THE OPIATE RECEPTOR AND OPIOID PEPTIDES

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ABSTRACT

Understanding the mechanism of opiate action in analgesia, euphoria, and addiction may answer several fundamental questions in pharmacology. The development of a method for the detection of highly specific opiate receptors in 1973, *opened the current era of explosively rapid progress in this field. The most recent achievement is the isolation of endogenous peptides having morphine-like action from the brain. These peptides may act as CNS neurotransmitters, and Neurobiologists hope that they will shed light on normal brain mechanisms regulating pain, emotion and behavioural abnormalities like Schizophrenia.*

Key words : Opiate receptor, Endorphin, Enkephalin, Morphine, Narcotics.

OPIUM ALKALOIDS

Introduction

Relief of pain is one of the greatest objectives in medicine. Drugs with a predominant pain relieving action are called analgesics. Analgesics are substances which reduce the sensation of pain without simultaneously reducing other sensations such as touch and pressure. This specific action on pain perception distinguishes the analgesics from the anesthetics, which block the perception of all incoming sensory information. Analgesics are categorised as (1) Narcotics and (2) Non-narcotics. The term " Narcotic " refers to the drugs having sedative and analgesic action and it is essentially restricted to opium and derivatives of morphine. The non-narcotic agents such as aspirin are mild analgesics and are not related to opium alkaloids.

Opium, the product of the juice of the poppy *Papaver somniferum*—is a drug that has a venerable place in history. Extracts of the poppy plant have been used since the days of the Homeric epics, either medicinally or recreationally to relieve pain, induce sleep, ease anxiety and simply to promote a sense of well-being.

Raw opium contains several alkaloids, including some which are clinically useful as analgesics. Opium alkaloids of interest in medicine are divided into two classes based on their chemical structure *viz.*, the Phenanthrenes, and benzylisoquinolines.

TABLE I

Class	Natural alkaloid	% in opium	Uses
Phenanthrene	Morphine	10·0	Potent analgesic
	Codeine	0·5	Potent analgesic
	Thebaine	0·2	Not an analgesic but produces convulsions in low doses
Benzyliso-quinoline	Papaverine	1·0	Smooth muscle relaxant
	Noscapine	6·0	Used as a cough suppressant

Narcotic antagonists

One of the most dramatic aspects of opiate pharmacology is the existence of opiate antagonists. These are drugs closely related in structure to agonists, and may have little or no analgesic or euphoric effects themselves, but can completely reverse or counteract the effects of opiate agonists. The antagonists are obtained by small chemical modifications of opiates, specifically the conversion of N-methyl substituent to an N-allyl, N-cyclopropyl methyl groups. Several antagonists are much more potent than the opiate agonists themselves. They are thought to act as competitive inhibitors for the narcotic receptor, displacing the agonist molecules already bound to the receptor site. The antagonists elicit withdrawal symptoms or an abstinence syndrome when given to a drug dependant person.

TABLE II

Representative narcotic analgesics of interest

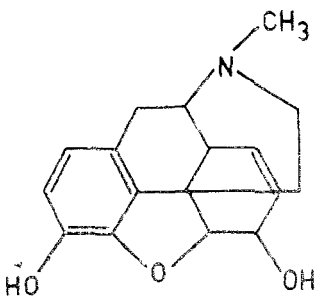
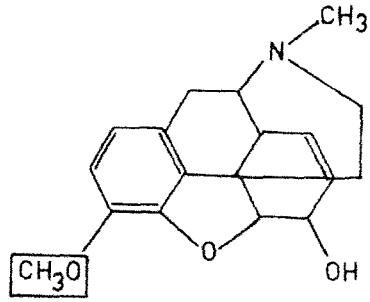
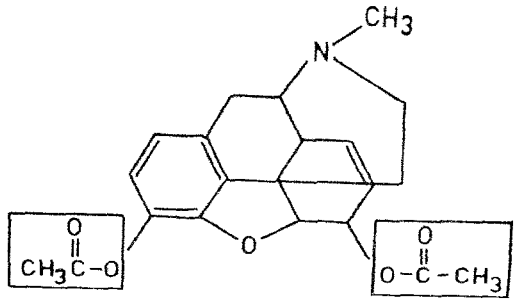
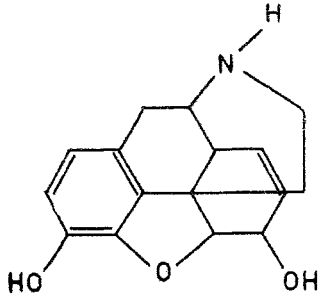
Name	Structure	Remarks
Narcotic agonists (drugs that bind to the receptor to produce a pharmacological response) :		
(a) Natural opium alkaloids		
1. Morphine		The standard against which all other narcotics are compared. A potent analgesic of all modalities of pain.

TABLE II (*Contd.*)

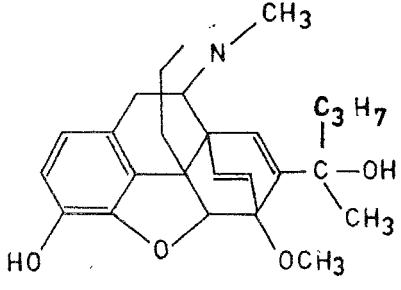
Name	Structure	Remarks
2. Codeine (Methylmorphine)		About half the analgesic potency of morphine. Useful for relief of mild pain and as an antitussive agent.
(*b*) *Semisynthetic derivatives of morphine* :		
3. Heroin (Diacetylmorphine)		Most popular drug of abuse among narcotic addicts. Effective analgesic but produces marked euphoria. The intravenous injection produces a peculiar orgastic sensation.

4. Normorphine



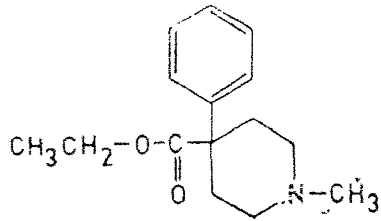
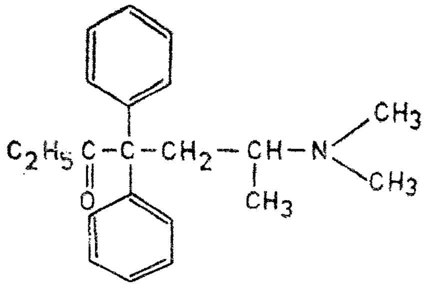
Ten times more potent than morphine.

5. Etrophine



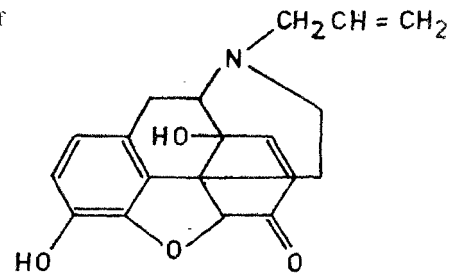
Most potent among the opiate drugs, 10,000 times more potent than morphine.

TABLE II (*Contd.*)

Name	Structure	Remarks
(c) *Synthetic derivatives of morphine* (prepared from a nonopiate base and some of which bear little chemical resemblance to natural drugs) :		
6. Meperidine		Most commonly used analgesic in medicine. It has an analgesic potency one-tenth of morphine with a short duration.
7. Methadone		Orally active analgesic equivalent to morphine but longer duration of action.

A. *Pure antagonist*

1. Naloxone (Narcan) (N-allyl derivative of oxymorphone)

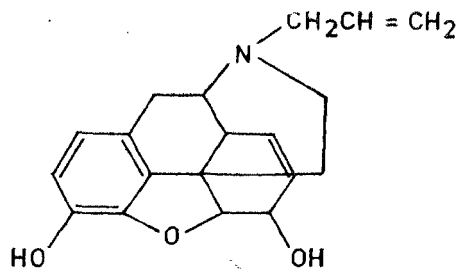


Naloxone is the only pure antagonist known. It has essentially no morphine like effects, *i.e.*, does not produce any physiological or psychological effects other than its role as a opiate antagonist. It has no effect in a normal person, but precipitates a withdrawal syndrome if given to an opium-dependant person.

B. *Antagonists with mixed properties*

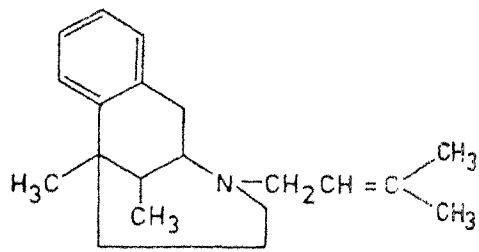
These antagonists are contaminated with varying range of agonist activity. The mixed antagonists in addition to antagonistic effect also elicit anxiety and psychomimetic effects at doses not much greater than those required to produce analgesia. The antagonists also cause physical dependance and tolerance.

2. Nalorphine (Nalline)-N-allyl-normorphine



Potent antagonist, and in a normal person behaves as a weak agonist

3. Pentazoicine



Pentazoicine has strong morphine-like effects and weak antagonistic action. It has considerable analgesic effect of short duration and is the most promising less addictive analgesic.

The uses of antagonists are

1. In the treatment of acute narcotic intoxication.
2. In the diagnosis of narcotic dependance.
3. Control of narcotic addiction.

Narcotic addiction

The phenomenon of addiction is difficult to define. It primarily involves tolerance, and dependance which are characteristic effects of opium and opiate-like substances and also of alcohol and barbiturates.

Tolerance means that the person who takes the drug requires progressively more of it to achieve the same effect, whether this be degree of analgesia or euphoria. It simply means that after prolonged administration of a drug the organism is now less sensitive to the drug, and can tolerate more of it. Addicts have been known to take as much as 4 g of opium in a day which is far greater than the lethal dose in a normal person.

Dependance : Dependance is of two types :

(i) *Psychic dependance* : Refers to the compulsive craving for the drug, to take it by some means or the other.

(ii) *Physical dependance* : Means that the person who stops taking the drug will suffer symptoms that are the inverse of the effects evoked

TABLE III

Effects of morphine

Target or parameter affected	Effect
Higher CNS structures	Analgesia: (*a*) Elevation of pain threshold. (*b*) Dissociation of pain perception from reaction to pain. (*c*) Sedation.
Other areas of CNS	Nausea and vomiting (medulla). Suppression of coughing. Meosis (occulomotor nucleus). Respiratory depression (medulla).
Behaviour	Euphoria (an exaggerated sense of well-being or a state of irrational happiness)/dysphoria. Excitation/depression.
Cardiovascular system	Depression of heart rate, postural hypotension, flushing of skin.
Smooth muscles	Contraction of sphincters: (*a*) Constipation. (*b*) Urinary retention.
Metabolism	Hyperglycemia.

by it. When the drug is withdrawn or when the person is treated with an opiate antagonist, withdrawal effects become evident which are usually in a direction opposite to the initial effects of the drug. Thus whereas morphine produces pupillary constriction, constipation and sedation, during withdrawal the pupils are dialated and there is diarrhoea and central excitation. These set of physiological and psychological symptoms following the withdrawal of a drug are termed " Abstinence syndrome ". A strong psychic dependance, an early development of physical dependance that increases in intensity paralleling with the dosage, and tolerance are the characteristic manifestations of opium addiction.

The question whether tolerance and dependance are different aspects of the same physiological phenomenon or are quite different events, has not been answered. The mechanism for the development of tolerance and physical dependance upon narcotic drugs is presently not known. The current theories concerning the mechanism of tolerance and dependance include enzyme induction and inhibition, an immune reaction to the morphine,[1] an increase in the narcotic receptor sites and an increased neuronal sensitivity due to a process similar to denervation supersensitivity. Of the above ideas the first two have received some support. The best evidence that macromolecular biosynthesis might be required, especially in the CNS, comes from the studies in which the inhibitors of RNA and protein synthesis were shown to block or delay the development of tolerance to chronic opiate treatment.[2] It is likely that the transient inhibition of protein biosynthesis is not a specific opiate effect, but is instead, related to a general depression of metabolism in brain.[3]

Several investigators have attempted to separate the opiate effects neurochemically to determine which, if any of the suspected neurotransmitters of cholinergic, adrenergic and sertonergic systems are involved in the analgesic action of morphine. It is impossible to ascribe chronic effects of opiate use to a single neurotransmitter system in the CNS although it is occasionally possible to define the neuronal pathway for a single effect of an opiate.[2]

A major goal of opiate research is to develop a non-addictive analgesic, *i.e.*, pain killers with the analgesic potency but without the addictive potential of the opiates now used. Although non-opiate pain killers such as Aspirin are useful in some situations, only opiates seem to be effective in the treatment of severe and protracted pain. This is the main reason for the current explosive research going on in this field.

Opiate Receptor

The main goal of pharmacology is to establish the complete chain of casuality between the combination of a drug with the cellular components and the manifest effects of that drug in the organism. The central concern is to understand the first step in that chain—the drug-receptor interaction.[4] Unusual interest attaches to opiate receptors because of the role they may play in analgesia, the euphorinogenic effects of narcotics, and other aspects of narcotic addiction such as tolerance and dependance.

A receptor for opiates—why ?

The naturally occurring narcotics are derived from plants and it is surprising that a receptor to it occurs in the brain. Narcotic analgesics are generally believed to exert their effects by interacting with specific receptors located in the CNS. The binding of a narcotic drug to the receptor site is a necessary step in the production of a behavioural effect. The notion that opiates exert their pharmacologic activities *via* a specific opiate receptor derives from several features of opiate pharmacology. The features which strongly indicate the existence of such a receptor in the brain are as follows :

(1) Despite some variations, all opiates possess a common chemical structure, in having a basic centre and aromatic features.

(2) Opiate actions are dramatically stereospecific. For most opiates virtually all pharmacological activity resides in the L (—) isomer, whereas D (+) isomers are inactive.

(3) The existence of specific antagonists, such as naloxone and others, which block not only the actions of morphine, but also of many other classes of narcotic analgesics.

(4) The fact that small structural changes, which are likely to alter the physical properties of the molecule in only minor degrees, may bring about changes in potency, or convert an agonist into an antagonist.

(5) Some opiates exert their actions in very small doses, much lower than could be accounted for, by non-specific membrane effects.

Inferential data about opiate receptors were first derived by Beckett and Casy[5] in 1954 from studies on structure-activity relationships in several series of analgesics. A receptor surface was formulated containing a flat surface, a cavity and an anionic group in the proper spatial relationship to elucidate the active compounds. Later workers recognized the stereochemical requirements for analgesic activity and Portoghese[6] in 1966 pointed out that to rationalize all the data, would require something better than the earlier model of a single rigid receptor. He introduced a concept of induced configuration (a flexible receptor) to explain the binding of different opiates.

Technological considerations

First and the most fundamental in receptor identification is the ensuring specificity. Merely because a ligand binds in a saturable fashion, with considerable affinity to brain tissue by no means establishes that the binding sub-

stance is a specific receptor for the ligand. Binding which is biologically irrelevant can often be extraordinarily deceptive. Thus Cuatrecasas and Hollenberg[7] noted that Insulin can bind to glass or talcum powder with affinity in manomolar range and with the capacity to discriminate among several insulin analogues according to their biological potency.

Opiate pharmacologic effects are stereospecific with the (−) isomer usually possessing all the activity. Certain glass filters can bind opiates stereospecifically with a preference for the (−) isomer.[8] Cerebrosides, the ubiquitous lipids can also bind opiates stereospecifically,[9] conceivably accounting for the properties of a soluble opiate binding substance from the brain tissue. Earlier attempts to demonstrate the presence of receptors specific for opiates had failed, because these drugs bind nonspecifically to many substances in the brain tissue. It was hard to detect the very small amount of specific binding against such a high background.

Criteria of stereospecificity

In 1971, Avram Goldstein of Stanford University Medical School came out with an elegantly worked out conceptualization for picking out the specific opiate binding from the background. Goldstein[10] proposed that opiate receptor binding should, like opiate analgesic effects themselves, be stereospecific and for pharmacologically relevant opiate receptor binding, one should attempt to identify stereospecific binding. This criterion of stereospecificity provided the basis for further studies.

It is assumed that there are three kinds of interaction between an opiate and membranes containing opiate receptors[11]: (1) A non-specific saturable binding consisting primarily of interactions between the protonated nitrogen atom of the opiate and anionic groups of membrane macromolecules. (2) A non-saturable interaction (trapped and dissolved) having the physical solution of lipophilic opiate molecule in the lipidic membranes. (3) The stereospecific interaction of L (−) opiates with opiate receptor.

Basis of the opiate receptor binding assay can be depicted as follows[11]:

The method is illustrated in the figure. In condition (A) the membranes are incubated with a radioactive opiate ligand (solid black symbols). The radioactivity associated with the membranes measures the sum of the three kinds of binding. In condition (B), prior incubation with a large excess of non-radioactive opiate drug in (+) (wrong) conformation—(Open L symbols)

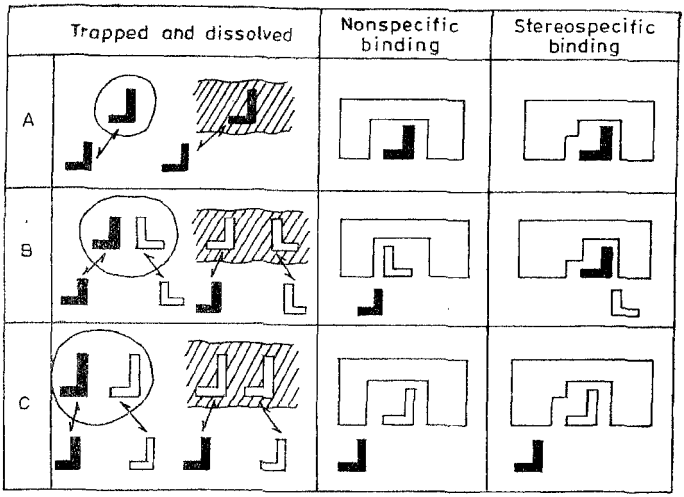


FIG. 1. The opiate receptor-ligand interactions.

excludes radioactive ligand from the non-specific saturable sites. The difference A—B measures the nonspecific saturable binding. In condition (C) the preliminary incubation is with a non-radioactive opiate of the (—) correct conformation. Now radioactive ligand is excluded from both nonspecific and stereospecific sites. Thus the difference B–C measures the stereospecific binding. Finally the residual radioactivity associated with the membranes in C measures the non-specific non-saturable interaction.

Demonstration of the opiate receptor

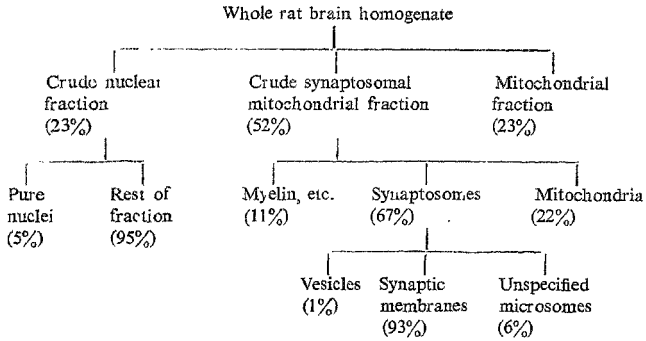
In order to amplify the specific binding, while minimising the non-specific component, the investigators used isotopically labelled opiates and opiate antagonists, and washed the treated tissues thoroughly to remove material that was bound non-specifically. The ligands used in these experiments were of very high specific activity. This assured that small quantities of the bound drug could still be detected, after the bulk of the extraneous material had been washed out. Using this approach, Snyder and Pert showed that labelled naloxone binds specifically to preparations of homogenized rat brain tissue.[12]

Researchers from three different laboratories identified the opiate receptors, almost simultaneously in 1973 based on the criteria of stereospecificity. They are Solomon H. Snyder and Candace B. Pert of Johns Hopkins,[12] Eric J. Simon *et al* of New York University School of Medicine[13] and Lars Terenius[11] of Uppsala University in Sweden. Simons group[13] used the very potent narcotic agonist ^{3}H etorphine in human brain. Terenius[11] employed ^{3}H dihydromorphine as the ligand. The stereospecific binding of opiates to the receptor has been confirmed in other systems.[15,16]

Stereospecific binding is necessary, but does not contribute sufficient evidence that one is dealing with binding to the pharmacologically relevant opiate receptor. It is of exceeding importance to show that there is a close parallelism between pharmacological potency and receptor binding in the same system. Accordingly a wide range of opiate drugs have been screened. In general there is a close parallel between pharmacologic potency and affinity for naloxone binding sites, as shown in the guineapig ileum assay.[17,18] This ensures that binding to brain membranes represents receptor interactions that operate *in vivo*.[17] Further assurance of the specificity of the opiate receptor is derived from studies showing that a wide range of 75–100 drugs which are not opiates have negligible affinity for the opiate receptor.[18] These observations also shed light on certain basic drug receptor concepts. Some receptor theories postulate that drugs can exert maximal pharmacological responses while occupying only a small fraction of the total number of pharmacologically active receptors, as classically exemplified by acetylcholine in guineapig ileum. If a substantial portion of opiate receptors does not mediate pharmacological responses and are thus " spare receptors ", then there should be major discrepancies between drug concentrations filling a given percentage of binding sites and concentrations required to produce the same per cent of a maximum pharmacological response. Opiate concentration occupying half the binding sites (ED_{50}) in the guineapig ileum correspond closely to those eliciting half maximal pharmacological response, whether agonist or antagonist. This suggests that opiate effects can be explained, without invoking " spare receptors ". Potent opiates like morphine and levorphanol have affinities in the nanomolar range, while weak opiates such as meperidine and propoxyphene have much less affinity for the receptor. Codeine which *in vivo* has 1/7th potency of morphine is extremely weak in binding to the opiate receptor with less than 1/1200th the affinity of morphine. Pasternak and Snyder[19] showed that there are high and low affinity sites in the receptor to a given opiate. Sodium at physiological concentration increased the binding of antagonists to the high affinity site.[19]

The opiate receptor is present in all vertebrates, but has not been detected in invertebrates[20], a fact wihch may be significant from the viewpoint of differences in neuronal connections. No increase in the number or binding affinity of opiate receptors was found in the dependant animals[15,21] which thus rules out one mechanism which had been widely advanced to explain opiate dependance.

Subcellular localization



Subcellular localization of opiate receptor binding in rat brain depicting the percentage of total binding in each fraction in relation to the fraction from which it was derived[22].

Using differential and sucrose density gradient centrifugation, it was shown that in rat brain homogenates, opiate receptor binding is most enriched in synaptosomal fraction, which contains primarily pinched off nerve ending particles[22]. Opiate receptor binding is recovered exclusively in the synaptic membrane fraction. This is an additional evidence in favour of the view that opiate receptors are actually receptors for an endogenous neurotransmitter. The synaptic membranes are the logical site for neurotransmitter receptors as exemplified by acetylcholine and norepinephrine receptors at the synapse.

Advances in isolating membrane bound receptors have been relatively slow. Goldstein[23] used Sephadex LH-20 columns and partially purified the opiate receptor and found the binding material to be a proteolipid.

Regional distribution and mapping

Regional studies offer considerable potential for elucidating relationship between opiate receptor action and pain-mechanisms in the brain. Binding of opiates to their pharmacologically relevant receptor *in vitro* autoradiographically shows dramatic regional variations in monkey[24], human, rat and calf[25] with the highest localization in limbic regions. The procedure for the autoradiographic localization of opiate receptors involves injecting a suitable radioactive antagonist or agonist, sacrificing the animal at a time when the bulk of the drug is specifically bound to receptors and processing the tissue in such a way as to prevent diffusion of drug from its binding site.

Amygdala shows the greatest amount of binding with the anterior portion displaying almost twice as much binding as the posterior amygdala. Binding in the periaqueductal area of the mid brain is about the same as in the posterior amygdala. The hypothalamus and medial thalamus, the next highest areas display only about 40% of the binding of anterior amygdala. No regional variations are detected within the hypothalamus, while the medial thalamus possesses about 3 times as much binding as lateral thalamus. Receptor binding is very low or not detectable in the cerebellum, spinal cord, and the white matter areas[24,27]. Interestingly the map of opiate receptor binding throughout the brain closely resembles the distribution of the paleospinothalamic and spinoreticulodiencephalic pathways which are involved in the affective component of pain perception. These pain pathways include areas of the brain such as periaqueductal gray, the medial nucleus of thalamus, the hypothalamus and several areas in the limbic system. Some of these regions, especially the periaqueductal gray correspond to those in which implantation of morphine most effectively elicits analgesia[26]. The amygdala apparently is an exception not associated with any pain pathways and may relate to affective components of pain. Morphine implantation in amygdala does not relieve pain[26]. Thus the wide anatomical distribution of opiate receptors suggests that opiates can influence a large variety of functional pathways.

Sodium effect

Sodium ion has a very specific effect on the opiate receptor. Concentrations of sodium as low as 1 mM enhance the binding of opiate antagonists to the receptor and decrease the binding of opiate agonists to a corresponding extent[28]. The influence of sodium is selective, and it can be reproduced to a lesser extent by lithium, but not by potassium, rubidium or

caesium[28]. Binding of mixed antagonists, some of which have less addictive potential than pure agonists is affected by sodium in a fashion, intermediate between agonists and antagonists. Thus at a practical level, the influence of sodium on opiate receptor binding provides a tool to determine whether a given drug is an agonist, pure antagonist or mixed antagonist.

Using this selective effect of sodium, Snyder and Pert[29] devised what they call the "sodium response ratio" to distinguish between agonists and antagonists. This is the ratio of the concentration of the test drug that inhibits by 50% the binding of labelled naloxone (a pure antagonist) in presence of sodium to the comparable concentration in the absence of sodium. In the presence of sodium, opiate agonists should have decreased potency in preventing the binding of naloxone, that is, more drug should be required to give a 50% inhibition and the ratio should be much greater than 1. Drugs that are predominantly antagonists should have the same capacity to inhibit the binding, whether sodium is present or not. Thus for pure antagonists the ratio expected is 1. Antagonists which are contaminated with some agonist activity have ratios greater than 1, but less than 3. The mixed agonist-antagonists having more agonist action have ratios between 3·3 and 6·4. The ratio for a variety of opiate agonists, ranges between 12 and 60—as shown in Table IV[30]. The results obtained agreed with the expected values.

Thus it is possible to predict the pharmacological properties of different opiates simply by measuring receptor interactions in the presence or absence of sodium, affording a rapid and inexpensive screen. Chemists need not synthesize grams of a drug for screening in intact animals, and there is no need to get the drug in labelled form[30].

The sodium effect explains the greater clinical potency of antagonists than of agonists. At normal body concentrations of sodium, the antagonist will bind to the opiate receptor, much more efficiently than the agonist and therefore exert pharmacologic effects at lower doses.

The differential effect of sodium on the binding of agonists and antagonists could be interpreted in several ways at the molecular level. One might assume that the receptor can bind both agonists and antagonists in different conformations. Further it has been shown that sodium accelerates the dissociation of agonists from receptor binding sites while it does not affect the dissociation rate of ^{3}H naloxone, which is an antagonist[28].

Divalent cations may also have a role in the normal function of opiate receptor. Low, physiological concentrations of Mn^{++} and Mg^{++} affect the

TABLE IV*

Effect of sodium on inhibition by opiate agonists and antagonists of stereospecific ^3H naloxone binding to rat brain homogenates

Nonradioactive opiate	ED_{50} of stereospecific ^3H naloxone binding (nM)		ED_{50}: 100 mM NaCl / No NaCl
	No NaCl	100 mM NaCl	
Naloxone	1·5	1·5	1·0
Naltrexone	0·5	0·5	1·0
Diprenorphine	0·5	0·5	1·0
Cyclazocine	0·9	1·5	1·7
Levallorphan	1·0	2·0	2·0
Nalorphine	1·5	4·0	2·7
Pentazocine	1·5	5·0	3·3
Etorphine	0·5	6·0	12
Meperidine	3,000	50,000	17
Levorphanol	1·0	15	15
Oxymorphone	1·0	30	30
Dihydromorphine	3·0	140	47
Propoxyphene	200	12,000	60

* Taken from Snyder, S. H. *et al*[28]

opiate receptor in a way diametrically opposite to sodium. They selectively increase the binding of opiate agonist by reducing receptor sensitivity to sodium[31]. Mn^{++}, Mg^{++} and Ni^{++} act selectively in the above fashion, while calcium fails to enhance opiate binding. The influence of chelating agent indicates that endogenous divalent cations regulate opiate-receptor functions. Thus treating brain membranes with EDTA which chelates most divalent cations, lowers the agonist binding, while EGTA which chelates Ca^{++} but not Mn^{++} and Mg^{++} has no influence on receptor binding.

Based on the sodium effect, it has been postulated that opiate receptor can exist in two interconvertible forms— an "antagonist" conformation which binds sodium and an agonist or no sodium conformation[28]. Antagonists have a high affinity for the sodium and a low affinity for the no sodium

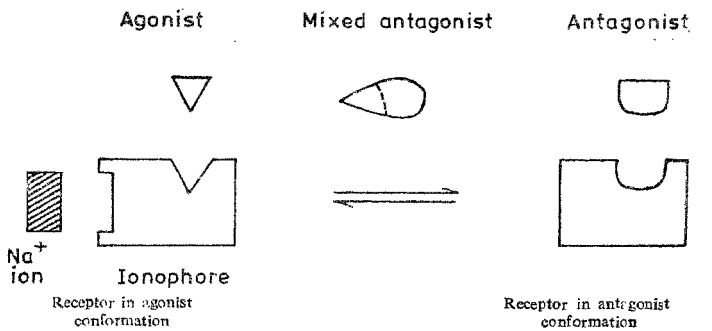


FIG. 2. A model of receptor function (From Snyder, S. H., *Biochem pharmac.*, 24, 1371, 1975).

conformation, while the reverse is true for agonists. Typical analgesic effects occur only if a drug binds to the agonist conformation, and this affords an explanation for the difference between the pharmacologic actions of agonists and antagonists. Conventional pharmacological theory holds that both agonists and antagonists bind to receptor, but antagonists cannot elicit pharmacologic effects due to lack of intrinsic activity and simply sit on the receptor, preventing the access of agonists. Antagonists block morphine action by occupying the sodium sites of the receptor, and shifting the equilibrium to reduce the number of no sodium receptors, available for agonists[28,32,33]. Scatchard analysis provides evidence for cooperative binding, a characteristic of allosteric systems. The evidences that both agonists and antagonists bind to the same receptor site are as follows:

(1) Displacement experiments between agonists and antagonists exhibit cooperative kinetics.

(2) The maximum number of binding sites is the same when estimated from agonist or antagonist binding.

(3) Sodium protects both agonist and antagonist sites against SH group inactivation.

(4) Both agonists and antagonists can in turn protect against NEM (N-ethylmaleimide) inactivation of binding sites.

Presumably interconversion of the two forms of the opiate receptor involves folding, unfolding, aggregation, disaggregation or such other modifications in protein structure. Consistent with the prediction is the obser-

vation that protein modifying agents and proteolytic enzymes at low concentrations do selectively reduce opiate agonist binding, with negligible effects on antagonist binding[28].

The model agrees with the generally accepted model for the interaction of neurotransmitters. It would seem reasonable to extrapolate the data with several brain receptors, to suggest, that all neurotransmitter receptors can exist in two forms which are interconvertible. This dual confirmation of the receptor is not required *in vivo*, because antagonists generally do not occur endogenously in the organism. Instead the transition between receptor states, mediated by ionic or other conductance modulators, may constitute the fundamental mechanism translating neurotransmitter signals into changes in the firing or other functions of the post-synaptic cell. Assuming that the opiate receptor is in fact a neurotransmitter receptor, the transition of the receptor from the antagonist to agonist state, caused by the endogenous neurotransmitter could be associated with a change in binding of the ion whose conductance is altered, resulting in firing of a specific neurone. This question of how, neurotransmitter recognition is translated into a change in ion conductance, or conceivably some other "second messenger" has remained a mystery in neurobiology.

Snyder[73] has proposed the concept of multiple receptor conformation to explain differential kinetics of binding and displacement. It is likely that more than one type of opiate receptor exists in the animal and human brain[31]. Hans Kosterlitz recently has[35] proposed the existence of a family of opiate receptors on the basis of binding anomalies and variations in rank, order of potency, for a series of opiates when tested in different peripheral assays.

Endogenous Opioid Peptides

Concept of an endogenous ligand for opiate receptor

Man was not made with morphine in him. Narcotics do not occur naturally *in vivo*, in the animal brain. If so, why there is an opiate receptor in all vertebrates, was the question next confronted with. It seemed very unlikely that nature has made such a stereospecific receptor to interact with the exogenous plant alkaloids like morphine. Naturally, neurobiologists hypothesized that there must exist some endogenous ligand or ligands which can bind to the opiate receptor and may act as a neurotransmitter or neuromodulator. The idea was first advanced by HOJ Collier in 1972[36], influenced

by the innovative thoughts of Davis[37]. The striking selectivity of specific opiate binding sites in the brain[12,13,14] in terms of their substrate specificity, regional distribution[34,37] and correlation with pharmacological activity[17] suggests that these sites do not exist fortuitously, but might represent receptor sites for a normally occurring opiate-like material.

Enkephalins and endorphins

The search for such an endogenous material which mimics morphine action began and positive results were reported in 1974 by Terenius and Wahlström[38]. John Hughes[39], working at the University of Aberdeen, Scotland, in 1975, identified a material from porcine brain, which mimicked the capacity of morphine to inhibit contractions in smooth muscle preparations such as guineapig ileum. Specificity was shown by the fact that low concentrations of opiate antagonist naloxone, reversed the effects of this brain extract. Another approach involves demonstrating that brain extracts inhibit opiate receptor binding[38,40]. Decrease in the activity of the material by carboxypeptidase A treatment and sensitivity to chymotrypsin indicated that the substance may be a peptide having aromatic amino acid residues[41]. Later Hughes, J. *et al*[42] characterized this material and found it to be a mixture of two pentapeptides—and coined the term "Enkephalin" to refer to these peptides which mimic the action of morphine. They sequenced these peptides, and found to have identical amino acids except the one at the carboxy terminal end. The sequences are :

H–Tyr–Gly–Gly–Phe–Met–OH (Methionine enkephalin).
H–Tyr–Gly–Gly–Phe–Leu–OH (Leucine enkephalin).

Met-enk and Leu-enk occur in the ratio of 4 : 1 in the porcine brain[42]. The enkephalin has a molecular weight of 1000, and even the synthetic peptides were shown to have the same activity as the natural ones[42]. Enkephalins have also been isolated from bovine,[45] guineapig, rat, mice,[44] rabbit and beef brains[43] and human cerebrospinal fluid[46]. Simantov and Snyder[43] showed that beef and bovine brain contains four times more leucine enkephalin than methionine enkephalin in contrast to pig brain, in which these ratios are reversed.

All the above enkephalins were isolated from brain extracts. Next came the discovery of peptides having morphine-like action from the pituitary. Teschmacher[47] and Cox *et al*[48] have reported a peptide material extracted from pituitary which is similar to enkephalins in inhibiting smooth muscle contraction, but chemically different from the morphine-like peptides of brain tissue. The pituitary substance has a molecular weight of 1700,

and is sensitive to trypsin digestion and insensitive to carboxypeptidase.[48] Moreover, Cyanogen bromide treatment inactivated only one of the peptides, suggesting the presence of basic amino acids and absence of methionine in one of the peptides. According to Goldstein[47], the pituitary peptide may be a precursor of enkephalins, but further work is needed to confirm this hypothesis.

The endogenous ligands for the opiate receptor have been variously called as MLF (morphine-like factor) by Terenius, Pasternak and Snyder, NRA (naloxone reversible activity) and enkephalins by Hughes and others.

The regional distribution of enkephalins in calf, rat, and rabbit brain is closely similar to that of opiate receptor itself[49] with high levels in corpus-striatum, hypothalamus, and negligible amounts in the cerebellum. Enkephalins and other opioid peptides behave as typical agonists in the bioassays and binding assay. Blockage of the effects of an opioid material by naloxone is one criterion for establishing that its binding to opiate receptor is necessary for its action.

TABLE V*

Regional localization of the MLF and opiate receptors in bovine and rat brain

	MLF, U/mg protein	Opiate receptor binding cpm/ 0·1 mg protein
Bovine :		
Caudate	480	320
Hypothalamus	250	282
Spinal cord	140	205
Pons	135	231
Cerebral cortex (Parietal)	70	173
Thalamus	75	179
Cerebellum	50	86
Medulla oblongata	50	88
Corpus collasum	10	61
Rat :		
Caudate	480	900
Brain stem (mid-brain)	140	220
Cerebral cortex	80	210
Cerebellum	None	None

* Taken from Goodman *et al*[48]

Endorphins

After the discovery of enkephalins, investigators came across some more peptides, having morphinomimetic activity. These are longer, naturally occurring peptides, that share similar pharmacological properties. The term "Endorphin" was coined by E. J. Simon in 1975 to designate the endogenous substances showing morphine-like effect and it is now widely accepted as a generic name of opioid peptides. It is analogous to the term "corticotropin" which denotes a biological activity rather a specific chemical structure. Thus enkephalins are the first characterized endorphins.

An area, intensively investigated in the neurosciences today, is the study of endorphins because of their possible implications in pain perception, etc. These endorphins occur naturally in the brain, or the pituitary gland and mimic the action of opiate drugs. Investigators at present have detected at least 7 such substances. These include a family of five structurally related peptides that are present in the brain, the pituitary or both, a pituitary peptide of unknown structure, that is apparently unrelated to others[48], and a low molecular weight peptide in the blood[50]. All these mimic at least some actions of opiates in the commonly used assays, but there are differences of opinion about which are more important physiologically.

Endorphins :
(1) Met-enkephalin
(2) Leu-enkephalin
(3) α-endorphin
(4) β-endorphin
(5) γ-endorphin
(6) A pituitary peptide of unknown structure[48]
(7) Anodynin—A low molecular weight peptide in the blood[50].

α, β and γ-endorphins were identified in the extracts comprising both pituitary and hypothalamus[51]. Later it was found that the sequences of α, β and γ-endorphins occur in the pituitary hormone called β-lipotropin. Pert *et al*[50] have identified an endorphin in human and rat blood. The material is apparently a peptide with a molecular weight of about 600, but it differs chemically and biologically from enkephalins. They have named it anodynin (from anodyne—a drug that calms and allays pain). Anodynin is degraded by brain much more slowly than the enkephalins and it produces long lasting

analgesia. Since naloxone blocks the analgesic effects of the material, it apparently interacts with opiate receptor to produce its effects. Hypophysectomy results in almost complete disappearance of anodynin from the blood, suggesting a pituitary origin. The authors[46] think that anodynin may be a hormone acting on opiate receptors. These investigators are now looking the effects of stress, pain, sleep and other physiological states on the concentration of anodynin in blood.

Methods of assaying opioid activity

Good assay systems are the keys to identify biologically active endogenous substances. Two assays are generally used to detect and quantitate opioid activity. The primary one is a bioassay, the electrically stimulated guineapig ileum myenteric plexus, longitudinal muscle preparation[52] and mouse vas deferens[53]. Electric field stimulation of the plexus causes the release of acetylcholine from the post-ganglionic cholinergic neurones resulting in a twitch of the longitudinal smooth muscle. This acetylcholine release is diminished in a dose related manner by opiates causing an inhibition of twitch amplitude. Many substances like catecholamines also inhibit the twitch amplitude, but only the opiate inhibition is blocked and reversed by opiate antagonist at low concentration (100 nM). It has been shown in the comprehensive studies of Kosterlitz[54] that naloxone reversible twitch inhibition with guineapig ileum preparation is nearly perfectly correlated with analgesic activity in whole animals. Thus opioid activity is defined as naloxone blocked or naloxone reversed inhibition in this system. This effect of narcotics is specific to guineapig ileum, and in rabbit ileum preparation morphine does not reduce transmitter release[55]. The myenteric plexus serves as a model of the CNS, because it contains neurones, satellite cells, glial elements and nerve fibres.

Electrically stimulated mouse vas deferens is another bioassay used to quantitate opioid activity.

The other routine assay is the opiate receptor binding assay, in which membranes from guineapig brain are used as the source of opiate receptors, along with the potent agonist ^{3}H etorphine. This mixture is incubated and then washed thoroughly. The pellet is treated with Triton X–100 and radioactivity counted. Inhibition of stereospecific binding in this assay is presumptive evidence of opioid activity. The principal advantage of the binding assay is its sensitivity which is approximately 5–10 times greater than that of bioassay.

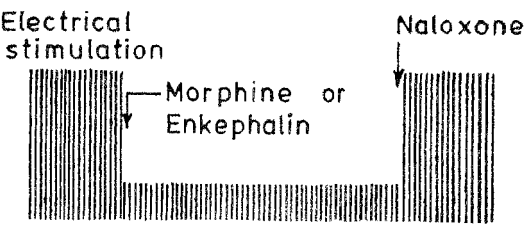


FIG. 3. Guineapig ileum assay

It appears that the morphine receptor in guineapig ileum is very similar to the one that mediates analgesia in man[54].

Origin and precursors of endorphins

β-Lipotropin : Hughes and Kosterlitz[42] recognized that the pituitary polypeptide β-lipotropin contains the sequence of methionine enkephalin. This touched off a series of investigations, and soon it became known that β-lipotropin has the sequences of several other endorphins.

β-Lipotropic hormone (βLPH) was isolated in 1964 from sheep pituitary glands by C.H. Li[56]. βLPH was subsequently isolated and characterized from the pituitary of a variety of species. In all cases, it is a homomeric 91 residue polypeptide with minor variations in its amino acid sequence. The physiological significance of βLPH has remained obscure to this day. It has lipolytic activity in several systems and animal melanophoretic and adrenocorticotropic activity[57]. It has been reported in the blood[58] and has been located in discrete cells of anterior and intermediate lobes of the pituitary by immunofluorescence[59]. This pituitary hormone has been long recognized to be the source of β-melanocyte stimulating hormone (βMSH)[56].

β-Lipotropin contains the sequences of methionine enkephalin (βLPH 61–65), α-endorphin (βLPH 61–76), βMSH (βLPH 41–58), β-endorphin (βLPH 61–91) also known as the ' C-fragment ', γ-endorphin (βLPH 61–77) and also of the fragment ^{4}Met to Gly^{10} of ACTH (βLPH 47–53). ACTH (4–10) was the shortest peptide with a behavioural potency comparable to that of parent molecule (whole ACTH). Shortening of the sequence of ACTH 4–10 step by step from COOH end revealed that the tetrapeptide 4–7 contains the essential elements required for behavioural effect of ACTH

analogues[60]. α, β and γ endorphins were isolated from the brain extracts[61,74]. The natural peptides were characterized and sequenced. Guillemin and his coworkers, to their surprise, found the same sequences in βLPH. The sequence of βLPH and endorphins embedded within it are shown :

H Glu Leu Thr Gly Glu Arg Leu Glu Gln Ala Arg Gly Pro
5 10
Glu Ala Gly Ala Asn Asp Gly Ala Ala Arg Ala Glu
15 25
Leu Glu Tyr Gly Leu Val Ala Glu Ala Glu Ala Ala Glu
30 35
Lys Asp Ser Gly Pro Tyr Lys Met Glu His Phe Arg Trp
40 45
Gly Ser Pro Pro Lys Asp Lys Arg Tyr Gly Gly Phe Met
60 65
Thr Ser Glu Lys Ser Gln Thr Pro Leu Val Thr Leu Phe
70 75
Lys Asn Ala Ile Ile Lys Asn Ala His Lys Lys Gly Gln OH
80 85 91

The primary structure of sheep and camel β-lipotropin (βLPH)$_T$. Data of Li and Chung, *Nature* **260**, 622, 1976.

Met-enk 61-65	α-endorphin 61-76
βMSH 41-58	β-endorphin 61-91
ACTH fragment 47-53	γ-endorphin 61-77

Endorphins have been found in extracts from pig, beef, sheep, rat and human pituitaries. The concentration per unit tissue weight is about 8 times higher in the posterior than in anterior pituitary. Thus the total activity is about equal in the two lobes[61]. Limited evidence from subcellular fractionation experiments suggests that endorphins and enkephalins may be contained within osmotically sensitive subcellular compartments probably synaptosomes[62]. In the bioassay, the whole βLPH molecule is virtually devoid of opioid activity[63]. α-Endorphin, the hexa decapeptide (βLPH 61–76) and γ-endorphin (βLPH 61-77) have opioid activity to a lesser extent but higher endorphin activity than the enkephalins[61]. Several groups have reported that the pituitary peptide corresponding to the 'C' terminal fragment of β-lipotropin or β-endorphin (βLPH 61–91) has high morphine-like activity in various test systems[62,64,65]. β-Endorphin is the most potent among all the endorphins. Methionine enkephalin consists of residues 61–65 of β-lipotropin and all the 3 endorphins (α, β, γ) share this common sequence at their N-terminal region. However, leucine enkephalin does not seem to

be derived from β-lipotropin. On elementary probabilistic grounds, it is most unlikely that an identical pentapeptide sequence could be synthesized by chance in different endorphins. So, it can be assumed that enkephalins are derived from the larger endorphins. There may be yet more morphine-like peptides, still to be characterized.

All these studies indicate that the parent of the whole family of endorphins may be the β-lipotropin. One school of thought holds that enkephalins are physiologically significant and that the larger peptides may serve as precursors. at least for methionine enkephalin. The fact that both the enkephalin sequence and βMSH are preceded by a pair of basic residues indicates the situation similar to proinsulin, prohormone and proenzymes. There are plenty of enzymes in the brain and elsewhere to split the larger peptides. Lazarus and Guillemin[66] have shown that incubating the β-lipotropin with rat brain homogenates at physiological pH generates peptides with opioid activity. Although the structure of the fragments has not yet been determined, this finding supports the hypothesis that β-lipotropin is a precursor of endorphins.

The other school of thought holds that the larger endorphins, principally β-endorphin are physiologically active, and enkephalins are mere degradation products of the former. The β-lipotropin could be the original precursor of any of these smaller peptides. Goldstein[67,68] on the other hand thinks that it is unlikely that the β-lipotropin derived peptides can account for all the opioid materials in the brain. Wahlström[17] has reported an opioid peptide from human CSF that appears chemically different from all the known fragments of β-lipotropin. The previously described anodynin[50] is also not an enkephalin. This is a unique situation among peptide hormones, because the precursors like β-endorphin and larger endorphins seem to be more potent than the enkephalins themselves in several test systems.

Many investigators think that the precursor of the brain endorphins may be synthesized in the pituitary and then secreted into the brain. Goldstein *et al*[69] have shown that the concentration of endorphins of rat brain does not decline after hypophysectomy, at least for a month. If the pituitary materials do not enter the brain, they would exert their effects only on those peripheral tissues that are innervated by nerves with opiate receptors.

While the biogenesis of the hypothalamic, hypophysiotropic peptides, TRF (thyrotropin releasing factor), LRF (lutenizing hormone releasing

factor), etc., is probably by protein synthesis of oligopeptides or more likely of protoforms from ribosomal templates, the likely biogenesis of endorphins and enkephalins discussed here is reminiscent of that of the angiotensins.

Enzymatic degradation of enkephalins

The enkephalins are rapidly destroyed by peptidases present in tissue homogenates[70]. In view of this, it is not surprising that *in vivo*, even after central administration, antinociceptive actions of peptides are at best transient. It is of interest to point out that in contrast to enkephalins, the plant alkaloid, morphine shows relative resistance to enzymatic degradation. This different behaviour of compounds derived from animal and plant kingdom is reminiscent of a similar relationship between the muscarinic agents—acetylcholine and muscarine. Hambrook *et al*[70] have demonstrated that the deactivation of enkephalins in rat and human plasma and in rat brain homogenates occurs by the cleavage of tyrosine-glycine amide bond. In support of this, Pert[71] has reported a simple chemical derivative, D ala D ala Met enkephalinamide which is resistant to enzymatic degradation and produces long lasting analgesia in rats. Other synthetic analogs in which tyrosine is replaced by D-amino acids, resistant to enzymatic deactivation have been reported[72].

Analgesia and physical dependance evoked by opioid peptides

A major reason for the interest in endorphins has been the possibility that they or their analogs might turn out to be nonaddictive pain killers that could replace opiates in medical practice. But recent studies indicate that enkephalins appear to be addictive similar to morphine.

Several investigators[73, 74] have found that, very large doses of enkephalin are needed to cause transient analgesia that lasts less than 5 minutes. In the same assays, much smaller doses of morphine produce more distinct effects that lasts up to 4 hours. The methionine enkephalin is at least 50 fold weaker than morphine as analgesic despite the fact that it has about half of the affinity of morphine for rat brain opiate receptors and microinjected directly into active brain sites. This has been ascribed to the potent enzymatic degradation *in vivo*[74].

β-Endorphin, on a molar basis, is 20 times more active than morphine as an analgesic[75] and has potent analgesic properties when injected to cerebral ventricles of rat and rodents[76].

Enkephalins and endorphins also produce both tolerance and dependance. The clearest evidence for this came from Wei[87] who reported that, following

the chronic infusion of very small amounts of β-endorphins or methionine enkephalin, rats exhibited typical withdrawal symptoms when challenged with naloxone. This has been confirmed by two other groups[77, 78]. These findings suggest that mechanisms involved in the production of tolerance may be important in regulating the actions of opiate peptides *in vivo*.

Structure and conformational relationships between opioid peptides and opiate alkaloids

It is of great interest to know the structural analogy between opium alkaloids and enkephalins which belong to very different classes of compounds. The enkephalins and opiates compete for the same receptor, and give a similar pharmacological response. Therefore it is likely that they have structural and conformational similarities.

The structure-activity relationship suggests that the tyrosine residue in enkephalins is essential for binding. Its substitution by tryptophan, phenylalanine and DOPA results in a loss of potency of 2 or 3 orders of magnitude[71]. Enkephalins bind slightly less tightly to the opiate receptor.

The most characteristic features of conformation, which could account for some of the physiological and biological properties of enkephalin and morphine, are as follows :

(1) A highly folded structure in both.

(2) A precise homology between morphine and methionine enkephalin as regards their functional groups—The phenol ring corresponding to the tyrosine residue, and the tertiary amine corresponding to the terminal ammonium function of peptide[80].

(3) A relative freedom of the N-terminal Tyr-Gly peptide bond.

All the opioid peptides like enkephalins, lipotropins, and synthetic peptides have in common the opioid N-terminal Tyr-Gly residues, but differ by the hydrophobic rest of the molecule. It can be assumed that in all these compounds, the Tyr–Gly moiety is in a state of relative freedom allowing the primary attachment of the opioid part and secondary adaptation of the different hydrophobic moieties of these peptides to the receptor. Thus the relative differences in affinity between all these compounds could be due to secondary interactions[79]. The rat brain opiate receptor has similar affinities for opioid materials from rat, turtle, fish and mouse brains, suggesting that the structure of opiate receptor and its ligand have been highly conserved during evolution.

Narcotic receptor as regulator of adenyl cyclase — implications in tolerance and dependance

Collier and Roy[81] in 1974 demonstrated that morphine and related opiates specifically inhibit PGE_1 stimulated adenyl cyclase activity in rat brain homogenates, in a way that correlates with agonist potency and receptor affinity. Results of experiments with homogenized brain tissue may be difficult to interpret, because such preparations contain several types of cells. Nirenberg *et al*[82] succeeded in developing a hybrid cell line NG 108-15 using the parent Glioma × Neuroblastoma of mouse brain. One of the hybrid cell lines, NG 108-15, was found to bind narcotic analgesics in a stereospecific manner. These hybrid cells are very rich in opiate receptor content, whereas little or no specific binding was observed with parent cell lines and other hybrid cell lines. The hybrid cells have numerous properties characteristic of normal nerve cells and they have approximately 300,000 opiate receptors per cell[82]. Next, two groups demonstrated[83, 84] the inhibition of both basal and PGE_1 (prostaglandin E_1) stimulated adenyl cyclase activity by morphine in hybrid cell homogenates with a concomitant decrease in cAMP in intact hybrid cells. Inhibition of adenyl cyclase activity in these hybrid cells is mediated by the opiate receptor. Opiate antagonists like naloxone completely reversed the adenyl cyclase inhibition. In other words, adenyl cyclase and morphine receptor are coupled together. The nature of this coupling is not clear. It exhibits positive cooperativity. However, the interaction of narcotic with the receptor is not a cooperative process[83]. Further, short and long term effects of narcotics on adenyl cyclase activity have given very interesting results. Morphine and other narcotics affect adenyl cyclase in two opposing ways, both mediated by the opiate receptor. The first process is the readily reversible inhibition of the enzyme. The second is a compensatory increase in enzyme activity which is delayed in onset, and relatively stable. Late positive regulation of the enzyme counteracts inhibitory influence of morphine and brings back the cAMP to the normal levels.

The changes that occur in cultured cells, incubated with opiates, indicate that the cells become tolerant to, and dependant on, the drug and thus constitute a model system for studying tolerance and dependance[85].

Cells exposed chronically to morphine become tolerant to the effects of morphine, so that previously active doses no longer antagonize the PGE_1 or basal adenyl cyclase activity. There is a gradual compensatory increase in specific activity of the enzyme and it comes to the normal levels by 4th day. At this stage, the cells are said to be tolerant to and dependant on

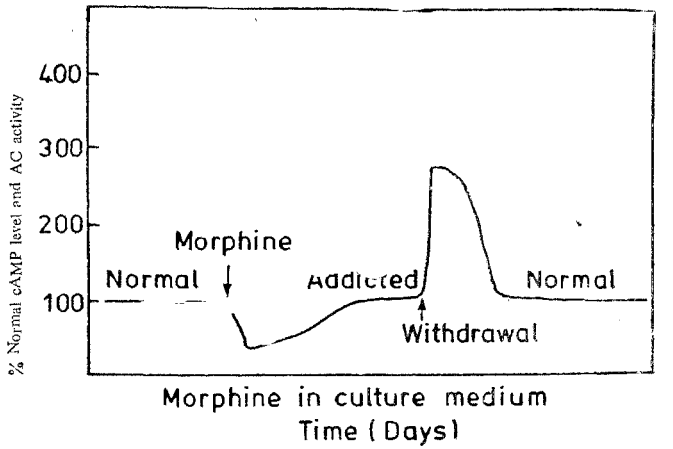


FIG. 4†

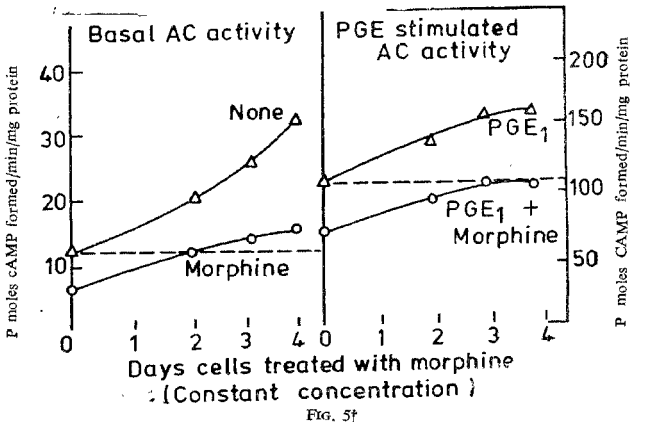


FIG. 5†

Tolerance in hybrid cells. AC = Adenylate cyclase, PGE_1 = Prostaglandin E_1.

morphine to maintain the normal levels of the enzyme activity. When the cells are put to withdrawal by removing morphine from incubation medium or treating with naloxone the cells become supersensitive to the ability of PGE_1 to stimulate adenyl cyclase and cAMP levels go abnormally high and comes to normal levels afterwards. These effects are opposite to that of morphine itself, which can be considered as analogous to or as biochemical counterpart of abstinence syndrome seen in animals after withdrawal. Thus the cells in culture show characteristic addictive properties like tolerance and dependance. The late positive regulation of the enzyme may be due to increase in the number of molecules of adenyl cyclase. Inhibition of adenyl cyclase and late positive regulation are coupled, since both are mediated by interactions of narcotics with opiate receptor. Enkephalins are also potent inhibitors of adenyl cyclase in hybrid tissue culture as demonstrated by Klee and Nirenberg[86]. Methionine enkephalin and leucine enkephalins are approximately 100 and 25 times more potent than morphine as inhibitors of adenyl cyclase[86]. Naloxone reverses this inhibition completely, once again showing that the effect is mediated by opiate receptor. Recently Nirenberg *et al*[87] have come out with the very interesting observation that methionine enkephalin and two other endorphins also cause tolerance and dependance in terms of adenyl cyclase activity identically similar to morphine. Methionine enkephalin evoked tolerance when incubated for 12 97 hours and adenyl cyclase activity showed an increase similar to the positive regulation effect of morphine. Naloxone had a similar effect in these experiments also and these results show that cells become tolerant and dependant upon enkephalins.

In addition to these effects involving cAMP, morphine and enkephalins also regulate cGMP levels[88]. Enkephalins and opiates at low concentrations, in the NG 108-15 hybrid cells, depressed the rate of formation of cAMP and caused an increase in the level of cGMP[89]. Such reciprocal effects on the concentration of cyclic nucleotides are common. Thus recent studies suggest that cyclic nucleotides may be involved in the cellular actions of opiate narcotics.

In summary, the effects of narcotics on a denyl cyclase have the following implications:

(1) Morphine receptors act as regulators of adenyl cyclase. Adenyl cyclase inhibition is the decisive biochemical consequence of opiate drug or opioid peptide binding with the receptor.

(2) The short and long term effects of narcotics and opioid peptides imply the biochemical correlates of tolerance and dependance. Tolerance

and dependance may be normal responses enabling the opioid peptides to regulate the efficiency of trans-synaptic communication[85].

(3) Endogenous opioid peptides seem to be neurotransmitters or hormones destined for neurones with opiate receptor. Opiate peptide-receptor complex is a potent inhibitor of adenyl cyclase and thus the activation of adenyl cyclase by other species of neurotransmitters is suppressed. In effect opiate peptides act as pleiotropic desensitizers of many kinds of receptors which in concert with their ligands activate adenyl cyclase.

(4) Tolerance and dependance as evoked by opiate peptides may regulate the perception of incoming messages by neurones with opiate receptors acting both in a positive and negative manner by regulating adenyl cyclase activity. Shifts in the levels of endogenous opiate peptides may increase or decrease the activity of specific neural circuits by regulating the responses of adenyl cyclase to different kinds of receptor mediated activators of the enzyme.

(5) cAMP and cGMP may be the second messengers mediating the action of endogenous opiate peptides.

(6) These studies support the suggestion of Goldstein and Goldstein[90] and Shuster[91] that drugs act as enzyme inducers.

Endorphins and schizophrenia

Schizophrenia includes a group of psychoses, in which there is a fundamental disturbance of personality, a characteristic distortion of thinking, often a sense of being controlled by alien forces, and he sees himself as the pivot of all that happens.

The wide range of variation in regional distribution of opioid peptides indicate that they may be concerned in several other functions. Bloom and Guillemin[92] have found that α, β and γ-endorphins at very low doses produce behavioral effects in addition to analgesia when they are injected to rat brains. The animals experience extreme muscular rigidity and they stop moving spontaneously. While in this stage, the animals stop blinking and lose their normal eyelids and corneal reflexes. Their rectal temperature drop by more than 2° C. Naloxone completely reverses these effects in a few seconds. The above effects are not found even when administered with large doses of morphine. The behavioral effects of α and γ-endorphins are less dramatic than those of β-endorphin. Bloom suggests that the endorphins

are involved in maintaining normal behaviour and that derangements in their activities could lead to the symptoms of illness.

The preliminary results of Terenius and his co-workers[92] indicate that endorphin levels are elevated in schizophrenics. Administration of naloxone to the schizophrenic patients improved their condition, by decreasing their hallucinations, and brought about an increased clarity in their thought processes. These results require further substantiation. Jacquet and Nelville[93] have also induced schizophrenic postures in rat by injecting the endorphins. They suggest that in schizophrenics the enzymes splitting β-lipotropin to give endorphins might be inefficient, or missing, leading to an imbalance in the concentration of the different endorphins. Thus endorphins might represent a system fundamentally involved in maintaining behavioral homeostasis.

Possible role of endorphins in pain perception

Pain is a protective mechanism alerting us, that our tissues are being damaged. It provides a signal to separate ourselves from the pain stimulus. The perception of pain depends upon the reception of the painful stimulus by a nociceptor (pain receptor) and the transmission of stimulus information to the brain. In the brain, the sensory information is analysed and integrated with the past experience and present context to result in the perpetual experience that we call pain. Since, powerful analgesia is a prominent and reliable phenomenon associated with the administration of both narcotic alkaloids and brain opioid peptides, it seems reasonable to suggest that the opioid peptides may normally function to modulate pain responsiveness. In support of this view, Mayer and Akil[94, 95] have shown that analgesia produced by electrical stimulation of the periaqueductal gray is blocked by naloxone, suggesting the existence of an endogenous pain inhibitory system, perhaps involving the endorphins. They postulate that such an endogenous system may be engaged by either environmental, sensory or other physiological events which lead to adaptive changes in pain responsiveness. For example noxious or stressful stimuli may recruit pain inhibitory mechanisms in the CNS bringing about alterations in enkephalins and endorphins with concurrent and subsequent changes in responses to pain. Consistent with this hypothesis, recently, Madden *et al*[96] have reported that inescapable acute stress causes a significant increase in the levels of opioid peptides with a concurrent decrease in pain responsiveness in the rat. On the contrary, Goldstein *et al*[97] were unable to show any significant effect of large doses of naloxone on shock escape threshold or temperature control under cold

stress in rats[98]. It is possible that endorphin system is normally in a quiescent stand by status, so that blockade by naloxone could be demonstrated, if the system is first activated by an appropriate stimulus.

Opioid peptides as neurotransmitters

Morphine causes a consistent inhibition of cell firing in the cerebral cortex in response to iontophoretically administered morphine, which can be antagonized by naloxone[99]. Several groups have studied the responses of single neurones in different brain regions to iontophoretically applied enkephalin, and in general, these peptides mimic the inhibitory action of morphine[100, 101]. Frederickson and Norris[102] have shown that methionine enkephalin applied microiontophoretically depressed spontaneous and glutamate induced firing of single neurones in frontal cortex, caudate nucleus, and periaqueductal gray matter where high concentration of opiate receptors and enkephalin are found. These data are compatible with a neurotransmitter or neuromodulator role for these brain peptides. Moreover, Hökfelt[103] of the Karolinska Institute of Stockholm has used antibodies to enkephalins, to show that they are located in nerve terminals, in the same regions of brain where opiate receptors occur.

The possibility that enkephalin may be a peptide neurotransmitter is interesting in light of recent findings suggesting that several small peptides are neurotransmitters in the brain. Substance P—a decapeptide—satisfies the criteria to be expected of the primary affarent neurotransmitter in the spinal cord[104]. The distribution, iontophoretic and brain receptor properties of TRF (thyrotropin releasing factor) indicates that besides its endocrine effects, TRF is probably a neurotransmitter[105].

Based on the morphine effect, if the enkephalin is a neurotransmitter, it would most likely function as an inhibitory transmitter. There are, in principle, three possibilities of control of an inhibitory mechanism by enkephalin[106]. The inhibition could be post-synaptic, pre-synaptic or could be due to a modulatory effect on the nerve terminal in which both enkephalin and morphine are present. Presynaptic inhibition or inhibitory modulation are the two possible models because morphine does not alter the membrane potential of ganglion cells or does not affect intraneural transmission[107].

Kosterlitz and Hughes[106] have given a model to explain tolerance and dependance, in terms of the inhibitory modulation of enkephalins. The interaction between endogenous enkephalins and exogenous opiate drugs to cause the addiction is the main attraction of this model.

Model

When morphine is first administered, enkephalin is still being released normally. So the brain cells are exposed simultaneously to both natural and exogenous sources, and this may be reason for powerful effects obtained. Excess of morphine, then, is supposed to exert a feedback inhibition of enkephalin release. The high levels of morphine cause the enkephalin release to be switched off. This makes morphine less effective (hence tolerance) and at the same time, necessary for normal brain function (hence dependance). On abrupt cessation of opiate administration or when naloxone is injected, the receptors would be temporarily deprived of both enkephalin and morphine. Withdrawal symptoms would result until the enkephalinergic neurones begin firing and release the peptides at a normal rate.

In support of the above model, Simantov and Snyder[108] have measured the levels of enkephalins present in the brain of rats, at different stages during the development of addiction by implanting a morphine pellet that slowly released morphine. They found a 75% increase in the levels of enkephalins by 5 days after implantation. The concentration of enkephalins returned to normal, within 1 hour after naloxone administration. The increase in the level of enkephalins is attributed to their storage in the terminals of synaptic cells, instead of being released normally. Data from several other authors indicate the presence of such an enkephalinergic system within the periaqueductal gray providing inhibitory modulation of the inhibitory interneuron[102].

The validity of this hypothesis obviously depends on the validity of assumptions that enkephalins are actually neurotransmitters. The resolution of this question requires the demonstration of the presence of enkephalin in nerve terminals from which it can be released. Confirmation must be obtained that the pharmacology of the released peptide is identical to that of the isolated natural peptide. The development of radioimmunoassay for enkephalins will facilitate such studies. Thus the criteria for establishing a neurotransmitter role for a substance are very strict, and no neurotransmitter has yet been unequivocally established for the supraspinal nervous system[109]. The opioid peptides are thus closely similar to morphine in terms of cellular responses like analgesia, physical dependance, tolerance and inhibition of adenyl cyclase activity.

Much additional work is required to sort out the roles of endorphins. Many questions remain to be answered. Which endorphins are physio-

logically active? By what mechanisms do they cause physiological effects? The relationship among endorphins, their role in an endogenous pain inhibitory system and as neurotransmitters, need to be investigated. If the progress is as rapid as it has been, since the discovery of endorphins, the answers to the above questions should be forthcoming soon.

REFERENCES

1. Cochin, J. — *Fed. Proc.*, 1970, **29**, 19.
2. Johnson, M. R. — *Ann. Reports in Med. Chem.*, 1975, **10**.
3. Yanagihara, T. — *J. Neurochem.*, 1974, **22**, 113.
4. Goldstein, A., Arnow, L. and Kalman, S. M. — *Principles of Drug Action—The Basis of Pharmacology*, John Wiley Inc., New York, 1974,
5. Beckett, A. H. and Casy, A. F. — *J. Pharm. Pharmacol.*, 1954, **6**, 986.
6. Portoghese, P. S. — *J. Pharm. Sci.*, 1968, **55**, 865.
7. Cuatrecasas, P. and Hollenberg, M. D. — *Biochem. Biophys. Res. Commun.*, 1975, **62**, 31.
8. Snyder, S. H., Pasternak, G. W. and Pert, C. B. — *Handbook of Psychopharmacology*, 1975.
9. Loh, H. H., Cho, T. M., Wu, Y. C. and Way, E. L. — *Life Sci.*, 1974, **14**, 2231.
10. Goldstein, A., Lowney, L. L. and Pal, B. K. — *Proc. Nat. Acad. Sci. U.S.*, 1971, **68**, 1742.
11. Goldstein, A. — *Science*, 1976, **193**, 77.
12. Pert, C. B., and Snyder, S. H. — *Science*, 1973, **179**, 1011.
13. Simon, E. J., Hiller, J. M. and Edelman, I. — *Proc. Nat. Acad. Sci. U.S.*, 1973, **70**, 1947.
14. Terenius, L. — *Acta Pharmacol. Toxicol.*, 1973, **32**, 317.
15. Klee, W. A. and Streaty, R. A. — *Nature*, 1974, **248**, 61.
16. Wong, D. T. and Horng, J. S. — *Life Sci.*, 1973, **13**, 1543.
17. Creese, I. and Snyder, S. H. — *J. Pharmacol. Exp. Ther.*, 1975, **194**, 205.

18. Kosterlitz, H. W., Waterfield, A. A. and Berthhood, V. — *Adv. Biochem. Psychopharmacol.*, 1973, **8**

19. Pasternak, G. W. and Snyder, S. H. — *Nature*, 1975, **253**, 563.

20. Pert, C. B., Pasthan, D. A. and Snyder, S. H. — *Brain Res.*, 1974, **75** 356

21. Huang, J. F. and Takemori, A. F. — *Pharmacologist*, 1974, **16**, 248

22. Pert, C. B., Snowman, A. M. and Snyder, S. H. — *Brain Res.*, 1974, **70**, 184

23. Goldstein, A. — *Life Sci.*, 1974, **14**, 615

24. Kuhar, M. J., Pert, C. B. and Snyder, S. H. — *Nature*, 1973, **245**, 447

25. Snyder, S. H. — *Nature*, 1975, **257** 185.

26. Pert, A. and Yaksh, T. — *Brain Res.*, 1974, **80**, 135

27. Pert, C. B., Kuhar, M. J. and Snyder, S. H. — *Life Sci.*, 1975, **16**

28. Pert, C. B. and Snyder, S. H. — *Mol. Pharmacol.*, 1974, **10**, 868

29. Pert, C. B., Pasternak, G. and Snyder, S. H. — *Science*, 1973, **182**, 135

30. Snyder, S. H., Pert, S. B. and Pasternak, G. — *Neuropsychopharmacology*, 1974, p. 307

31. Pasternak, G. W., Snowman, A. M. and Snyder, S. H. — *Molecular Pharmacol.*, 1974, **11** (6), 735

32. Pasternak, G. W. and Snyder, S. H. — *Nature*, 1975, **253**, 563.

33. Wilson, H. A., Pasternak, G. W. and Snyder, S. H. — *Nature*, 1975, **253**, 448

34. Simon, E. J., Hiller, J. M., Edelman, I. Groth, J. and Stahl, K.D. — *Life Sci.*, 1975, **16**, 1795

35. Kosterlitz, H. W. — *Opiates and Endogenous Opioid Peptides* (North-Holland Amsterdam), 1976.

36. Collier, H. O. J. — *Proc. 5th Int. Cong. Pharmac.*, 1973, **1**, 65.

37. Davis, V. E. and Walsh, M. J. *Science*, 1970, **167**, 1005.

38. Terenius, L. and Wahlström, R. *Acta Pharmac. Toxicol.*, 1974, **35**, 55.

39. Hughes, J. *Brain Res.*, 1975, **88**, 1.

40. Pasternak, G. W. and Snyder, S. H. *Neurosci. Res. Bull.*, 1975, **13**, 27.

41. Pasternak, G. W. and Snyder, S. H. *Life Sci.*, 1975, **16**, 1765.

42. Hughes, J., Smith, T. W., Kosterlitz, H. W., Fothergill, L. A., Morgan, B. A. and Morris, H. R. *Nature*, 1975, **258**, 577.

43. Simantov, R. and Snyder, S. H. *Life Sci.*, 1976, **18**, 781.

44. Hughes, J., Smith, T., Morgan, B. and Fothergill, L. *Life Sci.*, 1975, **16**, 1753.

45. Pasternak, G. W., Goodman, R. and Snyder, S. H. *Life Sci.*, 1975, **16**, 1765.

46. Terenius, L. and Wahlström, A. *Life Sci.*, 1975, **16**, 1959.

47. Teschemacher, H. T., Opheim, K. E., Teschemacher, H. and Goldstein, A. *Life Sci.*, 1975, **16**, 1971.

48. Cox, B. M., Opheim, K. E., Teschemacher, H. and Goldstein, A *Life Sci.*, 1975, **16**, 1777.

49. Goodman, R., Pasternak, G. W. and Snyder, S. H. *Life Sci.*, 1975, **16**, 1765.

50. Pert, C. B., Pert, A. and Tallmar, J. F. *Proc. Nat. Acad. Sci. U.S.*, 1976, **73**, 2226.

51. Guillemin, R., Ling, N. and Burgus, R. *C.r. hebd. Seance Acad. Sci.*, 1976, **282**, 783.

52. Kosterlitz, H. W., Lyden, R. J. and Watt, A. J. *Br. J. Pharmacol.*, 1970, **39**, 398.

53. Henderson, G., Hughes, J. and Kosterlitz, H. W. *Br. J. Pharmacol.*, 1972, **41**, 539.

54. Kosterlitz, H. W. and Waterfield, A. A. — *Ann. Rev. Pharmacol.*, 1975, **15**, 29

55. Lees, G. M., Kosterlitz, H. W. and Waterfield, A. A. — *Some Central and Intraspinal Action of Narcotic Analgesic Drugs*, 1975

56. Li, C. H. — *Nature*, 1964, **201**, 924

57. Yamashiro, D. and Li, C. H. — *Proc. Nat. Acad. Sci.*, 1974, **71**, 3041

58. Lehmann, P. and Li, C. H. — *Endocrinology*, 1968, **82**, 808

59. Moon, H. D., Li, C. H. and Jennings, B. M. — *Ann. Rev.*, 1973, **17**, 5829

60. Graven, H. M. and Wied, D. de — *Prog. in Br. Res.*, 1974, **39**, 430

61. Ross, M., Ehrenkranz, J., Cox, B. M. and Goldstein, A. — *Fed. Proc. Fed. Am. Soc. Exp. Biol.*, 1976, **35**, 844.

62. Hughes, J., Smith, T., Morgan, B. and Fothergill, L. — *Life Sci.*, 1976, **16**, 1753

63. Bradbury, A. F., Smyth, D. G., Snell, C. R., Birdsall, N. J. M. and Hulme, E. C. — *Nature*, 1976, **260**, 793.

64. Li, C. H. and Chung, D. — *Proc. Nat. Acad. Sci. U.S.*, 1976, **73**, 1145.

65. Cox, B. M., Goldstein, A. and Li, C. H. — *Proc. Nat. Acad. Sci. U.S.*, 1976, **73**, 1821.

66. Lazarus, L. H., Ling, N. and Guillemin, R. — *Proc. Nat Acad. Sci., U.S.*, 1976, **73**, 2156.

67. Wei, *et al*, — *Opiates and Endogeneous Opioid Peptides*, North-Holland, Amsterdam, 1976.

68. Goldstein, A. — *Science*, 1976, **193**, 1081.

69. Alphonsus, L., Cheung, and Goldstein, A. — *Life Sci.*, 1976, **19**, 1005.

70. Hambrook, J. M., Morgan, B. A., Rance, M. J. and Smith, C. F. C. — *Nature*, 1976, **262**, 782.

71. Pert, C. B. and Pert, A. — *Science*, 1976, **194**, 330.

72. Kangchang, J., Fong, B. T. W. and Pert, C. B. — *Life Sci.*, 1976, **18**, 1473.

73. (*a*) Snyder, S. H. — *Nature*, 1975, **257**, 185.
(*b*) Belluzi, J. D., Grant, W., Grassky, V. and Stein, L. — *Nature*, 1976, **260**, 625.

74. Bradbury, A. F. — *Opiates and Endogenous Opiates*, North Holland, Amsterdam, 1976.

75. Loh, H., Tseung, L. F., Wei, E. and Li, C. H. — *Proc. Nat. Acad. Sci. U.S.*, 1976, **73**, 2895.

76. Bradbury, A. F. and Hume, E. C. — *Nature*, 1976, **261**, 793.

77. Waterfield, A. A., Hughes, J. and Kosterlitz, H. W. — *Nature*, 1976, **260**, 624.

78. Wei, C. E. and Loh, H. — *Science*, 1976, **193**, 1262.

79. Bradbury, A. F., Smyth, D. G. and Snell, C. R. — *Nature*, 1976, **260**, 793.

80. Horn, A. S. and Rodgers, J. R. — *Nature*, 1976, **260**, 795.

81. Collier, H. O. J. and Roy, A. C. — *Nature*, 1974, **248**, 24.

82. Klee, W. A. and Nirenberg, M. — *Proc. Nat. Acad. Sci. U.S.*, 1974, **71**, 3474.

83. Sharma, S. K., Klee, W. A. and Nirenberg, M. — *Proc. Nat. Acad. Sci. U.S.*, 1975, **72**, 590.

84. Traber, J., Fisher, K., Larzin and Hamprecht, B. — *Nature*, 1975, **253**, 120.

85. Sharma, S. K., Klee, W. A. and Nirenberg, M. — *Proc. Nat. Acad. Sci. U.S.*, 1975, **72**, 3092.

86. Klee, W. A. and Nirenberg, M. — *Nature*, 1976, **263**, 609.

87. Lampert, A., Klee, W. A. and Nirenberg, M. — *Proc. Nat. Acad. Sci. U.S.*, 1976, **73**, 3165.

88. Minneman, L. L. and Iversen — *Nature*, 1976, **262**, 414
89. Brandt, M., Gillis, R. J., Fisher, K., Büchen, K., Büchen, C. and Hamprecht, B. — *Nature*, 1976, **262**, 311
90. Goldstein, D. B. and Goldstein, A. — *Biochem. Pharmacol.*, 1961, **8**, 48
91. Sinister — *Nature*, 1961, **189**, 314
92. Research report — *Science*, 1976, **193**, 1227.
93. — *Women's New Scientist*, 1976, **172**, 332.
94. Mayer, D. J. and Hayes, R. — *Science*, 1975, **188**, 941.
95. Akil, H., Mayer, D. J. and Liebeskind, J. C. — *Science*, 1976, **191**, 961
96. Madden, J., Akil, H., Patrik, R. T. and Barachas, J. D. — *Nature*, 1977, **265**, 358
97. Goldstein, A., Pycor, G. F., Otis, L. S. and Larsen, F. — *Life Sci.*, 1976, **15**, 809
98. Goldstein, A. and Loweny, P. J. — *Life Sci.*, 1975, **17**, 927
99. Satoh, Fries, W. and Herz, A. — *Brain Res.*, 1974, **82**, 378
100. Bradley, P. B., Briggs, I., Gayton, R. J. and Lambert, L. A. — *Nature*, 1976, **261**, 428
101. Gent, J. P. and Wolstencroft, J. H. — *Nature*, 1976, **261**, 426
102. Frederickson, R. C. A. and Norris, F. A. — *Science*, 1976, **194**, 40.
103. Hökfelt, T. — *Science*, 1976, **193**, 1227.
104. Takahasi, T. and Otusuk, M. — *Brain Res.*, 1975, **87**, 1.
105. Burt, D. C. and Snyder, S. H. — *Brain Res.*, 1976, **88**, 60.
106. Kosterlitz, H. W. and Hughes, J. — *Life Sci.*, 1975, **17**, 91.
107. North, R. A. and Henderson, G. — *Life Sci.*, 1975, **17**, 63.
108. Simantov, R. and Snyder, S. H. — *Nature*, 1976, **262**, 505.
109. Phillips, J. W. — *The Pharmacology of Synapses*, Pergamon Press, 1976, p. 6.