

Ryszard Przewlocki

Abbreviations

ACTH	Adrenocorticotropic hormone
CCK	Cholecystokinin
CPA	Conditioned place aversion
CPP	Conditioned place preference
CRE	cAMP response element
CREB	cAMP response element binding
CRF	Corticotrophin-releasing factor
CSF	Cerebrospinal fluid
CTAP	D-Phe-Cys-Tyr-D-Trp-Arg-Thr-Pen-Thr-NH ₂ (μ -opioid receptor antagonist)
DA	Dopamine
DOP	δ -opioid peptide
EOPs	Endogenous opioid peptides
ERK	Extracellular signal-regulated kinase
FSH	Follicle-stimulating hormone
GnRH	Gonadotrophin-releasing hormone
HPA axis	Hypothalamo-pituitary-adrenal axis
KO	Knockout
KOP	κ -opioid peptide
LH	Luteinizing hormone
MAPK	Mitogen-activated protein kinase
MOP	μ -opioid peptide
NOP	Nociceptin opioid peptide
NTS	Nucleus tractus solitarii
PAG	Periaqueductal gray

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PDYN	Prodynorphin
PENK	Proenkephalin
PNOC	Pronociceptin
POMC	Proopiomelanocortin
PTSD	Posttraumatic stress disorder
PVN	Paraventricular nucleus
SIA	Stress-induced analgesia
VTA	Ventral tegmental area

Brief History of Opioid Peptides and Their Receptors

Man has used opium extract from poppy seeds for centuries for both pain relief and recreation. At the beginning of the nineteenth century, Serturmer first isolated the active ingredient of opium and named it morphine after Morpheus, the Greek god of dreams. Fifty years later, morphine was introduced for the treatment of postoperative and chronic pain. Like opium, however, morphine was found to be an addictive drug. In search of a safe analgesic, chemists of The Bayer Company of Germany isolated heroin. Heroin was marketed as a nonaddictive medicine for cough and pain, free of abuse liability, and more potent than morphine. Soon it became clear that heroin was one of the most addictive compounds known, and its use became restricted. Furthermore, Weijlard and Erikson synthesized N-allylnormorphine (nalorphine), a mixed opioid agonist/antagonist, and Lewenstein of Endo laboratories developed naloxone, a potent morphine antagonist. The search for opiate receptors thus began. In 1971, the first attempt to demonstrate an opiate receptor by its capability to bind opiates in a stereospecific manner was performed by the Goldstein group, and in 1973, several other groups followed, e.g., the groups of Pert and Snyder and Terenius and Simon. These groups independently demonstrated the presence of stereospecific opiate binding in the central nervous system, suggesting the existence of specific opiate receptors in vertebrates. In 1972, Collier remarked that he "could not imagine natural receptors existing primarily for molecules of a drug that is foreign for the body." But, at that time, the existence of endogenous ligands for opiate receptors was not considered, given that the administration of the opioid antagonist naloxone to a normal animal produced little if any effect, although the drug was effective in inhibiting the effects of opiates. In the same year, Akil found that electrical stimulation of brain structures, which had been previously identified by the Herz group as sites of opiate analgesic action, induced analgesia that could be inhibited by the opioid antagonist naloxone. The discovery of stereospecific opioid receptors and the observation that brain stimulation induced naloxone-reversible analgesia were strong points in favor of the hypothesis of the existence of endogenous opioids. In 1974, Hughes and Kosterlitz demonstrated that extracts of guinea pig striatum could inhibit electrically induced contractions of the mouse vas deferens, an effect that was antagonized by naloxone. They soon after

identified two opiate-like brain factors, which they named enkephalins (*enkephalos* meaning "in the head"). The factors responsible for these effects proved to be two pentapeptides: Met-enkephalin (Tyr-Gly-Gly-Phe-Met) and Leu-enkephalin (Tyr-Gly-Gly-Phe-Leu). During the International Narcotics Research Conference (INRC) Meeting in Airlie House of Virginia, Eric Simon proposed the name "endorphine" from the term "endogenous morphine," and finally Avram Goldstein proposed to make it shorter by dropping the final "e."

INRC

COMMISSION ON NOMENCLATURE

June 15, 1983

We were appointed in May 1975 to bring forward proposals on nomenclature in the field of opioid peptides. This, our report, has been somewhat delayed by the distractions of research, to say nothing of past difficulties in achieving unanimity, but better late than never! Syd Archer's pointed remarks at the recent banquet at Churchill College prodded us into action.

1. We are agreed that "enkephalin" is the firmly established trivial name for the pentapeptides YGGFL and YGGFM. We recommend that all follow the IUPAC recommendations -- see also J.S. Morley, *Neuropeptides* 1, 231 (1981) -- using [Met]enkephalin and [Leu]enkephalin for the respective pentapeptides.

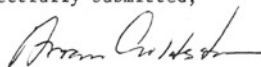
2. We recognize that the originally intended generic term "endorphin" has come to refer specifically to the opioid product(s) of the pro-opiomelanocortin gene. We recommend that this be accepted as a fait accompli.

3. Accordingly, we recommend use of the generic terms "endogenous opioid" and "opioid peptide" as descriptors for the range of gene products with affinities for opioid receptors. We object to "endogenous opiate" and "opiate peptide" as distorting the well accepted meaning of "opiate", which describes products of the opium poppy and related synthetic alkaloids. "Opioid", meaning "like an opiate", is appropriate.

4. In recognition of Nature's design, we recommend the term "opioid receptor" be used in place of "opiate receptor".

5. We recommend that μ , δ , κ , etc. be referred to as multiple types of opioid receptor, analogous to α and β adrenergic receptors, originally called "types" of adrenoceptor by Ahlquist. For further differentiation within a type (e.g., μ_1) we recommend the term subtype.

Respectfully submitted,



Avram Goldstein



Hans W. Kosterlitz

In 1976, Martin and colleagues postulated the existence of *multiple types of opiate receptors*, based on in vivo pharmacological studies in dogs. Thus, the concept of opioid receptor multiplicity arose. This event was followed by the discovery of multiple opioid peptides with high affinity to brain opioid receptors,

including a fragment of β -lipotropin isolated previously from pituitary extracts in C. H. Li's laboratory. Later, after the discovery of enkephalins, Li realized that the peptide he isolated contained Met-enkephalin and named it β -endorphin.

In 1979, dynorphin A was isolated from the porcine brain. Several atypical opioid peptides were found in frog skin, casomorphins derived from milk or exorphins derived from structural proteins. These were usually more resistant to enzymatic degradation than classical opioid peptides. In 1995, nociceptin or orphanin FQ, the endogenous ligand for the orphan opioid-like receptor (ORL1), was isolated and named nociceptin or orphanin FQ. A novel group of peptides was discovered in the brain in 1997 and named endomorphins (endomorphin-1 (Tyr-Pro-Trp-Phe-NH₂) and endomorphin-2 (Tyr-Pro-Phe-Phe-NH₂)) for their characteristic structure and high selectivity for the μ -opioid (MOP) receptor. Endomorphin peptides differ in their amino acid sequences from other known endogenous opioid peptides (EOPs), in which the Tyr residue is followed by Gly; endomorphins are related to the family of the previously discovered opioid peptides, including morphiceptin, hemorphin, and casomorphins, which contain Tyr-Pro residues.

Three members of the receptor family were cloned in the early 1990s, beginning with the mouse δ -opioid receptor (DOP) and followed the MOP and κ -opioid (KOP) receptors. A fourth member of the multiple opioid peptide receptor family (based on sequence homology to opioid receptors), the nociceptin/orphanin FQ (ORL1) receptor, was cloned in 1994 (Table 49.1). This last receptor does not interact with classical opioid ligands, however, and is not strictly opioid in function, although it possesses a nearly 70% sequence homology with opioid receptors. Naloxone, a nonselective antagonist, has a very low affinity for this receptor, and

Table 49.1 Opioid receptor type classification (IUPHAR database)

Current NC-IUPHAR- recommended nomenclature ^a	Previous nomenclature	Presumed endogenous ligands
μ , mu, or MOP	OP ₃	β -endorphin (not selective) Enkephalins (not selective) Endomorphin-1 ^b Endomorphin-2 ^b
δ , delta, or DOP	OP ₁	Enkephalins (not selective) β -endorphin (not selective)
κ , kappa or KOP	OP ₂	Dynorphin A Dynorphin B α -Neoendorphin
NOP	OP ₄	Nociceptin/orphanin FQ (N/PFQ)

^aThe well-established Greek terminology for opioid receptor types using the descriptors, μ (mu), δ (delta) or κ (kappa), is recommended, but the receptor type should be additionally defined as MOP, DOP, KOP, or NOP when first mentioned in a publication

^bNo mechanism for the endogenous synthesis of endomorphins has been identified; their status as endogenous ligands for the μ opioid receptor is tentative

opioid receptor ligands are ineffective. The endogenous ligand for the ORL1 receptor nociceptin does not directly interact with opioid receptors, and its pharmacological action is opposite to opioids in some tests.

Multiple Opioid Systems

Opioid Peptides

Over the last three decades, considerable advances have been made in our understanding of the biogenesis of various EOPs, their anatomical distribution, and functions. It has been shown that EOPs derive from three different precursor proteins: proopiomelanocortin (POMC), prodynorphin (PDYN), and proenkephalin (PENK) (Fig. 49.1). The main groups derived from PENK, PDYN, and POMC are enkephalins, dynorphins, and β -endorphin, respectively. PENK is the source of Met- and Leu-enkephalins and several longer opioid peptides, such as BAM22. The EOPs dynorphin A, dynorphin B, α - and β -neoendorphin, and several larger molecules can be generated from PDYN. POMC is the precursor of β -endorphin, α -endorphin, and several physiologically important non-opioid peptides, such as ACTH and MSH.

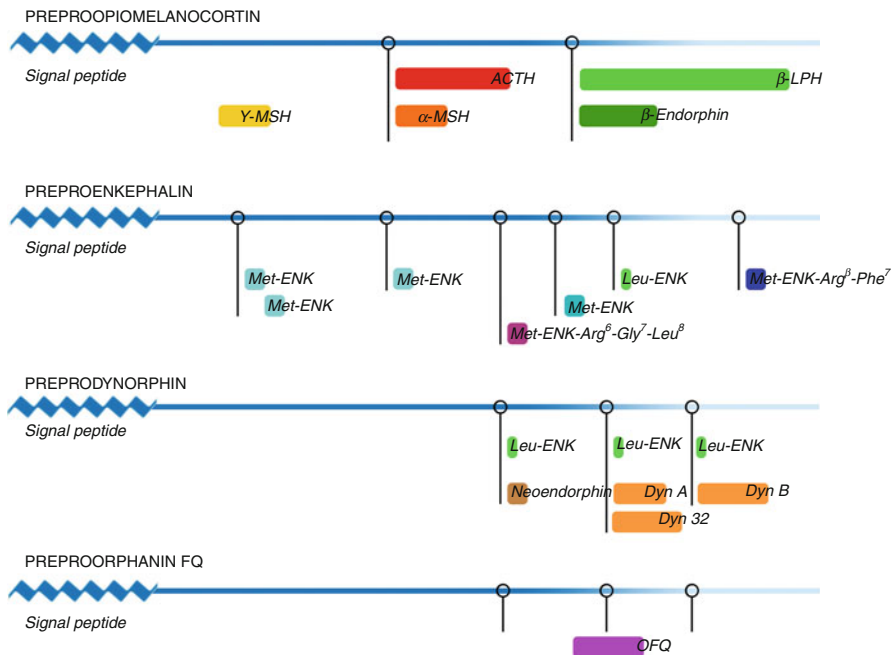


Fig. 49.1 Structural organization of opioid peptides prohormones

Another group that has joined the endogenous “opioid-like” peptide family is the pronociceptin (PNOC) system, comprised of the peptide nociceptin/orphanin FQ derived from PNOC and which is a natural ligand of an orphan ORL1 receptor (NOP receptor). Nociceptin is structurally homologous to dynorphin A. Sequence homologies to EOPs observed at the precursor and receptor levels place nociceptin in the family of opioid peptides.

Opioid Receptors and Their Endogenous Ligands

The discovery of such a large number of EOP ligands makes it unsurprising that multiple classes of opioid receptors were also identified. Three members of the opioid receptor family, DOP, MOP, and KOP, have been cloned. They differ in terms of both their distribution in the brain and their affinity to different opiate drugs and endogenous opioids. The MOP receptor is a morphine-like receptor, and endorphins and endomorphins can act as endogenous ligands. The enkephalins bind to the DOP receptor with great affinity and are therefore considered to be endogenous DOP receptor agonists. Notably, the affinity of β -endorphin binding to MOP and DOP receptors was found to be similar. Dynorphins bind to the KOP receptor and appear to function as its endogenous ligands. Opioid peptide systems generate a plethora of opioid peptides at presynaptic sites. These peptides interact with three opioid receptors. Several subtypes of the opioid receptors have been predicted on the basis of pharmacological studies, and several variants of opioid receptors have been suggested. Notably, the alternative splicing of MOP receptor subtypes has been shown in mice, rats, and humans. The particular MOP receptor domains encoded by various exons of the MOP gene can take part in different mechanisms of MOP activation. Additionally, the efficacy of various MOP ligands was shown to differ in relation to various MOP splice variants, such that different ligands demonstrated differential rank orders of efficacy. Finally, the expression of MOP receptor variants is structure specific, which suggests its potential functional significance. The MOP receptor appears to be highly polymorphic. Recent studies have suggested several polymorphisms in human MOP that may encode opioid receptor variants. The existence of these variants and polymorphisms could have important functional consequences in humans and may contribute to a wide spectrum of opioid effects. Although it is not known how those alternative variants and MOP polymorphisms correspond to the types of pharmacologically defined receptors, their identification may help to explain clinically observed phenomena, such as incomplete cross-tolerance of different MOP receptor agonists. Human genetic studies indicate that individuals carrying one or two copies of the A118G nucleotide substitution in exon1 of the MOP receptor may be at increased risk for opiate and alcohol addictions. Furthermore, a person with the altered MOP receptor allele coding a high affinity MOP receptor can be more vulnerable to stress and more prone to addiction as a consequence of stressful situations. Additionally, the pharmacologically defined subtypes of KOP opioid receptors are also proposed to have functional

significance. For example, the two types of KOP receptor, KOP1 and KOP2, were shown to selectively modulate dopamine and acetylcholine release in the rat neostriatum, respectively.

All three opioid receptors belong to the family of seven transmembrane G-protein-coupled receptors and share extensive structural homologies. A vast majority of opioid peptides do not bind exclusively to one specific opioid receptor type but have some affinity and may interact with other opioid receptors. The existence of several subtypes of opioid receptors has been suggested on the basis of functional studies. The opioid receptors cloned to date constitute a single receptor type, and the proposed subtypes are possibly alternative splicing products. In fact, molecular attempts to identify subtypes of opioid receptors have been undertaken, and the existence of several variants of opioid receptors has been reported. In addition, oligomerization of various opioid receptors generates unique functional properties. It has also recently been demonstrated that there is an association between DOP and MOP receptors and that the occupancy of DOP receptors (by DOP receptor antagonists) enhances MOP receptor binding and signaling activity, which indicates functional interaction between the opioid receptors.

Functional Anatomy of Opioid Peptides and Their Receptors

Opioid Peptides

Proopiomelanocortin System

Opioid peptides differ in terms of their distributions in the brain and periphery. POMC neuropeptides are present in the arcuate nucleus of the mediobasal hypothalamus and in an extensive nerve fiber system originating there, which terminates in many brain regions, e.g., the hypothalamic nuclei, limbic and raphe nuclei, and some pontine nuclei. Moreover, β -endorphin can also be found in the reward system of rodents, i.e., in the medial prefrontal cortex, nucleus accumbens, and ventral tegmental area. In addition, some of these structures may also be innervated by POMC system neurons located in the nucleus tractus solitarii (NTS) of the caudal medulla, which project laterally and also enter the spinal cord. Furthermore, POMC peptides are produced and secreted by endocrine cells of the pituitary, cells of some peripheral tissues, immunocytes, blood mononuclear cells, and mast cells in the skin.

Proenkephalin System

PENK neuropeptides are widespread throughout the central and peripheral nervous systems. They are predominantly found locally in interneurons, but some form longer tract projections. PENK neurons are abundant in the paraventricular nucleus and the nucleus arcuatus of the hypothalamus. A number of PENK neurons exist in limbic structures, e.g., in the hippocampus, septum, and bed nucleus of the stria terminalis. Septal PENK neurons project directly to the amygdala. PENK fibers also extend throughout the bed nucleus of the stria terminalis, projecting to the

paraventricular nucleus and median eminence. PENK neurons have also been found in the spinal cord, cranial sensory systems, and in the major pain-signaling network. PENK-containing cells are also present in the adrenal medulla.

Prodynorphin System

PDYN neuropeptides are present in the magnocellular neurons of the paraventricular nucleus of the hypothalamus, where they are costored with vasopressin. In addition, these peptides have been found in the NTS, an area usually associated with the regulation of vagal and other autonomic functions. Furthermore, PDYN neurons exist in the limbic system and in areas of the spinal cord involved in the transmission of nociceptive stimuli. Cells expressing PDYN mRNA are also present in a subpopulation of anterior lobe gonadotrophs, intermediate lobe melanotrophs, posterior pituitary pituicytes, and in the adrenals.

Opioid Receptors

Opioid receptors are differentially distributed in the brain. Moderate densities of MOP and KOP receptors have been observed in the periaqueductal gray, locus coeruleus, substantia nigra, ventral tegmental area, raphe nuclei, and the NTS, while low DOP receptor binding is present in the substantia nigra and the NTS. Furthermore, opioid receptor-containing neurons can be found in the limbic system, where they may mediate the emotional component of stress, and in areas of the spinal cord involved in the transmission of nociceptive stimuli. Dense MOP receptor binding is present in most hypothalamic nuclei. Hypothalamic nuclei, however, show little DOP receptor binding. In contrast, dense DOP receptor binding is detected in the median eminence. All three opioid receptors are present in the median eminence of the monkey, whereas KOP receptors predominate in the rat median eminence, which is consistent with the distribution of opioid receptors in the posterior lobe of the pituitary in the monkey and rat. Localization of opioid receptors in the hypothalamic nuclei is in line with the effects of opioids on the neuroendocrine system. Opioid receptors have also been found in the peripheral nervous system and are expressed by various immune cells.

Molecular and Cellular Mechanisms of Opioid Peptide Action

Opioid receptors are G-protein-coupled receptors. EOPs acting on opioid receptors via Gi/Go classes of G proteins inhibit cyclic AMP (cAMP) formation. Furthermore, the activation of opioid receptors leads to inhibition of Ca²⁺ current and the enhancement of potassium conductance, resulting in a suppression of cellular activity. This results in inhibition of neuronal excitability and synaptic transmission and represents a classic cellular mechanism of opioid action. Alternatively, opioids exhibit excitatory activity in the brain via both diminution of inhibitory transmission and by direct excitatory activities. In specific subpopulations of cells, opioids

stimulate inositol lipid hydrolysis and the production of IP₃ and diacylglycerol, which could lead to the mobilization of intracellular Ca²⁺ stores and an increase in intracellular Ca²⁺ concentrations. Thus, opioid exposure could further activate the mitogen-activated protein kinase (MAPK). The most abundant members of the MAPK family in neurons are extracellular signal-regulated kinases (ERKs). The activation of the ERK/MAPK pathway could further activate transcription factors, including the Ca²⁺/cAMP response element binding (CREB) protein. CREB is a transcription factor that binds to cAMP-responsive elements (CREs) in the promoter region of several target genes, regulating their expression. Thus, EOPs and exogenous opioids modulate gene expression, evoke a cascade of genomic changes, and affect cellular plasticity, among other basic neurobiological processes.

Genetic Alterations of Opioid Systems: Searching for Endogenous Opioid System Functions

Opioid Peptide Knockout Mice

β-Endorphin

EOP knockout (KO) mice have been generated by gene targeting to inactivate opioid genes in the CNS. These lines were produced to study the role of EOPs in physiology and behavior and to predict their importance in human physiology and pathology. Mice lacking β-endorphin showed normal locomotor behavior and lower sensitivity to nociceptive stimuli. They displayed normal anxiety but were less sensitive to anxiety-inducing stimuli. Some studies demonstrated that alcohol consumption was reduced in β-endorphin knockouts. In contrast, other research showed that these animals displayed unchanged sucrose and ethanol self-administration. Also unchanged were ethanol preference and opioid-induced conditioned place preference (CPP). Such data suggest that β-endorphin may not be involved in mechanisms of drug reinforcement. Furthermore, stress-induced ethanol consumption was clearly inhibited in β-endorphin knockouts, indicating a role for EOP in the stress effect. β-endorphin-deficient mice displayed slightly higher corticosterone levels following social conflict stress, suggesting that this peptide may play an inhibitory action on the hypothalamo-pituitary axis (HPA) during stress.

Proenkephalin

Mice lacking the PENK gene exhibited decreased locomotor activity and showed enhanced anxiety and aggression in novel environments. These mice exhibited hyperalgesia during the hot plate test, indicating a role of enkephalins in central antinociceptive processing. Enkephalins also appeared to be involved in hedonic behavior, given that naloxone-induced reduction of sucrose consumption in wild-type mice was absent in PENK KO mice. Alternatively, opiate-induced reinforcement and ethanol self-administration were not modified in these mice. Furthermore, nicotine withdrawal, stress-induced hyperthermia, and footshock stress-induced

freezing behaviors were attenuated in PENK KO mice. Such findings indicate that endogenous enkephalin neurons may regulate hedonic behavior and play a role in the control of stress-induced behaviors, pain, anxiety, and aggression.

Prodynorphin

Mice lacking the PDYN gene showed normal locomotor activity, although they also displayed heightened behavioral anxiety levels. PDYN KO mice are hyperalgesic in the spinal nociceptive test (tail-flick test). These data may suggest involvement of dynorphin in spinal nociceptive and pain mechanisms. Furthermore, although nicotine produced a normal CPP in PDYN KO mice, enhanced nicotine self-administration was observed in these animals. PDYN mutants showed an absence of cannabinoid-induced conditioned place aversion (CPA), indicating that endogenous dynorphins may mediate the dysphoric effects of cannabinoids. Alternatively, PDYN mutant mice were impaired in the potentiation of cocaine-induced CPP triggered by stress and in stress-induced reinstatement. Notably, basal extracellular dopamine (DA) levels were decreased, and cocaine-stimulated release of DA in the ventral striatum was reduced in PDYN KO mice. Stress-induced hyperthermia was also reduced in PDYN KOs. These studies suggest an interaction between stress, pain, addiction and endogenous PDYN system.

Opioid Receptor Knockout Mice

MOP

Basal locomotor activity of MOP KO mice was slightly lower relative to wild-type mice, and psychostimulant-induced locomotor activity was also reduced in these animals. These mice showed increased sensitivity to heat stimuli, suggesting that the MOP receptor is involved in a basic level of thermal nociception. MOP KO mice were insensitive to the analgesic effects of morphine, demonstrating that the receptors are essential targets of opioid analgesia. MOP KO mice also displayed a decreased motivation to eat. Furthermore, MOP mutant pups showed lower maternal attachment behavior. Such studies may suggest that MOP KOs have deficits in basic motivational behaviors. A plethora of studies show a critical role of the MOP receptor in mediating natural rewards. This receptor is also required for the rewarding effects of opioids. But the rewarding effects of various drugs of abuse were also diminished in MOP KO mice. Furthermore, behavioral and hormonal responses to various kinds of acute and chronic stresses were diminished in MOP KO mice, indicating that MOP may play a facilitatory role in emotional responses to stress. Furthermore, these KOs show reduced adaptive responses to various drugs of abuse, indicating that the MOP receptor can play a role in drug addiction.

DOP

Basal locomotor activity of DOP KO mice did not differ from that of wild-type mice, whereas the locomotor effect of psychostimulants was enhanced. DOP mutants showed an increase in anxiety- and depression-like behavior. Basal nociceptive

sensitivity was unaffected by disruption of the DOP receptor gene. DOP KO mice demonstrated normal opioid reward behavior, but disrupted cue-induced drug association learning. DOP KO mice exhibited an increased response to stress. Thus, the DOP receptor does not appear to be involved in the reinforcing effects of opioids, although it may facilitate both appetitive and aversive place conditioning.

KOP

KOP KO mice displayed normal locomotor activity and normal anxiety levels. They did not show changes in sensitivity to nociceptive stimuli, such as pressure, heat, and acute inflammation, but were hyperalgesic to visceral stimuli. KOP receptor gene knockout resulted in an absence of an analgesic effect following treatment with the KOP receptor agonist U50,488 H but did not modify the analgesic efficacy of other opioids. Deletion of the KOP receptor has inconsistent effects on the rewarding properties of drugs of abuse; it did not modify opioid-conditioned rewards but reduced ethanol-conditioned and enhanced cannabinoid-conditioned rewards. Notably, stress potentiated the rewarding effects of ethanol and cocaine in KOP KO mice, indicating that KOP receptor signaling counteracts reward processing upon stress. Alternatively, the aversive motivational effects of KOP receptor agonists were inhibited in KOP KO mice, and the effects of opioid dependence and withdrawal were reduced. This finding supports the involvement of KOP receptor in counteracting reward processes under stressful conditions.

Notably, none of the phenotypes of EOPs knockout mice clearly overlap with the respective opioid receptor mutants. Nevertheless, a few functional associations can be found. MOP KOs and PENK KOs display hypolocomotor activity and higher sensitivity to heat, suggesting that some PENK-derived peptides may regulate basal locomotion and control thermal nociceptive processing via the MOP receptor. Moreover, DOP, PENK, and PDYN KOs each exhibit high anxiety levels. Furthermore, the majority of opioid knockouts (excluding DOR KOs) reduce various reactions to stress. Thus, future studies using more selective, opioid inducible knockout mice are necessary to achieve a clearer picture of endogenous opioids involvement in physiology and behavior.

Involvement of EOPs in Behavioral and Physiological Responses

EOPs play an important role in a variety of behavioral and physiological processes, including nociception, reward, learning, memory, and mood. They are also involved in the modulation of endocrine, cardiovascular, respiratory, gastrointestinal, autonomic, and immune functions.

EOPs in Antinociceptive Processes

EOP systems are strongly implicated in antinociceptive processes and have been found in the brain regions involved in nociception. Opioid peptides deriving from

POMC are present in the nucleus arcuatus of the mediobasal hypothalamus. An extensive nerve fiber system originating in the arcuate nucleus terminates in many areas of the brain that have been implicated in nociception, e.g., the hypothalamic nuclei, limbic and raphe nuclei, and some pontine nuclei. In addition, some of the structures conveying nociceptive stimuli are innervated by POMC-containing neurons located in the NTS of the caudal medulla, which project into the spinal cord. Lesioning the arcuate nucleus of the hypothalamus, the primary location of β -endorphinergic cells, reduces the antinociceptive effect of electrical stimulation of the periaqueductal gray. β -endorphin administered both to the lateral brain ventricle and intrathecally evokes strong antinociceptive action, and these effects are blocked by naloxone.

PENK-containing neurons have been found in the spinal cord. A number of PENK neurons exist in limbic system structures (e.g., the hippocampus, septum, and bed nucleus of the stria terminalis) and may be involved in the emotional component of pain. PENK-derived peptides are susceptible to proteolytic action, so their central antinociceptive action is short lived. Enkephalin analogues are resistant to proteolytic enzymes and show strong antinociceptive activity. The augmentation of enkephalin action has been observed following the administration of inhibitors of enzymatic degradation of these peptides. A number of studies have indicated that longer PENK-derived peptides, bearing the enkephalin sequence, evoke stronger analgesic action in comparison to enkephalins themselves. However, enkephalin analogues poorly penetrate the blood-brain barrier following peripheral administration, which is a serious obstacle to their potential use in pain treatment.

PDYN neurons are also widely distributed in brain areas associated with nociception. A lack of antinociceptive action of dynorphin following its administration to the lateral brain ventricle and antinociceptive activity following intrathecal injection has been reported. The antinociceptive action of dynorphin in the spinal cord is hindered by its neurotoxic effects. They may result from the non-opioidergic mechanism of action of PDYN peptides mediated by NMDA glutamatergic receptors. Non-peptide KOP receptor agonists also possess some antinociceptive activity. However, the effect is much weaker than that evoked by similar levels of MOP or DOP receptor agonists.

Stress-Induced Analgesia

Akil and her colleagues were the first to report that exposure to footshock stress resulted in potent antinociception in rats. Furthermore, they found that the opioid antagonist naloxone partially reversed this analgesia. A number of subsequent studies showed that various kinds of stress resulted in antinociception, an effect that, under certain conditions, appeared to be mediated by EOPs. The analgesic opioid effects were comparable with the stress effects induced in rodents by analgesic doses of morphine, but the former usually occurred more rapidly and were shorter lasting. Additionally, there are data showing cross-tolerance between morphine and stress-induced analgesia (SIA). In rodents, mild stressors usually produced opioid-dependent analgesia, whereas severe stressors induced analgesia

independent of opioids. Thus, stress severity plays an important role in determining the neurochemical basis of SIA. Increasing the severity, duration, or intensity of stress causes a shift from opioidergic to non-opioidergic mechanisms in the mediation of the resultant analgesia. Furthermore, it has also been found that opioidergic analgesia following stress in mice depends on genetic factor(s). The potential involvement of the brain's β -endorphin systems in SIA has to be seriously considered. Early evidence showed that hypothalamic and midbrain β -endorphin levels were changed upon footshock stress. Stress was shown to evoke naloxone-sensitive analgesia and a marked decrease in β -endorphin levels in the plasma, hypothalamus, and both pituitary lobes. Lesions of the arcuate nucleus of the hypothalamus reduced opioid-mediated SIA but, notably, enhanced a form of footshock SIA that was not blocked by injections of the opiate receptor blocker naltrexone. Thus, arcuate lesions led to compensatory changes in the non-opioid analgesic system, resulting in enhanced non-opiate-mediated SIA. Furthermore, naloxone injections into the periaqueductal gray blocked analgesia in defeated mice. Moreover, micro-injections of antibodies against β -endorphin into the midbrain periaqueductal gray attenuated the antinociception elicited by electroacupuncture. Another study suggested the potential role of β -endorphinergic cells within the NTS in SIA. Electrical stimulation of this structure evoked opioid-mediated analgesia in the rat. Such results may indicate that β -endorphin-containing neuronal cells may be involved in SIA, at least in part. Additional studies of mice with a selective deficiency of β -endorphin clearly demonstrated the critical role of β -endorphin in SIA.

There is some evidence suggesting a role of brain PENK-derived peptides in SIA. Forcing mice to swim in cold water produced opioid-induced analgesia that was blocked by intrathecal pretreatment with antiserum to Met-enkephalin. The study suggests that SIA is mediated by spinal Met-enkephalin. Inhibiting PENK degradation has been reported to potentiate this phenomenon. When the enkephalinase inhibitor thiorphan was applied intraventricularly in mice, it evoked a dose-related potentiation of both the peak effect and the duration of SIA following exposure of rats to inescapable footshock. Moreover, it was shown that adrenal demedullation abolished the analgesic response, suggesting the participation of circulating PENK peptides. Unexpectedly, however, enkephalin-deficient KO mice exhibited normal SIA.

A recent study in mice demonstrated that psychological stress-induced analgesia was fully antagonized by the selective KOP receptor antagonist norbinaltorphimine, but this compound had no effect on footshock-induced antinociception. It has also been recently demonstrated that swim stress-induced analgesia was abolished in PDYN KO mice. The KOP receptor antagonist blocks the SIA elicited by the repeated forced swim test in mice, and PDYN KO mice fail to display SIA following the forced swim test. Such results provide evidence that the PDYN system, as well as KOP receptors, may be involved in the mechanisms of certain kinds of stress.

Thus, EOPs' role in antinociception depends on the type of stress and variables studied; some stressors activate EOP systems but others do not. Moreover, when EOPs are involved, there is frequently interaction with other neuronal systems.

Therefore, the EOP-mediated analgesic effects of stress are complex and difficult to characterize. Nevertheless, reactions to certain kinds of stress have been shown to be mediated by specific neuronal opioid systems.

Placebo Analgesia

Several studies have suggested that EOP systems mediate analgesia evoked by placebo treatment in humans. Placebo analgesia is apparently reversed by the opioid antagonist, naloxone. The study therefore suggests that released EOPs mediate placebo-induced analgesia. Results of several studies have demonstrated placebo-induced activation of opioid neurotransmission in neural networks in areas responsible for pain transmission, emotion and motivation, including the following regions: the cortex (orbitofrontal and dorsolateral), rostral anterior cingulate, insula, thalamus, periaqueductal gray, hypothalamus, amygdala, and nucleus accumbens. The placebo-responding brain network and EOP system are critical parts of neuronal processes that mediate placebo analgesia in humans.

Acupuncture Analgesia

Some studies has demonstrated that naloxone partially reversed the analgesic effect of electroacupuncture and indicated that electroacupuncture may evoke release of an EOP in the central nervous system. Extensive study by Han and his associates showed that electroacupuncture induced release of opioid peptides in animals and humans. In fact, electroacupuncture increased cerebrospinal fluid (CSF) levels of β -endorphin in chronic pain patients. Notably, low-frequency acupuncture appears to be associated with the release of enkephalins and to be mediated by MOP and DOP receptor activation, whereas high-frequency electroacupuncture stimulates the release of dynorphins in both rodents and humans and appears to be mediated by KOP receptors.

EOPs and Reward

EOP systems play a key role in modulating reward, mood, and in regulating neural hedonic homeostasis. Opioids, including EOPs, elicit CPP. For example, animals are inclined to choose an initially non-preferred environment associated with previous administration of an opioid. In contrast, the opioid antagonist naloxone and KOP receptor agonists evoke CPA. A recent study suggests that enkephalins, rather than β -endorphin, mediate the “opioid” part of the basal reward state. This was hypothesized because PENK KO mice failed to show an aversion to naloxone, whereas β -endorphin-deficient mice displayed CPA to the opioid antagonist. PENK-derived peptides acting on MOP and DOP receptors produce rewarding actions in several brain regions, including the ventral tegmental area and nucleus accumbens. In contrast, dynorphins appear to play a more general role as transmitters that limit drug-induced hedonic states.

PENK- and POMC-containing neurons located in the ventral tegmental area (VTA) of the dopamine system modulate activity of dopaminergic neurons.

The increase in their activity may indirectly enhance dopamine release. The administration of opioids into the VTA has been shown to enhance dopamine turnover in both the nucleus accumbens septi and the prefrontal cortex. Alternatively, the administration of opioid antagonists prevented stress-induced variations in dopamine turnover. Microinjection of enkephalin analogues into the VTA produced an increase in spontaneous motor activity, and this effect was antagonized by intra-accumbens or peripheral administration of dopamine receptor antagonists. The enkephalin-evoked increase in motor activity was associated with an increase in dopamine metabolism in the mesolimbic system and appeared to be mediated through the MOP receptor. These receptors are most likely located on local GABAergic interneurons in the VTA, given that GABA antagonists can antagonize the effects of DAMGO, a selective MOP agonist. Thus, the effects of opioids appear to be mediated via disinhibition of GABA neurons, thereby releasing the dopamine neurons from tonic GABA inhibition. Under normal conditions, the tonic activity of EOPs is minimal or absent, allowing profound GABA inhibition. During activation however, EOPs inhibit GABA interneurons, resulting in an activation of dopaminergic transmission. DOP and KOP receptors also appear to be involved in the modulation of VTA dopaminergic neuron activity. The activation of a DOP receptor in the VTA appears to facilitate brain reward. Conversely, KOP receptor agonists induce aversive effects, which may be mediated by the modulation of dopaminergic cell activity, at least in part.

Feeding Behavior

Opioids are said to influence the palatability of food and its relative reward value. Administration of opioid agonists increases and antagonists decreases food intake. Administration of the nonselective opioid antagonist naloxone decreases food intake in rodents, indicating involvement of multiple opioid receptors and EOPs. EOPs can influence feeding behavior by acting on central opioid receptors and may partly determine the rewarding aspects of eating. MOP and DOP, rather than KOP, receptors in the nucleus accumbens shell and VTA are postulated to mediate this effect of EOPs. Furthermore, secretion of enkephalins and dynorphins in the hypothalamus is modulated by the nutritional value of food. These observations argue that altered EOP activity may elicit food cravings, which in turn may influence food consumption. Support for this opioidergic theory of food cravings is provided by various clinical conditions (bulimia nervosa, anorexia nervosa, and eating-induced obesity), which are associated with altered EOP levels, intensified food cravings, and increased or decreased food intake. Furthermore, food deprivation results in alterations of EOP levels in the rodent brain and pituitary. The data support the notion that EOPs and MOP receptors play a role in the modulation of food intake. Taken together, MOP receptors, PENK-deriving peptides, and β -endorphin appear to modulate the motivational properties of food.

Locomotor Activity

The inhibition of opioid receptors by opioid antagonists had either no effect on or decreased motor activity. Naloxone treatment produced a decrease in locomotor activity and rearing in rats exposed to a new environment. The results may suggest the activation of EOP systems, which have been shown to underlie changes in exploratory behavior. Naloxone also inhibited both the emotional and motor components of stress, suggesting the involvement of EOPs in the behavioral responses. Notably, naloxone potentiated stress-evoked freezing in rats.

Administration of enkephalin analogues into the VTA of rats has been shown to increase spontaneous motor activity, and daily injection into the same area resulted in a progressive enhancement in the motor stimulant effect. It is therefore possible that the VTA's endogenous enkephalin system may participate in motor sensitization. Mice exhibited marked suppression of motility when they were placed in the same cage in which they had previously received electric shock, an effect that was associated with a decrease in striatal enkephalinergic activity. Inhibition of enkephalin degradation by treatment with thiorphan and bestatin attenuated the conditioned suppression of motility. This effect was antagonized by naloxone, indicating that it was mediated by an opioid receptor. These results suggest that attenuation of the conditioned suppression of motility evoked by thiorphan and bestatin may be directly proportional to the increase in endogenous striatal enkephalins.

Respiration

It is well documented that opioids and EOPs influence respiration. Local administration of naloxone on respiratory neurons increases while inhibition of enkephalin metabolism decreases their firing rate. The same study strongly suggests direct involvement of EOP in modulation of respiratory center in the brain. Rats exposed to inescapable footshock displayed an increased respiratory rate, and naloxone potentiated the footshock-induced increase in ventilation. These results strongly suggest that the induction of respiratory functions leads to activation of EOPs and the release of endogenous opioids as a compensatory mechanism that prevents excessive stimulation of respiration. Alternatively, pathological effects of EOP on breathing mechanisms may result in respiratory depression and sudden infant death syndrome.

Cardiovascular Effects

EOPs are present in the CSF and the cerebrovascular bed, and opioid receptors have been found in cerebral perivascular nerves. Their activation may directly modulate the function of vasoregulatory mechanisms involved in the control of cerebrovascular tone. Furthermore, EOPs have been found in cardiac tissue, and EOPs of myocardial origin have recently been shown to play a role in the regulation of heart

functions. PENK synthesis has been demonstrated in the isolated rat heart, and PDYN expression has been observed in cultured rat myocytes. Under resting conditions, EOPs do not appear to play an important role in the regulation of the cardiovascular system, but they become important under stress. Restraint stress evoked an increase in heart rate, blood pressure, and plasma catecholamine levels in rats. In situations of severe stress, however, opioid blockade increased ambulatory blood pressure in humans. These observations suggest that opioidergic mechanisms inhibit blood pressure responses to stress.

In the brain, POMC neurons are involved in the control of the NTS, the structure known to participate in the control of cardiac function. Intravenous or intracerebroventricular administration of β -endorphin, as well as injection into the NTS, has been shown to decrease blood pressure. Studies on cardiovascular responses to centrally administered PENK products are currently contradictory and provide little insight into the physiological role of central PENK in cardiovascular functions. However, adrenal PENK peptides released by the stimulation of the splanchnic nerve may induce bradycardia and hypotension, effects that were reported in reserpinized dogs. Dynorphin decreases blood pressure and produces bradycardia when applied intravenously or into the cisterna magna. In contrast, application of dynorphin into the NTS or cerebral ventricles does not alter cardiovascular function. PDYN peptides appear to modulate the release of vasopressin from the posterior lobe of the pituitary and may regulate diuresis in this way. In fact, KOP receptor agonists are powerful diuretics. Therefore, PDYN peptides and KOP receptors may influence the cardiovascular system by regulating diuresis. In humans, mental stress affects blood pressure and increases plasma levels of various EOPs. Subjects responding to stress with a low increase in blood pressure had high levels of β -endorphin, whereas those who responded to stress with a high increase in blood pressure had elevated levels of dynorphin and Met-enkephalin. Pretreatment with naloxone increased blood pressure in low responders but not in high blood pressure responders. Notably, naloxone decreased the blood pressure response in hypertensive subjects with acute stress-induced increases in blood pressure, suggesting the effects of some EOPs, possibly PDYN- or PENK-derived peptides, on blood pressure in hypertensive patients.

It is likely that some EOPs may counteract the cardiovascular effect of tachycardia and increased blood pressure, while others may be involved in hypertensive pathology. In contrast, EOPs appear to mediate cardiovascular depression, which occurs in response to severe stress. In fact, a number of studies have demonstrated that naloxone reverses the hypotension induced by most cardiovascular shock states.

Thermoregulation

Several opioid agonists, when administered directly into the hypothalamus, produce hyperthermia in rats via MOP opioid receptors. There is also indirect evidence of β -endorphin involvement in the hypothalamic mechanisms of the development

of fever and hyperthermia. In anesthetized rabbits, microinjection of β -endorphin in the preoptic/anterior hypothalamus resulted in the elevation of body temperature. It has been suggested that β -endorphin reduces the sensitivity of hypothalamic neurons to high ambient temperature. This reduction is hypothesized to result in increased peripheral vasoconstriction, inhibition of evaporative heat loss, and modification of behavioral thermoregulation, resulting in the elevation of body temperature. Plasma β -endorphin responses under three exercise-thermoregulatory stress conditions were measured in humans during stationary upright cycling. The β -endorphin response pattern closely paralleled rectal temperature changes under all conditions. These data suggest that conditions of increasing body temperature caused by exercise are associated with increased peripheral β -endorphin concentration. It has also been reported that sauna-induced fevers result in an increase in β -endorphin levels in normal volunteers. This report also describes the changes in plasma β -endorphin levels in cancer patients suffering from whole-body hyperthermia. The results show that there is a linear relationship between hyperthermia and the quantitative rise in plasma β -endorphin levels. In contrast, KOP receptor activation and injection of dynorphin into the hypothalamus resulted in hypothermia. Adler and his group proposed that a balance between MOP and KOP receptor activation by specific EOPs controls temperature homeostasis.

Involvement of EOPs in Modulation of the Hypothalamo-Pituitary-Adrenal (HPA) Axis

EOPs are known to be synthesized in and released from endocrine organs and hypothalamic neurons involved in the regulation of hormone secretion.

EOP-containing neurons have been found in regions involved in hormone regulation, e.g., the hypothalamus, pituitary, and adrenals. Notably, most EOPs are usually not tonically active, and therefore, opioid antagonists have little or no effect during homeostasis. Alternatively, EOP systems are activated by stressful stimuli. EOPs are closely associated with "classical" stress hormones, including adrenocorticotrophic hormone (ACTH), corticotrophin-releasing *factor* (CRF), and adrenaline. Dynorphins are costored with CRF in hypothalamic neurons. β -endorphin and ACTH are produced from the same prohormone molecule, POMC, and are cosecreted from pituitary corticotrophs and arcuate neurons in the brain. Enkephalins are synthesized and coreleased with adrenaline from the adrenals. Corticotrophs in the anterior pituitary react to different types of acute stress by enhancing the release of β -endorphin and ACTH in animals and humans. This release increases the rate of POMC synthesis and processing in the anterior lobe. Furthermore, POMC biosynthesis appears to be under the negative control of adrenal steroids, given that adrenalectomy induces an increase in hypothalamic POMC mRNA levels.

Several studies have clearly indicated that stressful stimuli enhance PENK gene expression in cells localized of the parvocellular region of the hypothalamic

paraventricular nucleus (PVN). Thus, the activity of hypothalamic PENK neurons may play a significant role in hormonal regulation. PENK is also present in the adrenals, and the biosynthetic activity of adrenal PENK cells increases upon stress, compensating for the enhanced release of PENK-derived peptides into the blood. In addition, plasma Met-enkephalin may derive not only from the adrenals but also from sympathetic peripheral neurons, at least in rodents.

The data strongly suggest the involvement of EOPs in the modulation of HPA responses. Enkephalins and low doses of β -endorphin were shown to stimulate CRF release from the hypothalamus *in vitro* in a naloxone-reversible fashion. An increase in CRF mRNA levels in the PVN and increased plasma ACTH concentrations were observed *in vivo* following the intraventricular injection of moderate doses of β -endorphin in rats. Both effects were mediated via opioid receptors. These results suggest that the injection of β -endorphin increases neuronal activity and the biosynthesis of CRF in the PVN, which results in an increase in CRF secretion and finally the secretion of ACTH. Alternatively, high doses of β -endorphin administered intraventricularly inhibited both basal and stimulated CRF release. Furthermore, intraventricular dynorphin induced a dose-related inhibition of CRF secretion into the hypophyseal portal circulation of rats, an effect that was antagonized by naltrexone, suggesting the involvement of opioid receptors. Moreover, both KOP and MOP (but not DOP) receptor agonists inhibited the stimulated release of CRF from rat hypothalamic cells *in vitro*, the effects being specifically reversed by opioid antagonists. Notably, naltrindole, a selective DOP receptor antagonist, clearly enhanced basal and stimulated CRF release. Thus, both *in vivo* and *in vitro* studies suggest that MOP and KOP receptors (but not DOP receptors) mediate the inhibitory effect of opioids on stimulated CRF release from the rat hypothalamus. Alternatively, CRF appears to be a potent secretagogue of the three major endogenous opioid peptides (β -endorphin, Met-enkephalin, and dynorphin), acting via specific CRF receptors and stimulating opioidergic neurons in the hypothalamus. Notably, CRF neurons seem to control EOP release tonically, given that the application of the CRF receptor antagonist, α -helical CRF9-41, lowered the rate of basal release of both β -endorphin and Met-enkephalin. Thus, a complex relationship appears to exist between the EOP and CRF systems.

Opioids regulate levels of luteinizing hormone (LH) and follicle-stimulating hormone (FSH). Acute opioid administration decreases plasma LH levels due to an inhibitory modulation of gonadotrophin-releasing hormone (GnRH) discharge from hypothalamic neurons. Naloxone administration in animals and humans produces an increase in LH and FSH levels. It has been shown that EOPs released within the hypothalamus inhibit GnRH and LH secretion and that this results in an inhibition of ovulation. A study on the involvement of EOPs in the direct control of the neuroendocrine mechanism modulating gonadotrophin secretion suggested the involvement of POMC and PDYN (but not PENK) neurons in the control of LH release. The data also strengthen the notion that dynorphin may play a role in reproductive functions. PDYN mRNA levels in posterior pituitary melanotrophs were upregulated three- to fourfold in the intermediate lobes of postpartum females when compared to pregnant or nonpregnant female rats. Alternatively, estrogen

treatment and stress both enhanced PENK gene expression in the ventromedial hypothalamus, and PENK expression in this region is associated with estrogen and progesterone concentrations during the estrous cycle.

Autonomic Nervous System

Circulating EOPs released from the pituitary and adrenals appear to modulate functions of the autonomic nervous system. An early study showed that tachycardia induced by stimulation of the accelerans nerve was reduced by ethylketocyclazocine, a preferential KOP receptor agonist. Moreover, it has been shown that KOP and DOP (but not MOP) receptor types are located on sympathetic nerves in isolated guinea pig atria. In several isolated arteries, KOP and DOP receptor agonists depressed the response to sympathetic stimulation. Another study in humans provided evidence that DOP receptors and possibly enkephalins may influence autonomic sympathetic reactivity. The selective DOP receptor agonist deltorphin failed to modify basal plasma levels of noradrenaline in control rats but completely suppressed insulin-evoked noradrenaline elevation and the release of noradrenaline elicited by cold stress. These findings provide evidence that DOP receptors and possibly enkephalins may control and regulate autonomic sympathetic output.

Immune System

Opioid peptides modulate the function of immune cells involved in host defense and immunity. Opioids produced by immune cells have paracrine and autocrine sites of action and demonstrate functional activities that are in part similar to cytokines. Several studies indicate that opioid receptors expressed by immune cells are the same as or similar to neuronal receptors, particularly KOP and DOP receptors. Some *in vitro* studies have suggested that EOP enhances immune responses, while other reports reached the opposite conclusion.

Enkephalins and β -endorphin are able to regulate many functions of granulocytes, mononuclear cells and T cells, in a manner similar to cytokines. It was found that stress resulted in the release of EOPs and suppressed natural killer cell cytotoxicity. Another study demonstrated that heat stress-induced immunosuppression during pregnancy was most likely mediated in a naloxone-dependent manner by placental β -endorphin release into the blood. Met-enkephalin itself affected the immune responses to stress by decreasing natural killer cell activity, the plaque-forming cell response, and by enhancing phagocytic activity.

The lymphoid organs are innervated by the autonomic nervous system, and there is a growing body of evidence that this system can have immunomodulatory effects. It appears that EOPs may influence peripheral noradrenergic nerves and noradrenergic innervation of lymphoid organs. Alternatively, there is direct evidence that

immune system cells can produce EOPs. The concentrations of β -endorphin in splenocytes, peripheral blood mononuclear cells, and lymph node cells were significantly increased following exposure to stress. It is likely that in situations such as inflammation, immunocytes may release EOPs and, through paracrine or direct communication, mediate peripheral effects.

EOPs Functions and Implications for Behavior and Human Pathology

Pain Control

Role of EOPs in Inflammatory Pain

The effects of opioids in animal models of inflammatory pain have been studied in great detail. In general, the antinociceptive potency of opioids is greater against various noxious stimuli in animals with peripheral inflammation than in control animals. However, this increase in antinociceptive potency is not the same for agonists of different opioid receptor types. Inflammation-induced enhancement of opioid antinociceptive potency is characteristic predominantly of MOP rather than KOP or DOP receptors.

EOP systems are well known to play important roles in pain mechanisms. The central β -endorphin system is activated by prolonged noxious stimulation. Injection of formalin into the rat paw caused an increase in β -endorphin levels in the periaqueductal gray (PAG), thalamus, and ventromedial hypothalamus. Alternatively, intraventricular administration of an antibody against β -endorphin potentiates formalin-evoked hyperalgesia. Several studies have demonstrated profound alterations in the spinal PDYN system, which occur in conditions of peripheral inflammation and chronic arthritis. Many forms of peripheral inflammation induce a dramatic upregulation of PDYN biosynthesis in nociceptive neurons of the spinal dorsal horn, which parallels the behavioral hyperalgesia. The most likely interpretation of this fact is that spinal dynorphin systems react to noxious stimulation by enhancement of their activity. In contrast, the majority of studies have found rather small changes in PENK peptide levels in the spinal cord upon peripheral hind limb inflammation.

Experimental data clearly demonstrate that opioids are able to inhibit nociception arising in inflamed tissues by acting locally on peripheral targets, presumably via opioid receptors on sensory nerves. The expression of opioid receptors in dorsal root ganglion neurons and the axonal transport of these receptors to the periphery are enhanced by peripheral inflammation.

EOPs, including β -endorphin and enkephalins, are released under noxious conditions from immune cells, a major source of EOP in an inflamed tissue. These and related peptides activate peripheral opioid receptors and induce analgesia by inhibiting the activity of peripheral sensory nerves. Although the mechanism of opioid peptide release from immunocytes is not fully understood, there is an indication that this process involves cytokines and CRF.

Role of EOPs in Neuropathic Pain

Neuropathic pain is defined as pain caused by an injury or dysfunction in the peripheral nerves or central nervous system. The endogenous dynorphin system changes under various conditions associated with neuropathic pain following damage to the spinal cord or peripheral nerve injury. The increase in PDYN-derived peptides has been observed in local spinal interneurons and in neuronal projections ipsilateral to the site of injury. In fact, the time and site of dynorphin level increase in the spinal cord following peripheral nerve injury appears to correlate with the appearance of neuropathic pain. Moreover, an intrathecal injection of dynorphin A to mice induced allodynia lasting several days. Another study has shown that the injection of anti-dynorphin antibody reversed hyperalgesia following nerve injury, again indicating the involvement of spinal dynorphin in neuropathic pain. Some of the observed effects of dynorphin appear to be mediated by NMDA receptors, given that these effects were inhibited by pretreatment with NMDA receptor antagonists. A model has been proposed in which dynorphin enhances neuronal excitability *via* the action of NMDA receptor sites, leading first to dorsal horn hyperexcitability and then to excessive depolarization and excitotoxicity. These data suggest that spinal dynorphin content, elevated as a consequence of peripheral nerve injury, may directly or indirectly govern sensitization of the spinal cord. Thus, studies suggest that dynorphin may be responsible for sensitization and expanding the receptive sites in neuropathic pain. However, a recent study on dynorphin KO mice demonstrated that dynorphin was not essential for the induction of neuropathic pain but for the maintenance of neuropathy.

A limited number of studies exist on the role of the β -endorphin system in neuropathic pain. Tsigos and his colleagues demonstrated that β -endorphin levels in CSF were reduced in patients with diabetic polyneuropathy but that these levels were not related to the presence of neuropathic pain. No significant variation was found in patients suffering from deafferentation pain. Another study suggests that changes occur in the spinal and supraspinal β -endorphin system, and that MOP opioid receptors may be at least in part responsible for the pathological plasticity accompanying neuropathic pain.

Notably, morphine lacks potent analgesic efficacy in neuropathic pain. In fact, other opioid analgesics are also less effective in relieving neuropathic pain than inflammatory pain. A vast body of clinical evidence suggests that neuropathic pain is not completely opioid resistant. The suggestion is rather that this condition presents reduced sensitivity to systemic opioids and that an increased dose is necessary to obtain adequate analgesia. Reduction of opioid antinociceptive potency was postulated to be due to the fact that nerve injury reduced the number and efficacy of spinal and central opioid receptors, reduced their signal transduction, or increased activity in physiological opioid antagonist systems (e.g., the cholecystokinin (CCK), nociceptin, or melanocortin systems). Notably, POMC-derived melanocortins also appear to be involved in neuropathic pain, given that melanocortin receptor antagonists display a strong antiallodynic effect in neuropathic rats when applied to the cisterna magna or intrathecally. The identification of

the mechanism involved may be of importance in understanding the molecular mechanism of opioid action in neuropathic pain and to the development of more effective drugs for the treatment of this condition in humans.

Stress-Related Diseases and EOPs

It is now clear that numerous stressors modulate behaviors that involve EOPs. Various stressors produce a wide range of behavioral responses, including motor suppression and catalepsy, which are both sensitive to opioid receptor antagonists. However, a number of studies have indicated that the type of stress employed, its duration, the frequency of stressor action, animal age and species, former stress experience, housing conditions, etc., are important variables determining the nature of the response and EOP involvement.

Systemic stressors act via hypothalamic factors, e.g., CRF, which induce the release of ACTH and β -endorphin in concert with other peptides. This in turn results in the release of glucocorticoids and adrenaline. EOPs appear to modulate HPA activity. EOPs can thus limit the HPA axis response to stress by dampening the adrenocortical system and by uncoupling the adrenal gland from hypothalamo-pituitary stimulation. Furthermore, EOPs attenuate stress-induced rise in plasma catecholamine levels.

In the brain, EOPs interact with catecholaminergic systems, specifically the noradrenergic system, which originates in the locus coeruleus, and the dopaminergic mesolimbic neurons of the VTA. Stressors activate locus coeruleus neurons and generate fear and induce anxiety. The coactivation of what are most likely β -endorphin neurons inhibits the activity of the locus coeruleus, favoring adaptive behavioral coping. Alternatively, EOPs derived from PENKs may enhance the activity of the mesolimbic dopaminergic system during stress, resulting in the reinforcement of a positive emotional state, a decrease in anxiety, and better adaptation. In contrast, PDYN peptides may have an opposite effect on the dopaminergic system during stress.

In general, the reactions of EOP systems tend to restore altered homeostasis. Alternatively, during acute severe stresses, including traumatic injury, circulatory shock, and hypoxia, EOPs appear to facilitate or mediate certain pathological responses and may even quicken death. It is possible that EOPs are responsible, in part, for the pathological effects of some forms of shock. Several experiments have shown that naloxone either blocked or reversed hypotension in conditions of circulatory shock and stress. Notably, it was found that a specific anti- β -endorphin antibody prolonged survival of animals following severe surgical stress. The last study suggested that circulating EOPs may have deleterious effects during severe stress. Several studies have shown that analogues of enkephalins attenuated, whereas dynorphin and naloxone potentiated, stress-induced immobility. Thus, it is likely that the endogenous PDYN system may act upon motor and emotional aspects of stress responses in a manner opposite to POMC and PENK systems. At present, however, it is not clear whether PDYN peptides mediate deleterious or adaptive effects in the nervous system.

Adaptation to chronic stress should enable an organism to cope with environmental demands, and opioids appear to be involved in this process. However, due to the fragmentary and sometimes inconsistent data available, their exact role remains unclear. EOP systems may contribute to dissociative symptoms in patients with personality disorder and posttraumatic stress disorder (PTSD). The increased activity of the opioid system may contribute to dissociative symptoms, including flashbacks, in borderline personality disorder. EOP systems appear to play an important role in the interaction of an organism with different stress factors, fulfilling stress-limiting and stress-protective functions. While relatively quiescent in the resting state, these peptides are released during intense stress and modify disturbed homeostasis in a number of ways. Stressors that are acute, mild, and short lasting appear to mobilize EOPs, which may in turn act to oppose stress-precipitated reactions and, in concert with other factors, counteract the initial response.

Eating and Gastric Disorders

Several results from animal studies indicate that there are close links between EOPs and feeding behavior. EOP systems are involved in central mechanism of feeding modulation and the pathogenesis of certain eating disorders, including eating-induced obesity, anorexia nervosa, and bulimia nervosa.

Hans Selye was the first researcher to show that acute gastric erosions could be induced in rats by morphine injections. Opioid antagonists appear to suppress the production of gastric ulcers. Further studies established that naloxone suppressed the production of gastric ulcers upon certain kinds of stress, indicating the involvement of EOPs. Notably, the central administration of opioid peptides also attenuated or inhibited these stress effects. Early studies suggested that neither adrenal- nor pituitary-derived EOPs were responsible for mediating the effects of stress upon feeding behavior. This observation points to the involvement of central pools of EOPs in these effects. It is also evident that various stress paradigms affect gastric, small intestine, and colonic transit. However, only a limited number of stressors, such as cold restraint or septic shock stress, appear to induce these gastric effects via EOPs. These findings suggest that peripheral EOPs released during stress may contribute to the production of gastric ulcers. Thus, although some reports are inconsistent, it appears that peripheral EOPs play a role in the pathogenesis of stress-induced ulcers, whereas the central pool of EOPs appears to have an opposite function.

Cardiovascular Disorders

There are data suggesting the participation of EOPs in the tonic regulation of blood pressure and the pathogenesis of hypertension. Recent research in humans and animals has described effects of opioids in the regulation of the circulatory stress response and has provided clues as to the significance of stress-induced opioid system dysregulation. In humans, mental stress affects blood pressure and increases

various opioid peptides in the plasma. Subjects responding to stress with a low increase in blood pressure had high levels of β -endorphin, whereas those who responded to stress with a high increase in blood pressure had elevated levels of dynorphin and Met-enkephalin. Pretreatment with naloxone enhanced blood pressure in low responders but not in high blood pressure responders. Notably, naloxone decreased the blood pressure response in hypertensive subjects with acute, stress-induced increases in blood pressure, suggesting that some EOPs, possibly dynorphins or PENK-derived peptides, affect blood pressure in hypertensive patients. Thus, it is likely that some EOPs may counteract cardiovascular effects, such as tachycardia and increased blood pressure, and others may be involved in hypertensive pathology. Alternatively, some EOPs appear to mediate cardiovascular depression, which occurs in response to severe stress. In fact, a number of studies have demonstrated that naloxone reverses the hypotension induced by most cardiovascular shock states.

EOPs in Psychiatric Disorders

Addiction

Although opioid agonists are self-administered by animals and humans, opioid receptor antagonists induce aversion, dysphoria, and withdrawal. The opioid antagonists naltrexone and naloxone (which bind to all opioid receptors) were shown to attenuate psychostimulant-induced CPP and cocaine and cannabinoid intake in rodents and monkeys. More selective compounds, such as MOP receptor antagonists (naloxonazine, CTAP) and a DOP receptor antagonist (naltrindole), also influence the rewarding effects of cocaine. Finally, in cocaine drug-induced reinstatement experiments, the administration of CTAP to the ventral pallidum inhibited reinstatement. Notably, CTAP does not exert rewarding or aversive effects. Naltrexone has been shown to decrease alcohol consumption under various experimental conditions. Furthermore, opioid antagonists can attenuate cocaine but not nicotine self-administration in rats, which suggests a role of EOPs acting via MOP and KOP receptors in psychostimulant, opioid, and ethanol reinforcement but not necessarily in the rewarding properties of other addictive drugs. Alternatively, activation of the KOP system antagonizes the acute reinforcing effects of drugs of abuse. Following drug use, the KOP system contributes to the aversive and dysphoric-like effects observed during withdrawal and participates in relapse occurrence. In the context of drug dependence, the KOP receptor appears to reduce increased drug intake. In summary, it appears that MOP and DOP receptors participate in the rewarding properties of drugs of abuse. Conversely, KOP receptors play the opposite role and are involved in mediating the withdrawal aspects of drug dependence via a stress mechanism.

The above observation indicates an involvement of EOPs in the effects of drugs of abuse and shows that EOP systems play a key role in both regulating the brain's hedonic homeostasis and in modulating reward and mood. Exposure to drugs of abuse modifies the activity of EOP systems and EOP release. Prolonged

administration leads to adaptations in EOP release, biosynthesis, and function. Thus, alterations of the EOP systems following exposure to drugs of abuse appear to contribute to the dysregulation of reward processes, an effect that may participate in the development of addiction. Acute administration of ethanol enhances the release of β -endorphin in the VTA, where dopaminergic cells are located. Alternatively, acute nicotine administration and self-administration of psychostimulants increased β -endorphin levels in the hypothalamus and nucleus accumbens, respectively. Biochemical experiments showed a small but consistent decrease in the levels of POMC and PENK in the nucleus accumbens/striatum pathways and hypothalamus, respectively, following the prolonged application of morphine. Biochemical studies showed that opiate withdrawal increased expression of the PDYN gene, PDYN-derived peptides levels, and their release in the nucleus accumbens. The above results therefore indicate that repeated morphine treatment leads to long-lasting upregulation of PDYN gene expression and possibly PDYN-dependent signaling in the nucleus accumbens and striatum. Other drugs of abuse have also been demonstrated to alter the activity of PDYN neurons, and the greatest exacerbation of changes was observed during withdrawal. Different addictive drugs induce similar effects on the direction of PDYN system activity and concomitantly decrease the density of KOP receptors, indicating an involvement of PDYN neurons in the VTA-nucleus accumbens system in shared neurochemical mechanisms of drug withdrawal and craving.

Fear and Anxiety

PENK KO mice show stronger anxiety than wild-type mice and display enhanced fear and anxiety in novel environments. In agreement with the above results, DOR receptor-deficient mice also show enhanced fear in novel environments. Furthermore, exposure to predator odor results in anxiety and an increase in PENK expression in CD-1 mice. Thus, the PENK system appears to be involved in regulating anxiety. In contrast, the PDYN system appears to generate anxiogenic peptides, while KOR receptor antagonists possess anxiolytic-like properties. In fact, the selective KOP receptor antagonist norbinaltorphimine has been reported to exert anxiolytic effects. Furthermore, anxiogenic properties of dynorphin have been suggested by a study of mice lacking the PDYN gene. Endorphin analogues have been shown to cause anxiolytic effects in the elevated plus-maze test in mice. Moreover, the relationships observed between brain levels of β -endorphin and anxious behavior in various anxiety tests suggest that β -endorphin inhibits anxiety under normal conditions. Other studies show that mice lacking β -endorphin displayed lower anxiety in the zero-maze test and an exaggerated anxiolytic response to ethanol. Thus, β -endorphin has been shown to play a role in anxiety-related behavior, but no clear conclusions can be drawn from the available research.

Interactions of EOPs with the brain catecholaminergic systems (i.e., the noradrenergic system originating in the locus coeruleus and the dopaminergic mesolimbic system derived from the VTA) appear to modulate the ability of an organism to cope with fear and anxiety. It has been shown that stressors activate the locus coeruleus neurons that generate fear and anxiety. The coactivation of EOP

systems (most likely including β -endorphin) inhibits the activity of the locus coeruleus, which favors adaptive behavioral coping. Alternatively, EOP derived from both POMC and PENK may enhance the activity of the mesolimbic dopaminergic system, resulting in the reinforcement of positive emotional state, a decrease in anxiety, and better adaptation. In contrast, PDYN peptides may have an opposite effect on the dopaminergic system. Furthermore, CRF-induced activation of the dynorphin/KOP receptor system in the mouse basolateral amygdala mediates anxiety-like behavior. Thus, it is likely that the endogenous PDYN system may act upon emotional aspects of the fear and anxiety in a manner opposite to the POMC and PENK systems.

Depression and Other Neuropsychiatric Disorders

Opioids are known to affect mood in animals and humans. MOP, and to some extent DOP, receptor activation induces euphoria, whereas opioid antagonists evoke aversion. Reduction in the activation of the endorphin/MOP system may therefore lead to dysphoria and depression. In fact, a decrease of MOR binding has been shown in depressive patients. Associations between variants of the MOP receptor and the antidepressant response and remission from major depressive disorders have been found, indicating that MOP receptor neurotransmission may be involved in major depressive disorder and antidepressant therapy. Notably, DOR activation has been shown to evoke antidepressant-like effects in preclinical studies, and DOR KO mice demonstrated an enhancement of depressive-like behavior.

Depressive symptoms appear to be at least partly KOP receptor and dynorphin dependent. A recent study proposed that PDYN neurons and their KOP receptors in the VTA-nucleus accumbens reward system and hippocampus may contribute to the pathology and possibly etiology of depression. The PDYN system seems to play an inhibitory role in the hippocampus. The hippocampus exerts inhibitory control of hypothalamic neurons, and reduced dynorphinergic inhibition in the hippocampus may therefore result in disinhibition of the hypothalamic network and an increase in hypothalamo-pituitary axis activity. The increase in HPA activity results in the increased levels of cortisol observed in depressed patients. Thus, enhanced release of dynorphin in the hippocampus may result in disinhibition of the HPA axis and may contribute to the development of depressive behavior. Furthermore, PDYN neurons and KOP receptors appear to be involved in tonic inhibitory regulation of mesolimbic dopaminergic neurons by controlling presynaptic dopamine and serotonin release. This effect may also be mediated by presynaptic inhibitory control of glutamatergic neurons. Activation of the dynorphin-KOP receptor system within the mesolimbic dopaminergic reward network results in an increased inhibition of adrenergic and serotonergic neurons and may lead to depressive-like behavior. In summary, EOPs appear to play unique roles in the development of depressive-like behavior in animals and depressive pathology in human. This is supported by findings that the PDYN/KOP system displays prodepressive activity, while PENK/endorphin neurons and MOP/DOP receptors display antidepressive activity.

Adaptation to chronic stress should enable an organism to cope with environmental demands. EOPs also appear to be involved in this process. The mean level

of β -endorphin in CSF was seen to be significantly greater in patients with PTSD when compared with the normal value. Thus, the increased endorphinergic activity in the central nervous system may exist in patients with PTSD, and hypersecretion of opioids may constitute an adaptive response to traumatic experience. However, there is a poor correlation between CSF and plasma β -endorphin levels when measured in combat veterans with PTSD. Some studies suggest that EOP systems may contribute to dissociative symptoms in patients with PTSD. The increased activity of the opioid system may contribute to such symptoms, including flashbacks. The role of EOPs in the development of the disorder appears to be significant, but the mechanism is not well understood. A role for EOPs in psychotic disorders is much less clear. The central administration of certain KOP receptor ligands, however, evokes dysphoria and hallucinations. Furthermore, KOP receptor agonists inhibit prepulse inhibition, a mechanism that is impaired in schizophrenia patients.

Outlook

Since the discovery of opioid peptides, tremendous progress has been made in functional and molecular characterization of EOPs and their receptors. This advance has opened pathways to understanding of how various neuropeptides may control behavior and complex neural functions. EOPs are involved in antinociception and central and peripheral pain mechanisms; play a critical role in reward and addiction, feeding behavior, and gastrointestinal diseases and obesity; regulate motor behavior and respiration, cardiovascular functions, body temperature. They are known to be released from endocrine organs and are involved in regulation of hormone release. EOPs are also synthesized by immune cells, modulate functions of immune system, and play a role in immune diseases. These peptides appear to be involved in the interaction of an organism with different stress factors and in the modulation of stress responses. Whereas relatively quiescent in the resting state, EOPs are released during intense stimulation and modify disturbed homeostasis. EOPs appear also to be involved in brain stress-related diseases such as in addiction, depression, anxiety disorders, and PTSD. Many questions concerning a specific role of EOPs in these disorders remain unanswered, but progress made during last years in genomics and complex genetics opens new avenues for investigating a role of the of EOPs and their receptors in a range of various brain diseases.

Further Reading

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