Pharmacology Biochemistry and Behavior, Vol. 45, pp. 615–617, 1993
Printed in the U.S.A.

0091-3057/93 $6.00 + .00

Picrotoxin-Induced Seizures Modified by Morphine and Opiate Antagonists

JOCELYN THOMAS,[1] WILLIAM L. NORES, VELGA KENIGS, GAYLE A. OLSON AND RICHARD D. OLSON

Department of Psychology, University of New Orleans, Lakefront, New Orleans, LA 70148

Received 6 November 1992

THOMAS, J., W. L. NORES, V. KENIGS, G. A. OLSON AND R. D. OLSON. *Picrotoxin-induced seizures modified by morphine and opiate antagonists.* PHARMACOL BIOCHEM BEHAV **45**(3) 615–617, 1993. – The effects of naloxone, Tyr-MIF-1, and MIF-1 on morphine-mediated changes in susceptibility to picrotoxin-induced seizures were studied. Rats were pretreated with naloxone, MIF-1, Tyr-MIF-1, or saline. At 15-min intervals, they received a second pretreatment of morphine or saline and then were tested for seizures following a convulsant dose of picrotoxin. Several parameters of specific categories of seizures were scored. Morphine increased the number of focal seizure episodes, duration of postseizure akinesis, and incidence of generalized clonic seizures. Naloxone tended to block the morphine-mediated changes in susceptibility. Tyr-MIF-1 had effects similar to naloxone on duration of postseizure immobility but tended to potentiate the effects of morphine on focal seizure episodes. The effects of morphine and the opiate antagonists on focal seizure episodes and postseizure duration suggest the general involvement of several types of opiate receptors in these picrotoxin-induced behaviors. However, the observation of antagonistic effects for Tyr-MIF-1 on immobility but agonistic effects for focal seizures suggests that the type of effect exerted by opiate agents may depend upon other neuronal variables.

MIF-1 Tyr-MIF-1 Seizures Naloxone Morphine

MORPHINE has been reported to have both pro- and anticonvulsant effects on seizure activity (5). In part, the apparently inconsistent effects of morphine may be due to methodological and procedural differences, such as the method used to induce seizures or the type or classification of seizures studied. For example, morphine successfully blocked the tonic component of maximal electric shock (MES)-induced seizures but was ineffective against the clonic component of MES seizures. In contrast, morphine had proconvulsant effects on a variety of seizure-related behaviors induced by the GABA antagonists, bicuculline and picrotoxin (4). These findings suggest that the type of modulation morphine exerts on seizure mechanisms may be dependent upon the brain sites or neurochemical systems involved in the precipation of the seizures and therefore is seizure dependent.

The involvement of opiate receptors in the effects morphine had on bicuculline- and shock-induced seizures was suggested because naloxone partially or completely blocked the morphine-mediated changes in seizure susceptibility (4). The effect naloxone on morphine potentiated picrotoxin-induced seizures was not reported, however, so the role of opiate receptors in the effects of morphine on picrotoxin-induced seizures is not known. One objective of the current study was to investigate the effect of morphine on seizures that have been previously reported to unfold after picrotoxin (12). A second objective was to measure the effects of naloxone and the endogenous opiate peptide antagonists, MIF-1 and Tyr-MIF-1, on morphine-mediated changes in susceptibility to picrotoxin-induced seizures.

METHOD

Subjects

Male Long-Evans hooded rats (260–375 g) were studied. Animals were maintained on a 12 L : 12 D cycle (light on at 0800 h) with food and water ad lib. Animals were handled daily for 2 weeks prior to the start of the experiment. Each animal was tested under one experimental condition.

Drugs and Procedure

Naloxone, Tyr-MIF-1, and MIF-1 were dissolved in 0.9% saline in concentrations of 3.0 mg/ml and injected in a volume of 1 ml/kg. Morphine was dissolved in saline in a concentration of 50 mg/ml and administered in a volume of 1 ml/kg. Picrotoxin (3.0 mg/kg in 1 ml; saline vehicle) was freshly mixed immediately prior to each test session and used within 3 h after mixing. Prior to seizure testing, animals were given two pretreatments. The first pretreatment was either saline, naloxone, MIF-1, or Tyr-MIF-1. Fifteen minutes later, animals received a second pretreatment of either saline (one

[1] To whom requests for reprints should be addressed.

TABLE 1

EFFECTS OF MORPHINE PRETREATMENT ON SEIZURES INDUCED BY PICROTOXIN

Parameter Scored	Saline/Saline/ Picrotoxin	Saline/Morphine/ Picrotoxin	p
Latency*			
Myoclonic seizures	11.82 ± 0.94	11.88 ± 0.89	n.s.
Focal seizures	13.65 ± 0.99	16.14 ± 1.49	n.s.
Akinesis	14.38 ± 1.04	16.69 ± 1.56	n.s.
Focal seizure episodes	1.71 ± 0.47	3.89 ± 0.61	<0.02
Duration of akinesis*	4.20 ± 0.34	7.59 ± 1.32	<0.05
GC seizure incidence†	0/7	6/9	<0.004

*Mean (± SEM) minutes.
†Number having seizures/number tested.

group) or morphine (four groups). Fifteen minutes after the second pretreatment, all animals were injected with picrotoxin and observed for 45 min for behavioral signs of seizures.

Four categories of seizure-related behavior that have been identified in association with picrotoxin include myoclonic jerks and twitches, focal seizures, postfocal seizure akinetic periods, and generalized clonic (GC) seizures. Behaviors associated with each category of seizures have been described previously (12). All behavior was monitored at the time of testing, as well as videotaped for later review and scoring.

Behavioral parameters scored included the latency to the first occurrence of myoclonic, focal, and GC seizures; the latency to the onset of akinesis following the first focal seizure; the incidence of GC seizures; the duration of the period of akinesis that followed the first focal seizure; and total number of focal seizure episodes.

Data Analyses

All data were analyzed by a one-way analysis of variance (ANOVA). Morphine's effects on picrotoxin-induced seizures and the effects of opiate antagonists on morphine-mediated effects were assessed in independent analyses.

RESULTS AND DISCUSSION

Table 1 summarizes the effects of morphine on picrotoxin-induced seizures. The data indicate that morphine significantly increased the mean duration of postseizure akinesis, $F(1, 14) = 4.80$, $p < 0.046$, significantly increased the mean number of episodes of focal seizures, $F(1, 14) = 7.18$, $p < 0.018$, and significantly increased the incidence of generalized clonic seizures, $F(1, 14) = 12.25$, $p < 0.004$. Together, the findings suggest that morphine has proconvulsant effects on several different types of seizures induced by picrotoxin. Morphine had no significant effect on the latencies to any of the different categories of seizures.

Table 2 shows differences among the opiate antagonists in their effects on postseizure immobility and on focal seizure episodes. None of the antagonists effectively blocked morphine-mediated increases in seizure susceptibility. However, as can be seen, there were tendencies for naloxone to antagonize the morphine-potentiated effects on focal seizures and postseizure immobility and on GC seizures. In contrast, Tyr-MIF-1 tended to antagonize morphine's effects on immobility but potentiate morphine's effects on focal seizures. Like Tyr-MIF-1, MIF-1 also tended to potentiate morphine's effects on focal seizures but did not affect the duration of postseizure immobility.

The interaction of opiate agents with picrotoxin in the modulation of postseizure akinesis and focal seizures suggests that one mechanism through which opiate agents affect seizure-related behaviors is interaction with the GABA receptor complex (10,11). Further, the effects of Tyr-MIF-1 and MIF-1 on focal seizures and postseizure immobility suggest that, like endogenous opiate agonists (2,7), endogenous opiate antagonist peptides may play a role in modulating seizure activity.

TABLE 2

OPIATE ANTAGONIST EFFECTS ON MORPHINE-POTENTIATED GC AND FOCAL SEIZURES AND POSTSEIZURE AKINESIS

Pretreatment	Focal Seizure Episodes*	Duration of Akinesis†	GC Seizures (%)‡
Saline-Morphine	3.89 ± 0.61	7.59 ± 1.32	67
Naloxone-Morphine	2.63 ± 0.57	5.52 ± 0.42	13
Tyr-MIF-1-Morphine	5.00 ± 0.73	5.06 ± 0.77	50
MIF-1-Morphine	4.25 ± 0.62	6.87 ± 1.19	63

*Mean (± SEM) number of episodes.
†Mean (± SEM) number of minutes.
‡Percent of rats having at least one episode.

These results extend findings from other studies showing that opiate receptors are specifically involved in focal seizures and postseizure immobility irrespective of the mechanisms by which they are induced (1,3,6,8,9). Differences among opiate antagonists in their effects on morphine-potentiated seizures suggest that in addition to the general or nonspecific involvement of multiple opiate receptors in focal seizures and immobility there may be selective effects that are manifested through interactions with different neurochemical systems in different brain regions.

REFERENCES

1. Cain, D. P.; Boon, F.; Corcoran, M. E. Involvement of multiple opiate receptors in opioid kindling. Brain Res. 517:236–244; 1990.
2. Cain, D. P.; Corcoran, M. E. Epileptiform effects of metenkephalin, B-endorphin and morphine: Kindling of generalized seizures and potentiation of epileptiform effects by handling. Brain Res. 338:327–336; 1985.
3. Cottrell, G. A.; Bohus, B. Immediate and long-term effects of opiate antagonists on postictal behavior following amygdala kindling in the rat. Eur. J. Pharmacol. 141:417–421; 1987.
4. Foote, F.; Gale, K. Morphine potentiates seizures induced by GABA antagonists and attenuates seizures induced by electroshock in the rat. Eur. J. Pharmacol. 95:259–264; 1983.
5. Frenk, H. Pro- and anticonvulsant actions of morphine and the endogenous opioids: Involvement and interactions of multiple opiate and nonopiate systems. Brain Res. Rev. 6:197–210; 1983.
6. Frenk, H.; Engle J., Jr.; Ackermann, R. F.; Shavit, Y.; Liebeskind, J. C. Endogenous opioids may mediate postictal behavioral depression in amygdaloid-kindled rats. Brain Res. 167:435–440; 1979.
7. Frenk, H.; Urca, G.; Liebskind, J. Epileptic properties of leucine- and methionine-enkephalin: Comparison with morphine and reversibility by naloxone. Brain Res. 147:327–337; 1978.
8. Fuller, T. A.; Olney, J. W.; Conboy, V. T. Naloxone blocks morphine enhancement of kainic acid neurotoxicity. Life Sci. 31: 229–233; 1982.
9. Holaday, J. W.; Belenky, G. L. Opiate-like effect of ECS in rats: A differential effect of naloxone on nociceptive measures. Life Sci. 27:1927–1938; 1980.
10. Miller, L. G.; Kastin, A. J.; Roy, R. B. Effects of Tyr-MIF-1 and MIF-1 at the $GABA_A$ receptor chloride channel site. Pharmacol. Biochem. Behav. 19:743–745; 1987.
11. Miller, L. G.; Kastin, A. J.; Roy, R. B. MIF-1 and Tyr-MIF-1 augment muscimol-stimulated chloride uptake in cerebral cortex. Pharmacol. Biochem. Behav. 23:413–415; 1989.
12. Thomas, J. Gender difference in susceptibility to picrotoxin-induced seizures is seizure- and stimulation-dependent. Brain Res. Bull. 24:7–10; 1990.