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A role for endocannabinoids in viral-induced dyskinetic and convulsive phenomena

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Abstract

Dyskinesias and seizures are both medically refractory disorders for which cannabinoid-based treatments have shown early promise as primary or adjunctive therapy. Using the Borna disease (BD) virus rat, an animal model of viral encephalopathy with spontaneous hyperkinetic movements and seizure susceptibility, we identified a key role for endocannabinoids in the maintenance of a balanced tone of activity in extrapyramidal and limbic circuits. BD rats showed significant elevations of the endocannabinoid anandamide in subthalamic nucleus, a relay nucleus compromised in hyperkinetic disorders. While direct and indirect cannabinoid agonists had limited motor effects in BD rats, abrupt reductions of endocannabinoid tone by the CB_1 antagonist SR141716A (0.3 mg/kg, i.p.) caused seizures characterized by myoclonic jerks time-locked to periodic spike/sharp wave discharges on hippocampal electroencephalography. The general opiate antagonist naloxone (NLX) (1 mg/kg, s.c.), another pharmacologic treatment with potential efficacy in dyskinesias or L-DOPA motor complications, produced similar seizures. No changes in anandamide levels in hippocampus and amygdala were found in convulsing NLX-treated BD rats. In contrast, NLX significantly increased anandamide levels in the same areas of normal uninfected animals, possibly protecting against seizures. Pretreatment with the anandamide transport blocker AM404 (20 mg/kg, i.p.) prevented NLX-induced seizures. These findings are consistent with an anticonvulsant role for endocannabinoids, counteracting aberrant firing produced by convulsive agents, and with a functional or reciprocal relation between opioid and cannabinoid tone with respect to limbic convulsive phenomena.

Keywords: Rat; Anandamide; Basal ganglia; Seizure; Borna

Introduction

Parkinson's Disease (PD) treatment has been based on dopamine (DA) replacement therapy for 35 years. Yet, side effects resulting from long-term use of DA agonists, namely dyskinesias and on–off responses, are prompting investigations of alternative neurotransmitter manipulations to modulate basal ganglia function and normalize motor activity. Dyskinesias often result from lesion or disturbance affecting the transcortical loop or indirect pathway, with disruption of balance between excitation and inhibition in the globus pallidus pars externa-subthalamic nucleus-globus pallidus pars interna (GPe-STN-GPi) circuit. Thus, dyskinesias reflect altered patterns of neuronal firing in this circuit, which result in the improper selection of specific motor programs and, eventually, in the development of hyperkinetic movements (Filion, 2000; Obeso et al., 2000).

Endocannabinoids, the endogenous ligands of cannabinoid (CB) receptors, are synthesized upon demand by neurons in response to depolarization (Freund et al., 2003), and, once released, diffuse backwards across synapses to suppress pre-synaptic GABA or glutamate release (Szabo et al., 1998; Wallmichrath and Szabo, 2002; Wilson and

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Nicoll, 2002). Because of these properties, the endocannabinoid system may offer new pharmacological targets for the treatment of neurologic conditions characterized by abnormal firing patterns. One application of cannabinoid-based therapeutics would be for dyskinetic syndromes, hyperkinetic disorders characterized by changes in pattern, synchronization, mean discharge rates, and somatosensory responsiveness of neurons in the direct and indirect extrapyramidal (pallidal) motor circuits (Vitek and Giroux, 2000). Further applications of cannabinoid-based therapeutics may extend to treatment of seizure disorders, changes in behavioral or cognitive state resulting from hypersynchronous excessive neuronal discharges in other, for example, limbic, cortical or thalamic circuits.

To test the hypothesis that endocannabinoids act as endogenous antidyskinetic agents with modulatory effects on abnormal basal ganglia circuits, we examined endocannabinoid production in specific areas of the basal ganglia of rats infected with Borna disease virus (BD rats) and how cannabinoid agonists and antagonists affect their motor behaviors.

Borna disease virus (BDV) is a negative strand RNA virus epidemiologically linked to patients with neuropsychiatric disorders and Parkinson's-plus syndromes (psychomotor syndromes with brainstem and cerebellar involvement) (Ikuta et al., 2002; Nakamura et al., 2000). After infection, BD rats develop an extrapyramidal disorder with spontaneous dyskinesias, hyperactivity, stereotypic behaviors, partial DA deafferentation, DA agonist hypersensitivity, and Huntington's-type striatal neuropathology (Solbrig et al., 1994, 1998). Our investigations revealed elevations in the endocannabinoid anandamide in the subthalamic nucleus (STN) of BD rats, associated with increased metabolic activity in this key basal ganglia relay nucleus. As pharmacological antagonism of CB_1 receptors caused BD rats to seize, we also evaluated the relationship between changes in anandamide levels and seizure phenomena. Our results suggest that anandamide acts as both an endogenous antidyskinetic and anticonvulsive compound, in part via interactions with the opioid system.

Materials and methods

Animals

Subjects were male Lewis rats (Charles River Labs, Wilmington, MA) group housed on 12 h light–dark cycles with ad libitum access to food and water. Ten-week-old male Lewis rats (n = 6–12 per experimental group) were segregated to either behavior, or electrophysiology or neurochemistry experiments. All experimental procedures were performed in compliance with institutional (University of California-Irvine Institutional Animal Care and Use Committee; Animal Welfare Assurance no. A3416-01) and National Institutes of Health guidelines.

Infection of animals

Under metofane anesthesia, 4-week-old males were infected intracerebrally (i.c.) with BD Virus (BD rats) by injection of 1.6×10^4 tissue culture infectious dose units, strain He/80-1, or sham infected with sterile PBS (NL rats), and tested or sacrificed 6 weeks after infection (Solbrig et al., 1994).

Drugs

Drugs used were: the CB agonist R (+)-WIN 55,212-2 (Sigma, St. Louis, MO) (doses 1, 2, or 4 mg/kg i.p.) suspended by sonication in sterile saline; the anandamide transport blocker AM404 (Tocris, Ellisville, MO) (doses 10, 20 or 40 mg/kg i.p.) dissolved in 50% DMSO and 50% saline; the CB_1 antagonist SR141716A (Research Triangle Institute, Research Triangle Park, NC) (dose 0.3 mg/kg i.p.) suspended in saline solution containing 5% Tween; and the general opioid antagonist naloxone (Sigma) (dose 1 mg/kg s.c.) dissolved in saline.

Motor behavioral testing

Each test drug or corresponding vehicle was given to animals according to a Latin Square design to control for conditioning and order effects. Activity testing began 5 min after drug administration. Animals were monitored for 3 h testing sessions once a day in 40 × 25 × 20 cm cages equipped with 2 equally spaced horizontal photocell beams across the long axis. Dyskinesias in BD rats are hyperkinetic behaviors, gross movements of the trunk, head, and neck, whose severity can be approximated by counting individual beam breaks (Solbrig et al., 1994). Dyskinetic behavior was assessed by the number of single beam breaks and direct observation. Locomotor activity was quantified by counting the number of crossovers (successive interruption of two photo-beams) (Solbrig et al., 1994). Dyskinetic and locomotor activity was recorded in 10-min time-bins over the test session.

Electroencephalography

BD and NL rats were anesthetized with ketamine + xylazine (87 mg/kg + 13 mg/kg, i.p.) (Western Medical Supply, Arcadia, CA). Stainless steel screw electrodes (Plastics One, Roanoke, VA) were implanted in the cranium over the right and left retrosplenial cortices (overlaying hippocampus), fixed to the skull with dental cement, and ground electrodes were placed in the frontal bones over the prefrontal cortex. Electroencephalographic (EEG) signals were recorded continuously for 1 h after injections of naloxone (1 mg/kg s.c.), or either SR141617A (0.3 mg/kg s.c.) or AM404 (20 mg/kg i.p.) followed in 5 min by naloxone (1 mg/kg s.c.). EEG signals were obtained from unrestrained rats using commutators with flexible recording cables (Plastics One, Roanoke, VA), and amplified and

displayed in a Grass Polygraph (Model P511) using manufacturer's settings, data acquisition (PolyVIEW/XL) and analysis software (Astro-Med, Inc./Grass Telefactor, W. Warwick, RI). After surgery, animals were allowed to recover for 1 week before being studied. Seizures were characterized by rhythmic spike or sharp wave discharges on the EEG tracings, with amplitudes at least 2–3 times higher than baseline, and accompanied by epileptic-like behaviors (staring spells, behavior arrest, twitches, chewing, wet dog shakes, clonus, rearing with loss of balance) (Racine, 1972).

Brain tissue preparation and HPLC/MS analyses

A separate group of animals were euthanized by inhaled methoxyfluorane followed by decapitation. Brains were rapidly removed 30 min after the last injection of drug or vehicle, frozen in cold 2-methylbutane (−50°C), cut into contiguous 2-mm coronal sections by razor blades using an ice cold aluminum alloy mould. The following regions were dissected from these sections: frontal cortex (cortex anterior to the genu of the corpus callosum), striatum, globus pallidus (medial and lateral), subthalamic nucleus, substantia nigra (compacta and reticulata), amygdala, and hippocampus. Tissue samples were thawed in 1 ml of methanol containing [$^2H_4$]-labeled anandamide (25 pmol) and homogenized. Acylethanolamides were extracted with methanol/chloroform (1:2, v/v) and quantified by isotope dilution HPLC/MS as described (Giuffrida et al., 2000a).

Cytochrome oxidase I histochemistry

Animals were euthanized and perfused with buffered 1% paraformaldehyde, 2% glutaraldehyde and cytochrome oxidase histochemistry was performed according to specifications of Crockett et al. (1993). Free floating 50 μm frozen sections were incubated in 100 ml of 0.1 M phosphate buffer containing 55 mg of 3,3′-diaminobenzidine (Sigma), 7.5 mg horse heart cytochrome *c* (Sigma), 5 g sucrose, 2 μg catalase (Sigma), 250 μl DMSO. 2.5 ml of 1% cobalt chloride and 2 ml of 1% nickel ammonium sulfate were added to the solution while stirring. Sections were then reacted for 30 min at 37°C until gray and white matters were easily distinguished (Crockett et al., 1993).

Immunohistochemistry

For detection of GABAergic neurons, free floating 50 μm frozen sections were collected into 0.1 M PBS, preincubated with 3% normal goat serum in PBS, incubated overnight at RT with 1:250 anti-glutamic acid decarboxylase (GAD) antibody (GAD-6 AP, 64 kDa subunit, Hybridoma Bank, Iowa City, IA), then incubated in biotinylated secondary antibody, processed by ABC histochemical method (Vector, Burlingame, CA) and developed with 3,3′-diaminobenzidine as chromagen. Negative controls included omission of primary or secondary antisera.

c-fos in situ hybridization

For c-*fos* in situ hybridization, animals were euthanized by inhaled methoxyfluorane 30 min after naloxone administration and perfused with buffered 4% paraformaldehyde. Brains were removed, postfixed and cryoprotected. Twenty micron coronal sections were collected onto Superfrost-Plus slides and hybridized with an ^{35}S labeled RNA probe (specific activity 2–6 × 10^7 cpm/mg; 5 ng/probe/slide) complementary to mRNA encoding cRNA probes generated from a 667 bp *Pst*I–*Pvu*II of pc-*fos*(rat)-1 (Curran et al., 1987). Optical densities of film autoradiograms were transferred to a computer-based image analysis system (MCID, Imaging Research Inc., Ontario, Canada) with calibration curves constructed using ^{14}C polymer standards (ARC, St. Louis, MO) and reproduced as pseudocolor images.

Data analysis

Neurochemical data was analyzed by one-way analysis of variance (ANOVA) followed by Student's *t* test with Bonferroni's correction. Photocell beam interruptions and crossovers were analyzed using repeated measures ANOVA. Beam break scores (photocell counts) were analyzed by two-way ANOVA with BD or NL groups as the independent factors and dose or time as the repeated measures. Differences among groups or treatments were verified by post hoc analysis (Newman–Keuls for comparisons among means). Numbers of animals observed with epileptic behaviors were analyzed using the Information Statistic for nonparametric independent samples (Kullback, 1968). Behavioral observations of epileptic behaviors were verified with recorded EEGs.

Results

BD-induced dyskinesias are associated with anandamide elevation in STN

Six weeks after infection, BD rats develop spontaneous dyskinesias (vacuous chewing, head bobbing, upper body tics), retrocollis, dystonia, locomotor hyperactivity, stereotypies, together with regional differences in anandamide levels in the basal ganglia circuit compared to NL (uninfected age-matched) rats [$F(9,99) = 3.682$ $P = 0.001$]. In BD rats, anandamide was significantly elevated in the subthalamic nucleus (STN) (BD 55.2 ± 8.2 vs. NL 17.3 ± 8.2 pmol/g, $P < 0.05$), whereas no changes were observed in other basal ganglia areas or in cortex (Fig. 1A).

Cellular effects of infection

To further characterize cellular bases for anandamide changes, we examined the effects of BDV infection on markers of neuronal metabolic activity, staining for Cyto-

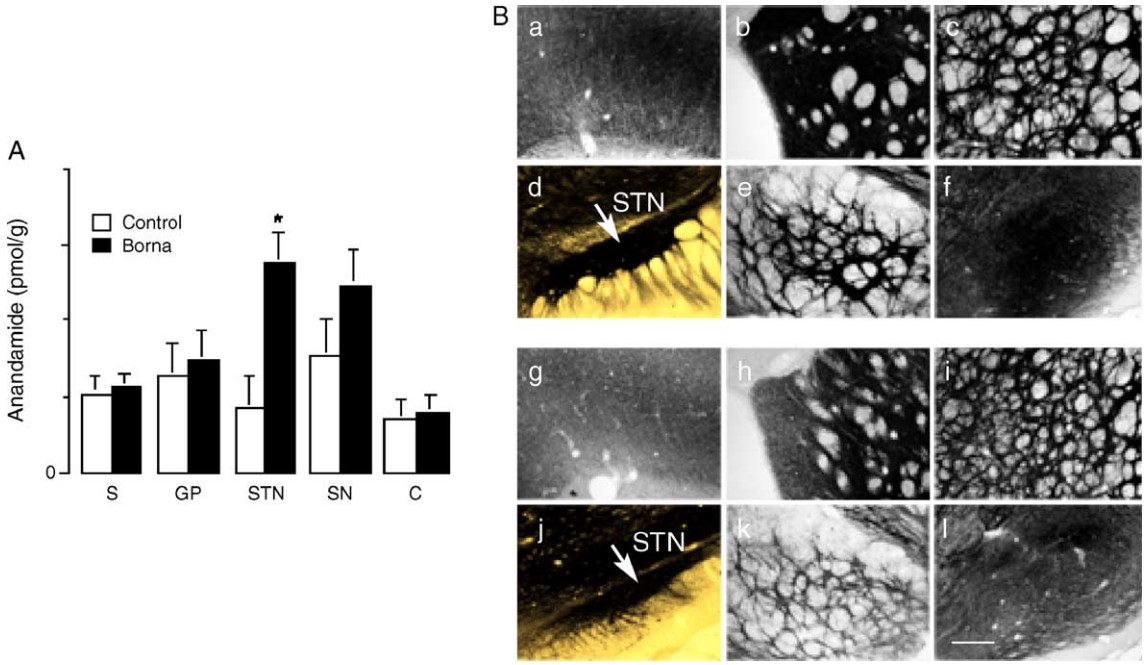

Fig. 1. (A) Regional anandamide levels in spontaneously dyskinetic BD rats. Anandamide tissue levels were significantly increased in STN. Values represent mean ± SEM *$P < 0.05$, compared to NL rats (Student's t test with Bonferroni's correction following significant ANOVA) ($n = 10$ per group). S, striatum; GP, globus pallidus; STN, nucleus subthalamicus; SN, substantia nigra; C, prefrontal cortex. (B) Regional cytochrome oxidase I (CoI) activity, as shown by enzyme histochemistry. An apparent increase in CoI activity is seen in STN of BD rats (j), compared to NL rats (d), while staining of all other BD structures appeared similar or reduced compared to normals. NL rat (a) motor cortex; (b) striatum, (c) globus pallidus externa; (d) STN, nucleus subthalamicus; (e) globus pallidus interna; (f) substantia nigra; and BD rat (g) motor cortex; (h) striatum; (i) globus pallidus externa; (j) STN, nucleus subthalamicus; (k) globus pallidus interna; (l) substantia nigra. Scale bar = 250 μm.

chrome oxidase I (CoI) in basal ganglia structures. CoI histochemistry staining, apparently increased in STN of BD rats, was consistent with subthalamic nucleus hyperactivity in these animals (Fig. 1B). In other areas, CoI appeared lower in BD rats, a result ascribed to regional neuronal loss. Anti-GAD staining for GABA neurons was diminished throughout the basal ganglia circuit (Fig. 2). Diffuse background staining replaced GAD-stained cells in motor cortex, cells and processes of striatum, pallidum, and STN output regions. The pattern of staining, similar to BD negative control sections processed without primary antibody, was attributed to increased blood brain barrier permeability and penetration of brain parenchyma by nonspecific IgGs.

Behavioral effects of cannabinoid agonists and antagonists

Neither the anandamide transport blocker AM404 nor the CB agonist WIN-55,212-2 had statistically significant effects on dyskinetic behavior over the 3-h test session. Administration of AM404 produced no significant group × dose effect [$g \times d$ $F(3,33) = 1.636$; $P = 0.1999$]. Likewise, administration of WIN-55,212-2 produced no significant group × dose effect over the 3-h test session [$g \times d$ $F(3,30) = 0.687$; $P = 0.5668$]. However, differing from AM404, WIN 55,212-2 produced a significant group × dose interaction effect 2 h after injection [$F(3,30) = 3.581$, $P < 0.05$ for the third test hour with a significant main effect of dose [$F(3,30) = 3.364$, $P < 0.05$].

On the other hand, BD rats treated with the selective CB_1 antagonist SR141716A (0.3 mg/kg i.p.) showed epileptic behaviors 15 min after drug administration. Observed activities included behavior arrest, staring spells, or myoclonic jerks of trunk or forepaws, which were time-locked to periodic spike or wave discharges on EEG recorded from electrodes overlaying the hippocampus (InfoStat, SR141716A BD 5/5 seizures vs. SR141716A NL 0/5 seizures, $2\hat{I} = 13.8629$, $df = 1$, $P < 0.001$) ($n = 5$ per group) (Fig. 3). The seizures began with a transition from background burst activity to continuous spike or sharp wave discharges. Seizures, approximately 20 s at first, increased in frequency and duration to last several minutes. Since epileptiform activity was consistently recorded after SR administration to all BD rats, the experiment was concluded after testing of a total of 5 BD rats and an equal number of NL controls.

The ability to reliably precipitate seizures in BD rats had been recognized previously. Similar seizures had been encountered during treatment of dyskinetic and self-injurious behaviors of the BD rats with naloxone (Solbrig et al., 1996). BD rats treated with the general opiate antagonist naloxone (1 mg/kg s.c.) develop epileptic behaviors within 10 min of drug administration with high amplitude periodic sharp wave discharges on hippocampal EEGs (Fig. 4A). c-*fos* expression mapping of neuronal networks involved in naloxone seizures showed prominent hippocampal (dentate gyrus) and amygdala in situ hybridization signal after drug

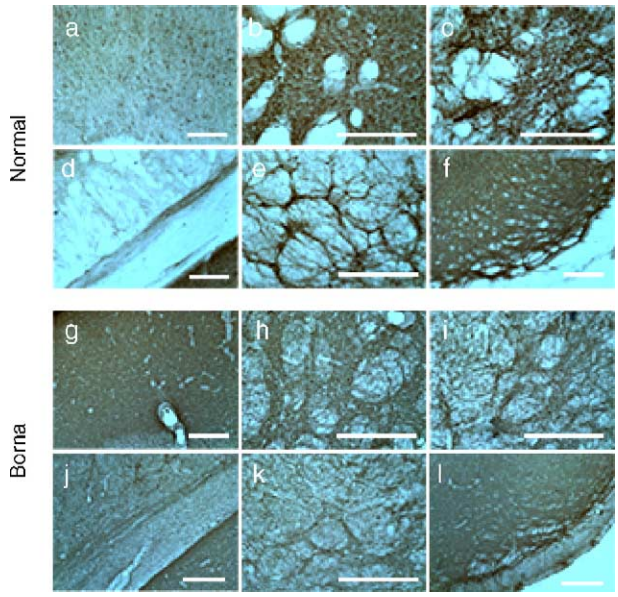

Fig. 2. Effects of BD viral infection on GABA circuits. Light microscopic analysis of GAD immunohistochemical staining in basal ganglia circuit shows reduced or absent immunoreactive cells or processes in BD rats compared to normal (NL) rats. NL rat (a) motor cortex; (b) striatum; (c) globus pallidus externa; (d) STN, nucleus subthalamicus; (e) globus pallidus interna; (f) substantia nigra; and BD rat (g) motor cortex; (h) striatum; (i) globus pallidus externa; (j) STN, nucleus subthalamicus; (k) globus pallidus interna; (l) substantia nigra. Scale bar = 250 μm.

administration (Fig. 4A). Elevated expression patterns of the immediate early gene c-*fos* 30 min from onset of seizures, thus revealed involvement of limbic structures in the convulsive syndrome. Demonstration of limbic involvement enabled further neurochemical analysis of the excitability phenotype.

Anandamide as an endogenous anticonvulsant

To determine if the convulsive phenomena observed in BD rats were associated with changes in anandamide levels in limbic areas, we measured anandamide concentrations in cortex, hippocampus, and amygdala of BD and NL rats 30 min after administration of naloxone (NLX). This time frame is sufficient to induce seizures in BD rats and immediate early gene c-*fos* activation in hippocampus and amygdala.

Significant increases in anandamide levels were found in hippocampus and amygdala of naloxone-treated NL animals, which did not seize, whereas no anandamide elevation was observed in the same areas of BD rats, which displayed behavioral and electroencephalographic seizures (hippocampus BD 13.2 ± 2.4; NL 59.4 ± 6.4 pmol/g, $P < 0.001$; amygdala BD 6.7 ± 1.6; NL 63.7 ± 12.1 pmol/g, $P < 0.001$) (ANOVA [$F(11,79) = 11.66$, $P < 0.0001$, $n = 12$ per group) (Fig. 4B). Anandamide levels in naloxone-treated BD rats were similar to basal anandamide levels of vehicle-treated BD and NL rats in which no seizures were observed (Fig. 4B). Interestingly, although naloxone administration did increase the number of sharp-wave transients on EEGs recorded from a separate group of NL rats (Fig. 4A, upper right), these rats did not seize.

Finally, to test if seizures were affected by manipulation of endocannabinoid tone, the anandamide transport blocker AM404 (20 mg/kg i.p.) was administered to BD rats 15 min before NLX administration. AM404, which has been shown to increase AEA levels extracellularly (Giuffrida et al., 2000b), prevented naloxone-induced seizures in all BD rats tested (InfoStat, BD AM404-NLX 0/5 seizures vs. BD NLX 8/8 seizures, $2\hat{I} = 17.3232$, $df = 1$, $P < 0.001$) ($n = 5$–8 per group) (Fig. 4C).

Discussion

Our results are consistent with a functional role for anandamide signaling as a natural mechanism to buffer abnormal firing patterns in various neural circuits. Using BD rats, a rodent model of viral-induced neurodegenerative syndrome and spontaneous dyskinesias, we showed significant anandamide elevations in the STN, a critical basal ganglia relay nucleus in which abnormal firing has been linked to dyskinesias, dystonia and hemiballismus (Vitek and Giroux, 2000). The characteristics of STN neurons, such as fast firing kinetics, short membrane refractory periods and ability to modify their firing pattern after small changes in impinging synaptic input (Kita et al., 1983; Kitai

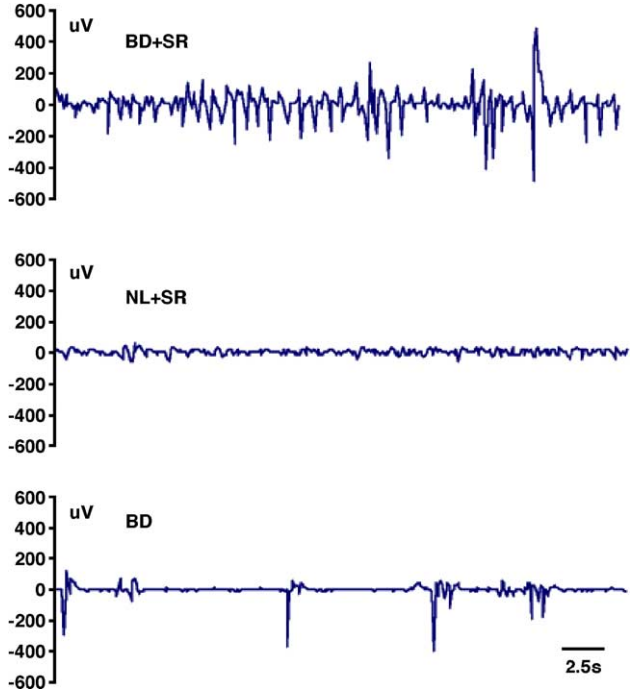

Fig. 3. Convulsive effects of SR141716A in BD rats. Representative EEGs recorded from hippocampal leads from BD (upper) and NL (middle) rats 40 min after SR141716A (0.3 mg/kg i.p.) injection. SR-treated BD rats had rhythmic (2–3/s) spike discharges recorded on EEG, distinct from vehicle or untreated BD rats whose encephalopathic tracings showed intermittent high amplitude bursts or sharp waves but not repetitive discharges (lower tracing). All (5) BD rats tested with SR showed epileptiform activity.

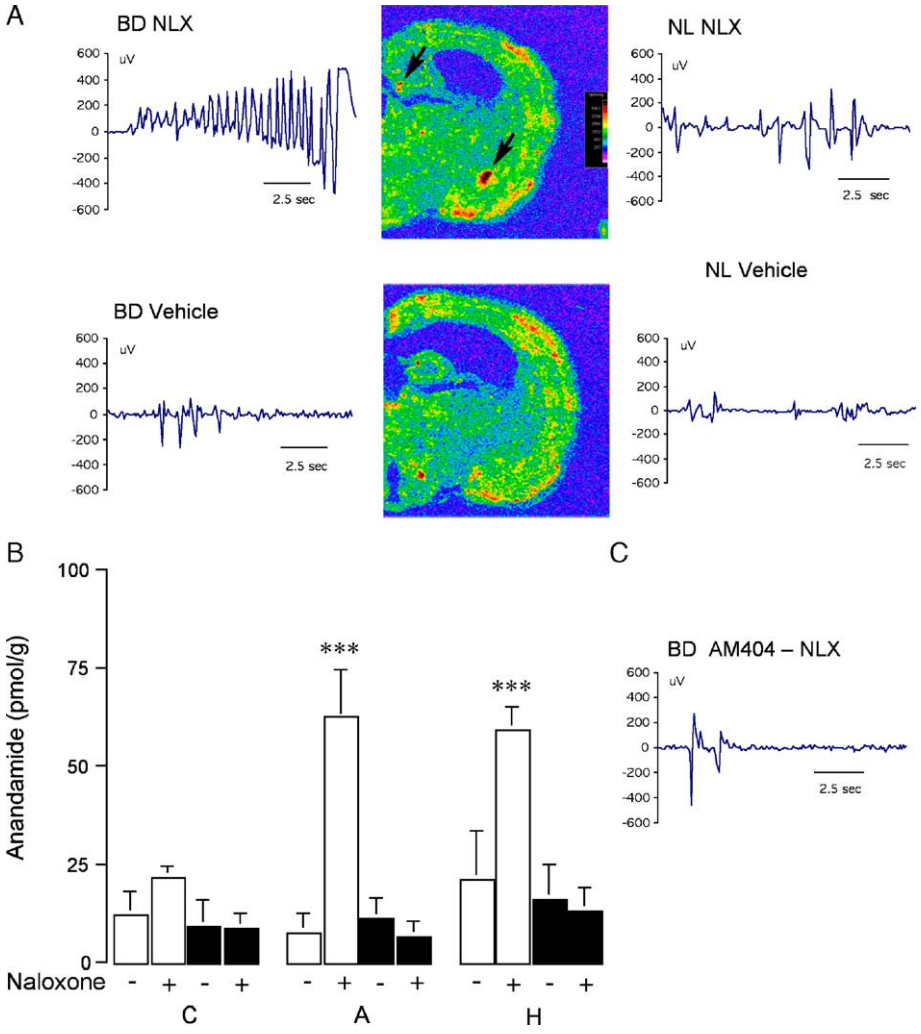

Fig. 4. Cannabinoid–opioid interactions. (A) Convulsive effects of naloxone (NLX) in BD rats. Representative hippocampal EEG recordings from BD (upper left) after NLX (1 mg/kg s.c.) injection, showing a seizure as high amplitude rhythmic sharp wave discharges. NLX-treated NL animals had increased numbers of sharp waves (upper right), but remained seizure-free. EEGs of vehicle-treated rats (lower left and right) are shown for comparison. Digitized images of film autoradiograms showing c-*fos* activation to drug treatments are shown beside corresponding treatment groups. Coronal sections hybridized with ^{35}S cRNA probe for detection of immediate early gene c-*fos* mRNA expression, shows dense hybridization signal in dentate gyrus and amygdala (arrows) of a NLX-treated BD rat 30 min after NLX administration and seizures. Limbic c-*fos* enhancement was not seen in vehicle-treated BD rats. (B) Anandamide levels in limbic areas of NL (open bars) and BD rats (filled bars) 30 min after systemic administration of naloxone (1 mg/kg, s.c.) or vehicle. Significant elevations in anandamide were present in amygdala and hippocampus of NL rats 30 min after drug administration. Values represent mean ± SEM ***$P < 0.001$ (Student's t test with Bonferroni's correction following significant ANOVA) (n = 12 per group). C, cortex; A, amygdala; H, hippocampus. (C) Pretreatment with AM404 (20 mg/kg i.p.) before a single dose of naloxone (1 mg/kg s.c.) prevented clinical and electrographic seizures.

and Deniau, 1981), render the STN well-suited to regulation by activity-dependent modulators such as endocannabinoids. In keeping with this hypothesis, CB_1 receptor protein (Mailleux and Vanderhaeghen, 1992) and functional CB_1 receptors (Miller et al., 1998) have been found in the STN of rats. However, neither WIN 55,212-2 nor AM404 had robust antidyskinetic effects in BD rats, which we attribute to loss of CB_1 receptors along with GABA neurons in other nuclei of the basal ganglia circuit, as indicated by striking loss of GAD immunoreactivity in BD rats.

STN hyperactivity is a recognized feature of PD (Porter et al., 1994; Vila et al., 1996), but may also signifying abnormal patterns of firing, as in dystonia (Vitek and Giroux, 2000). Thus, in BD rats, which display mixed Parkinsonian and Huntington's lesions, the anandamide and Col activity elevation observed in the STN may represent an important compensatory or modulatory reaction to abnormal input to STN, with the net effect of reducing pathologic or dyskinetic movements.

The convulsant effect of CB_1 receptor antagonist SR141716A was an unexpected result. Since abrupt reduction of endocannabinoid tone produced hippocampal seizures in BD rats, we suggest that anandamide, in addition to its compensatory function in the basal ganglia, may have a role in maintaining homeostatic or balanced activity in limbic networks.

To further evaluate the role of anandamide in convulsive phenomena, we used a seizure paradigm already developed in BD rats. In these rats, degenerative changes extend to hippocampus and amygdala and self-limited limbic seizures can be consistently produced within 5 to 10 min of administration of the general opiate antagonist naloxone (Solbrig et al., 1996). We found that administration of naloxone did not change anandamide levels in the hippocampus and amygdala of BD rats that seized, while naloxone did cause significant anandamide elevation in the same brain areas of normal rats that did not seize. The failure of BD rats to increase limbic region levels of anandamide in response to the opiate antagonist naloxone is consistent with the idea that decreased availability of anandamide on demand contributed to seizures induced by a chemoconvulsant.

Dynamic neurotransmitter buffering in rapid response to excitatory stimuli may be a general principal of endogenous anticonvulsants, applying to classic inhibitory neurotransmitters such as GABA and to neuromodulators such as opioids or endocannabinoids. For example, when opioid tone was reduced by naloxone, the result was increased EEG activity of both normal and BD rats. When BD rats developed increased or hypersynchronous EEG activity but could not increase anandamide levels, it was the anandamide transport blocker AM404 that limited or reversed naloxone excitability and rescued the animal from seizures. Anticonvulsant efficacy was most likely via elevation of anandamide or other endocannabinoid tone, an interpretation consistent with anecdotal reports by patients of improvement in seizure frequency or severity with marijuana use (Gross et al., 2004). However, at this time, we cannot exclude a vanilloid-mediated effect of AM404, since this drug binds to the TRPV1 receptor (Jerman et al., 2000).

Our study widens the role of potential cannabinoid–opioid interactions beyond substance abuse, tolerance-dependence phenomena, analgesia, hypothermia, and inflammation (Hine et al., 1975; Ledent et al., 1999; Manzanares et al., 1999; Navarro et al., 2001; Welch and Stevens, 1992) and suggests a reciprocal relation between these two systems with respect to convulsive phenomena. So far, cannabinoids and opioids have been implicated separately in seizures (Karler et al., 1973,1974; Simonato and Romualdi, 1996; Solbrig and Koob, 2004; Tortella, 1988; Wallace et al., 2002, 2003). While CNS opioid dysregulation has been considered a substrate for the interictal personality disorder (Engel and Rocha, 1992), CNS endocannabinoid signaling changes because of their association with schizophrenia and psychotic symptoms (Giuffrida et al., 2004) might also contribute to interictal cognitive or personality syndromes. Endocannabinoid upregulation during opiate withdrawal could explain the absence of seizures during opiate withdrawal (Brust, 1993).

The opioid system includes several families of related neuropeptides and μ, δ, κ opioid receptors. In other work (Solbrig and Koob, 2004), kappa opioid receptors (KOR) have been identified as a major contributor to anticonvulsant efficacy. When K and CB_1 receptors are compared, they are found to have convergent biochemical mechanisms. Both are members of Gi/o protein coupled receptor family and signal through cAMP-Protein Kinase A, inwardly rectifying K^+ channels and N, P/Q, R type Ca^{++} channels (summarized in Navarro et al., 2001). KOR and CB_1 receptors exhibit overlapping (regional but not cellular) neuroanatomic distribution in hippocampus. CB_1 receptors are found on CCK-expressing interneurons (Katona et al., 1999), while KOR receptors are found on mossy fiber terminals, principal neurons, perforant path and supramammillary afferents, and GABA/SOM/NPY containing interneurons (reviewed in Solbrig and Koob, 2004). The classic hippocampal circuit is a trisynaptic circuit utilizing glutamatergic neurotransmission. At each step, excitatory tone is modulated by a diverse group of inhibitory and excitatory neurons (Freund and Buzsaki, 1996). KOR or CB_1 stimulation of selective interneurons could desynchronize GABA inputs to a postsynaptic network. Desynchronization of signals from GABA-containing interneurons to their networks of pyramidal cells is one mechanism of enhanced inhibition of principal neurons. KOR stimulation at other sites, producing pre- or postsynaptic inhibitory effects on large pyramidal neurons, would also modulate excitability of principal neurons, with net effect the modulation of hippocampal outflow pathways.

Further studies in our model will investigate the effect of AM404 on endocannabinoid production in the limbic system. Greater understanding of the conditions for interaction between endocannabinoids and opioid system will enhance our knowledge of neural circuits that serve fundamental or broad homeostatic functions and also will be the goal of future studies.

In conclusion, knowledge of endocannabinoid distribution and function throughout basal ganglia circuits could lead to the identification of non-dopamine pharmacologic targets for dyskinetic disorders and a greater understanding of the role of output pathways in the genesis of motor behaviors and involuntary movements. Parallel advances in the knowledge of endocannabinoid function throughout limbic circuits should lead to identification of alternative strategies for the treatment of seizure disorders.

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