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Quipazine–Metoclopramide Inhibition of CB-154-Induced Prolactin Suppression in Rats: Neurotransmitter–Metabolite Correlations (42475)

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Abstract. The ability of quipazine and metoclopramide to protect rats from CB-154-induced suppression of serum prolactin concentrations was studied. These drugs affect whole brain concentrations of dopamine and serotonin, and their major metabolites dihydroxyphenylacetic acid, homovanillic acid, and 5-hydroxyindoleacetic acid. Serum prolactin concentrations have been correlated with the concentrations of the neurotransmitters and their respective metabolites. Differences in the metabolite/precursor ratios have been used to compare turnover rates of the neurotransmitters dopamine and serotonin. Increased turnover of dopamine and decreased turnover of serotonin correlate with elevated prolactin concentrations for quipazine and metoclopramide administered together. The combination of quipazine and metoclopramide protects rats against CB-154-induced prolactin suppresssion better than either of the drugs given alone. This study suggests that a quipazine–metoclopramide regimen may have therapeutic potential for combating ergotlike fescue and other similar toxicities observed in cattle grazing on endophyte-infected pasture grasses.

Reduced serum prolactin (PRL) concentrations have been associated with "fescue toxicity" in cattle and sheep grazing on fungus (endophyte)-infected fescue pastures (1–5). Reduced levels of both PRL and the dopamine (DA) metabolites dihydroxyphenylacetic acid (DOPAC) and homovanillic acid (HVA) have been observed in brains of rats administered extracts from endophyte-infected fescue seed (6). Other studies (7–11) have shown that poor animal performance (reduced weight gains, elevated body temperatures, reproductive problems) observed in animals grazing on endophyte-infected fescue pastures is also observed in ergot-type toxicoses associated with alkaloids produced by species of *Claviceps*. In addition, the endophyte from tall fescue has been shown to produce ergot alkaloids both *in vivo* and *in vitro* (12–16). Moreover, poor animal performance has been associated with consumption of grasses infected with other members of the Clavicipitaceae (i.e., *Balansia* sp.). These fungi are known to produce ergot alkaloids and to depress and/or inhibit prolactin release in cattle and other animals (14–19).

In order to study potential preventive measures for these toxicoses of cattle, 2-bromo-α-ergocryptine (CB-154), a synthetic ergot alkaloid, was used as a PRL-suppressant compound in laboratory rats. Ergot alkaloids suppress PRL by acting as a DA agonist and/or serotonin (5HT) antagonist (20). Therefore, metoclopramide (2-methoxy-5-chloroprocainamide hydrochloride), a DA antagonist, and quipazine (2-(1-piperazinyl)quinoline maleate), a 5HT receptor agonist, were used in this experiment to determine their effects on serum PRL concentrations and to correlate PRL values with whole brain DA and 5HT concentrations and their metabolites.

Materials and Methods. *Animals and drugs.* Male Sprague–Dawley rats (Harlan–Sprague–Dawley, Inc., Indianapolis, IN)[1] were housed in individual cages (22°C, 12 hr light/12 hr dark), and were fed (*ad libitum*) certified feed RMH 3000, Agway, Inc., Ithaca, NY) and water for 1 week prior to the experiment. After weighing (mean weight ± SD = 302 ± 9 g), the rats were randomly assigned to 8 groups

[1] Mention of a vendor, proprietary name, or trademark does not imply its approval by the U.S. Department of Agriculture to the exclusion of others that also may be suitable.

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TABLE I. EXPERIMENTAL GROUPS AND PROLACTIN CONCENTRATIONS (ng/ml) IN RATS (±SD)

Group no.	Group	Prolactin concentration
1	Saline controls	68 (55)
2	CB-154	18 (21)
3	Q	40 (12)
4	M	216 (185)
5	Q + M	165 (179)
6	Q + CB-154	27 (16)
7	M + CB-154	61 (37)
8	Q + M + CB-154	238 (267)

Note. Groups 1, 3, and 7 significantly different from Group 2 at $P < 0.01$; Group 5 significantly different from Groups 2 and 6 at $P < 0.01$ and Group 3 at $P < 0.05$; Group 4 significantly different from Groups 2, 3, and 6 at $P < 0.01$ and Groups 1 and 7 at $P < 0.05$; Group 8 significantly different from Groups 2, 3, and 6 at $P < 0.01$ and Groups 1 and 7 at $P < 0.05$. $N = 8$/group.

($N = 8$). All drugs were injected intraperitoneally in 0.85% sodium chloride solution (Table I). Control animals (Group 1) received only the largest volume of saline vehicle given with the test drugs.

Test drugs and rationales were as follows. CB-154 (Sigma Chemical Co., St. Louis, MO) was prepared as a 0.02% solution, and animals in Groups 2 and 6–8 received 0.2 mg/kg, a dose reportedly effective in inhibiting prolactin release in rats (6). Metoclopramide monohydrochloride (M; Sigma Chemical Co.) was prepared as a 0.05% solution and administered at a dose of 0.05 mg/kg (21). Quipazine maleate (Q) (Miles Scientific Co., Naperville, IL) was prepared as a 1% solution and given at a dose of 10 mg/kg (22).

Animals received their respective drugs 1 hr before sacrifice. In Groups 6–8, CB-154 was administered 30 min after initial drug(s) administration. These animals were then sacrificed 30 min after CB-154 was given. In Groups 5 and 8, both M and Q were administered simultaneously (i.e., quipazine was injected and immediately followed by metoclopramide).

Animals were anesthetized (diethyl ether) and exsanguinated (by cardiac puncture). Blood samples were allowed to clot and were stored overnight at 4°C. Serum was collected and stored (−20°C) until assayed for PRL. The whole brain of each animal was collected over ice, weighed, and stored (−80°C) in 0.5 *M* $HClO_4$–0.1% cysteine (150 mg/tissue/ml) solution until analyzed for the neurotransmitters and their metabolites described below.

PRL radioimmunoassay. The RIA technique used to determine the PRL concentrations was a modification of the NIAMDD rat prolactin-RP-2 described by Porter *et al.* (6). (This kit was supplied through the National Hormone and Pituitary Program, National Institutes of Allergic, Metabolic, and Digestive Diseases, National Institutes of Health, Bethesda, MD).

Neurotransmitter–metabolite Assay. Whole brains were analyzed for norepinephrine (NE), dopamine, dihydroxyphenylacetic acid, homovanillic acid, serotonin, and 5-hydroxyindolacetic acid (5HIAA) by high-performance liquid chromatography–electrochemical detection (Catecholamine Analyzer No. 3, Bioanalytical Systems, West Lafayette, IN). The tissues were thawed, homogenized (Polytron, Brinkman Co., Westbury, NY) in an ice–salt bath, and centrifuged (2°C; 20,000 rpm) for 30 min. The supernatant was filtered (0.2-μm nylon Gelman Acrodisc filter, Gelman Sciences, Inc., Ann Arbor, MI) and analyzed directly using a 20-μl injection loop. Guard (30 × 4.6-mm) and analytical (250 × 4.6-mm) columns were Biophase ODS 5 μ and the mobile phase consisted of acetonitrile–0.1 *M* citric acid (8:92 v/v) solution according to Lin and Blank (23) at 26°C with a flow rate of 1 ml/min. The W_1 and W_2 glassy carbon dual parallel electrodes were 800 and 700 mV, respectively (vs Ag/Ag Cl). Compound identification and quantification were based on comparison of chromatographic retention time of authentic standards, and the ratios for the parallel outputs at 800 mV (W_1) and 700 mV (W_2) were according to Mayer and Shoup (24). Standards were obtained from Sigma Chemical Co. and Bioanalytical Systems, Inc., and concentrations are reported for the free compounds.

Statistical analysis of data. Analyses of variance procedures were used on data concerning neurotransmitters and metabolite concentrations (SAS Institute, Inc., 1982). Duncan's multiple-range test was used for determining mean differences ($P < 0.05$). Figures 1, 2, and 3 represent concentration ratios of metabolites/neurotransmitter DOPAC/DA: HVA/DA and 5HIAA/5HT, respectively. Ratios are normalized relative to controls using

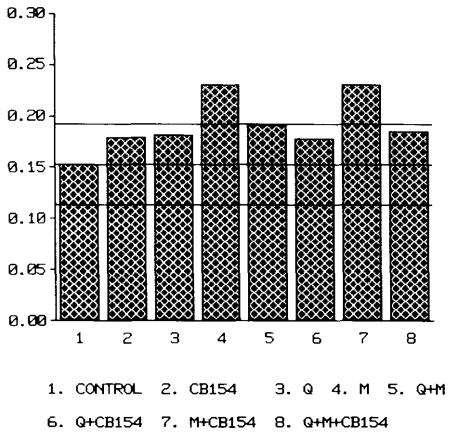

FIG. 1. Ratio DOPAC/DA concentrations (Group 1–8, see Table I).

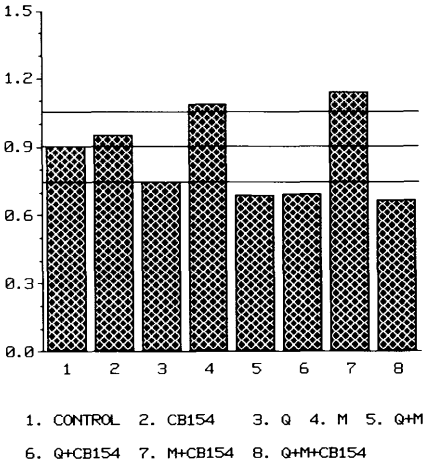

FIG. 3. Ratio 5HIAA/5HT concentrations (Groups 1–8, see Table I).

the critical range for a $P < 0.05$ confidence level.

Serum PRL concentrations were analyzed by one-way analysis of variance followed by the Student–Newman–Keuls multiple-range test of all possible mean contrasts at the 0.05 level of protection (25).

Results and Discussion. Experimental groupings, PRL data, and brain neurotransmitters and their respective metabolite concentrations are summarized in Tables I and II. The ratios for the metabolites/neurotransmitter precursors are depicted in Figs. 1, 2, and 3. PRL concentrations were significantly lowered in those rats given CB-154 (Group 2),

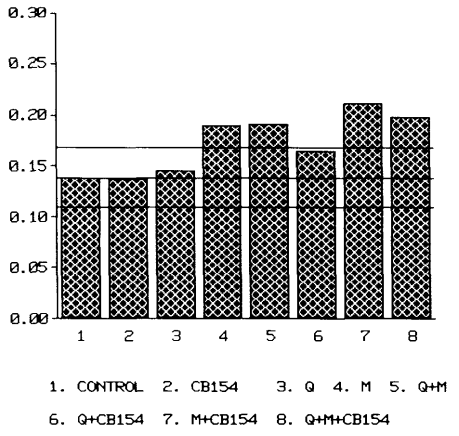

FIG. 2. Ratio HVA/DA concentrations (Groups 1–8, see Table I).

while there were no apparent effects on the levels of measured neurotransmitters and/or their metabolites. These results agree with previous investigations on serum prolactin and brain catecholamine metabolite depression observed in rats given extracts of endophyte-infected fescue seeds (6).

While quipazine (Group 3) did not significantly elevate PRL concentrations, it did significantly decrease concentrations of 5HIAA (major metabolite of 5HT) and had no effect on other compounds analyzed in whole brain tissue. In contrast, metoclopramide (Group 4) significantly elevated PRL, DOPAC, HVA, and 5HIAA concentrations with no effects on NE and/or 5HT concentrations. The quipazine and metoclopramide combination (Group 5) significantly increased PRL, HVA, and 5HT concentrations, and decreased 5HIAA concentrations. Although DOPAC concentrations were increased, the increases were not significant. Quipazine plus CB-154 (Group 6) decreased 5HT metabolism just as it did in rats given only quipazine (Group 3). Rats given metoclopramide plus CB-154 (Group 7) responded similarly to those in Group 4. Rats given quipazine, metoclopramide, and CB-154 (Group 8) responded with changes in both 5HT and DA metabolism, with HVA concentrations significantly increased and 5HIAA concentrations significantly decreased. Although 5HT concentrations were elevated, they were not significantly

TABLE II. NEUROTRANSMITTER-METABOLITE CONCENTRATIONS (ng/g BRAIN TISSUE) IN RATS (±SD)

Group no.	Group	NE	DOPAC	DA	HVA	5HT	5HIAA
1	Saline controls	712 (64)	139 (53)	908 (52)	126 (8)	533 (53)	476 (28)
2	CB-154	684 (68)	170 (6)	953 (76)	131 (6)	565 (97)	522 (49)
3	Q	676 (36)	167 (17)	925 (56)	135 (8)	555 (61)	410 (54)*
4	M	652 (30)	212 (30)*	920 (21)	175 (25)*	497 (43)	538 (39)*
5	Q + M	753 (83)	172 (20)	920 (108)	175 (28)*	624 (99)*	420 (48)*
6	Q +CB-154	710 (56)	155 (7)	882 (81)	145 (9)	528 (21)	365 (50)*
7	M + CB-154	744 (51)	204 (23)*	887 (72)	187 (25)*	495 (13)	563 (39)*
8	Q + M + CB-154	707 (70)	168 (33)	914 (84)	180 (30)*	591 (86)	385 (76)*

Note. Q, quipazine; M, metoclopramide; NE, norepinephrine; DOPAC, dihydroxyphenylacetic acid; DA, dopamine; HVA, homovanillic acid; 5HT, serotonin; 5HIAA, 5-hydroxyindoleacetic acid; CB-154, 2-bromo-α-ergocryptine.
* Significantly different from controls at $P < 0.05$.

different from the controls. These observations are in agreement with observations seen with the individual drug regimens (Groups 3 and 4) and support results of previous studies concerning dopaminergic and serotonergic mechanisms involved in the pharmacologic actions of metoclopramide and quipazine, respectively (22, 26–30).

Elevated serum PRL concentrations in rats given metoclopramide and/or quipazine appear to be related to the turnover rates of neurotransmitters and the prospective agonist-antagonist activities of these drugs. Significantly elevated DOPAC/DA ratios for the metoclopramide (Group 4) and the metoclopramide plus CB-154 (Group 7)-treated rats (Fig. 1) suggest that metoclopramide increased dopamine turnover. Elevated DOPAC/DA ratios (although not significantly different from the controls) in the quipazine plus metoclopramide (Group 5) and the quipazine, metoclopramide plus CB-154 (Group 8)-treated animals support this suggestion. These observations are more strongly substantiated by the HVA/DA ratios depicted in Fig. 2. Metoclopramide (Group 4), quipazine plus metoclopramide (Group 5), metoclopramide plus CB-154 (Group 7), and metoclopramide, quipazine, plus CB-154 (Group 8) all show significantly elevated ratios of HVA/DA, demonstrating an increased turnover rate of DA to HVA.

Figure 3 depicts a decreased 5HIAA/5HT ratio for the quipazine (Group 3)-treated animals (although not significantly different from controls). Animals dosed with quipazine plus metoclopramide (Group 5), quipazine plus CB-154 (group 6), and the quipazine, metoclopramide, plus CB-154 (Group 8) all showed significantly decreased 5HIAA/5HT ratios. This observation suggests that quipazine exerts its effects (PRL increase) by slowing or reducing the 5HIAA/5HT turnover and thereby increasing concentrations of 5HT. The significant decreases in 5HIAA concentrations measured in Groups 3, 5, 6, and 8 (Table II) support this suggestion. In addition, there was a significant increase in 5HT concentration in Group 5 (quipazine–metoclopramide) which appears to be an effect of quipazine on the prevention of the metoclopramide-induced increase of 5HIAA.

The changes that we observed in whole brain homogenates are consistent with previous studies (31, 32) where a decrease in 5HIAA concentration has been shown to correspond to a decrease in the 5HIAA/5HT ratio. These observations strengthen the proposed serotonergic activity of quipazine; the proposal is more strongly supported by the observed increase in 5HT concentration seen with concomitant decrease in 5HIAA concentration for the quipazine plus metoclopramide (Group 5)-treated animals. Also, quipazine appears to antagonize or reverse the metoclopramide effects on DOPAC concentrations and the DOPAC/DA ratios, respectively. This result supports the observations of Ponzio *et al.* (31) that quipazine in rat striatum reduces DOPAC and HVA concentrations and has a greater effect on DOPAC than on HVA.

Metoclopramide has been reported to stimulate rat pituitary PRL secretions by a serotonergic mechanism in addition to its dopa-

minergic antagonist properties (26). Although 5HT concentrations were lower than controls, the current studies showed no significant changes (Table II, Groups 4 and 7) in 5HT concentrations; however, its major metabolite 5HIAA was significantly elevated, a finding which would give added support to this proposed mechanism. The 5HIAA/5HT ratio (Fig. 3) was also significantly higher, suggesting that faster turnover may stimulate increased biosynthesis of 5HT. These differences might have been more pronounced if discrete brain sections had been studied instead of whole brain homogenates.

Mangoni *et al.* (28) demonstrated in rats that metoclopramide elevated HVA levels without affecting DA and/or NE concentrations, and thus concluded that metoclopramide induced acceleration of dopamine turnover. This is supported by our findings (and other investigations (32)) where both DOPAC and HVA levels in rats dosed with metoclopramide were elevated as were the DOPAC/DA and HVA/DA ratios. The increases in DOPAC/DA and 5HIAA/5HT ratios found with metoclopramide treated animals (Table II, Figs. 1 and 3, Groups 4 and 7) apparently were negated by quipazine (Table II, Figs. 1 and 3, Groups 5, 6, and 8). These observations along with the PRL data (Table I) substantiate previous studies that dopaminergic inhibition (and/or increased turnover of DA) has a more pronounced effect on prolactin secretion than serotonergic stimulation (22).

Conclusions. The combined drug regimen of quipazine and metoclopramide more effectively prevented the serum prolactin suppressant activity of CB-154 than either of the individual drugs given alone (Table I). A proposed mechanism for this response with these two drugs is supported by data showing an increased dopamine metabolism and a decreased 5HT metabolism. The current studies suggest that the metoclopramide–quipazine regimen may have therapeutic potential for combating the ergotlike fescue and other similar toxicities observed in cattle grazing on endophyte-infected pasture grasses.

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