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From Opiate Pharmacology to Opioid Peptide Physiology

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ABSTRACT

This is a personal account of how studies of the pharmacology of opiates led to the discovery of a family of endogenous opioid peptides, also called endorphins. The unique pharmacological activity profile of opiates has an endogenous counterpart in the enkephalins and β-endorphin, peptides which also are powerful analgesics and euphorigenic agents. The enkephalins not only act on the classic morphine (μ-) receptor but also on the δ-receptor, which often co-exists with μ-receptors and mediates pain relief. Other members of the opioid peptide family are the dynorphins, acting on the κ-receptor earlier defined as precipitating unpleasant central nervous system (CNS) side effects in screening for opiate activity, A related peptide, nociceptin is not an opioid and acts on the separate NOR-receptor. Both dynorphins and nociceptin have modulatory effects on several CNS functions, including memory acquisition, stress and movement. In conclusion, a natural product, morphine and a large number of synthetic organic molecules, useful as drugs, have been found to probe a previously unknown physiologic system. This is a unique development not only in the neuropeptide field, but in physiology in general.

INTRODUCTION

Historical background

Opiates are indispensible drugs in the pharmacologic armamentarium. No other drug family can relieve intense, deep pain and reduce suffering. Morphine, the prototypic opiate is an alkaloid extracted from the capsules of opium poppy. The use of opium extract as a euphoriant and as an analgesic has a long history. Some of the earliest documentation is from the Sumers in the 3rd millennium B.C. and use spread to the Egyptians, Greeks and disseminated into the whole known "civilized" world at the time, India, China and Europe. Opium abuse was common in the Orient, but also flourished in Europe from the 16th century with a culmination of opium "eating" in the 19th century.

Already in 1806, Sertürner isolated morphine, named after the Greek god of sleep, Morpheus, and found it to be the principal analgesic and addictive component in opium. The access of the pure substance, and the following technological invention of the injection syringe would revolutionize the clinical handling of pain, and together with the development of gaseous anesthetics open the way to surgery under humane conditions. Morphine has an intricate chemical structure and is impractical to synthesize. It is therefore necessary to cultivate opium poppy for access to morphine and to codeine (a weaker, broadly used analgesic and antitussive agent). The accompanying problems of illegal traffic stem from the fact that opium poppy is cultivated in geographical areas that are hard to access and control. A simple chemical process, acetylation turns morphine into heroin, which no longer has a medical use, but is preferred by the opiate addict.

Morphine is not a safe drug, it is easily overdosed and is strongly addictive. Attempts have therefore been made to produce equally efficacious, non-addicting drugs. In the late 1930s it was found that a synthetic atropine analogue, named pethidine was an opiate analgesic. This work was done in Germany, which during the 2nd World War became isolated from opium cultivation areas. German scientists synthesized another analgesic, methadone which was found to be equipotent with morphine and orally active. Both pethidine and methadone remain clinically important analgesics. Methadone is mainly used, however, as a substitute in maintenance therapy of chronic, relapsing heroin addicts. The early history and the development of opiate pharmacology, has been summarized by the leading German scientist, O. Schaumann (1).

The prototypic opiate

The identification of synthetic compounds with the same activity as morphine was a success for medicinal chemistry. It generated a tremendous amount of work in synthetic chemistry and virtually thousands of compounds were prepared and assayed for opiate activity. In fact, opiates became prototypes for pharmacologic assay evaluation. Besides behavioral tests for analgesia, such as the escape jump or paw-licking of a mouse on a hot plate, it was found that several isolated organ preparations, notably the electrically stimulated longitudinal muscle/nerve plexus from the guinea-pig ileum responded to opiates. The correlation between analgesic activity and activity in the ileum was excellent, taking into account pharmacokinetic differences. In fact, such comparisons were instrumental in defining activity at the site of action and indirectly gave a measure of how well an analgesic penetrated the blood-brain barrier. Synthetic work would generate compounds that when injected were 1000 to 10 000 times more potent than morphine (e.g. etorphine used by veterinarians for immobilizing large wild animals), but not much more active in the isolated preparation showing that the new compound distributed to the brain very

efficiently. Another synthetic derivative, fentanyl would find a use as an intravenous anesthetic (and of course at the same time a potent analgesic).

The access to in vitro organ preparations for fundamental studies was of principal importance. Apparently, a whole family of chemically unrelated compounds had a common mode of action in relatively simple systems, and not only in a behavioral assay where the end point seemed more obscure. The in vitro preparations also allowed correlation between chemical structure and biological activity. Thus, opiate pharmacology was equally accessible to the establishment of structure-activity relationships (SARs) as other fields of pharmacology, such as anticholinergics or antiadrenergics, where in a similar manner, organ preparations were available and the large families of compounds generated SARs. Based on SARs complementary receptor surfaces were depicted and used for activity predictions and guidance of the development of new agents. Opiates were, however, importantly unique. Whereas anticholinergics or antiadrenergics were known to antagonize acetylcholine and noradrenaline, respectively, there was no endogenous morphine-like substance known at the time. Since acetylcholine is the transmitter released by electric contractions of the guinea-pig ileum preparation, and opiates inhibited the contraction it was assumed that opiates acted by inhibiting acetylcholine release (2). This effect, was, however, thought to be a pharmacological artifact and therefore not relevant physiologically.

THE OPIATE RECEPTOR

It was recognized in the late 1960s that hormone receptors were proteins either in the cellular membrane or in a resting state in the cytosol ready to enter the cell nucleus in a complex with the hormone. These receptors could be identified with the use of hormones that had to be labeled with isotopes to very high specific activity since receptor numbers are very small. For protein hormones or other larger molecules labeling was easily done with radioactive iodine. With smaller molecules, receptor ligands had to be labeled with tritium to highest possible (theoretical) specific activity, which was not recommended, since such compounds were thought to decompose rapidly. Later work would show that this was not a problem if storage conditions (low temperature, no light, as little water as possible) were optimized.

Almost simultaneously, three groups published the successful demonstration of specific opiate receptor binding in rat brain membranes and in the guinea-pig ileum preparation (3-5). Interestingly, in the latter preparation it was found that the receptor was associated with the nerve plexi and not with the muscle, as was predicted from in vitro opiate pharmacology. The receptors, so defined showed the expected binding properties, i.e. they bound the labeled prototypic ligand and analgesically active opiates (measured as displacement of label) but not inactive

compounds. A critical comparison was the demonstration that of two optical antipodes (compounds that are identical except in their interactions with an asymmetric surface) the active antipode had affinity, the inactive not (6). The binding assay was found to be very robust and suitable for drug screening. The radioactive ligand could either be the morphine derivative dihydromorphine (3) or the "superanalgesic" etorphine (5) with equal results. As already pointed out etorphine derives its extreme potency from a more favorable kinetics and reaches the central nervous system in a larger proportion than morphine (or its close analogue, dihydromorphine).

By receptor autoradiography, it was soon found that the opiate receptor (or more accurately, binding sites) localize in CNS areas known to be relevant for the treatment of pain, the dorsal horn of the spinal cord, the periaqueductal gray and the raphe nuclei, but also in other areas such as the striatum or the amygdala, areas not associated with pain. Besides, it was obvious that there was no association with any particular CNS neurotransmitter (such as acetylcholine as suggested from the work in the guinea-pig ileum).

Another important route of investigation was opened with the finding that opiate receptors couple to a second messenger pathway, by acting on G-proteins to inhibit the activity of adenylate cyclase. Thus opiate receptors seemed to belong to a large class of receptors that included receptors for neurotransmitters such as acetylcholine, noradrenaline, dopamine, etc. yet with a unique specificity. Thus, from an obscurity as a pharmacological artifact the opiate receptor seemed to share a place with the "real" receptors.

It has already been pointed out that opiate overdose can be fatal. It was found quite early that certain semi-synthetic morphine derivatives could antagonize the toxic actions of opiates. The typical substance, nalorphine had some analgesic activity, in pharmacological terms acting as a partial agonist, but also induced unpleasant side reactions precluding its use as a safe analgesic. Further chemical work identified naloxone as a pure opiate antagonist with virtually no activity. Naloxone must, however, be given intravenously and is very short acting. A closely related congener, naltrexone, is also a pure opiate antagonist and has the additional advantage of being orally active. Naloxone has become a standard agent for treatment of opiate overdose and is also a pharmacologic probe for the opiate receptor. In fact, the definition opiate and opiate receptor is based on the antagonism by naloxone. It can of course be argued that this is a highly artificial definition and as will be apparent, this definition can be challenged.

In most tests naloxone alone was reported to be inactive (for a review on opiate antagonists see ref. 7). This seemed to confirm the notion that opiates are useful pharmacologic agents, but in physiology, artifacts.

THE ENDOGENOUS LIGANDS

Testing a hypothesis

Two groups independently entertained the idea, that there might be endogenous ligands for the opiate receptor. Terenius (8) used the binding assay with membranes prepared from rat brains and tritium-labeled dihydromorphine as a probe for the binding sites. Brain extracts were prepared and subjected to chemical fractionation. Partition into organic solvents did not recover any material that displaced the labeled probe, not even under alkaline conditions that would have extracted opiates. However, activity was consistently found in aqueous extracts from which salts and larger molecular weight proteins and other macromolecules had been eliminated. Separation in aqueous medium on a gel filtration column identified components with low molecular weight, approximately 700-1000. The activity was destroyed by treatment with proteolytic enzymes. The identity of these components was clear; they were low-molecular weight peptides. John Hughes and Hans Kosterlitz used a different approach (8). Kosterlitz had refined the guinea-pig ileum system for the assay of opiates. Various fractions of pig brain extracts were added to the preparation. Opiate activity was recorded as the suppression of the electrically induced twitch. Since this response is quite non-specific and could be due to "poisoning" they added the extra criterion, reversibility by naloxone. Initial positive experiments called for large-scale extractions of pig brain. They entered collaborations with a drug company, Reckitts & Coleman that had access to large-scale extraction facilities. Since the company also is the manufacturer of the superanalgesic, etorphine chemical characterization of some active fractions turned out etorphine, which apparently was around ubiquitously. After such setbacks they eventually succeeded in isolating and sequencing two homologous opioid peptides, Leu-enkephalin and Met-enkephalin (9). They soon recognized that the enkephalin sequence was also represented in the hormone β-lipotropin, of unknown physiologic significance. A fragment of this hormone (called the C-fragment, later β-endorphin) had also been isolated (10). This chemical triumph gave the astonishing result, not only was there one ligand for the opiate receptor, there were several. Hughes and Kosterlitz named their peptides enkephalins, since they had been isolated from brain (Gk. enkefalos). The structures of these peptides are:

Leu-enkephalin	YGGFL
Met-enkephalin	YGGFM
β-endorphin	YGGFMTSEKSQTPLVTLFKNAIIKNAYKKGE (human)

This discovery initiated extensive efforts both in the synthesis of analogues and derivatives and in pharmacologic experimentation. The enkephalin sequence was found to be the minimum active sequence. However, being peptides, they were easily degraded particularly by aminopeptidases that eliminated the N-terminal tyrosine, absolutely essential for activity. Replacement of

Gly^2 with $DAla^2$ and other modifications generated peptides with excellent stability and potency. At the receptor level, morphine and enkephalins were found to be approximately equipotent. The natural ligand, β-endorphin was found to be a potent analgesic if introduced in the CNS and also long-acting (11).

The discovery that an alkoloid and small peptides act on the same receptor was a conceptual breakthrough. Several neuroactive peptides were known at the time, for instance substance P and neurotensin, but in no case had a non-peptide agonist with activities similar to the peptide been discovered. The discovery of the opioid peptides set an example. Natural product and medicinal chemists screened large numbers of compounds against peptide receptors. Progress was initially slow but it would soon turn out that so-called peptidomimetics can be prepared if enough effort is given to the problem. Peptides are never going to be the favored chemical structures for drug development, but the possibility to obtain peptidomimetics opens the neuropeptide systems to future pharmacotherapies.

Early studies on opiate receptor ligands

The discovery of endogenous opioids initiated research in different directions, particularly in relation to their putative functional significance. Antibodies were generated to the newly discovered peptides and used for immunohistochemical studies. Already the first studies were rewarding (12). Enkephalin immunoreactivity was present in areas of particular relevance for pain and in juxtaposition to areas previously known to be rich in opiate receptors, including the dorsal horn of the spinal cord, the raphe nuclei, the periaqueductal gray, and also in large areas of the basal ganglia, the caudate nucleus, globus pallidus etc. Of particular interest in relation to the euphorigenic activity of opiates was the rich innervation of the nucleus accumbens, previously known to be central in the brain's reward system. It had been shown by Olds and others after him that a rat would activate electrodes implanted in this structure thereby releasing endogenous neurotransmitters. Such rats would neglect eating, grooming and were less interested in the opposite sex. A closer look at enkephalin distribution in the spinal cord and a comparison with the distribution of substance P revealed interesting differences (13). Substance P, a peptide with 11 amino acids was previously known to be present in thin primary afferents, C-fibers, known to be essential for nociception, and the first link in pathways eliciting pain. Enkephalin nerve terminals were present in areas overlapping the substance P terminals. The enkephalin neurons were, however, local interneurons available already at the first synapse. Studies of the finer architecture revealed that opiate receptors were present both on the primary afferent terminals and on the second order neurons. These receptors are accessible to local analgesic therapy via intrathecal or epidural treatment, which became an important therapeutic principle during this time pe-

riod, for instance in obstetrics. Apparently this is an intrinsically very powerful system and the extent to which it is operated by enkephalin release became an interesting area of study. Subsequent work would show that the distribution of β-endorphin immunoreactivity was much more limited, essentially only one diffuse projection from cell bodies in the brain stem.

The discovery of endogenous opioid peptides raised hopes that they would not be addictive. It seemed counter-intuitive that the body would generate compounds with such activity. However, an early study where rats were allowed to self-administer Leu-enkephalin via a catheter implanted in the cerebral ventricles revealed that the rats liked to take enkephalin as readily as morphine. In fact it was necessary to protect the rat from overdosing (14). This discovery was disappointing from a drug development perspective, but exciting from a physiological perspective. If the opioid peptides have such powerful properties could it be that they are intrinsic parts of the reward system and in fact involved in the regulation of mood? It has previously been emphasized that the opioid antagonists are practically inactive in pharmacologic experiments, thus suggesting that the tonic activity in opioid systems is low. In fact this may be the way natural selection operated. A powerful tonic release of the opioid peptides would not be functional, pain is a useful signal of injury and a feeling of good mood (pleasure) is not functional except as a reward. Therefore opioid peptides may only be released under special, perhaps more extreme conditions such as in "battlefield analgesia". the lack of pain a soldier feels when wounded and transported to hospital behind the front.

These types of extreme conditions are ethically hard to address in experiments. A physically exhausting, yet benevolent activity is running. Rats given access to a running wheel in their cages will readily run during their active period. Running activity is less if the animals are treated with naloxone. If they are habituated to running for several weeks and thereafter the wheel is locked, they will become nervous, aggressive with a peak maximum after 2 to 3 days. In parallel, there is a drop in their secretion of β-endorphin into cerebrospinal fluid from the elevated levels during running (15). It is interesting that the time course of the phenomenon is similar to that seen during detoxification of a heroin addict.

NEW FAMILY MEMBERS AND THE FAMILY TREE

As already pointed out, opiates constitute a group of compounds with a consistent activity spectrum. For instance, despite strong efforts it has not been possible to separate analgesic activity from dependence-producing liability. The newly discovered peptides apparently were no exception. It was found, however, that certain synthetic analogues produced side effects, from mild dysphoria to psychotomimetic effect. A prototypic compound with such effect is ketocyclazocine. To separate this activity Martin and collaborators suggested the existence of a receptor

distinct from the "morphine"-receptor (μ-receptor) and named it the κ-receptor. There were other differences, for instance whereas morphine causes urine retention, κ-agonists induced diuresis (16). The κ-receptor remained a pharmacological curiosity, until a new group of opioid peptides were discovered, the dynorphins. Dynorphin A, the first member found, has 17 amino acids and contains the Leu-enkephalin sequence in its N-terminus. Subsequently, two additional members also with the same N-terminal sequence were discovered. Immunohistochemical analysis revealed that the distribution was distinct from that of other opioid peptides. Dynorphin interneurons in the dorsal horn of the spinal cord are primarily found in deeper laminae than enkephalin interneurons, suggesting a greater role in modulating nociceptive signals from inner organs. In the brain there is a major presence in striatonigral neurons suggesting a role in motor control, in neurons projecting to the nucleus accumbens suggesting effects on reward and in mossy fibers of the hippocampus suggesting a role in learning. The distribution roughly matched that of κ-receptors identified by autoradiography. Clearly, in brain dynorphin peptides and κ-receptors are present in structures not primarily related to pain. It is therefore not surprising that κ-receptor agonists are analgesic when given intrathecally but not centrally into the brain.

Advances in molecular biology led to the cloning of the protein precursors to the opioid peptides. The first identified precursor was found to be pluripotent and contains three hormones, ACTH (adrenocorticotrophic hormone), MSH (melanocyte-stimulating hormone) as well as β-endorphin/β-lipotropin and was consequently named proopiomelanocortin. The other precursors, proenkephalin with no less than 7 enkephalin sequences (6 Met-enkephalin and 1 Leu-enkephalin) and prodynorphin (also named proenkephalin B) with 3 dynorphin sequences all containing Leu-enkephalin, were close to follow. The chemical complexity is thus bewildering and the number of chemical species that can be derived from these precursors by partial or full proteolytic processing is large (17).

For a long time the opioid receptors resisted attempts at cloning. The first receptor to be eventually cloned was the δ-opioid receptor (18, 19). This receptor had been postulated from experiments with the enkephalin peptides, which besides activity at the μ-receptor also had affinity for another receptor less sensitive to naloxone. This receptor seemed uniquely present in the in vitro preparation, the mouse vas deferens. (The name δ– is a homonym with d in deferens). When the sequence of the δ-receptor was known it became relatively simple to use homology screening to identify and clone the two other receptors predicted from pharmacologic experiments, the μ- and κ-receptors.

The entrance of molecular genetics had changed the opioid field dramatically. Homology screening identified yet another receptor, named ORLI (orphan receptor L-1) since no natural

ligand was known. A few years later, however, a ligand was found and named nociceptin (20) or orphanin FQ (21) since rats given this peptide intrathecally respond as if they have been exposed to a noxious stimulus. Nociceptin has subsequently been found to have a multitude of actions in the whole CNS. It is distributed in the dorsal horn of the spinal cord overlapping with the enkephalins and in the brain, for instance overlapping with dynorphin in the mossy fibers of the hippocampus. The discovery of nociceptin prompted a change in terminology for its receptor, now called the NOR-receptor.

In summary, there is now a superfamily of structurally related peptides and receptors. Peptides from three families have opioid activity, operationally defined as activity that can be blocked by the antagonist naloxone. Nociceptin has no affinity for the opioid receptors but interacts with the homologous NOR-receptor for which the opioids have no affinity. Still, nociceptin itself is structurally related to dynorphin A, and the NOR-receptor related to the opioid receptors indicating a common ancestral precursor to all these peptides and receptors. Since functional activity after all is the most relevant parameter in biomedical research the terminology opioid is likely to stay. Another synonym, endorphin (for endogenous morphine) is also frequently used. The following graph summarizes the properties of this family of peptides. Enkephalin and β-endorphin have morphine-like opioid activity, are potent analgesics and euphoriants, dynorphin is also an analgesic primarily at a spinal level whereas at higher levels it produces unpleasant effects and is not self-administered, and finally nociceptin, a non-opioid peptide acts on its own unique receptor modulating pain and other CNS activities.

Precursor	**Typical peptide**	**Preferred receptor**
Proenkephalin	Leu-enkephalin[1]	μ (=opiate), δ
	Met-enkephalin[1]	
Proopiomelanocortin[2]	β-endorphin[1]	μ + δ
Prodynorphin	Dynorphins A and B[1]	κ
Pronociceptin[3]	Nociceptin	NOR

1. Opioid peptides.
2. Also precursor to ACTH and MSH acting on non-opioid receptors.
3. Also precursor to other peptides with unknown receptors.

OPIOID PEPTIDES AND PAIN

Both from a principal and practical point of view, it would be of interest to determine the role, if any of opioid peptides in the modulation of clinical pain. One experimental approach is to test the influence of naloxone on pain thresholds. Under well-controlled conditions it is in fact

possible to demonstrate hyperalgesic effects in laboratory animals. In human healthy volunteers, pain thresholds were, however not much affected, partly because realistic pain conditions would be unethical. Other approaches have therefore been taken.

It seemed possible that opioid peptides would be released into the cerebrospinal fluid (CSF) in enough quantities to be measurable. Early studies indicated that this might be feasible. In these studies, the earlier described receptor-binding assay was used to identify opioid material in two chromatographic fractions, covering the molecular weight interval 700-1200, i.e. likely enkephalin-related peptides (22). Since CSF is usually sampled at a lumbar level contributions from release at the spinal level are likely substantial as also proven in an animal model. Already the first studies suggested the possibility that severe, chronic pain might in fact be associated with very low levels of opioid peptide secretion. This was confirmed (23) in a larger series of patients with chronic pain (defined as having lasted for at least 6 months). When compared with levels in a group of healthy volunteers, patients with neurogenic pain (causalgia, neuralgia etc.) tended to have lower levels of receptor-active opioids. It was also found that patients with non-neurogenic "idiopathic" pain had levels within or higher than those in volunteers. With a surprising degree of accuracy, the simple binding assay distinguished patients in the two populations.

During this time period, there was a general shift in the conceptualization of chronic neurogenic pain, from models where the pain was considered a consequence of nociceptive input from primary afferents (afferent pain) to the understanding of chronic pain as a disease state, probably more often of a central origin. Intuitively obvious procedures for pain treatment such as sectioning of afferent nerves were abandoned, since such approaches were frequently not only clinically ineffective but might even aggregate the condition. In fact, pain of neurogenic pain may derive from deafferentation, i.e. a failed neuronal input. It was also known that such pain was resistant to opiate therapy. In short, such chronic pain, which by definition is a therapeutic failure, does not respond to pharmacotherapies known to modulate acute or chronic afferent pain.

Parallel work suggested other approaches to the treatment of neurogenic pain. An old Chinese procedure for treatment of many ailments including pain, acupuncture was introduced in China as an alternative to conventional analgesic medication during surgery. The results were successful particularly if the acupuncture needles were stimulated electrically. Several clinical investigators attempted acupuncture-like techniques in patients with chronic pain. A frequently used technique Transcutaneous Electric Nerve Stimulation (TENS) with surface electrodes instead of needles started to gain interest. It would soon be recognized that different stimulation parameters produced different results, clinically some patients responded well on high-

frequency, low intensity TENS whereas others responded better to low-frequency high intensity stimulation. The latter paradigm elicited deeper stimulation and muscle contractions and was therefore considered "acupuncture-like". The clinical impression was that particularly the neurogenic cases responded better to low-frequency TENS than idiopathic cases. Moreover, naloxone antagonized the pain modulating effect of low-frequency TENS suggesting that opioid peptides were released. This was confirmed in a study where individual patients with severe chronic neurogenic pain donated lumbar CSF before stimulation and after stimulation. The stimulation gave an increase (24). Similar results were obtained in animal models. The historic background, some of the Chinese experience with acupuncture and these early results have been reviewed (25). In a later study, a comparison was made between patients given high- and low-frequency stimulations, respectively. On this occasion, two distinct peptide species were measured with radioimmunoassay in CSF samples taken before and 15 min after stimulation. The results were interesting since they suggested a differential effect. Low-frequency stimulation significantly elevated an enkephalin peptide, high-frequency stimulation a dynorphin peptide. The former interacts primarily with μ-receptors (which are highly sensitive to naloxone and with δ-receptors) whereas the latter interacts almost exclusively with κ-receptors with low naloxone sensitivity (26). These results are compatible with the previously described observation that only low-frequency stimulation produces naloxone-sensitive pain relief.

There are considerable interindividual differences in the amount of analgesic required to produce adequate pain relief, for instance during surgery and postoperatively. It has been assumed that everything else being equal, these differences are related to the individual's pain tolerance. Patient-controlled infusion of an opiate has been introduced for maximum comfort postoperatively. Interestingly, individual patients titrate their drug intake very accurately to a pseudo steady-state plasma level. This titrated level represents an indirect estimate of the pain intensity. In a series of patients who had to undergo major gastrointestinal surgery, a CSF sample was taken at the time for the induction of anesthesia, and another sample was taken postoperatively, when the patient was self-administering opiate. The CSF taken prior to surgery was used for opioid peptide analysis using a receptor assay and the sample taken post surgery for analysis of the concentration of opiate. Opiate consumption was recorded and the plasma concentration monitored at regular intervals. The study showed distinct differences in the opiate steady state levels, despite efforts to follow a standard protocol for premedication, anesthesia etc. Moreover, when the plasma (actually calculated CSF) opiate concentration was related to CSF opioid activity, an inverse relationship was found (27). Thus, a patient who demanded more opiate had lower peptide levels and vice versa, indirectly suggesting that the endogenous release of opioid peptides contributes to the overall pain modulation.

Parturition is a natural pain- and stressful process. A series of primagravidae selected for vaginal delivery participated in another study. These women were offered an epidural treatment with opiate to relieve pain. A lumbar CSF sample was taken at an early phase of the delivery and analyzed for two peptide markers, β-endorphin and dynorphin A. Women who demanded an epidural had significantly lower levels of dynorphin whereas there was no difference between the two groups regarding β-endorphin (28). Experiments in rats have confirmed that dynorphin peptides are relevant for pain modulation in parturition.

In conclusion, several experiments suggest that there is a significant contribution of the opioid peptide systems in the modulation of clinical pain. The systems can be activated by stimulation techniques either physical, acupuncture or electrical. The level of activity, particularly in the enkephalin system can be boosted by inhibition of enzymatic degradation. This offers an alternative to treatment with analgesic drugs for moderately severe pain (29).

OTHER ACTIONS

Although there is little direct evidence, enkephalins and β-endorphin probably play a role in reward, and in the human, in emotions such as euphoria and pleasure. Studying subjective experiences with biological tools is, however, experimentally very difficult. Besides, there are more stringent ethical constraints in studying completely normal reactions rather than pathologic deviations. There is, however, indirect evidence that chemically induced "pleasure" may be mediated via opioid peptides. Naltrexone has been introduced as a drug (Reviva®) to prevent relapse in alcoholics. It has been shown that this drug reduces alcohol-induced euphoria. An alternative approach to relapse prevention might be the activation of dynorphin systems (for a review see 30).

As already mentioned, agents acting on κ-receptors produce unpleasant mental side effects. These effects are reported as altered perception or even delusions and hallucinations (mainly auditory) and therefore, κ-agonists may be regarded as psychotomimetic. Other agents producing hallucinations, for instance LSD-25 mainly produce visual hallucinations that are not typically recorded in schizophrenia. It therefore seemed possible that schizophrenics might have an abnormal production of psychotomimetic opioids. To test this possibility, two experimental approaches were undertaken. A series of 6 patients with chronic schizophrenia and residual symptoms including auditory hallucinations were given naloxone intravenously. Several patients responded - one case with habitual hallucinations was essentially symptom-free after naloxone (31). This finding stimulated a WHO-multicenter investigation on the efficacy of the orally active naltrexone. Naltrexone was found to have a significant effect on residual symptoms in neu-

roleptic-treated patients, however not to an extent that clinical use was warranted. The other approach, chemical analysis of CSF opioid peptides confirmed an elevated secretion of opioid peptides, both using a receptor-assay and direct radioimmunoassay of dynorphin peptides (32). Taken together, these data indicate that dynorphin peptides, which are intimately associated with dopamine pathways which are the main targets of neuroleptics, in some way are involved in psychosis.

It has also been suggested that dynorphin peptides have a modulatory role in certain behaviors. Several investigators have shown that κ-agonists suppress self-administration of heroin or cocaine or self-selected intake of alcohol in rats. The site of action might be the nucleus accumbens that receives rich innervation of dynorphin fibers. A model where enkephalin and dynorphins have opposite roles on the activity of the mesolimbic dopamine system has been presented (32).

CONCLUDING REMARKS

The opiates constitute a unique family of substances with a well-defined pharmacologic profile. This remarkable and unusual family of compounds acts on mainly one receptor, the μ-receptor. The endogenous peptides, enkephalins and β-endorphin also act on this receptor and share all the classic actions of opiates, potent analgesia, euphorigenic activity etc. These peptides and their respective physiologic systems can therefore be identified with these specific actions; no other neurotransmitter or neuropeptide is so well related to a particular physiologic profile. It has therefore been possible to establish direct links between activity in these systems and the physiologic (or pathophysiologic) condition. Other peptides belonging to the same family, dynorphins and nociceptin cannot be attributed to any unique physiologic function yet, they seem to act as neuromodulators at different levels of the CNS.

The phylogeny of the opioid systems is not well characterized. Most studies have been done in mammals. However, also the mollusk, Mytilus edulis synthesizes opioid peptides and has an opioid receptor, 95% identical with the human μ-receptor. Thus from mollusk to man, nature has provided a system for protection against severe "pain" and "stress".

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