We have now identified the linkage for the genes that govern IdU-inducibility of xenotropic virus in two mouse strains inducible for this virus—C57BL/10J, studied chiefly as its *H-2*^{k} congenic derivative B10.BR/SgLi, and BALB/cN. In crosses of these mice with noninducible strains, virus inducibility is regularly expressed in F_1 hybrids, and the segregation ratios in the backcross generation are compatible with single gene control (Table 1). In all three cases, virus inducibility showed linkage to the *Dip-1* locus on chromosome 1 (linkage group XIII), a gene that codes for electrophoretically distinguishable forms of the enzyme dipeptidase-1 (*10*). No correlation was observed between the virus phenotype and several other gene markers tested, including *Gpd-1* (chromosome 4), *Gpi-1* (chromosome 7), *Es-1* and *Gr-1* (chromosome 8), *Mod-1* (chromosome 9), and *H-2* (chromosome 17).

The linkage estimates obtained with the C57BL/10 and B10.BR hybrids were not significantly different ($P > .25$), and we presume that the two strains carry the same xenotropic virus-inducing locus. In contrast, the difference in recombination frequency between the BALB/c and C57BL/10 crosses (11 percent and 23 percent, respectively; $P = .05$) suggests that the virus-inducing loci in these strains may not be at allelic sites, even though both are clearly on chromosome 1. This would not be unprecedented, since ecotropic virus-inducing loci are not at allelic sites in different laboratory mouse strains (*11*). Until this question of allelism of the BALB/c and C57BL/10 xenotropic virus-inducing loci is clarified, we recommend that for the present only one, that of C57BL/10, be named; we refer to it as *Bxv-1*.

Further studies are in progress to define precise map positions and to determine if inducible xenotropic viruses in other inbred strains show linkage to chromosome 1 markers.

Knowledge of the chromosomal location of these xenotropic virus-inducing loci should facilitate analysis of these complex viruses, particularly with regard to their regulation, their expression as viral antigen in differentiated cell types in the absence of virus production, their role in the generation of recombinant viruses (*12*), and their importance in tumorigenesis.

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Specific-Opiate-Induced Depression of Transmitter Release from Dorsal Root Ganglion Cells in Culture

Abstract. *The opiate etorphine depresses monosynaptic excitatory postsynaptic potentials (EPSP's) elicited in spinal cord cells by activation of dorsal root ganglion cells in murine neuronal cell culture. The depression is reversed by naloxone. Statistical analysis of the synaptic responses reveals that the opiate reduces EPSP quantal content at this synapse without altering quantal size. Therefore, the opiate action is presynaptic and affects transmitter release rather than postsynaptic responsiveness.*

Electrophysiological studies with intracellular and extracellular recording techniques coupled with microiontophoresis have revealed a multiplicity of opiate effects on neuronal excitability and on responses elicited by putative excitatory and inhibitory neurotransmitters (*1*). Several lines of evidence have suggested that a presynaptic locus might be involved in mediating opiate effects in the nervous system and, in particular, that primary afferent fibers in the spinal cord might be one site of opiate action (*2*). Cell cultures containing dorsal root ganglion (DRG) and spinal cord (SC) cells offer an opportunity to assess directly pre- and postsynaptic mechanisms of drug action by means of electrophysiologic techniques; in our studies, we used mouse dissociated DRG-SC cell cultures to assess opiate effects on synaptic transmission. We report (i) that the opiate etorphine reversibly depresses synaptic transmission between DRG and SC cells, (ii) that this depression is presynaptic, and (iii) that the depression in synaptic transmission is reversible with the specific opiate antagonist, naloxone.

We used mouse SC cell culture and electrophysiological methods as described (*3*); the cultures were treated with nerve growth factor (*4*) for at least 1 week prior to the electrophysiological experiments. Experiments were performed in normal control medium (90 percent Eagle's minimal essential medium and 10 percent horse serum) to which Ca^{2+} and Mg^{2+} were added (the final concentration being 6.7 and 10 m*M*, respectively). A DRG cell and an SC cell within the same microscopic field were penetrated with micropipette electrodes filled with 4*M* potassium acetate, and ex-

Table 1. The effect of etorphine on quantal parameters for the DRG to SC EPSP. The data taken during either control conditions or under etorphine + naloxone conditions are shown under the heading control. Data taken during the application of etorphine are shown under the heading E.

Quantal size (q)			Quantal content (m)			EPSP amplitude ($\bar{v}$)		
Control	E	Ratio	Control	E	Ratio	Control	E	Ratio
313	286	0.91	44	14.1	.32	14.4	4.7	.33
96	105	1.09	66.5	28.3	.43	7.3	3.0	.45
183	193	1.05	24.8	11.1	.45	4.5	2.1	.47
222	253	1.13	52	24.7	.48	11.5	6.3	.55
190	144	0.75	46	36	.78	8.8	5.3	.60
201*	196*	.99	47†	23 †	.49	9.3‡	4.3‡	.47

*$P > .20$. †$P < .02$. ‡$P < .05$.

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citatory synaptic connections from the DRG to the SC cell were accepted as monosynaptic when a directly elicited DRG action potential was followed after a short (< 3 msec) invariant latency by an excitatory postsynaptic potential (EPSP) of invariant configuration recorded in the SC cell. Rapid (> 10 per second) stimulation typically produced a diminution of EPSP amplitude with no change in latency or configuration and with no failures. Etorphine hydrochloride and naloxone hydrochloride, 1 to 10 m*M* in distilled water (*4*), were applied by iontophoresis from theta or fiber-filled microelectrodes (> 100 megohms resistance); the pipettes were sometimes broken to 1- to 2-μm tip diameter. The EPSP amplitude histograms were constructed both from hand analyses of penwritten records and by a computer program (*5*). A correction for nonlinear summation of the postsynaptic potentials was made on the assumption that the equilibrium potential for the EPSP was zero (*6*, *7*). Correction for the system noise was made on the assumption that 300 μV was the root-mean-square noise value (*7*). The mean and variance of a series of 150 to 200 EPSP's evoked at 1 to 2 per second were determined during control periods or with drug applications when steady state conditions had been reached.

Etorphine iontophoretically applied to regions of contact between the DRG and SC cells reversibly depressed the monosynaptic DRG-SC EPSP in 12 of 12 cell pairs tested, and the degree of depression was dependent on the amount of etorphine current in a graded fashion. The etorphine depression could also be reversed by simultaneous application of naloxone in 10 of 12 cases; the reversal was usually nearly complete (80 percent in five cell pairs), and no reversals to > 100 percent of control were observed. Naloxone when applied alone with similar currents had no effect on EPSP amplitude (eight of eight cases).

The mean quantal amplitude (q) and quantal content (m) of a series of EPSP's were determined from the relationship $\bar{m} = \bar{v}^2/\text{var } v$ and $q = \text{var } v/\bar{v}$, where $\bar{v}$ is the average EPSP amplitude and var v is the variance of the series of evoked EPSP's (*7*). The data format is shown in Figs. 1 and 2, while a summary of data from several cells in the control condition and in the presence of etorphine with and without naloxone is given in Table 1. It is evident that q is essentially unaffected by etorphine ($P > .20$), while m is depressed ($P < .02$), and that the reduction in m quantitatively accounts for the depression of EPSP amplitude. Naloxone reverses this etorphine-induced depression of quantal content.

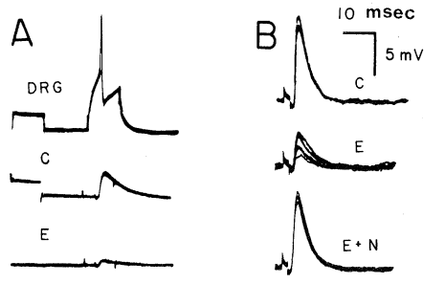

Fig. 1 Etorphine antagonism of synaptic transmission from DRG to SC. Stimulation of DRG (5-msec duration depolarizing current) evoked a typical action potential (A-DRG) that was followed by a 10- to 12-mV EPSP recorded in the SC with a 2.5-msec latency (A-C and B-C). Iontophoresis of etorphine hydrochloride (1 m*M*, 5 nA) produced clear attenuation in EPSP amplitude (A-E and B-E), and iontophoresis of naloxone hydrochloride during etorphine application at a point adjacent to the etorphine pipette reversed this antagonism, restoring the EPSP to 80 percent of its control value (B-E+N). The Ca pulse in (A) is 10 mV and 5 msec.

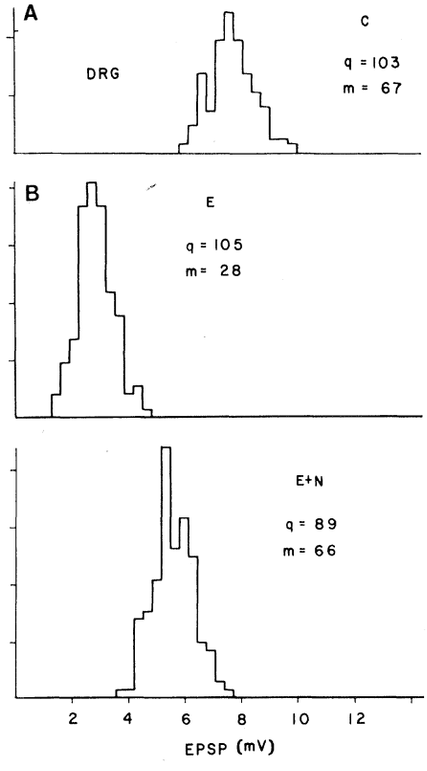

Fig. 2. Etorphine produces naloxone-reversible presynaptic inhibition of DRG transmitter release. Evoked EPSP amplitude histograms were obtained during a control run (A), during steady application of etorphine hydrochloride (5 nA) (B), and during coincident etorphine and naloxone (30 nA) iontophoresis (C). The values for quantal size (q) and quantal content (m) were calculated by the variance method (*7*) and were corrected for nonlinear summation of the EPSP's and for system noise (assuming a root-mean-square noise level of 300 μV). The results demonstrate presynaptic inhibition of transmitter release (decrease in m) with minimal postsynaptic effect (no change in q), an effect which was largely reversible by naloxone. The ordinate scale represents ten responses per division.

The quantal model of synaptic transmission attributes the determinants of quantal number to the presynaptic element, and a large body of evidence demonstrates that this is correct. The fact that etorphine changes m rather than q is evidence for a presynaptic action of the opiate. Two possible presynaptic mechanisms must be considered. (i) Axon terminal excitability might be depressed by etorphine, resulting in partial blockade of spike propagation into the terminals; transmitter release would therefore be diminished. It is difficult to exclude this possibility, but synaptic transmission was depressed after doses of etorphine that had no detectable effect on spike threshold or configuration when applied directly to the neuronal soma. (ii) Etorphine may have a direct depressant effect on the transmitter release mechanism. A number of mechanisms for such an effect might be suggested, but our data do not distinguish between such alternatives as altered Ca^{2+} ingress or partial "sealing" of the presynaptic membrane to vesicle release.

In addition to this presynaptic action, postsynaptic effects of the opiates are well documented (*1*), and a naloxone reversible facilitation of acetylcholine release by opiates at the neuromuscular junction has been described (*8*). Opiate effects thus appear to be multiple, but the possibility exists that a general membrane-opiate interaction may underlie the diverse phenomena that have been described.

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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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