HPLC Method for Detection of Ergotamine, Ergosine, and Ergine after Intravenous Injection of a Single Dose

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A simple, rapid method of liquid solid extraction coupled with a sensitive HPLC procedure with fluorescence detection to measure ergot alkaloid levels in bovine blood samples is presented. Single injections of either ergosine, ergotamine, or ergine were given to Holstein steer calves (140 kg), and serum concentrations of the ergot alkaloids were monitored over a 2-h period. Serum samples were loaded on a Bond Elut (100 mg) C_{18} column that had been washed with methanol and distilled water. Ergot alkaloids were then eluted with methanol and analyzed by HPLC. Recovery of ergosine, when added to serum at concentrations of 0.5−24.0 ng/mL, ranged from 95 to 98%. The data fit previous reports of three phases in elimination of drug. The method reported has excellent repeatability, a detection limit of 0.5 ng/mL, and provides an alternative to quantitate ergosine, ergotamine, and ergine in bovine blood samples at therapeutic dosages.

Keywords: *Ergosine; ergotamine; ergine; blood; assay; HPLC*

INTRODUCTION

The elucidation of mammalian biochemical pathways is important in attempting to rationalize detoxification and to evaluate potentially active metabolites of ergot alkaloids. Human and small animal experiments suggest that ergot alkaloids rapidly disappear from blood and tissue (Kanto, 1980, 1983), whereas their physiological effects remain for lengthy periods of time. Perrin (1985) suggested that good responses to migraine and cluster headache are associated with plasma concentrations of 0.2 ng/mL or above within an hour of administration of ergotamine. The naturally low level of ergot alkaloids, low gastrointestinal absorption (Meier and Schreier, 1976; Little et al., 1982), and the high first-pass clearance by the liver (Aellig and Nuesch, 1977) have delayed progress in understanding the pharmacokinetics of ergot alkaloids or any of their active metabolites.

Numerous analytical methods have been developed to detect and measure the levels of ergot alkaloids in a variety of samples (Edlund, 1981; Axelrod et al., 1957; Savary et al., 1990; Cox et al., 1992); however, most methods for detection in the blood have been limited to human and small monogastric animals. Savary et al. (1990) reported an analytical method for ergovaline and Cox et al. (1992) for ergonovine in bovine plasma. Both methods require organic solvents for extraction and involve a lengthy and complicated procedure. Pharmacokinetics information on ergot alkaloids in bovine animals has been hampered by the lack of simple, sensitive methods to evaluate the levels of these alkaloids in blood samples. In the present study, our objectives were to develop a simple, rapid, and sensitive method to detect and measure a ng/mL level of ergot alkaloid in bovine blood samples and to monitor the changes in ergosine, ergotamine, and ergine serum concentration in bovine blood after single intravenous injection.

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EXPERIMENTAL PROCEDURES

Materials. Ergotamine tartrate was obtained from Sigma Chemical Company, St. Louis, MO; ergosine and ergine were gifts from Dr. M. Flieger (Institute of Microbiology, Prague, Czechoslovakia). Organic solvents were HPLC grade, and the C_{18} concentration and cleanup column was a Bond Elut (100 mg) column from Varian (Harbor City, CA).

Methods. Holstein steer calves (140 kg) were given intravenous injections (jugular vein) of either ergosine, ergotamine, or ergine, and blood samples were collected from each calf over a 2-h period. Ergosine was injected at two levels (1.8 or 7.0 μg/kg of BW) to one calf each and analyzed in triplicate to determine the precision of the assay. Ergotamine and ergine were injected, separately, at 14 μg/kg of BW into four calves each to determine the assay response to animal variability. Levels of ergosine, ergotamine, and ergine used were selected based on the physiological responses produced in preliminary tests. Blood samples from the ergosine and ergotamine trials were collected before injection; 5, 10, and 15 min after injection; and then every 15 min up to 120 min. Samples from the ergine trial were collected before injection and 5, 10, 20, 30, 60, 90, and 120 min after injection. All blood samples were allowed to clot at room temperature, and the serum was separated by centrifugation, held at 4 °C, and analyzed within 12−24 h of collection for ergotamine, ergosine, or ergine.

HPLC Analyses. Serum (5 mL) samples were centrifuged (800*g* for 30 min) or filtered with 13 mm × 4″ filter sampler (Porex Medical Technologies Corp., Fairburn, GA) to remove any residual protein and then loaded on a Bond Elut, 100 mg, C_{18} cleanup and concentration column that had been previously washed with 10 mL of methanol followed by 15 mL of distilled water. Ergot alkaloids were then eluted with 0.5 mL of methanol and loaded onto the HPLC. A 20-μL sample loop was fitted to an ISCO 2350 pump and 2360 gradient programmer, and detection was accomplished with a Shimadzu RF-551 spectrofluorometric detector (excitation at 250 and 425 nm). Separation was conducted on a 3 × 3 CR C_{18} cartridge column (Perkin-Elmer) with a linear gradient elution as described by Moubarak et al. (1993); that is, acetonitrile (A), 2.5 mM ammonium carbonate (B), and methanol (C):

time, min	flow rate, mL/min	event
0.0	1.0	20% A, 70% B, 10% C
5.0	1.0	50% A, 40% B, 10% C
10.0	1.0	20% A, 55% B, 25% C
16.0	1.0	20% A, 70% B, 10% C
16.1	0.3	end

This gradient program was used to separate and estimate

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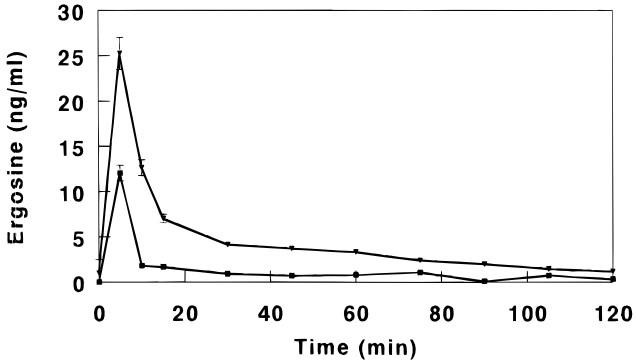

Figure 1. Mean serum ergosine concentration and standard deviation following a single intravenous injection at two dosage levels [7.0 (▼) and 1.8 (■) μg/kg of BW) in calves.

areas of peaks associated with ergotamine (retention time, 10.2 min) and ergosine (retention time, 9.8 min) and their respective isomers. Analysis of ergine was not possible with the same gradient conditions because of its early elution; therefore, an isocratic mobile phase of 15:80:5 acetonitrile:2.5 mM ammonium carbonate:methanol was used. This gradient gave ergine a retention time of 5.4 min. Standard curves were generated for ergotamine, ergosine, and ergine with freshly prepared standard solutions and were used to calculate the alkaloid concentration in serum samples. The isomers of ergotamine (ergotaminine) and ergosine (ergosinine) were identified by allowing ergotamine and ergosine solutions to isomerize during storage. Isomerization takes place fairly rapidly (3−5 h) under benchtop conditions at pH 7.4 and is particularly fast when the ergopeptide is added to the serum at low concentration (0.5−24 ng/mL). The area measured for each isomer was added to the area of the parent compound to estimate the total area of each alkaloid in the serum samples.

Verification of Recovery. Ergosine was added to the calf serum at concentrations ranging from 0.5 to 24 ng/mL. Immediately after addition of ergosine to the serum, it was centrifuged and loaded on the packed column, and ergosine were eluted with methanol and injected onto the HPLC. Rates of recovery and isomerization were calculated, and the concentration found was compared with that added by linear regression procedures.

Statistical Analyses. Assay precision for ergosine is illustrated by a plot of means and standard deviations (Figure 1). Intraclass correlations were calculated for each dosage level of ergosine. The intraclass correlation would be the correlation between serum concentrations measured on the same blood sample and reflects the repeatability. Differences in serum concentration of ergotamine and ergine in the blood of calves over time were examined by analysis of variance for each alkaloid with a model that included terms for animal and time. Differences among means over time were determined by LSD when analysis of variance indicated a significant ($p < 0.05$) time effect. Significant differences among animals and (or) time would indicate that the assay is capable of picking up differences among treatment (time after injection).

RESULTS

The Bond Elut C_{18} column appears to have a high affinity for the ergopeptide alkaloids in 25% methanol: ammonium carbonate and for lysergic acid amide derivative at 10−15% methanol. As shown in Table 1, when filtered serum was loaded directly onto the C_{18} column, the amount of recovery of ergosine from serum samples to which it was added ranged from 95 to 98%. The amount of isomerization (ergosine to ergosinine) ranged from 30.6 to 34.4%. The relationship between concentration of ergosine added and ergosine recovered was linear for concentrations used in this study, with a regression coefficient of 0.959 ± 0.005 and an R^2 value of 0.997. Ergotamine and ergine recovery and isomerization followed the same pattern as that of ergosine.

Table 1. Percent Recovery and Isomerization of Ergosine Added to Calf Serum[a]

concn added, ng/mL	concn found, ng/mL	isomerization, %	recovery, %
0.5	0.47 ± 0.004	33.4	95.0
1.5	1.46 ± 0.005	33.1	97.1
3.0	2.89 ± 0.035	30.6	96.6
6.0	5.80 ± 0.016	34.4	96.6
12.0	11.40 ± 0.103	33.4	95.0
18.0	17.56 ± 0.030	32.8	97.6
24.0	22.84 ± 0.205	32.5	95.2

[a] $n = 4$.

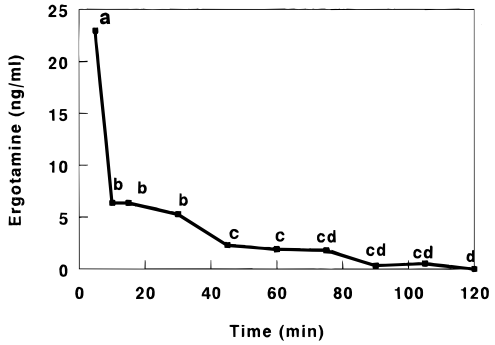

Figure 2. Mean serum ergotamine concentration following a single intravenous dose (14 μg/kg of BW) of ergotamine in calves.

Presented in Figure 1 are means and standard deviations for serum concentration of ergosine at times up to 2 h after injection of 1.8 or 7.0 μg/kg of BW of ergosine. Small standard deviations (0.02−0.84 for 1.8 μg/kg of BW and 0.08−1.77 for 7.0 μg/kg of BW injections of ergosine, respectively) indicate good precision of the assay when repeated in triplicate. Intraclass correlations (repeatability) were high (0.99 and 0.98 for ergosine injections of 1.8 and 7.0 μg/kg of BW, respectively).

Serum concentrations of all three alkaloids were highest immediately after injection and dropped significantly within 30 min (Figures 1−3). Ergosine concentrations peaked within 5 min and declined rapidly within 10−15 min after injection (Figure 1). Concentration of ergosine in serum from calves that received the higher dosage of injected ergosine (7.0 μg/kg of BW) peaked at a higher level and did not fall as rapidly as concentration of ergosine in serum from calves that received the lower level of ergosine. Serum concentrations of ergosine at the low dosage reached a high of 12 ng/mL at 5 min postinjection and dropped to 1.8 ng/mL at 10 min after injection. The highest concentration of ergotamine (23 ng/mL) was measured 5 min postinjection (Figure 2); this high concentration was followed by a significant drop to 6 ng/mL by 10 min. Serum levels plateaued for the next 20 min, then fell to near zero over the next 1.5 h. Ergine serum concentrations exhibited the same pattern as ergotamine and ergosine, with a mean peak concentration at 5 min of 2.5 ng/mL.

Significant effects ($p < 0.05$) were found for both animal and time for serum concentrations of ergotamine and ergine. In both trials, there was one animal with a significantly higher level of serum concentration of ergot alkaloids than the other animals. Serum concentrations of both ergotamine and ergine dropped significantly ($p < 0.05$) after 5 min, followed by a second significant ($p < 0.05$) drop at 30−45 min and remained very low but detectable up to 2 h (Figures 2 and 3). Isomers of both ergotamine and ergosine were in an

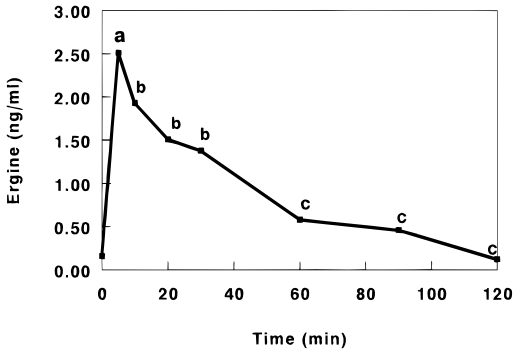

Figure 3. Mean serum ergine concentration following a single intravenous dose (14 μg/kg of BW) of ergine in calves.

equilibrium concentration with the parent compound (35/65) 12−24 h after collection. The ergine isomer was detected, but only total ergine was measured.

DISCUSSION

Detection and measurement of the serum concentration of ergot alkaloids in blood is essential for the understanding of the mode of action and the rate of clearance of these substances. Although a number of methods for determining ergot alkaloids in the blood have been reported, no information is available on the pharmacokinetics of these alkaloids in bovine animals. Bobik et al. (1980) and Kanto et al. (1981) described the disappearance of dihydroergotamine from plasma of human subjects in a biexponential manner, supporting the presence of three different compartments. Similarly, in this paper the disappearance of ergosine, ergotamine, and ergine from the blood (Figures 1−3) can be characterized by at least three separate phases. The first phase (0−10 min) reflects a volume distribution equilibrium, which is followed by the second phase (starting immediately after injection and lasting up to 60 min) representing an equilibrium between blood and tissue. The third and final phase represents the reverse equilibrium between tissue-to-blood movements and elimination by the liver.

In the present study, the disappearance of all three alkaloids (ergotamine, ergosine, and ergine) from the blood follows similar trends to that reported by Ibraheem et al. (1985) for ergotamine in humans. The ability of any assay to detect any of these alkaloids may depend on several factors, such as the level of intake, the rate of uptake by the tissue, the affinity of the binding, and the rate of clearance. Such factors have not been characterized in bovine animals. Our data show that this method can detect 12.5, 7.0, and 1.25% of the total ergotamine, ergosine, and ergine injected, respectively, at 5 min postinjection. On the one hand, the similarity of the chemical structure of ergotamine and ergosine may explain the relatively higher circulating level in the blood for these two alkaloids. On the other hand, the presence of the phenyl ring in ergotamine and the lack of it in ergosine may be the factor in the difference between these two alkaloids. Ergine is lacking the tricyclic amino acid portion of the molecule when compared with ergotamine or ergosine, and this difference may explain the very low detection level for this alkaloid. Physiological responses to ergot alkaloids have been reported in a variety of areas in both humans and animals (Kanto, 1983; Perrin, 1985), and research suggests that peak physiological response occurs sometime after the peak blood concentration starts to decline. Data from our laboratory reported elsewhere (McCollough et al., 1994), together with data from this report, suggest that decreased blood flow in calves as measured by tail temperature occurs 30−150 min after the blood concentration of ergotamine reaches a maximum.

In conclusion, we presented a simple, rapid, and sensitive procedure to detect and measure 0.5−1.0 ng/mL levels of ergosine, ergotamine, and ergine in bovine blood samples following administration of these agents at therapeutic dosage.

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