Cocaine Pharmacokinetics/ Pharmacodynamics in Awake Freely Moving Rats

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INTRODUCTION

Cocaine pharmacological effects are primarily caused by blocking neuronal uptake of catecholamines in both the peripheral and the central nervous systems (CNS). Peripheral accumulation of catecholamines leads to vasoconstriction, hypertension and tachycardia. In the CNS, higher dopamine concentration is responsible for cocaine euphoric and locomotor effects. After cocaine administration, 45% of the dose is metabolized to benzoylecgonine and 40% to ecgonine methyl ester. Other minor metabolites including the pharmacologically active metabolite norcocaine can be detected after cocaine administration (1).

The changes in cocaine effects after different treatment regimens have been explained by different functional mechanisms including sensitization and tolerance. Very little attention has been paid to the correlation between cocaine pharmacokinetics and the resulting pharmacological and toxicological effects after cocaine administration. Previous reports have clearly documented good correlation between brain cocaine concentration and locomotor activity (2), and between serum cocaine concentration and locomotor activity (3). The primary objective of this study was to investigate how the plasma and brain cocaine concentration-time profiles after cocaine administration are correlated with the time-course of cocaine pharmacological effects in awake freely moving rats. In this study we examined the relationship between cocaine brain extracellular fluid (ECF) concentration and the neurochemical response measured as the change in dopamine level in the nucleus accumbens utilizing the microdialysis system. Also, the relationship between cocaine plasma concentration and the change in the mean arterial blood pressure was examined. This is the first study that investigated the pharmacokinetics and pharmacodynamics of cocaine in the same animals.

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ABBREVIATIONS: CNS, central nervous system; ECF, extracellular fluid; ip, intraperitoneal; AUC, area under the curve; $t_{1/2}$, half life; Cp_{max}, the maximum concentration; all results are presented as mean ± SE.

MATERIALS AND METHODS

Animal Preparation

Male Wistar rats weighing 300–350 gm (Simonsen Laboratories, Gilroy, CA), were used. Surgical preparation was done while the animals were fully anesthetized, under aseptic condition in two phases. Brain surgery for the placement of the microdialysis guide cannula was performed first, followed 5 days later by cannulation of the femoral artery, femoral vein and insertion of the abdominal catheter as described previously in details (4). The only difference in our procedures is that the microdialysis guide cannula was inserted into the nucleus accumbens according to the coordinates of the rat brain atlas (5). Also, an abdominal catheter made of PE-50 tubing was inserted inside the abdominal cavity. The abdominal catheter was pulled under the skin to exit in the back of the neck. The cannulae were passed through a tether attached to an anchor button sutured in the back of the neck, and were connected to a three channel swivel to allow free movement for the rat during the experiment. All animal experiments were performed in compliance with the "Principles of Laboratory Animal Care" (NIH publication #85-23, revised 1985) and all procedures were approved by the institutional animal care and use committee.

Monitoring the Mean Arterial Blood Pressure

The mean arterial blood pressure was measured utilizing a portable physiological monitoring device (Model VSM1, Physio-Control Corp.; Redmond, WA). The femoral artery cannula was connected to a pressure transducer connected to the monitoring device which continuously displays the mean arterial blood pressure. The mean arterial blood pressure was recorded at predetermined time points after drug administration. The pressure transducer was balanced and calibrated periodically to ensure accurate measurements.

Cocaine Pharmacokinetic and Pharmacodynamic Experiment

A group of Wistar rats (n = 12) was used in this study. During the experiment, the animal cage was placed in a quite area and was covered with aluminum foil to minimize external stimulation. The femoral artery cannula was connected to the blood pressure monitoring system. The microdialysis probe effluent was injected into the HPLC immediately after collection, for dopamine analysis until stable basal dopamine concentration was detected (less than 10% difference in dopamine concentration in three consecutive collections). When stable basal dopamine concentration was achieved, a single dose of cocaine (30 mg/kg, ip) was administered via the abdominal catheter. Ten blood samples each of 0.2 ml were collected through the femoral vein cannula over 240 minutes in heparinized tubes containing 20 μl of saturated sodium fluoride solution. The effluent of the microdialysis probe (1 μl/min) was collected every 20 minutes, in vials containing 20 μl of the mobile phase used for dopamine analysis to increase dopamine stability during the collection period. After mixing the vial content, 5 μl were injected immediately into the HPLC for dopamine determination. The rest of the vial content was analyzed for cocaine and its metabolites by HPLC. The blood

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pressure was continuously monitored during the entire experiment.

The brain ECF concentrations of cocaine and its metabolites were calculated after correction for microdialysis probe recovery determined from in vitro experiment (6). The probe recovery is the extent of equilibration of cocaine between dialysate and brain ECF expressed as percent. The average recoveries of the microdialysis probe were 23.1 ± 2.3%, 23.9 ± 2.6%, and 26.9 ± 2.2% for cocaine, norcocaine and benzoylecgonine, respectively. Dopamine microdialysis probe recovery was not determined because the change in dopamine concentration after cocaine administration was expressed as percent change in baseline dopamine concentration. This was calculated from the ratio of the dopamine dialysate concentration after cocaine administration to the baseline dopamine dialysate concentration.

Sample Analysis

Plasma and microdialysis probe effluent were analyzed for cocaine and its metabolites utilizing the HPLC assay developed in our laboratory (7). The microdialysis probe effluent was analyzed for dopamine by HPLC with electrochemical detector utilizing the method described previously (8).

Pharmacokinetic/Pharmacodynamic Analysis

The plasma and brain AUC of cocaine, norcocaine, and benzoylecgonine after cocaine administration were calculated by the linear trapezoidal rule. The E_{max} pharmacodynamic model (9) was used to describe the relationship between cocaine brain ECF concentration and the neurochemical response. The brain ECF concentration was used in this analysis because it is the best estimate for cocaine concentration at its site of action. PCNONLIN was used to determine the pharmacodynamic model parameters. Also, the relationship between cocaine plasma concentration and the change in the mean arterial blood pressure was examined.

RESULTS

After ip administration, cocaine was rapidly absorbed reaching Cp_{max} in 10–15 minutes and its metabolites were detected shortly after administration (Figure 1.A). In the brain ECF, cocaine and its metabolites reached their maximum values at slightly longer time compared to plasma, then the concentrations declined parallel to the plasma profile. However, the relative magnitude of the brain ECF/plasma concentrations of cocaine and its metabolites were different, due to the differences in their brain distribution characteristics (Figure 1.B).

After cocaine administration, dopamine concentration in the nucleus accumbens reached its maximum value in 30 min, then declined back to the baseline at the end of the 4-hour experiment. Dopamine concentration-time profile followed the cocaine concentration-time profile in the nucleus accumbens (Figure 2). Cocaine brain ECF concentration and the corresponding change in dopamine concentration in the nucleus accumbense for each rat were fitted to the E_{max} pharmacodynamic model to estimate the model parameters. The baseline dopamine level was kept constant (with value of 100%). Figure 3 is a representative example of the E_{max} model description of the relationship between cocaine brain ECF concentration and the change in dopamine concentration. In three rats, it was not

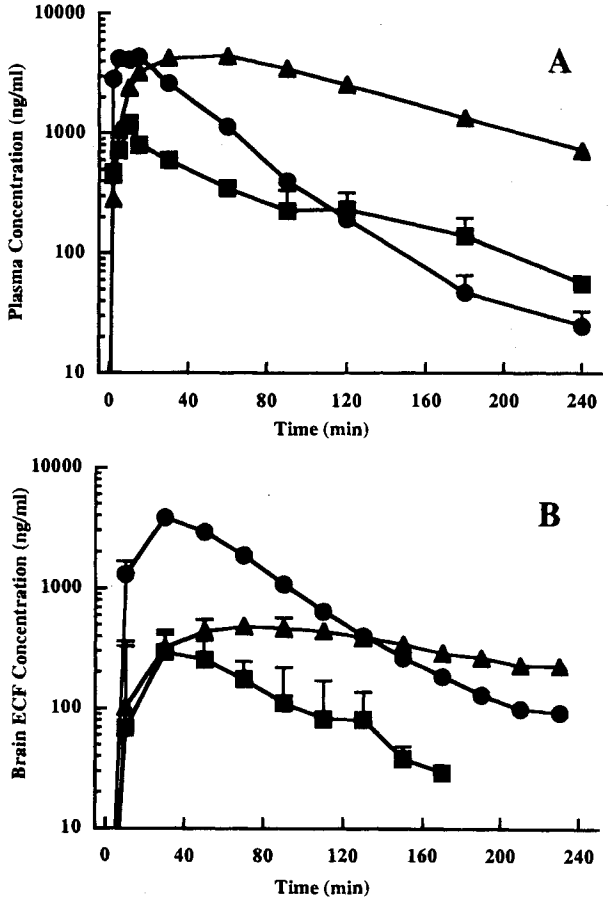

Fig. 1. Plasma (A) and brain ECF (B) concentration-time profiles for cocaine (●), norcocaine (■), and benzoylecgonine (▲) after administration of 30 mg/kg cocaine, ip. The plasma AUC for cocaine, norcocaine and benzoylecgonine were 3450 ± 494 μg-hr/L, 1010 ± 238 μg-hr/L, and 9930 ± 1090 μg-hr/L, respectively, while the brain ECF AUC for cocaine, norcocaine and benzoylecgonine were 4130 ± 644 μg-hr/L, 343 ± 78.3 μg-hr/L, and 1260 ± 230 μg-hr/L, respectively. The terminal half lives for cocaine, norcocaine and benzoylecgonine were 22.8 ± 2.1 min, 54.6 ± 9.3 min, and 64.1 ± 5.5 min, respectively. Results are presented as mean ± SE, n = 12.

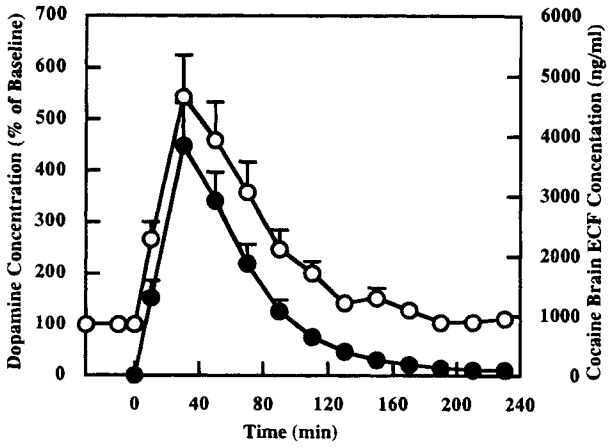

Fig. 2. Brain concentration-time profile of cocaine (●) and percent change in dopamine baseline (○), after administration of 30 mg/kg cocaine, ip. Results are presented as mean ± SE, n = 12.

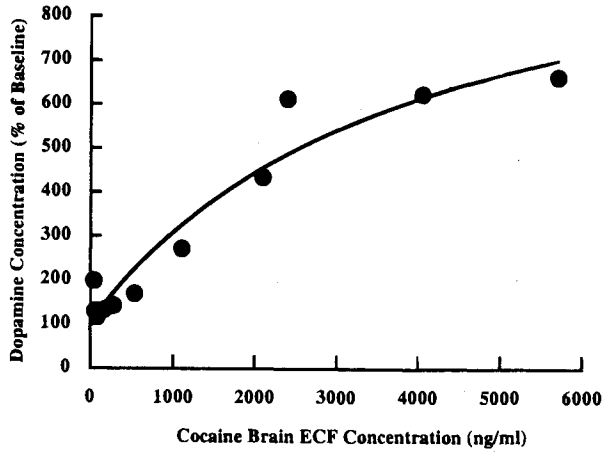

Fig. 3. A representative example of the relationship between percent change in dopamine baseline concentration versus brain ECF cocaine concentration. The solid circles represent the observed values and the line represent the E_{max} model-fitted curve.

possible to obtain precise estimates for E_{max} as indicated by the very large confidence interval around the estimated parameter. The estimated parameters for the E_{max} pharmacodynamic model in the other nine rats were 770 ± 133% and 3380 ± 541 ng/ml for E_{max} and EC_{50}, respectively.

The mean arterial blood pressure reached its maximum value in 15–20 min after cocaine administration, then declined at slower rate compared to cocaine concentrations and did not return back to its baseline value in all rats (Figure 4). When the relationship between plasma cocaine concentration and the change in mean arterial blood pressure was examined an anticlockwise hysteresis loop was observed. Despite this hysteresis, the change in the mean arterial blood pressure followed the plasma concentration-time profile.

DISCUSSION

The animal model used in this study allowed administration of cocaine and determination of plasma and brain concentrations of cocaine and its metabolites in awake freely moving rats. This is in addition to monitoring the neurochemical response and the blood pressure without the need to hold the rat. This was necessary to avoid animal handling which may cause animal excitation leading to changes in dopamine level and blood pressure. This is an excellent model for pharmacokinetic/pharmacodynamic studies specially for centrally acting drugs.

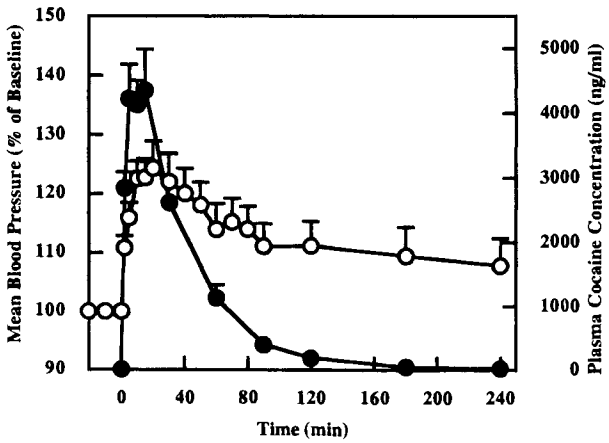

Fig. 4. Plasma concentration-time profile of cocaine (●) and percent change in mean arterial blood pressure (○), after administration of 30 mg/kg cocaine, ip. Results are presented as mean ± SE, n = 12.

Cocaine and its metabolites were distributed rapidly into the brain. The average brain ECF/plasma distribution ratios of cocaine, norcocaine, and benzoylecgonine measured as the corresponding brain ECF/plasma AUC ratios were 1.2, 0.33, and 0.13, respectively. This difference in the brain distribution of cocaine and its metabolites is due to the difference in their lipophilicity which affects their blood brain barrier permeability. This is because the estimated difference in brain distribution cannot be explained only by the difference in the plasma protein binding of these compounds (unpublished data from our laboratory). These results imply that the contribution of cocaine metabolites to the observed CNS effects after cocaine administration may be limited by their lower brain distribution.

This study is the first investigation that examined the pharmacokinetics and pharmacodynamics of cocaine simultaneously in individual rats. Our results showed good correlation between dopamine concentration-time profile and cocaine concentration-time profile in the nucleus accumbens. Although the linear concentration-effect model can reasonably describe the relationship between cocaine concentration and the change in dopamine level as reported previously (10), the E_{max} model provided a better description of this relationship. Examination of the residual plots, and the sum of squared residuals, suggested that the E_{max} model was superior than the linear model in characterizing this concentration-effect relationship in nine out of the twelve rats under investigation. It was not possible to obtain good estimates for the E_{max} model parameters in three rats. In these three rats, good linear relationship existed between the brain ECF cocaine concentration and the change in dopamine concentration. It is possible that all the obtained samples were in the range where dopamine concentrations increase linearly with cocaine concentrations, so it was not possible to obtain good estimates for E_{max}.

Cocaine primarily inhibits the reuptake of dopamine at the nerve endings leading to its accumulation. Our results suggest that as cocaine concentration increases, dopamine reuptake is inhibited resulting in higher dopamine concentration. However at very high concentrations of cocaine when the reuptake process is completely inhibited, dopamine concentrations will reach a plateau that is dependent on the rate of dopamine synthesis, release and metabolism. This is in agreement with the physiological factors that govern dopamine concentration at the nerve endings.

Cocaine plasma concentrations were used to describe the effect of cocaine on the mean arterial blood pressure. This is because the effect of cocaine on the blood pressure is primarily due to cocaine peripheral effects. Examination of the concentration-effect relationship showed anticlockwise hysteresis. This hysteresis may suggest the presence of a slowly equilibrating effect compartment for the effect of cocaine on blood pressure. The contribution of benzoylecgonine, which has been reported to possess vasoconstriction effect (11), to the change in blood pressure after cocaine

administration may be another explanation for this hysteresis. The existence of this hysteresis is not contradictory with the fact that higher cocaine plasma concentrations resulted in higher mean blood pressure. Further investigation of this observation is currently underway.

The results of this investigation indicate that good correlation exists between cocaine pharmacokinetics and pharmacological effects. This means that factors that may affect cocaine absorption, distribution, metabolism and excretion can also alter the duration and/or intensity of cocaine effects. This also emphasizes the contribution of the pharmacokinetic changes to the resulting effects after administration of different cocaine regimens. After repeated administration, the higher plasma and brain cocaine concentrations observed may contribute, at least partially, to the augmented cocaine effects (12). Also, the higher cocaine concentrations achieved after cocaine and alcohol abuse, may explain the more intense and longer lasting euphoric effects described after abusing this drug combination (13,14). Furthermore, conditions which are known to alter the rate of cocaine metabolism such as during pregnancy (15), may increase the susceptibility to cocaine toxicity. Because of its clinical significance, it is important to study the different disease states, drug interactions, and conditions that can alter cocaine pharmacokinetics, and how the changes in cocaine pharmacokinetics are reflected on cocaine pharmacological effects and toxicities. This may help in identifying additional risk factors that increase the susceptibility of cocaine abusers to greater incidence of toxicity.

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