Table 1  Perforant path tetanization depresses heterosynaptic e.p.s.p.s

<table>
<thead>
<tr>
<th>Prior history of tetanization</th>
<th>None</th>
<th>None</th>
<th>MPP</th>
<th>LPP</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pathway most recently tetanized</td>
<td>LPP</td>
<td>MPP</td>
<td>LPP</td>
<td>MPP</td>
</tr>
<tr>
<td>Pathway being tested</td>
<td>MPP</td>
<td>LPP</td>
<td>MPP</td>
<td>LPP</td>
</tr>
<tr>
<td>Synaptic I/O regression slope (mean % change from pre-tetanus value)</td>
<td>$-14.4^* -20.5^* +3.1 -0.6$</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* $P < 0.05$, one-way analysis of covariance.

Can depress synaptic function of converging untetanized afferents for periods exceeding 3 h. Many aspects of this long-term depression remain to be elucidated, including the duration and synaptic specificity of the effect. Given that in 3/8 animals LPP trains produced decreases in LPP synaptic strength, the depression may be nonspecific, occurring in both tetanized and untetanized pathways but usually being masked in the stimulated pathway by concurrent LTD. If so, such nonspecific depression must have maximized easily, because a second series of trains on a separate pathway failed to produce reductions in synaptic strength in either pathway. Regardless of the cellular explanation, the functional effect of heterosynaptic depression is complementary to that of LTD, that is, enhancing the relative strength of the recently active synapses, but in this case at the expense of other synapses not protected by previous LTD.

We thank Dr T. V. P. Bliss for his collaboration and advice during early phases of this experiment. Dr Neil Naughton provided valuable assistance in the statistical analyses and made useful comments on a previous version of this manuscript. This work was supported by grants from the New Zealand MRC and New Zealand Neurological Foundation to G. V. G. W. C. A. was supported by postdoctoral fellowships from the University of Otago, New Zealand, and NINCDS, USA.

Received 17 June; accepted 10 August 1983.


Intracellular injections of EGTA block induction of hippocampal long-term potentiation

Gary Lynch, John Larson, Stephen Kelso, German Barrionuevo & Frank Schottler

Center for the Neurobiology of Learning and Memory, University of California, Irvine, California 92717, USA

Hippocampal long-term potentiation (LTP) is a remarkably stable facilitation of synaptic responses resulting from very brief trains of high-frequency stimulation. Because of its persistence and modest induction conditions, LTP represents a promising candidate for a substrate of memory. Some progress has been made in localizing the changes responsible for the effect; for example, it has been shown that LTP is not accompanied by changes in the fibre volleys of the test afferents or by general alterations of the dentrites of their target cells. However, it is unknown whether the potentiation is due to presynaptic or postsynaptic changes and there is evidence in favour of each (for example, see refs 5, 6). We now report that intracellular injections of the calcium chelator EGTA block the development of LTP. These results strongly suggest that LTP is caused by a modification of the postsynaptic neurone and that its induction depends on the level of free calcium.

Slices of rat hippocampus were prepared and maintained using methods described elsewhere. A stimulating electrode was placed in the stratum radiatum to activate the Schaffer-commissural fibres and an extracellular recording electrode was lowered into the apical dendritic zone of field CA1 or, in a few cases, into the pyramidal cell body layer. An intracellular electrode filled with 2 M potassium acetate (control) or potassium acetate plus 0.2-0.5 M potassium acetate-EGTA (80-200 M0 impeding) was then placed into the cell body layer of CA1 and attempts made to penetrate neurones. Only cells having a resting membrane potential of $\geq 55$ mV ($\pm$ s.e.m. $66.8 \pm 1.3$ mV), a spike of $\geq 60$ mV, and a membrane impedance of $\geq 15$ M0 ($\pm$ s.e.m. $30.6 \pm 2.4$ M0) were used. In a few cases, a small hyperpolarizing current was used to increase the size of the excitatory postsynaptic potential (e.p.s.p.). If a satisfactory impalement was achieved, stimulation pulses were delivered at a rate of one per 10 s to the Schaffer-commissural fibres. The stimulation current (20-70 $\mu$A) was adjusted to a level at which the e.p.s.p. amplitude could be reliably measured but was sub-threshold for spike discharge, and at which the dendritic field potential was not maximal.

The following measurements were made by computer following each stimulus: (1) rise time and amplitude of the e.p.s.p.; (2) resting membrane potential; and (3) slope and amplitude of the extracellular dendritic field potential (or amplitude of the population spike). If these measurements proved stable, 5-7 min of 200-ms 0.5 nA pulses given at 2 per s were applied to EGTA electrodes and several of the regular electrodes. Following this, testing was resumed for $\geq 10$ min and, if the responses proved stable, a series of high-frequency trains were applied to the stimulation electrode. These consisted of five 300 s$^{-1}$ bursts, each of 35 ms duration, or 100 s$^{-1}$ bursts each of 300 ms duration. Testing was resumed within 15 s and recordings taken for 10-90 min, depending on the stability of the neurone. Finally, the recording pipette was withdrawn from the neurone and extracellular responses collected.

Intracellular data were used if the resting membrane potential did not vary by more than 2.5 mV from the control period to at least 10 min after the high-frequency stimulation trains. Only intracellular recordings from slices which exhibited extracellular LTP were included in the study. LTP was defined as a non-decremental increase of greater than 10% of the slope and amplitude of the dendritic response recorded by the extracellular electrode during the period 5-15 min after the high-frequency stimulation. If an extracellular response was reliably detected by the intracellular electrode after leaving the cell, it was averaged and subtracted from the stored e.p.s.p.

Thirty-three cells were studied with regular electrodes from slices with extracellular LTP (mean of 28.5 $\pm$ 2.2% increase of field potential slope). Figure 1A, C illustrates one of these. Five
Fig. 1 A, Intracellular recording with a regular electrode. Each trace is the average of six successive responses 10 s apart collected immediately before and 15 min after five bursts of 300 s\(^{-1}\) stimulation, each lasting 35 ms, delivered through a bipolar electrode in the trajectory of the Schaffer-commissural fibres. Calibration bars: 5 mV and 5 ms for this and subsequent panels. B, Intracellular recording with an EGTA-filled electrode. Averaged responses to six single-pulse stimulations collected immediately before and 15 min after four high-frequency stimulation trains are superimposed. C, Per cent change in amplitude of the e.p.s.p. after high-frequency stimulation (arrow) for the cell illustrated in A. The x-axis is time in minutes. Each line represents a single response and the average amplitude for the control period is expressed as 100%. The high-amplitude responses are spikes which have been chopped in preparation of the figure. D, Amplitude of individual e.p.s.ps expressed as percentage of the averaged responses collected before high-frequency stimulation for the cell described in B. EGTA was injected before collecting control data. E, Intracellular recording from a neuron with an EGTA-filled electrode. Calibration as in A. F, Recording as in E but after withdrawal from the cell.

of the neurons recorded with regular electrodes did not show increased e.p.s.ps after the high-frequency train, five spiked to each single stimulus pulse, and 23 exhibited stable potentiation of the type illustrated in Fig. 1. Thus, intracellular LTP was obtained in 28 of 33 cells. Table 1 summarizes the mean percentage increase in e.p.s.p. for the cells (n = 28) which did not spike. Twenty-six cells were studied with EGTA-filled electrodes from slices which exhibited LTP (mean increase of 28.3 ± 4.4% in slope of dendritic field potential); 20 did not exhibit evidence of LTP (Fig. 1B, D), four showed at least a 10% increase in e.p.s.p., while two neurons spiked to single stimulation pulses after high-frequency stimulation. Thus, LTP was obtained in only six of 26 neurons recorded with EGTA-filled electrodes. Table 1 summarizes per cent change in the 24 cases in which e.p.s.ps could be recorded following high-frequency stimulation.

In agreement with previous reports, EGTA injections had no detectable effect on resting membrane potential or membrane impedance. As seen in Table 1, the mean e.p.s.p. amplitude before high-frequency stimulation was comparable in the two groups. It is worth noting that similar dendritic field potential amplitudes were obtained in both groups (control, 2.2 ± 0.1 mV;...
Table 1. e.p.s.p. amplitudes of cells which did not spike to single pulse stimulation after induction of extracellular long-term potentiation

<table>
<thead>
<tr>
<th>e.p.s.p. amplitude before high-frequency stimulation (mV)</th>
<th>Time (min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>10</td>
</tr>
<tr>
<td>7.0 ± 0.6</td>
<td>30 ± 5%</td>
</tr>
<tr>
<td>EGTA</td>
<td>7.1 ± 0.6</td>
</tr>
<tr>
<td>6 ± 3%*</td>
<td>30 ± 4%</td>
</tr>
<tr>
<td>5 ± 4%*</td>
<td>28 ± 4%</td>
</tr>
</tbody>
</table>

The e.p.s.p. amplitudes (mean ± s.e.m.) for 28 of 33 cells recorded with regular electrodes (control) and 24 of 26 cells recorded with EGTA-filled electrodes.

EGTA, 2.1 ± 0.1 mV), indicating that the stimulation current used to obtain similar sized e.p.s.p.s in the two groups was not different. Furthermore, excellent paired-pulse facilitation was found in these cells (Fig. 2A) and they typically spiked to single pulse stimulation for 30-45 s after high-frequency stimulation, indicating post-tetanic potentiation (PTP) was present. Additional evidence of the selective action of EGTA has been documented in studies of burst after-hyperpolarizations (AHPs) of the CA1 region. We have found that in slices incubated in medium containing 20 μM picrotoxin (used to block the inhibitory postsynaptic potential, i.p.s., i.e., which obscures the initial part of the AHP), EGTA blocks the AHP following bursts induced by depolarizing current injection, but does not block the AHP following repetitive firing induced by stratum radiatum stimulation (Fig. 2B, C). As other evidence indicates that the depolarization-induced AHP is a Ca(2+)-dependent potential5-11, we suggest that the only effect of EGTA in these neurons was on Ca(2+)-mediated processes.

These results indicate that selective manipulation of the postsynaptic neurone blocks the induction of long-term potentiation. As EGTA did not produce evident disturbances in several physiological parameters in our experiments or those reported by others8,9, it is reasonable to assume that it interfered with the induction of LTP via its known actions as a calcium buffer. Therefore, the present findings strongly support the hypothesis that LTP is due to a calcium-stimulated modification of the postsynaptic neurone12. Previous studies have established that low levels of calcium cause an increase in glutamate binding sites in purified membranes from hippocampus13, apparently by activating a calcium-sensitive proteinase14-16. Furthermore, LTP is correlated with an increase in glutamate binding to hippocampal membranes17-18. We propose that EGTA, by buffering intracellular calcium, prevents the activation of enzymatic machinery controlling postsynaptic receptors and thereby blocks the development of LTP.

This work was supported by research grants from NIMH (MH 19793-12) and NSF (BSNS-7617370), and an NIH research scientist award (MH 00358-03) to G.L.

Received 6 June; accepted 16 August 1983.


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**Novel C-terminally amidated opioid peptide in human phaeochromocytoma tumour**

Hisayuki Matsuo, Atsuro Miyata & Kensaku Mizuno

Department of Biochemistry, Miyazaki Medical College, Miyake, Miyazaki 880-16, Japan

As has often been observed in hypothalamic releasing factors and gastrointestinal hormones, the carboxy-terminal amide structure is a unique feature of peptides exhibiting hormonal or physiological activities. Although a variety of opioid peptides have hitherto been identified8-10, such a C-terminal amidated species has never before been discovered in mammals. Here we present the first identification of a novel opioid octapeptide with a C-terminal amide structure, henceforth designated as "adrenorphin", in human phaeochromocytoma tumour derived from adrenal medulla. The complete amino acid sequence of adrenorphin was determined by microsequencing and corresponds to the sequence of the first eight amino acids of peptide E which is derived from proenkephalin A. Adrenorphin has also been identified chromatographically in normal human and bovine adrenal medulla. Adrenorphin exhibits potent opioid activity in guinea pig ileum assay, suggesting a specialized physiological function.

Adrenorphin was purified from human phaeochromocytoma using a technique similar to that used previously in the isolation of opioid peptides such as BAM-12P from bovine adrenomedullary gland3. A portion corresponding to the lower molecular weight (Mr, < 2,000) that was separated from acid extracts of human phaeochromocytoma by gel filtration on Sephadex G-50 was treated batchwise with CM-cellulose in a buffer of 10 mM ammonium formate (pH 6.6). After washing the resin with the same buffer, the basic peptides adsorbed on the column were eluted with 1 M formic acid and pooled. The basic peptide pool thus obtained was then subjected to HPLC on a reverse-phase column of TSK LS-410 ODS-SIL (Fig. 1). An aliquot of each fraction was trypsinized and then generation of [Arg²]-enkephalin was analysed by radioimmunoassay utilizing anti-[Arg²]-Leu-enkephalin antiserum, having 5% cross-reactivity with [Arg³]-Met-enkephalin. As seen in Fig. 1, five immunoreactive peaks (A-E) were obtained. The present purification of adrenorphin is concerned with peak B, which was eluted close to the position corresponding to the authentic BAM-12P.

The subsequent reverse-phase HPLC of peak B on a different column of µ-Bondapak C-18, yielded adrenorphin as an unidentified peptide peak that was eluted slightly ahead of the standard BAM-12P. Rechromatography afforded purified adrenorphin, as shown in Fig. 2a. Only a tyrosine residue was identified by dansylation as the amino-terminal residue of adrenorphin thus purified, confirming its homogeneity. Attempted C-terminal analyses of the peptide utilizing carboxypeptidase A and B revealed that the C-terminus of adrenorphin is blocked.

On hydrolysis with 6 M HCl, adrenorphin afforded the following amino acid composition: Gly₂, Val₁, Met₉, Tyr₁, Phe₁, ArB₂₁, suggesting its octapeptide structure. Based on quantitative amino acid analysis of adrenorphin, it is estimated that 20 nmol of the pure peptide were isolated from 42.6 g of the tumour. Trypsinization of the peptide generated [Arg³]-Met-enkephalin, which was identified with the standard specimen by chromatographic comparison on reverse-phase HPLC (Fig. 3). The primary structure of adrenorphin was determined by stepwise Edman-dansyl sequencing of the native peptide. The complete amino acid sequence of adrenorphin thus ascertained is H-Tyr-Gly-Gly-Phe-Met-Arg-Arg-Val-NH₂. The presence of C-terminal valine amide was verified by thermolytic digestion, followed by dansylation in a manner similar to that described by Tatemoto et al.10.