Neuropeptides 5: 213-216, 1984

ELECTROENCEPHALOGRAPHIC ASSESSMENT OF THE ROLE OF δ RECEPTORS IN OPIOID PEPTIDE - INDUCED SEIZURES

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ABSTRACT

DPDPE ([D-Pen^2, D-Pen^5]-Enkephalin) and DPLPE ([D-Pen^2, L-Pen^5]-Enkephalin) are conformationally-constrained cyclic analogs of enkephalin with high selectivity for δ opioid receptors. Intracerebroventricular (i.c.v.) administration of each analog acutely produces a complex EEG response in rats characterized by a dose-related increase in spectral power and HVSA (peak frequency of 5.0 Hz) during behavioral stupor, and a theta driving (5.25-8.0 Hz) associated with intense behavioral arousal. These effects were antagonized by high (10 mg/kg), but not low (1.0 mg/kg), doses of naloxone. Both analogs failed to cause EEG or convulsive seizures. In contrast, i.c.v. administration of DADLE ([D-Ala^2, D-Leu^5]-Enkephalin), an enkephalin analog with activity at both μ and δ binding sites, caused initial nonconvulsive EEG seizures followed by HVSA (3.0 Hz); theta driving was not evident. The incidence of the seizures was dose-related and antagonized by very low doses of naloxone (0.01-1.0 mg/kg). Collectively, the inability of DPDPE and DPLPE to cause seizure activity, and the marked sensitivity of DADLE-induced EEG seizures to naloxone, suggest that δ receptors are not directly responsible for DADLE-induced EEG seizure activity. Furthermore, these data implicate μ opioid receptors as the primary sites responsible for enkephalin-induced seizures.

INTRODUCTION

Central administration of opioid peptides causes a sequence of electroencephalographic (EEG) responses characterized by an initial nonconvulsive EEG seizure, a subsequent sustained period of high-voltage slow-wave activity (HVSA) and a final short phase of low-amptitude desynchronized EEG activity prior to sleep (1,2,3). The seizurogenic properties of opioid peptides have received considerable attention, and it has been suggested that opioid peptide-induced seizure activity is mediated by the δ-receptor subtype of cerebral opioid binding sites (1,4,5,).

Whereas the existence of multiple opioid receptors has been firmly established (6,7), it remains difficult to establish with certainty that a particular response is mediated by a single subtype of opioid receptor, the major obstacle being the unavailability of highly selective agonist ligands. Recently, cyclization of the enkephalin molecule and conformational constrainment with a bis-penacillamine bridge in positions two and five has resulted in highly selective ligands for the δ opioid receptor subtype (8). In the present study, we have used these novel compounds to assess the role of δ receptors in opioid peptide-induced seizures. In addition, we have studied the EEG effects of DADLE,

a less selective enkephalin analog which binds equally well to μ and δ sites (9).

METHODS

Male Sprague-Dawley rats (250-300g), maintained on a timer-regulated lights-off period (0600hr-1800hr), were surgically prepared with chronic bipolar epidural frontoparietal electrodes and a right lateral (i.c.v.) cannulae(2). Animals were allowed 5-7 days to recover from surgery before testing.

During testing all rats were housed in individual recording chambers. Each chamber was equipped with a specially designed mercury swivel commutator (Walter Reed Div. of Instrumentation), providing noise-free recording contacts and permitting unrestrained movement of the rat during EEG monitoring. EEG activity, filtered to pass frequencies between 1 and 35 Hz, was continuously recorded during each test session on a Grass Model 7 polygraph. The EEG was simultaneously stored on FM tape for subsequent analysis. Power spectral analysis was performed online during each experiment using a Nicolet Pathfinder II computer system. Briefly, sequential EEG power spectrum arrays were derived from consecutive 12-sec epochs of cortical activity obtained after saline and drug treatment. The EEG was digitized at a rate of 256/sec, and spectral densities were estimated at 0.25 Hz intervals from zero to 20 Hz.

DADLE, DPDPE and DPLPE were dissolved in sterile saline and injected i.c.v. over 45 sec using a Hamilton microliter syringe. Total injection volumes never exceeded 10 μl. Ten to 30 min prior to drug injections, each rat received an i.c.v. saline injection and thereby served as its own control. In the naloxone studies, the antagonist (0.01-10 mg/kg, s.c.) was given 10 min prior to the agonist. All rats were given only a single i.c.v. injection series (saline and peptide), and each experiment was monitored until the appearance of slow-wave sleep (SWS).

RESULTS

Within 1 min following its injection, the cyclic enkephalins (35-140 nmol, i.c.v.) produced an HVSA with a maximal increase in total power of 253% to 286% of control. At the highest dose tested (140 nmol), the HVSA lasted approximately 15-30 min and had a mean peak frequency of 4.75 Hz to 5.0 Hz with very little EEG activity above 13 Hz. At these doses the rats were awake but stuporous. The HVSA was followed by a prolonged period of EEG theta-driving (peak frequency ranging from 6.75-7.5Hz) which was associated with an intensely aroused behavioral state characterized by locomotor activity and rearing with no grooming or eating. The effects of DPDPE and DPLPE were dose-related and antagonized by high doses of naloxone. These data are summarized in tables 1 and 2.

In contrast, the i.c.v. administration of DADLE (4.4-35 nmol) caused an initial nonconvulsive EEG seizure temporally associated with wet-dog shake behavior. At the highest dose tested (35 nmol) the seizure activity had a peak frequency of 1.75 Hz and a marked increase in spectral power in the 13 to 30 Hz band. The EEG seizures were followed by HVSA with a maximal increase in total power to 451% of control and a peak frequency of 3.0Hz. During the HVSA, the animals were immobile with extreme muscle rigidity. A short period of intermittent arousal dominated by eating and grooming followed the HVSA. However, no theta activity was seen (table 1). DADLE's effects lasted approximately 40 min. Unlike the cyclic analogs, the EEG effects of DADLE were very sensitive to naloxone. In particular, the DADLE-induced seizures were attenuated by doses of naloxone as low as 0.01 mg/kg, s.c. These data are summarized in table 2.

Table 1. Summary of the EEG effects of i.c.v. administered DADLE, DPDPE and DPLPE in rats.[1]

Compound	Dose Range (nmol)	EEG Seizures (% Responding)	HVSA[2] (% Power)	Theta Driving (5.25-8.0 Hz)
DADLE	2.2-35	71 (1.75Hz)[3]	451* (3.00Hz)	---
DPDPE	35-140	0	253* (5.00Hz)	+++ (6.75Hz)
DPLPE	35-140	0	286* (4.75Hz)	+++ (7.50Hz)

[1] n=14 rats per group.
[2] Maximal increase in total power (% of control) during high-voltage slow-wave activity (HVSA).
[3] Mean peak frequency.
* P<0.01 with respect to saline control (Paired t-test).

Table 2. The effect of naloxone on DADLE- and DPDPE-induced EEG responses in rats.[1]

Agonist (nmol,i.c.v.)	Naloxone (mg/kg, s.c.)	EEG Seizure (% Responding)	HVSA[2] (% Power)	Theta Driving (5.25-8.0 Hz)
DADLE	---	100	451*	---
(35)	0.01	66	290*+	---
	0.1	25	213*+	---
	0.5	0	177*+	---
	1.0	0	156*+	---
DPDPE	---	0	253*	+++
(140)	1.0	0	201*	+++
	10.0	0	153*+	---

[1] n=3-5 rats per group.
[2] Maximal increase in total power (% of control) during high-voltage slow-wave activity (HVSA).
* P<0.01 with respect to saline control (Paired t-test).
+ P<0.05, at least, with respect to agonist alone (Dunnett's test for multiple comparisons).

DISCUSSION

DPDPE and DPLPE have been described as selective δ opioid receptor agonists both *in vitro* (8,9) and *in vivo* (10). Using these peptide ligands we have attempted to ascertain the contribution, if any, of δ-receptor activation in opioid peptide-induced seizures. Our results have failed to demonstrate any direct role for activation of δ opioid binding sites mediating the nonconvulsive EEG seizures reported here. Moreover, the EEG seizure activity produced by DADLE, a μ-and δ-directed ligand, was highly sensitive to naloxone antagonism, suggesting that these seizures are possibly the result of activation of μ (naloxone-sensitive) binding sites.

Previous reports have suggested that the EEG seizures produced by opioid peptides may be a consequence of δ receptor activation (1,4,5,). However, the "prototypical" δ ligand used has been DADLE which, as noted earlier (9,10), shows selectively for μ sites as well as δ sites. Additionally, thorough naloxone dose-response studies have not been carried out such that a true determination of the relative naloxone-sensitivity of DADLE-induced seizures could be established. In the present study, doses of DADLE as low as 4.4 nmol caused epileptiform EEG activity which could be antagonized by very low doses of naloxone (minimal effective dose was 0.01 mg/kg).

All three ligands tested caused a similar HVSA and stuporous behavior in rats. However, power spectral analysis of the EEG clearly revealed discrete differences in spectral patterns evoked by DADLE and the cyclic analogs with regards to power and frequency distribution. Also, higher doses of naloxone were required to antagonize DPDPE- and DPLPE-induced HVSA than DADLE-induced HVSA. The δ selective cyclic enkephalins also differed from DADLE in producing a marked theta driving. This inability of low doses of naloxone to antagonize this DPDPE- and DPLPE-induced activity is suggestive of a non-μ (possibly δ) receptor involvement.

In summary, while i.c.v. administration of DPDPE, DPLPE or DADLE produces a complex EEG response in rats, there is no evidence to suggest that the DADLE-induced nonconvulsive EEG seizures are a result of δ receptor activity. Furthermore, given the results obtained with DPDPE and DPLPE, and the naloxone sensitivity studies, it appears that μ receptors represent the primary binding sites responsible for enkephalin-induced seizures.

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