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Opioid Antagonism of Electroshock-Induced Seizures

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PUGLISI-ALLEGRA, S., C. CASTELLANO, V. CSÁNYI, A. DÓKA AND A. OLIVERIO. *Opioid antagonism of electroshock-induced seizures.* PHARMACOL BIOCHEM BEHAV **20**(5) 767–769, 1984.—Morphine, β-endorphin and [D-Ala², D-Leu⁵] enkephalin administered intracerebroventricularly exerted a protective effect on electroconvulsive shock (ECS)-induced seizures in mice. This effect was reversed by intraperitoneal injections of naltrexone. The role of μ and δ receptors in ECS-induced convulsions is discussed.

Morphine β-Endorphin [D-Ala², D-Leu⁵] enkephalin Electroconvulsive shock Seizures Mice

THE role of endogenous opioids in different kinds of behavioural or electrographic convulsions has been assessed by considering rats subjected to volatile convulsants [3], to electroshock (ECS)-induced or kindled-induced seizures as well as audiogenic seizures [4, 6, 11].

Recently it has been shown that mice subjected to immobilization stress are protected against electroconvulsive shock-induced seizures and that naltrexone antagonizes the effects of stress. The findings were interpreted in terms of a stress-induced release of endogenous opioids [9]. The actions of different opioids on volatile convulsants-induced seizures have been interpreted in terms of μ, δ, and σ receptors [3].

In the present research we wanted to assess the role of morphine, β-endorphin and [D-Ala², D-Leu⁵] enkephalin (DADL) on ECS-induced behavioural convulsions at doses which do not result in electrographic spiking phenomena which are evident after very high doses of opioids [5].

METHOD

C57BL/6 inbred mice (Charles River, Como, Italy) were used since the effects of opioids have been studied extensively in this strain [8]. The animals were males, weighing 25–28 g at the beginning of the experiment. Seven days before testing, a guide cannula (26 gauge) was implanted stereotaxically 1.5 mm over the right lateral ventricle. The cannula was attached to the skull with acrylic dental cement reinforced by two stainless steel screws. A dummy needle was kept in place at all times except during injection. Awake mice, briefly restrained by wrapping to put in the injection tubing (0.25 mm diameter) into the cannula, received 2.5 μl of the material injected over 60 sec. Injections were performed with a Hamilton microsyringe. Placements were confirmed by subsequent injections of 2.5 μl of toluidine blue dye when the animals were killed. ECS-induced tonic-clonic seizures were produced by administering through two ear clips electrodes a DC current level of 45 mA for 200 msec. When convulsing tonically, the animals were reanimated through artificial respiration by gently inflating their lungs by applying the rubber bulb of a dropper to the nose area in order to avoid asphyxia. Ten mice per group were used one time only. Drugs were dissolved in 0.9% NaCl, the pH adjusted to 7.4, and injected intracerebroventricularly (ICV) by using a stainless-steel chronically implanted cannula [12]. Control animals were injected with 0.9% NaCl (pH adjusted to 7.4).

Naltrexone (HCl) was dissolved in 0.9% NaCl and injected intraperitoneally (IP) in a volume of 10 ml/kg. Data were analyzed statistically with the two-tailed Kolmogorov-Smirnov test. Morphine sulphate was purchased from Carlo Erba, Milan; β-endorphin from Beckman, Palo Alto, CA; [D-Ala², D-Leu⁵] enkephalin from Bachem, AG, Bubendorf, Switzerland; naltrexone (HCl) from ENDO, Garden City, NY.

RESULTS

Table 1 indicates that different doses of morphine exerted a protective effect on ECS-induced seizures. Also β-endorphin and DADL exerted a protective effect at different doses.

The protective effects exerted by the three opiates had a different time course (see Table 1). In fact the effects of morphine were evident after a longer time interval (60 min after the injection) and lasted longer than β-endorphin and DADL. Table 1 shows that the effects of β-endorphin were short-lasting since the drug was ineffective when injected 60 min before the test. Finally, 125 ng of DADL exerted a 50% protective effect 60 min after the injection of the drug. It must be pointed out that no cataleptic effect occurred after the three opioids and that only the tonic phases of convulsions were effected. Naltrexone (7.5 mg/kg, IP), at a dose

TABLE 1

EFFECTS OF DIFFERENT DOSES OF MORPHINE, β-ENDORPHIN AND [D-Ala², D-Leu⁵] ENKEPHALIN (DADL) ON ECS-INDUCED CONVULSIONS

		Time Intervals (in min)					
	ng	5	15	30	60	90	120
Morphine	6.2	10	10	10	10	10	10
	12.5	10	10	7	5	7	9
	25.0	10	10	7	2†	5	6
	50.0	10	10	4	0†	0†	4
	100.0	10	10	1†	0†	0†	3*
β-Endorphin	25.0	10	10	10	10	10	10
	50.0	10	10	10	10	10	10
	125.0	8	6	8	10	10	10
	200.0	6	2†	6	10	10	10
	250.0	6	0†	5	10	10	10
DADL	2.5	10	8	10	10	10	10
	6.2	10	7	10	10	10	10
	12.5	10	5	9	10	10	10
	62.2	9	2†	6	10	10	10
	125.0	3*	0†	2*	5	9	10

ECS was administered at time intervals following ICV injections.

The numbers represent number of convulsing mice versus the total of 10 mice.

*$p<0.05$; †$p<0.01$ in comparison with saline injected mice (two tailed Kolmogorov-Smirnov test).

Saline injected mice showed a 100% percentage of tonic seizures.

which was previously found not to affect the ECS seizure thresholds, injected 30 min before the ICV injection completely antagonized the protective effects of morphine, β-endorphin and DADL (number of convulsing mice vs. a total of 10 animals: Morphine=1; β-endorphin=0; DADL=0).

DISCUSSION

In general the present findings indicate that the three opioids considered antagonize ECS-induced convulsions. Previous findings indicate that enkephalin analogues antagonize behavioural seizures in relation to volatile convulsants [3]. Our findings indicate that also morphine and β-endorphin exert a protective effect, though administration of higher doses of morphine were reported to lower the chemoconvulsive thresholds in mice [7]. However an anticonvulsant effect of opioids was reported in relation to kindled seizures [1] or to flurothyl-induced seizures [3, 4, 13].

The binding of these opioids to different receptor sites is well known. In particular, morphine shows a higher affinity to μ receptors, DADL presents a higher δ receptor affinity while β-endorphin is characterized by affinity for both receptor types [2].

A different onset of the effects observed with the three opioids may be explained (1) by a different capability of these drugs to diffuse into the brain or (2) by a different distribution of μ or δ receptors in brain structures involved in behavioral seizures [14].

In the present study an antagonism of ECS induced seizures was evident after injection of μ or δ agonists (morphine and DADL respectively) and of β-endorphin also. Therefore one could suggest that (1) the protective effect on seizures is related to both receptor types or (2) that the protective effects of DADL on seizures might also be related to its binding to μ receptors. In fact, as suggested by Pert *et al.* [10], DADL can bind in the patchy as well as the diffuse pattern depending on the incubation conditions used, suggesting that the μ and δ are functionally different conformations of the same type I opiate receptor.

Our findings are in contrast with a number of data indicating that high doses of morphine or other opioids exert a convulsant or proconvulsant effect [1, 7, 14]. This dual action of opioids may be explained not only by the route of administration employed (peripheral vs. ICV in this study) which may involve different brain structures, but also by the dose used and by the animal species considered. In fact opioids generally exert a cataleptic effect in rats, where high or toxic doses of morphine are convulsive, while morphine and endogenous opioids exert a behavioural stimulation in mice and may result in anticonvulsive effect, as shown by our findings. Further studies will possibly ascertain the significance of these differences.

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