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Pert C B & Snyder S H. Opiate receptor: demonstration in nervous tissue. *Science* 179:1011-4, 1973.
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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 *SCI*® indicates that this paper has been cited in over 1,300 publications.]

The Naloxone Methodology and the Discovery of Opiate Receptors

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So much has been written about the "discovery of the opiate receptor" that I am grateful for the opportunity to share my perspective.

When I entered the Johns Hopkins School of Medicine Department of Pharmacology in September 1970, I was already committed to doing my doctoral dissertation with Dr. Solomon H. Snyder, who had in fact recruited me into the department. My goal was to provide an integration of biochemistry with behavior, and Sol was already pioneering in psychoneuropharmacology. After three requisite laboratory "rotations," I learned that I not only shared Sol's scientific interests but also harmonized well with his research style: one of unbridled optimism about what one scientist—and even one experiment—can accomplish.

When Dr. Pedro Cuatrecasas joined the department, Sol recognized the importance of Pedro's receptor-binding techniques, urged me to study with him, and had us all to dinner. My painful horseback riding injury, which had in fact been cured by bed rest and opiates, had been one of that social evening's topics of conversation. When Sol offered the "opiate receptor" ("it's just like the insulin receptor—only for morphine!") as one of my dissertation options, I was excited, inspired, and determined. Sol gave me two articles as a basis for initiating my literature search. The first was a recent (1971) *Proceedings of the National Academy of Sciences* paper by the famous pharmacologist Avram Goldstein[1] that set out a strategy for determining "stereospecific binding" and claimed a minute (1-2 percent) signal of detection with this method. The second was a review by Vincent P. Dole,[2] proponent of methadone maintenance therapy. I was convinced of the existence of opiate receptors by the section of Dole's review dealing with Hans Kosterlitz's work on opiate receptors in the guinea pig ileum: the ability of a large series of opiate analogs with similar rank potency to both suppress contraction of the guinea pig ileal smooth muscle *in vitro* and to elicit analgesia in rodent models offered compelling evidence that the opiate receptor was a biochemical component with a definite stereospecificity that was used by the organism in both the brain and the intestine. My library research (e.g., the Sumerian hieroglyphic for opium consists of two characters, one for "joy" and one for "juice") fired me with the enthusiasm I would need. Opiate receptors had been hinted about for decades and more firmly posited by Beckett and Casey in the 1950s and Phil Portoghese later on. I was not at all dismayed by several papers that had failed to demonstrate opiate receptors even though they concluded that their failure to find them proved that opiate receptors did not exist!

Still, it was tough going when my daily experiments with tritiated morphine and minced guinea pig ileum and the rapid filtration I learned during my rotation with Pedro failed to give any hint of a signal of "stereospecific binding." Still, I believed there had to be opiate receptors if I could find the proper combination of times, temperatures, and buffers, i.e., "conditions" to make the experiment "work." One day during this disappointing period, Sol had Dr. Eric Simon visit me in the laboratory.

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From him I learned that he, like me, could not detect any stereospecific binding even when replicating "Goldstein's system." Eric's interest in the strategies I was developing as well as his sharing of the fact that he was hoping to make a breakthrough himself spurred my efforts further. I was running out of ideas and Sol, always generous, began to be concerned that I would never finish my doctoral dissertation research.

A critical decision to change the radioactive ligand to the narcotic antagonist naloxone, based upon Paton's pharmacological theory about slow off rates for antagonists, as well as a stimulating six-week psychopharmacology summer program in Nashville, Tennessee, provided the break. Returning to the lab after the summer break, I applied myself with renewed energy and with the tritiated naloxone on October 22, 1972, produced a signal-to-noise ratio of greater than 50 percent! Sol was ecstatic and instantly assigned Ms. Adele Snowman as my full-time technician. Having found the right combination of experimental conditions, every experiment thereafter "worked," and we quickly learned which parts of brains had the most opiate receptors (striatum) and which the least (cerebellum). A very satisfying and important result was that the ability of a number of opiate analogs to inhibit tritiated naloxone binding correlated closely with their potencies in modulating analgesic thresholds. In other words, potent analgesics were also potent in the binding assay, while weaker analogs were less active in binding inhibition.

Sol's literary productivity is legendary and, suffering no "writer's block" whatsoever, he was upon me almost instantly to complete a submission to *Science* magazine. Together, in his office, less than two months after the first good experiment, the first draft was dictated, and Dr. Paul Talalay, then chairman of the department, and Pedro were kind enough to provide a thorough and essential critical review and editing.

The paper came out the first week in March 1973, and in the absence of much other news (right after Vietnam, right before Watergate) Hopkins held a press conference to put this discovery on the front page of newspapers throughout the world. I had the great privilege of continuing my working relationship with Sol for two more years during which period we published a number of further articles on opiate receptors to exploit the original technology described in this first critical paper.[3-7] Later on with my colleagues at the National Institute of Mental Health, a similar technology revealed how opiate receptors ("recognition molecules") have been conserved in evolution[8] and yet are profoundly enriched in newly evolved areas of the primate brain.[9]

For my personal scientific development, this one of my over 200 publications is the most important because I learned an important lesson. One must believe with all one's heart first in an idea in order to provide the energy for the repeated experiments usually needed to find a reproducible methodology that works. Experiments that fail are worthless, i.e., absence of proof is not proof of absence, of the opiate receptor—or anything else.[10] The Pert/Snyder opiate receptor demonstration methodology was simple and reproducible. This ability to study brain receptors led eventually to a greater understanding of the molecular basis of mind and its role in health and disease.[11,12]

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