Effects of Morphine and d-Ala²-D-Leu⁵-Enkephalin in the Seizure-Susceptible El Mouse

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(Accepted December 23, 1991)

Opioid agonists were used to investigate the modulation of seizures in the seizure-susceptible El mouse. Morphine and D-Ala²-D-Leu⁵-enkephalin (DADLE) were injected subcutaneously or intracisternally as prototypic agonists for μ and δ opioid receptors. Systemic or intracisternal injection of both morphine and DADLE decreased the incidence of seizures and the seizure score in El mice in a dose-dependent manner. The anticonvulsant effects of morphine and DADLE were reversed by naloxone (2 mg/kg, s.c.). This implies that opioid agonists have anticonvulsant properties which are mediated by μ and δ opioid receptors. In conclusion, a deficit in endogenous opioid peptides, which act as anticonvulsants may play a significant role in the etiology or pathophysiology of seizures in the El mouse.

KEY WORDS: El mouse; morphine, enkephalins; seizure; epilepsy.

INTRODUCTION

Opioid peptides have manifold physiological effects on pain (1), stress (2), and behavior (3). Exogenous opioid peptides which produced epileptiform EEG activity were first reported as proconvulsants in 1977 (4, 5). Despite extensive research, such as maximal electroshock (6) and chemical treatment (7-9), it remains uncertain whether opioid peptides have pro- or anticonvulsant properties on seizures (10). In the kindling model of epilepsy, morphine with little or no effect on the rate of kindling or on the expression of fully kindled seizures, exacerbated the spontaneous interictal spiking and the postictal behavioral depression seen after kindled seizures (11, 12). Naloxone decreased the behavioral depression seen after a kindled seizure (13). These findings suggested that endogenous opioids mediate postictal events in kindled animals. However, these are experimental models of epilepsy which are different from natural models of epilepsy, and therefore might not entirely reflect the true pathogenesis of epileptic seizures. On the other hand, in genetically epileptic animals such as au-

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diogenic mice (14), photosensitive baboons (15), and the seizure-sensitive Mongolian gerbil (16-18), exogenous opioid peptides have been shown to act as anticonvulsants.

The El mouse is an genetically epileptic model (19, 20). The seizures occur in response to vestibular stimulation. By repeating such stimulation El mice have developed generalized convulsion. In most adult El mice, spontaneous seizure can occur without repeated tossing stimulation during cage changing or when placed in an unfamiliar environment. Because of these findings taken together with EEG findings (21), the El mouse can be considered an excellent animal model for complex partial seizures or temporal lobe epilepsy in human (20).

We previously determined the distribution density of opioid delta receptors (22) and the concentration of methionine enkephalin-like immunoreactivity (ME-LI) (23) in the El mouse brain to elucidate the relation between seizures and the opioid system. We found that the up-regulation of opioid delta receptors in the brain of El mice compared to seizure-nonsusceptible (ddY; the mother strain of El) mice is correlated with the decrease of ME-LI in the brain of El mice compared with ddY mice during the interictal period. These findings suggest that the hypofunction of enkephalinergic neurons in the brain...
of El mice play an important role in the pathogenesis of seizure diathesis and seizure manifestations. Subsequently, we speculate that endogenous opioid peptides act as anticonvulsants in the El mouse brain.

In this study, we investigated the role of μ and δ opioid agonists in the modulation of seizures in the El mouse to determine whether opioid peptides act as pro- or anticonvulsants in this species.

EXPERIMENTAL PROCEDURE

Animals. Seizure-susceptible (El) mice were allowed to inbreed in our animal center as described previously (22). The El mice were tossed-up once a week from the 28th day after birth. By repeating such stimulation once a week, El mice first exhibited abortive seizures at the 50th day after birth, and developed generalized convolution upon stimulation starting after the 75th day after birth. Abortive seizures, whereby El mice become immobile, give the characteristic squeak, and perhaps experience brief running fits without the tonic-clonic phase of a fullscale seizure. We evaluate the abortive seizures by such characteristics, especially we distinguish abortive seizures from tonic-clonic seizures by having whether the tonic-clonic phase or not. Experiments were carried out on adult from 100-day-old to 200-day-old El mice which exhibited tonic-clonic convulsions within 40 tossing-stimulations. Drugs were administered 7 days after the convulsions developed prior to the tossing-stimulation.

Drugs. Morphine and d-Ala2–D-Leu5-Enkephalin (DADLE) were injected subcutaneously (s.c.) or intraventricularly by the method of intracisternal administration (i.c.) (24). The intracisternal injection technique into conscious mice produced no damage whatever to brain tissues and the mice maintained spontaneous, normal activity immediately after the administration of physiological saline or distilled water. These substances were diluted with sterile saline and administered 7 days after the convulsions developed prior to the tossing-stimulation. Drugs s.c. were administered 45 min prior to stimulation. Drugs i.c. were injected 15 min prior to stimulation in a volume of 10 μl. Naloxone (2 mg/kg) was injected s.c. 10 min prior to stimulation.

Seizure Score. The seizures of the El mice were classified into grade 0, no seizures with 80 tossing-stimulations; grade 1, abortive seizures within 80 tossing-stimulations; grade 2, abortive seizures within 40 tossing-stimulations; grade 3, tonic-clonic seizures within 80 tossing-stimulations; and grade 4, tonic-clonic seizures within 40 tossing-stimulations.

Statistics. Comparisons were made using Fisher's exact probability test for the data of the incidence and Wilcoxon-Mann-Whitney test for the data of seizure score. The data of seizure score are the means ± SEM. The significance level was P<0.05. Line of dose dependency was determined by least squares linear regression analysis; slope was significant at p<0.05.

RESULTS

Subcutaneous injection of morphine at doses of 1 to 100 mg/kg reduced the incidence of epileptiform seizures and the seizure score in El mice in a dose-dependent manner. Subcutaneous injection of morphine at doses of 10 to 100 mg/kg reduced the incidence of seizures significantly compared to saline control. The anticonvulsant effect was prevented by prior administration of naloxone (Figure 1).

Subcutaneous injection of DADLE at doses of 0.1 to 40 mg/kg reduced the incidence and the seizure score in a dose-dependent manner. Subcutaneous injection of DADLE at doses of 20 to 40 mg/kg decreased the incidence significantly compared with saline control. The anticonvulsant action was reversed by naloxone (Figure 2).

Intracisternal injection of morphine at doses of 1 to 50 μg/animal decreased the incidence and the seizure score in a dose-dependent manner and that at doses of 20 to 50 μg/animal significantly decreased the incidence compared to saline control. Naloxone antagonized the anticonvulsant effect (Figure 3).

Intracisternal administration of DADLE at doses of 0.01 to 100 μg/animal reduced the incidence and the seizure score in a dose dependent manner and that at doses of 1 to 100 μg/animal significantly reduced the incidence of seizures. Naloxone antagonized the anticonvulsant effect (Figure 4).

A larger does of morphine s.c. (150 and 200 mg/kg), DADLE s.c. (80 mg/kg), morphine i.c. (100 μg/animal), and DADLE i.c. (150 and 200 μg/animal) produced abnormal tonic limb extension seizures which were different from the characteristic seizures of the El mice. Naloxone did not block such abnormal seizures.

DISCUSSION

This is the first report to demonstrate that subcutaneous or intracisternal injection of μ and δ opioid agonists clearly produced anticonvulsant actions which were reversed by opioid antagonist in the El mouse. This implies that exogenous opioid peptides have a protective role against seizure activity mediated by opioid receptors in the El mouse, as suggested in our previous studies in which the concentration of ME-LI in the El mouse brain was reduced compared to the ddY mouse (23) and the opioid delta receptor density in the El mouse brain was higher than that of the ddY mouse during the interictal period (22).

Chen et al. (14) found that morphine has anticonvulsant effects on priming-induced audiogenic seizures in BALB/c mice. Meldrum et al. (15) also reported anticonvulsant actions of morphine and FK 33.824, the enkephalin analogue, in the photosensitive baboons. Bajorek et al. (16–18) reported that several opioid agonists have anticonvulsant effects in the seizure-sensitive Mon-