Pharmaceutical Research, Vol. 22, No. 4, April 2005 (© 2005)
10.1007/s11095-005-2488-8

Research Paper

Pharmacodynamic Assessment of the Benztropine Analogues AHN-1055 and AHN-2005 Using Intracerebral Microdialysis to Evaluate Brain Dopamine Levels and Pharmacokinetic/Pharmacodynamic Modeling

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Received May 7, 2004; accepted January 12, 2005

Purpose. The benztropine (BZT) analogues bind with high affinity to the dopamine transporter (DAT) and demonstrate a behavioral and pharmacokinetic profile unlike that of cocaine. The development of a predictive pharmacokinetic/pharmacodynamic (PK/PD) model to characterize the concentration-effect relationship between the BZT analogues and brain dopamine (DA) levels is an important step in the evaluation of these compounds as potential cocaine abuse pharmacotherapies. Hence, the objective of this study was to mathematically characterize the PD of BZT analogues and cocaine, using appropriate PK/PD models.

Methods. Dialysis probes were stereotaxically implanted into the nucleus accumbens of Sprague-Dawley rats (275–300 g). Extracellular fluid (ECF) DA levels were measured after intravenous administration of the BZT analogues AHN-1055 and AHN-2005, as well as cocaine using high performance liquid chromatography-electrochemical detection (HPLC-ECD). PD models were used to describe the relationship between the BZT analogues or cocaine and brain microdialysate DA, and suitability was based on standard goodness-of-fit criteria.

Results. The BZT analogues produced a sustained increase in brain microdialysate DA levels in comparison to cocaine. The time of maximum concentration (T_{max}) for brain microdialysate DA was 2 h for AHN-1055 and 1 h for AHN-2005 compared to a T_{max} of 10 min for cocaine. The duration of brain microdialysate DA elevation was ~12–24 h for the BZTs in comparison to 1 h for cocaine. An indirect model with inhibition of loss of response and a sigmoid E_{max} model best described the PK/PD for the BZT analogues and cocaine, respectively. The 50% of maximum inhibition (IC_{50}) of the loss of DA was lower for AHN-2005 (226 ± 27.5 ng/ml) compared to AHN-1055 (321 ± 19.7 ng/ml). In addition, the EC_{50} for cocaine was 215 ± 11.2 ng/ml.

Conclusions. The slow onset and long duration of BZT analogue–induced DA elevation may avoid the reinforcing effects and craving of cocaine. Further, the developed models will be useful in characterizing the PK/PD of other analogues and aid in the assessment of the therapeutic efficacy of the BZT analogues as substitute medications for cocaine abuse.

KEY WORDS: benztropine; dopamine; cocaine; pharmacokinetics; pharmacodynamics.

INTRODUCTION

Medication development for cocaine abuse therapeutics targets the neurotransmitter systems [i.e., dopamine (DA), serotonin, norepinephrine] that are thought to play a significant role in the reinforcing effects of cocaine. The substitute therapy approach for the treatment of cocaine abuse involves administration of a substitute agent (DA uptake inhibitor) so that the use of cocaine can be minimized and subsequently discontinued. In general, an ideal substitute therapeutic agent should possess a high affinity for dopamine transporter (DAT), slowly enter the brain or slowly bind to DAT, produce a slow increase in brain dopamine (DA) levels, inhibit DA reuptake, and enhance and prolong DA levels over baseline but to a lesser extent than cocaine (1,2). As such, the pharmacokinetic (PK) and the pharmacodynamic (PD) profile of a potential substitute therapeutic agent should be significantly different from cocaine. For this reason, a comparison of the pharmacokinetic (PK) and pharmacodynamic (PD) profiles of cocaine vs. the substitute therapeutic agents would be a useful predictor of therapeutic efficacy and abuse liability.

A novel class of DA uptake inhibitors, the benztropine (BZT) analogues (Fig. 1), have been designed, synthesized, and characterized as potential substitute therapeutic agents for cocaine abuse (3–10). The most potent BZT analogues inhibit DA uptake with a higher potency than cocaine, are not

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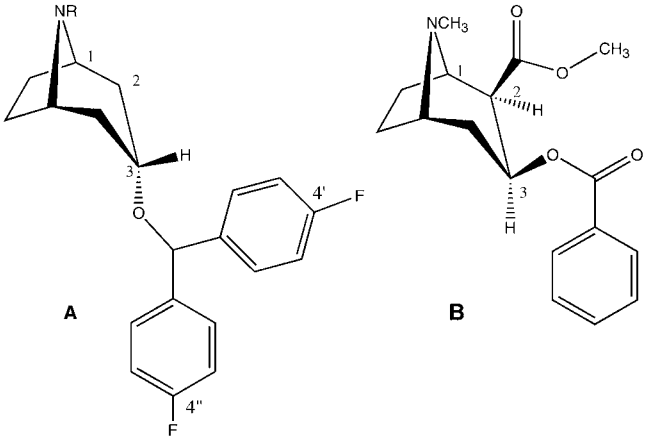

Fig. 1. Chemical structures of (A) 3α(bis (4′fluorophenyl) methoxy) tropane BZT analogues [structural substituents (R-) presented in Table I]; (B) cocaine.

efficacious locomotor stimulants in mice, and do not fully substitute for cocaine in drug discrimination studies (6,7). BZT analogues bind to DAT more selectively [K_i for DAT <<< K_i for serotonin transporter (SET), norepinephrine transporter (NET), and muscarinic M_1 receptor (M_1)] and with a higher affinity higher than cocaine (K_i for $DAT_{BZT\ analogue}$ <<< K_i for $DAT_{cocaine}$) (Table I) (5,7). In addition, they inhibit DA uptake with a higher potency in comparison to cocaine ($IC_{50BZT\ analogue}$ <<< $IC_{50cocaine}$). *In vitro* and *in vivo* studies with the BZT analogues have demonstrated that they possess certain characteristics that support their potential as substitute agents (7). Studies have shown the following: 1) BZT analogues display a slower ($p < 0.05$) net transport (higher efflux ratio) across the BBB vs. cocaine, 2) BZT analogues displayed a slower entry into the brain as compared to cocaine, and 3) BZT analogues brain-to-plasma ratios were higher ($p < 0.05$) than cocaine (10). Pharmacokinetic studies showed that the BZT analogues have a lower clearance and longer half-life in comparison to cocaine. Based on their pharmacokinetic, binding and behavioral properties, it would seem feasible that the BZTs may be potential substitute medications for cocaine abuse.

Because the BZT analogues display favorable transport and pharmacokinetic characteristics as substitute agents, the next step is to determine whether DA levels are elevated at a slower rate and remain elevated for a longer period of time in comparison to cocaine after their administration. Understanding the DA response pattern will be greatly enhanced when predictive PK/PD models are defined that describe the concentration- effect relationship for the BZT analogues. The PK/PD profile will indicate if the BZT analogues' plasma levels are directly or indirectly correlated with the DA levels as well as facilitate optimizing dosage regimens. This approach has proven useful for a number of CNS drugs such as antipsychotic medications, neuromuscular blockers, antihypertensives, and anesthetics (11,12).

In view of this, the objectives of this study were 1) to determine the extracellular brain DA levels in rats after i.v. bolus administration of the BZT analogues AHN-1055 and AHN-2005 and of cocaine, using intracerebral microdialysis, 2) to develop a PK/PD model to describe the relationship between plasma BZT analogue and cocaine concentrations and brain DA levels. AHN-1005 and AHN-2005 were selected based on the following specifications that may be indicative of potential pharmacodynamic properties (10). These agents were found to display significant permeability across an *in vitro* model of the blood-brain barrier (BBB). *In vivo* studies results suggested that these two analogues had brain uptake ratios higher than cocaine, plasma clearance lower than cocaine, and an elimination $t_{1/2}$ longer than cocaine. Based on these observations, AHN-1055 and AHN-2005 were identified as the most promising analogues for the PD studies.

MATERIALS AND METHODS

Potassium chloride, sodium chloride, magnesium chloride heptahydrate, calcium chloride dihydrate, sodium phosphate monobasic monohydrate, sodium phosphate dibasic anhydrous (Na_2HPO4) formaldehyde, cresyl violet, glacial acetic acid, cocaine hydrochloride, and ethanol were purchased from Sigma Chemical Co. (St. Louis, MO, USA). Permount, slide holder, coverslips (24 × 60 # 1 thickness), and transfer pipettes were purchased from Fischer Scientific (Fair Lawn, NJ, USA). The BZT analogues were synthesized as previously described (3,4,8). Cocaine, AHN-2005, and AHN-1055 were dissolved in artificial cerebrospinal fluid (aCSF: 5 KCl mM, 140 NaCl mM, 1.2 $MgCl_2$ mM, 1.4 $CaCl_2$ mM, phosphate-buffered saline, pH 7.2) for the microdialysis studies. All chemicals and solvents were ACS analytical grade or high performance liquid chromatography (HPLC) grade.

Animals

Adult male Sprague-Dawley rats (Taconic Laboratories, Germantown, NY, USA) weighing 275–300 g were used in the studies. All animals were housed individually in a tempera-

Table I. Structural Substituents, Physicochemical Properties, DAT Binding, and DA Uptake Inhibition Data on BZT Analogues and Cocaine (8)

Compound	R	MW	ClogP[a]	(^{3}H) WIN Binding[b] K_i (nM)	DA Uptake[c] IC_{50} (nM)
AHN 1-055	CH_3	393.16	3.95	12	71
AHN 2-005	$CH_2CH=CH_2$	405.92	4.52	30	14
Cocaine		303.31	2.72	300–400	150–200

[a] Calculated log of the partition coefficient (cLogP).
[b] Affinity for the dopamine transporter (DAT) by determination of the displacement of (^{3}H) WIN 35,428, an analogue of cocaine.
[c] Inhibition of (^{3}H) dopamine (DA) uptake.

ture and humidity controlled environment under a 12:12 light-dark cycle. They were given *ad libitum* access to food and water. Animals were maintained in facilities fully accredited by the American Association of the Accreditation of Laboratory Animal Care. The protocol was approved by the Institutional Animal Care and Use Committee, National Institutes on Drug Abuse, NIH, and all experiments were performed in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals. All necessary measures were taken before, during, and after surgical procedures to minimize pain and discomfort in the animals.

In Vivo Microdialysis Methods

Rats were anesthetized for jugular vein cannulation by intraperitoneal administration of a combination of ketamine/xylazine (80 mg/kg:12 mg/kg). Concentric dialysis probes were constructed as previously described with slight modifications (12). After cannulation, each rat was placed in Kopf stereotaxic apparatus, and a plastic intracerebral guide cannula (CAN 12; CMA microdialysis, Acton, MA, USA) was implanted. The guide cannula were stereotaxically implanted into the nucleus accumbens on both the right-hand and left-hand side using the following coordinates relative to bregma: 1.4 mm anterior to bregma, 2.5 mm lateral to bregma, and 4.4 mm below the surface of the skull [based on atlas by Paxinos and Watson, 4th edition (13)]. Guide cannulas were cemented in place using stainless steel screws and dental acrylic. After surgery, animals were singly placed in cages with *ad libitum* food and water and allowed a minimum of 5 days to recover from surgery. A microdialysis probe was lowered into the guide cannula and was continuously perfused with aCSF at a flow rate of 0.5 μl/min during the recovery period. On the day prior to the experiment, each animal was placed into its own plastic container and connected to a tethering system that allowed for motor activity. On the morning of the experiment, a 500-μl Hamilton syringe filled with aCSF and connected to an infusion pump, perfused the microdialysis probe at a flow rate of 2 μl/min for at least 2 h prior to sample collection. After the 2-h stabilization period, four consecutive baseline dialysate samples were collected. At the end of this 20-min period, vehicle was injected via the jugular vein cannula and dialysate samples were collected at 10, 20, 30, and 60 min after injection. After this 1-h period, rats were administered AHN-1055 (10 mg/kg), AHN-2005 (10 mg/kg), or cocaine (5 mg/kg) intravenously via the jugular vein cannula. The dose was followed by a saline flush to ensure accurate dosing. Dialysate samples were collected at 5, 10, 20, 30, 60, 120, 240, 360, 600, 720, 1440, 1800, and 1920 min for AHN-1055. Samples were collected up to 240 and 1440 min for cocaine and AHN-2005, respectively. Samples were collected over a 10-min period for all time points, except the 5-min time point. Brain microdialysate DA levels were assumed to reflect brain ECF DA levels. Dialysate samples were placed on dry ice immediately after collection and stored at –70°C until HPLC analysis. Verification of the positions of the probes implanted in the brain was determined after termination of the experiment by staining of coronal slices (100 μm thick) with cresyl violet.

HPLC Analysis of Dialysate Samples

Dialysate samples were analyzed for DA content by HPLC with electrochemical detection (ECD) using a previously described method (14,15). Stock solutions of 200 nM DA and 100 nM dihydroxybenzylamine (DHBA) were prepared in mobile phase. Standard solutions of DA ranged from 1 to 100 nM. The internal standard, 3,4,DHBA hydrobromide (1×10^{-7} M) was added to the aforementioned dialysate samples. The mobile phase was composed of 90 mM NaH_2PO_4, 50 mM citric acid, 1.7 mM octanesulfonic acid, 50 μM EDTA, and 10% acetonitrile, (pH 3.0) and pumped at a flow rate of 0.5 ml/min. The HPLC system consisted of an ESA Model 582 solvent delivery system (ESA, Chelmsford, MA, USA), CMA 200 refrigerated autosampler, ESA Model 501 Coulochem-II electrochemical detector, ESA Model 5014A microdialysis high performance analytical cell, ESA Model 5020 guard cell, Model 501 data analysis software, and ESA MD-150 analytical column (C_{18}, 150 × 3 mm, 3 μm). The guard cell electrode was set at +350 mV, the oxidation electrode was set at + 300 mV, and the reduction electrode was set at – 250 mV. Isocratic separation of DA and the internal standard, was achieved at ambient temperature and the run time was 9.5 min. The response values for the assay were determined by calculating the ratio of peak area of DA to that of the internal standard, DHBA, and the response was correlated against analyte concentration by least squares regression. The standard curve was found to be linear in the above mentioned range with an $R^2 > 0.99$ and intra- and inter-day variability and error were ≤10%.

Pharmacokinetic and Pharmacodynamic Modeling

A significant temporal delay was observed in the production of effect for both the BZT analogues. (AHN-1055: T_{max} for concentration = 5 min, T_{max} for effect = 120 min; AHN-2055: T_{max} for concentration = 5 min, T_{max} for effect = 60 min). Based on this observation, two PK/PD models were evaluated to determine which one best described the concentration-effect relationship. These models were an indirect physiologic response (IPR) model with inhibition of loss of response and a sigmoid E_{max} model (16). This inhibition of loss of response pharmacodynamic model describes drug response that results from inhibition of the factors controlling the dissipation of response. The selection of the indirect response model was based on the known mechanism of action of the BZT analogues. AHN-1055 and AHN-2005 bind to the DAT and inhibit the reuptake or loss of DA, thereby resulting in elevated brain DA levels. Brain microdialysate DA levels were assumed to be immediately and directly related to inhibition of the loss of DA as a result of blockade of the DAT. The pharmacokinetic model in both cases was a two-compartment model with an i.v. bolus dose and first-order elimination. The selection of the indirect response model was based on the known mechanism of action of the BZT analogues (4,8). AHN-1055 and AHN-2005 bind to the DAT and inhibit the reuptake or loss of DA, thereby resulting in elevated brain DA levels. The rate of change of response was defined according to the following equation:

$$\frac{dR}{dt} = K_{in} - K_{out}\left(1 - \frac{C_p}{IC_{50} + C_p}\right) R \qquad (1)$$

where R (response) is the brain DA level, K_{in} is a zero-order input rate constant, K_{out} is a first-order response dissipation rate constant, C_p is the plasma concentration of AHN-1055 or AHN-2005, and IC_{50} is the drug concentration producing

50% of maximum inhibition. DA levels were assumed to be immediately and directly related to inhibition of the loss of DA as a result of blockade of the DAT.

The possibility of an "effect" compartment which represents the drug that is actually available at the synapses for inhibition of the DAT was investigated by using a PK/PD E_{max} link model, which links the plasma compartment to a hypothetical "effect" compartment by a first order rate constant K_{eo}. The concentration-effect relationship was described by the following equation:

$$E = E_0 + \frac{(E_{max-E_0}) \times C_e}{EC_{e_{50}} + C_e} \quad (2)$$

where E_0 is the baseline DA level in the absence of drug, E_{max} is the maximum effect produced, C_e is the concentration in the effect compartment, and EC_{e50} is the effect compartment concentration required to produce 50% of E_{max}. Similar models were also used to characterize the concentration-effect relationship observed after cocaine administration as well as the sigmoid emax model. This model was first described by Hill and is an extension of the E_{max} model.

$$E = \frac{E_{max} \times C^n}{EC_{50} + C^n} \quad (3)$$

where n is the sigmoidicity factor and provides a further degree of flexibility in the sensitivity of the effect-concentration relationship. The larger the value of n, the more the curvature (i.e., the line is steeper). A high exponent results in an all or none effect. Value of n can be calculated from the slope of the center of the curve.

The pharmacokinetics of the BZT analogues evaluated in this study were determined in a previous study (Table II; Ref. 10). In order to select the best model, pharmacokinetic parameters from our previous study and brain extracellular fluid (ECF) DA levels from the microdialysis study were used and parameters were obtained using the nonlinear least squares regression analysis program, WINNONLIN. Pooled BZT analogue or cocaine PK data and pooled DA concentrations were modeled using both the IPR and linked PK/PD model. The BZT analogues and cocaine concentrations vs. time were described using a two-compartment model (10). Various weighting schemes such as uniform weighting (weight = 1), inverse of the observed effect (1/y), inverse of the square of the observed effect ($1/y^2$), inverse of the predicted effect (1/y^), and inverse of the square of the predicted effect ($1/y^{\wedge 2}$) were investigated to obtain the most reliable pharmacodynamic estimates. The choice of the PK/PD model was based on standard goodness of fit criteria which included

Table II. Plasma Pharmacokinetic Parameters (Mean ± SD) for the BZT Analogues and Cocaine After Intravenous Administration to Male Sprague-Dawley Rats (10)

Parameter	AHN-1055	AHN-2005	Cocaine
α (h^{-1})	7.7 (±0.25)	3.3 (±0.08)	69.3 (±2.10)
βα (h^{-1})	0.09 (±0.02)	0.17 (±0.014)	1.41 (±0.17)
t1/2-α (h)	0.09 (±0.003)	0.21 (±0.005)	0.01 (±0.0003)
t1/2-β (h)	7.69 (±1.76)	4.12 (±0.33)	0.49 (±0.06)
Vss (L/kg)	18.7 (±2.3)	12.3 (±3.2)	0.9 (±0.1)
Cl (L h^{-1} kg^{-1})	1.8 (±0.5)	2.6 (±0.6)	3.1 (±0.2)

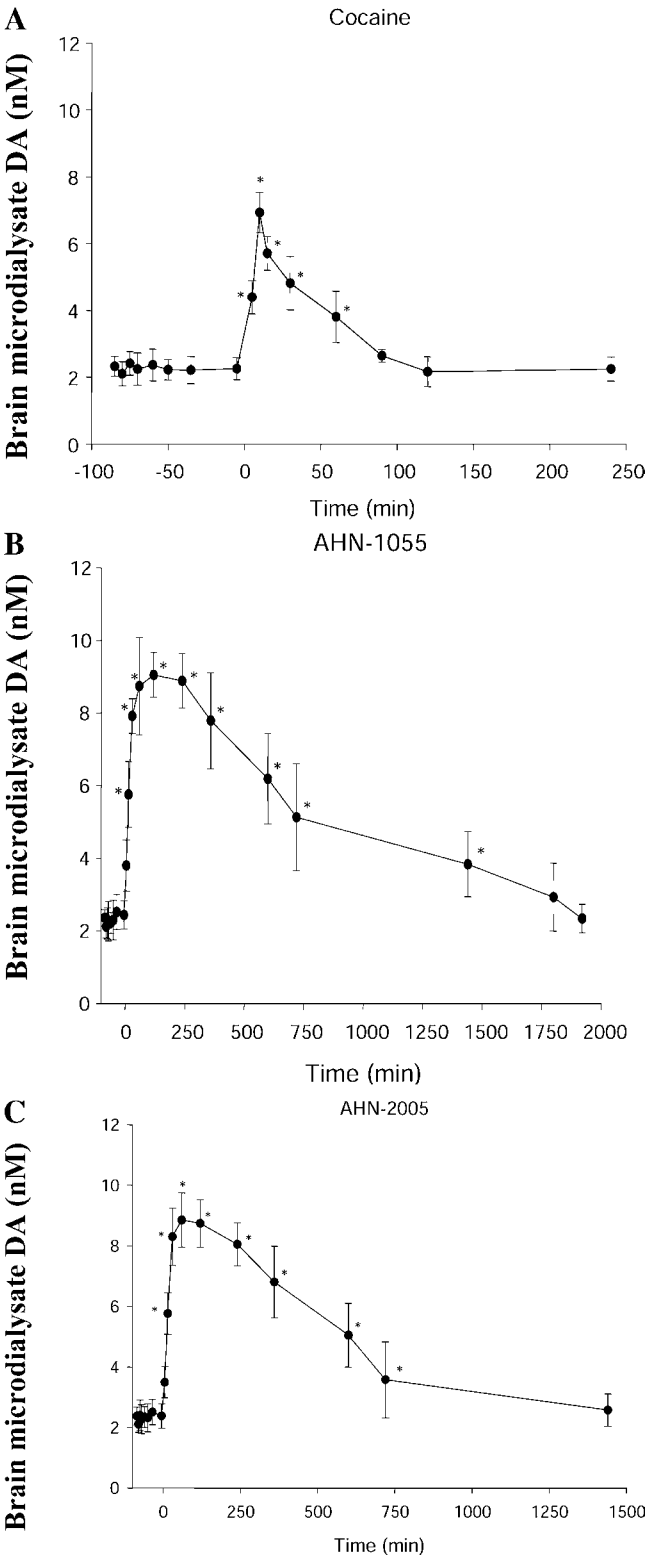

Fig. 2. Brain microdialysate dopamine levels seen after i.v. administration of (A) cocaine (5 mg/kg), (B) AHN-1055 (10 mg/kg), and (C) AHN-2005 (10 mg/kg) to Sprague-Dawley rats (cocaine, n = 7; AHN-1055, n = 6; AHN-2005, n = 8; *significant difference from baseline).

weighted sum of squares of residuals (WSSR), Akaike's information criteria (AIC), Schwarz criteria (SC), residual plots, and plots of observed and model-predicted concentration vs. effect. The model with the smallest values for AIC, SC, and WSSR was chosen as the best model.

Dopamine Data Analysis and Statistics

Mean baseline brain microdialysate DA concentration for an animal was defined as the mean of the DA concentration of the four samples preceding vehicle treatment. The brain microdialyste DA concentration in each sample was expressed as a percentage of the mean basal value. For each analogue, brain microdialysate DA levels at each time were compared to baseline levels by one-way ANOVA followed by Dunnett's post-hoc test and significance was set at $p < 0.05$. Pharmacodynamic parameters obtained for AHN-1055, AHN-2005, and cocaine were compared by one-way ANOVA followed by Dunnetts *post hoc* analysis ($p < 0.05$).

RESULTS

HPLC Analysis and Baseline DA Levels

The HPLC-ECD method for quantification of DA in rat brain extracellular fluid was found to be sensitive and specific for the analyte with no interfering peaks from the matrix. The mean basal brain mcirodialysate DA levels for cocaine, AHN-1055, and AHN-2005 were 2.10 nM, 2.31 nM, and 2.34 nM, respectively. Stable basal DA levels were seen after the 2-h stabilization period after increasing the aCSF flow rate to 2 μl/min. Basal levels of DA were stable over the 20-min monitoring period with <15% inter-animal variability. The probe placement displayed that the location of cannulas was well-defined, extending completely into the nucleus accumbens. Injection of vehicle produced no significant changes in ECF DA levels. Stable basal DA levels were maintained for the entire 1-h period after injection.

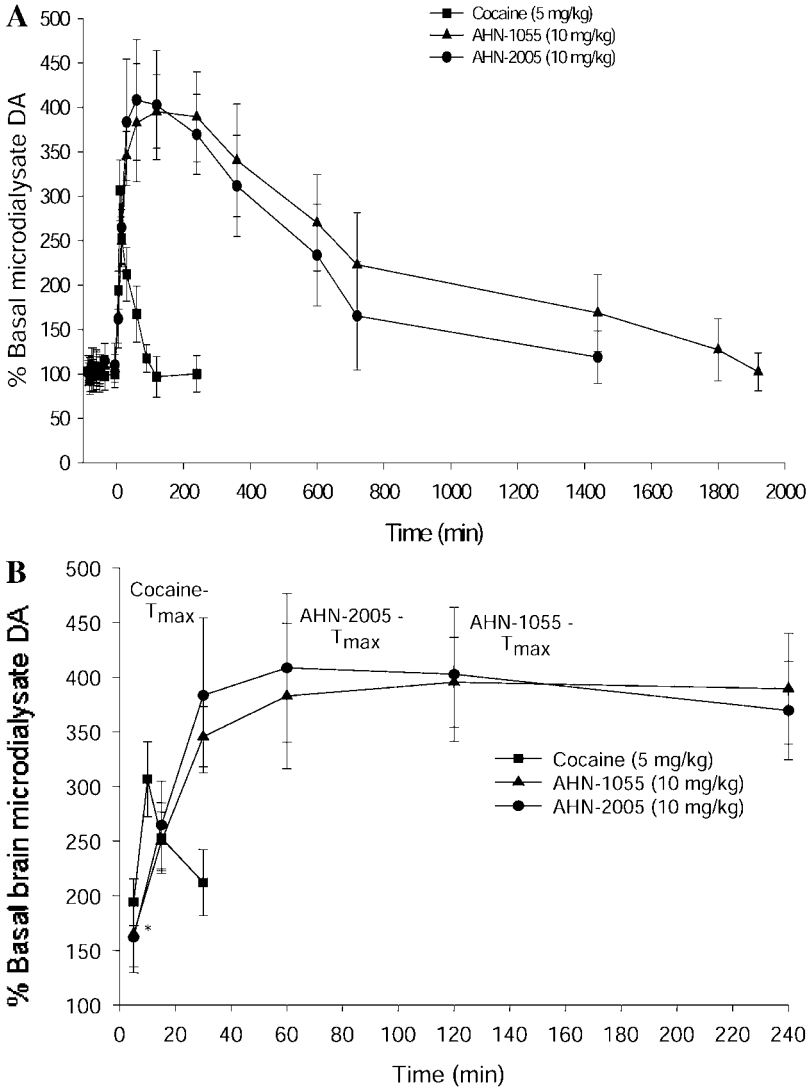

Fig. 3. Comparison of effects of cocaine, AHN-1055, and AHN-1005 on basal brain microdialysate DA levels after single-dose administration to Sprague-Dawley Rats: (A) percent basal DA levels over time-course of sample collection and (B) percent DA basal DA levels up to 240 minutes after dosing. Data presented as mean ± SD; for cocaine n = 7, AHN-1055, n = 6, AHN-2005 n = 8.

Effect of Cocaine, AHN-1055, and AHN-2005 on DA Release

The effects of cocaine, AHN-1055, and AHN-2005 on brain extracellular fluid (ECF) DA concentrations are illustrated in Figs. 2A–2C, respectively. Brain microdialysate DA levels were significantly higher than baseline at 5 min and remained so for up to 60 min after cocaine administration and brain microdialysate DA levels returned to baseline within 2 h post-injection (Fig. 2A). AHN-1055 significantly increased brain microdialysate DA levels (Fig. 2B) with DA concentration at 15 min being statistically higher ($p < 0.05$) than baseline. The brain microdialysate DA levels remained significantly higher ($p < 0.05$) than baseline for up to 24 h after administration of AHN-1055 and returned to baseline at 30 h. AHN-2005 produced significantly higher brain microdialysate DA levels 60 min (Fig. 2C) after dosing and these levels remained higher than baseline for up to 12 h. Brain microdialysate DA levels returned to baseline 24 h post-dosing.

Figure 3A illustrates comparative brain microdialysate DA vs. time profiles after cocaine, AHN-1055, and AHN-2005 dosing. Figure 3B displays the up-slope or "rate" for each compound in relation to its ability to elevate brain microdialysate DA levels. After cocaine administration, there was a rapid, almost instantaneous increase in brain brain microdialysate DA levels with peak elevations of 306.5% of baseline being achieved in 10 min. AHN-1055 increased brain microdialysate DA levels to 395.3% of baseline ($p < 0.05$ compared to cocaine) and the increase in brain microdialysate DA levels was more sustained, with peak levels occurring in 120 min in comparison. AHN-2005 increased brain microdialysate DA levels to 408.4% of basal levels ($p < 0.05$ compared to cocaine) and similar to AHN-1055, the increase was more sustained, with peak levels occurring in 60 min.

Pharmacokinetic/Pharmacodynamics of the BZT Analogues and Cocaine

Figure 4 illustrates the plasma concentration vs. time and effect vs. time curve plots for AHN-1055 (Fig. 4A) and AHN-2005 (Fig. 4B). The time at which maximum BZT analogue concentration is achieved was 5 min for both compounds, however, a significant delay is observed in the effect (brain microdialysate DA levels) produced. The maximum brain microdialysate DA levels were seen at 120 min after AHN-1055 administration and 60 min after AHN-2005 administration. Due to the delay in effect, a counterclockwise hysteresis was observed for both compounds. Based on this observation, brain microdialysate DA levels were modeled using a PK/PD link model with an effect compartment and an indirect response model with inhibition of loss of DA.

The indirect response PK/PD model was found to best characterize brain microdialysate DA release after AHN-1055, and AHN-2005 administration based on the lowest AIC, SC, and WSSR. Figure 5 displays the pooled observed and model predicted brain microdialysate DA vs. time profile for AHN-1055 (Fig. 5A) and AHN-2005 (Fig. 5D), respectively. Random scatter was apparent in the weighted residual brain dopamine vs. time profiles (Figs. 5B and 5E) for AHN-1055 and AHN-2005, respectively. Excellent and correlation was observed in the observed vs. model-predicted brain dopamine levels for AHN-1055, however the model displayed

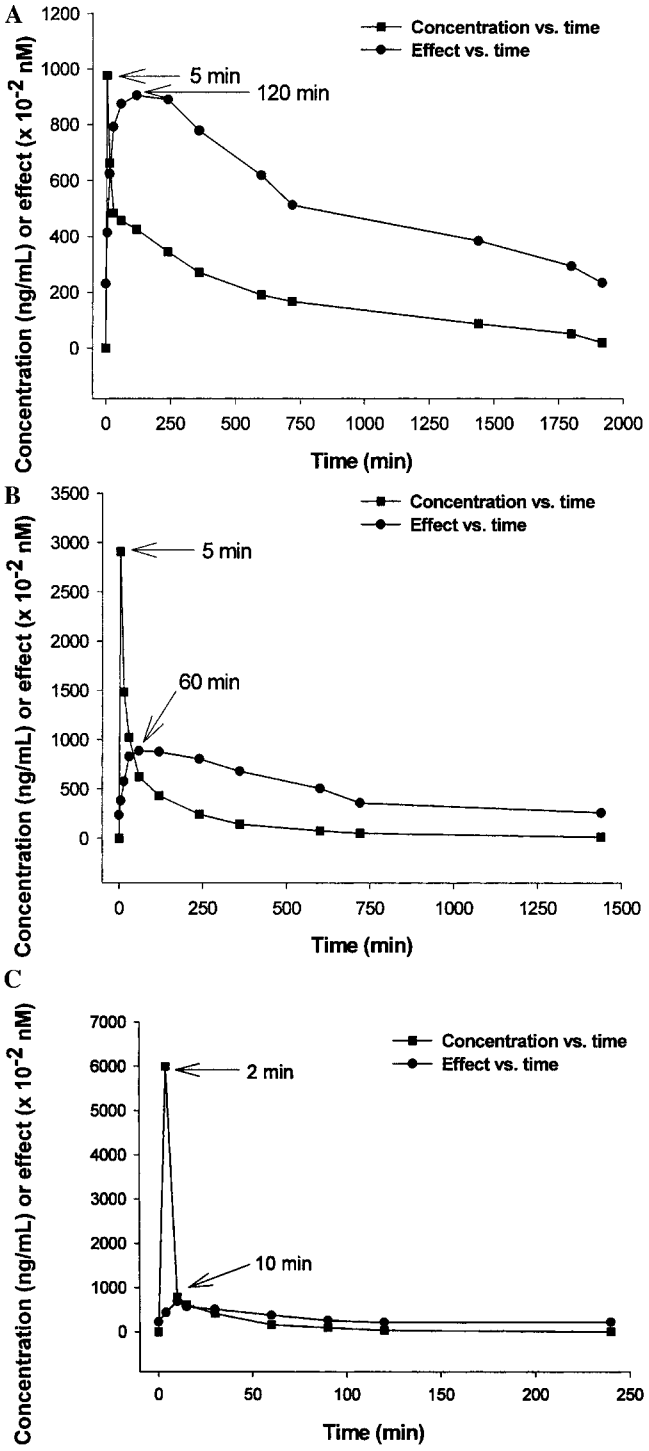

Fig. 4. Concentration and effect (brain microdialysate DA levels) *vs.* time curve plots for (A) AHN-1055 (10 mg/kg), (B) AHN-2005 (10 mg/kg), and (C) cocaine after single-dose i.v. administration to Sprague-Dawley rats.

moderate correlation for AHN-2005. The pharmacodynamic parameters for both AHN-1055 and AHN-2005 are summarized in Table III. The rate constant, K_{in}, for production of response (brain microdialysate DA levels), was comparable for both compounds (AHN-1055: 0.19 nM/min, AHN-2005: 0.07 nM/min). Also, the rate constants for loss of response, K_{out}, were 0.17 min^{-1} and 0.06 min^{-1} for AHN-1055 and

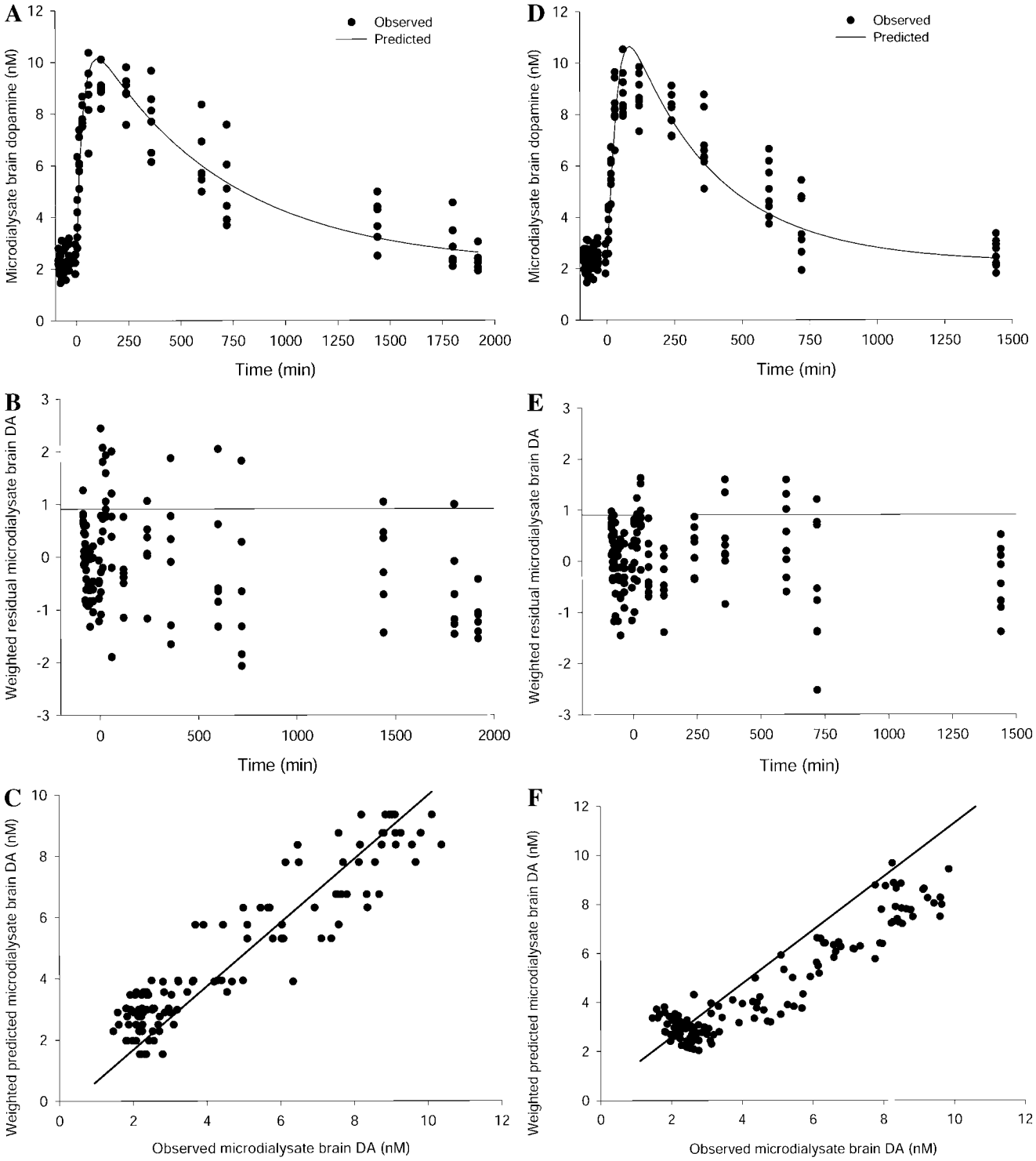

Fig. 5. Brain microdialysate dopamine level vs. time fits for AHN-1055 (A, B, C) and AHN-2005 (D, E, F) (pooled data set) after single-dose i.v. administration (10 mg/kg) to rats according to the indirect response model II (WINNONLIN): (A and D): Model-predicted and observed dopamine level vs. time; (B and E): weighted residual brain dopamine vs. time; and (C and E): observed vs. model-predicted brain dopamine (AHN-1055, n = 6; AHN-2005, n = 8).

AHN-2005, respectively. The plasma concentration required to produce 50% of maximum inhibition (IC_{50}) of the loss of brain microdialysate DA was lower for AHN-2005 (226 ng/ml) compared to AHN-1055 (321 ng/ml).

Figure 6 shows the plasma concentration vs. time and effect vs. time curves for cocaine. As can be seen, the time of maximum concentration was observed to be two minutes and the maximum brain microdialysate DA levels were observed seen at 10 min after cocaine administration. Thus, there was no significant delay between the concentration and effect for cocaine. Based on this observation, brain microdialysate DA levels were modeled using direct pharmacodynamic models

Table III. Mean (CV%) Pharmacodynamic Parameters for AHN-1055 and AHN-2005 Using the Indirect Response Model (Model II) and for Cocaine Using the Sigmoid E_{max} Model

Compound	K_{in} (nM/min)	K_{out} (min^{-1})	IC_{50} (ng/ml)	
AHN-1055	0.19 (15.3)	0.07 (8.4)	321 (19.7)	
AHN-2005	0.17 (13.4)	0.06 (14.9)	226 (27.5)	
	E_{max} (nM)	EC_{50} (ng/ml)	E_0 (nM)	n
Cocaine	5.72 (4.2)	215 (11.2)	2.17 (6.1)	3.37 (34.8)

(i.e., linear, E_{max} and sigmoid E_{max}). Based on the lowest AIC, SC, and WSSR, the sigmoid E_{max} model was chosen as the best model. The observed and model predicted cocaine concentration vs. DA release is presented in Fig. 6A, and the pharmacodynamic parameters for this model is presented in Table III. The residuals for brain microdialysate DA levels were randomly scattered with no obvious pattern (Fig. 6B) and there was good correlation between observed and model-predicted brain microdialysate DA levels (Fig. 6C). The maximum brain microdialysate DA level (E_{max}) was 5.72 nM and a concentration of 215 ng/ml (EC_{50}) was required to produce 50% of E_{max}. The baseline DA level (E_0) was calculated as 2.17 nM and a value of 3.36 was obtained for the sigmoidicity factor (n). The estimates were reliably determined with % CVs < 11.5% for E_{max}, EC_{50}, and E_0.

DISCUSSION

The aim of this research was to develop a predictive PK/PD model for AHN-1055, AHN-2005 and cocaine that characterized the DA release after single dose administration. After a 5 mg/kg i.v. dose of cocaine, brain microdialysate DA levels increased to approximately 306% of basal levels. The peak brain microdialysate DA levels were observed at 10 min post-dose and brain microdialysate DA levels declined rapidly returning to baseline in ~90–120 min. These results are consistent with studies reporting elevation of brain microdialysate DA after cocaine administration. Studies have shown DA levels to increase within 5-10 min after i.v. administration of cocaine (17,18). Microdialysis experiments observed a 300% increase in DA levels over baseline within 10 min after intramuscular dosing of cocaine to squirrel monkeys (19). In another study, increases in DA levels in the order of ~250% from baseline were observed within 20 min after ip cocaine (20). Striatal ECF dopamine has been reported to increase by 210, 450 and 630% of baseline after acute cocaine dosing (0.5, 2 and 5 mg/kg, i.v., respectively) in Rhesus monkeys with peak levels obtained 15 min post dose (20). In all studies, DA levels returned to baseline within 50- 60 min post administration.

Substitute therapeutic agents should produce a slow and sustained increase in DA levels in order to minimize the euphoria associated with cocaine administration (1,21). The BZT analogues, AHN-1055 and AHN-2005, produced a maximum increase of 395 and 408.4%, respectively of basal DA after a 10 mg/kg i.v. dose. Peak brain microdialysate DA levels were observed 60 min and 120 min after dosing with AHN-2005 and AHN-1055, respectively. In terms of duration

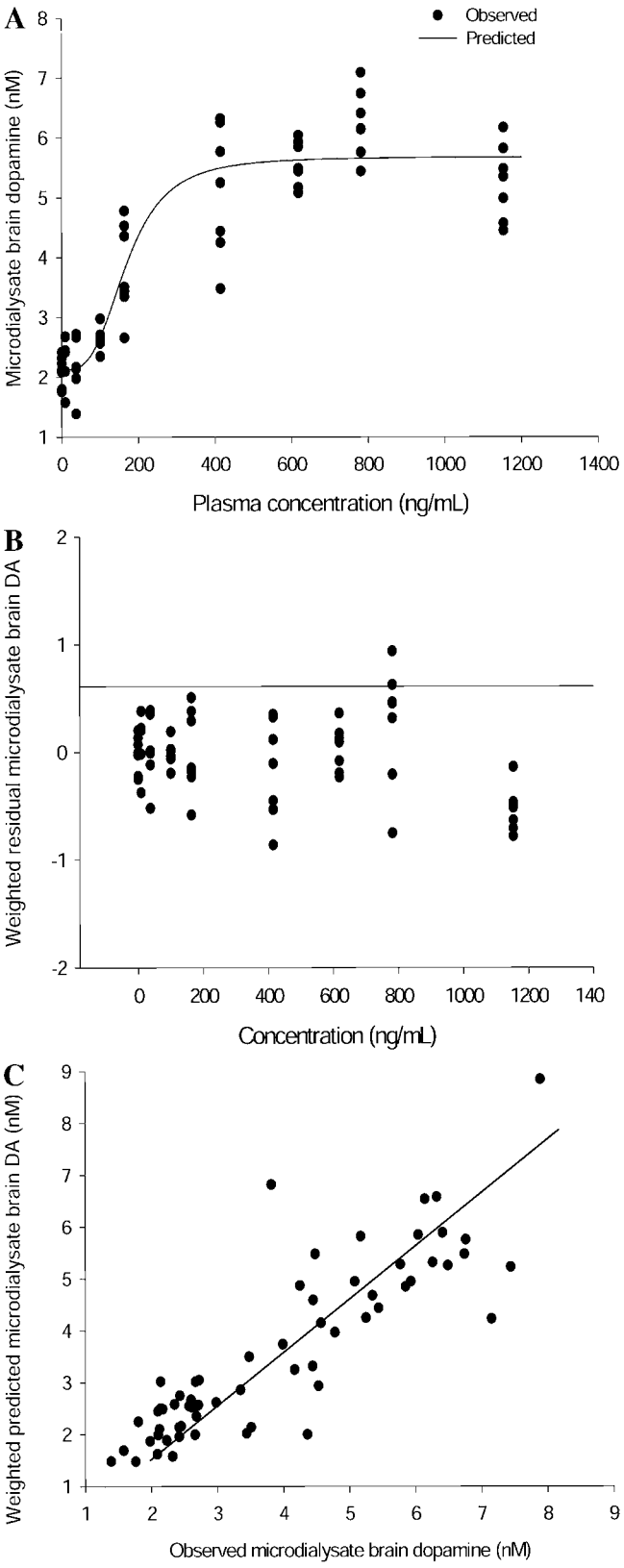

Fig. 6. Plasma concentration vs. brain microdialysis DA fits for cocaine (pooled data set) after single-dose i.v. administration (5 mg/kg) to rats according to sigmoid E_{max} model (n = 7, WINNONLIN): (A) Model-predicted and observed brain microdialysate dopamine level vs. concentration, (B) weighted residual brain dopamine vs. concentration, and (C) observed vs. model-predicted brain-microdialysate dopamine.

of effect, both AHN-2005 and AHN-1055 produced sustained brain microdialysate DA levels that ranged from 60 to 120 and 60 to 240 min with a return to baseline at 12 and 24 h post-dosing, respectively. These results are consistent with observations with other potential substitute therapeutic agents. GBR12909 produced a 225% increase in brain microdialysate DA levels over baseline at 60 min post dose (25 mg/kg) and the levels remained elevated over 4 h (22). Another promising DA uptake inhibitor, 4-chloro-3α-(diphenylmethoxy)tropane, has been shown to produce a slower and lesser increase in DA levels as compared to cocaine after ip dosing with maximum DA levels observed at 60-70 min post dosing (23). The brain microdialysate DA profile observed with the analogues suggest a slow rate of increase as well as a longer duration of action, both are suggested characteristics of a potential substitute agent. Although both cocaine and the BZT analogues produced significant increases in basal DA, the time-course of effect was significantly different. Peak levels in dopamine release were observed at 10 min with cocaine, vs. 120 min for AHN-1055 and 60 min for AHN-2005. In addition to the time of brain microdialysate DA peak levels being significantly different for the BZT analogues vs. cocaine, brain microdialysate DA levels remained elevated over 12 (AHN-2005) and 24 h (AHN-1055) as compared to cocaine (2 h). The effect associated with cocaine, AHN-1055, and AHN-2005 on the duration of DA release correlated strongly with their terminal half-life ($t_{1/2}$) (0.49, 7.69 and 4.12 h, respectively) and clearance (Cl) (3.1, 1.8, and 2.6 L h^{-1} kg^{-1}, respectively). This highlights the importance of the pharmacokinetic profile on the duration and extent of DA release. Further, it supports the premise that a lower clearance and longer half-life are critical parameters required for a potential cocaine abuse therapeutic agent.

As previously stated, an important objective of this investigation was to characterize the PK/PD relationship between the BZT analogues and brain microdialysate DA brain concentrations. Such information can be used to compare the BZT concentration-response relationships with those for cocaine by comparing PD parameters as opposed to behavioral profiles. The indirect model with loss of effect was found to best characterize the concentration-response relationship for the BZT analogues. There was a distinct time lag in the BZT plasma concentration vs. time profiles vs. the ECF dopamine vs. time profiles. The suitability of the indirect model for the BZT analogues vs. the linked PK/PD lined model was supported by lower AIC, SC, and WSSR. In addition, the adequacy of the indirect model fit was evidenced by the tight scatter illustrated in the observed vs. predicted profiles as well as the random pattern observed in the residual plots. The rate constants for production of response, K_{in}, were found to be similar for both AHN-1055 (0.19 nM/min) and AHN-2005 (0.17 nM/min). Because the BZT analogues act on the inhibition of loss of DA and not on its production rate, it would be expected that the rate constant for the production of DA would be similar for the two compounds. Our results were in agreement with this expectation. The major factor influencing the rate of change of response would be the inhibitory function, specifically IC_{50}. This parameter was not significantly different for the two BZT analogues, however AHN-2005 (IC_{50} = 226 ng/ml) was found to be more potent than AHN-1055 (IC_{50} = 315 ng/ml).

A direct sigmoid Emax model was used to describe the concentration-relationship observed between cocaine and brain microdialysate DA concentrations seen after i.v. administration of cocaine were related to the plasma concentrations using a direct response model. Examination of the residual plots and the AIC, SC, and WSSR values showed that this model was superior than other pharmacodynamic models such as the linear or E_{max} model in describing the concentration-effect relationship. The baseline DA level was included in the estimation of the total effect as a function of concentration. The baseline DA was estimated from the model (E_0 = 2.21 nM) and was found to be very close to the observed value of 2.10 nM. A maximum effect of 5.87 nM was seen and the plasma concentration required to produce 50% of this effect was 250.6 ng/ml. The sigmoid E_{max} assumes that as cocaine concentration increases, brain microdialysate DA level at the nerve endings will increase until it reaches a maximum value at very high cocaine concentration. The same PD model has been used to describe the relationship between cocaine plasma concentration and locomotor activity (24). These results are consistent with our findings since it is well documented that the locomotor activity observed after cocaine administration is well correlated with the DA concentrations in the nucleus accumbens (25).

In conclusion, the pharmacodynamics and PK/PD relationship for two BZT analogues, AHN-1055 and AHN-2005 were characterized. In agreement with their mechanism of action, brain microdialysate DA concentrations were seen to increase significantly from basal values after intravenous administration of AHN-1005, AHN-2005, as well as cocaine. Unlike cocaine, the increase in brain microdialysate DA concentrations was much slower after administration of the BZT analogues. Maximum brain microdialysate DA concentrations were seen after two hours for AHN-1055 and after one hour for AHN-2005 in comparison to a peak level time of 10 min for cocaine. These results indicate that the BZT analogues would probably not produce the euphoria associated with cocaine abuse and the slow increase in brain microdialysate DA concentrations, in addition to several other factors, may be responsible for the lack of cocaine-like behavioral effects in rats. In addition, brain microdialysate DA concentrations were seen to remain elevated for a duration of time much longer than cocaine. Thus, the BZT analogues satisfy the desirable property of slow onset and long duration of effect for substitute therapeutics and may be useful in the treatment of cocaine. An IPR model with inhibition of loss of response was able to satisfactorily characterize the PK/PD relationship for these DA uptake inhibitors. A sigmoid E_{max} model was used to characterize concentration-effect relationship for cocaine. The PK/PD models developed for BZT analogues are predictive of the *in vitro* DA uptake IC_{50} data (Table I) and can serve as a foundation for future models characterizing the concentration-response relationship for similar DA uptake inhibitors.

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