SHORT COMMUNICATION

Key role for enkephalinergic tone in cortico–striatal–thalamic function

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Abstract

Whereas the role of dopaminergic tone in the cortico-striatal-thalamic system is well-established, the role of endogenous opioids in the function of this system is less understood. We show that Borna disease virus infection of adult rats results in an increase in preproenkephalin transcripts in the striatum of Borna-infected rats, a region important for forming coordinated sequential motor actions and in developing programmes of thought and motivation. Stereotypic behaviours and dyskinesias, the clinical hallmarks of infection in adult Lewis rats (BD rats), are accompanied by a disrupted pattern of immediate early gene c-fos activation in the motor thalamus, with significance for the breakdown in coordinated sequential motor actions. We also find increased preproenkephalin in infected cultured neuroblastoma and rat foetal glial cells. The expression pattern of enkephalin mRNA in vivo and in vitro suggest that increased enkephalin function is one of the neuropharmacological means by which Borna disease virus causes motor disease of animals and possibly cognitive and affective disease in man, and further suggest that enkephalins play a critical role in the maintenance of a balanced tone of activity in the cortico-basal ganglia-thalamo-cortical loops.

Introduction

Borna disease virus (BDV) is a neurotropic negative-strand RNA virus, worldwide in distribution, that causes disturbances of movement and behaviour in a wide range of animal species (Narayan et al., 1983; Ludwig et al., 1988; Solbrig et al., 1994). The virus is a natural pathogen of several domestic mammalian and bird species, and has been linked by serology, detection of viral nucleic acid and virus isolation to neuropsychiatric disorders of man (de la Torre, 2001; Ludwig et al., 1988; Solbrig et al., 1994). The purpose of the present study was to characterize the effect of BDV infection on CNS enkephalin expression in specific components of the cortico-striatal-thalamic system in rats at the time their movement and behaviour disorder appeared.

Materials and methods

Adult male Lewis rats infected experimentally with this virus (BD rats) develop a movement and behaviour disorder characterized by stereotypic patterns of grooming, sniffing, rearing, gnawing, self-biting and dyskinesias (vacuous chewing and retrocollis) 6 weeks after infection (Solbrig et al., 1994). Preproenkephalin (PPE) mRNA expression in brain was examined by in situ hybridization after 6 weeks of infection, using [35S]cRNA probes synthesized from PPE cDNA clones (Yoshikawa et al., 1984) following published methods (Solbrig et al., 1994). All procedures were performed on animals anaesthetized deeply with inhaled methoxyflurane in compliance with institutional (UCI IACUC–University of California-Irvine Institutional Animal Care and Use Committee) and National Institutes of Health guidelines.

Results

The PPE mRNA signal was higher in the caudate putamen of BD rats than in uninfected rats (Fig. 1A). Autoradiograms were analysed using a computer-based image analysis system (MCID; MicroComputer Imaging Device, Imaging Research Inc., St. Catharines, Ontario, Canada) with calibration curves constructed using [14C]-polymer standards (American Radiolabelled Chemicals, St. Louis, MO, USA). The PPE signal, expressed as d.p.m./mg, was increased significantly in the caudate putamen of BD rats relative to uninfected rats (BD 6633.87 ± 876.81 vs. normal 3775.98 ± 743.26; values in d.p.m./mg, mean ± SEM; t = 2.486, d.f. = 10, P < 0.05, two-tailed t-test, n = 6 per group). Increases in signal were also found in nucleus accumbens, and central and basolateral amygdala. Increased caudate putamen signal was attributed to increased in situ hybridization signal per cell. Numbers of cells expressing enkephalin mRNA per high power (280 × 176 µm) field were similar in BD and normal rats (approximately 35–40 per field); however, numbers of silver grains per cell were increased throughout the caudate putamen in BD rats (Fig. 1B).
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Double-labelling immunofluorescence histochemistry showed colocalization of virus and methionine (met)-enkephalin, a biologically active product of the PPE gene, in caudate putamen (Fig. 1C). A total of 63 ± 4% of cells expressing BDV phosphoprotein also expressed met-enkephalin; 37 ± 4% of met-enkephalin-expressing cells also expressed BDV phosphoprotein (mean ± SEM of counts obtained from 306 BDV-infected cells, 544 met-enkephalin cells in three animals). The increase in PPE expression in BD rat brain (Fig. 1A and B) and coincidence of BDV and met-enkephalin immunoreactivity (Fig. 1C) is consistent with the hypothesis that infection directly influences met-enkephalin expression.

The effect of infection on c-fos expression was examined by in situ hybridization as an index to integrity of circuitry. Instead of the well-circumscribed ventrolateral nuclear signal in the thalamus of uninfected rats, BD rats had a chaotic pattern of gene activation in the ventrolateral region, with irregular clustering of c-fos signal in rostral and central thalamic nuclei, and increased palidal signal (Fig. 1D). Although cortico-basal ganglia-thalamo-cortical loops participate in the learning and maintenance of sequential motor actions dependent on sensorimotor integration (Graybiel & Rauch, 2000a), our results are consistent with enkephalin overexpression effecting a desynchronization of signal through the motor thalamus, breaking up motor programmes of coordinated sequential actions.

In vitro effects of BDV on PPE transcription in BDV (strain V)-infected cell lines (Pauli & Ludwig, 1985) were examined by Northern hybridization. Fifteen micrograms total RNA extracted from cells in Tri-Reagent (Molecular Research Centre Inc., Cincinnati, OH, USA) were size-fractionated in 2.2 M formaldehyde/1% agarose gels, transferred to nylon membranes, UV cross-linked and hybridized to random primed [32P]DNA fragments generated from cloned DNA representing enkephalin or BDV sequence. Levels of PPE mRNA, the enkephalin precursor, were increased in two neural cell lines infected with BDV: LAN (human neuroblastoma) and C6 (rat astroglia). The differential effect on enkephalin was more pronounced in LAN cells than C6 cells (Fig. 2A). Total RNA loaded per lane was standardized using a probe for the cellular gene glyceraldehyde-3-phosphate dehydrogenase (GAPDH) and autoradiographic signal quantified by phosphorimaging (Storm 840 Phosphorimager; Molecular Dynamics, Sunnyvale, CA, USA). Persistent infection of LAN cells caused an increase in PPE mRNA of 2.4-times control (uninfected) values (Fig. 2B).

Specificity of viral effects for PPE in striatum was assessed by measuring levels of dopamine D2 receptor, c-fos and preprodynorphin (PDP) mRNA in caudate putamen by in situ hybridization using [35S]cRNA probes synthesized from dopamine D2 receptor (Bunzow et al., 1988), c-fos (Curran et al., 1987), and PPD (Civelli et al., 1985) clones. Levels were similar in BD and uninfected rats for D2 receptor (BD 3282.96 ± 362.53 d.p.m./mg vs. normal 3489.67 ± 556.24 d.p.m./mg; t = 0.3113, P > 0.05), c-fos (BD 84.14 ± 1.01 d.p.m./mg vs. 74.97 ± 11.94 d.p.m./mg; t = 0.7653, P > 0.05) and dynorphin (BD 2997.21 ± 44.78 d.p.m./mg vs. 3266.65 ± 254.61 d.p.m./mg; t = 1.042, P > 0.05).

Although the basis for enkephalin induction by BDV remains unknown, a potential mechanism is suggested by in vitro experiments indicating that BDV biases cells toward kinase reactions through mitogen-activated protein kinase pathway activation (Hans et al., 2001; Planz et al., 2001). These reactions, hypothesized to enhance viral replication and infectivity (Planz et al., 2001), would terminate with phosphoCREB (cAMP response element binding protein) elevations, increasing transcription factors important for striatal enkephalin expression (Konradi et al., 1993), in turn increasing enkephalin mRNA. Induction could be restricted to enkephalin because there is limited infection of striatal dynorphin-expressing cells. Although partial dopamine denervation occurs in BD rats (Solbrig et al., 1994), increases in both striatal dopamine D2 receptor and PPE mRNA that accompany complete 6-hydroxydopamine deafferentation (Gerfen et al., 1990) are not seen. Thus, enkephalin...
upregulation as a result of pure dopamine lesion is excluded. Although manipulations that drive phosphorylating kinase reactions to phospho-CREB might induce both striatal PPE (Konradi et al., 1993) and PPD (Cole et al., 1995), only 14 ± 1% of cells expressing dynorphin B express BDV phosphoprotein (values represent mean ± SEM of counts obtained from 214 BDV-infected cells, 173 dynorphin B cells in three animals) whereas 37 ± 4% of met-enkephalin-expressing cells also express BDV phosphoprotein. Thus, virus-driven kinase reactions are more likely to influence striatal expression of met-enkephalin than dynorphin.

Our analyses of the BD rat model are consistent with a role for enkephalin upregulation in the production of stereotypic behaviours. Enkephalin analogues are known stimulants of motor behaviour (Stinus et al., 1980; Kalivas et al., 1983). Morphine sensitization produces oral stereotypies (Pollock & Kornetsky, 1996) and endomorphin-1 injected into the globus pallidus induces oroal dyskinesias in rats (Mehta et al., 2001), behaviours similar to those observed in the context of BDV infection.

In the normal striatum, a greater percentage of neurons in patches/striosomes than matrix contain enkephalin (Gerfen & Young, 1988). Assuming a similar striosome : matrix distribution for enkephalin in BD rats, the most parsimonious interpretation of data presented here is that viral infection has resulted in increased activity of the striosomal compartment relative to the matrix. A striosome-dominant gene activation pattern is another hypothesized determinant of stereotypy (Graybiel et al., 2000b).

These results with a neurotropic virus demonstrate a key role for enkephalinergic tone in the highly sensitive and exquisitely balanced cortico-basal ganglia–thalamo–cortical loops. By increasing transcription or stability of an enkephalin precursor mRNA, BDV causes a behavioural disease characterized by repetitive or fragmented motor actions. Further evaluation of pathways and mechanisms of the viral effect on neuropeptide expression should lead to enhanced understanding of the role of neuropeptides in movement disorders, and of neuropeptides in the aetiopathology (Gillberg et al., 1985; Engel & Rocha, 1992) and treatment of neuropsychiatric diseases.

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Abbreviations

BDV, Borna disease virus; CREB, cyclic AMP response element binding protein; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; PPD, pro- dynorphin; PPE, preproenkephalin.

References