CRC Handbook of Comparative Opioid and Related Neuropeptide Mechanisms

Volume II

Editor

George B. Stefano, Ph.D.
ADAMHA-MARC Project Director
Associate Professor and Chairperson
State University of New York/
College at Old Westbury
Old Westbury, New York

CRC Press, Inc.
Boca Raton, Florida

Library of Congress Cataloging-in-Publication Data
(Revised for vol. 2)
Main entry under title:

Handbook of comparative opioid and related neuropeptide mechanisms.

Bibliography: p.
Includes indexes.
1. Neuropeptides. 2. Opioids. 3. Physiology, Comparative. I. Stefano, George B., 1945— .
QP552.N39H35 1986 591'.0188 85-16225
ISBN 0-8493-3257-5 (v. 1)
ISBN 0-8493-3258-3 (v. 2)

Direct all inquiries to CRC Press, Inc., 2000 Corporate Blvd., N.W., Boca Raton, Florida, 33431.

International Standard Book Number 0-8493-3257-5 (Volume I)
International Standard Book Number 0-8493-3258-3 (Volume II)

Library of Congress Card Number 85-16225
Printed in the United States

TABLE OF CONTENTS

RECEPTORS

Immunocytochemical Studies on Neuropeptides and Their Precursors with Special Reference to Opioids 3
Opioid Receptor Properties in Invertebrates 13
Multiple Mu Opioid Receptors in the Central Nervous System 25
Lectin and Ligand Affinity Chromatography of Solubilized Opioid Binding Sites 31

INTERACTION OF PEPTIDERGIC AND NONPEPTIDERGIC NEURONS

Neurophysiological Characterization of Opioid Peptide Actions on Neurohumoral Transmission in the Mammalian Central Nervous System 41
Responses of *Aplysia* Cerebral Ganglion Neurons to Leucine-Enkephalin 49
Morphine Enhancement of the Cholinergic Response of Anterior Byssus Retractor Muscle of *Mytilus edulis* 59
The Regulation by β-Endorphin and Related Peptides of Collagen-Tailed Acetylcholinesterase Forms in the Skeletal Muscles of Vertebrates 65
Brain-Gut Peptides in the Cockroach with Special Reference to Midgut 81
Enkephalins Modulate Chronotopic Responses and Calcium Flux in Rat and Guinea Pig Atria 91
Interaction of Opioid Peptides and Low-Molecular-Weight Neurotransmitters in *Helix* Neurons 103

MULTIPLE ROLES OF NEUROPEPTIDES

Modulation of Sensory and Motor Activity in the Horseshoe Crab *(Limulus polyphemus)* by a Substance P-Like Peptide 117
Peptide Modulation of Neuronal Activity and Behavior in *Aplysia* 129
The Effects of Met-Enkephalin and FMRFamide on the Excitability of the Neurosecretory Caudodorsal Cells of the Pond Snail 145
Characterization of Substance P Release from the Myenteric Plexus: Effects of Opiates and Opioid Peptides 159
Peripheral Endocrine Functions of TRH- and Somatostatin-Like Molecules in Fresh-Water Gastropods 173

AGING

Aging and Opioid Responsiveness 191
Opioid Aging and Seasonal Variations in Invertebrate Ganglia: Evidence for an Opioid Compensatory Mechanism 199
Altered Mechanisms of Neurotransmitter Action During Aging: The Striatal Dopaminergic System as a Model 211
The Vasopressin Neuron in the Aging Human and Rat Brain 221

BEHAVIORAL EFFECTS

An Opioid Mechanism Modulates Dopaminergic Control of Ciliary Activity in the Marine Mussel *Mytilus edulis* 233
Investigations on the Effects of Neuropeptides on Chromatophore Responses in the Land Crab *Gecarcinus lateralis* 243
Effects of Opiates in Bryozoa 249
Peptide Modulation of Mammalian Learning and Memory: Behavioral Mechanisms of Action 259

Conformational Matching: A Possible Evolutionary Force in the Evolvement of Signal Systems 271

INDEX 281

NEUROPHYSIOLOGICAL CHARACTERIZATION OF OPIOID PEPTIDE ACTIONS ON NEUROHUMORAL TRANSMISSION IN THE MAMMALIAN CENTRAL NERVOUS SYSTEM

W. Zieglgänsberger, N. Mercuri, P. Stanzione, and B. Sutor

INTRODUCTION

In the mammalian central nervous system, opioid agonists induce changes in physiological parameters, and alter behavior, mood, and mental processes as well as endocrine and autoregulatory functions. The most extensive studies have certainly been carried out on the analgesic actions of opioids. The effects of systemically or topically applied opioid agonists are dose dependent, show stereoselectivity, and are reversed by opioid antagonists such as naloxone or naltrexone. The stereoselectivity of opioid effects suggested that stereoselective receptors existed on neuronal tissue where endogenous ligands are operative under physiological conditions.

Since the discovery of the first endogenous ligand for the opioid receptor, some 20 endogenous opioid peptides have been isolated and characterized that mimic the naloxone reversible effects of opioids in bioassays for opioid activity or compete at low concentrations with opioid ligands at opioid binding sites. The various endorphins probably serve diverse physiological functions.[1] This view is supported by their different and rather specific distribution in the nervous system and their origin from different precursor molecules.[2] Furthermore, different opioid peptides interact with a different spectrum of opioid receptor-subclasses,[3] which are also distributed in a rather specific manner in the nervous system.

A survey of present data suggests that no simple anatomical or functional link exists between the endorphinergic system and a particular neurotransmitter system in the mammalian central nervous system. The enkephalins are mainly contained in short-axoned interneurons which are present at all levels of the neuraxis. In contrast, β-endorphin-reactive material is almost exclusively found to originate from the basal hypothalamus and adjacent tissue, and project paraventricularly to mesencephalic sites. Dynorphin fibers from the hypothalamus project to cortical and limbic areas, the midbrain and mesencephalon, and are also contained in groups of interneurons including spinal cord dorsal horn neurons.[1,4] The rather restricted distribution of, e.g., β-endorphin does not, however, preclude an action on remote neurons carrying opioid receptors. It has been postulated that opioid peptides are released into the circulation, e.g., after psychic or physical stressors have been applied, from brain sites as well as the adrenal medulla.[5] Their specific distribution in nerve terminals, their release after synaptic stimulation, and the distinct actions exerted by some of the opioid peptides on neuronal systems suggest that they are involved in information transfer as intercellular messengers in certain parts of the mammalian central nervous system. There is evidence from various investigations in the vertebrate as well as the invertebrate nervous system that, besides fast synaptic transmission triggered by the rapid opening and closing of ionic channels, processes which have a significantly slower time course take place at synaptic sites. Neuropeptides are considered to be major candidates for involvement in such modulatory mechanisms where, e.g., a substance without directly influencing membrane potential or membrane conductance transiently alters the responsiveness of a neuron to transmitters released from adjacent terminals. Such findings support the notion that the borderline between neurosecretion and neurotransmission is less clear than previously thought.

In general, the activation of opioid receptors in the mammalian central and peripheral nervous system evokes an inhibition of spontaneous and chemically or synaptically induced neuronal discharge activity. Considerable uncertainties still exist regarding the mode of action of opioids on single neurons. In this chapter, results of studies on the action of opioid

peptides on neurons from the adult rat cortex, from hippocampus, and from the adult spinal cord of the rat will be reported.

MATERIALS AND METHODS

Single Unit Recording

Most of the information we have gathered so far about the actions of neuropeptides in the central nervous system (CNS) derive from extracellular recording of single unit activity in combination with iontophoretic administration from micropipettes or by microsuperfusion from multibarreled electrodes. It is obvious that extracellular recordings only show the influence of a substance on suprathreshold neuronal phenomenon. In order to analyze the ionic mechanisms underlying the action of neuropeptides, it is necessary to record intracellularly and to apply the compounds in the near vicinity of the neuron under study. These technically difficult studies have been limited so far to a few types of mammalian neurons.

The introduction of in vitro preparations of the mammalian has introduced interesting possibilities in the analysis of peptide actions and their interactions with classical neurotransmitters (for technical details see Reference 6). In these preparations, a large proportion of the afferent fibers remains intact so that some of the neurons can be identified by afferent stimulation. Furthermore, the pharmacological actions of the neuroactive compounds can be analyzed without the interference of anesthesia or paralyzing agents. Lowering the Ca^{2+} concentration and increasing the Mg^{2+} concentration of the superfusion medium or adding the spike-blocking neurotoxin tetrodotoxin effectively reduces or blocks synaptic transmission so that drug actions can be studied on a synaptically isolated neuron. Together with recordings from invertebrates, whose neurons are accessible to even more elaborate electrophysiological techniques and of which some species possess enkephalin-containing neurons (see this volume), this approach will gain considerable relevance for the analysis of opioid actions.

Microiontophoresis and Microsuperfusion from Multibarreled Pipettes

The microiontophoretic application of a neuroactive substance circumvents the diffusional limits set by the blood-brain barrier. Furthermore, it reduces indirect effects arising from influences upon remote neurons and from actions secondary to metabolic and circulatory disturbances after, e.g., systemic applications. The concentrations reached at the receptor site by phoretic application of the opioid peptides (or other substances) are virtually unknown, although the number of molecules transported is linearly related to the current flowing through the micropipette.[7]

This major disadvantage of the microiontophoretic administration technique can be at least partially overcome by ejection of diluted solutions from micropipettes by pressure application. This microsuperfusion technique does not require high pressure, typically 10 to 50 kPa or measurement of the volume ejected. It was established recently that the effective concentrations of opioid peptides reached at the receptor sites with the application of commonly used phoretic currents (50 to 300 nA) from pipettes filled with 10 m*M* solutions of the opioid compare favorably to those opioid concentrations used in various bioassays such as the guinea pig ileum and the mouse vas deferens[8] (Figure 1). The effective concentrations for the excitatory amino acid neurotransmitter L-glutamate (GLU) were established to be in the range of 10^{-7} to 10^{-6} *M* (Figure 2).

RESULTS

Cortex

Most areas of the neocortex of mammals contain enkephalin or other opioid peptide-reactive material and the presence of multiple opioid receptor subtypes has been demonstrated

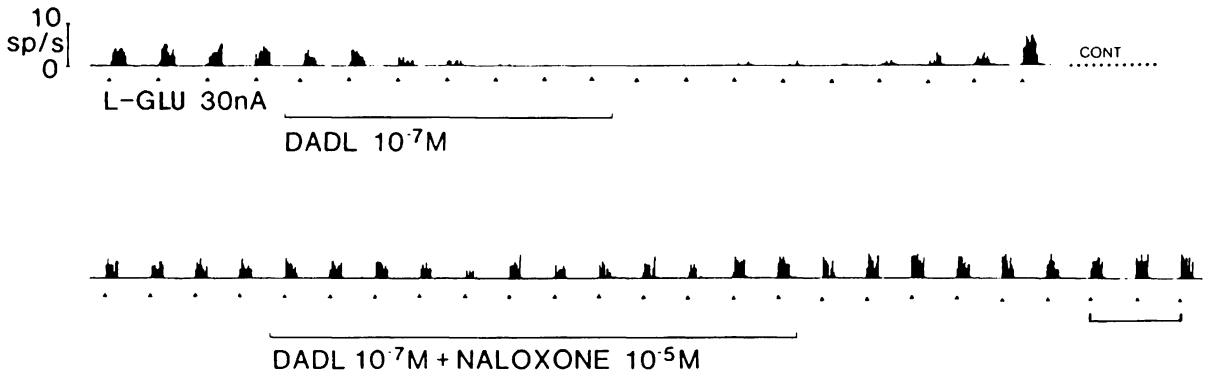

FIGURE 1. Depressant effect of DADL on a neuron in the dorsal horn of the rat spinal cord. The activation of this neuron by short pulses of GLU (30 nA/20 sec) was reversibly blocked by microsuperfusion (20 kPa) of DADL (10^{-7} *M*). This effect was prevented by simultaneous administration of naloxone (10^{-5} *M*) ejected from the same multibarreled micropipette. Time calibration: 2 min.

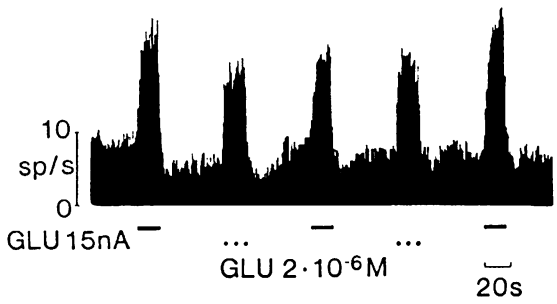

FIGURE 2. Alternate microiontophoretic and pneumatic application of GLU. The spontaneously discharging dorsal horn neuron receiving multimodal input from cutaneous receptive fields was recorded in rat anesthetized by halothane (for technical details see Reference 37). To establish the effective concentrations of microiontophoretically applied GLU (from 0.5 m*M* solution), the amino acid was ejected alternately by phoresis (bars) or by pressure (30 kPa) (dotted lines) from micropipettes containing GLU at different concentrations. The effective concentration reached by a phoretic current of 15 nA for GLU corresponded to concentrations of 2×10^{-6} *M*. Note: this concentration is the maximal concentration at the orifice of the pipette; the concentration at the receptor is probably lower.

in cortical structures (see "Introduction"). The prominent initial response of neurons recorded in various sites in the cortex to microiontophoretically applied opioid peptides is a reduction in their spontaneous firing rate or in the discharge activity of these neurons induced by short iontophoretic pulses (5 to 100 nA/5 to 20 sec) of excitatory neurotransmitters such as GLU or acetylcholine.[9,10] In a microiontophoretic study, where morphine was used as a selective mu-agonist and D-Ala^2-D-Leu^5-enkephalin (DADL; a synthetic enkephalin congener which is resistant to enzymatic degradation) was used as a prototypic delta-agonist, convincing evidence was obtained that these inhibitory responses were mediated by various subtypes of the opioid receptor.[10]

Short-term iontophoretic applications of either opioid resulted in adaptive processes which were similar to tolerance to and dependence on opioid agonists occurring after chronic treatment. When the delta-receptor agonist was applied after the cell had been desensitized to the mu-agonist, the potency of DADL to produce inhibition of firing was unchanged, i.e., no cross-desensitization was exhibited. These studies on the single unit level showed for the first time that different subtypes of opioid receptors can be located on the same cell.[8,10] Multiplicity of opioid receptors was initially suggested by the different patterns of opioid and opioid peptide activity in various assay systems.[11]

In a subsequent study, the effect of DADL on neurons in a slice preparation[12] obtained from the rat sensory-motor cortex was examined. In these in vitro studies, intracellular recordings were obtained from neocortical cells that displayed membrane potentials between 65 to 75 mV, input resistances between 10 to 20 MΩ, and action potentials with amplitudes between 80 to 120 mV. Stable, long-lasting intracellular recordings could be obtained from these neurons. The enkephalin-analog DADL and the opioid antagonist naloxone were applied iontophoretically, by ejection from pressurized micropipettes, or added to the superfusion medium, after a postimpalement control period during which the membrane potential was stable for 15 to 30 min. In none of the neurons tested did the application of DADL change the membrane potential more than 2 mV or alter the membrane resistance of the neuron. DADL applied topically in concentrations of 10^{-8} to 10^{-6} *M* reduced the amplitude and the rate of rise of the EPSP evoked by stimulation in superficial layers about

1 to 2 mm remote to the recording site. Bath-applied naloxone (10^{-6} *M*) reversed or prevented this effect which was clearly dose dependent. Short microiontophoretic pulses of GLU or aspartate (10 to 80 nA/10 to 20 sec) were clearly reduced or blocked by DADL applied phoretically (40 to 200 nA/1 to 5 min) or added to the medium (10^{-8} to 10^{-6} *M*). Most importantly, this anti-GLU effect of DADL was not altered when the neuron was synaptically uncoupled by superfusing the slice with a medium that contained 9 m*M* Mg^{2+} and no Ca^{2+}. These latter findings indicate that the opioid receptors activated by DADL are located on the neuron recorded from.

To test for the reversal potential of this opioid peptide the effect of DADL was tested at various membrane potential levels. As under resting conditions, DADL had no effect on the membrane potential displaced by intracellular current injection. When DADL was applied during depolarizations (5 to 20 mV) induced by continuous phoretic application of GLU, the membrane potential was repolarized by DADL. Findings similar to these described for DADL have also been obtained with morphine.[12] The sum of these results suggests that DADL and morphine have a modulatory effect on the actions of excitatory amino acid neurotransmitters like GLU which preferentially increases the permeability of the cell to Na^+ ions.[13]

In contrast to the modulatory effect of DADL, the phoretic application of GABA (5 to 150 nA) was associated with a marked reduction of neuronal input resistance. Depending on the membrane potential, the decrease in input resistance was associated with depolarizing or hyperpolarizing changes in membrane potential. The reversal potential for this action of GABA was found to be essentially identical to the Cl^- equilibrium potential (between -69 and -74 mV) and the reversal potential was shifted in the depolarizing direction by intracellular injection of Cl^-. These findings suggest that the decrease in cell input resistance produced by GABA is due to a selective increase in membrane permeability to Cl^-. These actions of GABA were dose dependent and could be reversibly blocked by bicuculline (10^{-5} *M*) added to the superfusion medium.[14]

Hippocampus

Unlike most other CNS structures, the CA1 region of the hippocampus (HC) predominantly responds to the application of opioid agonists with a profound increase in hippocampal pyramidal cell (HCP) excitability[15,16] which might be the basis for the observed limbic seizure activity.[17,18] It was first postulated from extracellular recordings that this excitatory response results from an inhibition of inhibitory GABAergic interneurons.[19,20] This inhibitory action of opioid agonists on adjacent interneurons which leads to a disinhibition of HCP neurons was substantiated by subsequent intracellular studies in in vitro slice preparations[21,22] and organotypic cell culture systems[23] where it was shown that opioid agonists markedly reduce the IPSP following alveus or stratum radiatum stimulation. The excitation evoked in HCP by the various opioid alkaloids and opioid peptides is mediated by multiple receptor mechanisms.[24] In a recent study, it was observed that DADL and the dynorphin A (1-17) (DYN A) fragment, DYN A (1-13), which is considered a kappa-preferring ligand, increase the excitability of HCP in the CA1 and CA2 regions. This increase in excitability is associated with a reduction of the inhibition produced by orthodromic stimulation of the stratum radiatum. DYN A (1-17) at concentrations between 10^{-5} and 10^{-7} *M* reduced the excitability of HCP in CA2 and decreased the excitatory effect of DADL in CA1 pyramidal cells in vitro. At low concentrations (10^{-10} *M*), DYN A (1-17) preferentially increased HCP excitability and mimicked the actions of the other opioid peptides. The excitatory effect of DADL and DYN A (1-13) was antagonized by bath-applied naloxone (10^{-6} to 10^{-5} *M*). The depressant actions of DYN A (1-17) on HCP excitability were not antagonized by naloxone added to the superfusion medium. DYN A (1-13) was shown to cause excitation at sites in the hippocampal pyramidal layer where DYN A (1-17) evoked a depression of the population

spike amplitude. The observation that DYN A (1-17) potentially modulates the activity of DADL on the same neuron population is suggestive of a partial antagonistic action of DYN A (1-17).[38] From the present results, however, the possibility of a functional antagonism cannot be excluded.

Spinal Cord

Opioid peptides and most of the other recently characterized neuropeptides have been localized in cell bodies and terminals of the substantia gelatinosa (SG). Peptidergic mechanisms have been implicated in the control exerted by interneurons in the SG over cells which give rise to spinofugal projections. Most spinofugally projecting neurons receiving multimodal synaptic input from peripheral receptive fields were inhibited by opioids[13] (Figure 1).

Extracellular recording performed from neurons of the SG in an in vitro preparations from the spinal cord of adult rats[11] showed that about 40% of the neurons was spontaneously active. Interestingly, they could be synaptically influenced by stimulation of fibers entering the spinal cord through either the dorsal or ventral roots. Repetitive low threshold stimulation transiently activated a substantial number of these neurons, whereas high intensity stimulation predominately reduced the excitability of SG neurons. These effects were slow in onset and had a slow time course for recovery. As in other structures in vivo and in vitro, activity evoked by phoretically administered GLU as well as synaptically induced and spontaneous activity was reduced or abolished by the opioid peptides (and GABA). The actions of DADL (30 to 200 nA/1 to 5 min) were blocked by phoretically applied naloxone (50 to 100 nA/2 to 3 min).

Several mechanisms have been proposed by which the depressant effects of opioids are produced. One is a presynaptic effect where the release of excitatory transmitters is decreased by opioids; a second is a postsynaptic modulatory effect on the efficacy of an excitatory neurotransmitter substance; and a third suggests a hyperpolarizing action associated with a conductance change at the target neuron. At present, most electrophysiological and ultrastructural data indicate that the major effects of opioids on central neurons are probably mediated via postsynaptically located opioid receptors. Before the morphological demonstration of axodendritic, axosomatic, or dendrodendritic enkephalinergic synapses,[25-31] a postsynaptic site of action of opioids was indicated solely by electrophysiological investigations demonstrating that depolarizations produced by iontophoretic application of GLU could be markedly reduced by opioid peptides.[7,32] Opioid peptides reduce the rise time of EPSPs both in spinal cord neurons and cortical neurons, without influencing the membrane potential of the membrane resistance.[13] Such a decrease in rise time of the EPSP induced by opioids would be much more effective in blocking spike initiation triggered in dorsal horn neurons by slowly rising synaptic potentials (mainly repetitive firing in C-fibers) than those produced by powerful fast-rising excitatory postsynaptic potentials evoked by stimulation of large fiber inputs. Observations compatible with the assumption of such a preferential effect of opioids on excitatory postsynaptic potentials have been made in cortical, striatal, and bulbar respiratory neurons,[33] and such an effect appears to be a characteristic feature of opioid action. The rather selective effect of opioid agonists on noxious mechanical, thermal, and chemical stimulation may be explained in terms of the characteristics of changes in the rise time of postsynaptic potentials. A substantial number of neurons in the substantia gelatinosa of the dorsal horn of the rat spinal cord studied in vitro were found to be hyperpolarized by opioid peptides. An increase in κ-conductance has been suggested to underlie this effect.[36]

SUMMARY AND CONCLUSIONS

Opioid peptides seem to be involved in many diverse functions in the vertebrate and

invertebrate nervous system.[34] For many classes of mammalian CNS neurons, it remains to be established whether the preferentially inhibitory action of opioids on neuronal activity is exerted by a modulatory effect on the action of excitatory transmitters, e.g., GLU,[13] by a transmitter-like hyperpolarizing effect which involves potassium-conductance increase[35] or by other possible mechanisms. It should be pointed out that the mechanism of action of different opioids is not necessarily the same, and that the mechanism of action of any single opioid may be different for different types of CNS neurons. Although immunohistochemical and ultrastructural data do not support a presynaptic interaction of opioid peptides with primary afferents or other afferent terminals via axo-axonic synapses, evidence for a presynaptic action of opioids has been presented and actions of opioids at nonsynaptic receptors are of course possible.

Before a biologically active agent can be established as a neurotransmitter or neuromodulator, several criteria must be satisfied: location in nerve terminals, release after stimulation, machinery for synthesis, and termination of action. As further evidence for a neurotransmitter role of a certain substance in the CNS, ligand binding experiments are used. To prove the identity of action of an exogenous compound with synaptically released substance, intracellular recordings are necessary and selective stimulation of a given pathway has to be achieved. Such technically difficult studies have been tried only in a few groups of mammalian neurons so far. At present, no peptidergic system in the mammalian CNS can be stimulated selectively. In the case of the opioid peptides, the reversibility of an evoked response by naloxone or the absence of action in the morphine-tolerant state is often the only indication for the involvement of an endorphinergic link. The relatively simple nervous system of the invertebrates might offer new experimental perspectives in the analysis of the physiological role of opioid peptides. Opioid binding sites do exist in some invertebrate species[34] and opioid peptides have been found in distinct cell groups (see Leung and Stefano, this volume). The accessibility of these neuronal circuits to elaborate stimulation and recording techniques could provide important experimental tools for the elucidation of opioid peptide actions and the opioid syndrome in general.

ACKNOWLEDGMENT

The helpful criticism of Dr. J. Howe is gratefully acknowledged.

REFERENCES

1. **Bloom, F. E.**, The endorphins: a growing family of pharmacologically pertinent peptides, *Ann. Rev. Pharm. Toxicol.*, 23, 151, 1983.
2. **Höllt, V.**, Multiple endogenous opioid peptides, *Trends Neurosci.*, 6, 24, 1983.
3. **Zukin, R. S. and Zukin, S. R.**, The case of multiple opiate receptors, *Trends Neurosci.*, 7, 160, 1984.
4. **Watson, S. J., Khachaturian, H., and Akil, H.**, Comparison of the distribution of dynorphin systems and enkephalin systems in brain, *Science*, 218, 1134, 1982.
5. **Akil, H., Watson, S. J., Young, E., Lewis, E. L., Khachaturian, H., and Walker, J. M.**, Endogenous opioids: biology and function, *Ann. Rev. Neurosci.*, 7, 223, 1984.
6. **Kerkut, G. A. and Weal, H. V.**, *Electrophysiology of the Isolated Mammalian CNS Preparations*, Academic Press, New York, 1981.
7. **Zieglgänsberger, W. and Tulloch, I. E.**, The effects of methionine- and leucine-enkephalin on spinal neurons of the cat, *Brain Res.*, 167, 53, 1979.
8. **Zieglgänsberger, W., French, E., Mercuri, N., Pelayo, F., and Williams, J. T.**, Multiple opiate receptors on neurons in the mammalian central nervous system. *In vivo* and *in vitro* studies, *Life Sci.*, 31, 2343, 1982.
9. **Satoh, M., Zieglgänsberger, W., and Herz, A.**, Actions of opiates upon single unit activity in the cortex of naive and tolerant rats, *Brain Res.*, 115, 99, 1976.

10. **Williams, J. T. and Zieglgänsberger, W.**, Neurons in the frontal cortex of rat carry multiple opiate receptors, *Brain Res.*, 226, 304, 1981.
11. **Martin, W. R.**, Pharmacology of opioids, *Pharmacol. Rev.*, 35, 283, 1984.
12. **Zieglgänsberger, W. and Sutor, B.**, Opioid peptides depress neuronal activity of neocortical neurons *in vitro* by reduction of excitatory input, *Naunyn-Schmiedeberg's Arch. Pharmacol. Suppl.*, 325R, 72, 1984.
13. **Zieglgänsberger, W.**, Opioid actions on mammalian spinal neurons, *Int. Rev. Neurobiol.*, 25, 243, 1984.
14. **Sutor, B. and Zieglgänsberger, W.**, A GABA-mediated, chloride-dependent depolarizing IPSP in neocortical neurons of the rat *in vitro*, *Pfluger's Arch. Eur. J. Physiol. Suppl.*, 40, R37, 1984.
15. **Hill, R. G., Mitchell, J. F., and Pepper, C. M.**, The excitation and depression of hippocampal neurones by iontophoretically applied enkephalins, *J. Physiol. (London)*, 272, 50, 1977.
16. **Nicoll, R. A., Siggins, G. R., Ling, N., Bloom, F. E., and Guillemin, R.**, Neuronal actions of endorphins and enkephalins among brain regions: a comparative microiontophoretic study, *Proc. Natl. Acad. Sci. U.S.A.*, 74, 2548, 1977.
17. **Henriksen, S. J., Bloom, F. E., McCoy, F., Ling, N., and Guillemin, R.**, Beta-endorphin induces nonconvulsive limbic seizures, *Proc. Natl. Acad. Sci. U.S.A.*, 75, 5521, 1978.
18. **Henriksen, S. J., Chouvet, G., and Bloom, F. E.**, In vivo cellular responses to electrophoretically applied dynorphin in the rat hippocampus, *Life Sci.*, 31, 1785, 1982.
19. **Corrigall, W. A.**, Opiates and hippocampus: a review of the functional and morphological evidence, *Pharmacol. Biochem. Behav.*, 18, 255, 1983.
20. **Zieglgänsberger, W., French, E. D., Siggins, G. R., and Bloom, F. E.**, Opioid peptides may excite hippocampal pyramidal neurons by inhibiting adjacent inhibitory interneurons, *Science*, 205, 415, 1979.
21. **Nicoll, R. A., Alger, B. E., and Jahr, C. E.**, Enkephalin blocks inhibitory pathways in the invertebrate CNS, *Nature (London)*, 287, 22, 1980.
22. **Siggins, G. R. and Zieglgänsberger, W.**, Morphine and opioid peptides reduce inhibitory synaptic potentials in hippocampal pyramidal cells *in vitro* without alteration of membrane potential, *Proc. Natl. Acad. Sci. U.S.A.*, 78, 5235, 1981.
23. **Gähwiler, B. H.**, Excitatory action of opioid peptides and opiates on cultured hippocampal pyramidal cells, *Brain Res.*, 194, 193, 1980.
24. **French, E. D. and Zieglgänsberger, W.**, The excitatory response of *in vitro* hippocampal pyramidal cells to normorphine and methionine-enkephalin may be mediated by different receptor populations, *Exp. Brain Res.*, 48, 238, 1982.
25. **Aronin, N., DiFiglia, M., Liotta, A. S., and Martin, J. B.**, Ultrastructural localization and biochemical features of immunoreactive Leu-enkephalin in monkey dorsal horn, *J. Neurosci.*, 1, 561, 1981.
26. **Bennett, G. J., Ruda, M. A., Gobel, S., and Dubner, R.**, Enkephalin immunoreactive stalked cells and lamina IIb islet cells in cat substantia gelatinosa, *Brain Res.*, 240, 162, 1982.
27. **Glazer, E. J. and Basbaum, A. I.**, Immunohistochemical localization of leucine-enkephalin in the spinal cord of the cat: enkephalin-containing marginal neurons and pain modulation, *J. Comp. Neurol.*, 196, 377, 1981.
28. **LaMotte, C. C. and de Lanerolle, N. C.**, Ultrastructure of chemically defined neuron systems in the dorsal horn of the monkey. II. Methionine-enkephalin immunoreactivity, *Brain Res.*, 274, 51, 1983.
29. **Ruda, M. A.**, Ultrastructural demonstration of direct enkephalinergic input onto medullary and spinal dorsal horn projection neurons, *Pain*, 1, 19, 1981.
30. **Ruda, M. A.**, Opiates and pain pathways: demonstration of enkephalin synapses and dorsal horn projection neurons, *Science*, 215, 1523, 1982.
31. **Sumal, K. K., Pickel, V. M., Miller, R. J., and Reis, D. J.**, Enkephalin-containing neurons in substantia gelatinosa of spinal trigeminal complex: ultrastructure and synaptic interaction with primary sensory afferents, *Brain Res.*, 248, 223, 1982.
32. **Zieglgänsberger, W. and Bayerl, H.**, The mechanism of inhibition of neuronal activity by opiates in the spinal cord of the cat, *Brain Res.*, 115, 111, 1976.
33. **Zieglgänsberger, W.**, Actions of amino acids, amines and neuropeptides on target cells in the mammalian central nervous system, in *Chemical Transmission in the Brain*, Buijs, R. M., Peyet, P., and Swaab, D. F., Eds., Elsevier Biomedical Press, Amsterdam, 1982, 55 and 297.
34. **Stefano, G. B.**, Comparative aspects of opioid-dopamine interaction, *Cell. Mol. Neurobiol.*, 2, 167, 1982.
35. **Duggan, A. W. and North, R. A.**, Electrophysiology of opioids, *Pharmacol. Rev.*, 35, 219, 1984.
36. **Yoshimura, M. and North, R. A.**, Substantia gelatinosa neurones *in vitro* hyperpolarized by enkephalin, *Nature (London)*, 305, 529, 1983.
37. **Stanzione, P. and Zieglgänsberger, W.**, Action of neurotensin on spinal cord neurons in the rat, *Brain Res.*, 268, 111, 1983.
38. **Vidal, C., Maier, R., and Zieglgänsberger, W.**, Effects of dynorphin A (1-17), dynorphin A (1-13), and D-ala^2-D-leu^5-enkephalin on the excitability of pyramidal cells in CA_1 and CA_2 of the rat hippocampus *in vitro*, *Neuropeptides*, 5, 237, 1984.