Cocaine Sensitivity in Borna Disease Virus-Infected Rats

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SOLBRIG, M. V., G. F. KOOB AND W. I. LIPKIN. Cocaine sensitivity in Borna disease virus-infected rats. PHARMACOL BIOCHEM BEHAV 59(4) 1047–1052, 1998.—Borna disease virus (BDV) is a neurotropic RNA virus that infects warm-blooded animals to cause disturbances of movement and behavior. Studies in infected rats have demonstrated behavioral sensitivity to direct and indirect dopamine (DA) agonists; however, behavioral responses to an indirect DA agonist with a pure presynaptic effect have not been analyzed. Rats infected with BDV had an enhanced response to the locomotor, behavioral, and convulsant effects of cocaine at intraperitoneal doses of 7.5, 15, and 30 mg/kg. The basis for this sensitivity was examined by striatal DA uptake site and D₁ and D₂ receptor autoradiography. DA uptake sites, labeled with [3H]mazindol, were reduced in medial caudate-putamen (CP), and binding of [3H]raclopride to D₂ sites was reduced in medial and ventral striatal areas. The topography of DA uptake and D₂ site loss corresponds to the distribution of BDV viral nucleic acids in CP and overlays the medial striatal areas that function in conditioned reward. The BDV-infected rat provides a model of cocaine sensitivity based on viral central nervous system infection and may have relevance for studies of cocaine abuse in the context of other viral encephalopathies, such as those associated with HIV infection. © 1998 Elsevier Science Inc.

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BORNA disease (BD) Virus (BDV) is a neurotropic negative strand RNA virus that causes movement and behavior disorders in a wide range of animal species (25,27,34). A pathogenic role for BDV in human disease has not been confirmed; however, serum antibodies to viral proteins have been found in patients with schizophrenia and affective disorders (6,11,31,37) and BDV nucleic sequences were recently amplified from peripheral blood mononuclear cells of neuropsychiatric subjects by RT-PCR (6,19). Experimentally infected rats (BD rats) have hyperactivity, dyskinesias, and stereotyped behaviors (34) and are the most frequently used model system for studies in BD pathogenesis. BD rats have enhanced behavioral responsiveness to dopamine (DA) agonists (34), increased nigrostriatal (34), and prefrontal cortical (35) DA turnover, and decreased numbers of striatal D₂ receptors (34), and reduced nucleus accumbens D₁ receptor radioligand binding (36). To determine whether defects in the high-affinity DA reuptake system may also play a role in expression of the BD behavioral syndrome, we examined the behavioral effects of cocaine, a drug that potentiates DA neurotransmission by binding to DA transporter and inhibiting reuptake of synaptic DA (30), and quantitated numbers of DA reuptake sites in caudate-putamen (CP) using [3H]mazindol.

METHOD

Infection of Animals

Under metofane inhalation anesthesia, 54 1-month-old male Lewis rats (Charles River Labs, Wilmington, MA) were inoculated intracerebrally (IC) into the right lateral ventricle with either 1.6 × 10⁴ tissue culture infectious dose units of BDV (BD rats) or phosphate-buffered saline (PBS, normal control, NL rats) in a total volume of 30 μL. Virus stock was a 10% wt/vol BD rat brain homogenate in PBS (9). Infection was confirmed by the appearance of a clinical syndrome consistent with BD and the presence of serum antibodies reactive with viral proteins by Western immunoblot. Animals were tested or sacrificed 15, 30, or 45 days after IC inoculation with either virus or PBS.

Drugs

Each experimental group consisted of eight animals 45 days post-IC infection. Cocaine HCl (Sigma, St. Louis, MO) dissolved in saline was used at doses of 0, 7.5, 15, and 30 mg/kg intraperitoneal (IP). One of three doses of test drug or its vehicle control was given to each animal on four successive days.

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according to a Latin square design to control for conditioning and order effects.

**Behavior Analysis**

Behavior was monitored continually for 90 min in 40 \( \times \) 25 \( \times \) 20 cm cages with two equally spaced horizontal beams across the long axis. Locomotor activity was quantified by numbers of crossovers (the successive interruption of the two photobeams). At 10-min intervals stereotyped behavior was scored according the method of MacLennan and Maier (26) by an experienced observer blind to drug dose. Photocell beam interruptions and crossovers were analyzed by using a repeated measures analysis of variance (ANOVA) design: infected or noninfected groups formed the independent factor, dose and time were the repeated measures. Subsequent group effects were analyzed with simple main effects (39). Significance was taken at \( p < 0.05 \). Observational data was analyzed using the Information Statistic (21).

**Quantitative Receptor Autoradiography**

Forty-five days after IC inoculation, metofane-anesthetized BD and NL rats were perfused with cold PBS followed by 0.1% paraformaldehyde to limit infectivity and maintain tissue integrity (23). Comparable specific binding values for 0.1% paraformaldehyde-fixed and unfixed tissue were verified for each radioligand.

Twenty-micron coronal sections were collected onto gelatin-subbed slides through the CP at Bregma \(-1.04\) mm according to atlas coordinates of Paxinos and Watson (28). DA uptake sites were detected by incubating slide-mounted tissue sections with 8.0 nM \([3H]\) mazindol (New England Nuclear, Boston, MA) in the presence of 300 nM DMI (Sigma). Non-specific binding was defined by incubating adjacent sections with 30 \( \mu \)M benztropine (33).

Slide-mounted tissue sections were incubated with 2 nM \([3H]\) SCH23390 (New England Nuclear) in the presence of 1 \( \mu \)M mianserin (Sigma) to detect D\(_1\) receptors. D\(_2\) receptors were detected with 1 nM \([3H]\) raclopride (New England Nuclear). Ligand concentrations approximately equal to the \( K_d \) for each receptor type were used. Nonspecific \( D_1 \) binding was defined by incubating adjacent sections with 30 \( \mu \)M benztprine (33).

Tissue sections from BD and NL animals were processed simultaneously, then apposed to \([3H]\)-sensitive film (Amersham, Arlington Heights, IL). Autoradiograms were subjected to densitometry using a computer-based image analysis system (MCID, Imaging Research Inc., St. Catherine, Ontario, Canada). Measurements were

**TABLE 1**

| Specific Binding of High-Affinity DA Uptake Sites to [H] Mazindol in Caudate Putamen and Each of Its Four Subregions in Normal (NL) and Borna Disease Virus-Infected (BD) Rats |
|---|---|---|
| Site | NL (\( n = 5 \)) | BD (\( n = 6 \)) |
| CP | 205.62 \( \pm \) 18.40 | 198.55 \( \pm \) 17.80 |
| DM | 213.50 \( \pm \) 23.11 | 139.81 \( \pm \) 10.69* |
| DL | 235.15 \( \pm \) 16.10 | 219.91 \( \pm \) 9.93 |
| VM | 206.84 \( \pm \) 22.95 | 152.85 \( \pm \) 26.52 |
| VL | 198.05 \( \pm \) 11.87 | 176.18 \( \pm \) 17.07 |

Specific binding values in fmol/mg of wet weight of tissue, mean \( \pm \) SEM. Caudate putamen, CP; subregions of CP: dorsomedial, DM; dorsolateral, DL; ventromedial, VM; ventrolateral, VL.

* \( p < 0.05 \) (t-test), significantly different from normal rat group.
FIG. 2. DA uptake site labeling, and dopamine D_{1} and D_{2} receptor binding in striatum of normal (NL) and BD rats. Twenty-micron coronal sections through striatum of NL and BD rats were incubated with 8 nM [³H] mazindol (DA uptake), 2 nM [³H] SCH23390 (D_{1} receptors) 1 nM [³H] raclopride (D_{2} receptors). Specific binding of [³H] mazindol was significantly decreased in DM quadrants in BD rats. Specific binding of [³H] SCH23390 was not significantly different in NL vs. BD rats. Specific binding of [³H] raclopride was decreased in DM and VM quadrants of BD rats.
taken across coronal sections of the entire CP and each of four quadrants. Calibration curves were constructed using $[^{3}H]$ polymer standards (Amersham, Arlington Heights, IL). After autoradiographic exposure, slides were stained with cresyl violet for histology. Each mazindol experimental group contained five or six animals. Statistical significance between groups was determined by t-test, with significance set at $p < 0.05$.

**In Situ Hybridization**

Fifteen, 30, and 45 days post-IC inoculation, BD rats and NL rats were anesthetized with metofane and perfused with buffered 4% paraformaldehyde. Brains were removed, postfixed overnight, then cryoprotected using 19% sucrose in PBS. Twenty-micron coronal sections were collected as described above. Sections were hybridized with a $[^{35}S]$-labeled RNA probe (specific activity $2-6 \times 10^{7}$ cpm/μg; Amersham, Arlington Heights, IL; 5 ng probe/slide) complementary to mRNA encoding the BDV phosphoprotein. Detailed protocols for hybridization, autoradiography, and analysis have been described (10). Each experimental group contained three animals.

**RESULTS**

**Behavior**

Forty-five days after infection BD rats had enhanced responsiveness to cocaine relative to NL rats with increases in locomotion (Fig. 1A) and stereotyped behavior ratings at each dose level (Fig. 1B). Two-way ANOVA with repeated measures over time revealed a significant overall group difference in locomotor activity [Main effect: $F(1, 14) = 19.678, p < 0.05$]. Cocaine-induced stereotyped behavior ratings were significantly increased in BD rats overall and at each dosage level (Table 1). Generalized or atonic seizures were observed in each of the BD rats during the test interval. Generalized seizures were rhythmic, symmetric jerking of the trunk and limbs followed by a period of inactivity. Atonic seizures were rearing and falling followed by inactivity. No seizures were observed in NL rats. Epileptic events were excluded from measurements of stereotyped behavior.

**Quantitative Receptor AR**

BD rats had reduced binding of $[^{3}H]$ mazindol to DA uptake sites in all quadrants of CP (Table 2). The greatest loss of DA uptake sites occurred in the dorsomedial subregion (Fig. 2) and overlapped the dorsomedial and ventral areas where significant reductions were also observed for $[^{3}H]$ raclopride binding to D$_2$ receptors. Specific binding of $[^{3}H]$ SCH23390 to D$_1$ sites was unchanged from control values.

**In Situ Hybridization**

Viral nucleic acids colocalized with areas of decreased mazindol and D$_2$ radioligand binding in CP. In CP, viral RNA was first detected in subventricular dorsal medial subregion between 15 and 30 days postinfection (Fig. 3). At 45 days, highest levels of viral RNA were found in subventricular zones: dorso- and ventromedial quadrants. Hybridization signal was associated with both cell bodies and processes (Fig. 4).

**DISCUSSION**

The rewarding and locomotor stimulant effects of cocaine in rodents are attributed to inhibition of DA reuptake in the nucleus accumbens (20). High doses of cocaine (12,13,29), sensitization (17), and potentiation of DA transmission in dorsal CP (2) result in dyskinesias and stereotyped behaviors.

**FIG. 3.** Borna disease virus (BDV) nucleic acids in coronal sections of caudate-putamen from adult Lewis rats 15, 30, or 45 days after intracerebral infection. Sections were hybridized with a $[^{35}S]$ RNA probe complementary to mRNA encoding the BDV P-protein.
Our results indicate that BD rats have heightened response to cocaine. Relative to NL rats, BD rats had increased locomotive and stereotyped behaviors in response to cocaine at doses of 7.5, 15, and 30 mg/kg. Sensitivity to cocaine did not depend on increases in DA reuptake sites in BD rats. Mazindol binding was reduced in all striatal regions, and significantly reduced in the dorsomedial subregion. Because mazindol has at least fivefold selectivity for the DA uptake sites over the serotonin uptake sites (16), in a DA-rich structure such as striatum, the reduction in mazindol binding is likely to reflect a reduction in DA reuptake binding.

D_2_ radioligand binding was also reduced in dorso- and ventromedial subregions.

It is unlikely that a single mechanism is responsible for the enhanced behavioral activation with cocaine because both pre- and postsynaptic sites of DA system pathology were identified in BD rats. Instead, we propose that enhanced behavioral activity in BD rats reflects disturbances at multiple levels. 1) The loss or functional inactivation of reuptake sites in the dorsomedial CP of BD rats could result in prolonged DA extracellular action in that area; this may be reflected in a decrease in DA_D_2_ receptor binding. 2) Surviving neurons and terminals may have increased DA release; the administration of cocaine to BD rats may unmask this increased release phenomenon. 3) DA may act nonsynaptically and over considerable distances. Disruption of classical synapses, formation of syncitia, or new axo-axonic interactions would be configurations favorable for (nonsynaptic) ephaptic DA transmission.

Viral containment requirements have precluded evaluation of dynamic aspects of DA transmission by dialysis and direct testing of the first two hypotheses. Indirect support for the third may be based on finding viral nucleic acids to be abundant in a highly plastic region of the CNS. The rat striatum is not functionally mature at the time of infection (1 month of age) (8). Because striatal DA circuitry undergoes developmental changes into adulthood, the presence of virus in striatum between 15 and 45 days after infection could interfere with development of the DA system. High levels of viral nucleic acids were found in the subventricular zone of the medial CP, overlaying the distribution of a multipotent population of cells that retain characteristics of a proliferative layer into adulthood (24). Viral nucleic acids were also seen outside of cell bodies, in the neuropil. In this location, virus is perhaps found in DA axons and terminals, presynaptic DA elements that also continue to grow into adulthood (24). Therefore, infection of immature rats with BDV during a critical period in DA system development may interfere with the normal development of the pre- and postsynaptic DA system and produce alterations in DA circuitry that enhance nonsynaptic actions of DA.

We cannot exclude the participation of other receptor systems in the behavioral effects of cocaine in BD rats. Seizures, for example, could reflect serotonergic (32) or anesthetic effects (38). However, self-biting is considered to be a DA-specific behavior. The neonatal 6-OHDA-lesioned rat shows self-mutilatory behavior when challenged with L-DOPA (7). Furthermore, BD rats displaying high-intensity stereotyped behaviors did not have such serotonin-mediated behaviors as tremor, wet dog shakes, or forepaw treading (3,15).

While an understanding of the precise neurotransmitter mechanisms underlying cocaine sensitivity in the BD rat is incomplete, the striatal distribution of the virus itself has implications for behavior. These subregions, in addition to the nucleus accumbens, mediate conditioned reward learning: the learning stimulus-reward associations and fixed-action patterns in conditioned reinforcement paradigms (18). In humans, subregions of the CP have also been recognized to perform different functions (1). For example, the head of the caudate, thought to support associative and cognitive functions, is functionally abnormal in Obsessive Compulsive Disease (4,5), a chronic anxiety disorder characterized by ritualistic behaviors, abnormal ideation sets, and failure to integrate reward-related information.

Over the past decade, two epidemics that have had severe impact on public health in this country are cocaine abuse and Acquired Immunodeficiency Syndrome (22). The linkage of cocaine and AIDS epidemics may extend beyond behavioral risk factors and altered immune status to a third type of association. It is intriguing to consider the possibility that vulnerability to cocaine use or craving may be a consequence of virus-induced neurochemical changes. As appreciation for the overlap in the AIDS and cocaine epidemics grows, the BD rat model may have implications for understanding a role for CNS viral infection in human drug-seeking behavior and dependence.

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