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# RAPID COMMUNICATION

## Selective Effects of Enkephalin on Electrical Activity of the *in Vitro* Hippocampal Slice

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The effect of infusion of small concentrations of D-Ala–D-Leu-enkephalin on electrical responses to stimulation of the *in vitro* hippocampal slice preparation was investigated. Concentrations of enkephalin as low as 10 n*M* dramatically increased the magnitude of the population spike response of pyramidal cell neurons to stimulation of their Schaffer-commissural afferents. This effect had a rapid onset and was quickly eliminated when the peptide was removed from the perfusion medium. Enhancement of the population spike was blocked by the opiate receptor antagonist naloxone, which, by itself, did not affect either synaptic potentials or the population response. In contrast even relatively high concentrations of the enkephalin produced no detectable effect on either the synaptic potentials recorded in the dendrites or the antidromic response of the pyramidal cells to stimulation of their axons. These findings suggest that enkephalin attenuates the effect of either a feedback or feed-forward inhibitory system which is usually activated by stimulation of the Schaffer-commissural pathways.

A rapidly expanding body of evidence points to the conclusion that enkephalins exert a powerful influence on the ongoing operation of the hippocampal formation, but the identification of the cellular targets of these peptides is uncertain (Nicoll, Siggins, Ling, Bloom, & Guillemin, 1977; Zieglgänsberger, Siggins, French, & Bloom, 1978; cf. Fry, Zieglgänsberger, & Herz, 1979). In an effort to provide data on this point, we investigated the effects of low levels of superfused enkephalin on

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electrical activity of the *in vitro* hippocampal slice preparation. Our findings indicate that the peptide has an extremely specific and naloxone-reversible effect in the hippocampus and provide some clues about the anatomical locus of this effect.

Slices were prepared and maintained using procedures described elsewhere in detail (Dunwiddie, Madison, & Lynch, 1978). They were continuously perfused with media containing in 1 liter of distilled $H_2O$, 7.25 g NaCl, 0.248 g KCl, 0.17 g $KH_2PO_4$, 0.29 g $MgSO_4$, 0.277 g $CaCl_2$, 2.16 g $NaHCO_3$, and 1.8 g D-glucose. D-Ala–D-Leu-enkephalin (Bachem, Torrance, Calif.) and naloxone (Endo Laboratories, Long Island, N.Y.) were added by continuous injection into the perfusion lines. Bipolar stimulation electrodes (twisted 120-$\mu$m wires) were placed in the Schaffer-commissural projections from the regio inferior to the apical dendrites of the regio superior as well as in the alveus, the fiber bundle carrying the axons of the regio superior pyramidal cells. These two electrodes thus allowed for orthodromic and antidromic activation of the regio superior. Recording micropipets (1–5 M$\Omega$ impedance) were placed in the regio superior dendritic zones containing the Schaffer-commissural inputs (*s. radiatum*) as well as in the densely packed layer of pyramidal cell bodies (*s. pyramidale*). The first electrode recorded the extracellular concomitants of the dendritic excitatory postsynaptic potentials (EPSPs) generated by the Schaffer-commissural inputs, while the cell body layer electrode measured the response, in terms of cell spiking, to those EPSPs (see Dunwiddie & Lynch, 1978 for a complete description). Stimulation voltages for the Schaffer-commissural afferents were adjusted to produce a small (0.5- to 1.0-mV) "population spike" in the cell body layer response. This population spike is a sharp negative potential that is an envelope of the extracellular currents generated by several simultaneously spiking neurons. It should be emphasized that postsynaptic components are usually stable for a number of hours in this preparation.

Figure 1 illustrates the results of a typical experiment and as can be seen in Fig. 1A the enkephalin had no detectable effect on the synaptic potentials generated by stimulation of the Schaffer-commissural projections. The same was true of the antidromic potential recorded at the cell bodies in response to stimulation of the alveus. This apparent lack of effect on orthodromic and antidromic responses was obtained even with enkephalin concentrations in the micromolar range. Conversely, the population spike was exquisitely sensitive to the peptide (Fig. 1B). This measure showed dramatic increases in each of six separate experiments and these effects were evident with concentrations as low as 10 n*M*. The onset of the effect of enkephalin was usually apparent within 2–3 min of the time it was added to the perfusion lines, and as seen in Fig. 1, the enkephalin effect was completely reversed by continued perfusion with

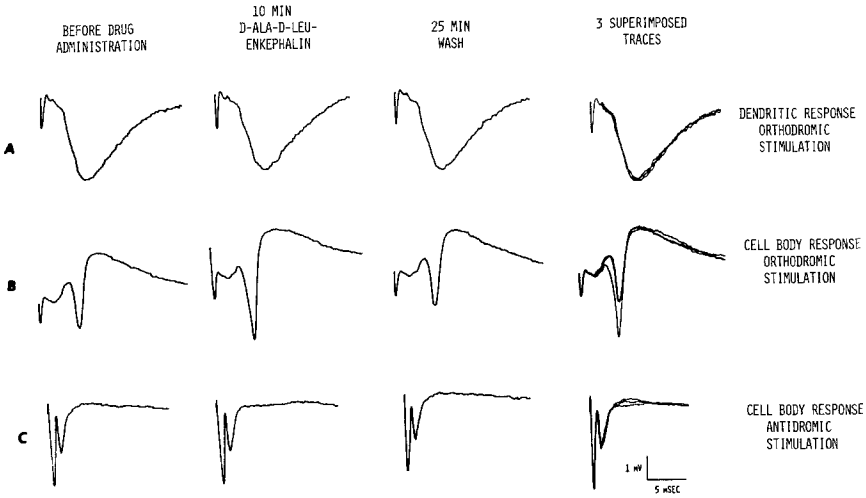


FIG. 1. Effects of 50-n*M* D-Ala–D-Leu-enkephalin on orthodromic and antidromic potentials recorded in the regio superior of the continuously perfused *in vitro* hippocampal slice. (A) The top four traces were recorded with a microelectrode located by physiological criteria to be within the apical dendritic subfield containing the activated synaptic contacts generated by stimulation of the Schaffer-commissural projections. (B) The middle four traces are responses to Schaffer-commissural stimulation collected by a micropipet in the densely packed cell body layer of the regio superior. The sharp negative deflection is the population spike referred to in the text. (C) Same electrode as in B but the potentials are antidromic responses to stimulation of the axons of the regio superior pyramidal cells. The extreme right panels (three superimposed traces) show the superimposed responses collected before and during drug administration as well as after a 25-min wash period.

peptide-free medium. Put simply, enkephalin had a rapid onset and offset of physiological action.

As shown in Fig. 2, the augmentation of the population spike produced by enkephalin was blocked by naloxone. Naloxone by itself had no effects on either synaptic potentials or the population spike.

The present findings can be summarized simply. Enkephalin has no effects on synaptic potentials or antidromic responses, but produces a reversible naloxone-sensitive increase in the synchrony and/or number of the spiking responses of the target cells. This pattern of results eliminates a number of possible modes of action of the peptide, as well as providing a hypothesis about its anatomical targets. It appears that the enkephalin acts either to change the spike thresholds of the pyramidal cells (possibly by direct action or the removal of tonic inhibition) or to suppress a phasic stimulation-locked inhibition of the cell body. Since the antidromic potentials were minimally affected or unchanged even by relatively large concentrations of enkephalin, it would appear that the first hypothesis is

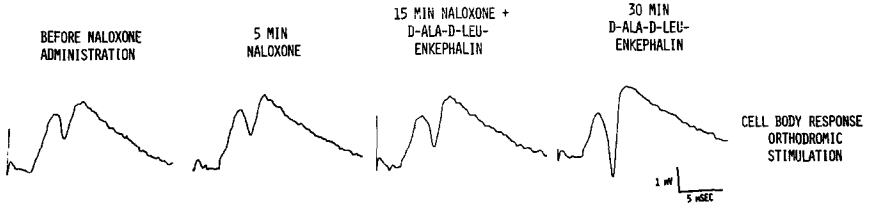


FIG. 2. The effects of 0.5 $\mu M$ naloxone and 50 nM enkephalin on the response recorded at the cell body layer of the regio superior to stimulation of the Schaffer-commissural projection. The naloxone, applied for 5 min before and for the first 15 min of enkephalin infusion, had no detectable effects by itself but did largely, though not completely, block the usual enkephalin-produced enhancement of the population spike. For the last 30 min of the experiment, enkephalin influsion was continued but without simultaneous treatment with naloxone and, as is evident from the last panel of the figure, enkephalin produced its typical effects on the population spike.

unlikely. Thus, we suggest that the peptide influences either a feedback inhibitory (e.g., basket-cell recurrent inhibition; see also Zieglgänsberger et al., 1978) or a feed-forward system usually brought into play by stimulation of the Schaffer-commissural pathways.

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