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Morphine and opioid peptides reduce inhibitory synaptic potentials in hippocampal pyramidal cells *in vitro* without alteration of membrane potential

(opiates/intracellular recording/disinhibition/inhibitory postsynaptic potential/excitatory postsynaptic potential)

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ABSTRACT **We used intracellular recording in the hippocampal slice *in vitro* to characterize further the mechanisms behind the unusual excitatory action of opiates and opioid peptides on hippocampal pyramidal cells *in vivo*. No significant effect on resting membrane potential, input resistance, or action potential size in cortical area 1 (CA1) pyramidal cells was observed with morphine sulfate, β-endorphin, [Met^5]enkephalin, or [D-Ala^2, D-Leu^5]enkephalin at 1–50 μM. However, in all cells studied, these agents markedly reduced the size of inhibitory postsynaptic potentials generated by stimulation of the stratum radiatum or alveus. Excitatory postsynaptic potentials were also diminished in many of these cells. The effects of the opioids were antagonized by naloxone. These results are consistent with excitation of pyramidal neurons by a disinhibitory mechanism.**

The discovery of opioid peptides has provoked considerable interest in their possible function. The most common action of exogenous opiates and opioid peptides is naloxone-sensitive inhibition of neuronal activity (1, 2). However, several studies have shown naloxone-sensitive excitation with iontophoresis of opioids onto hippocampal pyramidal cells (3–5). The hippocampus also exhibits epileptiform activity with opioid peptide administration (6, 7). On the basis of extracellular studies on the *in vivo* hippocampus (4, 8), it was suggested that the pyramidal cell may be activated by the opiates indirectly through a disinhibitory mechanism—by depression of the firing of inhibitory interneurons. Results of more recent extracellular studies (9–14) of the hippocampus *in vivo* and *in vitro* have generally corroborated this view.

However, recent reports of intracellular studies on hippocampal pyramidal neurons *in vitro* reveal several inconsistencies. Thus, Deadwyler and Robinson (15) have found direct effects of high concentrations (1 mM) of morphine on membrane potentials of neurons in the hippocampal slice. Still, by virtue of the ability of pentabarbital to antagonize these potential changes, these authors suggest that a disinhibitory mechanism is likely to account for this action (14). Recent studies on the same preparation from three other laboratories (16–18) have revealed no effect of lower doses of the opiate peptides on resting membrane properties. However, whereas Haas and Ryall (16) and Dingledine (17) found that opioids enhance excitatory postsynaptic potentials (EPSPs) with no change in inhibitory postsynaptic potential (IPSP) size, Nicoll *et al.* (18) showed a dramatic reduction of both recurrent and feedforward IPSPs. In contrast, Gähwiler (19) reported that perfusion of opioids onto hippocampal pyramidal cells in culture has no effect on resting membrane properties but enlarges spontaneous EPSPs and reduces evoked IPSPs, leading to paroxysmal depolarization shifts.

The reason for these discrepancies is unknown, although they may arise from the complicated feedback circuitry of the hippocampus and the close interrelationship between excitatory and inhibitory mechanisms in the pyramidal cell. Thus, it is difficult to explain how opiates might elevate EPSP size in the pyramidal cell without concomitant increases in IPSPs. To help clarify these discrepancies, we pursued ongoing studies of the action of opioids on both EPSPs and IPSPs in the same pyramidal cells of the hippocampal slice preparation (20, 21).

METHODS

Male albino rats of 130- to 200-g body weight were decapitated, and hippocampal formations were rapidly removed. Transverse hippocampal slices of 360- to 400-μm thickness were cut on a Sorval brain slicer and immediately placed in cold (6–10°C; gassed with 95% O_2/5% CO_2) 124 mM NaCl/3.3 mM KCl/1.25 mM KH_2PO_4/2.4 mM $MgSO_4 \cdot 7H_2O$/2.5 mM $CaCl_2$/25.7 mM $NaHCO_3$/10.0 mM glucose. This standard solution henceforth will be referred to as "CSF." The slices were then transferred and incubated in a chamber for 1 hr in CSF that was continuously perfused at 0.5–2 ml/min and gradually warmed from 31°C to 35°C. Field potentials in response to stimulation of the stratum radiatum or the alveus were recorded in cortical area 1 (CA1) with an extracellular pipette (1 MΩ, 3 M NaCl) (Fig. 1*A*). Slices with population spikes less than 3 mV (Fig. 1*B*) were discarded.

Slices selected for intracellular recording were then perfused at about 1–2 ml/min, with complete immersion and superfusion of the slice to facilitate uniform drug penetration and to prevent artifacts likely to occur with methods that leave the top surface exposed. The chamber volume was about 1 ml; together with the "dead space" of the tubing, this provides a fluid replacement time of about 2–3 min. Intracellular electrodes were pulled from fiber-filled tubing and filled with 3 M KCl or 1 M KCl/1.6 M potassium citrate, 1:10 (vol/vol). Electrode resistances with KCl were 60–80 MΩ. CA1 neurons were penetrated and identified as pyramidal cells on the basis of their visible location in the pyramidal layer and their antidromic and orthodromic synaptic responses to stratum radiation and alveus stimulation (Fig. 1 *A* and *D*). Intracellular voltage recording and current injection (0.1–1 nA; 200- to 300-msec pulses) were performed by standard techniques. Recordings were stored on polygraph paper and on magnetic tape (38 cm/sec, bandpass to 5 kHz), and relevant periods were filmed from a storage oscilloscope. Mea-

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Abbreviations: IPSP, inhibitory postsynaptic potential; EPSP, excitatory postsynaptic potential; CA1, cortical area 1.

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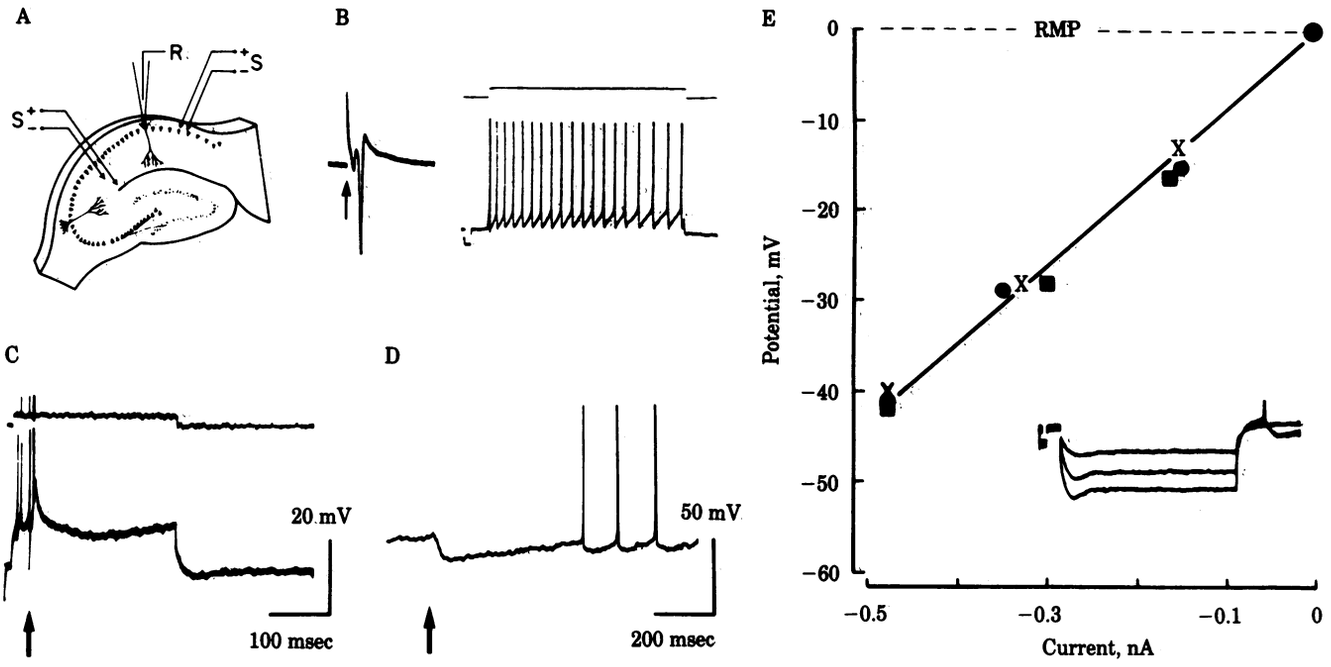

FIG. 1. Recording configuration and potentials obtained from the *in vitro* hippocampal slice preparation. (*A*) Schematic of the stimulation (S) and recording (R) arrangement. Left-most stimulating electrode is positioned on the stratum radiatum and right-most on the alveus. Pyramidal neurons were recorded in CA1 cells. (*B Left*) Extracellular recording of population spike recorded in pyramidal cell layer after stimulation of stratum radiatum (arrow and artifact). Spike is 5.2 mV peak to peak; recording is 22 msec in duration. (*B Right*) Intracellular recording. Uninterrupted trains of action potentials generated by intracellular injection of depolarizing current pulse (0.15 nA, top trace). Calibration pulse at the beginning of the record is 10 mV for 10 msec. (*C*) Another cell: stimulation of stratum radiatum at a strength supramaximal for spike generation (arrow), superimposed on a train of spikes evoked by intracellular injection of depolarizing current (0.15 nA). Note hyperpolarization and abolition of spiking after stratum radiatum stimulation. (*D*) Another cell: elicitation of a large IPSP by stratum radiatum stimulation (arrow) without spike generation. The apparent lack of an EPSP may be due to the shunting effect of the IPSP, perhaps indicating a feedforward inhibitory mechanism (22). (*E*) Current-voltage curve generated by intracellular injection of constant-current, square-wave pulses of incrementally increasing intensity (roughly 0.15, 0.33, and 0.48 nA), through a bridge circuit and the recording electrode. ●, CSF control; X, during 10 μM β-endorphin perfusion; ■, 30-min CSF washout; RMP, resting membrane potential. (*Inset*) Superimposed voltage pulses generated by such current before β-endorphin perfusion. Note the time-dependent rectification of the hyperpolarizing pulses always seen in hippocampal pyramidal neurons; neither the fast-changing nor the flat portions of the pulse were altered by the peptide. Current–voltage plot was measured from the flat portion; thus, input resistance is not changed. Calibration pulse at the beginning of the *Inset* record was 10 mV for 10 msec.

surements of current–voltage relationships, EPSPs, and IPSPs were taken from filmed records.

Drugs perfused were morphine sulfate, β-endorphin, [D-Ala^2,D-Leu^5]enkephalin, [Met^5]enkephalin and naloxone·HCl. Most of the cells were tested two or more times with the same agonist.

RESULTS

The CA1 pyramidal cells accepted for inclusion in this study satisfied several criteria for lack of injury by electrode penetration (23), including steady membrane potentials above 50 mV, input resistance above 30 MΩ, large action potentials (70–95 mV), and the capability of generating continuous trains of action potentials of undiminished size in response to weak depolarizing stimulation (Fig. 1*B*; see ref. 23). Thus, 24 cells were recorded for durations of 1–4 hr. In addition, several other less stable cells were recorded that showed essentially the same responses.

Most of the neurons utilized in this study (about 60%) did not display action potentials unless stimulated by intracellular current injection or by activation of the alveus or stratum radiatum. In all such quiescent cells, there was no change in membrane potential in response to perfusion of the slice with morphine sulfate (5–50 μM; eight cells), with [Met^5]enkephalin or [D-Ala^2, D-Leu^5]enkephalin (1–20 μM; eight cells), or with β-endorphin (1–10 μM; five cells). Likewise, there were no significant changes in input resistance in these 21 cells as assessed by generation of current–voltage curves (Figs. 1*E* and 2*A*). In three other cells, a slight (1–3 mV) change in membrane potential (one hyperpolarized, two depolarized) was seen on occasion with perfusion of the opioids. However, these changes were always in the direction expected for, and probably caused by, changes in the spontaneous firing rate of these neurons: (*i*) a reduction in firing (associated with hyperpolarization) was evoked by [Met^5]enkephalin (one cell) and by β-endorphin (one cell) and (*ii*) increased firing (with depolarization) was seen with morphine sulfate (two cells) and β-endorphin (two cells). Neither the changes in firing nor in membrane potential were associated with any detectable change in input resistance. Three cells showed abrupt, prolonged depolarization shifts (10–15 mV) lasting up to 8 sec with β-endorphin, suggestive of epileptiform activity (24).

As shown elsewhere (25), stimulation of the stratum radiatum with low intensities usually evoked an EPSP that was capable of triggering a single spike at greater stimulus intensities. The spike or EPSP was usually followed by a hyperpolarization (2–8 mV) identified as an IPSP because of the following properties: (*i*) ability to reverse the potentials with cell hyperpolarization and increase them with depolarization; (*ii*) long inhibition by concurrent alveus or stratum radiatum stimulation of spike trains produced by weak depolarizing currents (see ref. 23; compare Fig. 1 *B* and *C*); (*iii*) increase in membrane conductance during the hyperpolarization; and (*iv*) occasional elicitation of such hyperpolarizations without preceeding EPSPs or spikes

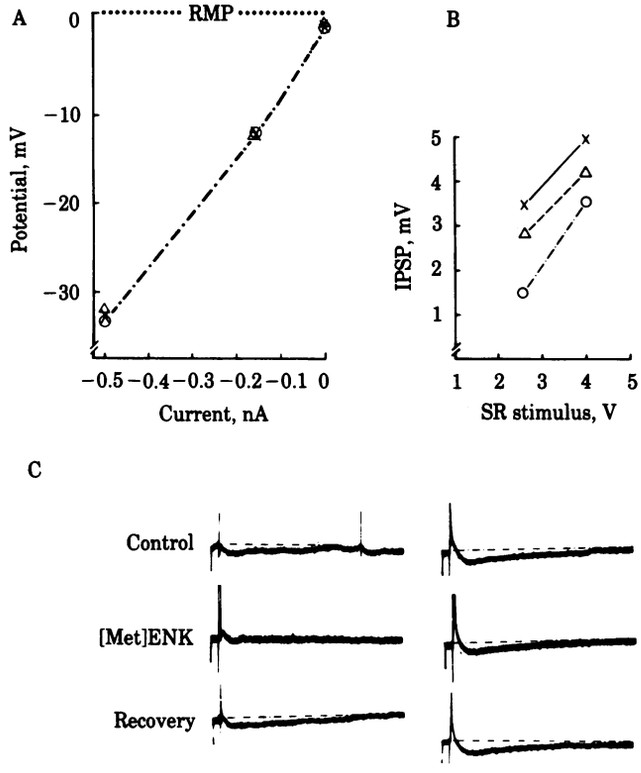

FIG. 2. Effects of [Met^5]enkephalin ([met]ENK) on a CA1 pyramidal neuron *in vitro*. (*A*) Superimposition of points (measured at the flat portion of the electrotonic potential) of the current–voltage relationship show a lack of effect of 10 μM enkephalin perfusion (○) compared to the CSF control (X) and 15-min washout with CSF (△) on input resistance. Resting potential (RMP) remained constant throughout the tests. (*B*) Same cell: stimulus–response (input–output) relationship of IPSP amplitude with two strengths of low-intensity stratum radiatum (SR) stimulation. (*C*) The actual oscillographic records of the IPSPs at 2.6 V SR stimulation (*Left*) and at 4 V SR stimulation (*Right*). Note that the enkephalin reduces the IPSP by 25–60%, with recovery at the 15-min washout. Tops of spikes were cut off for display. First negative pulse at beginning of each trace was 10 mV; total trace duration was 1000 ms.

(Fig. 1*D*). These IPSPs were 3–7 mV in amplitude and appear identical in form to those described elsewhere (18, 23, 25).

Perfusion of the slices with β-endorphin (1–10 μM) reduced the peak size of the IPSPs (Fig. 3 *A* and *B*) by 35 ± 21% (mean ± SD), in all seven cells studied, with slow recovery to the original size on washout with artificial CSF. Likewise, [Met^5]enkephalin or [D-Ala^2, D-Leu^5]enkephalin (2–10 μM) always reduced the IPSPs [by 41% ± 16% (mean ± SD), six cells; Fig. 2 *B* and *C*] as did morphine sulfate [5–50 μM; by 47 ± 22% (mean ± SD), 10 cells]. The extent of opioid antagonism of IPSPs ranged from 17% to 100%. Diminution of the IPSPs occurred without alteration of spike sizes or other membrane properties, even with perfusion of 40 μM morphine. Moreover, the opioid-induced reductions in the IPSP were completely blocked in all of the eight neurons so tested (Fig. 3*A*) by concomitant perfusion of naloxone at doses (10–20 μM) that had little or no effect on IPSPs or membrane properties.

In many of the same neurons the EPSPs also seemed to be sensitive to the opiates and peptides, although reproducibly stable EPSPs without spikes were difficult to obtain. Whether recorded at resting potential or superimposed on hyperpolarizing pulses to prevent spike generation (Fig. 3*C*), the EPSPs usually were reduced in size: by 55% and 78% with β-endorphin in two of four testable cells, by 25% and 100% with [Met^5]enkephalin in two of four cells, and by 8%, 33%, and 15% with morphine sulfate in three of four cells. EPSP size was increased (by 33%) in only one cell with morphine sulfate and in two cells by β-endorphin (by 4% and 78%). EPSPs of one cell were not affected by [Met^5]enkephalin. The inhibitory effect of the opioids on EPSP size was reduced or abolished by concomitant perfusion of naloxone (20 μM) in two cells; in three others, the actions of the opioids were not affected.

DISCUSSION

In this study we attempted to determine (*i*) whether the opiates and opioid peptides act directly on the pyramidal cell postsynaptic membrane or only on synaptic potentials and (*ii*) how the opioids activate pyramidal cells and elicit epileptiform activity *in vivo*. With regard to the first question, we have found no evidence for a direct action of opioids on the resting membrane properties of the pyramidal cell because perfusion of high concentrations of opiate agonists did not significantly alter either membrane potentials or membrane resistance.

In contrast, the opioids do appear to alter synaptic potentials. The most striking of these effects in our study is the clear reduction in the size of the IPSPs evoked by stratum radiatum stimulation. Such a reduction of inhibition *in vivo* would favor excitatory mechanisms, perhaps even culminating in epileptiform activity (6, 7, 15, 19). The involvement of such a disinhibition (4, 8) is generally supported by unit and field recordings of pyramidal cells and interneurons in the hippocampus slice (9, 11–14). In this regard, our data are most in accord with the recent intracellular results of Nicoll *et al.* (18) and Gähwiler (19) who observed opioid-induced diminutions in IPSP size in hippocampal pyramidal cells. However, Gähwiler (19) also observed a concomitant increase in EPSP size in hippocampal cultures; Nicoll *et al.* (18) did not report effects of opioids on EPSPs in the slice preparation. Our results are in strongest contrast to those of Haas and Ryall (16) and Dingledine (17) who report opioid-induced augmentation of EPSPs without effect on IPSPs. Although the reason for these discrepancies is not known, we speculate that differences in methods of measuring EPSPs (e.g., with vs. without spike generation) or in methodologies of drug perfusion (e.g., with the upper surface of the slice submerged or exposed) could be responsible.

The reduction of EPSPs often seen in the present study with perfusion of the opioids parallels the findings of Zieglgänsberger and coworkers (26, 27) and may suggest a mechanism by which the IPSPs are reduced. It is likely that the pyramidal cells activate inhibitory interneurons by releasing the same excitatory transmitter as that with which they activate each other (25). There is good evidence that this transmitter is glutamate (see 28). The opioids may antagonize a glutamate-induced postsynaptic activation of Na-channels in the inhibitory interneurons, as suggested for spinal neurons (26–29), although a presynaptic blockade of transmitter release (30–33) or hyperpolarization of the inhibitory interneuron cannot be ruled out by the present studies. A similar action at synapses between one pyramidal cell and another would account for our frequent observation of opioid-induced reduction in EPSPs. Although it is possible that some proportion of the presumed EPSPs were contaminated by depolarizing feedforward IPSPs (22) sensitive to the opioid agonists (18), our preliminary studies show that [DAla^2, D-Leu^5]enkephalin also frequently antagonizes the depolarizing actions of glutamate on the hippocampal pyramidal cell.

The occasional changes in firing rate produced by the opioids (and small membrane potential changes sometimes associated with these) may be a reflection of the changes in IPSPs and EPSPs. Thus, the direction of change in firing rate for a given cell may be dependent on the synaptic drive to that cell and the

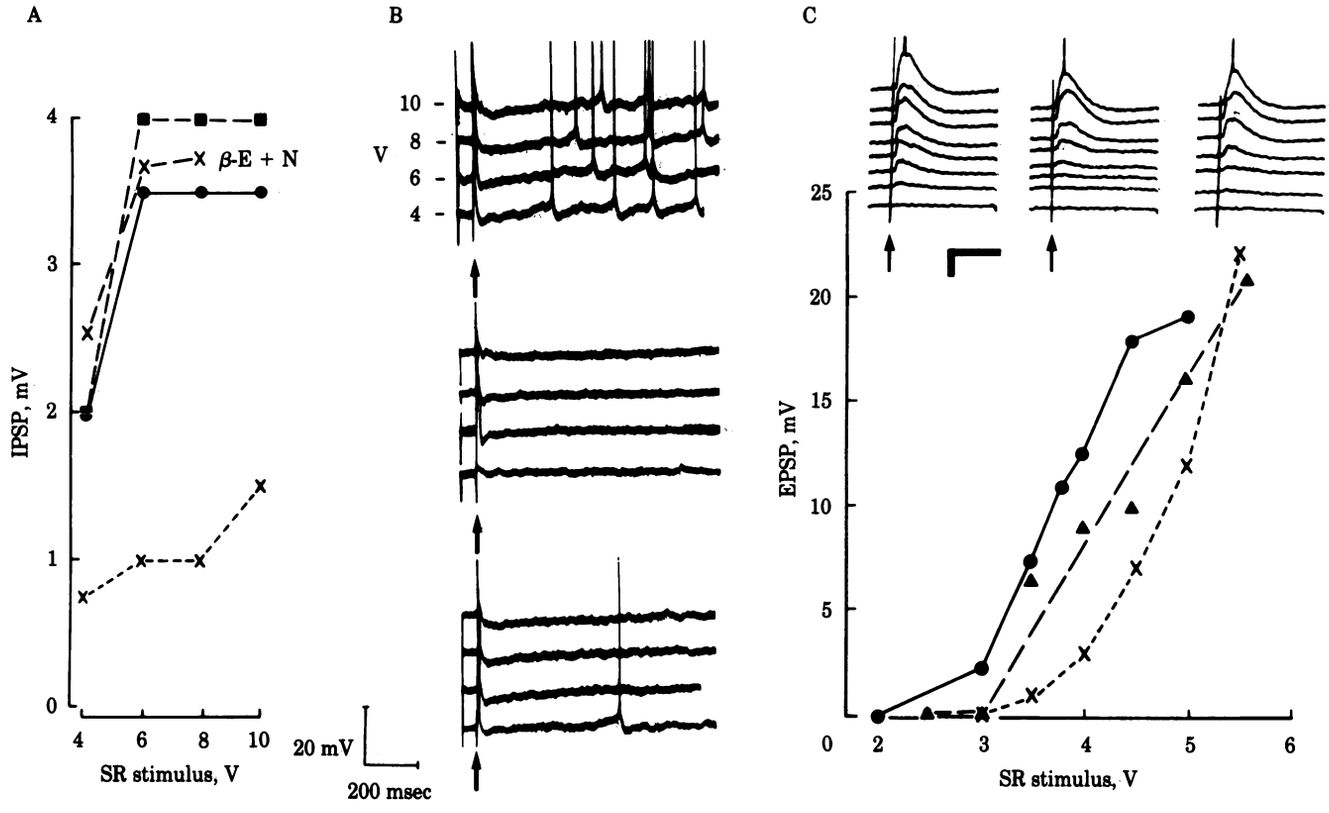

FIG. 3. Effects of 10 μM β-endorphin on synaptic potentials of a CA1 neuron. (*A*) Stimulus–response curve of peak IPSP amplitudes after stimulation of stratum radiatum (SR) by four different intensities. ●, CSF control; x, β-endorphin (10 min); ■, 20-min washout; larger x, 10 μM β-endorphin plus 20 μM naloxone (β-E + N). (*B*) Same test: oscillographic data of IPSPs before drug perfusion (CSF control; *Top*), during β-endorphin perfusion (*Middle*), and at 20-min washout (CSF recovery; *Bottom*). Note dramatic reduction in the IPSPs and abolition of spiking in this cell by β-endorphin. Whereas IPSPs show recovery, spiking does not. The effect on IPSPs is blocked by 20 μM naloxone (shown in *A* only). Neither membrane potential nor resistance were altered by β-endorphin. (*C*) Stimulus–response curve for EPSPs from the same cell. In this cell all EPSPs were superimposed on a constant 0.35-nA hyperpolarizing current pulse to prevent contamination by spikes. ●, Control; x, β-endorphin; ▲, recovery (≈30 min). (*Insets*) Oscillograph records of EPSPs, with increasing stimulus intensity from bottom to top, before (*Left*), during (*Middle*), and 30 min after (*Right*) 10 μM β-endorphin perfusion. The SR-stimulating voltages used to generate each EPSP is shown in the stimulus–response plot. Largest stimulus intensity generates a spike (not plotted). Note reduction in both the peak and rise-time of the EPSPs by the β-endorphin. Calibration bars, 20 mV, 50 msec; arrows, SR stimulus. Tops of spikes in *B* and *C* were cut off for display.

balance between inhibitory and excitatory influences: a relatively greater or more widespread reduction in IPSPs than in EPSPs would favor increases in firing. The strong reduction in EPSPs sometimes seen in our study could account for the observation of occasional inhibitory actions of iontophoretically applied opioids in hippocampus *in vivo* (e.g., ref. 5).

During β-endorphin perfusion, several cells exhibited rapidly reversible, paroxysmal depolarization shifts suggestive of epileptiform activity (19, 23). These shifts may represent an *in vitro* intracellular correlate of the interictal episodes produced by β-endorphin *in vivo* (6, 7) and *in oculo* (34). Evidence is accumulating (35) that these epileptiform episodes, like the unit excitations evoked by iontophoretically applied opioids (3–5), are also produced by a disinhibition of pyramidal cells. These electrographic phenomena may represent only pathological or pharmacological curiosities, or they may signify some physiological role of the opioid peptides in hippocampal function. Recent immunohistochemical studies of hippocampus have demonstrated enkephalin-like substances in structures resembling nerve fibers, especially in regio inferior of hippocampus, but also to a lesser degree in CA1 (36–38). However, ultrastructural elucidation of the target cell(s) for these enkephalin-containing fibers will be required to support a disinhibitory function for the endogenous peptides in hippocampus.

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1. Zieglgänsberger, W. & Fry, J. P. (1978) in *Development in Opiate Research*, ed. Herz, A. (Dekker, New York), pp. 193–239.
2. North, R. A. (1979) *Life Sci.* **24**, 1527–1546.
3. Nicoll, R. A., Siggins, G. R., Ling, N., Bloom, F. E. & Guillemin, R. (1977) *Proc. Natl. Acad. Sci. USA* **74**, 2584–2588.
4. Zieglgänsberger, W., French, E. D., Siggins, G. R. & Bloom, F. E. (1979) *Science* **205**, 415–417.
5. Hill, R. G., Mitchell, J. F. & Pepper, C. M. (1977) *J. Physiol.* **272**, 50–51P (abstr.).
6. Frenk, H., Urca, G. & Liebeskind, J. C. (1978) *Brain Res.* **147**, 327–337.
7. Henriksen, S. J., Bloom, F. E., McCoy, F., Ling, N. & Guillemin, R. (1978) *Proc. Natl. Acad. Sci. USA* **75**, 5221–5224.
8. Siggins, G. R., Zieglgänsberger, W., French, E., Ling, N. & Bloom, F. (1978) *Neurosci. Abstr.* **4**, 414 (abstr.).
9. Dunwiddie, T., Mueller, A., Palmer, M., Stewart, J. & Hoffer, B. (1980) *Brain Res.* **184**, 311–330.
10. Stanley, J. C., DeFrance, J. F., Taber, K. & Dafny, N. (1979) *Neurosci. Abstr.* **5**, 282 (abstr.).

11. Jensen, R. A., Martinez, J. L., Craeger, R., Veliguette, J., McGaugh, J. L. & Lynch, G. (1979) *Neurosci. Abstr.* **5,** 530 (abstr.).
12. Lee, H. K., Dunwiddle, T. & Hoffer, B. (1980) *Brain Res.* **184,** 331–342.
13. Corrigal, W. A. & Linseman, M. A. (1980) *Brain Res.* **192,** 227–238.
14. Robinson, J. H. & Deadwyler, S. A. (1980) *Fed. Proc. Fed. Am. Soc. Exp. Biol.* **39,** 281.
15. Deadwyler, S. A. & Robinson, J. H. (1979) *Brain Res. Bull.* **4,** 609–613.
16. Haas, H. L. & Ryall, R. W. (1980) *J. Physiol.* **308,** 315–330.
17. Dingledine, R. (1980) *Neurosci. Abstr.* **6,** 612 (abstr.).
18. Nicoll, R. A., Alger, B. E. & Jahr, C. E. (1980) *Nature (London)* **287,** 22–25.
19. Gähwiler, B. H. (1980) *Brain Res.* **194,** 193–203.
20. Skrede, K. K. & Westgaard, R. H. (1971) *Brain Res.* **35,** 589–593.
21. Yamamoto, C. (1972) *Exp. Brain Res.* **14,** 423–435.
22. Alger, B. E. & Nicoll, R. A. (1979) *Nature (London)* **281,** 315–317.
23. Schwartzkroin, P. A. (1977) *Brain Res.* **128,** 53–68.
24. Schwartzkroin, P. A. & Prince, D. A. (1978) *Brain Res.* **147,** 117–130.
25. Anderson, P., Eccles, J. C. & Loyning, Y. (1964) *J. Neurophysiol.* **27,** 608–619.
26. Zieglgänsberger, W. & Bayerl, H. (1976) *Brain Res.* **115,** 111–128.
27. Zieglgänsberger, W. & Tulloch, I. F. (1979) *Brain Res.* **167,** 53–64.
28. Zieglgänsberger, W. & Fry, J. (1976) in *Opiates and Endogenous Opioid Peptides,* ed. Kosterlitz, H. W. (Elsevier/North Holland, Amsterdam), pp. 213–238.
29. Barker, J. L., Neale, J. H., Smith, T. G., Jr. & McDonald, R. C. (1978) *Science* **199,** 1451–1453.
30. Jessell, T. M. & Iversen, L. L. (1977) *Nature (London)* **268,** 549–551.
31. Mudge, A. W., Leeman, S. E. & Fischbach, G. D. (1979) *Proc. Natl. Acad. Sci. USA* **76,** 526–530.
32. Bornstein, J. C. & Fields, H. L. (1979) *Neurosci. Lett.* **15,** 77–82.
33. Konishi, S., Tsunoo, A. & Otsuka, M. (1979) *Nature (London)* **282,** 515–516.
34. Taylor, D., Hoffer, B., Zieglgänsberger, W., Siggins, G., Ling, N., Seiger, A. & Olson, L. (1979) *Brain Res.* **176,** 135–142.
35. French, E. D. & Siggins, G. R. (1980) *Regul. Pept.* **1,** 127–146.
36. Sar, M., Stumpf, W. E., Miller, R. J., Chang, K.-J. & Cuatracasus, P. (1978) *J. Comp. Neurol.* **182,** 17–38.
37. Bloom, F., Bayon, A., Battenberg, E., French, E., Koda, L., Koob, G., Le Moal, M., Rossier, J. & Shoemaker, W. (1980) in *Neural Peptides and Neuronal Communication,* eds. Costa, E. & Trabucchi, M. (Raven, New York), pp. 619–632.
38. Gall, C., Brecha, N. & Karten, H. J. (1980) *Neurosci. Abstr.* **6,** 354 (abstr.).