

Naloxone-induced seizures in rats infected with Borna disease virus

Article abstract—The opioid antagonist naloxone is widely used in the emergency treatment of nontraumatic coma. Although it is uncommon for serious side effects to result from administration of opiate antagonists, we report that naloxone can have epileptogenic effects in the context of encephalitis. In an experimental model of viral encephalitis, rats infected with Borna disease virus developed myoclonic, generalized clonic, or atonic seizures; behavior arrest; and staring spells when treated with naloxone. These findings suggest a novel neuropharmacologic link, through opioid peptide systems, between epilepsy and encephalitis and disclose a potential contraindication to use of opioid antagonists in nontraumatic coma.

NEUROLOGY 1996;46:1170-1171

Marylou V. Solbrig, MD; George F. Koob, PhD; and W. Ian Lipkin, MD

Borna disease virus (BDV) is a neurotropic RNA virus that causes movement and behavior disorders in immunocompetent animals. Previous research established that rats experimentally infected with BDV at 1 month of age have orofacial dyskinesias and stereotyped behaviors.¹ This syndrome reflects dopamine system lesions and appears similar in phenotype, neuropharmacology, and neurochemistry to neuroleptic-induced tardive dyskinesia (TD), the hyperkinetic movement disorder caused by long-term treatment of patients with neuroleptics. The host range for BDV may extend to humans. There are antibodies reactive with BDV proteins in patients with neuropsychiatric diseases. Furthermore, BDV proteins and nucleic acids were reported in peripheral blood mononuclear cells of some of these patients.²

Evidence of opiate-induced activation of nigrostriatal dopamine (DA) pathways in rodents³ suggested that opiate antagonists may improve conditions aggravated by DA stimulation, such as TD. Indeed, improvements in the orofacial movements of TD with naloxone were reported in several patients.⁴ The current study was undertaken to examine the efficacy of naloxone in the treatment of orofacial dyskinesias in the Borna disease (BD) rat model of TD. The results yielded information relevant to the treatment of CNS infections with opiate antagonists.

Methods. One-month-old male Lewis rats were anesthetized with metofane and inoculated intracerebrally (IC) with either 1.6×10^4 tissue culture infectious dose units of BDV (BD rats) or phosphate-buffered saline (PBS) (normal [NL] rats) in a total volume of 30 μ L. Virus stock was a 10% wt/vol BD rat brain homogenate in PBS. Infection was confirmed by the appearance of a clinical syndrome consistent with BD and the presence of antibodies reactive with viral proteins by Western blot. All animal procedures conformed to the *Guide for the Care and Use of Laboratory Animals* endorsed by the National Institutes of Health.

Experiments were initiated 45 days after IC inoculation with either virus or PBS. Animals were habituated to the test cages on the day before testing and then tested during the light portion of the cycle at the same time each day. Naloxone (Sigma, St. Louis, MO) at doses of 0, 0.04, 0.2, and 1.0 mg/kg was dissolved in 0.09% saline and injected subcutaneously. One of three doses of test drug or its vehi-

cle control was given to each animal according to a Latin square design to control for conditioning and order effects. Each experimental group contained eight animals. Behavior was monitored continually for 30 minutes in 40 \times 25 \times 20-cm activity cages. Stereotypic behavior was scored through direct observation by an experienced observer who was blind to drug dose. Observational data were analyzed using the Information Statistic as previously described.¹

Results. BD rats showed dramatic differences in their behavioral responses to naloxone (at doses 0.04, 0.20, and 1.0 mg/kg) than age-matched NL controls. In the first 10 minutes of the test session, BD rats showed signs of opiate withdrawal: irritability, restlessness, leaping, twitches, piloerection, hunched posture, and Straub tail. Frequent or continuous seizures followed for the remaining 20 minutes of the test session and included myoclonic, generalized clonic, and atonic seizures; behavior arrest; and staring spells. Myoclonic seizures were defined as head nods or drop attacks, with immediate righting. Generalized clonic seizures were rhythmic symmetric jerking of the trunk and limbs followed by a period of inactivity. Atonic seizures were rearing and falling followed by inactivity. Behavior arrest or staring spells were pauses in activity, punctuated by myoclonic jerks or wet dog shakes. Frequent seizures masked a naloxone effect on perioral movements.

With epileptiform effects as the measure, naloxone precipitated a convulsive syndrome in the BD group. A dose-response relationship was not observed in that each dose level revealed a significant difference in behavior rating at doses 0.04, 0.20, and 1.0 mg/kg (table). In contrast, all naloxone doses were without epileptogenic effect in the uninfected control group.

Discussion. Although BDV nucleic acids and proteins are present in high concentration in potentially epileptogenic cortical and subcortical sites, there are only infrequent reports of seizures in either natural or experimental infection. Facial twitching and head nodding occur in infected horses. Bilateral runs of spikes and sharp waves time-locked to clonic movements occur in infected rabbits.⁵ Paroxysms of locomotor hyperactivity, peaking at hourly intervals and falling off abruptly, occurred in infected tree shrews.⁶ Although no electrical recordings were reported, the periodic bursts of motor activity described are consistent with epilepsy.

In BD rats, naloxone-induced myoclonic, generalized

Table Seizure responses to naloxone in BD and NL rats ($n = 8$ for each group).

Naloxone dose (mg/kg)	No. of rats with seizures		InfoStat
	BD	NL	
0.00	0/8	0/8	$2\hat{I} = 0, df = 1, NS$
0.04	7/8	0/8	$2\hat{I} = 15.90, df = 1, p < 0.001$
0.20	8/8	0/8	$2\hat{I} = 22.18, df = 1, p < 0.001$
1.00	8/8	0/8	$2\hat{I} = 22.18, df = 1, p < 0.001$
Overall			$2\hat{I} = 60.2632, df = 4, p < 0.001$

Significance was determined by InfoStat for observational data \hat{I} = information statistic; NL = normal; BD = Borna disease.

clonic, and atonic seizures and staring spells mask any drug effect on orofacial dyskinesias. Seizures occurred at low (μ opioid receptor) doses of naloxone. These are doses that block opiate effects and are below the doses that produce nonspecific effects on other neurotransmitter systems.⁷

Opioid peptides have variable effects in different models of epilepsy.⁸ Although initial studies suggested that opioid peptides had convulsant activity, subsequent studies found both pro- and anticonvulsant effects for opioids and their antagonists. For example, parenteral naloxone enhanced audiogenic seizures in mice and amygdaloid-kindled seizures in rats and precipitated physical signs of withdrawal together with electrocortical epileptiform activity in morphine-dependent rats.⁹

Behavior, posture, and autonomic signs resembling opiate withdrawal were present in naloxone-treated BD rats before the onset of seizures. The observation that naloxone elicited a syndrome resembling opiate withdrawal suggests that infection stimulates the synthesis of a pharmacologically active opiate or increases the sensitivity of opioid receptors. Indirect support for the former hypothesis is found in studies reporting elevated levels of enkephalin precursor mRNA in striatum of BD rats.¹⁰ Because BDV infection in rats causes behavioral effects that can be modified by opioid drugs, we began to explore the possible interactions with other neurotransmitter systems that interact with opioid systems. Preliminary results indicate that a D1 agonist, SKF82958 (Research Biochemicals Inc., Natick, MA) prevents seizures in BD rats treated with naloxone (Solbrig MV, Koob GF, Lipkin WI, unpublished results).

Our results complement the structural hypotheses of epilepsy in encephalitis (electrotonic coupling, synaptic degeneration, and reduction of inhibitory processes or cells) by suggesting a neuropharmacologic link through opioid peptide systems. From a clinical vantage point, recognition that opioid antagonists can precipitate seizures in the context of encephalitis may suggest the need for caution in the use of these agents for treatment of nontraumatic coma.

From the Laboratory for Neurovirology, Department of Neurology (Drs. Solbrig and Lipkin), University of California, Irvine, CA; and the Department of Neuropharmacology (Dr. Koob), The Scripps Research Institute, La Jolla, CA.

Supported by grants from the Pew Charitable Trusts, the Wayne and Gladys Valley Foundation, and the National Institutes of Health, NS29425 and MH 47680.

Received July 17, 1995. Accepted in final form August 14, 1995.

Address correspondence and reprint requests to Dr. M.V. Solbrig, Department of Neurology, 148 Med-Surg I, University of California, Irvine, CA 92717-4290.

References

- Solbrig MV, Koob GF, Fallon JH, Lipkin WI. Tardive dyskinesic syndrome in rats infected with Borna disease virus. *Neurobiol Dis* 1994;1:111-119.
- Bode L, Zimmermann W, Ferszt R, Steinbach F, Ludwig H. Borna disease virus genome transcribed and expressed in psychiatric patients. *Nature Med* 1995;1:232-236.
- Iwamoto ET, Way EL. Circling behavior and stereotypy induced by intranigral opiate microinjections. *J Pharmacol Exp Ther* 1977;203:347-359.
- Lindenmayer JP, Gardner E, Goldberg E, et al. High-dose naloxone in tardive dyskinesia. *Psychiatry Res* 1988;26:19-28.
- Gierend M. Zur Pathogenese Der Bornaschen Krankheit Beim Kaninchen: Untersuchungen Über Die Zelluläre Immunantwort, Die Wirkung von Immunsuppressiver Behandlung und Die Elektroencephalographie (EEG) [thesis]. Berlin: Freie Universität, 1982:83.
- Sprankel H, Richarz K, Ludwig H, Rott R. Behavior alterations in tree shrews (*tupaia glis*, Diard 1820) induced by Borna disease virus. *Med Microbiol Immunol* 1978;165:1-18.
- Dingledine R, Iversen LL, Breuker E. Naloxone as GABA antagonist: evidence from iontophoretic, receptor binding and convulsant studies. *Eur J Pharmacol* 1978;47:19-27.
- Ramabadran K, Bansinath M. Endogenous opioid peptides and epilepsy. *Int J Clin Pharmacol Ther Toxicol* 1990;28:47-62.
- Pinsky C, Dua AK, LaBella FS. Peptidase inhibitors reduce opiate narcotic withdrawal signs, including seizure activity, in the rat. *Brain Res* 1982;243:301-307.
- Fu ZF, Weihe E, Zheng YM, et al. Differential effects of rabies and Borna disease viruses on immediate-, early- and late-response gene expression in brain tissues. *J Virol* 1993;67:6674-6681.