Published in final edited form as:

Nature. 1993 June 3; 363(6428): 451–454.

Endogenous dynorphins inhibit excitatory neurotransmission and block LTP induction in the hippocampus

John J. Wagner^{*},

Department of Pharmacology, University of Washington, Seattle, Washington 98195, USA

Gregory W. Terman, and

Department of Anesthesiology, SJ-30, University of Washington, Seattle, Washington 98195, USA

Charles Chavkin

Department of Pharmacology, University of Washington, Seattle, Washington 98195, USA

Abstract

Although anatomical and neurochemical studies suggest that endogenous opioids act as neurotransmitters^{1–7}, their roles in normal and pathophysiological regulation of synaptic transmission are not defined. Here we examine the actions of prodynorphin-derived opioid peptides in the guinea-pig hippocampus and show that physiological stimulation of the dynorphin-containing dentate granule cells can release endogenous dynorphins, which then activate κ_1 opioid receptors present in the molecular layer of the dentate gyrus. Activation of κ_1 receptors by either pharmacologically applied agonist or endogenously released peptide reduces excitatory transmission in the dentate gyrus, as shown by a reduction in the excitatory postsynaptic currents evoked by stimulation of the perforant path, a principal excitatory afferent. In addition, released dynorphin peptides were found to block the induction of long-term potentiation (LTP) at the granule cell-perforant path synapse. The results indicate that endogenous dynorphins function in this hippocampal circuit as retrograde, inhibitory neurotransmitters.

Whole cell recordings were made from granule cells in the guinea-pig hippocampal slice (Fig. 1) and excitatory postsynaptic currents (e.p.s.cs) were evoked by afferent stimulation either in the molecular layer (to activate perforant path fibres from the entorhinal cortex) or in the hilus (to activate commissural/associational afferents). Opioid receptor activation by the κ_1 selective agonist U69,593 (refs 8,9) at 500 nM significantly reduced (by $41 \pm 3\%$; $n = 8$) the amplitude of the CNQX-sensitive e.p.s.cs evoked by perforant path stimulation without affecting granule cell input conductance (Fig. 1b). This effect was reversed by the κ_1 selective antagonist 10, 11, norbinaltorphimine at 100 nM (Fig. 1b). In contrast, the CNQX-sensitive component of the e.p.s.cs evoked by hilar stimulation was not significantly affected by κ_1 receptor activation ($n = 4$) (Fig. 1b). Thus, κ_1 receptors appear to be selectively expressed on perforant path terminals and to inhibit glutamate release rather than directly affecting the postsynaptic cell or its response to glutamate.

To determine whether endogenous opioids also modulate the release of glutamate from perforant path afferents, we stimulated granule cells using a paradigm previously shown to release dynorphins by antidromic activation of granule cell axons in the hilus of the dentate gyrus⁶. Perforant path-evoked e.p.s.cs were monitored before and after dynorphin release, and e.p.s.c. amplitudes were found to be significantly reduced (by $21 \pm 2\%$, $n = 15$) following hilar

Correspondence to: Charles Chavkin.

^{*}Present address: Department of Physiology, University of Maryland School of Medicine, Baltimore, MD 21201, USA,

Note added in proof: A report on a similar topic was submitted and published³¹ since submission of this letter. D

stimulation. The onset of e.p.s.c. inhibition was evident in the first minute after antidromic stimulation and was maximal by 1.7 ± 0.1 min post-stimulation (range, 1.0–2.3 min; $n = 15$). For the representative granule cell recording shown in Fig. 2a, e.p.s.c. amplitude was reduced by ~24% following a hilar stimulus train, and a second stimulus train to the hilus evoked a similar (27%) depression in e.p.s.c. response. The duration of the inhibition was more variable (Fig. 2).

The reduction in e.p.s.c. amplitude caused by hilar stimulation was blocked by $1 \mu\text{M}$ naloxone ($0 \pm 5\%$ change; $n = 5$) at 2 min after hilar high-frequency stimulation (HHFS). In the representative cell shown, hilar stimulation reduced perforant path e.p.s.c.s by 26%, whereas in the presence of naloxone, perforant path e.p.s.c. amplitude was reduced only 4% following hilar stimulation (Fig. 2b). This antagonism of the inhibitory effects of hilar stimulation indicates that, under these conditions, endogenously released opioids, like exogenously applied opioids, can regulate excitatory neurotransmission in the dentate gyrus.

The temporal characteristics of the inhibitory effects of endogenous opioids were further defined using population spike amplitude measurements because this extracellular response is more stable. Antidromic granule cell activation by high-frequency hilar stimulation also significantly inhibited the population response evoked by glutamate release from the perforant path (Fig. 3a). The onset was within 2 min and the duration was 4.1 ± 0.4 min (range, 2 to 8 min; $n = 17$) following hilar stimulation. The κ_1 antagonist norbinaltorphimine at 100 nM also significantly blocked the reduction in population response amplitudes caused by hilar stimulation (Fig. 3b).

To identify the opioid peptide responsible for the inhibition of granule cell excitability, the effects of polyclonal antisera raised against the prodynorphin-derived opioid peptides were tested (antisera were a gift from C. Evans). A mixture of antisera against dynorphin A(1–8), dynorphin B, and α -neoendorphin was found significantly to antagonize the hilar stimulation-evoked inhibitory effect when added to the buffer perfusing the hippocampal slices (Fig. 3c). Population spike amplitude was not directly affected in the absence of hilar stimulation by norbinaltorphimine, dynorphin antisera, or control rabbit antisera (Fig. 3). The ability of dynorphin selective antisera to block hilar stimulation-induced inhibition of synaptic transmission supports the conclusion that endogenous dynorphins are released to suppress the response evoked by perforant path stimulation.

The inhibitory effects of endogenous κ opioids on excitatory input to the granule cell indicated that opioid peptides might also modulate long-term sequelae from such afferent input. High-frequency perforant path stimulation consistently produced long-term potentiation; but if the perforant path stimulation train was immediately preceded by hilar high-frequency stimulation, LTP production was blocked (Fig. 3a). This blockade was attenuated by either norbinaltorphimine (100 nM; Fig. 3b) or dynorphin antisera (Fig. 3c), indicating that endogenous dynorphins released by hilar stimulation can act through κ_1 receptors to inhibit LTP at the perforant path-granule cell synapse. Data from a series of replicates are summarized in Table 1.

Our results demonstrate an inhibitory effect of endogenously released dynorphins on excitatory neurotransmission and synaptic plasticity in the dentate gyrus of the guinea-pig hippocampus. Functionally, the dynorphins released by high-frequency stimulation appear to act as retrograde transmitters, providing negative feedback to the presynaptic terminal. The sites of dynorphin release were not defined by these experiments, but are likely to be either from granule cell dendrites or recurrent collaterals 1–4. Following stimulated release, the effects of endogenous dynorphins were slow and prolonged, reaching a peak within 2 min and lasting for several

minutes. These temporal characteristics are similar to those of other neuropeptides acting in the peripheral nervous system^{12,13}.

The finding that dynorphin can block LTP production suggests that this peptide powerfully affects synaptic plasticity and, presumably, learning and memory, for which the phenomenon of LTP has been proposed to be a cellular substrate^{14–16}. Indeed, exogenous opioids inhibit learning and memory^{17,18}. Elevated hippocampal dynorphin levels correlate with impaired spatial learning in aged rats¹⁹, an impairment attenuated by naloxone²⁰. A naloxone-sensitive learning deficit can also be produced by mossy fibre stimulation²¹, similar to that reported here. Nonetheless, the role of opioid peptides in modulating learning and memory is likely to be complicated as μ and δ opioids (which suppress GABA, not glutamate, release) facilitate LTP induction in the dentate gyrus^{22,23}.

The regulation of dentate gyrus excitability by dynorphin may also be important in seizure disorders. κ agonists inhibit seizure activity in several animal models^{24,25}; mossy fibre stimulation can cause a naloxone-reversible elevation in seizure threshold²⁶; and seizures have been correlated in humans²⁷ and in animal models^{28,29} with increases in granule cell dynorphin expression and mossy fibre sprouting. Thus dynorphin, by providing feedback control of afferent input to the hippocampus, may normally act to modulate hippocampal activity during excitation involved in learning and during pathological excitation resulting in seizures or cell death.

Acknowledgements

We thank C. Evans for dynorphin antisera. The work was supported by the National Institute on Drug Abuse. G WT. was supported by a NRSA grant from NIGMS.

References

1. Gall C, Brecha N, Karten HJ, Chang K. J comp Neurol 1981;198:335–350. [PubMed: 6263955]
2. McGinty JF, Henriksen SJ, Goldstein A, Terenius L, Bloom FE. Proc natn Acad Sci USA 1983;80:589–593.
3. Chavkin C, Shoemaker WJ, McGinty JF, Bayon A, Bloom FE. J Neurosci 1985;5:808–816. [PubMed: 3838345]
4. McLean S, Rothman R, Jacobson A, Rice K, Herkenham M. J comp Neurol 1987;255:497–510. [PubMed: 2880880]
5. Chavkin C, Bakhit C, Weber E, Bloom FE. Proc natn Acad Sci USA 1983;80:7669–7673.
6. Wagner JJ, Evans C, Chavkin C. J Neurochem 1991;57:333–343. [PubMed: 1675664]
7. Wagner JJ, Caudle RM, Chavkin C. J Neurosci 1992;12:132–141. [PubMed: 1345943]
8. Lahti R, Mickelson M, McCall J, von Voightlander P. Eur J Pharmac 1985;109:281.
9. Zukin R, Eghbali M, Olive D, Unterwald E, Tempel A. Proc natn Acad Sci USA 1988;85:4061–4065.
10. Portoghesi PS, Lipkowski AW, Takemori AE. Life Sci 1987;40:1287–1292. [PubMed: 2882399]
11. Nock B, Giordano L, Cicero T, O'Connor L. J Pharmac exp Ther 1990;254:412–419.
12. Jan LY, Jan YN. J Physiol London 1982;327:219–246. [PubMed: 6181250]
13. Lundberg JM, Rudehill A, Sollevi A, Theodorsson-Norheim E, Hamberger B. Neurosci Lett 1986;63:96–100. [PubMed: 3005926]
14. Bliss TVP, Lomo T. J Physiol 1973;232:331. [PubMed: 4727084]
15. Lynch G, Baudry M. Science 1984;224:1057–1063. [PubMed: 6144182]
16. Madison DV, Malenka RC, Nicoll RA. Rev Neurosci 1991;14:379–397.
17. Martinez, JL.; Janak, PH.; Weinberger, SG.; Schulties, G.; Derrick, RE. NIDA Res Monogr. Erinoff, L., editor. 97. US Govt Printing Office; Washington DC: 1990. p. 48-78.
18. Decker MW, McGaugh JL. Synapse 1991;7:151–168. [PubMed: 1672782]
19. Jiang HK, Owyang V, Hang JS, Gallagher M. Proc natn Acad Sci USA 1989;86:2948–2951.

20. Gallagher M, Bostock E, King RA. Behav Neurosci Biol 1985;44:374–385.
21. Collier TJ, Routtenberg A. Brain Res 1984;310:384–387. [PubMed: 6488030]
22. Bramham C, Milgram N, Srebro B. Brain Res 1991;567:42–50. [PubMed: 1667745]
23. Xie CW, Lewis DV. J Pharmacol Exp Ther 1991;256:289–296.
24. Tortella FC. Trends Pharmacol Sci 1988;9:366–372.
25. Singh L, Vass CA, Hunter JC, Woodruff GN, Hughes J. Eur J Pharmacol 1990;191:477–480.
26. Jones LS. Brain Res 1991;564:336–340.
27. Houser CR, Miyashiro JE, Walsh GO, Rich JR, Delgado-Escueta AV. J Neurosci 1990;10:267–282. [PubMed: 1688934]
28. Sloviter RS. Neurosci Lett 1992;137:91–96.
29. Cronin J, Obenaus A, Houser CR, Dudek FE. Brain Res 1992;573:305–310. [PubMed: 1504768]
30. Evans C, Erdelyi E, Hunter J, Barchas J. J Neurosci 1985;5:3423–3427. [PubMed: 3908623]
31. Weisskopf MG, Zalutsky RA, Nicoll RA. Nature 1993;362:423–427. [PubMed: 8096624]

FIG. 1.

Granule cell responses to molecular layer and hilar stimulation and the effects of U69,593 on excitatory synaptic currents (e.p.s.cs). *a*, In a representative cell, e.p.s.cs produced by increasing stimulus intensities of perforant path (20, 25, 30 μ A) or hilar (100, 150 μ A) stimulation in the presence of bicuculline (10 μ M) were both largely blocked by 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX; 5 μ M). U69,593 affected neither the membrane conductance of granule cells at membrane potentials from -120 to -60 mV (data not shown), nor the holding current required to clamp the cell at -70 mV. *b*, In another representative granule cell in the presence of bicuculline, the κ opioid agonist U69,593 (500 nM) inhibited CNQX-sensitive e.p.s.cs from from perforant path. This inhibition was completely reversed by the κ antagonist NBNI (100 nM; $n = 4$). The slight reduction in e.p.s.c. amplitude in the presence of U69,593 seen after hilar stimulation was neither reversed by NBNI nor statistically significant in 4 replicates ($P > 0.05$). Individual traces shown in *a* and *b* are the averages of two sequential sweeps; the experiment was done 3 times with similar results. Sweep length is 110ms; vertical scales are current amplitudes (pA); calibration is 10ms (horizontal), 40 pA (vertical) for all traces.

METHODS. Guinea-pig hippocampal slices (500 μ m) were prepared as described⁷ and were perfused with Krebs bicarbonate buffer containing (mM): NaCl (125), KCl (3), CaCl_2 (2), MgCl_2 (1), NaH_2PO_4 (1.25), NaHCO_3 (26), and glucose (10), saturated with 95% O_2 , 5% CO_2 , pH 7.4. Patch pipettes (3–5 M Ω) contained (mM): CsCl (120), CaCl_2 (1), MgCl_2 (2), ATP (5), tetraethyl-ammonium-Cl (20), EGTA (10), HEPES (10), adjusted to pH 7.2 with CsOH. Whole-cell voltage clamp recordings were obtained using an Axopatch 200 amplifier and analysed using FastLab software. Uncompensated series resistance was checked throughout the recording period, and the data excluded from analysis if the drift exceeded 20%. To study isolated e.p.s.cs, bicuculline (10 μ M) was added to the bath to block GABA_A receptors in all studies reported here and CsCl was included in the intracellular recording pipettes to block GABA_B receptor-gated potassium currents. Spontaneous activity was virtually eliminated after GABA_A receptors were blocked by bicuculline (45 of 45 cells). For statistical analysis we used analysis of variance with or without repeated measures as appropriate. Tukey's tests were used for *post-hoc* comparisons; $P < 0.05$ was considered to be significant.

FIG. 2.

Hilar high-frequency stimulation (HHFS) induces inhibition of perforant path-evoked excitatory postsynaptic potentials (PP e.p.s.cs) recorded in the granule cell, *a*, HHFS causes a 24% reduction in the amplitude of the granule cell e.p.s.cs. Following recovery (>90% of control) from the initial HHFS, a second HHFS was again able to inhibit PP e.p.s.c. amplitude (compare sweeps 3 and 4). *b*, HHFS causes a 26% reduction in the PP e.p.s.c. amplitude (compare sweeps 1 and 2). Twelve minutes after addition of 1 μ M naloxone to the superfusion buffer, the PP e.p.s.c. amplitude returned to a pre-HHFS level and another HHFS reduced PP e.p.s.c. amplitude by only 4% (compare sweeps 3 and 4). Vertical scales for figure insets are current amplitudes (pA). Stimulus artefacts are truncated. Group data: HHFS significantly

decreased e.p.s.c. amplitude at two ($-15 \pm 2\%$), three ($-13 \pm 3\%$) and four ($-16 \pm 3\%$) min post-stimulus in normal Krebs bicarbonate buffer ($n = 15$); it had no effect at any time point when naloxone ($1 \mu\text{M}$) was added to the perfusate at least 10 min before HHFS ($n = 5$).

METHODS. The opioid-mediated effects of HHFS were monitored by measuring granule cell e.p.s.c. amplitudes evoked by a perforant path test pulse in the presence or absence of naloxone. PP e.p.s.cs were elicited at 0.1 Hz and 6 sweeps averaged into 1 min bins (bars are means $\pm$ s.e.m. of the 6 sweeps). For each cell tested, the mean of 3 or 4 min of pre-HHFS e.p.s.c. amplitudes was determined, and the per cent of that control value calculated. Hilar stimulation at high frequency (50 Hz, 1 s train of 0.3 ms, $150\text{-}\mu\text{A}$ pulses) was applied using a concentric bipolar electrode (Kopf, SNE 100). Calibration is 10 ms (horizontal). 25 pA (vertical) for all traces.

FIG. 3.

Both norbinaltorphimine and dynorphins antisera block the excitatory effects of hilar high-frequency stimulation on granule cell population spike amplitude and long-term potentiation produced by perforant path high-frequency stimulation on granule cell population spike amplitude and long-term potentiation produced by perforant path high-frequency stimulation (PPHFS). *A*, Representative experiment showing the effects of HHFS, HHFS followed immediately by PPHFS, and PPHFS on granule cell population spike responses. HHFS (6 1-s, 50 Hz trains of 0.3-ms 300 μ A pulses given at a rate of 1 every 10s) decreased spike amplitude by 40% and then 38%, showing nearly identical inhibitory effects of repeated HHFS. Whereas HHFS followed immediately by PPHFS resulted in a minimal change (+2%) in spike amplitude

30 min after stimulation, PPHFS by itself produced a 46% increase 30 min after stimulation (that is, LTP). Inset shows representative traces from a replicate experiment at different times during the protocol: *a*, control; *b*, 1 min after HHFS; *c*, 20 min after HHFS; *d*, 1 min after HHFS immediately followed by PPHFS; and *e*, 30 min after PPHFS-induced LTP. Responses were evoked at 55 μ A (the $S_{1/2}$, or half-maximal spike amplitude, for this preparation); calibration bars for the inset are 5 ms (horizontal), 1 mV (vertical). *B*, Effect of 100 nM NBNI on the HHFS-induced attenuation of the population spike and PPHFS-evoked LTP. HHFS produced a 29% decrease in spike amplitude initially, but only an 8% decrease after NBNI was added to the perfusate. Unlike the results shown in *A*, HHFS given immediately before PPHFS in the presence of NBNI did not block LTP (+31%). *C*, Effect of dynorphin antisera on HHFS-induced reduction of the population spike and LTP following PPHFS. With normal rabbit serum (1:125) in the perfusate, HHFS decreased spike amplitude 25% (similar in magnitude to *A* and *B*). After dynorphin antisera was added to the perfusate, HHFS reduced spike amplitude by only 7% and, unlike the results shown in *A*, a 22% potentiation was seen with HHFS immediately followed by PPHFS.

METHODS. Extracellular recordings were made under the conditions described in Fig. 1 legend, except that the concentrations of CaCl_2 and MgCl_2 in the extracellular buffer were each increased to 4 mM to inhibit hyperexcitability in the presence of the 10 μ M of bicuculline. Recording pipettes were filled with 3 M NaCl. The perforant path stimulation-induced dentate granule cell population response amplitude was measured from peak to peak using a digitizing oscilloscope. A stimulus intensity was chosen that evoked a half-maximal granule cell population spike amplitude ($S_{1/2}$). The LTP induction paradigm consisted of a total of 9 pulses (0.3 ms, 300 μ A) to the perforant path given as three trains (each with three stimuli) applied at 10-s intervals with 10 ms between stimuli. LTP was assessed at 25–30 min post-PPHFS. Pooled dynorphin antisera included rabbit polyclonal antisera raised against dynorphin A_{1-8} , dynorphin B and α -neoendorphin. The preparation and characterization of this antisera has been described^{6,30}. All three antisera were added to the perfusate at the following dilutions: 1:300, dynorphin A_{1-8} ; 1:250, dynorphin B; 1:1,000, α -neoendorphin (for a final litre of 1:120 serum in the medium).

TABLE 1

Effects of NBNI and dynorphin antisera on perforant path-evoked granule cell population response amplitude after high-frequency stimulation of the hilus and/or perforant path

	Per cent change from prestimulation spike amplitude		
	1 min post-HHFS	Perforant path LTP	HHFS/perforant path LTP
Control buffer	-31.9 ± 3.5 (29)	52.0 ± 6.7 (13)	-5.1 ± 3.3 (9)
100 nM NBNI	-11.4 ± 4.4 (9)*	40.6 ± 11.6 (6)	34.7 ± 5.3 (8)*
Anti-dynorphin	-6.5 ± 3.7 (6)*	ND	44.1 ± 11.8 (5)*

Data are expressed as per cent change from the mean population spike amplitude measured during the five minutes before high-frequency stimulation. In control buffer, high-frequency stimulation of the hilus reduced the amplitude of the population spike by 32% (1 min post-HHFS column). This effect was significantly attenuated by pretreatment with 100 nM norbinaltorphimine (NBNI) or dynorphin antisera (anti-dynorphin) 15 min before hilar high-frequency stimulation. Perforant path LTP was induced by high-frequency stimulation of the perforant path, resulting in a 52% increase in response to low-frequency perforant path stimulation 30 min post-induction (mean amplitude 25–30 min after high-frequency stimulation). NBNI had no effect under these conditions. Administering the hilar high-frequency stimulation immediately before the perforant path high-frequency stimulation (HHFS/perforant path LTP column) eliminated any potentiation measured 30 min after stimulation in control buffer. Pretreatment with either NBNI or dynorphin antisera resulted in significant LTP following perforant path high-frequency stimulation in spite of preceding hilar high-frequency stimulation. Control buffer data includes slices treated with normal rabbit sera (1:125) 15 min before high-frequency stimulation. Responses of this treatment group showed no significant differences from slices in normal buffer. Population spike amplitudes were chosen as the most robust measure of the changes observed; field e.p.s.p. slopes were also measured and showed the same κ -mediated inhibition of synaptic transmission and LTP. Data are means $\pm$ s.e.m. from the number of independent experiments given in parentheses. Asterisks indicate statistical difference from similarly stimulated control buffer group ($P < 0.05$). ND, not determined.