Evaluation of the Blood-Brain Barrier Transport, Population Pharmacokinetics, and Brain Distribution of Benztropine Analogs and Cocaine Using in Vitro and in Vivo Techniques

SANGEETA RAJE, JIANJING CAO, AMY HAUCK NEWMAN, HUANLING GAO, and NATALIE D. EDDINGTON

Pharmacokinetics Biopharmaceutics Laboratory, Department of Pharmaceutical Sciences, School of Pharmacy, University of Maryland, Baltimore, Maryland (S.R., H.G., N.D.E.); and Medicinal Chemistry Section, National Institute on Drug Abuse-Intramural Research Program, National Institutes of Health, Baltimore, Maryland (J.C., A.H.N.)

Received April 24, 2003; accepted June 11, 2003

ABSTRACT

The N-substituted 3α-[bis(4′-fluorophenyl)methoxy]tropanes (AHN 2-003, AHN 1-055, AHN 2-005, and JHW 007) bind with high affinity to the dopamine transporter and inhibit dopamine uptake more potently than cocaine, but they demonstrate behavioral profiles in animal models of psychostimulant abuse that are unlike that of cocaine. The objective of this study was to characterize the in vitro permeability, brain distribution, and pharmacokinetics of the benztropine (BZT) analogs. Transport studies of cocaine and the BZT analogs (10⁻⁴ M) were conducted across bovine brain microvessel endothelial cells. Male Sprague-Dawley rats (300 g) were administered BZT analogs (10 mg/kg) or cocaine (5 mg/kg) via the tail vein. Blood and brain samples were collected over 36 h and assayed using UV-high-performance liquid chromatography. Transport of both AHN 1-055 (2.15 × 10⁻⁴ cm/s) and JHW 007 (2.83 × 10⁻⁴ cm/s) was higher (p < 0.05) than that of cocaine (1.63 × 10⁻⁴ cm/s). The volume of distribution (12.3–30.5 l/kg) of the analogs was significantly higher than cocaine (0.9 l/kg). The BZT analogs displayed a ≥8-fold higher elimination half-life (4.12–16.49 h) compared with cocaine (0.49 h). The brain-to-plasma partition coefficients were at least two-fold higher for the BZTs versus cocaine, except for AHN 2-003. The BZT analogs are highly permeable across the blood-brain barrier and possess a pharmacokinetic profile different from that of cocaine. These characteristics, in addition to their distinctive behavioral profiles, suggest that the BZT analogs may be promising candidates for the treatment of cocaine abuse.

Cocaine exerts its psychomotor stimulant and reinforcing effects primarily through the blockade of the dopamine transporter (DAT) (Ritz et al., 1987; Carroll et al., 1992; Self and Nestler, 1995; Wise, 1996). The blockade of the DAT by cocaine yields synaptic accumulation of dopamine due to a reduction in the reuptake of dopamine. Significant synthetic efforts for the development of effective therapeutic agents to treat cocaine abuse have focused on chemical entities that are unlike that of cocaine. The objective of this study was to characterize the in vitro permeability, brain distribution, and pharmacokinetics of the benztropine (BZT) analogs. Transport studies of cocaine and the BZT analogs (10⁻⁴ M) were conducted across bovine brain microvessel endothelial cells. Male Sprague-Dawley rats (300 g) were administered BZT analogs (10 mg/kg) or cocaine (5 mg/kg) via the tail vein. Blood and brain samples were collected over 36 h and assayed using UV-high-performance liquid chromatography. Transport of both AHN 1-055 (2.15 × 10⁻⁴ cm/s) and JHW 007 (2.83 × 10⁻⁴ cm/s) was higher (p < 0.05) than that of cocaine (1.63 × 10⁻⁴ cm/s). The volume of distribution (12.3–30.5 l/kg) of the analogs was significantly higher than cocaine (0.9 l/kg). The BZT analogs displayed a ≥8-fold higher elimination half-life (4.12–16.49 h) compared with cocaine (0.49 h). The brain-to-plasma partition coefficients were at least two-fold higher for the BZTs versus cocaine, except for AHN 2-003. The BZT analogs are highly permeable across the blood-brain barrier and possess a pharmacokinetic profile different from that of cocaine. These characteristics, in addition to their distinctive behavioral profiles, suggest that the BZT analogs may be promising candidates for the treatment of cocaine abuse.

onset of action, long duration of action, and behavioral effects that may be distinctive from those of cocaine (Rothman and Glowa, 1995; Newman and Kulkarni, 2002). A slow onset of effect, characterized by a gradual increase in dopamine synaptic levels may not produce the profound psychomotor stimulant and mood-enhancing effects associated with cocaine. Furthermore, a long duration of action characterized by sustained and elevated dopamine levels would thus reduce the repeated administrations associated with cocaine abuse. Numerous therapeutic agents have been developed based on the substitute therapy strategy, including, GBR 12909 and RTI 112, dopamine uptake inhibitors currently in clinical trials (Sogaard et al., 1990; Rothman and Glowa, 1995; Carroll et al., 1999; Newman, 2000). However, these agents demonstrate cocaine-like actions in animal models and thus may have abuse liability themselves or lack effect in treating cocaine abuse or maintenance of abstinence (Tutton and Crayton, 1993).

This research was funded in part by National Institutes of Health/National Institute on Drug Abuse-Intramural Research Program and National Cancer Institute Grant CA87654-02.

Article, publication date, and citation information can be found at http://jpet.aspetjournals.org.

DOI: 10.1124/jpet.103.053504.

ABBREVIATIONS: DAT, dopamine transporter; BZT, benztropine; BBB, blood-brain barrier; BBMEC, bovine brain microvessel endothelial cell; DMEM, Dulbecco’s modified Eagle’s medium; PBS, phosphate-buffered saline; MEM, minimal essential medium; HPLC, high-performance liquid chromatography; A → B, apical-to-basolateral; B → A, basolateral-to-apical; P-gp, P-glycoprotein; P appellant permeability coefficient; AUC, area under the curve.
A series of the N-substituted 3α-[bis(4'-fluorophenyl) methoxy]tropanes, or benztprine (BZT) analogs, have been developed as potential substitute therapeutic agents for cocaine abuse (Newman et al., 1994, 1995, 2001; Agoston et al., 1997; Robarge et al., 2000). The BZT analogs bind with higher affinity to the DAT (K_i = 11–30 nM versus K_i cocaine = 187 nM) and inhibit dopamine uptake with a higher potency, in comparison with cocaine (Table 1) (Agoston et al., 1997; Katz et al., 2001). These analogs are not efficacious locomotor stimulants in mice and also do not fully substitute for cocaine in drug discrimination studies (Katz et al., 1999). Hence, these analogs may have potential as substitute therapeutic agents for the treatment of cocaine abuse. As such, it is necessary for them to reach the brain in sufficient concentrations to produce this desired effect in vivo.

The BZT analogs are highly lipophilic (cLogP_BZT = 3.1–5.5) compared with cocaine (cLogP_cocaine = 2.72), a physiochemical property known to promote blood-brain barrier (BBB) transport. However, the BBB transport characteristics of the BZT analogs have not been characterized. Furthermore, it should be noted that the presence of efflux proteins such as P-glycoprotein may limit entry of these agents into the brain (Ambudkar et al., 1999). As stated, another important characteristic of a potential “substitute therapeutic” agent is a slow onset and long duration of action (Gorelick, 1998). As such, the ideal pharmacokinetic properties of a substitute agent would be slow input especially into the brain as well as a slow rate of elimination out of the body. This pharmacokinetic profile would most likely allow for a gradual increase in brain dopamine levels resulting in slow inhibition of dopamine reuptake along with a sustained elevation of dopamine. Cocaine is rapidly absorbed and metabolized after administration (Wilkinson et al., 1980; Mets et al., 1999). Thus, a pharmacokinetic profile different from cocaine might also be a determining factor in predicting the therapeutic efficacy of a potential substitute agent.

Based on the above-mentioned observations, investigations of the BBB permeability, brain distribution, and pharmacokinetics of the BZT analogs are warranted to determine whether they possess the requisite dispositional characteristics identified as ideal for substitute therapeutic agents. To investigate this, the following two objectives were pursued: 1) determine the in vitro permeability of BZT analogs across bovine brain microvessel endothelial cell (BBMEC) monolayer and 2) determine the brain distribution and pharmacokinetics of BZT analogs after intravenous administration to male Sprague-Dawley rats using destructive sampling techniques. The physiochemical and pharmacological properties of the BZT analogs evaluated in this study are presented in Table 1 and Fig. 1.

### Materials and Methods

#### Materials

Cell Culture medium components included Dulbecco’s modified Eagle’s medium (DMEM/F-12), phosphate-buffered saline (PBS), gentamycin (100 μg/mL), horse serum (from platelet-poor plasma), polyethylene glycol (PEG, 1 000,000 molecular weight), and heparin (50 μg/mL). All chemicals and solvents were American Chemical Society analytical grade or high-performance liquid chromatography (HPLC) grade.

#### Bovine Brain Microvessel Endothelial Cells Isolation and Seeding

BBMECs were isolated manually from the gray matter of cerebral cortices as described previously (Audus and Borchardt, 1986, Cox et al., 2001). Primary bovine microvessel fragments were seeded onto 12-well Costar inserts (Transwell; Costar, Cambridge, MA), 1 cm² in diameter, consisting of a 0.4-μm pore polycarbonate filter. Filter membranes (apical surface) of the inserts were treated with 0.5 ml of rat tail collagen type 1, excess was aspirated off and the filters were allowed to dry. Fibronectin (0.5 ml) was then added to each insert, remixed shortly thereafter (40 min), and allowed to dry. BBMECs were then seeded onto the collagen/fibronectin-coated membranes at a density of approximately 70,000 to 80,000 cells/cm² after suspending the cells in plating medium consisting of DMEM/F-12 (1×) and gentamycin (50 μg/mL), supplemented with horse serum, polyethylene glycol (PEG, 1 000,000 molecular weight), and heparin (50 μg/mL). The upper (apical, A) compartment received 0.5 ml of plating medium, and the lower (basolateral, B) compartment received 1.5 ml for the first 3 days of growth. On the 3rd day after plating, the changing medium, which consisted of DMEM/F-12 (1×), gentamycin (50 μg/mL), horse serum, heparin, and amphotericin (50 μg/mL), was added to the cells. The changing medium was added every other day. Cells were placed in an incubator maintained at 37°C, with 5% CO₂ and 95% humidity until confluence was reached within 10 to 14 days. Integrity of the BBMEC monolayer was assessed by determination of flux of the radiolabeled markers [14C]sucrose (paracellular) and [3H]propranolol (transcellular) as well as transepithelial electrical resistance values (100–150 ohms/cm²) for each 12-well Costar plate before the transport study.

#### Benztprine Analogos and Cocaine Transport Studies

Transport experiments were performed in triplicate in both the apical-to-basolateral (A→B) as well as basolateral-to-apical (B→A) pathways of the BBMEC monolayer.

### Table 1

<table>
<thead>
<tr>
<th>Compound</th>
<th>R</th>
<th>Mol. Wt.</th>
<th>cLogP</th>
<th>[3H]WINBinding (K_i)</th>
<th>Dopamine Uptake (IC₅₀)</th>
</tr>
</thead>
<tbody>
<tr>
<td>AHN 2-003</td>
<td>H</td>
<td>365.85</td>
<td>3.10</td>
<td>11</td>
<td>9.7</td>
</tr>
<tr>
<td>AHN 1-055</td>
<td>CH₉</td>
<td>393.16</td>
<td>3.95</td>
<td>12</td>
<td>71</td>
</tr>
<tr>
<td>AHN 2-005</td>
<td>CH₉CH=CH₂</td>
<td>405.92</td>
<td>4.52</td>
<td>30</td>
<td>14</td>
</tr>
<tr>
<td>JHW 007</td>
<td>(CH₉)₂CH₃</td>
<td>421.96</td>
<td>5.53</td>
<td>25</td>
<td>370</td>
</tr>
<tr>
<td>Cocaine</td>
<td></td>
<td>303.31</td>
<td>2.72</td>
<td>300–400</td>
<td>150–200</td>
</tr>
</tbody>
</table>

* Calculated log of the partition coefficient (cLogP).
* Affinity for the DAT by determination of the displacement of [3H]WIN 35,428, an analog of cocaine.
* Inhibition of [3H]DA uptake.
directions. BZT analogs and cocaine solutions were prepared in PBS at a concentration of $1 \times 10^{-4}$ M. On confluence of BBMECs, complete culture medium was removed from both the A and B sides, and both were phenolized with sterile PBS. The inserts were then placed into tissue culture plates (Costar) containing 1.5 ml of prewarmed PBS. All the transport studies were performed in triplicate, and BBMECs were continuously agitated during the experiments on a shaker plate (100 rpm) at 37°C. For the A→B transport study, 0.4 ml of BZT analogs/cocaine/radiolabeled marker was added to the apical side at time $t = 0$. After 5 min, the insert was removed and transferred to a new transwell containing fresh PBS. This procedure was repeated up to 120 min, with samples being collected from the basolateral compartment at 5, 10, 15, 20, 30, 45, 60, 75, 90, and 120 min. For the B→A transport study, 1.5 ml of the test agent was placed on the basolateral side at time $t = 0$. The apical side contained 0.4 ml of fresh PBS. After 5 min, the PBS sample from the apical side was removed and replaced with an equal volume of fresh PBS. Samples were collected at similar time points and the samples were stored at −70°C until HPLC analysis.

**Benztropine Analogs and Cocaine Inhibition Studies.** To evaluate the possible interaction of BZT analogs with the efflux protein P-gp, inhibition studies with a known P-gp inhibitor, PSC833, were carried out. PSC833, a nonimmunosuppressive analog of cyclosporin A, has been shown to be a potent and specific inhibitor of P-gp (Boesch et al., 1991). Rhodamine 123, a known P-gp substrate, was used to evaluate the functional activity of the efflux protein in BBMECs (Fontaine et al., 1996). Bidirectional transport of rhodamine 123 (3.2 μM) was determined across the BBMEC monolayer, and the efflux ratio was determined as a measure of P-gp activity. Rhodamine levels in the transport study samples were determined by HPLC. For inhibition studies with BZT analogs, medium was removed and inserts were washed with PBS. Cells were then preincubated with 0.5 μM PSC833 on the apical side for 30 min (Smith et al., 1998), after which PSC833 was removed, cells were washed, and PSC833 was added again along with the BZT analog/cocaine solution (1 × 10^{-4} M) at $t = 0$. Apical-to-basolateral transport studies were carried out in the manner stated above, and samples were collected at various time points up to 120 min. Samples were stored at −70°C until HPLC analysis.

**Animal Pharmacokinetic Studies.** Adult male Sprague-Dawley rats (250–275 g) were used in this study and were purchased from Hilltop Animal Laboratories (Scottdale, PA). The protocol was approved by the Institutional Animal Care and Use Committee of the School of Pharmacy (University of Maryland). All the animals were approved by the Institutional Animal Care and Use Committee of the National Institutes of Health specifications. Animals were housed in the animal facility at a room temperature of 72°F, and a dose of 10 mg/kg free base was administered i.v. (via tail vein). The chromatographic conditions consisted of a Supelcosil LC-ABZ Plus column (250 × 4.6 mm, 5 μm), UV detector ($\lambda = 220$ nm), gradient mobile phases [methanol/0.05 M Na2HPO4 (pH 3.0), 40:60 (v/v)] (A) and methanol/0.05 M Na2HPO4 (pH 3.0), 80:20 (v/v) (B), and a flow rate of 1 ml/min pumped over a 15-min gradient profile. The internal standard was oxepenolol. Without any interference from endogenous peaks, the calibration curves were linear in the range of 100 to 50,000 ng/ml, 25 to 10,000 ng/ml, and 50 to 10,000 ng/ml ($r^2 > 0.999$) for cell culture, plasma, and brain, respectively.

**Cocaine HPLC Analysis.** A slight modification of a previously described HPLC method was used to quantitate cocaine in plasma and brain tissue samples (Pan and Hedaya, 1997). Reverse phase chromatography with ultraviolet detection ($\lambda = 235$ nm) was used to quantitate the eluate. The mobile phase consisted of 0.05 M monobasic ammonium phosphate, methanol, and acetonitrile [76:12:12 (v/v)] pumped at a flow rate of 1.0 ml/min. Oxprenolol served as the internal standard. Brain tissue was homogenized and diluted with an equal volume of phosphate-buffered saline. To the brain homogenate mixture 100 μl of triethylamine was added after which the samples were vortexed briefly for 20 to 30 s. For plasma samples, 6 ml of hexane was added to 0.6 ml of plasma, and samples were vortexed (30–45 s) and centrifuged at 3000 rpm for 5 min. The supernatant was evaporated to dryness at 40°C under a gentle stream of nitrogen. The resulting residue was reconstituted with 210 μl of mobile phase (0.05 M monobasic ammonium phosphate/methanol/acetonitrile [76:12:12 (v/v)], gradient form A to B in 10 min), and 210 μl was injected onto the HPLC system. The calibration curves were found to be linear in the range of 10 to 5000 ng/ml for plasma and 25 to 1000 ng/g for brain ($r^2 > 0.999$). The intra- and interday variability and error were <10%.

**Rhodamine 123 HPLC Analysis.** Rhodamine transport study samples were analyzed by an HPLC system consisting of a Waters 2690 liquid chromatograph supplied with a LC 20 fluorescence detector (PerkinElmer Life Sciences, Boston, MA) set at an excitation and emission wavelength of 498 and 525 nm, respectively. The chromatographic data were recorded using a model 3390A integrator (Hewlett Packard, Rockville, MD). Separation was achieved at ambient temperature using a reverse phase C8 column (150 × 4.6 mm, 5 μm; Phenomenex, IL) fitted with a 5 μm LC-8, 2-cm guard column (Phenomenex). The mobile phase consisted of a mixture of 0.025 M Na2HPO4 and methanol [50:50 (v/v)] pumped at a flow rate of 0.5 ml/min, after filtration and degassing. The chromatographic run time was 15 min, and 150 μl of the sample was injected onto the system. The calibration curve was found to be linear in the range of 62.5 to 5000 ng/ml ($r^2 > 0.999$).

**Permeability Data Analysis.** Apparent permeability coefficients (Papp) alone or in the presence of inhibitor were calculated for each compound after triplicate experiments (n = 3). Permeability coefficients were determined at sink conditions from the following equation:

\[
\text{Papp} = \frac{V}{A t} \left( \frac{C_{in}}{C_{out}} - 1 \right)
\]
where $P_{\text{app}}$ is the apparent permeability coefficient in centimeters per second; $\frac{dC}{dt}$ is the flux of drug across the monolayer, calculated as the linearly regressed slope through linear data; $V_r$ is the volume in the receiver compartment; $SA$ is the diffusion area (1 cm$^2$); and $C_0$ is the initial BZT analog or cocaine concentration in the donor chamber at $t = 0$. All values are represented as mean and standard deviation of the triplicate experiments performed under identical conditions and from the same preparation of cells.

Efflux ratios ($Re$) were calculated according to the following equation:

$$Re = \frac{P_{\text{app}}(B \rightarrow A)}{P_{\text{app}}(A \rightarrow B)}$$  \hspace{1cm} (2)

Pharmacokinetic Analysis of Destructive Sampling Data. Data obtained after the administration of the BZT analogs and cocaine to rats were initially analyzed by the naive pooled data method. Plasma concentration versus time data from a given compound were pooled and analyzed according to nonlinear least-squares. Compartment modeling was used to estimate various pharmacokinetic parameters ($V_d$, $k_e$, $AUC_{\text{inf}}$ (0 to infinity) $t_{1/2}$, and clearance) using WinNonlin version 3.1 (Pharsight, Cary, NC). Both one- and two-compartment analysis were evaluated to determine the best model fit. Various weighting schemes evaluated included a weight of 1, $1/y$, ($y$ is the drug concentration), $1/y^2$, 1/predicted concentration (iterative reweighting) and 1/predicted concentration$^2$.

Goodness of fit was based on visual inspection, final residual sum of squares, weighted residual sum of squares, random distribution of residuals, Akaike's information criteria, and Swartz criteria.

To evaluate interanimal variability and to statistically compare the pharmacokinetic parameters of the BZT analogs and cocaine, population pharmacokinetic analysis was performed with WinNonlin version 2.01 (Pharsight) (Cox et al., 2002). A two-stage analysis (naive pooled data method) with the individual pharmacokinetic data was performed initially, and it was determined that a two-compartment model best described the disposition of both the BZT analogs and cocaine after a single i.v. bolus dose. Each structural parameter for the two-compartment model, i.e., $V_1$, $K_{10}$, $K_{12}$, and $K_{21}$ was expressed as a function of the fixed effects as well as random effects, i.e., interanimal variability $\sigma$ using additive, proportional, and exponential relationships. The effect of the weight of each animal as the covariate was also assessed. The residual error ($e$) was assumed to be identically and independently distributed with a mean of 0 and a variance of $\sigma^2$. The residual error was modeled using additive, proportional, or exponential models, “goodness of fit” was based on WSSR, %CV for parameters, Akaike’s information criteria, Schwarz criteria, and plots of observed and model-predicted concentration versus time, time versus residual concentration, and observed versus model-predicted concentrations. Local minima in the sum of squares surface were avoided by changing the values of the initial estimates and repeating the iterative process to achieve convergence.

Brain Distribution Analysis. Total brain concentrations of BZT analogs were determined and partition coefficients ($R_i$) were calculated based on the ratio of drug in brain to drug in plasma. The $AUC_{\text{inf}}$ from time 0 to infinity were used to calculate $R_i$, according to the following formula:

$$R_i = \frac{AUC_{\text{inf,brain}}}{AUC_{\text{inf,plasma}}}$$  \hspace{1cm} (3)

The $AUC_{\text{inf}}$ values for both plasma and brain tissue were determined by noncompartmental methods (WinNonlin) using the linear trapezoidal rule.

Statistical Analysis. Permeability coefficients obtained from the transport studies, and the pharmacokinetic parameters were compared by one-way analysis of variance followed by Tukey's post hoc analysis. Statistical significance was set at $p < 0.05$.

Results

Benztropine Analogs and Cocaine Transport across BBMECs. Permeability studies were conducted to evaluate the bidirectional transport of BZT analogs across the BBMEC monolayer. The flux of the BZT analogs as well as cocaine was linear as a function of time. $P_{\text{app}}$ values of BZT analogs ranged from $1.54$ to $2.83 \times 10^{-4}$ cm/s in comparison with a $P_{\text{app}}$ value of $6.13 \times 10^{-4}$ cm/s for cocaine (Table 2). The $P_{\text{app}}$ values for the BZT analogs ranked in the following order: AHN 2-003 < AHN 2-005 < cocaine < AHN 1-055 < JHW 007. The BZT analogs showed very high permeability in the A → B direction. The A → B transport of AHN 1-055 and JHW 007 was significantly higher than that of cocaine ($p < 0.05$). There was no significant difference between the A → B $P_{\text{app}}$ for AHN 2-003 and AHN 2-005 versus cocaine.

The BZT analogs as well as cocaine were significantly effused across BBMEC monolayers with $P_{\text{app}}$ values in the B → A direction being significantly higher than $P_{\text{app}}$ values in the A → B direction ($p < 0.05$) and B → A/A → B ratios > 4. The B → A $P_{\text{app}}$ values ranged from $8.51$ to $22.8 \times 10^{-4}$ cm/s in comparison with a $P_{\text{app}}$ value of $6.88 \times 10^{-4}$ cm/s for cocaine with the rank order: cocaine < AHN 2-003 < AHN 2-005 < AHN 1-055 < JHW 007. Except for JHW 007, there was no significant difference between the B → A $P_{\text{app}}$ for cocaine and the other BZT analogs. The B → A/A → B ratio was smallest for cocaine (4.2) and highest for JHW 007 (8.0).

Transport of rhodamine 123 was evaluated to confirm the functional activity of P-gp in the cultured BBMEC monolayers. The B → A/A → B ratio for rhodamine 123 was ~4, a value that is in accordance with literature (Lee et al., 1994).

### Table 2

Bidirectional $P_{\text{app}}$ of BZT analogs and cocaine (1 × 10^{-4} M) and $P_{\text{app}}$ of BZT analogs with PSC833 across BBMEC monolayers

| Compound | A → B | B → A | B → A/A → B | A → B with PSC833
<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Cocaine</td>
<td>1.63 ± 0.05</td>
<td>6.88 ± 0.20</td>
<td>4.2</td>
<td>2.12 ± 0.07</td>
</tr>
<tr>
<td>AHN 2-003</td>
<td>1.54 ± 0.10</td>
<td>8.51 ± 0.90</td>
<td>5.5</td>
<td>1.84 ± 0.13</td>
</tr>
<tr>
<td>AHN 1-055</td>
<td>2.15 ± 0.10*</td>
<td>11.5 ± 1.40</td>
<td>5.3</td>
<td>3.10 ± 0.10</td>
</tr>
<tr>
<td>AHN 2-005</td>
<td>1.56 ± 0.03</td>
<td>9.88 ± 1.08</td>
<td>6.2</td>
<td>2.91 ± 0.15</td>
</tr>
<tr>
<td>JHW 007</td>
<td>2.83 ± 0.10*</td>
<td>22.8 ± 2.00**</td>
<td>8.0</td>
<td>3.87 ± 0.02</td>
</tr>
</tbody>
</table>

* Significant difference ($p < 0.05$) for A → B.
* Significant difference ($p < 0.05$) for B → A versus A → B for all compounds.
* Significant difference ($p < 0.05$) for A → B versus A → B with PSC833.

$$\text{P}_{\text{app}} = \frac{(dC/dt) \cdot V_r}{SA \cdot C_0}$$  \hspace{1cm} (1)

** Significant difference ($p < 0.05$) for A → B.
To evaluate involvement of P-gp, cells were preincubated with PSC833 (0.5 μM). As seen in Table 2, A → B transport of all compounds, including cocaine, increased in the presence of PSC833 (p < 0.05). The A → B P_app values in the presence of PSC833 ranged from 1.84 to 3.87 × 10⁻⁸ cm/s in comparison with a P_app value of 2.12 × 10⁻⁸ cm/s for cocaine (Table 2).

**Benztropine Analogs and Cocaine Pharmacokinetics.** Figure 2A presents the mean plasma concentration versus time profile for the BZT analogs and cocaine after single i.v. bolus dosing in Sprague-Dawley rats. Pharmacokinetics of the BZT analogs seemed to follow a biexponential decay, with a two-compartmental model providing the best fit. Figure 2B shows the WinNonlin-predicted and observed concentration versus time fits for the BZT analogs (best fits shown). The parameter estimates obtained from WinNonlin along with one concentration data point from each animal were used to obtain individual concentration versus time profiles using the mixed effects population modeling program WinNonmix. A two-compartment model provided the best fit for each animal. The interanimal variability was best described by an exponential model and the residual error was described by an additive model.

**Benztropine Analogs and Cocaine Comparative Pharmacokinetics.** The primary goal in using population analysis was to statistically compare the pharmacokinetic parameters obtained after the administration of the BZT analogs and cocaine given the destructive sampling study design. Table 3 summarizes the population pharmacokinetic parameters for the BZT analogs and cocaine after single dose i.v. administration. Dose-normalized AUC_∞ for cocaine (5932 mg/l h) was not significantly different from the AUC_∞ in the presence of PSC833 (p < 0.05). The A → B P_app values in the presence of PSC833 ranged from 1.84 to 3.87 × 10⁻⁸ cm/s in comparison with a P_app value of 2.12 × 10⁻⁸ cm/s for cocaine (Table 2).

The steady-state volume of distribution (Vdss) of the BZTs was ≥12-fold higher compared with that of cocaine (p < 0.05), with values ranging from 12.3 to 30.5 l/kg, in comparison with a value of 0.9 l/kg for cocaine. AHN 2-003 had the highest Vdss which is about 30 times higher than that of cocaine. In general, the BZTs are more lipid-soluble (cLogP 3.10–5.53) compared with cocaine (cLogP 2.72) (Table 1). The significantly lower Vdss of cocaine versus the BZT analogs may be explained by the differences in lipophilicities of the compounds.

The BZT analogs displayed a ≥8 fold longer elimination t½-β with the half-life of cocaine being significantly shorter than the BZT analogs (p < 0.05). The elimination half-lives ranged from 4.12 to 16.49 h for the BZT analogs compared with a t½-β of 0.49 h for cocaine and ranked in the following order: AHN 2-005 < JHW 007 < AHN 1-055 < AHN 2-003. There was no significant difference between the half-lives of AHN 2-005 (4.12 h) and JHW 007 (5.35 h). Clearance for all the compounds in this series was significantly slower than that of cocaine (p < 0.05), except for JHW 007, which was found to have a clearance value of 3.29 l/h/kg, compared with 3.05 l/h/kg for cocaine.

**Brain Distribution of Benztropine Analogs and Cocaine.** Figure 3 illustrates the brain concentration versus time profiles for the BZT analogs after naive pooled averaging of the data, whereas the Ri values and brain t½-β values are specified in Table 3. In general, higher concentrations of BZTs as well as cocaine were seen in the brain tissue compared with plasma. Peak brain levels for all compounds were seen at the first sampling time after i.v. administration, indicating that there is no lag time for the entry of these compounds to their effect site. Except for AHN 2-003, the BZT analogs showed a ≥2-fold higher brain-to-plasma ratio compared with cocaine. Maximum brain-to-plasma ratios were seen after 1 h for JHW 007 and AHN 2-005, 2 h for AHN 1-055 and 4 h for AHN 2-003, compared with 15 min for cocaine. The ratio for AHN 2-003, the least lipophilic of the series, was similar to that for cocaine (R_i = 2.1 for AHN 2-003 versus 2.0 for cocaine). AHN 2-005 was found to have the highest brain uptake (R_i = 6.6), followed by JHW 007 (R_i = 5.6), and AHN 1-055 (R_i = 4.8). Brain half-lives for all the compounds were comparable with that of plasma half-lives (Table 3).

![Chemical structures of 3α-[bis(4′-fluorophenyl)methoxy]tropane BZT analogs (A) and cocaine (B); structural substituents (R-) presented in Table 1.](image-url)
The BZT analogs may be considered as potential substitute therapeutic agents and display significantly higher binding affinity to the DAT compared with cocaine (Agoston et al., 1997). Despite potent inhibition of dopamine uptake, in vitro, these agents have not been found to display significant cocaine-like behavioral effects in animal models of cocaine abuse (Agoston et al., 1997, Katz et al., 1999). It should be noted that the in vitro binding affinities of these compounds may not necessarily reflect their abilities to inhibit dopamine uptake, in vivo, because the BBB penetrability of these compounds, after dosing is of critical importance. As such, studies were performed to evaluate the BBB permeability, brain distribution, and pharmacokinetics of the BZT analogs and cocaine.

The low molecular weight (365–422 Da) and the high lipophilicity (cLogP >3.0) of the BZT analogs would suggest passive permeability across the BBB. As expected, the transport was very high for the analogs corresponding to permeability of other central nervous system agents that display good uptake into the brain (P_app, of $0.5 \times 10^{-4}$–$2 \times 10^{-4}$ cm/s) (Pagliara et al., 1999). Both AHN 1-055 and JHW 007 had significantly higher $A \rightarrow B$ transport in comparison with cocaine. This may be due to the fact that both AHN 1-055 and JHW 007 are much more lipophilic than cocaine. Although more lipophilic than AHN 1-055, there was no significant difference between permeability of AHN 2-005 and cocaine. However, differences in the N-substituents (R group; Table 1; Fig. 3) may alter their permeability across the BBB.

### Table 3
Population pharmacokinetic parameters (mean ± S.D.) for the BZT analogs and cocaine after intravenous administration to male Sprague-Dawley rats

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Cocaine</th>
<th>AHN 2-003</th>
<th>AHN 1-055</th>
<th>AHN 2-005</th>
<th>JHW 007</th>
</tr>
</thead>
<tbody>
<tr>
<td>AUC (ng/h/l)</td>
<td>593 ± 444</td>
<td>30,304 ± 8477</td>
<td>21,397 ± 6643</td>
<td>14,592 ± 3324</td>
<td>11,132 ± 1449</td>
</tr>
<tr>
<td>$C_{\text{max}}$ (ng/ml)</td>
<td>64,283 ± 2163</td>
<td>1239 ± 349</td>
<td>1477 ± 162</td>
<td>3389 ± 725</td>
<td>984 ± 95</td>
</tr>
<tr>
<td>$V_s$ (l/kg)</td>
<td>0.9 ± 0.1</td>
<td>30.5 ± 11.9*</td>
<td>18.7 ± 2.3*</td>
<td>12.3 ± 3.2*</td>
<td>23.2 ± 3.2*</td>
</tr>
<tr>
<td>Clearance (l/h/kg)</td>
<td>3.1 ± 0.2</td>
<td>1.3 ± 0.5*</td>
<td>1.8 ± 0.5*</td>
<td>2.6 ± 0.6*</td>
<td>3.2 ± 0.4</td>
</tr>
<tr>
<td>$t_{1/2-\alpha}$ (h)</td>
<td>0.01 ± 0.0003</td>
<td>0.17 ± 0.001*</td>
<td>0.09 ± 0.003*</td>
<td>0.21 ± 0.005*</td>
<td>0.31 ± 0.004*</td>
</tr>
<tr>
<td>$t_{1/2-\beta}$ (h)</td>
<td>0.49 ± 0.06</td>
<td>16.49 ± 0.11*</td>
<td>7.69 ± 1.76*</td>
<td>4.12 ± 0.33*</td>
<td>5.35 ± 0.48*</td>
</tr>
<tr>
<td>$AUC_{\text{m}}/AUC_{\text{plasma}}$</td>
<td>2.1</td>
<td>2.0</td>
<td>4.8</td>
<td>6.6</td>
<td>5.6</td>
</tr>
<tr>
<td>$\text{Brain}-t_{1/2}$ (h)</td>
<td>0.6</td>
<td>12.5</td>
<td>6.2</td>
<td>5.8</td>
<td>8.5</td>
</tr>
</tbody>
</table>

* Significant difference ($p < 0.05$).
theless, the high permeability observed for the BZT analogs indicates that they should effectively bind to DAT and inhibit dopamine uptake because the BBB does not minimize their transport.

Our in vitro studies suggest that the transport of the BZT analogs and cocaine may involve a multidrug resistance protein system that effluxes the agents out of the BBB (Ambul- kar et al., 1999). The transport of BZT analogs and cocaine increased in the presence of the P-gp inhibitor PSC833, indicating the possibility that these compounds may be substrates of an efflux membrane transporter. Nonetheless, their high passive permeability and high brain-to-plasma ratios (2.1–6.6) would suggest that the efflux system does not effectively minimize brain uptake. Furthermore, it should be noted that the BZT binding affinities (Table 1) are at least 14-fold higher than cocaine. Although the efflux of BZT analogs and cocaine seems to be similar, they should more effectively block dopamine uptake based on their higher binding affinity.

According to the pharmacokinetic or rate theory, it would be of therapeutic importance for an ideal substitute therapeutic to possess a pharmacokinetic profile different from cocaine (Gorelick, 1998). It is clear that the high abuse potential of cocaine is in part due to its rapid onset and short duration of action, which reflect its pharmacokinetic and pharmacodynamic properties (Quinn et al., 1997). Positron emission tomography studies have shown that the rate at which cocaine enters the brain and blocks the DAT is the variable associated with the “high” rather than the presence of the drug in the brain (Volkow et al., 1997, 1999). Thus, a substitute therapeutic agent should provide a slower onset of action to decrease the rapid mood-enhancing effects associated with cocaine. Another critical property required for a substitute therapeutic agent is a longer residence time and slower elimination to minimize repeated cocaine administration. Together, the potential substitute therapeutic should possess a slow input into the central nervous system as well as a slow clearance in comparison with cocaine.

The BZT analogs entered the brain rapidly with peak levels occurring at the first sampling time point. Maximum brain levels were observed within 2 min for cocaine. Maximum brain-to-plasma ratios were observed after 1 h for JHW 007 and AHN 2-005, 2 h for AHN 1-055, and 4 h for AHN 2-003, in comparison with 15 min for cocaine. Interestingly, in drug discrimination studies wherein rats are trained to discriminate 10 mg/kg cocaine from saline, cocaine dose dependently and fully substitutes for the cocaine cue, with a pretreatment time of 5 min (Katz et al., 1999). Conversely, at a pretreatment time of 5 min, AHN 1-055 does not produce cocaine-like discriminative stimulus effects, even at the highest doses tested. However, at a pretreatment time of 90 min., AHN 1-055 fully generalizes to the cocaine discriminative stimulus (Katz et al., 1999). Thus, although, AHN 1-055 seems to penetrate the BBB rapidly, perhaps the achievement of higher brain-to-plasma ratios is required to result in in vivo activity.

Dose-normalized AUCbrain was significantly higher for the BZT analogs compared with cocaine. In addition, AUCbrain/AUCplasma ratio for cocaine was ~2.0, a value in accordance with previously reported values (Pan and Hedaya, 1998; Javaid and Davis 1993). AHN 2-003, was found to have the smallest AUC ratio of 2.1 in comparison with 2.0 for cocaine, although this compound had the highest Vdss. This points to the possibility of AHN 2-003 being extensively distributed to tissues other than the brain. The in vivo brain uptake results are in agreement with our in vitro permeability studies. The transport of AHN 2-003 across BBMECs was not significantly different from that of cocaine. Alternatively, AHN 2-005 was seen to have the highest brain uptake ratio (Ri = 6.6) compared with cocaine. Interestingly, it displayed the smallest Vdss (12.3 l/kg) of the analogs and had an in vitro permeability similar to cocaine. The brain uptake ratios for AHN 1-055 and JHW 007 were in accordance with their in vitro permeabilities; both compounds had significantly higher BBB permeability and brain uptake compared with cocaine. It is important to point out that although our in vitro studies suggested a very high efflux for JHW 007 across the BBMEC system, a very high brain uptake for this compound was observed in our in vivo studies.

The disposition of the BZT analogs are in accordance with the requirements of a substitute therapeutic agent. The elimination of BZT analogs was significantly slower compared with cocaine as can be seen from their long half-life (4.12–16.49 h) and slow clearance (1.3–3.2 l/h/kg). The clearance for all analogs, except JHW 007, was significantly slower than that for cocaine. Cocaine has two ester groups that are highly susceptible to hydrolysis by plasma esterases. The BZT analogs have a 3o-diphenyl methoxy group that is metabolically more stable that the ester groups of cocaine (He et al., 1995). As a result, the elimination t1/2β is longer for the BZT analogs than for cocaine. It should be noted that among the BZT analogs, clearance increased and elimination t1/2 decreased as the steric bulk of the N-substituent increased. AHN 2-003, the parent compound (NH), exhibited the lowest clearance (1.32 l/h/kg). The longer elimination half-life for the analogs is supported by in vivo studies with AHN 1-055 that showed long-acting locomotor stimulation, compared with cocaine (Katz et al., 1999).

In conclusion, the BZT analogs are highly permeable across the BBB but are also transported by an efflux system out of the cells. The net transport of the BZTs, although slightly lower, was not significantly different from cocaine. The BZT analogs possess a pharmacokinetic profile different from cocaine, with a significantly higher distribution, and most importantly a longer elimination half-life. Entry of these compounds into the brain was rapid, with a Tmax value of 5 min. Thus, a distinctive behavioral profile, as seen with the BZTs, may not be due to a slow entry into the brain but may rely on the achievement of high brain-to-blood ratios or some other yet to be determined pharmacodynamic factor. In light of the desirable pharmacokinetic properties for substitute therapeutic medications, the differences in distribution and elimination properties of the BZTs and cocaine identify the BZT analogs as promising candidates for treatment of cocaine abuse. Furthermore, because some of the BZTs do not seem to produce cocaine-like behaviors in animal models (Katz et al., 1999, 2001, Woolverton et al., 2000, 2001), their abuse liability may be limited and this further supports their continued development toward a cocaine abuse medication. Because the frequency of craving associated with cocaine abuse is influenced by the rapid clearance of this drug and subsequent rapid disappearance of the elevated dopamine levels, a slow clearance and much longer half-life as seen with these BZT analogs may be beneficial in reducing re-
peated drug administration associated with cocaine abuse. Studies are underway to evaluate the relationship between the pharmacokinetics of the BZT analogs and their pharmacodynamics (i.e., dopamine versus time profile) in relation to cocaine.

Acknowledgments

We thank Drs. Nigel Greig and Jonathan Katz for helpful discussions and review of this manuscript.

References


Address correspondence to: Dr. Natalie D. Eddington, Pharmacokinetics Biopharmaceutics Laboratory, Department of Pharmaceutical Sciences, School of Pharmacy, University of Maryland, 20 Penn St., HSF II, Baltimore, MD 21201. E-mail: neddingt@rx.umm.umd.edu