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Neurotransmitter abnormalities in Borna disease

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Borna disease (BD) agent is an infectious pathogen that causes progressive central nervous system (CNS) dysfunction in a wide range of vertebrate hosts. The course of BD in adult rats is biphasic. The acute phase is characterized by aggressive behavior and inflammatory cell infiltrates in brain. With chronic infection animals become listless and inflammation resolves. BD antigens are similarly distributed in neurons in hippocampus, neocortex, cerebellum and brainstem in acutely and chronically infected animals. We have recently examined brain levels of neuronal transcripts in rats with acute and chronic BD. Levels for 3 of these mRNAs, cholecystokinin, glutamic acid decarboxylase and somatostatin, were decreased in acutely infected rats and increased toward control values in chronically infected rats. A fourth transcript, MuBr8, correlated in distribution with BD antigen, was persistently decreased throughout the course of infection. These data may have implications for understanding the pathogenesis of neurologic disturbances in BD and other inflammatory CNS diseases.

Mammals infected with a variety of central nervous system (CNS) pathogens have neurologic dysfunction and abnormal levels of activity for enzymes associated with synthesis or degredation of neurotransmitters[2,3,10,11,23,26,30]. We have recently shown that viral infections can selectively alter behavior and steady state brain levels of neurotransmitter mRNAs[24]. These findings have prompted us to look for infectious agents which can be used as probes to map anatomic and functional domains in the CNS.

Borna disease (BD) agent is a poorly characterized infectious pathogen with tropism for limbic system neurons. Though BD is a natural infection only in sheep[38] and horses[16,25], intracerebral or peripheral inoculation of BD infected brain homogenates into rodents[5,17–20,22,25,28,29,33] and primates[6,22,25,36] leads to accumulation of BD antigens in hippocampal neurons and prominent behavioral disturbances. In rats, the onset of BD is heralded by hyperactivity, aggression and in most cases, ataxia (acute phase). This coincides with the appearance of BD antigens in hippocampal CA3 pyramidal neurons. Within several days, brains of infected animals show inflammatory cellular infiltrates and BD antigens in neurons throughout hippocampus, layers 4 and 5 of cortex, deep cerebellar nuclei and brainstem. The acute phase lasts 2–4 weeks and is followed by listlessness and less frequently, by paralysis and obesity (chronic phase). Brains of chronically infected rats show resolution of inflammation in spite of a similar distribution of BD antigens to that seen in acutely infected animals[5,28,29]. In an effort to explore the neurochemical basis for neurologic dysfunction in BD we have examined steady state brain levels of mRNA for 4 neuronal products in acutely and chronically infected rats.

Four- to 6-week-old inbred male Lewis rats (Charles River, Wilmington, MA) were inoculated intracerebrally with 10^5 tissue culture infectious doses of BD agent and sacrificed in the acute or chronic phase of BD (4 or 8 weeks following inoculation, respectively)[5]. Individual brains from acutely infected, chronically infected or uninfected (control) animals were removed for RNA extraction by homo-

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genization in guanidinium isothiocyanate and centrifugation through cesium chloride. RNA concentration and purity was assayed through optical density measurements and pilot hybridization experiments with a probe to 28S ribosomal RNA[24]. Ten μg aliquots of brain RNA from individual animals were slot blotted, fixed to nitrocellulose membranes and serially hybridized with ^{32}P-labeled cDNA probes to detect mRNAs for two peptide neurotransmitters, cholecystokinin (CK)[9] and somatostatin (SOM)[14], the enzyme glutamic acid decarboxylase (GAD)[21] which synthesizes the inhibitory amino acid neurotransmitter γ-aminobutyric acid (GABA), MuBr8 (ref. 4), which encodes a CNS-restricted axonal membrane associated 25 kDa protein and actin[7], an ubiquitous pol II transcript not confined to neurons. Probes to CK, GAD and SOM were selected for two reasons. First, reduced CNS concentrations of CK, GAD, SOM and associated markers have been reported in human disorders characterized by altered behavior including Alzheimer's disease, Huntington's disease, schizophrenia and bipolar affective disorders[12,31,34,35,37]. Second, mice persistently infected with lymphocytic choriomeningitis virus have abnormal behavior and reduced whole brain levels of SOM mRNA yet normal brain levels of CK mRNA[24]. The probe to MuBr8 was chosen because in situ hybridization studies in rat brain have demonstrated that this transcript, like BD antigens in infected rats, is concentrated in neurons in hippocampus, layers 4 and 5 of neocortex, deep cerebellar nuclei and brainstem[4]. Following autoradiography, hybridization signals for individual slots (animals) were quantitated by scanning films with a laser densitometer (LKB, Sweden). The values obtained were used to determine the mean and standard error of hybridization signal with each probe for each group of animals. Differences in mean hybridization were determined by unpaired Student's t-test. Ten μg aliquots of rat spleen RNA were used in slot blot experiments as controls for the specificity of neuronal cDNA probe hybridization.

Fig. 1 is a composite of slot blot experiments with brain RNA extracted from 6 uninfected, 6 acutely infected and 6 chronically infected animals. Each slot along the vertical axis represents RNA from an individual animal which has been hybridized in sequence with probes to CK, GAD, SOM, MuBr8 and actin.

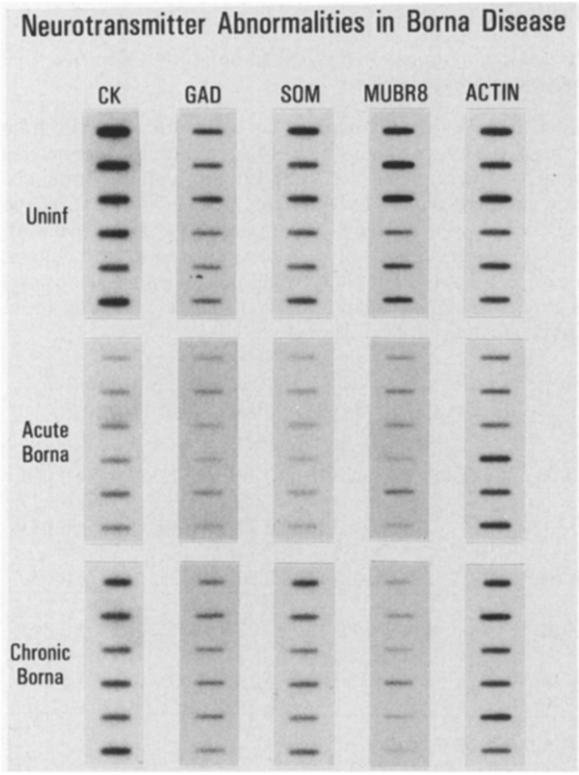

Fig. 1. Slot blot analysis of brain RNA extracted from rats with acute or chronic Borna disease. Whole brains were homogenized in guanidinium isothiocyanate and centrifuged over cesium chloride gradients. RNA was quantitated spectrophotometrically and normalized to 28S ribosomal RNA concentration in pilot hybridization experiments. Ten μg aliquots of RNA from individual uninfected, acutely infected or chronically infected rats were applied to slots and fixed to a nitrocellulose membrane. Membranes were hybridized in sequence for autoradiography with ^{32}P-labeled cDNA probes to cholecystokinin (CK), glutamic acid decarboxylase (GAD), somatostatin (SOM), murine brain 8 (MuBr8) and actin.

The reproducibility with which BD agent affected whole-brain levels of each mRNA species is indicated by the consistency of hybridization signal seen with each probe for each group of uninfected, acutely infected or chronically infected rats. Qualitatively similar data was obtained from northern blot hybridizations of size fractionated RNA, revealing a single appropriately sized transcript for each mRNA probed (not shown). Densitometric and statistical analysis of larger cohorts of animals (8 uninfected, 8 acutely infected and 8 chronically infected rats) is shown in Table I. Brain levels of three of these mRNAs, CK, GAD, and SOM dropped dramatically with acute infection and returned toward normal with

TABLE I

Hybridization of neuronal probes to brain RNA extracted from rats with Borna disease

Densitometric measurements of slot blot autoradiographs from hybridization experiments with RNA extracted from brains of uninfected control rats, rats with acute or chronic Borna disease and probes to cholecystokinin, glutamic acid decarboxylase (GAD), somatostatin, MuBr8 and actin. (See legend for Fig. 1.) Values are given in arbitrary densitometric units as mean ± S.E.M. Parentheses indicate percentage of control (uninfected). Data analyzed by unpaired Student's *t*-test. GAD, glutamic acid decarboxylase.

Probe	*Control (n = 8)*	*Acutely infected (n = 8)*	*Chronically infected (n = 8)*
Cholecystokinin	0.846±0.131	0.183±0.012 (25)*	0.415±0.066 (68)*
GAD	0.529±0.069	0.221±0.017 (36)*	0.366±0.053 (68)**
Somatostatin	0.856±0.144	0.261±0.023 (26)*	0.667±0.074 (88)***
MuBr8	0.971±0.141	0.219±0.031 (23)*	0.250±0.042 (26)*
Actin	0.356±0.069	0.291±0.033 (80)***	0.325±0.044 (97)***

*$P < 0.002$, **$P < 0.02$, ***P not significant.

chronic infection. In contrast, levels of MuBr8 mRNA were persistently decreased. Acutely infected animals showed a mean reduction in whole brain levels of two neurotransmitter mRNAs, CK and SOM, to 25% of control values ($P < 0.002$). Mean brain levels of mRNA for the neurotransmitter synthesis enzyme GAD were decreased to 36% of control values ($P < 0.002$). MuBr8 mRNA levels were decreased to 23% of control values ($P < 0.002$). In chronically infected rats, mean neurotransmitter (CK and SOM) and neurotransmitter-related (GAD) mRNA levels returned toward normal: CK and GAD mRNA levels were 68% of control values; SOM mRNA levels were not significantly different from levels in uninfected animals. In contrast, mean levels for MuBr8 mRNA remained depressed (26% of control values; $P < 0.002$). Actin mRNA levels in acutely and chronically infected animals did not differ significantly from control animals (Table I). Spleen RNA showed no hybridization signal with probes to CK, GAD, SOM or MuBr8, although the level of actin probe hybridization was comparable to that seen with brain RNA.

Inoculation of neonatal rats with BD agent results in persistent CNS infection without inflammation or neurologic defects in spite of BD antigen immunoreactivity in distribution identical to that described in rats infected as adults[5]. In order to separate the direct effects of BD infection from effects mediated by immune response to infection, we examined brain levels of SOM, MuBr8 and actin mRNAs in six 4-month-old rats infected with BD as neonates. Levels of these mRNAs in neonatally infected animals were no different than in uninfected littermate controls (not shown).

Rats infected with BD agent have a biphasic neurologic illness. Aggression and hyperactivity in the first phase is attended by prominent inflammatory cell infiltration throughout the CNS. Inflammation resolves with loss of neuropil during the clinically depressive second phase of the illness[5,28,29]. We have now shown that these biphasic disturbances in behavior and histology are mirrored by abnormalities in steady state levels of three neuronal mRNAs, CK, GAD, and SOM. Levels of each of these transcripts were profoundly depressed in acute infection but returned toward normal in chronic infection. In contrast, brain levels of MuBr8 mRNA were reduced during both the acute and chronic phases of the disease. The finding that brain actin mRNA levels were not altered in either the acute or the chronic phase of BD, implies that the effects of infection were not generalized but were instead, restricted to subpopulations of cells within the CNS.

The mechanism(s) by which BD agent causes neurologic dysfunction and abnormalities in brain levels of neuronal mRNAs is probably different in acute and chronic phases of infection and may reflect the presence or absence of inflammation. Inflammation is associated with a myriad of soluble factors including glucocorticoids and polypeptides which could affect either the rate of transcription or the stability of neuronal mRNAs[13,15]. Rates of transcription for several rat genes including albumin, transthyretin, α_2-microglobulin and α_1-inhibitor III are decreased in vivo by acute inflammation[13,15,32]. In addition, transcription in vitro of prolactin[1] and somatostatin[8] is down-regulated by glucocorticoids. In this model then, with resolution of inflammation in chronically infected animals, mRNA levels would reflect only neuronal loss due to immune mediated cytopathology. The patterns of neuronal mRNA disturbance in

BD fit this model. Neurotransmitter-associated mRNAs (CK (R. Haun and J. Dixon, personal communication), GAD (D. Kaufman and A. Tobin, personal communication), and SOM[27]), which decrease in acutely infected animals and rise again in chronically infected animals are all inducible in vitro through activation of adenylate cyclase. In contrast, MuBr8 mRNA, which shows no biphasic disturbance in BD, may be constitutive in its regulation (M.C. Wilson, unpublished observations). Depression of MuBr8 mRNA levels in rats with BD likely reflects direct tropism of the BD agent for regions that contain a high steady-state level of this transcript. Understanding how inflammation effects these neurochemical disturbances, the basis for BD agent tropism for select brain regions and for its overlap in distribution with MuBr8 and finally, the characterization of this novel infectious pathogen will be foci for future work.

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