6. Preliminary data on polarization of the evoked DRG-SC EPSP with the reversal potential estimated by extrapolation indicate that 0 mV is a reasonable approximation, although we have not demonstrated reversal of this EPSP, and support the assumption that this is likely to be a chemical synapse rather than an electrotonic synapse.
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Opiate Peptide Modulation of Amino Acid Responses Suggests Novel Form of Neuronal Communication

Abstract. *Mouse spinal neurons grown in tissue culture were used to study the electrophysiological pharmacology of the opiate peptide leucine-enkephalin. Enkephalin depressed glutamate-evoked responses in a noncompetitive manner independent of any other effects on membrane properties. The results demonstrate a neuromodulatory action of opiate peptide functionally distinct from the conventional neurotransmitter class of operation.*

Opiate peptides are distributed in neuronal pathways throughout the central and peripheral vertebrate nervous systems (*1*), and behavioral (*2*) and biochemical (*3*) pharmacology experiments have demonstrated various actions suggesting important roles for opiate peptides in neuronal function. Also, extracellular recordings obtained in vivo in the central nervous system (CNS) have shown that enkephalin depresses both spontaneous and glutamate-evoked activity (*4*). The membrane mechanisms underlying these actions have yet to be elucidated. We have used tissue-cultured mouse spinal neurons to examine the effects of the opiate peptide leucine-enkephalin ([Leu^5]enkephalin) and report here that the peptide has an action distinct from conventional neurotransmitters. The peptide effects may appropriately be characterized as "neuromodulatory" (*5*).

Spinal cord neurons derived from 13-day-old mouse embryos were grown as a monolayer in tissue culture as described (*6*). Intracellular recordings of membrane properties were made on the modified stage of an inverted phase microscope by conventional methods with one microelectrode and voltage clamp techniques with two microelectrodes. Glutamate (0.5*M*, *p*H 8; Sigma), [Leu^5]enkephalin, (0.01*M*, *p*H 3.5; Pierce) and naloxone (0.05*M*, *p*H 3.5) (*7*) were introduced by iontophoresis with extracellular micropipettes; 10 m*M* $MgCl_2$ was added to the extracellular medium to suppress ongoing synaptic activity and to allow clearer examination of postsynaptic pharmacology.

[Leu^5]enkephalin depressed responses to iontophoresis of the putative transmitter glutamate on 28 of 32 neurons tested, independent of any other effects on neuronal membrane properties (Figs. 1A and 2B). The depressant effects were rapid in onset, sustained for the duration of the peptide iontophoresis, and readily reversible. Peptide depression of the glutamate response was current-dependent and ranged from 20 to 80 percent of the control amplitude, with maximum depression averaging 50 percent (Fig. 1, C1). The depression of glutamate response amplitude was associated with a current-dependent change in the kinetics of the response, both time-to-peak and decay time being measurably prolonged (Fig. 1, A2 and C2; Fig. 2, B and D). Partial reversal of enkephalin-induced depression of glutamate responses by coincident iontophoresis of naloxone was observed in six of ten cells tested (Fig. 1B). The reversal was never complete, responses being restored on the average to about 80 percent of control.

A pharmacological analysis of the interaction between enkephalin and glutamate was carried out with the use of glutamate current-response curves (N = 7). On every occasion, glutamate responses evoked by higher iontophoretic currents were depressed relatively more than those evoked by lower currents (Fig. 2A). Analysis of the log-log plots of the current-response data, which give a minimum estimate of the number of molecules involved in the unitary conductance (*8*), suggests that the peptide does not alter the number of glutamate molecules involved in the unitary conductance (Fig. 2C). If we assume that one glutamate molecule participates in the response, analysis of the double-reciprocal plots derived from the current-response data indicates that the peptide has apparently not altered the affinity of the receptors for glutamate. From these results we conclude that enkephalin depresses glutamate responses through a noncompetitive mechanism. The results do not allow us to specify further the site of interaction on the receptor-coupled conductance mechanism. The molecular mechanisms involved in the enkephalin-glutamate interaction at the single channel level remain to be investigated.

Our results suggest that the effects of this peptide are functionally distinct from conventional neurotransmitter action. Neurotransmitters are released from synaptic terminals in close apposition to postsynaptic membranes. Neurotransmitter action involves activation or inactivation of specific receptors which are coupled to one or more ionic conductances (*9*). The time course of such action is brief milliseconds to seconds and subject to "desensitization"; and the functional effect is to alter cellular excitability indirectly. Neurotransmitter-induced excitation results either when the driving force of the ionic conductance activated is depolarized relative to threshold for firing or when the drive force of the ionic conductance inactivated is hyperpolarized relative to threshold. Conversely, neurotransmitter-induced inhibition results either when the driving force of the ionic conductance activated is hyperpolarized relative to threshold or when the drive force of the ionic conductance inactivated is depolarized relative to threshold.

Although the physiology of enkephalinergic neurons is unknown, as is the nature of the anatomical contacts made by such neurons, it is clear from the pharmacology presented here that enkephalin does not directly dominate membrane excitability by altering a specific ionic conductance like a neurotransmitter but rather modulates the subsynaptic actions of a neurotransmitter-coupled event. The modulation effect does not appear to fade or desensitize during sustained application. This would provide a subtle yet effective modulation of glutaminergic pathways without alteration in other transmissions in which the same cellular elements are utilized. Whether enkephalin modulates other neurotransmitter-coupled conductances remains to be determined. From these observations we may tentatively define neuromodulation as the alteration of synaptic receptor-coupled conductances without direct activation of such conductances.

Neuromodulation by peptides appears to differ functionally from the neurohormonal communication by peptides described in several invertebrate systems (*10*). The latter involves peptide-mediated communication between a specific cluster of neurons remote from, and not in synaptic contact with, target cells (*11*). The actions thus far described are mul-

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tiple and long-lasting and include alteration in both voltage-independent and voltage-dependent conductances (*10*). Such a mode of intercellular communication allows one substance to regulate the excitability of diverse and distant neurons.

Neurohormonal communication might be likened to information broadcast in a public manner by radio where only those receivers with antennas properly tuned to the correct wavelength receive information. Neurohormones, although widely dispersed in the extracellular fluid, only affect the excitability of those target neurons with receptors properly "tuned" to the peptide structure. Neurotransmission is analogous to a telephone conversation in that it is essentially private and requires hardwiring. Neurotransmitters, released at intimate synaptic junctions between fixed, contiguous elements, convey excitability changes from axon to postsynaptic membrane. (To continue the analogy, neuromodulation would appear to be a form of gain control imposed on private conversations.) Neuromodulators appear to function at synapses where they alter

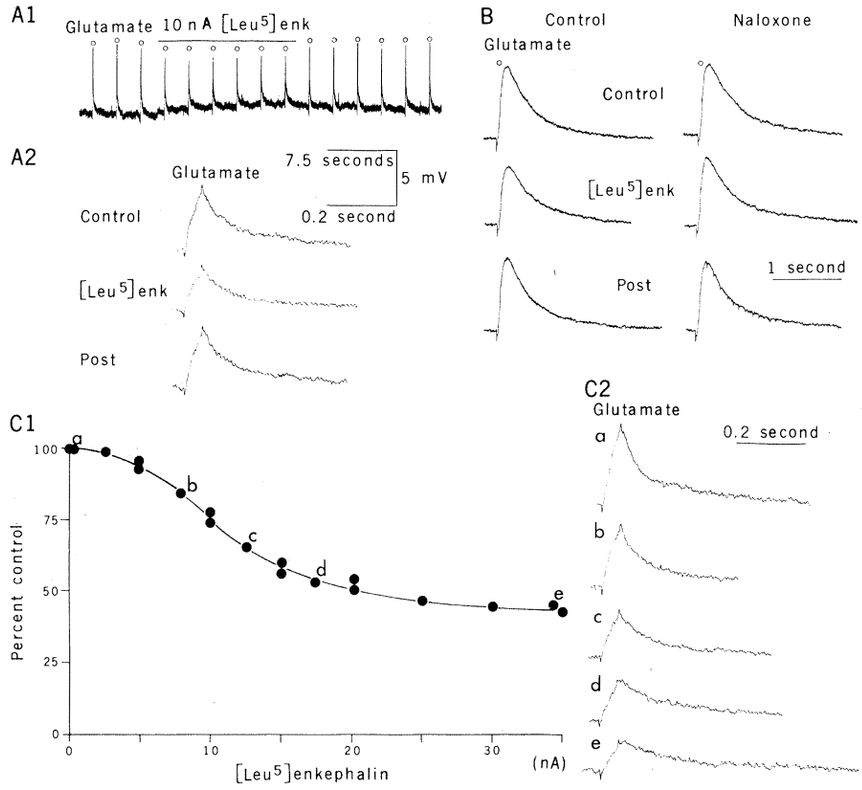
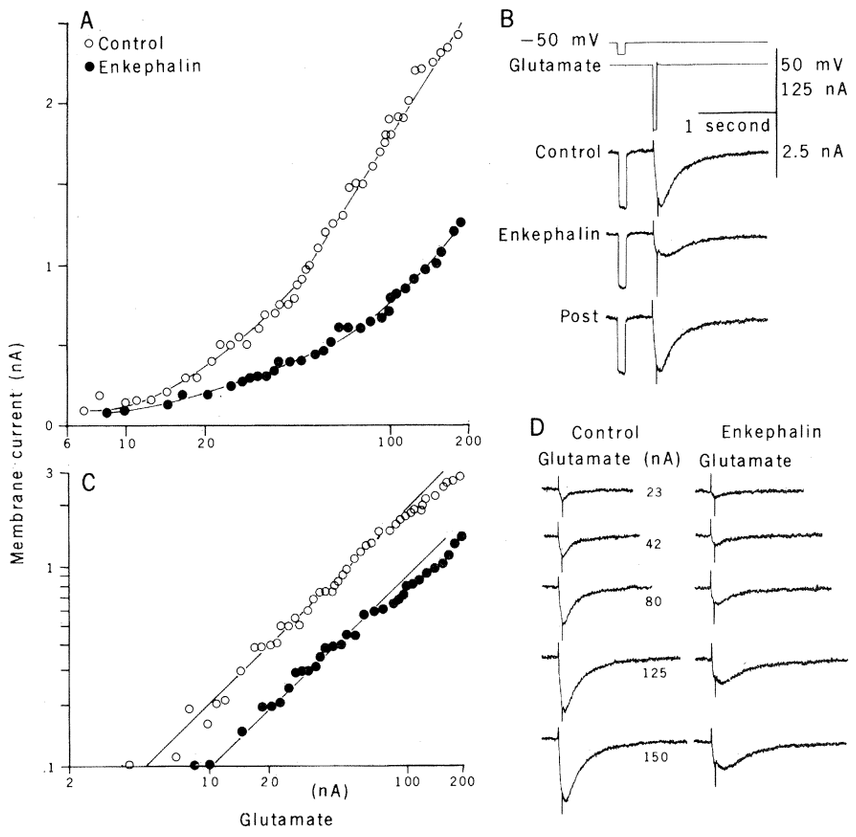


Fig. 1 (top). Leucine-enkephalin modulation of glutamate responses of cultured mouse spinal neurons. Rectilinear penwriter records of intracellular recordings were made with KCl pipettes. Enkephalin and glutamate were introduced by iontophoresis from extracellular pipettes placed at the same site on the cell body. (A1) Iontophoresis of enkephalin, marked by the bar above trace, rapidly and reversibly depresses depolarizing voltage responses to 50 msec–25 nA glutamate pulses with little change in membrane potential. The membrane potential was held hyperpolarized at −80 mV by a steady current through the bridge circuit. (A2) Faster chart speed of events; 0.2-second time calibration applies. (B) Partial naloxone reversal of enkephalin depression of responses to 100 msec–40 nA glutamate pulses. The control sequence (left) was repeated in the presence of coincident iontophoretic application of naloxone at 50 nA (right). The membrane potential was −75 mV. (C1) Depression of glutamate responses by increasing the enkephalin iontophoretic current with specimen records of some data points (a to e) shown on right (C2). Same cell as in (A). Increasing the enkephalin current produces a decrease in the amplitude of the glutamate response, but the effect saturates at about 50 percent depression. A slowing of the glutamate response kinetics at higher iontophoretic currents of enkephalin is evident. Fig. 2 (bottom). Analysis of the enkephalin-glutamate interaction on cultured spinal neurons observed with the voltage-clamp technique. The spinal neuron was impaled with two independent KCl micropipettes and the voltage was clamped to −50 mV. The experimental paradigm is illustrated in (B). A 10-mV hyperpolarizing command followed by 100 msec–100 nA glutamate iontophoretic pulse before (*Control*), during (*Enkephalin*), and after (*Post*) application of enkepalin (20 nA) at the same site. Depression and alteration in kinetics of glutamate-evoked inward membrane current during enkephalin application without change in current response to voltage command is evident. (A) The dose-response curve of the membrane current as a function of the glutamate iontophoretic current in control and during enkephalin application. Specimen records are shown in (D) and a log-log plot is shown in (C). Numbers in (D) refer to glutamate iontophoretic currents.



pre- and postsynaptic events. Whether one substance can have multiple, operationally distinct actions as transmitter, modulator, or hormone, depending on engagement of specific receptors, remains to be determined. Our observations indicate only that one action of [Leu^5]enkephalin falls outside two other previously described functional classes of communication in nervous systems.

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Cellulose Digestion in the Midgut of the Fungus-Growing Termite *Macrotermes natalensis*: The Role of Acquired Digestive Enzymes

Abstract. *The midguts of adult workers of the higher termite species* Macrotermes natalensis *contain the entire set of digestive enzymes required for the digestion of native cellulose. The C_x-cellulases and the β-glucosidases are produced, at least in part, by the termite's own midgut epithelium and salivary glands. The C_1-cellulases, on the other hand, are acquired by the termites when they feed on a fungus that grows in their nests. We propose that the involvement of acquired digestive enzymes could serve as the basis for a general strategy of resource utilization and further suggest that the acquisition of digestive enzymes may be a widespread phenomenon among mycophagous invertebrates.*

The nutritive regime of termites is based upon the exploitation of cellulosic materials. In the lower termites (*1*) the digestion of cellulose is brought about by flagellate protozoa resident in the paunch, an enlarged region of the gut posterior to the midgut (*2*). The higher termites lack this assemblage of xylophagous protozoa, and the mechanism by which ingested cellulose is degraded remains a matter of speculation. It has been suggested that, in some species, paunch bacteria have taken over the role of the protozoa, but evidence in support of this notion is meager (*3*). "Cellulases" have been reported from the guts of several higher termites, but in only one species has the full set of enzymes required to digest native cellulose been demonstrated (*4*). It is also possible that some species simply restrict their diets to wood that has already experienced extensive fungal decay.

Termites of the higher termite subfamily Macrotermitinae (*5*), common in Africa and Asia, have long intrigued biologists because of their symbiotic association with fungi that grow in their nests on structures referred to as "fungus combs." The combs, which are spongelike in appearance and corklike in texture, are derived from chewed but undigested plant fragments (*6–8*). The surface of the comb is covered with a sparse growth of mycelium and numerous small white spheres or nodules, 0.5 to 2 mm in diameter (see Fig. 1). These nodules, or synemata, are the conidia or conidiophores of a fungus, *Termitomyces* sp., believed to be restricted to Macrotermitinae nests (*9*). The mycelium is a mixture of *Termitomyces* sp. and various xylariaceous species (*10*).

The termites eat both the comb material (*11*) and the nodules (*12*). It is not known whether in nature they also eat the various cellulosic materials they collect (*6*), or whether these materials are all first incorporated into the fungus combs. The material from which the comb is constructed contains plant fragments with intact cells and cell walls, while extensive deterioration of cellular structure is evident in the contents of the midgut, paunch, and rectum (*8*), suggesting that the digestion of cell wall constituents occurs in the termite's gut. However, laboratory studies have shown that the termites starve if provided only with cellulosic materials, such as sound wood or filter paper, whereas they survive for extended periods of time if provided with fragments of fungus comb, complete with the nodules (*13, 14*).

Our investigation was directed at the question of how the fungus-growing termites digest cellulose and what the basis is for the dependence of the termites on their symbiotic fungal partner.

The enzymatic degradation of cellulose requires the concerted action of several enzymes, the C_1 enzymes (active against crystalline cellulose), the C_x enzymes (active against noncrystalline cellulose and soluble derivatives or degradation products of cellulose), and a β-glucosidase (active against cellobiose) (*15*). We have found that the entire set of enzymes required for cellulolysis is present in the midguts of adult *Macrotermes*

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