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Short communication

Activators of potassium M currents have anticonvulsant actions in two rat models of encephalitis

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Abstract

Opioid systems in hippocampus regulate excitability and kappa opioids have a role in anticonvulsant protection, but their mechanisms of action are incompletely understood. We examined the ability of opioid and nonopioid agents with overlapping ionic mechanisms and actions similar to kappa opioid agonists, to block seizures in rat models of encephalitis due to Borna Disease virus and Herpes Simplex Virus Type-1. Naltrindole, a delta antagonist and thus a kappa opioid sparing agent, (10 mg/kg s.c.) blocked spontaneous and naloxone (opioid antagonist)-induced seizures in the models, but produced somatic signs similar to opioid withdrawal. Given that delta antagonists as well as kappa opioid agonists in hippocampus enhance potassium M currents (I_M), we tested the effect of the I_M augmenter flupirtine. Flupirtine (20 mg/kg i.p.) prevented seizures in Borna and herpes infected rats, without signs of withdrawal, hypotonia or sedation. The results support the efficacy of opioid and nonopioid drugs in modulating naloxone-induced seizures in critical illness due to viral encephalitis and by analogy, opioid withdrawal seizures.

Keywords: Opioid; Seizure; Epilepsy; Virus; Herpes; Borna

1. Introduction

Seizures often accompany alcohol or barbiturate withdrawal, but are not typically part of narcotic withdrawal or abstinence syndromes. Although the adaptive changes in excitatory synaptic transmission during acute opiate withdrawal do not usually produce seizures, there are exceptions. Seizures occur in up to 8% of neonates exposed to heroin or methadone *in utero* (Osborn et al., 2005) and one-third of critically ill adults after sudden withdrawal of narcotic agents (Wijdicks and Sharbrough, 1993).

An interesting parallel to opiate withdrawal seizures has been seen in encephalitic rats. Systemic injection of the competitive opioid antagonist naloxone precipitates seizures in rats with chronic encephalitis due to Borna Disease (BD) virus (Solbrig et al., 1996, 2006a) and in rats with acute herpes simplex virus type-1 (HSV-1) encephalitis (Solbrig et al., 2006b). Restoration of dynorphin tone by administration of a kappa opioid agonist blocks seizures (Solbrig et al., 2006b). If dominant principles of encephalitic models define aspects of human seizure vulnerability, such as neurobiologic factors during stress of critical illness, the similarities afford an opportunity to learn from encephalitic models. Results from these unique rodent models can be applied to precipitated opiate withdrawal and to other refractory seizure states in man.

The present study was designed to explore treatments for naloxone-induced seizures in the BD virus-infected and HSV-1-infected rat encephalitis models, testing the hypothesis that opioid and nonopioid drugs with overlapping (ionic) mechanisms have anticonvulsant actions during opioid withdrawal. In hippocampal models, kappa selective agonists or delta opioid antagonists each increase voltage-dependent potassium (K^+) M currents (I_M) of CA3 neurons (Moore et al., 1994), currents that

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are important stabilizers at or near resting membrane potentials (Wickenden, 2004). In CA3 pyramidal neurons, delta agonists reduce voltage-dependent potassium M currents (Moore et al., 1994), providing a basis for convulsant activity. Given the recent report of delta opioid agonists as convulsants in rats (Torregrossa et al., 2004), we investigated the effect of naltrindole, a delta opioid antagonist and kappa sparing agent, on naloxone seizures. Finding naltrindole prevented seizures but elicited somatic signs similar to opioid withdrawal, we elected to examine another compound for a more beneficial spectrum of biologic responses. Since compounds that increase I_M diminish neuronal excitability, we examined the I_M enhancer flupirtine for anticonvulsant efficacy in naloxone seizures.

2. Materials and methods

2.1. Animals

Subjects were male Lewis rats (Charles River Labs, Wilmington, MA, USA) group housed on a 12 h light–dark cycle with *ad libitum* access to food and water. 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.

2.2. Virus

Borna Disease virus preparations have been described (Solbrig et al., 1994). Wild-type McKrae HSV-1 virus was triple plaque purified and passaged in rabbit skin cells, titrated as described (Perng et al., 1994).

2.3. Infection of animals

Under methoxyflurane anesthesia, 4 week old males were infected intracerebrally (i.c.) with Borna Disease virus (BD virus-infected rats) by injection of 1.6×10^4 tissue culture infectious dose units, strain He/80-1, in a total volume of 30 μl or sham infected with sterile phosphate buffered saline (PBS) (uninfected normal [NL] rats) (Solbrig et al., 1994). Neuropharmacologic testing was 6 weeks after infection, at 10 weeks of age (Solbrig et al., 1994). Infection was confirmed by the appearance of a clinical syndrome consistent with BD and by immunohistochemical detection of viral antigen in post-mortem specimens (Solbrig et al., 1994, 2006a).

For HSV-1, nine week old male Lewis rats were anesthetized with ketamine+xylazine (87 mg/kg+13 mg/kg, i.p.) (Western Medical Supply, Arcadia, CA, USA) and infected by placing, as eye drops, 3×10^6 PFU of virus into the right eye and conjunctival sac, then manually closing and opening the eye. Uninfected control rats were anesthetized with ketamine+xylazine and sham infected using sterile Eagle minimal essential medium as eye drops. Animals were tested on days 5 and 6 of illness, and sacrificed on day 6, at 10 weeks of age. Infection was confirmed by immunohistochemical detection of HSV-1 in brain (by a polyclonal anti-HSV-1, 1:200, B0114 to envelope glycoproteins and a core protein, Dako, Glostrup, Denmark).

2.4. Drugs

Drugs used were the general opioid antagonist naloxone (1 mg/kg s.c.) (Sigma, St. Louis, MO, USA) dissolved in saline, the delta opioid receptor antagonist naltrindole (10 mg/kg s.c.) (Sigma) dissolved in saline, and the nonopioid analgesic flupirtine (20 mg/kg i.p.) (Sigma) dissolved in 10% Tween 20/saline. Naloxone is a dose-dependent convulsant in encephalitic rats, with 1 mg/kg s.c. causing seizures in both BD virus-infected and HSV-1-infected rats (Solbrig et al., 1996, 2006a,b). Ten mg/kg s.c. naltrindole is a dose that blocks delta agonist mediated increases in brain derived neurotrophic factor mRNA expression after seizures (Torregrossa et al., 2004). Twenty mg/kg i.p. flupirtine is a median effective dose in rodent maximal electroshock and pentylenetetrazol seizure models (Porter et al., 1983). Flupirtine is the only anticonvulsant potassium M current enhancer available commercially.

2.5. Electroencephalography (EEG)

Eight week old BD virus-infected or NL animals were anesthetized with ketamine+xylazine (87 mg/kg+13 mg/kg, i.p.) (Western Medical Supply, Arcadia, CA, USA). Stainless steel screw electrodes (Plastics One, Roanoke, VA, USA) were implanted in the cranium over the right and left retrosplenial cortices (overlaying hippocampus) as described (Solbrig et al., 2006a). After surgery, BD virus-infected animals were allowed to recover for 1 week before study. NL animals were allowed to recover 1 week, then infected with HSV-1 or sham-infected with sterile eye drops and retained as uninfected study animals.

EEG signals were continuously recorded from freely moving rats for up to 1.5 h after drug injections using Grass Polygraph (Model P511), data acquisition (PolyVIEW/XL) and analysis software (Astro-Med, Inc./Grass Telefactor, W. Warwick, RI, USA). (Solbrig et al., 2006a). Seizures were rhythmic spike or sharp wave discharges on the EEG tracings, with amplitudes at least 2 times higher than baseline, and accompanied by epileptic-like behaviors (staring spells, behavior arrest, twitches, chewing, clonus, rearing with loss of balance) (Racine, 1972).

2.6. Experimental treatment

Behavioral and EEG response to drug was assessed in 10 week old animals, at 6 weeks of infection for BD virus-infected rats. For HSV-1 experiments, behavioral and EEG activity was assessed in 10 week old animals with clinically apparent illness 5–6 days after infection. Also, rats were observed for classical somatic signs based on those observed during opioid withdrawal: tremors, escape jumps, wet dog shakes, facial fasciculations, abdominal constrictions, genital licks, eye blinks, diarrhea, vocalizations, abnormal posture, ptosis (Maldonado et al., 1992). Behaviors were recorded on a checklist throughout the 90 min test session. Anticipating

difficulty differentiating withdrawal behaviors from the underlying condition or seizures, we reported as withdrawal behaviors only behaviors distinct from BD virus-infected rat hyperactivity, stereotypies (Solbrig et al., 1994) and epileptiform behaviors. For example, wet dog shakes, which can be epileptic (Schwarcz et al., 1984), were excluded from the final report.

In experiment 1 BD virus-infected rats were injected with saline or naltrindole 10 mg/kg s.c., 15 min prior to injection of saline vehicle or naloxone 1 mg/kg s.c. ($n=6$ per group). NL uninfected age-matched rats served as controls. Animals were simultaneously observed and EEG monitored for convulsions for 1.5 h following the last injection.

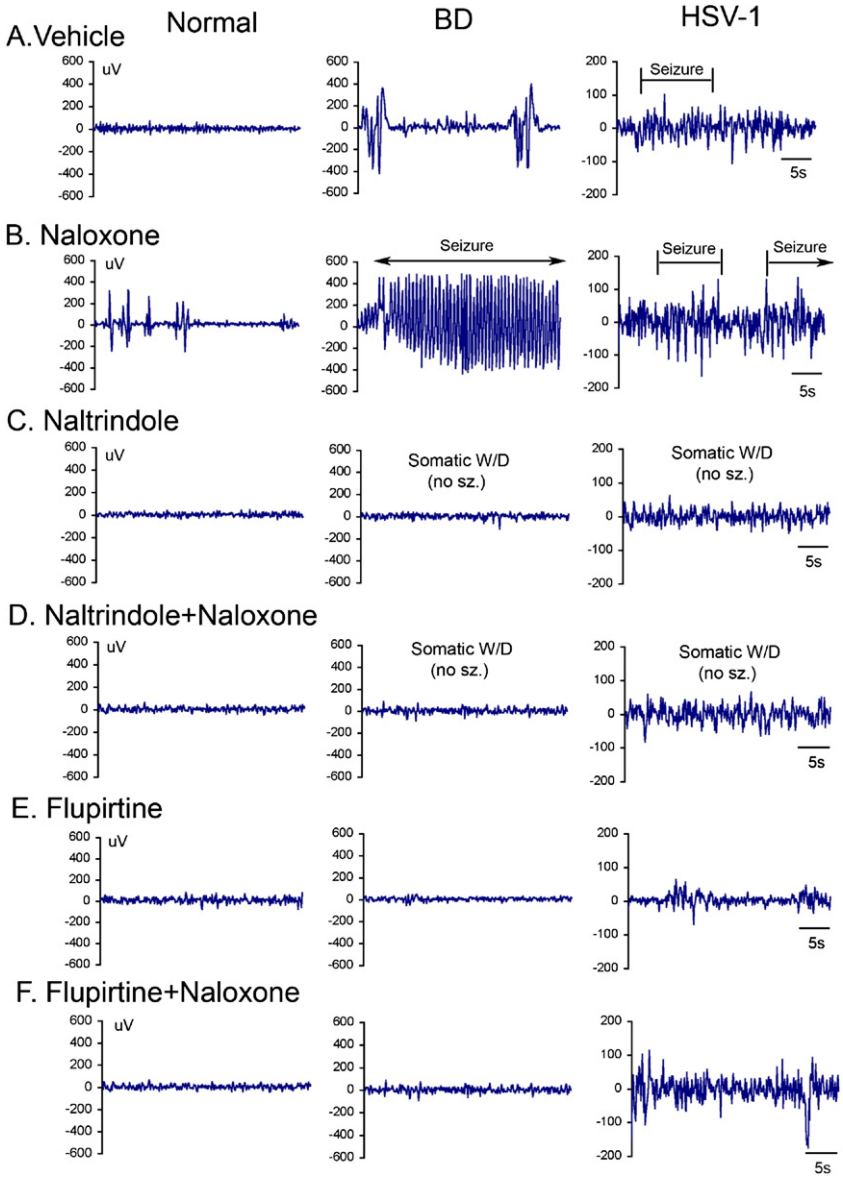

Fig. 1. Representative EEGs recorded from freely moving normal uninfected rats, BD rats and HSV-1 rats. A. Vehicle treatment produced low amplitude desynchronized activity with little variation in normal rats, burst-suppression patterns in BD rats, intermittent spikes or sharp waves against a disorganized background and a brief spontaneous seizure with rhythmic sharp waves in HSV-1 rats. B. Naloxone (1 mg/kg s.c.) treatment elicited transient isolated sharp waves in normal rats, rhythmic 2 per second high amplitude spike or sharp wave discharges in BD rats, and in HSV-1 rats, more synchronous EEGs, with extended epileptiform activity as bursts of rhythmic sharp waves or grouped rhythmic discharges, compared to vehicle treated HSV-1 rats. These EEG segments were recorded 15 min after drug administration. In BD rats, this electrographic seizure is typical of patterns recorded from 5 to 45 min after naloxone administration. In HSV-2 rats, this electrographic seizure is typical of patterns recorded from 5 to 35 min after naloxone administration. C. Naltrindole (10 mg/kg s.c.) treatment elicited neither behavioral nor EEG changes in NL rats. Infected rats were agitated and tremulous. Naltrindole blocked high amplitude polyphasic bursts of BD rat EEGs, and produced irregular high frequency discharges in HSV-1 rats, blocking their high amplitude sharp waves. These EEG segments were recorded 15 min after drug administration. D. Naltrindole (10 mg/kg s.c.) blocked naloxone seizures in both BD and HSV-1 rats. Irregular electrocortical activity was nested in lower frequency oscillations approximating tremor frequency of HSV-1 rats. These EEG segments were recorded 15 min after naloxone administration. E. Flupirtine (20 mg/kg i.p.) treatment elicited neither behavioral nor EEG changes in normal rats, and decreased overall EEG amplitude of BD and HSV-1 rats. F. Flupirtine (20 mg/kg i.p.) blocked naloxone seizures in both BD and HSV-1 rats. These EEG segments, recorded 15 min after naloxone administration, were representative of 45 min of monitoring. BD — Borna Disease, HSV-1 — herpes simplex virus type 1, W/D — withdrawal, s — seconds.

In experiment 2 HSV-1-infected rats were injected with saline or naltrindole 10 mg/kg s.c., 15 min prior to injection of saline vehicle or naloxone 1 mg/kg s.c. and monitored as in experiment 1 ($n=6$ per group).

In experiment 3 BD virus-infected rats were injected with vehicle or flupirtine 20 mg/kg s.c., 15 min prior to injection of saline vehicle or naloxone 1 mg/kg s.c. ($n=6$ per group). NL uninfected age-matched rats served as controls. Animals were simultaneously observed and EEG monitored for convulsions for 1.5 h following the last injection.

In experiment 4 HSV-1-infected rats were injected with vehicle or flupirtine 20 mg/kg i.p., 15 min prior to injection of saline vehicle or naloxone 1 mg/kg s.c. and monitored as in experiment 3 ($n=6$ per group).

2.7. Neuropathology

Animals were sacrificed after EEG recordings, euthanized then perfused with buffered 4% paraformaldehyde. Brains were removed, postfixed, cryoprotected using sucrose in PBS. Brains and dural surfaces were examined during brain removal for presence of extraaxial fluid collections and hemorrhage.

2.8. Data analysis

Observations of epileptic-like behaviors were verified with recorded EEGs. Numbers of animals observed with epileptic behaviors were analyzed using a chi square for multiple comparisons of equal samples in a binomial distribution (Zar, 1984), and Tukey's *post hoc* tests. Significance was set at $P<0.05$. Seizure times were analyzed by t test with significance set at $P<0.05$ and group measures expressed as mean±S.E.M.

3. Results

3.1. Experiment 1 Effects of general opioid and specific deltoid opioid antagonists on behavioral and EEG responses in BD virus-infected rats

EEGs obtained from drug-naïve and vehicle-treated BD virus-infected rats had burst suppression patterns — high voltage bursts of slow waves intermingled with sharp transients or spikes against depressed background (Fig. 1A). The animals were hyperactive and showed stereotypical behaviors, but spontaneous seizures were not seen.

Systemic injection of the competitive opioid antagonist naloxone (1 mg/kg s.c.) precipitated seizures in all BD virus-infected animals, with a transition from background burst activity to rhythmic spike or sharp wave discharges within 10 min of drug administration (Fig. 1B). There was little variation in EEG response between animals. Seizures were staring spells, or episodes of automatisms such as lip smacking, blinking, and head nodding that increased in length during the experimental period, from several seconds to multicomponent episodes of 2 min. Convulsive behaviors were distinct from infected rat baseline behaviors (locomotor hyperactivity, grooming, self-licking or self-biting stereotypies) seen in all 6/6 vehicle-treated BD virus-infected rats. The onset of naloxone seizures was rapid, within 5 to 10 min of drug administration in all cases, and not accompanied by signs of physical withdrawal: forepaw treading, salivation, chromodacryorrhea/rhinorrhea, salivation, wet dog shakes, ptosis vocalization, diarrhea, or jumping. Seizures peaked in frequency and duration 30–40 min after drug administration, and tapered thereafter, to prolonged postictal periods or sleep by 70 min. Crescendo seizures ended in generalized clonic movements 30 min after naloxone administration in 1 of 6 BD virus-infected rats. Epileptiform activity was not observed in NL uninfected rats, that showed awake and sleep patterns during the sessions.

Naltrindole blocked the convulsant effects of naloxone (Fig. 1D). Administration of the delta opioid receptor antagonist naltrindole (10 mg/kg s.c.) 15 min before naloxone (1 mg/kg s.c.) prevented naloxone-induced seizures in all BD virus-infected rats tested (Fig. 1D). $\chi^2_3=10.00$ $P<0.01$ for multiple comparisons, Tukey $P<0.001$ for comparisons between naloxone and other BD virus-infected groups ($n=6$ BD virus-infected rats per group) (Table 1). However, naltrindole also produced signs of physical withdrawal: whole body tremors, jumps, and vocalizations. Naltrindole behaviors were classified as nonconvulsive withdrawal behaviors and were distinct from baseline stereotypies and epileptiform behaviors. All BD virus-infected rats showed coarse, whole body tremors 5–10 min after naltrindole administration. Tremors, observed in all BD virus-infected rats (6/6 BD virus-infected rats in naltrindole+vehicle group), were pronounced early in the recording session, but still present at 90 min. Animals were fatigued and the tremors fragmented by the end of the session. Jumps were observed in 3/6 BD virus-infected rats from 10 to 25 min, and vocalizations in 1/6 between 15 and 20 min during the observation period after naltrindole administration. Nonconvulsive withdrawal behaviors appeared to worsen after naloxone. More violent tremors that impaired locomotion, were observed in all BD virus-infected rats (6/6 BD virus-infected rats in naltrindole+naloxone group).

3.2. Experiment 2 Effects of general opioid and specific deltoid opioid antagonists on behavioral and EEG responses in HSV-1-infected rats

Drug-naïve or vehicle-treated HSV-1-infected rats were spontaneously epileptic, with staring spells, blinking, lip smacking, tongue protrusions, head bobbing or scratching automatisms time-locked to rhythmic spike or sharp wave EEG discharges (Fig. 1A). Epileptic patterns were similar in all HSV-1-infected rats in the group.

A single dose of 1.0 mg/kg naloxone significantly increased seizure duration, compared to untreated HSV-1-infected animals (Fig. 1B). Values for total time spent with behavioral and electrographic seizures for 20 min recorded session were (in seconds, mean±S.E.M.): vehicle 133.83±12.9; naloxone 1 mg/kg 403.33±48.65; $t(1,10)=5.354$, $P<0.05$, Student's t test, $n=6$ per group]. Changes in seizure phenotype or secondary generalization were not seen. Seizures, observed from 5–30 min

Table 1
Effects of naloxone, naltrindole, and flupirtine on seizure occurrence

Treatment	Normal (# rats)	Borna Disease (# rats)	Herpes Simplex Virus Type 1 (# rats)
Vehicle	0/6	0/6	6/6 [b]
Naloxone (1 mg/kg, s.c.)	0/6	6/6[c]	6/6 [b]
Naltrindole (10 mg/kg, s.c.)	0/6	$\chi^2_3 = 10.00$, $P < 0.01$ — 0/6	$\chi^2_3 = 24.00$, $P < 0.001$ — 0/6
Naltrindole (10 mg/kg, s.c.) + Naloxone (1 mg/kg, s.c.)	0/6	0/6	0/6
Vehicle	0/6	0/6	6/6 [b]
Naloxone (1 mg/kg, s.c.)	0/6	6/6[c]	6/6 [b]
Flupirtine (20 mg/kg, i.p.)	0/6	$\chi^2_3 = 10.00$, $P < 0.01$ — 0/6	$\chi^2_3 = 24.00$, $P < 0.001$ — 0/6
Flupirtine (20 mg/kg, i.p.) + Naloxone (1 mg/kg, s.c.)	0/6	0/6	0/6

χ^2_3 denoted Chi Square value for multiple comparisons for proportions. b, c indicates significant difference for *post hoc* comparisons. [b]$P<0.01$, [c]$P<0.001$, Tukey following significant χ^2. Naltrindole and flupirtine experiments contain separate sets of vehicle and naloxone-treated rats.

after drug administration, were immediately followed by prolonged postictal periods or sleep.

As in BD virus-infected rats, naltrindole blocked convulsant effects of naloxone and produced signs similar to opioid withdrawal (Fig. 1C and D). $\chi^2_3=24.00$ $P<0.001$ for multiple comparisons, Tukey $P<0.01$ for comparisons between vehicle and naltrindole, as well as between vehicle+naloxone and naltrindole+naloxone ($n=6$ HSV-1-infected rats per group) (Table 1).

3.3. Experiment 3 Effect of a potassium M current enhancer on naloxone-induced seizures in BD virus-infected rats

Flupirtine reduced the bursting patterns of BD virus-infected rat EEGs to restore a more normal pattern, without causing sedation or sleep (Fig. 1E). Flupirtine also blocked the convulsant effects of naloxone (Fig 1F). Administration of flupirtine (20 mg/kg i.p.) 15 min before naloxone (1 mg/kg s.c.) prevented naloxone-induced seizures in all BD virus-infected rats tested. $\chi^2_3=10.00$ $P<0.01$ for multiple comparisons, Tukey $p<0.001$ for comparisons between naloxone and other BD virus-infected groups ($n=6$ BD virus-infected rats per group) (Table 1). Animals remained active during the recordings and showed no unusual or withdrawal behaviors.

3.4. Experiment 4 Effect of a potassium M current enhancer on naloxone-induced seizures in HSV-1-infected rats

Flupirtine reduced higher amplitude rhythmic discharges of HSV-1-infected rat EEGs without causing sedation or sleep (Fig. 1F). Flupirtine prevented naloxone seizures in 6 HSV-1-infected rats. $\chi^2_3=24.00$ $P<0.001$ for multiple comparisons, Tukey $P<0.01$ for comparisons between vehicle and flupirtine, as well as between vehicle+naloxone and flupirtine+naloxone ($n=6$ HSV-1-infected rats per group) (Table 1). Animals remained active during the recordings and showed no unusual or withdrawal behaviors.

3.5. Effect of HSV-1 infection on gross brain pathology

EEGs of HSV-1-infected rats were lower in amplitude than BD virus-infected rats or NL controls in each treatment group. To further investigate structural causes for low voltage EEG activity of HSV-1-infected animals, animals were sacrificed and brains removed for gross pathology inspections. No effusions or extra-axial hemorrhages were seen on post-mortem dissections of HSV-1-infected animals.

4. Discussion

Endogenous opioids of the central nervous system (CNS) may signal overall health and disease of an organism, with unstable or decompensated systems associated with stress of severe illness or recurrent seizures (reviewed in Simonato and Romualdi, 1996; Schindler et al., 2004). In rats with hippocampal dynorphin systems disrupted by virus, administration of the general opioid antagonist naloxone leads to limbic seizures (Solbrig et al., 2005, 2006a,b). Naloxone treatment elicited rhythmic EEG discharges in BD virus-infected rats and grouped, synchronous discharges in HSV-1-infected rats. Lower voltage EEGs of HSV-1-infected rats signified severe cortical illness, similar to that described in encephalitis in man (Hosoya et al., 2002). Since opioid receptor and potassium ion channel systems have been implicated as modulatory substrates for seizures, the present study was undertaken to examine anticonvulsant efficacy of these classes of compounds on naloxone seizures in rats with severe encephalitis. Naltrindole, a delta opioid antagonist, and flupirtine, a K^+ M current (I_M) enhancer, were tested using anticonvulsant doses reported previously. Both drugs were effective anticonvulsants in BD virus- and HSV-1-infected rat models. Naltrindole induced somatic withdrawal but protected from seizures and flupirtine suppressed seizures with no somatic signs. The results support the effectiveness of a delta antagonist or an I_M enhancer in blocking convulsive behaviors in encephalitis and should have relevance to medically refractory seizures associated with opioid destabilization during critical illness or opiate withdrawal.

The hippocampus, one of the most seizure-prone brain regions, is specifically targeted by the viruses we studied. In this area, naltrindole can oppose convulsant actions of delta agonists and preserve kappa opioid tone. Previous work has shown that reduced dynorphin expression in the dentate gyrus of hippocampus due to virus exposure leads to epileptic responses Solbrig et al., 2006a,b). Since delta agonists are convulsants (Torregrossa et al., 2004), a net effect of naltrindole or

naltrindole plus naloxone would be rebalance of hippocampal opioid tone in favor of kappa opioids, providing a basis for its anticonvulsant effect. A key role for kappa opioids in anticonvulsant protection in several models has been reported (Moore et al., 1994; Simmons and Chavkin, 1996; Bausch et al., 1998; Solbrig et al., 2006a,b).

The existence of opioid-sensitive ion channels in hippocampus is supported by electrophysiologic analyses, with potassium channels underlying kappa opioid receptor inhibition of hippocampal neurons. Dynorphin has presynaptic inhibitory effects in the hippocampus through activation of Shaker type potassium (K) channels on mossy fibers (Simmons and Chavkin, 1996) thereby limiting calcium influx and neurotransmitter release. Also, dynorphin acts as a postsynaptic neuromodulator, augmenting voltage-dependent K^+ I_M of principal hippocampal neurons (Moore et al., 1994; Madamba et al., 1999).

In hippocampal models, delta agonists reduce K^+ M currents (Moore et al., 1994), providing a basis for their convulsant activity. Accordingly, delta antagonists would have an opposite, anticonvulsant effect by enhancing I_M. M currents are important stabilizers at or near resting membrane potentials (Wickenden, 2004), and potassium M channel coding regions have genetic associations with epilepsy (Biervert et al., 1998; Singh et al., 1998). Administration of flupirtine prevented seizures in the encephalitis models reported here, supporting anticonvulsant efficacy of this class of compound.

Naltrindole-induced somatic signs are an interesting feature of our models. The phenomenon may signify a type of physical dependence on endogenous released endorphins with stress of viral infection and recruitment of multiple circuits in the behavioral responses to naltrindole. The presence of somatic signs raises the possibility that naltrindole's anticonvulsant action is also a function of opioid effects in extrahippocampal sites. Drug-induced tremor, locomotor and stereotypic behaviors suggest an interaction of naltrindole with subcortical sites and monoaminergic circuits. A role for extrahippocampal sites in anticonvulsant protection, particularly brainstem or extrapyramidal sites, is consistent with reports of anticonvulsant efficacy of vagal or *substantia nigra* stimulation (Velisek et al., 2002). If naltrindole-driven tremor and hyperactivity are markers for monoaminergic stimulation or fluctuation, anticonvulsant actions of naltrindole might be linked to the actions of some newer anticonvulsants such as zonisamide, an agent with novel dopaminergic and serotonergic effects (Okada et al., 1992). Mapping of sites of anticonvulsant protection with electrophysiologic or immediate early gene probes can be a subject of further investigations.

There is increasing experimental and clinical evidence to implicate opioid destabilizations in seizure pathogenesis. Recently, *in utero* opioid exposure in rats was reported to produce long-lasting changes in neurons associated with hippocampal opioid circuits and sensitize to naloxone seizures (Schindler et al., 2004). Subsequently, we reported BDV infection of rats, through similar changes in hippocampal dynorphin, were sensitized to naloxone seizures (Solbrig et al., 2006a). Moreover, HSV-1 infection in rats reduces hippocampal dynorphin to produce naloxone seizures (Solbrig et al., 2006b), further supporting the possibility of common seizure mechanisms in opioid withdrawal seizures and naloxone seizures in viral models (Solbrig et al., 2006b).

Finally, hypotheses of opioid involvement in seizures need not be limited to animal models. In man, in newborns, a role for hypofunction or imbalance of endogenous opioid systems in seizure genesis is supported by recommendations for treating neonatal abstinence seizures with paregoric, a mixture of opium and alcohol (Osborn et al., 2005). A logical next step is to exploit opioid actions at a cellular level on membrane stabilizing currents, for therapeutic benefit.

We suggest that *in utero* opioid exposure or passive drug administration for critical care of patients, by challenging or collapsing CNS opioid homeostasis, stresses and produces hyperexcitability via mechanisms similar to viral models. The rat experimental paradigms mimic conditions under which opioid drugs are likely to be administered in man. These include naloxone for emergency room treatment of nontraumatic coma, and naloxone for prolonged post-op or post-analgesia stupor. While there may be fewer opioid withdrawal seizures in neonates today, there is a growing number of critical care patients receiving morphine and fentanyl for analgesia and sedation. Their illness and treatment puts them at risk for seizures. Based on the results of the present study, an experimental manipulation that preserves or enhances kappa opioid receptor tone can be anticonvulsant, and any experimental manipulation that enhances K^+ M current is likely to be anticonvulsant. An expanding knowledge of neuromodulatory roles of opioids and other analgesics should translate to improved seizure management in select patients.

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