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Distribution and Clearance of Cocaine in Brain Is Influenced by Genetics

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AZAR, M. R., N. ACAR, C. L. HEIST, V. G. ERWIN, G. F. BARBATO, A. C. MORSE AND B. C. JONES. *Distribution and clearance of cocaine in brain is influenced by genetics*. PHARMACOL BIOCHEM BEHAV **59**(3) 637–640, 1998.—The purpose of this study was to examine the pharmacokinetics of cocaine in two inbred mouse strains, C57BL/6 (B6) and DBA/2 (D2). Male and female mice were administered 30 mg kg^{-1} cocaine IP and killed after 5, 15, 30, or 60 minutes postinjection. Brains were removed quickly and assayed for total brain cocaine concentration. Quantification of cocaine was conducted using gas chromatography and mass spectrometry. The results of this study revealed a strain difference in total brain cocaine kinetics. Specifically, we observed that at 5 min onward, B6 mice cleared cocaine from the brain with a $t_{1/2}$ estimated at 22.3 min, while distribution in D2 mice appeared to be incomplete until 15 min with a subsequent $t_{1/2}$ estimated at 11.2 min. These results show that despite faster clearance by D2 mice, the prolonged time to distribution in this strain may help explain why D2 mice show initial greater locomotor activation by cocaine, compared to B6s. © 1998 Elsevier Science Inc.

Cocaine Pharmacokinetics Inbred strains Mice

THE conduct of pharmacological studies using genetically defined mice offers an insight into pinpointing genetic variables that may contribute to differences in drug effects (3,5). For example, inbred strains of mice have been used frequently to investigate locomotion stimulation caused by cocaine (8,12, 14). Cocaine-induced hyperlocomotion has been measured in many different strains of mice (6,11,15,17) and at doses ranging from 0.1 to 75 mg kg^{-1} (2,6,10,12). At low doses, cocaine may depress locomotor activity in mice (6) and rats (7), while doses ranging from 5 to 30, mg kg^{-1} increase locomotor activity (9,10). Much of the research to date has focused on the pharmacodynamics of cocaine, while paying little attention to the pharmacokinetics, i.e., absorption, distribution, and elimination. Several attempts have been made to associate brain levels of cocaine with locomotor activity; however with measurement at one time point only (16,17). The problem this presents is that while measuring brain cocaine concentrations at a single time period, the ability to accurately interpret the difference in locomotor activity due to absorption, distribution, and clearance of cocaine may be overlooked. The study presented here explored the distribution and clearance of cocaine between C57BL/6 (B6) and DBA/2 (D2) mice. Brain cocaine levels were measured at four time points 5, 15, 30, and 60 min postinjection.

METHOD

Animals

Male and female B6 and D2 mice, 60–80 days of age, were used in this study. Mice were born and raised in our own colony at the Pennsylvania State University. Subjects were weaned into unisex groups of two to three mice/cage at 21 days of age, and housed in standard 30 × 26 × 23 cm polycarbonate boxes until testing. All subjects were maintained on a

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12 L:12 D (lights on 0700–1900 h), with temperature and humidity maintained at 22°C and 20%, respectively. Free access to food and water were available continuously.

Drug Administration and Analysis

Male and female B6 and D2 mice were randomly assigned to one of four time groups, administered cocaine intraperitoneally (IP) at 30 mg kg^{-1} and sacrificed at 5, 15, 30, or 60 min postinjection. To avoid any litter-specific effects, each subject within a litter was assigned to a different time point. Cocaine HC1 was obtained from the National Institute on Drug Abuse (Rockville, MD) and prepared by dissolving in sterile saline. After sacrifice by cervical dislocation, brains were removed quickly and placed in 15 × 100-mm polypropylene tubes containing 4.5 ml of 0.1 N HC1 and weighed. The brains were then homogenized with a Tissuemizer^R (Tekmar, Co., Cincinnati, OH) for 10 s at a power setting of 60%. The homogenates were centrifuged at 20,000 × *g* for 15 min and the supernatant was transferred into 12 × 75 mm polypropylene tubes. Supernatants were frozen, lyophilized, and stored at −70°C.

Brain Cocaine Extraction

Brain cocaine concentrations were determined by the method developed in our lab according to Acar et al. (1). Samples were reconstituted 4.0 ml of phosphate buffer (pH 6.0). Fifty microliters of 1.0 M KOH was then added to adjust the pH up to 5.0. The suspensions were then transferred into Salivette filter tubes (Sarstedt Inc., Research Triangle Park, NC) and centrifuged at 2,000 × *g* for 4 min to remove excess lipids. Tropacocaine was obtained courtesy of the National Institute on Drug Abuse (Rockville, MD) and used as internal standard. Twenty-five microliters of tropacocaine dissolved in phosphate buffer (0.24 mg/ml, pH 6.0) was added into each tube after centrifugation. Solid phase extraction (SPE) was performed on Isolute^R SPE (Jones Chromatography, Lakewood, CO) columns (HCX 130 mg/10 ml XL column reservoir). The SPE columns were conditioned with 2.0 ml of methanol, 2.0 ml of deionized water, followed by 2.0 ml of 0.1 M phosphate buffer (pH = 6.0) before samples were loaded into columns. Samples were washed through the SPE columns utilizing a vacuum manifold at a flow rate of 1–2 ml/min. The SPE column was then washed with 6.0 ml of deionized water, followed by 3.0 ml of 0.1 M HC1. The columns were then dried under full vacuum for 5.0 min, followed with a final wash of 9.0 ml of methanol, and dried for an additional 2.0 min under full vacuum. Cocaine and tropacocaine were eluted from the column with 4.0 ml of 80/20 (v/v) dichloromethane/propan-2-ol containing 2% ammonium hydroxide, then evaporated to dryness with N_2. Analytes were reconstituted in 0.5 ml of dicloromethane and again evaporated to dryness with N_2. All samples were derivatized using 100 μl BSTFA containing 1% TMCS at 90°C for 8 min and transferred into GC sample vials. The derivation was conducted in case there were measurable levels of benzoylecgonine in the brain.

Standards

Standards for calibration curves were prepared by adding cocaine to brain homogenates to obtain 0.586, 1.17, 2.34, 4.68, and 9.36 μg of cocaine per 5 ml. Extraction of standards were conducted the same way as the experimental samples.

Instrumentation

Detection and quantification of cocaine were performed using a Hewlett-Packard 5890 gas chromatograph (GC) with a 5971A mass selective detector (MSD) (HP Co., San Fernando, CA). Samples were injected automatically via an HP 7673A autoinjector. Data processing and quantification were performed using Hewlett-Packard Chem Station^R software. A HP-5MS capillary column (5% phenyl methyl silicone with 30 m × .25 mm i.d., .25 μ film thickness) was used to separate cocaine and tropacocaine.

Chromatographic Conditions

The injector port was operated at 250°C utilizing the splitless mode option. The initial oven temperature was 150°C held for 30 s, programmed at 290°C at 20°C/min and held for 4 min. The transfer line temperature was maintained at 250°C. The carrier gas was ultrapure grade helium, programmed to be constant at 9.0 psi with a flow rate at 1 ml/min. The MSD was calibrated with perfluorotributylamine (PFTBA) using the mid-mass autotune option by monitoring mass fragment ions (*m/z*) 69, 131, and 264. The electron multiplier was operated at autotune values, plus 400 EMV. The selected ion monitoring (SIM) mode was used to detect cocaine (*m/z*) 82, 182, 303, and tropacocaine (*m/z*) 67, 124, 245 fragments, respectively. Injections (1 μl) were made automatically into the vaporization chamber.

Data Analysis

Brain cocaine levels were evaluated by analysis of variance for a three between-subjects variables (strain, sex, time) experiment. Post hoc tests for time effects were conducted using Tukey's HSD method (13).

RESULTS

Figure 1 illustrates mean brain cocaine concentrations across the four time periods for male and female B6 and D2 mice. Analysis of variance revealed significant effects of strain (D2 > B6), $F(1, 80) = 18.814$, $p < 0.0001$, and time, $F(3,80) = 70.004$, $p < 0.0001$. There were no significant effects of sex or any interaction. The proportion of variance in brain cocaine concentration accounted for by strain was 6% and that accounted for by time was 65% (est ω^2).

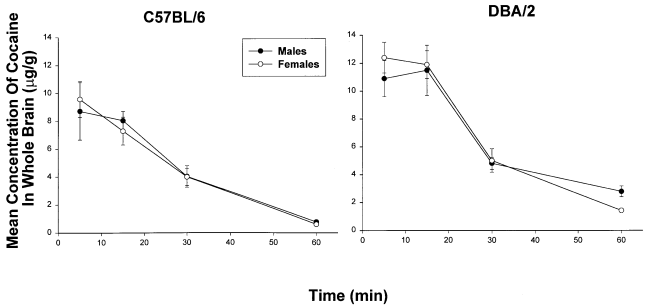

FIG. 1. Brain cocaine concentrations in male and female C57BL/6 and DBA/2 (n = 5–7) mice. Male and female mice were administered 30 mg kg^{-1} cocaine IP and killed after 5, 15, 30, or 60 minutes postinjection. Brains were removed quickly and assayed for total brain cocaine concentration as described in the text. Estimated $t_{1/2}$ in C57BL/6 and DBA/2 mice were 22.3 and 11.2 min, respectively. Data are mean (±SEM) cocaine concentrations in whole brain.

As can be seen, cocaine begins to disappear from the brain after 5 min in B6 mice but peaks at 5 to 15 min in D2 mice, and then begins to disappear (Fig. 1). Furthermore, cocaine had a longer half-life in B6 than in D2 mice. The estimated half-life of cocaine in B6 was 22.3 min (estimated from 5–30 min). Because the plot of the values appeared linear we used a linear equation with a corresponding correlation coefficient of −0.993 to obtain this value. The estimated half-life in D2 mice was 11.2 min (estimated from 15–60 min). This value was calculated using a double log transformation of the data. Correlation coefficients for all such transformed data exceeded $r = 0.99$, indicating linearity of the transformed function.

DISCUSSION

The results show a significant difference in brain cocaine levels between B6 and D2 mice, with B6 mice reaching peak brain concentrations at or before 5 min compared to D2 mice, which showed peak concentrations between 5 and 15 min. Expanding the time course of measuring brain cocaine concentrations up to 60 min extends the findings of Womer et al. (18), and presents a clearer picture of the distribution and fate of cocaine. Although it appears from previous research that B6 mice clear cocaine from the brain at a much faster rate than D2 mice, such is not the case. Brain cocaine concentrations measured at 5, 15, 30, and 60 min clearly show that while B6 mice reach peak levels at or before 5 min and then decline, after distribution, and clearance of cocaine is actually nearly twice as fast in D2 mice.

The present results may help explain why D2 mice appear to be more activated by cocaine than B6 mice when measured at short intervals postinjection. These data also support previous studies conducted by Wiener and Reith (17), which suggests that differences between cocaine-induced locomotor stimulation, in part, may be due to differences in the distribution of cocaine in the brain following IP administration of cocaine. A number studies have explored the relationship between dopamine receptors in the brain and differences in cocaine-induced activation in these two strains. Erwin et al. (4) reported differences in D_2 receptor densities, but not D_1 densities in the striatum with B6 showing greater densities than D2 mice. Concerning the dopamine transporter, Womer et al. (18) showed no differences in dopamine transporter binding between the two strains. Genetically based differential sensitivity to cocaine's locomotor activating effects, therefore, appears to be a combination of pharmacodynamic and pharmacokinetic factors.

The difference between B6 and D2 mice in the distribution and clearance of cocaine has important implications regarding pharmacological studies. For example, if one were to measure locomotor activity for a duration of 10 min postinjection in B6 and D2 mice, a comparative difference in activation between strains, at peak cocaine concentrations, would be overlooked, because D2 mice attain peak levels at 15 min compared to B6 mice after 5 min. The difference in cocaine pharmacokinetics between strains leads us to believe that initial greater locomotor activation in D2 mice compared to B6 mice (4) is, in part, influenced by cocaine pharmacokinetics. The significance of our research adds insight into variables that must be considered when exploring cocaine's actions in genetically defined mice.

Our data clearly show that absorption and distribution of cocaine to brain differ considerably between our two strains. How this effect affects clearance of cocaine is not entirely clear. Within the limitations of this study, we need to assume that peak concentrations of cocaine indicate absorption/distribution of cocaine to be complete or nearly complete, with little effect on clearance. If absorption and distribution are not complete, one would expect an underestimate of $t_{1/2}$. This would certainly be the case for D2 mice if we had attempted such in our earlier study (18). In the present study, by starting a peak concentration with the D2 strain, we observed exactly the opposite, i.e., a much shorter $t_{1/2}$ for the D2 strain than that observed in the B6s. It is clear that further study into the genetics of pharmacokinetics of cocaine is warranted to solve the problem of differential absorption and distribution.

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