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Note

High-performance liquid chromatographic assay of bromocriptine in rat plasma and brain

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Bromocriptine (2-bromo-α-ergocriptine) (Fig. 1) is an ergot derivative with agonist activity at cerebral dopamine receptors [1]. It is used clinically to suppress pituitary tumors that secrete prolactin or growth hormone, and as an adjunct to levodopa therapy in the management of Parkinson's disease [2–5].

Administered to experimental animals, bromocriptine produces hypomotility followed by stereotypic behavior (sniffing and gnawing) [6,7]. A previous study in our laboratory correlated these behaviors with local cerebral glucose utilization after bromocriptine was administered to three-month-old Fischer-344 rats [8]. At 2 h after injection, hypomotility was found to be correlated with reduced glucose utilization in 50% of the brain regions examined. At 4 h, on the other hand, stereotyped behavior was accompanied by increased glucose utilization in motor regions of the brain. In order to interpret this biphasic effect of bromocriptine on behavior and cerebral metabolism, pharmacokinetic profiles of bromocriptine in plasma and brain were determined.

Several analytical procedures for quantitating bromocriptine levels have been published. Radioimmunoassay has been used to measure bromocriptine in rat tissue and in human plasma [9–11]. Although high sensitivity is achieved with

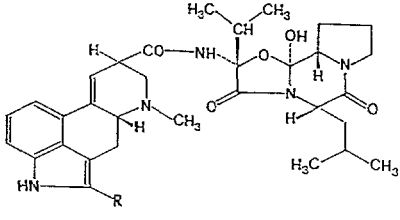

Fig 1 Structures of bromocriptine (R = Br) and ergocriptine (R = H)

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radioimmunoassay, specificity is poor, due to extensive metabolism of bromocriptine Radiotracer techniques, some in conjunction with high-performance liquid chromatography (HPLC), have been used successfully in animal and human studies [12–14]. However, this analytical procedure has the multiple disadvantages of high cost and contamination of instruments. Larsen *et al.* [15] reported the use of gas chromatographic, mass fragmentographic and liquid chromatographic techniques for the determination of bromocriptine in plasma. These methods, however, were not applicable for extraction of the compound from rat brain It was necessary, therefore, to develop a sensitive and specific assay by which bromocriptine levels could be measured conveniently in both rat plasma and brain.

EXPERIMENTAL

Materials

Bromocriptine mesylate, α-ergocriptine and lactic acid were obtained from Sigma (St Louis, MO, U.S.A.). Acetonitrile, isopropyl alcohol and methanol were of HPLC grade and were supplied by Burdick and Jackson (Muskegon, MI, U.S.A). Ammonium bicarbonate was purchased from Aldrich (Milwaukee, WI, U.S.A.) and potassium carbonate was supplied by J. T. Baker (Phillipsburg, NJ, U.S.A.). Distilled, deionized water was prepared using a Milli-Q reagent water system (Millipore, Bedford, MA, U.S.A.). Adult male Fischer-344 rats, three months old and approximately 250 g weight, were obtained from Charles River Breeding Labs. (Wilmington, MA, U.S.A.). Blank rat plasma and blank rat brain tissue used for preparation of standard curves were obtained from Bioproducts For Science (Indianapolis, IN, U.S.A.).

Chromatography

Separation was performed on a Waters (Milford, MA, U.S.A.) HPLC system, equipped with a WISP 712 sample processor maintained at 10°C, a variable-wavelength detector (Model 484) set at 310 nm and two Model 6000 dual-piston pumps. Samples were injected onto a 70 mm × 4.6 mm I.D., 3-μm Ultrasphere XL C_8 reversed-phase column (Beckman Instruments, San Ramon, CA, U.S.A.). Data were collected using a Waters 860 computer system.

The mobile phase was composed of 25.3 m*M* (2.0 g/l) aqueous ammonium carbonate–acetonitrile–isopropyl alcohol (54:40:6, v/v), pH 9.0. The mixture was filtered through a 0.45-μm pore size filter (Rainin Instrument, Woburn, MA, U.S.A.) and degassed by sonication. The column was allowed to equilibrate with the mobile phase for at least 30 min before any injections were made. Flow-rate was maintained at 1.2 ml/min, resulting in a column back-pressure of 710 p s.i. Retention times of ergocriptine and bromocriptine were 5.5 and 10.5 min, respectively. After each 20-min run, a second mobile phase of acetonitrile–water (80:20), with a flow-rate of 2.0 ml/min, eluted a non-polar contaminant within 15 min.

Standards

Stock solutions of bromocriptine or ergocriptine (internal standard) were prepared by dissolving 10 mg of either drug in 10 ml of methanol. Stock solutions were stored at −70°C and were stable for at least eight weeks Working solutions of bromocriptine were obtained by serial dilution of the stock solution into a water–acetonitrile–methanol (5:4:1) mixture. Plasma and brain standards were prepared by spiking blank plasma (1.0 ml) and blank brain tissue (300 mg) with 100 μl of the corresponding working solution, providing concentrations of 19.5–2500 ng/ml of plasma and 19.5–625 ng per 300 mg of brain tissue. Two working solutions of ergocriptine, one for plasma and one for brain, were obtained similarly. The final ergocriptine concentrations in plasma and brain samples were 500 ng/ml and 100 ng per 300 mg, respectively The working solutions of bromocriptine and ergocriptine were stable for at least eight weeks when stored at −70°C.

Animal studies

Male Fischer-344 rats, three months old and approximately 250 g, were injected intraperitoneally (2 ml/kg) with a suspension of bromocriptine methane sulfonate (10 mg/ml) in 1% lactic acid. At various times following bromocriptine administration, the animals were killed, trunk blood was collected, and the brain was recovered. The blood was placed in a heparinized tube and centrifuged at 10 000 *g* for 2 min. The plasma was removed and, together with the brain, stored temporarily on dry ice. All samples then were stored at −70°C until analysis.

Plasma extraction

Borosilicate glass test tubes (100 mm × 13 mm) and disposable centrifuge tubes (5.0 ml) were treated with 5% (v/v) trimethylchlorosilane in hexane (5.0 ml) by vortex-mixing for at least 10 min The silanized tubes were emptied and rinsed with methanol (5.0 ml, 2×). A 1.0-ml aliquot of blank plasma, plasma standard or unknown plasma specimen was placed in a silanized test tube with 10 μl of internal standard solution (ergocriptine, 50 μg/ml). Plasma was vortex-mixed with 2.5 *M* aqueous potassium carbonate (1 0 ml), followed by extraction with acetonitrile (4.0 ml) which was added slowly, while vortex-mixing, for 30 s. The mixture was centrifuged (Sorvall Instruments, Wilmington, DE, U.S.A.) at 2200 *g* for 15 min at −10°C and was maintained at this temperature for an additional 30 min to separate the organic and aqueous layers. The organic phase was removed and evaporated to dryness (Savant Instruments, Farmingdale, NY, U.S.A.). The residue was dissolved in 100 μl of a water–acetonitrile–methanol (5:4:1) mixture and was filtered through a 0.45-μm filter (Millipore) into a 200-μl conical glass vial. A 50-μl sample was injected onto the HPLC system for analysis.

Brain extraction

Blank brain tissue (300 mg), brain standard (300 mg) or unknown brain tissue

specimen (one half rat brain, longitudinally divided) was sonicated (Heat Systems–Ultrasonics, Farmingdale, NY, U.S.A.) in water (1.0 ml) in a silanized test tube and was spiked with 10 μl of ergocriptine (10 μg/ml). The homogenate was treated with 2.5 *M* aqueous potassium carbonate (1 0 ml) followed by extraction with acetonitrile (4.0 ml). The remainder of the procedure was carried out as described for the plasma extraction.

Calibration and quantitation

Plasma and brain concentrations of bromocriptine were quantified by using calibration curves. Standard curves of eight points (plasma) or six points (brain) were run daily at the beginning and end of each run. Drug-free plasma (1.0 ml) and brain tissue (300 mg) were spiked with known, increasing amounts of bromocriptine, as described above. A known concentration of the internal standard, ergocriptine, was also added. Least-squares linear regression between bromocriptine concentration and peak-height ratios (bromocriptine/ergocriptine) was performed for each standard curve The mean regression coefficient for six curves, run over a one-month period, was 0.992 for plasma and 0.984 for brain. The intercept was negligible. Plasma standard curves were linear over the range 19.5–2500 ng/ml. Brain standard curves were linear over the range 19.5–625 ng per 300 mg.

RESULTS

Recovery

Recovery was determined by comparing the absolute peak height of bromocriptine from extracted samples to the peak height obtained by direct injection of a neat standard containing a known amount of bromocriptine. The acetonitrile extraction of alkalinized blank rat plasma spiked with bromocriptine (19.5–2500 ng/ml) yielded a recovery (mean ± S.D.) of 95.2 ± 7.0% (coefficient of variation, C.V. = 7.4%). Similar extraction of blank rat brain spiked with bromocriptine (19.5–625 ng per 300 mg) yielded a recovery (mean ± S.D.) of 70.0 ± 11.1% (C.V. = 15.6%).

Stability

Sample stability was assessed by analyzing, over a three-week period, preparations of plasma and brain spiked with bromocriptine and stored at −70°C. No decomposition was measured in either plasma or brain stored for seven days. However, an 18% reduction in peak-height ratios was observed for plasma samples, whereas a 10% decrease was seen for brain samples at the three-week time point. Both plasma and brain reference standards were stable at 4°C for 30 min.

Reproducibility and sensitivity

The within-day reproducibility of the assay was determined by replicate assays

(n = 6 for plasma and n = 5 for brain) of standards containing low (39 ng/ml of plasma, 39 ng per 300 mg of brain) and high (312 ng/ml of plasma, 312 ng per 300 mg of brain) concentrations of bromocriptine. The C.V. values for plasma were 6.3% (39 ng/ml) and 6.5% (312 ng/ml). The C.V. values for brain were 10.3% (39 ng per 300 mg) and 4.2% (312 ng per 300 mg). The between-day reproducibility for plasma over a five-day period had C.V. values of 12.8% (39 ng/ml) and 7.9% (312 ng/ml). Inter-day measurements for brain over a five-day period had C.V values of 5.1% (39 ng per 300 mg) and 6.3% (312 ng per 300 mg).

The lower limits of linear detection for this assay were found to be approximately 19.5 ng/ml of plasma and 19.5 ng per 300 mg of brain.

Animal studies

A single dose of bromocriptine (20 mg/kg) was administered intraperitoneally to three-month-old male Fischer-344 rats. Plasma and brain concentrations were measured at time points 5 to 480 min later. Fig. 2 shows the chromatograms of an unspiked blank plasma sample (A) and of a plasma sample taken 30 min after the administration of bromocriptine (B). Fig. 3 shows chromatograms of blank rat brain (A) and a brain sample taken 30 min after the drug administration (B) (from the same animal as in Fig. 2B). Calculations against standards gave bromocriptine concentrations of 286.8 ng/ml and 73.2 ng/g for plasma and brain, respectively. The plasma and brain concentration *versus* time profiles are seen in Fig. 4.

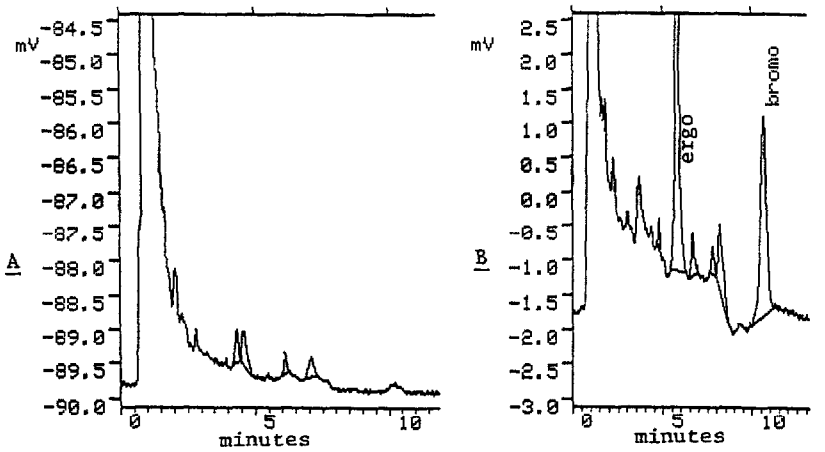

Fig. 2 Chromatograms of a 50-μl injection of (A) a blank plasma sample and (B) a plasma sample containing 286.8 ng/ml bromocriptine taken from a rat at 30 min after injection of 20 mg/kg bromocriptine intraperitoneally Peaks ergo = ergocriptine, bromo = bromocriptine

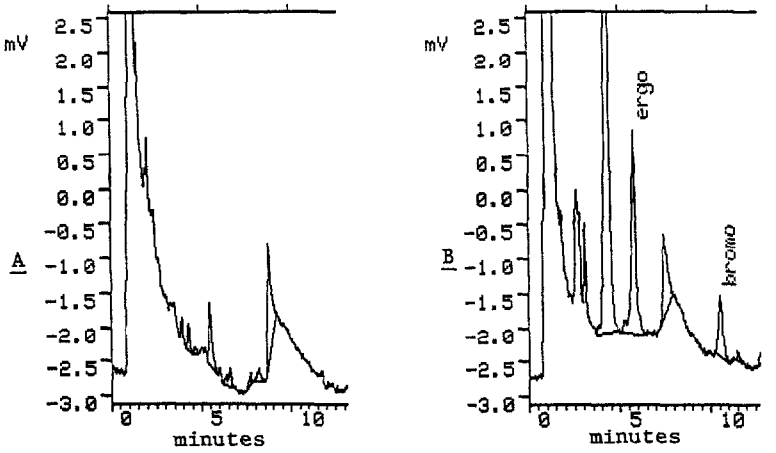

Fig 3 Chromatograms of a 50-μl injection of (A) a blank brain sample and (B) a brain sample containing 73.2 ng/g bromocriptine taken from a rat at 30 min after injection of 20 mg/kg bromocriptine intraperitoneally Peaks: ergo = ergocriptine, bromo = bromocriptine

DISCUSSION

The method described is a simple, sensitive and reproducible assay for the quantitation of bromocriptine in plasma and brain. The method was used to analyze plasma and brain concentrations of bromocriptine in rats at various times after drug administration. All samples were alkalinized with potassium carbonate and extracted into acetonitrile. Extraction efficiency was improved by

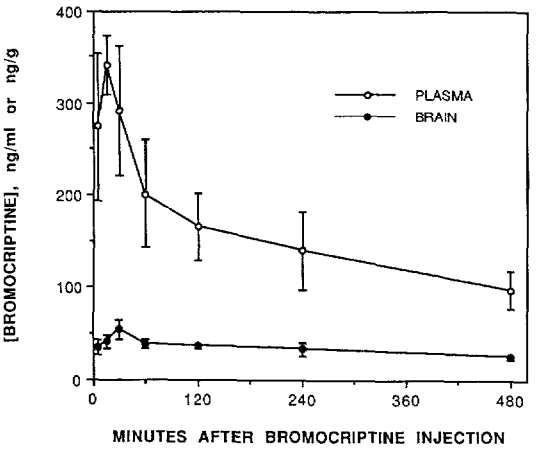

Fig 4. Mean (± S E.M) plasma and brain concentrations of bromocriptine following injection into awake rats of 20 mg/kg intraperitoneally (n = 5–7 animals for each time point)

30% when acetonitrile, rather than ethyl acetate, was employed, and by an additional 15% with use of silanized tubes.

Initially, an attempt was made to quantify samples using a conventional 254-nm fixed-wavelength ultraviolet detector. However, at this setting, several endogenous contaminants were detected that coeluted with bromocriptine and ergocriptine. Bromocriptine displays ultraviolet maxima at 230 and 310 nm, whereas the interfering compounds do not absorb at 310 nm Therefore, all HPLC analyses were carried out at 310 nm using a variable-wavelength detector. Ergocriptine was chosen as the internal standard due to its structural similarity to bromocriptine and because it is not an endogenous metabolite of bromocriptine in rats.

Radioimmunoassay has previously been used to measure bromocriptine levels [9–11]. Although the minimum detectable concentration in plasma of bromocriptine is low utilizing a radioimmunoassay method [16] (25 pg *versus* 9.75 ng using this HPLC assay), the specifity of the radioimmunoassay technique is compromised due to cross-reactivity with metabolites. An earlier HPLC method [15] reported a sensitivity similar to that of the current assay, but the extraction procedure was not applicable to rat brain.

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