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Opiate Receptor Revisited

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Opiate receptor: Demonstration in nervous tissue. By Candace B. Pert and Solomon H. Snyder. *Science* 1973; 179:1011–4. Reprinted with permission.

Tritiated naloxone, a powerful opiate antagonist, specifically binds to an opiate receptor of mammalian brain and guinea pig intestine. Competition for the opiate receptor by various opiates and their antagonists closely parallels their pharmacological potency. The opiate receptor is confined to nervous tissue.

OUR identification of opiate receptors 34 yr ago[1] seems distant. Today, receptors for drugs and neurotransmitters are taken for granted. Almost all receptors have been cloned, providing intimate understanding of signal transduction. Modern drug development uses robotics to screen millions of chemicals at dozens of receptors and their subtypes. By contrast, when we began our work, how drugs and neurotransmitters signaled was a mystery, with receptors and second messengers essentially black boxes. Drug development usually required screening in intact rodents with no way to discriminate whether differential drug potency stemmed from receptor affinity, metabolism, or target penetration.

Identification in 1970 of the nicotinic acetylcholine receptor in the electric organ of the electric eel by the binding of radiolabeled α-bungarotoxin was an important advance. Paradoxically, this success convinced most workers in the field that it would never be possible to monitor receptors for drugs and most neurotransmitters in mammalian brain. Here is the reasoning. The acetylcholine receptors comprise 20% of the protein in the electric organ, yet detecting these receptors required the extraordinarily potent and specific α-bungarotoxin labeled with ^{125}I to high specific radioactivity. By contrast, the opiate pharmacologist Vincent Dole, M.D. (Rockefeller University, New York, New York; 1913–2006), had calculated that opiate receptors would likely constitute no more than one millionth by weight of mammalian brain, and no magic toxins existed for the receptors. A few laboratories had attempted to monitor the binding of [^{3}H]opiates and other drugs to brain membranes, but nonspecific binding to tissue lipid, carbohydrates, and proteins was great, and no specific receptor interactions had ever been demonstrated.

I became interested in receptors at a time that my faculty colleague Pedro Cuatrecasas, M.D. (Professor, Johns Hopkins University, Baltimore, Maryland), had demonstrated insulin receptors by monitoring the binding of ^{125}I-insulin to tissue membranes. Pedro used a custom-made vacuum manifold in which he could trap tissue membranes with receptor-bound insulin on glass fiber filters and wash away nonspecific binding vigorously but so rapidly that receptor-bound insulin was not also removed. I read a publication describing the sequencing of nerve growth factor, an impressive technical feat in those days, which reported a close similarity in amino acid sequence to insulin. I suggested a collaboration in which my postdoctoral fellow Shailesh Banerjee, Ph.D. (Department of Pharmacology, Johns Hopkins University), would use Pedro's equipment and attempt to identify nerve growth factor receptors, a project in which we were eminently successful.[2]

In 1971 President Nixon had declared "War on Heroin" and appointed my friend, Jerome Jaffe, M.D. (Professor of Psychiatry, University of Maryland, Baltimore, Maryland), as his czar of drug abuse. After importuning by myself and some colleagues, Jaffe established a series of

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Table 1. Drug Inhibition of Stereospecific [^{3}H]Naloxone Binding

Drug	IC_{50}, nM
(−) Naloxone	10
(−) 3-Hydroxy-*N*-allyl-morphinan (levallorphan)	1
Levorphanol	2
(−) Nalorphine	2
(−) Morphine	6
(−) Methadone	20
(±) Pentazocine	50
(+) Methadone	200
(±) Propoxyphene	1,000
(+) 3-Hydroxy-*N*-allyl-morphinan	5,000
Dextrorphan	8,000
(−) Codeine	20,000

No effect at 0.1 mM: hexobarbital, serotonin, norepinephrine, carbachol, choline, atropine, histamine.

Adapted and reprinted from Pert and Snyder[1]; with permission.

Drug Abuse Research Centers, and Johns Hopkins received one. Although I had never previously worked with opiates, I could see that finding their receptors would be the most effective way to learn how they work. Accordingly, I was willing to spend $3,000, which seemed an astronomic amount of money, to obtain a custom preparation of [^{3}H]naloxone, the potent, pure opiate antagonist. My graduate student Candace Pert, Ph.D. (Johns Hopkins University), had been studying the uptake of choline as an acetylcholine precursor into preparations of the guinea pig ileum. Accordingly, she monitored binding of [^{3}H]naloxone in these preparations and at the same time examined binding in rat brain membranes using Pedro's vacuum manifold. After a relatively few manipulations of washing conditions, we obtained what seemed to be specific receptor interactions which were approximately twofold to threefold greater than nonspecific binding, assessed in the presence of large concentrations of nonradioactive naloxone.[1]

Our experimental system enabled us to examine hundreds of samples a day and address diverse questions. The pharmacologic actions of opiates are stereospecific, and binding was intensely stereospecific with the pharmacologically active (−) isomers thousands of times more potent than (+) isomers (table 1). Codeine was essentially inactive. Codeine is morphine with a methyl group covering up what we soon learned to be a critical hydroxyl on the benzene ring of morphine. This finding helped explain the pharmacology of codeine, which acts more gradually than morphine, because it first must be metabolized in the liver to morphine.

In the initial experiments in rat brain, we found marked regional differences in densities of opiate receptors. Together with Michael Kuhar, Ph.D. (Professor of Pharmacology, Johns Hopkins University), we dissected monkey brain into many small areas and measured receptor binding in each of them.[3] We then used recently developed autoradiographic techniques to visualize receptor localization at a microscopic level.[4] We were astounded at the extremely high densities of receptors in selected nuclei. These discrete localizations could explain the major actions of opiates. In the thalamus, receptors were highly concentrated in lateral nuclei mediating deep, aching pain that is selectively alleviated by opiates rather than medial nuclei that mediate sharp, prickly pain. Areas that regulate emotional behavior, such as the locus ceruleus and various limbic structures, were greatly enriched, which could account for the euphoriant influences of the drugs. Vagal nuclei in the brainstem were loaded with receptors, especially the respiratory nuclei, fitting with the respiratory depression elicited by opiates. Tectal nuclei with high densities of receptors presumably explained the meiotic influences of the drugs.

One of the most fundamental quests of pharmacologists has been to distinguish agonists and antagonists. In initial receptor studies, they behaved the same. In a routine screen of ions, my M.D.–Ph.D. student Gavril Pasternak (Department of Pharmacology, Johns Hopkins University) saw effects of sodium ions on receptor binding that differed from Candace's observations. My technician Adele Snowman set up experiments to resolve the controversy. She found that both students were correct. One had been using a [^{3}H]agonist and the other a [^{3}H]antagonist. Adele noted that sodium selectively decreased the affinity of agonists for receptors while antagonist binding was unaffected by sodium.[5] Mixed agonist–antagonists such as pentazocine (Talwin) were influenced in an intermediate fashion. This provided a simple means of screening for pentazocine-like drugs, which offer potential as less-addicting opiates. Monitoring opiate receptor binding in the presence and absence of sodium was soon adopted by the pharmaceutical industry as a strategy for developing new analgesics. We still do not understand exactly what the "sodium effect" represents, but it is synergistic with influences of guanosine triphosphate, which decrease receptor binding of agonists but not antagonists. The sodium–guanosine triphosphate effects indicate that receptor binding monitored in brain membranes not only reflects interactions at the ligand recognition site but also reveals links between the receptor and associated G proteins.

Man was not born with morphine in him. The properties of opiate receptors so resemble those of classic neurotransmitter receptors that it was reasonable to search for an endogenous ligand, an opiate-like neurotransmitter. In our laboratory, Gavril Pasternak identified a small peptide-like substance in brain extracts that competed with [^{3}H]naloxone for binding to opiate receptors and whose concentrations varied throughout the brain in proportion to regional variations in opiate receptor levels.[6] Lars Terenius, M.D.[7] (Professor, Center for Molecular Medicine, Karolinska Hospital, Stockholm, Sweden), also showed that brain extracts could compete for radiolabeled opiate binding to receptors.

The Scottish investigators John Hughes, Ph.D. (Professor, Imperial College, London, United Kingdom), and Hans Kosterlitz, M.D. (Professor, University of Aberdeen, Scotland; 1903–1996), showed that brain extracts contained a substance that mimics the ability of morphine to inhibit electrically induced contractions of the guinea pig ileum and mouse vas deferens in a fashion that was blocked by naloxone.[8]

Using their smooth muscle assay, the Scottish group isolated two opioid pentapeptides, methionine-enkephalin and leucine-enkephalin.[8] In our laboratory, my postdoctoral fellow Rabi Simantov, Ph.D. (Department of Pharmacology, Johns Hopkins University), also isolated the two enkephalins and confirmed their structure in a study published a few months after the Hughes–Kosterlitz report.[9] Our immunohistochemical maps of enkephalin were virtually identical to autoradiographic maps of opiate receptors, providing compelling evidence that enkephalins were the endogenous ligands for the receptors.[10] Subsequently, several other opioid peptides have been isolated, termed *endorphins* or *dynorphins*, all containing the enkephalin sequence.

When we had identified opiate receptor binding, we developed tritiated preparations of drugs that we hoped would interact potently with receptors for numerous neurotransmitters. In each case, it was critical to select a ligand of high potency so it would dissociate slowly and remain bound to the receptor despite vigorous washing that was required to remove nonspecific binding. Also, optimal ligands had to be hydrophilic, because lipophilic ligands tended to bind to nonspecific sites. Within 2–3 yr, we were able to identify and characterize receptors for glycine, norepinephrine, dopamine, serotonin, γ-aminobutyric acid, angiotensin, bradykinin, neurotensin, cholecystokinin, histamine (H1), acetylcholine (the muscarinic receptor), and thyrotropin-releasing hormone.[11] Receptor identification enabled us to explain actions of various drugs. Thus, an extraordinarily close correlation between the affinity of antipsychotic neuroleptic drugs for dopamine receptors labeled with [^{3}H]spiperone but not [^{3}H]dopamine established that the therapeutic actions of these drugs involved blocking a subtype of dopamine receptors labeled by spiperone, a receptor type now designated D2.[12] Steve Peroutka, an M.D.–Ph.D. student (Department of Pharmacology, Johns Hopkins University), combined receptor binding and behavioral studies to provide the first demonstration of subtypes for serotonin receptors, 5-HT$_1$ and 5-HT$_2$.[13]

Subtypes of opiate receptors were first discriminated by Hans Kosterlitz based on pharmacologic differentiation of drugs in smooth muscle studies as well as ligand binding.[14] His discovery of discrete μ, δ, and κ receptors has stood the test of time, as molecular cloning has shown that these are the three principal classes of opiate receptor genes. The "alchemist's gold" of the opiate field would be a nonaddicting opiate, conceivably one that binds selectively to a receptor subtype that mediates analgesia while not affecting receptors that cause addiction. Drugs that are agonists at κ receptors tend to be less addicting but also cause dysphoria and psychotomimetic effects. Buprenorphine is the closest approximation of a much improved, though not ideal drug. It is a mixed agonist–antagonist at μ receptors and, because of its antagonist influences, buprenorphine is less addicting than pure agonists. For commercial reasons, buprenorphine was not developed as an analgesic in the United States but has emerged as the drug of choice for the maintenance of heroin addicts. Being less addicting than methadone, buprenorphine can be prescribed in general medical practice, whereas methadone treatment is restricted to specialized clinics.

Despite the many advances over the past 35 yr in molecular studies of opiate receptors, we still do not have a "nonaddicting" opiate. Moreover, the molecular basis of addiction to opiates and other drugs has not been unraveled. In the early days of the field, we expended vast energies looking for changes in addicted animals of opiate receptors and enkephalin dynamics but came up empty-handed. Perhaps addiction lies at the level of second, third, and fourth messengers and/or in the interface of neurotransmitter systems that regulate rewarding behavior. I have faith that answers will be forthcoming.

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