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An In Vitro Study on the Hippocampal Electrophysiological Properties of Enkephalinase Inhibitors in Rats

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PROIETTI, M. L., S. SAGRATELLA, C. FRANK, M. TRAMPUS AND A. SCOTTI DE CAROLIS. *An in vitro study on the hippocampal electrophysiological properties of enkephalinase inhibitors in rats.* PHARMACOL BIOCHEM BEHAV **39**(1) 119–121, 1991.—The effects of two enkephalinase inhibitors were studied on the CA1 and dentate hippocampal extracellular field potentials (FPs). The enkephalinase inhibitors thiorphan and SCH 32615, at a concentration of 1–500 μM, failed to significantly affect CA1 and dentate FPs. Thiorphan and SCH 32615, at a concentration of 150 μM, were able to potentiate the enkephalin-induced epileptiform bursting, inducing an increase in the bursting duration and in the number of spikes per burst due to 3.5 μM DAEAM or 0.20 μM DAGO. The results suggest: 1) the potentiation of an electrophysiological opiate receptor-mediated response by enkephalinase inhibitors; 2) the inability to show a direct effect on the basal CA1FP as a result of the inhibition of the endogenous enkephalinase.

Enkephalins Endopeptidase Epileptiform bursting Hippocampal slices

EXOGENOUS opiates, interacting through the various recognition sites (i.e., mu, delta, kappa and sigma receptors) are able to interfere with epileptic and convulsive phenomena (4,9). In fact, morphine and enkephalins lower the epileptic threshold and induce the appearance of electrographic seizures in rats (2,13). The epileptogenic effect of these compounds is well-characterized especially in the limbic cerebral system, where hippocampal EEG seizures, accompanied by a wet-dog shake behaviour, develop after topical application of opiates (5,6). In fact, in the hippocampal area, opiates present an excitatory effect, which is well established in electrophysiological experiments in vitro on brain slices. Enkephalins, in particular, as from a nanomolar concentration increase the amplitude of CA1 population spikes, without affecting the excitatory postsynaptic potential of extracellular field potentials evoked after electrical stimulation of the stratum radiatum (11,14).

In order to assess the actual involvement of the endogenous enkephalinergic system in this property of opiates, the effects of two inhibitors of enkephalinase—a catabolic endopeptidase, and the major degrading enzyme of enkephalinergic system—have been investigated.

METHOD

Slice Preparation Session

Male Wistar rats (200–300 g) were killed by decapitation, the skull was opened and the hippocampus rapidly removed. Hippocampal slices (450 μm thick) were cut with a tissue chopper (McIlwain) and immediately placed in the recording chamber, where they were constantly perfused (at a rate of 1–2 ml/min) with an artificial cerebral spinal fluid (ACSF) saturated with 95% O_2–5%CO_2.

The ACSF had the following composition: 122 mM NaCl, 0.4 mM KH_2PO_4, 3 mM KCl, 1.2 mM $MgSO_4$, 25 mM $NaHCO_3$, 1.3 mM $CaCl_2$, 10 mM glucose (pH 7.3). A 60–90-min period elapsed after the slices were cut and before the recording session started.

Recording Session

Field potentials (FP) were recorded in the hippocampal CA1 and dentate areas after electrical stimulation (0.1 Hz, 70 μs, 200–400 μA) of the stratum radiatum or stratum moleculare in

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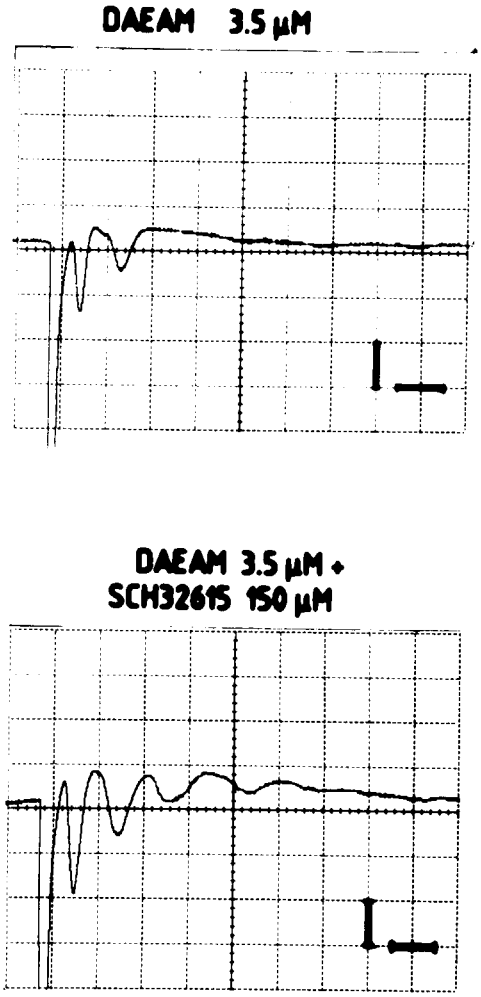

FIG. 1. Potentiation of the DAEAM effects by an enkephalinase inhibitor. Upper side: the recording shows the epileptiform bursting induced after a 60-min slice perfusion with 3.5 μM DAEAM. Lower side: after a 60-min slice perfusion with 3.5 μM DAEAM plus 150 μM SCH 32615 an increase in the bursting duration and in the number of population spikes is recorded. Calibrations: 5 ms, 2 mV.

order to stimulate the enkephalinergic terminals from the perforant path (10). Electrical potentials were amplified, monitored on oscilloscope, recorded on tape (Racal 4DS), graphically plotted (Gould DSO 1604) and analyzed on-line on a PS2 IBM computer. After 60 min, a stable FP (max 0.2 mV–1 ms changes between stimulations) was attained before beginning the drug session.

Drug Session

The drugs were added directly into the perfused solutions. After a 60-min period, the effects of the drugs were assessed with reference to 1) the amplitude of the CA1 and dentate population spikes (PS); 2) the stimulus-response curves of the CA1 and dentate areas; 3) the occurrence (number of spikes × burst) of additional epileptiform PSs; and 4) FP duration (measured from the first to the last PS, whose amplitude was higher than 0.2 mV). In another separate series of experiments, the combined superfusion of enkephalins and enkephalinase inhibitors was tested for 60-min periods.

TABLE 1

IN VITRO ELECTROPHYSIOLOGICAL ACTIVITIES OF ENKEPHALINASE INHIBITORS

Drug	(N)	Conc.	%EB	EBD (ms)	No. Spikes × EB
None	(6)	—	—	2.8 ± 0.4	—
THIO	(6)	150/μM	—	3.2 ± 0.2	—
SCH 32615	(6)	150/μM	—	2.8 ± 0.4	—
DAGO	(10)	0.2/μM	40%	5.0 ± 0.5	1.4 ± 0.1
DAGO	(10)	0.5/μM	100%	8.9 ± 0.7	2.2 ± 0.6
DAEAM	(10)	3.5/μM	50%	5.5 ± 0.6	1.4 ± 0.2
DAEAM	(10)	5/μM	85%	7.8 ± 1.1	2.0 ± 0.1
DAEAM	(10)	10/μM	100%	11.2 ± 0.9	2.8 ± 0.2
THIO + DAGO	(10)	150/μM 0.2/μM	60%	* 7.6 ± 0.5	* 2.1 ± 0.3
SCH 32615 + DAGO	(10)	150/μM 0.2/μM	70%	* 8.1 ± 1.0	* 2.3 ± 0.2
THIO + DAEAM	(10)	150/μM 3.5/μM	100%	† 11.4 ± 1.5	† 2.5 ± 0.2
SCH 32615 + DAEAM	(10)	150/μM 3.5/μM	100%	† 10.2 ± 1.1	† 2.3 ± 0.3

The table shows the value (± SE) of the parameters of the epileptiform bursting after a 60-min slice perfusion with enkephalins plus enk'ase inhibitors or enkephalins alone.

Abbrev.: N = number of experiments; %EB = % incidence of epileptiform bursting; EBD = epileptiform bursting duration; No. spikes × EB = number of spikes per epileptiform bursting; THIO = thiorphan. *Significantly different from DAGO alone $p<0.05$ according to Student's t-test; †significantly different from DAEAM alone $p<0.05$ according to Student's t-test.

[D-Ala^2-N-methyl-Phe^4-Gly^5-ol]Enkephalin (DAGO) and [D-Ala^2-Met^5]Enkephalinamide (DAEAM) were obtained from Sigma (St. Louis, MO); thiorphan and N[L(1-carboxy-2-phenyl)-ethyl]-L-phenylalanyl-alanine (SCH 32615), from Schering-Plough SpA (Milan, Italy).

RESULTS AND DISCUSSION

Control CA1 and dentate FPs consisted of an excitatory postsynaptic potential and a single superimposed PS (2–4 ms, 3–5 mV). Slice perfusion with 1–500 μM thiorphan (N = 6) or SCH 32615 (N = 6), within 30–60 min, affected neither control CA1 or dentate FPs, nor PS amplitude (+10 ± 5% and +6 ± 8%, respectively) or PS duration (+6 ± 5% and +9 ± 8%, respectively) (n = 10). The stimulus-response curve of the CA1 and dentate hippocampal areas remained unchanged as well. DAGO (0.2–0.5 μM) and DAEAM (2.5–10 μM) increased, in a concentration-dependent manner, the pyramidal neuronal excitability, inducing the appearance of a CA1 epileptiform bursting constituted by multiple epileptiform additional PSs (Table 1).

Slice perfusion with 150 μM thiorphan or SCH 32615 plus 0.2 μM DAGO significantly increased the incidence of the CA1 epileptiform burstings (+50% and +48%), the CA1 bursting duration (+40 ± 10% and +65 ± 6%), and the number of spikes per burst (+35 ± 10% and +35 ± 7%) as compared to slice perfusion with the enkephalin alone (Table 1).

Slice perfusion with 150 μM thiorphan or SCH 32615 plus 3.5 μM DAEAM significantly increased the incidence of the CA1 epileptiform burstings (+50% and +48%), the CA1 burst-

ing duration ($+72 \pm 12\%$ and $+70 \pm 10\%$) and the number of spikes per burst ($35 \pm 11\%$ and $32 \pm 7\%$) as compared to slice perfusion with the enkephalin alone (Table 1, Fig. 1).

In order to determine the actual involvement of the naturally occurring enkephalinergic system in the hippocampus we investigated the effects of two inhibitors of the catabolic endopeptidase, enkephalinase. This is the trivial name given to the neutral endopeptidase (E.C. 3.4.24.11) that cleaves either Met^5 or Leu^5 enkephalin at the glycinylphenyl-alanyl amide bond (3).

There is now substantial evidence that this peptidase participates in the inactivation of endogenous enkephalins both in vitro (8,12) and in vivo (3, 7, 16). Therefore, the use of enkephalinase inhibitors could facilitate the evaluation of the role of endogenous neuropeptides in the electrophysiological response of the CA1 pyramidal neuron-Schaffer collateral interconnections.

Our data indicate that enkephalinase inhibitors failed to affect the extracellular hippocampal CA1 and dentate FPs. However, they were able to potentiate the epileptogenic activity of enkephalins, thus confirming the pharmacological properties of these compounds. In fact, in vivo and in vitro studies have reported enkephalinase inhibitors to potentiate the biochemical and behavioural effects of enkephalins. In particular, they potentiate or prolong the analgesic activity of DAEAM (3) or D-Ala-Met^5-enkephalin (15) in mice. In addition, enkephalinase inhibitors potentiate the DAEAM-induced analgesic and catatonic effects in rats (1). Our data show a significant increase in the DAGO- or DAEAM-induced hippocampal epileptiform bursting duration produced by thiorphan and SCH 32615, which demonstrates that enkephalinase inhibitors potentiate the enkephalin-induced electrophysiological effects.

This potentiation might depend on the blockade of the peptide's catabolism and thereby on the subsequent increase in the amount of enkephalin available to the postsynaptic receptors. The inability of enkephalinase inhibitors to affect the extracellular field potentials might be related to an incapability of the compounds to raise the basal extracellular levels of enkephalins in rat hippocampal slices. In agreement with this hypothesis, Patey et al. (12) showed in slices of rat striatum that thiorphan enhanced the potassium-stimulated increase of Met^5-enkephalin, while it did not affect the resting levels of enkephalins.

In conclusion, the present data show a potentiation of an opiate receptor-mediated electrophysiological response by enkephalinase inhibitors, but fail to demonstrate that these drugs affect the basal synaptic transmission in an in vitro brain preparation.

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