This Week's Citation Classic®

Simon E J, Hiller J M & Edelman I. Stereospecific binding of the potent narcotic analgesic [^{3}H]etorphine to rat-brain homogenate. *Proc. Nat. Acad. Sci. USA* 70:1947-9, 1973.

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This report provided evidence for the existence of stereospecific binding sites for opiate drugs in rat brain homogenate. Stereospecific binding, representing 40-60 percent of total binding, was sensitive to sulfhydryl reagents, high salt, and proteolytic enzymes. Binding was saturable and reversible, and the affinity of a number of opiates paralleled their pharmacological potency. [The *SCI*® indicates that this paper has been cited in over 790 publications.]

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This paper was one of three, published simultaneously and independently, that reported the discovery of stereospecific opiate binding sites that have since been shown to be the pharmacologically relevant opiate receptors. This discovery led to another important finding, namely, that the brain and a number of other tissues of animals and man contain substances with opiatelike properties that are natural ligands for these receptors, the endogenous opioid peptides. These two discoveries opened up a new field of research that has become one of the most active in biology, an important reason for the frequent citation of this paper.

As senior author I became interested in the biochemical demonstration of the existence of opiate receptors when I first entered this field in the early 1960s. Such receptors had been postulated based on the structural and steric specificity of many of the actions of opiate drugs. A paper describing our efforts to demonstrate nalorphine-displaceable ^{3}H-dihydromorphine binding was published by Dina Van Praag and me in 1966.[1] No displaceable binding was found. In retrospect, this was due to the use of labeled dihydromorphine that had much too low a specific activity. I was encouraged to try again by a paper from Avram Goldstein's laboratory[2] in 1971. Though these authors found only 2 percent of total ^{3}H-levorphanol binding to be stereospecific, their work suggested that it might be possible to observe significant stereospecific binding by using a centrifugation method to separate bound drug from free.

The key changes we made in the Goldstein procedure were the use of ligands with high specific activities (curies/millimole) and washing of the pellet by careful rinsing or recentrifugation to lower the background. This technique was not very successful with labeled dihydromorphine, yielding positive results only occasionally. This suggested to us that the affinity of dihydromorphine might be too low (off rate too high) to withstand washing. We therefore obtained etorphine, the most potent opiate known at the time, and had it tritiated by the New England Nuclear Corporation (NEN). With this drug we immediately observed stereospecific binding, which represented 40-60 percent of total binding, and went on to characterize specific etorphine binding in some detail. This work, published in the paper under discussion, was performed with my longtime collaborator Jacob M. Hiller, who has played a key role in all of our subsequent work, and with my technician at the time, Irit Edelman.

The following is an interesting sidelight that illustrates the importance of luck in research. We had asked NEN to catalytically reduce the double bond in etorphine. Since the resulting product had a lower specific activity than expected, we retained 1 mCi and had the rest (about 9 mCi) retritiated. The result was that all of the radioactivity was lost. The explanation: the double bond of the ethene bridge of etorphine was too hindered to be reduced under the mild conditions employed. The tritium was therefore incorporated by catalytic exchange of hydrogens in the molecule. After tritiation, the technician at NEN usually flushes with hydrogen in the presence of catalyst to exchange out exchangeable tritium. The material we utilized (the mCi we retained) for all of the early studies was labeled because the technician forgot to flush with H_2. We were lucky in several ways: the technician made a fortunate omission, we retained sufficient labeled material, and the tritium label was stable in the absence of catalyst.

I had the honor to be the first to present an oral report on the biochemical demonstration of opiate receptors at a scientific meeting. This occurred at the symposium on "Current Status of Pharmacological Receptors" at the Federation of American Societies for Experimental Biology meeting in Atlantic City, New Jersey, on April 18, 1973. Sol Snyder and Goldstein, who had wanted to speak on the same topic, were each given five minutes as discussants. It was an exciting and very lively symposium attended by a standing-room-only audience.

Our laboratory has continued to contribute actively in the area of opioid research and particularly to our knowledge concerning the receptors. Three recent papers[3-5] and a review[6] illustrate the direction and progress of our more recent research.

I and my laboratory have received several honors for this work, including the National Institute on Drug Abuse Research Pacesetter Award in 1977, the Louis and Bert Freedman Foundation Prize from the New York Academy of Sciences in 1980, an honorary doctorate from the University of Paris in 1982, and the Nathan B. Eddy Memorial Medal from the Committee on Problems of Drug Dependence in 1983.

1. **Van Praag D & Simon E J.** Studies on the intracellular distribution and tissue binding of dihydromorphine-7,8-H^3 in the rat. *Proc. Soc. Exp. Biol. Med.* 122:6-11, 1966. (Cited 25 times.)
2. **Goldstein A, Lowney L I & Pal B K.** Stereospecific and nonspecific interactions of the morphine congener levorphanol in subcellular fractions of mouse brain. *Proc. Nat. Acad. Sci. USA* 68:1742-7, 1971. (Cited 345 times.)
3. **Gioannini T L, Howard A D, Hiller J M & Simon E J.** Purification of an active opioid binding protein from bovine striatum. *J. Biol. Chem.* 260:15117-21, 1985. (Cited 15 times.)
4. **Howard A D, Sarne Y, Gioannini T L, Hiller J M & Simon E J.** Identification of distinct binding site subunits of mu and delta opioid receptors. *Biochemistry—USA* 25:357-60, 1986.
5. **Hiller J M, Itzhak Y & Simon E J.** Selective changes in μ, δ and κ opioid receptor binding in certain limbic regions of the brain in Alzheimer's disease patients. *Brain Res.* 406:17-23, 1987.
6. **Simon E J & Hiller J M.** Solubilization and purification of opioid binding sites. (Pasternak G W, ed.) *The opiate receptors.* Clifton, NJ: Humana Press, 1988. p. 165-94.

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