Opiate Receptors: Implications and Applications

Scientists who are looking for examples of basic research that could pay off with practical benefits have one in the current investigations on the mechanisms of opiate action. The investigations were sparked by the discovery about 2 years ago of opiate receptors in the brain. The results, which include the discovery of what may be a new neurotransmitter (a chemical that transmits nerve impulses from one neuron to another), are leading to a better understanding of how opiate drugs act and of how the brain itself works. They are providing an explanation of the biochemical changes underlying addiction. And they are pointing the way toward achievement of a goal long sought by pharmacologists—that is, the design of pain-killers with the potency, but without the addictive potential, of the opiates now used.

Many drugs and also natural agents such as hormones are thought to produce their effects by first combining with specific cell components called receptors. Investigators suspected for many years that opiates such as morphine and heroin acted in this manner, but they were unable to directly demonstrate the existence of receptors until 1973 when researchers in three laboratories did it independently. The researchers are Solomon Snyder and Candace Pert of Johns Hopkins University School of Medicine, Eric Simon of the New York University School of Medicine, and Lars Terenius of Uppsala University in Sweden.

Earlier attempts to demonstrate the presence of receptors specific for opiates had failed because these drugs will bind nonspecifically to many substances, including brain tissue. It was hard to detect the very small amount of specific binding against such a high background. However, in 1971 Avram Goldstein of Stanford University Medical School and the Addiction Research Foundation in Palo Alto did establish a criterion for picking out the specific opiate binding from the background. The criterion depends on the fact that only certain forms of opiates have biological activity. Most of the drugs exist in at least two isomeric forms. The structures of the isomers are mirror images of one another and only one of the pair is active. Goldstein proposed that only the active form should bind to the specific receptor. This criterion of stereospecificity provided a basis for further experiments.

In order to amplify the specific binding while minimizing the nonspecific component, the other investigators used isotopically labeled opiates or opiate antagonists (drugs that resemble opiates chemically but block their usual effects) and washed the treated tissues thoroughly to remove material that was nonspecifically bound and therefore more loosely held than that attached to biologically active receptors. The radioactive opiates and antagonists used in these experiments were of high specific activity, which means that a large percentage of the molecules carry the radioactive label; this assured that small quantities of 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 naloxone, an opiate antagonist, binds specifically to preparations of homogenized rat brain. Opiates and other antagonists decreased binding of naloxone because they themselves could bind to the receptors and prevent naloxone from doing so. The agents with the greatest physiological activity were most effective in preventing naloxone binding, whereas drugs unrelated to opiates had no effect on it.

The investigators observed similar effects in a preparation of guinea pig intestine. Such preparations contract when they are stimulated electrically, and opiates are known to inhibit these contractions. This activity of the drugs correlated well with their affinities for receptors in guinea pig intestine when both were measured in the same preparations. Moreover, the binding sites appeared to be in nerves, because when Snyder and Pert removed the nerves from the intestine, the opiates no longer bound specifically to the material.

The existence of opiate receptors has a number of implications for further research. For one, it has spurred the search for an endogenous material with opiate activity. Opiates do not occur naturally in animal brains, so the assumption is that the receptors function somehow in the transmission of nerve impulses. This requires the presence of a corresponding neurotransmitter.

John Hughes, working in the laboratory of Hans Kosterlitz at the University of Aberdeen, Scotland, recently identified a material in the brains of pigs, cows, guinea pigs, rats, rabbits, and mice that mimics the capacity of morphine to inhibit contractions in smooth muscle preparations such as guinea pig intestine. According to Hughes, the material, which he calls "enkephalin," is a peptide having a molecular weight of about 1000; it probably contains seven amino acids, including three residues of glycine, one each of phenylalanine, methionine, and tyrosine, and possibly one of tryptophan.

Snyder and Terenius have identified peptides with opiate activity in brain, and Goldstein has identified another in extracts of the pituitary gland. The molecular weight of Snyder's peptide is similar to that of Hughes', but, according to Goldstein, the pituitary opioid peptide has a molecular weight of 1800 and also differs from enkephalin in that it contains basic amino acids. Goldstein thinks that the pituitary peptide may be a precursor of enkephalin, but further work is needed to confirm this hypothesis.

The relationship of these materials to one another will not be certain until the amino acid sequence of each peptide has been determined. Since all the peptides are small, this should be relatively easy. The main problem is isolating adequate quantities for the analysis. Some investigators have suggested that naming the peptide (or peptides) should wait until it is known whether a family of materials, rather than a single substance, is involved. Another name that has been suggested, besides enkephalin, is "endorphin"—for endogenous morphine-like substance.

The small size of the peptide also means that synthesis should be readily achieved. This would increase the availability of the material for further study and for potential therapeutic use.

The physiological role of the endogenous material is now uncertain, as is its mechanism of action. Studies of the distribution of opiate receptors in brain, however, give some clues about the possible role of the material and about how opiates produce their effects. According to Snyder and to Simon, the number of receptors in different parts of human and monkey brains varies markedly. They are most concentrated in those parts concerned with perception of pain, especially the diffuse and chronic types of pain (as opposed to sharp, acute pain such as that caused by a pinprick), with emotional reaction to pain, and with other emotional centers. Both investigators find that many of the structures constituting the limbic system, which is sometimes called the emotional or visceral brain and is involved in pain perception, have large numbers of receptors. This distribution of receptors is consistent with the production of two of the major opiate effects, analgesia and euphoria. Investigators have also shown that the distribution

of endogenous morphine-like materials parallels that of the opiate receptors. Hughes says that it seems possible that the brain does possess a pain suppressive system and that enkephalin plays a role in it.

There is additional evidence in favor of the hypothesis that opiate receptors are actually receptors for an endogenous neurotransmitter. When Snyder and Pert separated brain tissues into subcellular fractions, they found the receptors in the fraction containing synaptic membranes. These are the membranes located around nerve synapses—the region of contact between neurons through which nerve impulses are transmitted—and they are the logical site for location of receptors for neurotransmitters. Receptors for the neurotransmitters acetylcholine and norepinephrine, for example, are located at the synapse. All these observations support the hypothesis that the endogenous morphine-like material is a neurotransmitter, although, according to Hughes, enkephalin and the opiate receptors are found in certain smooth muscle preparations; consequently, its function may not be restricted to brain.

Studies of the receptors itself are not only yielding information about how opiates interact with their receptors but they are also providing a more efficient approach for designing potent analgesic drugs that have no addictive potential. Despite the fact that opiates are very effective pain-relievers, their use is limited because they produce euphoria and are addictive.

Some opiate antagonists completely prevent all the effects of opiates. For this reason they are useful in treating overdoses of heroin because they reverse its effects within minutes. They may also be useful for treating heroin addicts because the narcotic does not elicit a "high" in the presence of antagonist and should no longer be attractive to the addict. Other antagonists, however, retain some of the usual effects of opiates. The idea is to design a drug for therapeutic use that still relieves pain effectively but which has just enough antagonist activity to prevent the addictive effects. The drug pentazocine is one example of an agent with such mixed properties.

Predicting what chemical features will produce the desired characteristics is difficult. Screening the drugs in animals is both time-consuming and expensive, and the results may not be borne out in human trials. But differences in the way sodium ions affect the binding to receptors of the three classes of agents—that is, opiates, complete antagonists, and drugs with mixed properties—appears to provide a method for distinguishing between them.

Sodium ions increase the binding of antagonists but decrease the binding of opiates by receptors. Based on this effect, Snyder and Pert devised what they call the sodium response ratio. This is the ratio of the concentration of the test drug that inhibits by 50 percent the binding of labeled naloxone (a pure antagonist) in the presence of sodium to the comparable concentration in the absence of sodium. The assay is convenient because the test drug need not be labeled.

In the presence of sodium, opiates should have decreased potency in preventing the binding of naloxone; that is, more drug should be required to give 50 percent inhibition and the ratio should be much greater than one. Drugs that are predominantly antagonists should have the same capacity to inhibit the binding of naloxone whether sodium is present or not; for these the ratio should be equal to one. And those drugs with mixed characteristics should have ratios greater than one but less than those of the more potent opiates. When Snyder and Pert tested a variety of drugs with known pharmacological properties, the ratio values they found agreed well with those predicted. Although this test should be valuable in screening for agents with mixed properties, additional testing in animals and humans will be required to determine whether they have the right mix of effects.

It's Not Mars, But It's Still a Big Drop

The Viking spacecraft is scheduled to be launched this week toward a July 1976 rendezvous with Mars. The lander, which is to be dropped into the uncertain atmosphere of the red planet, was put through its paces earlier this year at the world's largest indoor parachute test facility, the huge Vehicle Assembly Building at Cape Canaveral.—A.L.H.

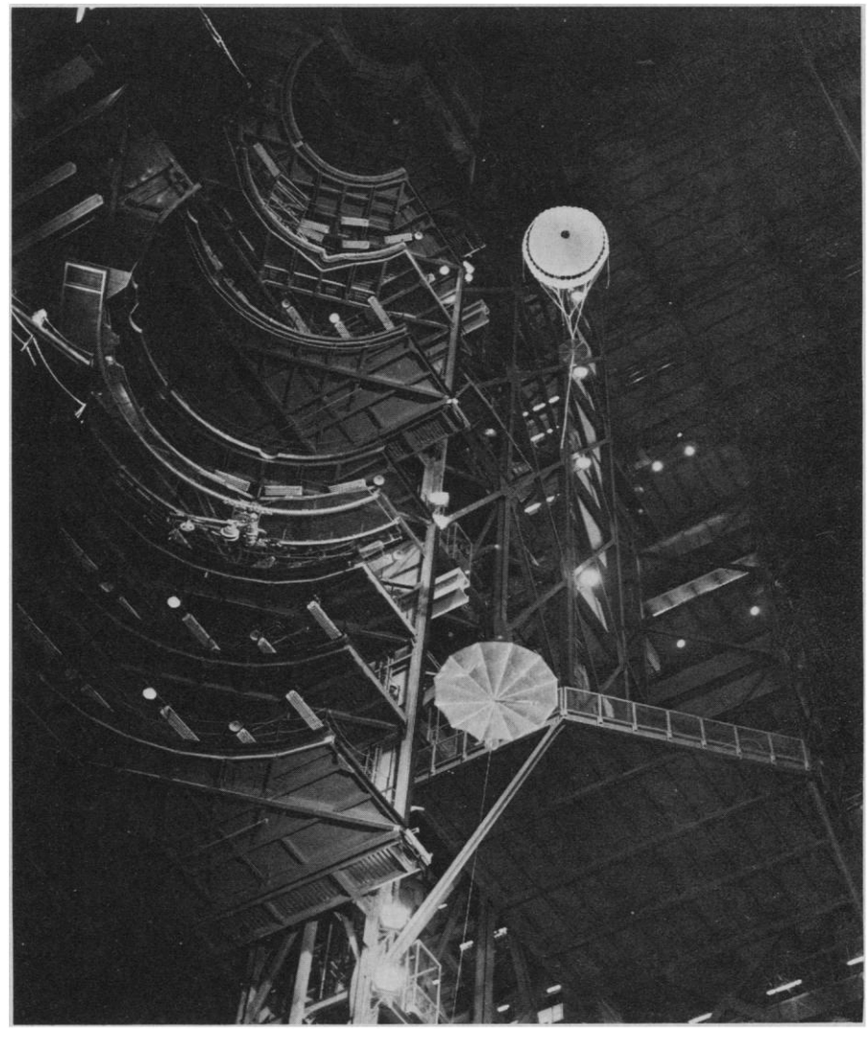

Photo courtesy of NASA

The current consensus among investigators of opiate receptors is that sodium ions act as an allosteric regulator of the receptor. Allosteric effects occur when complex substances—such as enzymes or, in this case, the opiate receptor—can exist in at least two conformations that are in equilibrium with one another. One conformation of the receptor has a higher affinity for antagonists than for opiate drugs whereas the reverse is true for the other conformation. Binding of sodium ions to the receptors shifts the equilibrium so that they are in the conformation that preferentially binds antagonists. Normal body concentrations of sodium ions are high enough to produce this effect. This fact helps to explain why antagonists are effective in blocking opiate action in vivo at lower concentrations than those of the opiates themselves.

The model agrees with a general model for the interaction of neurotransmitters with receptors that has been suggested by a number of investigators. It includes a mechanism for altering the movements of ions across the neural membrane. Generation of nerve impulses depends on such changes in ion movements. If most of the receptors are in the "off" position, that is, in the sodium-bound form which binds antagonist, binding of opiate drugs—or normal neurotransmitter molecules—to the small quanitity of the sodium-free form would shift the equilibrium in the direction of the latter conformation. The release of sodium ions might then affect nerve firing patterns.

The chain of biochemical events following combination of opiates with receptors is another subject of intense scrutiny. These events probably are part of the mechanism by which opiates alter neural activities to produce analgesia and euphoria, and they may also be involved in tolerance and dependency, two heretofore unexplained phenomena characteristic of opiate addiction.

The evidence thus far indicates that opiates combining with receptors alter the concentrations of adenosine 3′,5′-monophosphate (cyclic AMP) and of guanosine 3′,5′-monophosphate (cyclic GMP) in nerve cells. Prostaglandins may also be involved, according to Harry Collier and Aschim Roy of Miles Laboratories in Slough, England. These investigators found that the prostaglandins PGE_1 and PGE_2 stimulate the formation of cyclic AMP from adenosine triphosphate (ATP) in homogenized rat brain and that opiates such as morphine inhibit the stimulation without inhibiting the basal production of cyclic AMP in the absence of the prostaglandins. Naloxone antagonized the effects of morphine. Since PGE_1 and PGE_2 produce pharmacological effects, including pain and diarrhea, that are the reverse of the effects of opiates, Collier and Roy hypothesized that opiates produce their effects by inhibiting the stimulation by the prostaglandins of cyclic AMP formation, presumably by inhibiting stimulation of adenylate cyclase in neurons. Adenylate cyclase is the enzyme that catalyzes the formation of cyclic AMP from ATP.

Results with Cultured Cells

Results of experiments with homogenized tissue may be difficult to interpret because such preparations contain several different cell types and are quite heterogenous in composition. Cells in culture may be cloned, however, to give a homogenous cell population. Recently, a group of investigators at the National Institutes of Health (NIH), including Werner Klee of the National Institute of Mental Health and Shail Sharma and Marshall Nirenberg of the National Heart and Lung Institute, found that a line of cultured cells having a high density of opiate receptors responds to opiates with a decrease in the activity of adenylate cyclase and a consequent decrease in cyclic AMP concentrations. The cell line, derived by Nirenberg with Bernd Hamprecht of the Max Planck-Institut für Biochemie in Munich, Germany, is a hybrid formed by fusing cells from a neuroblastoma (a tumor of embryonic neurons) with those from a glioma (a tumor of glial cells). All experiments with cultured cells are open to the criticism that they are not "physiological"; nevertheless, the hybrids have numerous properties characteristic of normal nerve cells. In addition, they have a large number of receptors, approximately 300,000 per cell.

The NIH investigators find that PGE_1 stimulates adenylate cyclase in hybrid cells. But morphine inhibits the enzyme activity in these experiments both in the presence and absence of added prostaglandin. Naloxone blocks the effects of the morphine. According to Klee, Nirenberg, and Sharma, the parent neuroblastoma cells have roughly one-third the number of receptors as the hybrids whereas the parent glioma cells have none. The degree of response of the three lines to morphine correlates with the number of receptors of the cells.

Hamprecht and his colleagues have also been studying the effects of morphine on the hybrid cells. They find that the effects of low concentrations of the drug on cyclic AMP concentrations are similar to those described by the other investigators studying the hybrids. In addition, they showed that morphine produces an increase in the concentration of cyclic GMP in these cells. Such reciprocal effects on the concentrations of the two nucleotides are common.

The changes that occur in cultured cells incubated with opiates indicate that the cells may become addicted to the drugs and that they thus constitute a model system for studying tolerance and dependency. Tolerance refers to the phenomenon in which addicts become less sensitive to a drug and require progressively higher doses of it to achieve the desired effect, such as euphoria in the case of heroin addiction. Dependency means that an addict who stops taking the drug or is given an antagonist to block its effects will suffer from physical symptoms that are the reverse of the effects evoked by the agent. These phenomena are characteristic of addiction to opiates and also to a number of other drugs, including barbiturates and alcohol.

According to Sharma, Klee, and Nirenberg, the activity of adenylate cyclase of the hybrid cells cultured with morphine at first decreases as expected and then gradually increases during the period of exposure to the drug. After 2 or 3 days of incubation the activity is as high in the presence of the morphine as it was in its absence. The cells are then dependent on morphine to maintain normal cyclic AMP concentrations for when the morphine is removed from the medium—analogous to drug withdrawal in addicts—the activity of the enzyme increases to well above its original value. Addition of naloxone to the medium in the presence of morphine causes a similar increase. The investigators think that the increased enzyme activity during exposure to morphine is the biochemical basis of tolerance and that the further increase following withdrawal is that of the withdrawal syndrome. Nirenberg says that it is not known whether the increased activity represents an actual increase in the number of adenylate cyclase molecules, but that the time required for the activity to build up and its relative stability is consistent with this hypothesis. Moreover, the investigators did not detect a change in the number or the properties of the receptors themselves.

One question of great interest is whether the endogenous morphine-like material will have effects on adenylate cyclase and cyclic nucleotides like those of opiates. Nirenberg points out that the relative persistence of high enzyme activity following morphine withdrawal may be a form of memory. The change could alter the transmission of nerve impulses and has the potential for affecting nerve connections. If the endogenous material is in fact a natural neurotransmitter and if it produces effects like those of opiates, such speculations should make an active area of research even more lively.—JEAN L. MARX