Prefrontal Cortex Dysfunction in Borna Disease Virus (BDV)–Infected Rats

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Viruses have been proposed to play a role in the pathogenesis of schizophrenia; however, the mechanisms by which infection could cause the affective, cognitive, and movement disorders of schizophrenia are not understood. The neurotropic RNA virus, Borna disease (BD) virus, linked to schizophrenia by serologic studies, causes movement and behavior disorders in a wide variety of mammalian and bird hosts. BD rats have hyperactivity and stereotyped behaviors similar to those that follow neurotoxic or electrolytic lesions in frontal cortex or its catecholamine afferents in rats. BD rats have high levels of viral nucleic acid in the prefrontal cortex (PFC), abnormal mesocortical dopamine activity (elevated levels of DOPAC in PFC), yet no alteration in specific binding of D1 or D2 receptor radioligands in PFC. Since frontal lobe dysfunction is frequently reported in schizophrenia, the BD rat model may provide insights into pathogenesis and management of this debilitating psychiatric disease.

Key Words: Prefrontal, dopamine, schizophrenia, rat model, Borna disease, neurotropic virus

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Introduction

Borna disease virus (BDV) is an RNA virus that causes Borna disease, a multiphasic central nervous system (CNS) disorder in immunocompetent animals (Narayan et al 1983; Ludwig et al 1988). Rats experimentally infected with BDV develop a progressive movement and behavior disorder with hyperactivity (Narayan et al 1983; Bautista et al 1994), stereotyped behaviors, and dyskinesias (Solbrig et al 1994), followed by apathy and senescence in late stages (Narayan et al 1983). BDV was once considered to be a pathogen only for horses and restricted to regions of Southern Germany; however, the virus has been found to have worldwide distribution and to naturally infect a wide variety of vertebrate hosts, including sheep, cattle, cats, and domestic fowl (Lipkin and Koprowski 1995). The mode of transmission for natural infection is not known. Prosimians and primates, such as tree shrews and rhesus monkeys, are susceptible to experimental disease (Sprankel et al 1978; Stitz et al 1980). The presence of antibodies reactive with BDV proteins has been reported in patients with schizophrenia (Waltrip et al 1993) and bipolar depression (Rott et al 1985; Fu et al 1993), suggesting that the viral host range may extend to humans. BDV is tropic for the limbic system in several species (Ludwig et al 1988), strengthening the connections...
between animal behavioral and human psychiatric diseases.

While an early hypothesis of schizophrenia proposed that psychosis is the result of an overactive mesolimbic dopamine (DA) system (Stevens 1973), emphasis is now shifting to a developmental syndrome of abnormal prefrontal function. Neuroimaging studies of brain metabolism and blood flow, increased ventricle/brain ratio, and poor prefrontal activation during frontally challenging neuropsychological tasks suggest dysfunction of prefrontal cortex in schizophrenia (Weinberger et al 1988; Buchsbaum et al 1992). In animals, prefrontal lesions lead to hyperactivity, hyperreactivity to sensory stimuli, increased distractibility, perseveration, and behavioral and appetite hyperactivity, hyperreactivity to sensory stimuli, increased ventricle/brain ratio, and disorganized and stereotyped behaviors (Narayan et al 1983; Bautista et al 1994; Solbrig et al 1994).

In the present study we investigate the prefrontal system of BDV-infected rats. Infected animals had high concentrations of viral nucleic acid in the prefrontal cortex (PFC) and mesocortical DA circuit. DA metabolic activity in the mesocortical DA system was significantly increased, yet numbers of D1 or D2 receptors in PFC were unchanged. These findings may provide insight into mechanisms for prefrontal lobe dysfunction and have relevance to the pathophysiology of schizophrenia.

**Methods**

**Infection of Animals**

Under metofane anesthesia, 1-month-old male Lewis rats (Charles River Labs, Wilmington, MA) were inoculated intracerebrally (i.c.) into the right lateral ventricle with either 1.6 x 10⁴ tissue culture infectious dose units of Giessen He/80 strain of BDV (BD rats), or PBS in a total volume of 30 μL. Virus stock was a 10% wt/vol BD rat brain homogenate in PBS (normal [NL] rats) (Carbone et al 1987). Animals were monitored for the appearance of signs of disease, including hyperactivity, hyperreactivity to acoustic and tactile stimuli, and disorganized and stereotyped behaviors (Fuster 1980). Animals, particularly primates, with prefrontal lesions are now studied as experimental models of the human psychotic state (Goldman-Rakic 1994). The early stages of Born disease in rats are similarly characterized by hyperactivity, hyperreactivity to sensory stimuli, and disorganized and stereotyped behaviors.

**Neurochemistry**

Six BD rats and seven NL rats were sacrificed for high-performance liquid chromatography (HPLC) analysis of brain catecholamines. After decapitation, brains were removed and dissected over ice. A 2 mm thick coronal section was taken between 1 and 3 mm posterior to the anterior genu of the corpus callosum. The prefrontal cortex was dissected from this section by separating it from the corpus callosum. Tissue pieces were immediately frozen using dry ice. Tissue levels of dopamine and its primary metabolite, DOPAC, were determined using HPLC with electrochemical detection (Jakubovic 1987). Tissues were homogenized for 30 sec in 0.2 M HClO₄ containing 0.15% Na₂S₂O₅, 0.5% Na₂ EDTA and 1 μM isoproterenol (internal standard). Following centrifugation at 16,000 x g for 15 min. at 4°C, the supernatant was removed and filtered with 0.2 μm Nylon-66 membrane filters (Whatman, Hillsboro OR) and stored at -80°C. The HPLC system consisted of a Rainin HPXL solvent Delivery System, Macintosh with Dynaxas Software, ESA Coulomet Model 5100A Electrochemical detector with Model 5011 High-Sensitivity Analytical Cell, and Beckman Ultrasphere C18 column (3 μm, 75 x 4.6 mm). The detector potential was set at +0.45 V, sensitivity 10 nA/V. The mobile phase consisted of 100 mM sodium phosphate (monobasic) (pH 3.0), with 0.1 mM EDTA, 1.0 mM heptane sulfonic acid, 5.0% acetonitrile, 0.01% triethanolamine. The detection limit of the system was approximately 0.4 pmol/100 μL of tissue sample. Standard solutions of norepinephrine (NE), 3,4-dihydroxyphenylalanine (DOPA), vanillylmandelic acid (VMA), epinephrine (E), 3-methoxy-4-hydroxyphenylglycol (MHPG), normetanephrine (NM), dopamine (DA), 5-hydroxytryptophan (5-HTP), 3,4-dihydroxyphenylacetic acid (DOPAC), isoproterenol, 3-methoxytyramine (3-MT), serotonin (5-HT), 5-hydroxyindoleacetic acid (5-HIAA) and homovanillic acid (HVA) (Sigma) were individually injected to verify elution times. Calibrations were run daily before and after sample analyses.

**Quantitative Receptor Autoradiography**

Under metofane anesthesia, four BD rats and four NL rats were perfused with cold PBS followed by 0.1% paraformaldehyde to limit infectivity and maintain tissue integrity (Lidow et al 1991). D1 receptors were detected by incubating slide-mounted tissue sections with 2 nM [³H]SCH2339) (New England Nuclear, Boston, MA) in the presence of 1 μM mianserin; D2 receptors with 1 nM [³H]raclopride (New England Nuclear). Ligand concentrations approximately equal to the kd for each receptor type were used. Nonspecific D1 binding was defined by incubating adjacent sections with 1 μM SKF83566 (Research
Biochemicals International, Natick, MA), nonspecific D2 binding with 1 μM (+)-butaclamol (Research Biochemicals International) (Lidow et al 1991). Tissue sections from BD rats and NL rats were processed simultaneously then exposed to tritium-sensitive film for 3.5 weeks when using the D1 receptor ligand, or for 5 months when using the D2 ligand. Autoradiograms were analyzed with a computer-based image analysis system (MCID, Imaging Research Inc., St. Catherine, Ontario, Canada). Calibration curves were constructed using [3H]polymer standards (Amersham, Arlington Heights, IL). Sample measurements were taken from deep cortical layers (V, VI) of prefrontal cortex, area 32 in the frontopolar cortex. These measurements were compared to those of a nearby region, the superficial layers (I–III) of dorsolateral frontopolar cortex. Specific binding was measured at an additional ligand concentration of 0.5 times the kd for each receptor.

Neuroanatomy
Under metofane anesthesia, BD rats and NL rats were perfused with buffered 4% paraformaldehyde, postfixed overnight, and cryoprotected using 19% sucrose in PBS. Twenty micron coronal sections were collected onto gelatin-coated slides. Sections from adult Lewis rats 45 days post intracerebral inoculation were hybridized with an [35S]labeled RNA probe complementary to mRNA for the BDV phosphoprotein, as previously described (Carbone et al 1991) and stained with cresyl violet for microscopic analysis.

For immunohistochemistry, brainstem sections were incubated first with rabbit antibodies to tyrosine hydroxylase (TH) (Eugene Tech, Allendale, NJ) at dilutions 1:1000 overnight and then with an avidin-biotin-peroxidase complex (ABC) system, following the Vectastain Elite ABC system protocol (Vector Labs, Burlingame, CA). Sections were developed with 3,3′-diaminobenzidine. TH-positive neurons were counted using 100 μm microscope eyepiece grids and recorded as cells per (right or left) side per 20 μm coronal section.

Results
Within 10 days of infection, rats became hyperactive, aggressive, vigilant, and startled easily. Forty days after infection, rats had, in addition, stereotyped behaviors (repetitive sniffing, chewing, scratching, or grooming), genital grooming, hoarding, ingestive disturbances (hyperphagia, coprophagia, polydipsia), and self-mutilation (chewing digits or tail).

Neurochemistry
The neurochemistry of the syndrome was investigated by measuring tissue levels of DA and its primary metabolite, DOPAC, in prefrontal cortex of NL and BD rats by HPLC. DA was eluted at 4.4 minutes and DOPAC at 7.0 min. Peaks were monophasic with baseline separation under the column conditions. DA and DOPAC values in micrograms per gram wet weight of tissue ± SEM are shown in Table 1. Infection resulted in an insignificant (6.4%) DA depletion in PFC of BD rats, but a significant (630%) increase in DOPAC. The DOPAC/DA ratio, an index of DA metabolic activity, was also significantly increased.

Receptor Autoradiography
Changes in D1 and D2 receptors in the prefrontal cortex were examined by quantitative receptor autoradiography. Figure 1 shows labeling of NL and BD rats of D1 sites by 2 nM [3H]SCH23390, and D2 sites by 1 nM [3H]raclopride. In BD rats, specific binding of 2 nM [3H]SCH23390, expressed as fmol/mg tissue, in layer V, VI of cortex did not significantly differ from NL values (p > 0.05, t test). The difference in specific binding between layers V, VI and layers I–III was also similar. In BD rats, specific binding of 1 nM [3H]raclopride in layers V, VI of frontopolar cortex, as well as the difference in specific binding between layers V, VI and layers I–III, did not differ significantly from control values (p > 0.05, t test) (Table 2). Differences in ligand binding between deep and superficial layers using 0.5 kd concentrations (1 nM [3H]SCH23390 and 0.5 nM [3H]raclopride) were also similar between NL and BD groups.

Neuroanatomy
The distribution of viral nucleic acids in BD rat brain was determined by in situ hybridization. Highest levels of viral RNA were detected in the prefrontal system (prefrontal/
BD rats 45 days after i.c. infection. The locus coeruleus extends approximately 1.2 mm through the pontine tegmentum. The densest concentration of TH-positive neurons in this region were located at the coronal level designated Bregma -10.04 (Fig 58) of Paxinos and Watson (1986). The average numbers of TH-positive cells per right or left side per section at this level for three animals ± SEM in BD group were 19.89 ± 4.69 cells. Corresponding values for NL rats were 48.50 ± 16.40 cells (p < 0.05, t test).

Discussion

The results show that rats infected with BDV have a movement and behavior disorder that is associated with pathological changes in the prefrontal cortex and prefrontal dopamine (DA) system. Hyperactivity and stereotyped perseverative behaviors were accompanied by high indices of prefrontal cortex DAergic activity yet stable numbers of D1 and D2 receptors. High levels of BDV nucleic acid were detected in PF cortex and the PF system by in situ hybridization.

BD in rats has been described as a biphasic illness with an acute, aggressive, active phase and a chronic, docile, apathetic phase with premature senescence, performance deficits in maze-learning paradigms, and pathologic weight gain. In addition to these descriptions, a recent report elaborated an intermediate phase, beginning 40 days after i.c. infection, characterized by hyperactivity and extreme stereotyped behaviors (Solbrig et al 1994), reminiscent of the motor perseveration or stimulus bound behaviors of frontal-lesioned animals (Fuster 1980).

Dopamine in the prefrontal cortex is important in the organization and execution of motor behavior. Anatomic studies across species revealed a distinct DA pathway (mesocortical pathway) originating in A10 cells of VTA and innervating the PFC (Divac et al 1978). In the rat, partial lesions of mesocortical DA system at the cortical (Carter and Pycock 1980) or subcortical (LeMoal et al 1976) levels induce spontaneous hyperactivity and general disorganization of behavior. Whereas in other studies such lesions resulted in marked reductions of PFC DA (52%--
68%) (Tassin et al 1986; Carter and Pycock 1980), in the present study BD rats had only 6% reductions in PFC DA.

Intriguingly, these modest changes in PFC DA in BD rats were accompanied by a sixfold increase in DOPAC levels and high DOPAC/DA ratios in PFC, indicating increases in mesocortical DA activity, utilization, or metabolism. One possible explanation for this metabolic hyperactivity may be found in the observation that BD rats have reduced DA levels in nucleus accumbens (NA) (Solbrig et al 1994). DA terminal lesions in NA could induce secondary hyperactivity in surviving bifurcations of the mesocortical-mesotelencephalic DA system, the highly collateralized SN-VTA DA neurons projecting to striatum and frontal cortex (Tassin et al 1978; Fallon 1981). Mesocortical DA neurons lack synthesis and impulse-modulating DA autoreceptors (Chiodo et al 1984; Talmaciu et al 1986); thus, high firing rates and DA turnover might also reflect the absence of negative-feedback mechanisms.

A third possibility is that differential damage to efferent projections from striatum may augment mesocortical DA activity. BD rats show preferential loss of caudate-putamen D2 receptors (Solbrig et al 1994). Striatal D2 lesions in BD rats may reduce transmission through the indirect (striatopallidal) pathway (Gerfen et al 1990), leading to disinhibition of the negative-feedback pathway to A10 DA.
neurons in substantia nigra-ventral tegmental area (SN-VTA) (Alexander and Crutcher 1990).

A fourth possibility is selective activation of mesocortical DA neurons by stress (Thierry et al 1976). Endogenous opioids released during stress directly excite midbrain A10 DA neurons (Matthews and German 1984). BD rats show signs of stress associated with chronic illness, including disheveled appearance, growth retardation, chronic hyperactivity, self-biting, and other maladaptive behaviors.

Finally, hyperactivity in mesocortical DA circuits may be a virus-induced enzymatic effect, i.e., increased activity of monoamine oxidase (MAO) A and/or B leading to increased DOPAC levels. Such a mechanism may be responsible for monoamine disturbances known to occur in two other viral encephalitides. Brains of excitable, aggressive mice infected with herpes simplex virus (HSV) have six times normal levels of HVA and twice normal levels of 5-HIAA in the presence of normal levels of DA and 5-HT (Lycke and Roos 1974). When mice experimentally infected with Newcastle Disease virus, a paramyxovirus that causes a respiratory and CNS disease in birds, with paresis, twitching, and torticollis (Jungherr 1964), their hypothalami have high DOPAC/DA, 5-HIAA/5-HT and MHPG/NE ratios (Dunn and Vickers 1994; Dunn and Chuluyan 1994). Although measures of DA transmission and release would contribute significantly to our understanding of mesocortical DA dysfunction, viral containment requirements preclude dialysis at this time.

D1 receptor denervation supersensitivity in PFC following VTA lesions depends on presence of intact ascending NE fibers. PFC D1 receptor numbers, which increase after electrolytic lesions of VTA, fail to increase in 6-OHDA-lesioned animals, due to concomitant destruction of cortical NA innervation (Tassin et al 1986). In BD rats, specific binding of D1 and D2 receptor radioligands are comparable to controls, despite presynaptic DA hyperactivity. Failure of activity to downregulate D1 DA receptors may similarly reflect disturbed heteroregulation of D1 receptor sensitivity, due to partial NA deafferentation. BD rats have viral nucleic acid in LC cells as early as 7 days after infection; cells are lost from the LC by day 45 (Solbrig et al 1995).

In a previous study we reported a 50% decrease of DA neurons in the SNc, and only a 9% decrease of DA neurons in the VTA of infected animals (Solbrig et al 1994). Based on the topography of DA neuron projections and the presence of near-normal levels of DA in the prefrontal cortex, it appears that the DA neurons of the VTA which give rise to the mesoprefrontal projections are spared in BD rats. Preservation of these cells may be related to the differential distribution of a number of compounds with neuroprotective or regulatory functions in the dorsal (mesocortical, mesolimbic projection) and ventral (predominantly nigrostriatal) tier DA neurons of the SN-VTA. For example, some peptides, including calbindin (Vincent et al 1986), glutamate dehydrogenase (Aoki et al 1987), neuropeptide (Seroogy et al 1987), and others, are localized to the dorsal, rather than ventral, tier of the SN-VTA.

BDV is a neurotropic virus that spreads transsynaptically (Carbone et al 1987; Morales et al 1988). It is possible that the virus concentrates in PFC because BDV is relatively nonlytic in the mesocortical DA circuit. In addition, the virus may concentrate in PFC because of the widespread afferent, efferent, and reciprocal connectivity of this structure.

Schizophrenia is a heterogeneous condition. Multiple environmental and genetic factors have been implicated in its expression. The viral hypothesis of schizophrenia is based on reports of antibodies to viruses in serum or cerebral spinal fluid from schizophrenic patients (Halonen et al 1974; Torrey et al 1982, 1991), increased numbers of schizophrenic births during months of viral epidemics, increased numbers of schizophrenics with history of exposure to viruses during gestation (O’Callaghan et al 1991), and the association of psychosis with encephalitis lethargica (Carter et al 1987). Stronger support for linkage between BDV and schizophrenia is provided by recent findings of immunoreactivity to BDV-specific proteins in deficit syndrome schizophrenics (schizophrenics with organic features and poor response to pure D2 antagonists) (Waltrip et al 1993). To date, there has been no documentation of heterologous host transfer to humans.

These studies have focused on investigation of DA activity, because of the strength of behavioral and pharmacologic bioassays for DA systems, and because prefrontal DA is critical to organization and execution of motor behavior; however, the BD rat, with parallels to certain human psychoses, renders many other aspects of prefrontal cortex pathophysiology experimentally accessible. Determining the mechanisms by which BDV produces CNS disease, understanding its primary and secondary effects on DA systems, and how BDV “recognizes” the heterogeneity of ascending DA systems and DA receptors, could improve our understanding of schizophrenia as a condition of transmitter imbalance and as a degenerative or developmental disease. The distribution and selective pathologic effect of BDV may also have implications for the design of antipsychotics with differential actions on different DA circuits.

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