Pharmacology Biochemistry & Behavior, Vol. 36, pp. 699–701. © Pergamon Press plc, 1990. Printed in the U.S.A. 0091-3057/90 $3.00 + .00

BRIEF COMMUNICATION

Correlation Between Cocaine-Induced Locomotion and Cocaine Disposition in the Brain Among Four Inbred Strains of Mice

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WIENER, H. L. AND M. E. A. REITH. *Correlation between cocaine-induced locomotion and cocaine disposition in the brain among four inbred strains of mice.* PHARMACOL BIOCHEM BEHAV **36**(3) 699–701, 1990.—BALB/cByJ, C57BL/6ByJ, CXBH/By, and CXBK/By mice differed in their locomotor response to cocaine measured 1–10 min after administration of 25 mg/kg IP of the compound. These differences were paralleled by differences in the disposition of cocaine (measured at 12 min) in the brain. Among all individual animals taken together, there was a significant correlation between locomotor stimulation and the brain concentration of cocaine. These results suggest that the differences between strains in their locomotor responsiveness to cocaine are determined, in part, by the disposition of cocaine in the brain following IP administration of cocaine.

Cocaine Locomotion Drug disposition Mice Inbred strains

GENETIC factors can play a role in the effects of psychostimulant drugs. Thus, different inbred strains of mice, each with their own homogeneous genetic material, display different behavioral sensitivities to d-amphetamine (8), phencyclidine (6,15), and cocaine (12,14). In three of the above studies (6, 8, 14) the same recombinant inbred strains (derived from BALB/cByJ and C57BL/6ByJ mice) were studied, and the distribution of the locomotor responsiveness among the strains was different for d-amphetamine, phencyclidine, and cocaine. Because the stimulation of locomotion by both d-amphetamine and cocaine involves dopaminergic systems (7,13) [for phencyclidine in mice this is still under debate (5)], the different patterns of locomotor stimulation among strains for the two drugs may implicate genetic differences beyond the dopaminergic system. One possible candidate responsible for such differences is the pharmacokinetics of the drugs involved. This is potentially important in the case of cocaine because there is a correlation between locomotor stimulation and brain cocaine level (1,9). In the present study, cocaine-induced locomotor stimulation and brain cocaine levels were measured in four inbred mouse strains: BALB/cByJ, C57BL/6ByJ, and two recombinant strains derived from these progenitors, CXBH/By and CXBK/By. The data were analyzed for strain differences and for correlation between locomotion and brain cocaine concentration among individual animals. This study focused on the 12-min period after cocaine injection because it encompasses peak brain cocaine concentrations after injection (1,9). Furthermore, brain levels of only cocaine were considered because the cocaine metabolites benzoylecgonine, ecgonine and ecgonine methylester are inactive towards the dopamine carrier (11), and the N-demethylated metabolite, norcocaine, reaches concentrations in the brain less than 20% of those of cocaine after IP administration of cocaine (1,2). A dose of 25 mg/kg of cocaine (IP) was used to obtain sufficient but not maximal stimulation; at this dose of cocaine, the locomotor effect is not yet at its maximum as determined in BALB/cBy mice (10) and C57BL/6ByJ mice (submitted).

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METHOD

Animals and Drugs

The experiments were conducted in the Institute on Ward's Island. Male BALB/cByJ and C57BL/6ByJ mice were obtained from the laboratory's own breeding colonies derived from the stocks of Jackson Laboratories (Bar Harbor, ME). Male CXBH/By and CXBK/By mice were purchased from Jackson Laboratories. Animals weighed between 21 and 33 g, and were 11–12.5 weeks old. Prior to inclusion in the experiment, all animals were held for at least one week on a 12-hr light/dark cycle (7 a.m./7 p.m.), with food and water available ad lib.

Cocaine hydrochloride (Sigma, St. Louis, MO) was dissolved in saline and injected IP in a volume of 0.15 ml per 20 g body weight.

Spontaneous Locomotor Activity and Cocaine Determination

Mice were housed in individual plastic cages (27 × 17 × 12 cm) from one day before testing in the same darkened room in which the behavioral measurements were made at 22–24°C. Behavioral testing was performed as described previously (10) between 9 a.m. and 5 p.m. Shades were drawn to reduce the light from the windows, and no artificial light was used. For each animal, a session started with the removal of the cage lid (along with food and water), injection of cocaine hydrochloride (25 mg/kg IP), and covering the cage with a flat lid without food and water. The cage was then placed in an Opto-Varimex-Minor activity monitor (Columbus Instruments, Columbus, OH). All interruptions of the left to right and front to back infrared beams (3 cm spaced from each other) were counted and totaled for a 9-min period (from 1 to 10 min after injection of cocaine). Twelve min after injection the animals were decapitated; the cerebral cortex was dissected, weighed (on the average 130 mg), and placed in 1.13 ml of ethanol. Tetracaine hydrochloride (Sigma) was added as an internal standard (final concentration 10 μM), and the samples were subjected to sonication and were centrifuged. A 20-μl aliquot was injected onto a Versapack C-18, 10-μm column, 300 × 4.1 mm (Alltech Associates; Deerfield, IL). Elution was performed at room temperature with an isocratic mobile phase consisting of 0.25 M potassium phosphate buffer (pH 2.7) containing 25% (v/v) acetonitrile. The flow rate was 1 ml/min and the absorbancy at 235 nm was monitored. The retention times for cocaine and tetracaine were 4.8 and 8.0 min, respectively. Quantitation was based on peak height or area relative to that of standards, estimated with the Hewlett Packard 3392A integrator, corrected for recovery of the internal standard, tetracaine (on the average 97%). Other details of the procedures used are as described by Benuck *et al.* (3).

Data Analysis

Locomotor data were subjected to logarithmic transformation for homogeneity of variance prior to ANOVA or correlation analysis with two-tailed p values. The accepted level of significance was 0.05. The data of Fig. 2 were analyzed in three ways. First, locomotor data were tested for strain differences by two-way ANOVA with the strain as factor A and the locomotor activity in 3-min blocks as factor B. Second, brain cocaine levels were tested for strain differences by one-way ANOVA. Third, 9-min locomotor scores and brain cocaine levels were analyzed together in a two-way ANOVA with the strain as factor A and the measured attribute (locomotor score or brain cocaine) as factor B; for this purpose the locomotor counts for each individual animal were expressed in the units of the y-axis representing the brain cocaine concentration, i.e., the locomotor data and cocaine levels were compared exactly as shown in Fig. 2.

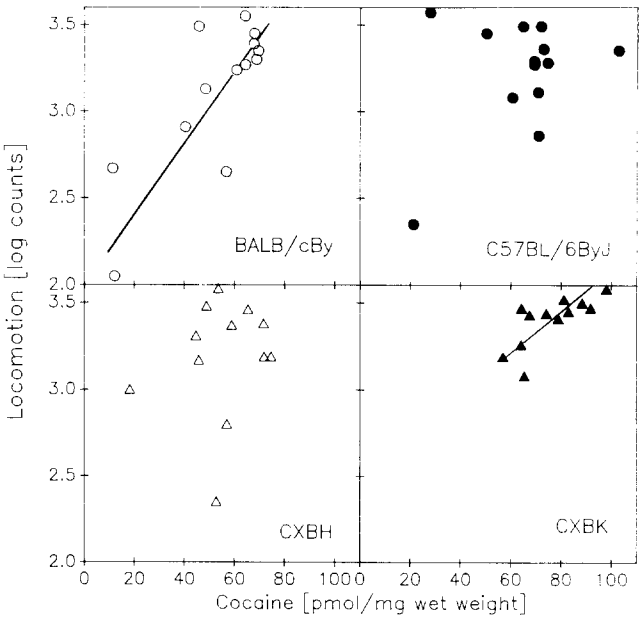

FIG. 1. Correlation between locomotor activity and cocaine level in brain. Animals were injected with cocaine (25 mg/kg IP) and monitored for locomotor activity between 1 and 10 min after injection. At 12 min, animals were decapitated and the cerebral cortex was dissected for determination of cocaine. Each point represents one animal. Straight lines are linear regression estimates. For correlation coefficients see the Results and Discussion section.

RESULTS AND DISCUSSION

Individual Animals

There was a significant correlation between the locomotor stimulation after injection of cocaine and the brain cocaine concentration among individual BALB/cByJ mice ($r = .78$; $n = 13$; $p < 0.001$) and CXBK/By mice ($r = .73$; $n = 12$; $p < 0.01$) (Fig. 1). No significant correlation was observed for animals of the C57BL/6ByJ strain ($r = .35$; $n = 13$) or CXBH/By strain ($r = .19$; $n = 12$) (Fig. 1). When animals of all strains were taken together, the correlation was significant ($r = .57$; $n = 50$; $p < 0.001$). Thus, in consonance with our previous work on BALB/cByJ mice (1,9), the concentration of cocaine in the brain is a factor in determining the locomotor stimulation.

Comparison of Strains

There was a significant difference between strains in locomotor activity [$F(3,138) = 4.16$, $p = 0.007$; two-way ANOVA with strain as factor A and locomotion in 3-min blocks as factor B] and also in brain cocaine levels [$F(3,46) = 4.41$, $p = 0.008$; one-way ANOVA] (Fig. 2).

The differences between the four mouse strains in cocaine-induced locomotor stimulation were paralleled by the differences in brain cocaine concentrations (Fig. 2). When locomotor activity and brain cocaine concentration were compared as shown in Fig. 2 in a two-way ANOVA (strain as factor A and measured attribute, locomotion or brain cocaine, as factor B), there was again a significant difference between strains, $F(3,96) = 2.98$, $p = 0.035$, but not between locomotor activation and brain cocaine concentration, $F(1,96) = 0.43$, $p = 0.51$. Moreover, there was no interaction between strains and the two measures locomotion and brain cocaine, $F(3,96) = 0.03$, $p = 0.99$. These results are consonant

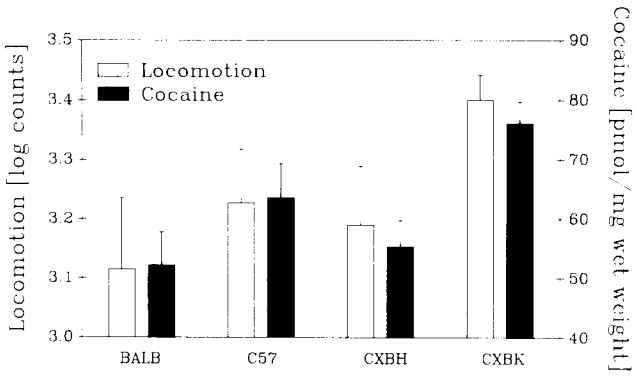

FIG. 2. Comparison of locomotor activity and brain cocaine in BALB/cByJ (BALB), C57BL/6ByJ (C57), CXBH/By (CXBH) and CXBK/By (CXBK) mice. Procedures were as in Fig. 1. Results are mean ± S.E.M. (vertical bar) for 12–13 animals. For ANOVA see the Results and Discussion section.

with the suggestion that the differences between strains in their locomotor responsiveness to cocaine are determined, in part, by the disposition of cocaine in the brain following IP administration of cocaine.

Strains and Cocaine Pharmacokinetics

Differences between mouse strains in their locomotor response to cocaine have been attributed mostly to differences in the responsiveness to cocaine. Recent evidence indicates that mouse strains (C57BL/6Ibg, DBA/2Ibg, C3H/2Ibg, and BALB/cByJ) can indeed differ in their sensitivity towards the inhibitory effect of cocaine on neuronal uptake of [^{3}H]norepinephrine, [^{3}H]dopamine, and [^{3}H]serotonin (4). It has been suggested that differences in the locomotor effects of psychostimulant drugs among strains are attributable to a more general effect: strains with a relatively lower baseline of spontaneous locomotor activity respond to the drug with a greater amount of locomotion, i.e, there is an inverse relationship between baseline locomotion and drug-induced locomotion (15). In a separate study, we have indeed observed rather low baseline activities of C57BL/6ByJ mice compared with BALB/cByJ mice, whereas the locomotor response to cocaine shows the opposite trend (Fig. 2) (unpublished observations). Attempts at expressing locomotor stimulation by cocaine relative to the baseline were unsuccessful because of the large variability introduced by relating an appreciable number of locomotor counts following cocaine to a very small number of counts of predrug baseline activity. The underlying mechanism for a relationship between baseline activity and psychostimulant-induced locomotion is unknown. Differences between strains in their locomotor response to stimulants must involve other variables as well. If baselines were the sole determinants, how could we explain the different pattern of cocaine- (14) and d-amphetamine-(8) induced locomotor activation observed among mouse strains of the same recombinant inbred system derived from the progenitors BALB/cByJ and C57BL/6ByJ lines? One variable to be considered is the pharmacokinetics of cocaine. A recent study concluded that this possibility was unlikely for the differences in cocaine sensitivity observed among C57BL/6Ibg, DBA/2Ibg, C3H/2Ibg, and BALB/cByJ mice (12). However, the small number of animals used in that study for the determination of brain cocaine ($n = 3–4$) does not constitute sufficient evidence to discard a role of cocaine pharmacokinetics. The present study indicates that such a role should be considered seriously. Of course, the results of the present study do not rule out the involvement of variables other than cocaine pharmacokinetics in observed strain differences in cocaine sensitivity.

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