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Behavioral Sensitization to Cocaine in the Absence of Altered Brain Cocaine Levels

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BONATE, P. L., A. SWANN AND P. B. SILVERMAN. *Behavioral sensitization to cocaine in the absence of altered brain cocaine levels.* PHARMACOL BIOCHEM BEHAV **57**(4) 665–669, 1997.—We conducted experiments investigating the role of altered cocaine distribution in behavioral sensitization. The first was designed to determine whether carry-over from one injection to the next occurs after acute cocaine administration. Female, Sprague–Dawley rats were administered 5 mg/kg ^{3}H-cocaine and 24 h later were challenged with either 5 mg/kg unlabeled cocaine or saline. Animals were sacrificed 15 min after drug administration. There was no difference between groups in cocaine levels in brain, liver, or plasma, thus indicating that carry-over did not occur following acute cocaine administration. The second experiment was designed to determine whether bound cocaine could be released following acute or multiple dose cocaine administration. In the acute dose study, animals were administered either 20 mg/kg cocaine or saline, challenged 24 h later with 5 mg/kg ^{3}H-cocaine, and sacrificed 5 min after drug administration. Animals with previous cocaine experience exhibited a significant increase in the number of rearings. The groups did not differ in brain or plasma cocaine levels. In the multiple dose study, animals were injected daily for 4 days with 20 mg/kg cocaine or saline, challenged with 5 mg/kg ^{3}H-cocaine on day 5, and sacrificed 10 min after drug administration. Animals with previous cocaine experience exhibited significantly greater locomotor activity and number of rearings. There was no difference between groups in cocaine levels in various brain regions, plasma, or liver. Brain cocaine content in various regions was significantly correlated, though heterogeneously distributed within the various regions. The highest cocaine levels were found in hippocampus, striatum, thalamus/hypothalamus, and cortex. These results provide further evidence that behavioral sensitization is not the result of cocaine redistribution following repeated administration.

Drug distribution Pharmacokinetics Pharmacodynamics Drugs of abuse Stimulants

REPEATED intermittent administration of cocaine and amphetamine results in a progressive increase in exploratory behavior in animals, a process known as sensitization or reverse tolerance (20,10). Numerous studies have led to the conclusion that sensitization is most likely related to increased dopaminergic transmission following repeated cocaine administration, although the exact mechanism is unknown.

Alternatively, increased response after repeated administration may be due to increased availability of the drug upon subsequent administration (19,11,14). Possible mechanisms for this include:

1. increased absorption from the injection site (15),
2. persistence of drug in the biophase, such as observed with repeated haloperidol administration (3,9), or
3. persistence of drug in peripheral binding sites that may be displaced or mobilized upon subsequent drug administration.

In order to investigate the role of increased brain cocaine content in behavioral sensitization after repeated cocaine administration, we have measured cocaine content in various brain regions under conditions that produce behavioral sensitization and carried out a study to determine whether repeated cocaine administration results in displacement of peripherally bound cocaine.

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METHODS AND MATERIALS

Drugs and Reagents

Drug standards (cocaine hydrochloride, ^{3}H-cocaine, and RTI-31) were obtained courtesy of the National Institute on Drug Abuse (Research Triangle Park, NC). Solvents were at least HPLC grade and inorganic chemicals were reagent grade or better. Drug standards and doses were calculated as the free base. ^{3}H-cocaine was at least 95% pure as determined by HPLC with fraction collection. The major contaminant in the standard was norcocaine based on retention time confirmation and co-chromatography. The radiolabeled cocaine standard was diluted with unlabeled cocaine in 0.1 N hydrochloric acid to generate a solution of 5 mg/kg cocaine.

Subjects

Subjects were female, Sprague–Dawley rats (Harlan Sprague Dawley, Indianapolis, IN) weighing between 200–270 g. They were group housed, 4–5 animals per cage, with a 12-L:12D cycle. Food and water were freely available except that food was made unavailable the night prior to sacrifice because previous research suggested that food delays the intraperitoneal absorption of cocaine and blunts the maximal cocaine plasma concentration. All injections were intraperitoneal.

Behavioral Assessment

Locomotor activity was monitored using a Digiscan Animal Activity Analyzer (Omnitech Electronics, Columbus OH). Animals were placed in a Plexiglas box (16 × 16 × 8 inches) with 12 photocell detectors along 2 perpendicular sides of the box and 12 photocell detectors along the other 2 sides for the measurement of horizontal activity. The beams were placed approximately 2 inches above the floor of the locomotor box. Photocell counts, produced by interruption of the infrared beams, were counted and displayed. The boxes were placed in a hood to minimize outside interference and were isolated from one another by dividers. To maximize the difference between the test cage and home hanging wire cages, wood chips were placed in the bottom of the test cage.

Analysis of Cocaine by HPLC and Liquid Scintillation Spectroscopy

Parent cocaine was analyzed by HPLC with ultraviolet detection (2). The limit of detection (LOD) was 25 ng/ml and 25 ng/g in plasma and brain, respectively, and was linear from 50 to 4000 ng/ml or ng/g, respectively. A modification of the method using a smaller dilution factor decreased the LOD to approximately 5 ng/g in brain.

Due to lack of a validated HPLC method for cocaine in tissues other than brain, cocaine levels were measured in other tissues by radioactivity using a Packard Tri-Carb Liquid Scintillation Counter (Merriden, CT). Counting efficiency was verified by spiking blank plasma, liver, and brain with a known amount of ^{3}H-cocaine. After background correction, quantification using radioactivity offered greater sensitivity than HPLC: the LOD for cocaine in plasma and brain was approximately 10 ng/ml and 10 ng/g, respectively. However, because radioactivity measures parent drug and any metabolites that carry the radiolabel, it suffers from lack of specificity.

Data Analysis

Differences between groups were tested using analysis of variance (ANOVA). When the assumptions of the ANOVA were violated, the Kruskal–Wallis test was substituted. Differences between groups in regional brain cocaine levels were tested using multivariate analysis of variance (MANOVA). Differences between brain regions were tested using Scheffe's test. Data are expressed as mean ± SEM. Radioactivity data was transformed to ng-equivalents of cocaine using the following formula:

$$\frac{\text{ng – equivalent}}{\text{g of tissue or ml}} = \frac{\text{dpm}}{\text{ml or g}} * \text{dilution factor} * \text{specific activity}$$

Test statistics were considered statistically significant if the p-value was less than 0.05.

STUDY 1: IS THERE CARRY-OVER OF COCAINE FROM ONE INJECTION TO THE NEXT?

Study Design

Ten animals were taken to the testing room and weighed. Animals were isolated in individual plastic cages for 30 min prior to injection. Animals were then given 5 mg/kg ^{3}H-cocaine (0.4 μCi) and placed in the locomotor boxes where their locomotor activity was recorded for 30 min. Immediately afterward the animals were returned to their home cage. The next day, animals were randomized into two groups. The control group was administered saline, sacrificed by decapitation 15 min after injection, and whole blood, brain, and liver were collected. The test group was taken to the testing room, weighed, and isolated in individual plastic cages for 30 min. Animals were then given 5 mg/kg unlabeled cocaine and placed in the locomotor boxes where their locomotor activity was recorded for 15 min. Immediately afterward the animals were carried to another room, where they were sacrificed by decapitation and whole blood, brain, and liver removed. Tissues were diluted 1:3 in 0.2 N perchloric acid and radioactivity in a 1.0 g aliquot counted by liquid scintillation spectroscopy. Whole blood was centrifuged and radioactivity in a 1.0 ml aliquot of plasma was counted. Animals in both groups were sacrificed 24 ± 1 h after their initial radiolabeled cocaine challenge.

Results

Figure 1 shows the radioactive cocaine plasma, liver, and brain levels 15 min after the second of 2 cocaine injections spaced 24 h apart. Negligible radioactivity was observed in brain and liver. Plasma levels were very close to the limit of detection and for all practical purposes were undetectable. Using an average weight of 5.0 and 1.7 g for liver and brain, respectively, approximately 310 and 66 ng of cocaine-equivalents was observed in the liver and brain, respectively. This represents less than 0.01% of the injected dose. Because the liver and brain cocaine levels within groups were highly skewed, the Kruskal–Wallis test was used to test for differences between groups. Although brain and liver levels were increased in the group challenged with cocaine 24 h after cocaine pretreatment the difference between groups was not significant. These results suggest that cocaine does not carry-over from one injection to the next.

STUDY 2: CAN BOUND COCAINE BE MOBILIZED FOLLOWING MULTIPLE DOSE COCAINE ADMINISTRATION?

Acute Study

Twenty animals were taken to the test room, weighed, and placed in isolated plastic cages for 30 min. Animals were then

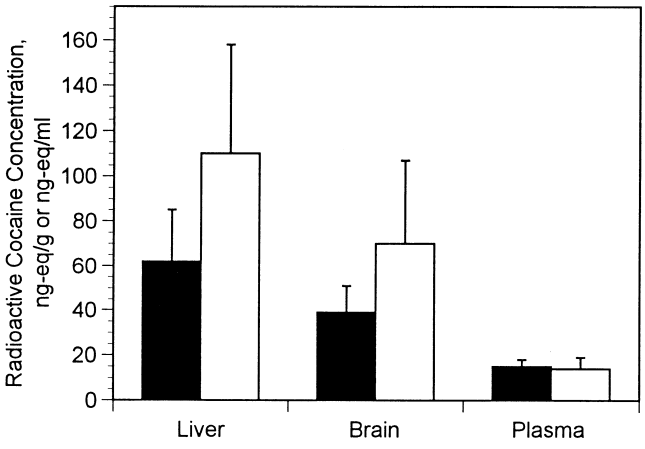

FIG. 1. Cocaine liver, brain, and plasma radioactivity levels 15 min after administration of either 5 mg/kg cocaine (open) or saline (solid). All animals were treated 24 h earlier with 5 mg/kg ^{3}H-cocaine. There was no difference in liver, brain or plasma levels between groups. Error bars are standard error of mean (n = 5/group).

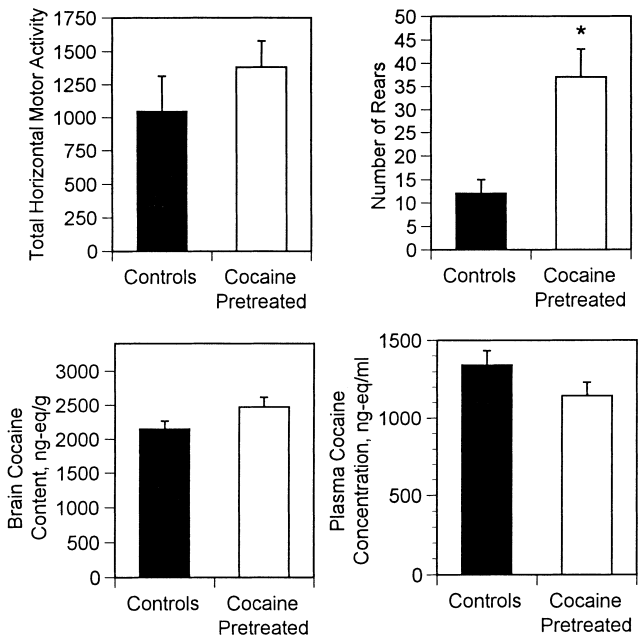

FIG. 2. Total horizontal locomotor activity and number of rears in 5 min after administration of 5 mg/kg ^{3}H-cocaine (top). Animals were treated 24 hours previously with either 20 mg/kg cocaine or saline controls. There was a significant difference in number of rears ($p < 0.001$) in animals with previous cocaine experience, but not in total locomotor activity, compared to saline-treated controls. There was no difference in cocaine brain or plasma levels (bottom). Error bars are standard error of mean (n = 9–10/group).

administered either 20 mg/kg cocaine or saline, immediately placed in the locomotor cage for 30 min, and then returned to their home cage. The next day animals were taken to the test room, weighed, and placed in isolated plastic cages for 30 min. They were then administered 5 mg/kg ^{3}H-cocaine (0.4 μCi) and placed into the locomotor boxes. Five min after drug administration, the animals were sacrificed and whole blood and brain removed. In Experiment 1 quenching had occurred because brains were homogenized in perchloric acid. To minimize quenching in this study, brain tissue was homogenized in the buffer recommended by Bonate et al. (2). Radioactivity in 1.0 g of brain homogenate and 1.0 ml of plasma was counted. Brain homogenate was assayed for parent cocaine using HPLC with UV detection.

Multiple Dose Study

The multiple dose study was similar to the acute study. Sixteen animals were given either 20 mg/kg cocaine or saline daily for 4 days. On the fifth day animals were injected with 5 mg/kg ^{3}H-cocaine (0.4 μCi). In the acute study, increased locomotor activity was not observed in animals with previous cocaine experience, possibly as a result of the short (5 min) interval between injection and sacrifice. Therefore in the multiple dose study animals were sacrificed 10 minutes after the final injection of radiolabeled cocaine in an attempt to further delineate behavioral differences between groups. Immediately after sacrifice the brain was dissected into the striatum, cerebellum, hippocampus, thalamus/hypothalamus, midbrain, hindbrain, and cortex using the modified method of Glowinski and Iversen (7). Radioactivity in each brain region was counted. Liver was removed, diluted 1:3 with 100 mM potassium phosphate monobasic/0.5% sodium fluoride, pH 4.5, and radioactivity in 1.0 g was counted.

Results

Figure 2 shows the plasma and brain cocaine levels 5 min after the second of 2 cocaine injections spaced 24 h apart. Figure 2 also shows the horizontal locomotor activity and number of rearings in each group. Animals with previous cocaine experience had greater exploratory activity than controls as evidenced by an increased number of rearings ($F(1, 17) = 13.81, p < 0.001$) in the cocaine treated group, thus suggesting that behavioral sensitization had occurred in the cocaine treated group. There was no difference in total locomotor activity between groups nor were there significant differences in brain or plasma cocaine levels between groups. There was a marginally significant correlation between whole brain cocaine levels and number of rears (Pearson's $r = 0.48$, $p < 0.048$), whereas the correlation between whole brain levels and locomotor activity was not significant. Also, there was a significant increase in the brain to plasma ratio ($p < 0.05$) in cocaine treated animals (2.3 ± 0.2) compared to saline treated animals (1.7 ± 0.15). HPLC analysis of the whole brain homogenates revealed that all of the radioactivity could be accounted for by parent cocaine and was not due to the presence of any radiolabeled metabolites such as norcocaine or benzoylecgonine.

Figure 3 shows the cocaine concentration in each brain region, in plasma, and in liver after repeated cocaine administration, along with total horizontal locomotor activity and number of rears. Animals with previous cocaine experience had greater exploratory activity than controls as evidenced by both total locomotor activity ($F(1, 11) = 20.1, p < 0.001$) and number of rears ($F(1, 11) = 29.9, p < 0.001$), thus indicating behavioral sensitization. No difference between groups was observed in plasma or liver cocaine levels.

Although there was good correlation in cocaine content among brain regions (range: 0.94–0.99, $p < 0.001$), MANOVA revealed that cocaine was heterogeneously distributed in the

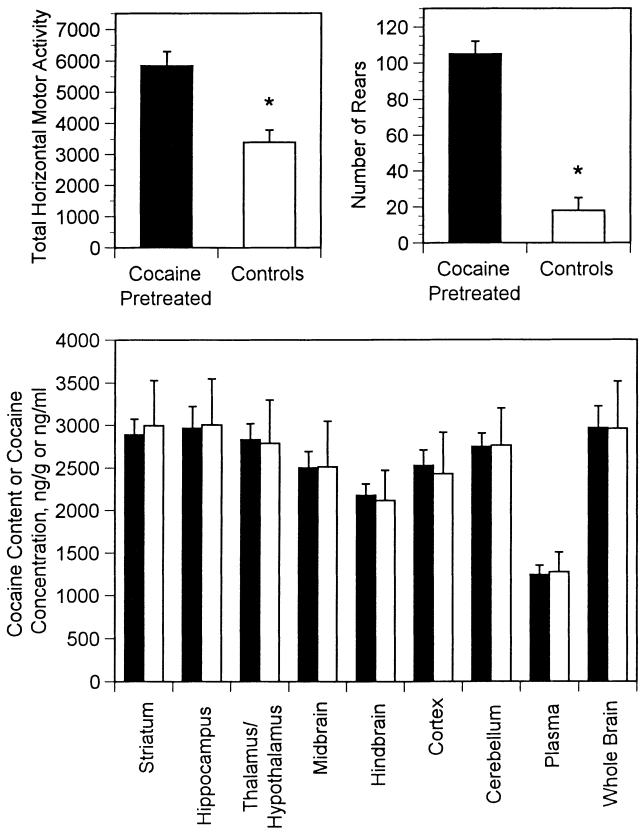

FIG. 3. Total horizontal locomotor activity and number of rears in 10 min after administration of 5 mg/kg ^{3}H-cocaine (top). Animals were treated daily for 4 days with either 20 mg/kg cocaine (open) or saline (solid). There was a significant difference in locomotor activity ($p < 0.001$) and number of rears ($p < 0.001$) in animals with previous cocaine experience compared to saline-treated controls. There was no difference between groups in regional cocaine brain levels, plasma, or liver levels (bottom). Error bars are standard error of mean (n = 7–8/group).

brain (Wilks' $\Lambda = 0.0179$, $F(6, 66) = 54.7$, $p < 0.0001$). Using Scheffe's test, there was no difference in cocaine levels in hippocampus, striatum, thalamus/hypothalamus, and cortex. Significantly less cocaine was found in hindbrain ($p < 0.01$), midbrain ($p < 0.05$), and cerebellum ($p < 0.01$). Plasma cocaine levels were significantly correlated with brain cocaine levels (range: 0.64–0.73, $p < 0.02$). No significant correlation was observed between brain cocaine levels and locomotor activity or between brain cocaine levels and number of rears. No difference was observed in the brain to plasma cocaine ratio between cocaine pretreated and saline pretreated rats. These results suggest that bound cocaine is not mobilized or released by subsequent cocaine administrations.

DISCUSSION

The results indicate that sensitization occurred following repeated cocaine administration but alterations in cocaine brain levels did not. Due to differences in time of sacrifice it is difficult to make comparisons between the studies. In Study 1, carry-over of cocaine did not occur from one injection to the next. In study 2, bound cocaine did not appear to be mobilized or released following acute or multiple dose cocaine administration. The results of this study indicate that behavioral sensitization occurs in the absence of altered brain cocaine levels suggesting that sensitization is independent of cocaine distribution.

Previous studies have reported that repeated cocaine administration could result in increased cocaine levels compared to an acute administration (8,17,18,19). In contrast, none of our studies demonstrated increased brain cocaine levels following either acute or multiple dose cocaine administration. Two possibilities may explain this discrepancy. First, the dose of cocaine used in our study is at least 50% less than the dose used in other studies. It may be that the pharmacokinetics of cocaine are nonlinear such that multiple administration of high cocaine doses results in increased brain cocaine levels whereas multiple administration of low cocaine doses does not.

Common mechanisms of nonlinearity include saturable protein binding or saturable tissue binding. Since it is generally believed that only unbound drug penetrates the blood-brain barrier, it follows that an increase in the unbound drug fraction after saturation of peripheral binding sites may increase the amount of drug in the brain to maintain equilibrium. Parker et al. (16) showed that in human serum free cocaine levels significantly increase when serum cocaine concentrations are greater than 1000 ng/ml. In fact, an increase from 1000 ng/ml to 3000 ng/ml resulted in a 40% increase in the free fraction. It is easy to conceive a scenario wherein the first and subsequent cocaine administrations saturate peripheral binding sites such that the final challenge dose results in an increase in the free fraction. Thus, in humans the possibility exists that disproportionately more cocaine may reach the brain after administration of higher doses than lower doses, if the corresponding drug concentrations are greater than the plasma protein saturation point. The question remains "at what level do cocaine levels saturate protein binding sites in the rat"?

Alternatively, our study used female rats whereas previous studies used male rats. It is widely recognized that the pharmacokinetic disposition of many drugs differs between females and males due to many factors, including alterations in metabolism and protein binding (1). It may be that female rats have either greater protein binding capability at a given cocaine concentration or have a higher saturation point than males. Thus females may require more cocaine to saturate peripheral binding sites than males with less cocaine available for diffusion into the brain. Behavioral data do not support this hypothesis; female rats tend to sensitize more readily than male rats (6) suggesting that, if anything, they may have greater free cocaine levels than males.

The brain to plasma ratio in animals pretreated with a single cocaine injection was elevated compared to saline controls but was not elevated following multiple dose cocaine pretreatment. Pharmacokinetic studies have shown that it is brain to plasma partition coefficient, not the observed brain to plasma ratio, which is an indicator of the degree of binding between a tissue and plasma (5). The brain to plasma partition coefficient is a constant, specific for a drug and the brain, whereas the brain to plasma ratio is a nonlinear function of many variables, including time, organ blood flow and the brain partition coefficient (4). It is only when an animal is dosed via continuous infusion and steady-state is reached, that the brain to plasma partition coefficient equals the brain to plasma ratio. Whether an increase in the brain to plasma ratio results in an increase in the brain to plasma partition coefficient is unclear in the absence of brain blood flow data. Hence, alterations in

the observed brain to plasma ratio can not be interpreted physiologically. The increase in brain to plasma ratio is apparently transient and not maintained upon multiple administration and/or is increased in the first 5 min after drug administration, but returns to control values within 10 min. Future studies are needed to further define the temporal characteristics of the brain to plasma ratio.

Animals pretreated with either warfarin (21) or the angiotensin converting enzyme inhibitor, enalaprilat (22) demonstrate elevated drug levels upon repeated administration. Levy (12) proposed that this could result from high-affinity, low-capacity binding in tissues whereupon subsequent drug administration results in altered drug distribution. Levy further speculated that drug redistribution might lead to an "unexpected and undesired resurgence of a pharmacologic effect", such as LSD flashbacks. Nayak et al. (13) demonstrated persistent radioactivity in liver and kidney of rats administered radiolabeled cocaine 24 hours earlier. We therefore hypothesized that repeated cocaine administration was either mobilizing this bound form of cocaine from liver to plasma, and subsequently to brain, or that once saturation of liver and/or fat binding sites was complete, subsequent drug administration would result in higher brain levels. Either effect could result in behavioral sensitization. Our results suggest that neither occurs. Although there was radioactivity in liver and brain 24 hours following administration of radiolabeled cocaine, there was no increase in brain radioactivity after challenge with unlabeled cocaine. This suggests that bound cocaine is not mobilized by subsequent cocaine administration.

In summary, our results suggest that changes in cocaine brain levels as a result of repeated administration are minimal and that sensitization is not the result of such changes. Future studies should be done studying the linearity of cocaine pharmacokinetics in rats and whether differences occur between males and females in cocaine's pharmacokinetics.

REFERENCES

1. Bonate, P. L.: Gender-related differences in drug metabolism. J. Clin. Pharmacol. 31:684–690; 1991.
2. Bonate, P. L.; Davis, C. M.; Silverman, P. B.; and Swann, A.: Analysis of cocaine in biological matrices: application to plasma and brain tissue. J. Liquid Chrom. 18:3473–3494; 1995.
3. Campbell, A.; Baldessarini, R. J., Teicher, M. H., Kula, N. S.: Prolonged antidopaminergic actions of single doses of butyrophenones in the rat. Psychopharmacol. 87:161–166; 1985.
4. Chen, H. S.; Gross, J. F.: Estimation of tissue-to-plasma partition coefficients used in physiological pharmacokinetic models. J. Pharmacokin. Biopharm. 7:117–125; 1979.
5. Gibaldi, M.; Perrier, D.: Pharmacokinetics, second edition. Marcell Dekker, New York, 1982.
6. Glick, S. D.; Hinds, P. A.: Sex-differences in sensitization to cocaine-induced rotation. Eur. J. Pharmacol. 99:119–121; 1984.
7. Glowinski, J.; Iversen, L. L.: Regional studies of catecholamines in the rat brain - I. The disposition of [^{3}H]-norepinephrine and [^{3}H]-DOPA in various regions of the brain. J. Neurochem. 13: 655–669; 1966.
8. Ho, B. T.; Taylor, D. L.; Estevez, V. Z.; Englert, L. F.; Mc Kenna, M. L.: Behavioral effects of cocaine - metabolic and neurochemical approach. In: Cocaine and Other Stimulants (Eds: E.H. Ellinwood and M.M. Kilbey). Plenum Press, New York, 1979.
9. Hubbard, J. W.; Ganes, D.; Midha, K. K.: Prolonged pharmacologic activity of neuroleptic drugs. Arch. Gen. Psychiatry 44:99–100; 1987.
10. Kalivas, P. W.; Stewart. J.: Dopamine transmission in the initiation and transmission of drug- and stress-induced sensitization of motor activity. Brain Res. Rev. 16:223–244; 1991.
11. Kuhn, C. M.; Schanberg, S. M.: Metabolism of amphetamine after acute and chronic administration in the rat. J. Pharmacol. Exp. Ther. 207:544–554; 1978.
12. Levy, G. : Pharmacologic target-mediated drug disposition. Clin. Pharmacol. Ther. 56:248–252; 1994.
13. Nayak, P. K.; Misra, A. L.; Mule, S. J.: Physiological disposition and biotransformation of [^{3}H]-cocaine in acutely and chronically treated rats. J. Pharmacol. Exp. Ther. 196:556–569; 1976.
14. Numachi, Y.; Yoshida, S.; Inosaka, T.; Sato, M.: Changes of drug distribution in the methamphetamine sensitized rat brain. Clin. Neuropharmacol. 15 Suppl 1A, 644A–645A; 1992.
15. Pan, H.-T.; Menacherry, S.; Justice, J. B.: Differences in the pharmacokinetics of cocaine in naive and cocaine-experienced rats. J. Neurochem. 56:1299–1306; 1991.
16. Parker, R. B.; Williams, C. L.; Lazure, S. C.; Lima, J. J.: Factors affecting serum protein binding of cocaine in humans. J. Pharmacol. Exp. Ther. 275:605–610; 1995.
17. Pettit, H. O.; Pan, H-T.; Parsons, L. H.; Justice, J. B.: Extracellular concentrations of cocaine and dopamine enhanced during chronic cocaine administration. J. Neurochem. 55:698–804; 1990.
18. Pettit, H. O.; Pettit, A. J.: Disposition of cocaine in blood and brain after a single pretreatment. Br. Res. 651:261–268; 1994.
19. Reith, M.; Benuck, M.; Lajtha, A.: Cocaine disposition in the brain after continuous or intermittent treatment and locomotor stimulation in mice. J. Pharmacol. Exp. Ther. 243:281–287; 1987.
20. Robinson, T. E.; Becker, J. N.: Enduring changes in brain and behavior produced by chronic amphetamine administration: a review and evaluation of animal models of amphetamine psychosis. Brain Res. Rev. 11:157–198; 1986.
21. Takada, K.; Levy, G.: Comparative pharmacokinetics of coumarin anticoagulants XLIII: dose dependent pharmacokinetics of warfarin in rats. J. Pharm. Sci. 69: 9–14; 1980.
22. Till, A. E.; Gomez, H. J.; Hichens, M.: Pharmacokinetics of repeated single oral doses of enalapril maleate (MK-421) in normal volunteers. Biopharm. Drug Disp. 5:273–280; 1984.