significantly ($P < 0.01$). [h] Values are calculated for NE on the basis of the bitartrate salt and for DA and SHT on the basis of their HCl salts. Values for DOPAC, HVA, and 5HIAA are for the free acids.

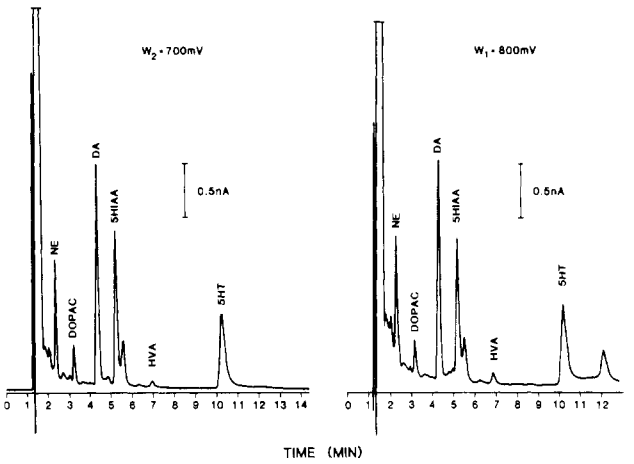

Figure 1.

Extracts of fungus-infected seeds significantly reversed the prolactin increases resulting from reserpine administration (Meltzer et al., 1979), and depressed the HVA levels in brain tissues as did the CB-154 (group 2, Tables I and II). However, there were no significant differences in the DOPAC concentrations between the control (group 1) animals and the CB-154 (group 2) animals.

Monitoring the fate of DA in brain tissues requires determination of its two primary catabolites—DOPAC and HVA. Both compounds are formed by the sequential enzymatic reactions of monoamine oxidase (MAO), catechol *O*-methyltransferase (COMT), and alcohol dehydrogenase. Since DOPAC was significantly lowered in rats dosed with extracts from endophyte-infected seeds, more so than concentrations of HVA, it is tempting to speculate that a monoamine oxidase inhibitor may be present more so in fungus-infected vs. fungus-free grass, thereby elevating dopamine levels (i.e., slowing turn over rates at both the DOPAC and HVA levels) and thus suppressing prolactin concentrations.

Low-level mixtures of the toxic components of fungus-infected fescue, along with environmental stress conditions, may be a more insidious detriment to animals than "toxic levels" of the individual compounds. It is unknown at present what the combined effects of 1-halostachine, harmane, and norharmane (found in tall fescue) (Davis and Camp, 1983) and the ergot alkaloids produced by *E. typhina* in the grass (Porter et al., 1981; Plattner and Yates, 1984) are to the animal. The monoamine oxidase activity of the β-carbolines could conceivably potentiate both the pressor activity of halostachine (Davis and Camp, 1983) and the activity of the ergot alkaloids (i.e., ergovaline). Then, too, the combined activities of the loline and perloline alkaloids produced by the grass must be considered (Robbins et al., 1972; Jackson et al., 1984).

The results of this study indicate that monitoring prolactin, catecholamine levels, and catecholamine metabolites in rats is potentially a useful bioassay for toxic fungus-infected fescue. Additionally, we have developed insights into possible mechanisms of fescue toxicity. Future studies involving both the fractionation and synergistic effects of these compounds at levels at which they exist in vivo should provide more insight toward solving the problems associated with this important forage grass.

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Registry No. Prolactin, 9002-62-4; dihydroxyphenylacetic acid, 102-32-9; homovanillic acid, 306-08-1; norepinephrine, 51-41-2; dopamine, 51-61-6; 5-hydroxy-3-indoleacetic acid, 54-16-0; serotonin, 50-67-9.

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Membrane-Degrading Enzymes in the Tubers of Various Cultivars of *Solanum tuberosum*

Robert A. Moreau

Tubers of 41 potato cultivars were surveyed for phospholipase and *p*-nitrophenyl palmitate hydrolase activities. Phospholipase levels ranged from 2.2 to 29.6 μmol min^{-1} (g of fresh weight)$^{-1}$ and *p*-nitrophenyl palmitate hydrolase ranged from 0.7 to 16.3 μmol min^{-1} (g of fresh weight)$^{-1}$. There was no apparent correlation between the two enzymatic activities among the cultivars tested, thus indicating that the popular *p*-nitrophenyl palmitate hydrolase assay is not an accurate assessment of overall lipolytic activity in potato tubers. A European cultivar, Désirée, which had previously been singled out because of its low levels of lipolytic activity, was found to contain as much phospholipase and *p*-nitrophenyl palmitate hydrolase activities as many of the other cultivars.

Galliard (1970) was the first to report that homogenization of potato tubers resulted in a rapid enzymatic breakdown of endogenous phospholipids and galactolipids. Even at 0 °C, 26% of the lipids were hydrolyzed after 10 min. At least three different acyl hydrolases have been identified in potato tubers (Galliard, 1971; Hasson and Laties, 1976a; Hirayama et al., 1975; Shepard and Pitt, 1976). One European cultivar, Désirée, was reported to

Eastern Regional Research Center, Agricultural Research Service, U.S. Department of Agriculture, Philadelphia, Pennsylvania 19118.