The Involvement of Cytochrome P450 in Ergot Alkaloid Metabolism

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Story in Brief

This study was conducted to investigate the involvement of cytochrome P450 3A4 (CYP3A4) in the metabolism of ergotamine in beef liver microsomes. In addition, the effects of ergonovine and dihydroergotamine on CYP3A4 induction were examined in rats. When incubated with beef liver microsomes, ergotamine and its isomer were hydroxylated to their respective metabolites M1, M2, M1-Iso, and M2-Iso (8-hydroxy-derivatives). Maximum formation of metabolites was reached after 20 min, and ergotamine and its isomer were almost totally metabolized after 60 min of incubation. Ergonovine and dihydroergotamine treatments did not produce any increase ($P > 0.05$) in the induction of CYP3A4 activity over the control treatment in rats.

Introduction

Fescue toxicosis has been a problem for farmers and subject to extensive scientific investigation for some time. The presence of low levels of highly toxic ergot alkaloids in endophyte-infected (Acremonium coenophialum) tall fescue has been implicated in causing fescue toxicosis. There is good pharmacological evidence that some of the ergot alkaloids are linked to modulation of physiologi-cal mechanisms. An approach to solving the problem of fescue toxicosis would be to manipulate the process used by animals to eliminate the ergot alkaloids from circulation. The cytochrome P450 (CYP3A4) enzyme system plays a significant role in the elimination ergot alkaloids by extensive hepatic biotransformation (Pollock, 1994). Cytochrome P450 exists mainly in the liver, but is found in other tissues such as intestines, lungs, and kidneys (Krishna and Klotz, 1994).To our knowledge, no research has been done to evaluate the presence of CYP3A4 in beef liver microsomes. Furthermore, it is not known whether fescue toxins will induce such enzyme systems or inhibit them. Therefore, the objective of this study was to obtain preliminary information on the link between CYP3A4 and the metabolism of ergotamine, a representative ergot alkaloid, in beef liver. In addition, the effects of ergonovine (EN) and dihydroergotamine (DH) on the induction and the inhibition of cytochrome P450 activity were examined.

Experimental Procedures

The following chemicals were obtained from Sigma Chemical Co. (St. Louis, MO): bovine serum albumin (BSA), dexamethasone, ergotamine (ET), NADP+, D-glucose-6- phosphate, magnesium chloride, glucose-6-phosphate dehydrogenase, and EDTA. All chemicals and reagents used were of the highest quality commercially available.

Experiment 1: Five steers (992 to 1323 lb BW) were processed at the University of Arkansas abattoir. These steers had not been on any sort of fescue feed prior to necropsy. Liver tissues (0.11 to 0.22 lb) were collected and microsomes were prepared according to Kremers et al. (1981). Livers were diced with scissors and then washed with 150 mM sodium chloride buffer. Diced tissue was then ground at 1 g/10 ml of buffer (250 mM sucrose, 100 mM Tris-HCL, 1 mM EDTA, pH7.4) with ice-cold medium using a precooled blender for 10 to 20 sec, followed by homogenization with Potter-Elvehjem (5X). The homogenate was successively centrifuged at 800 x g for 10 min, at 13,500 x g for 20 min before collecting the supernate. The supernate was then centrifuged at 105,000 x g for 60 min, collecting the pellet containing the microsomal fraction. The pellet (microsomal fraction) was washed with 100 mM sodium pyrophosphate pH 7.5 and resuspended in buffer containing 100 mM sodium phosphate and 20% V/V glycerol to give 50 mg protein/ml concentration. Protein concentration was determined using BSA as a standard. Microsome suspensions were aliquoted and stored at -90oC and were used within 20 to 30 days.

Experiment 2: Male Sprague-Dawley rats (0.44 to 0.55 lb) were provided with laboratory chow and water ad libtium. After 4 days of adaptation, a total of 20 rats (five for each treatment) were treated intraperitoneally for 4 days with 100 mM levels of each of the following: a) dexamethasone (DXM), b) dihydroergotamine (DHET), c) ergonovine (EN), or d) control. Control rats received 0.5 ml of corn oil as the delivery vehicle. The dexamethasone was included as a reference treatment. Peyronneau (1994) reported that dexamethasone produced maximum induction of P450 activity at 4 days. Rats were sacrificed, livers were collected, and microsomes were prepared using the same procedure used for the bovine liver microsomes preparations.

The CYP3A4 activity in animals from both experiments was measured using ergotamine as a substrate. The metabolism of ergotamine was assayed in medium containing 100 mM Tris-HCl, 10 mM potassium phosphate, 0.1 mM EDTA, pH 7.5, cofactor generating system (NADPH), 20% glycerol, 1.0 mg/ ml ergotamine that had been fully isomerized, and 0.1 mg/ml microsomal protein in a total volume of 500 ml. The cofactor generating system was: 26 mM NADP+, 66 mM D-glucose-6- phosphate, 66 mM magnesium chloride, and 1 U Glucose-6-Phosphate dehydrogenase in sodium citrate. Bovine liver microsomes were diluted in assay buffer to a working concentration of 2.5 mg protein/ml and kept on ice. The reactions were started by adding the NADPH generating system and were terminated after 30 min by adding 100 ml of 94% acetonitrile and 6% glacial acetic acid and centrifuged at 12,000 x g for 5 min pH 7.5.

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Twenty ml of each of the supernatants from the enzyme assays were examined for the disappearance of ergotamine and its isomer and also for the appearance of the metabolites by using a modification of the high pressure liquid chromatography (HPLC) method described by Moubarak et al. (1993, 2000). A 20 ml sample loop was fitted to a Millennium32 Workstation HPLC system with auto-sample injector and a gradient programmer. The detection was accomplished by a Gilson 121 fluorescence detector (excitation at 250 nm and emission at 370 nm long pass filter). Separation was conducted on a 3x3 CR C18 cartridge column using acetonitrile and 2.6 mM ammonium carbonate in 10% methanol gradient elution at 1 ml/min flow rate. The gradient program was used to separate and quantitate the peak area of ergotamine, its isomer, and the metabolites with no carry over effects from run to run.

Results of experiment 1 are described and presented graphically. Data from experiment 2 were examined by one-way analysis of variance.

Results and Discussion

In experiment one where enzyme preparations from beef liver microsomes were used, results indicated the presence of CYP3A4 which is capable of metabolizing ergotamine. Figure 1 shows a representative HPLC chromatogram where ergotamine was metabolized by beef liver enzyme preparation (CYP3A4) to more water soluble metabolites M1 and M2. Similar chromatograms were obtained from all five steers. Similarly, the ergotamine isomer was also hydroxylated to M1-Iso and M2-Iso (8 or 9-hydroxy-derivatives). Further incubation resulted in a second hydroxlation of M1 and M2 to more water soluble metabolites M3 and M4 with 8,9-dihydroxy derivatives. The formation of these metabolites (M1, M2, M1-Iso and M2-Iso) was dependent on the presence of NADPH or the NADPH generating system and was also dependent on microsome concentration. Figure 2 shows the results of a time dependent incubation of ergotamine with beef liver microsomes. Values shown are means of evaluations for the five steers done in triplicate. Ergotamine was hydroxylated first to metabolites M1 and M2; then metabolites M1 and M2 were converted to M3 and M4 by the addition of a second hydroxyl group. Ergotamine and its isomer were almost totally metabolized after 60 min of incubation and the metabolites M1, M2, M3, and M4 appeared to be formed in a time dependent fashion with M1 and M2 formed first reaching a maximum level after 30 min. Similar data were reported in dexamethasone treated rats (Peyronneau et al., 1994) and human liver microsomes (Christians et al., 1996). Under normal conditions, animals possess a base-line level of CYP3A4 activity; however when they consume or are injected with drugs or toxins, one of the body's responses will likely be to elevate the level of CYP3A4 to speed up the clearance of such compounds from circulation.

The CYP3A4 activity in liver microsomes from rats treated with 100 mM DXM, DHET, or EN and controls is shown in Figure 3. Enzyme preparations from control animals produced a base line activity of 0.139 and 0.221nM/ mg protein/min (SE = 0.040) for ET and ET isomer. Dexamethasone treatment (100 mM) of rats for 4 days produced a significant ($P < 0.05$) 3.8-fold increase (0.535 nM/ mg protein/min) over control rats in CYP3A4 activity as determined by the conversion of ET to its metabolites. Treatment of rats with DHET or EN at a concentration of 100 mM did not produce any significant ($P > 0.05$) increase in CYP3A4 activity over the control rats. The enzyme system which is involved in metabolism of ergot alkaloid found in endophyte-infected tall fescue has not been fully characterized in beef animals, yet structurally related ergot alkaloids such as bromocriptine and dihydroergotamine have been found to be metabolized by animals and man, in vivo and in cell cultures by the CYP3A4 enzyme system (Maurer et. al., 1983). Our data documented the presence of CPY3A4 activity in liver of beef steers and clearly show its involvement in ergotamine metabolism. The first experiment demonstrated that beef steer liver microsomes have the enzymatic capability to metabolize the ergot alkaloid ergotamine. The second experiment demonstrated that ergot alkaloids of other families (DHET, as a representative of the ergopeptide, or EN, as representative of the lysergic acid amid derivative) did not induce additional, or above base line, CYP3A4 activity after 4 days of treatment. The lack of additional activity of CYP3A4 in liver microsomes collected from rats treated with EN or DHET raises the question as to whether they are not exerting any effects, are they interacting with CYP3A4 at the protein level hindering its activity, or could they be affecting the induction process upstream.

Implications

If we have a better understanding of the mechanisms by which ergot alkaloids are metabolized, then we can use this information to define markers that can be used to select animals that are resistant to fescue toxins. Part of those mechanisms are through the cytochrome P450 system.

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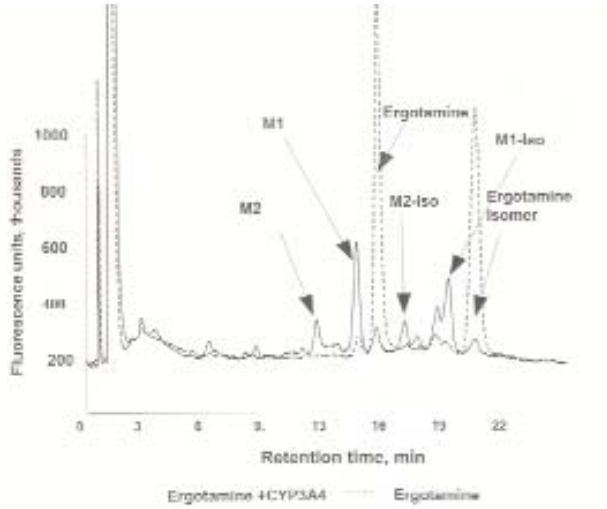

Fig. 1. A representative HPLC chromatogram of products from ergotamine incubation with beef liver microsomes. For incubation conditions see Materials and Methods.

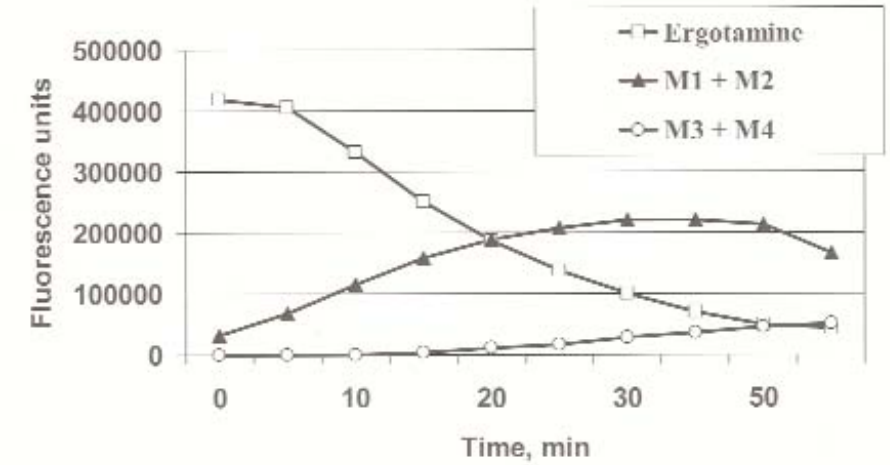

Fig. 2. Time dependent disappearance of ergotamine and appearance of metabolites when incubated with beef liver microsomes. Mean of determinations from five animals done in triplicate.
□=Disappearance of ergotamine; △=Formation of metabolites M1 and M2; o=Formation of metabolites M3 and M4.

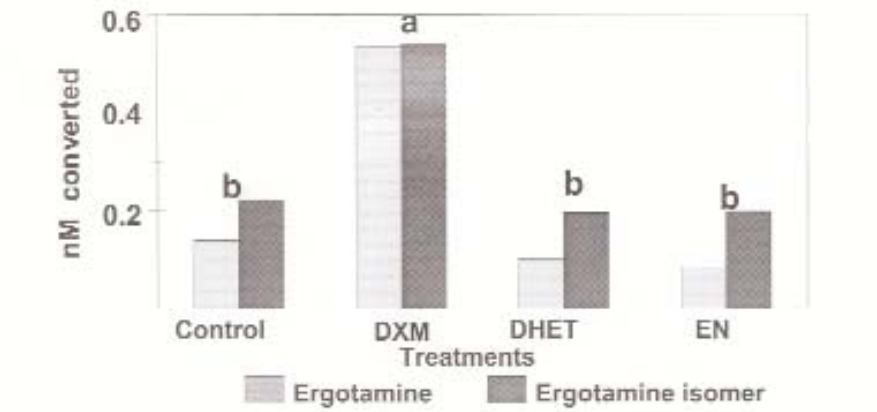

Fig. 3. Incubation of ergotamine and its isomer with liver microsomes from rats treated with the following: a) control, b) dexamethasone (DXM, 100 mM), c) dihydroergotamine (DHET, 100 mM), or d) ergonovine (EN, 100 mM).
a,b Means with no letters in common are different (P < 0.05) for both ergotamine and the ergotamine isomer.