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Review

Serotonin receptors in brain revisited

José M. Palacios

Frontera Biotechnology S.L., Barcelona 08013, Spain

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ABSTRACT

In the early 1980's, the dispute on the existence of a multiplicity of receptors for neurotransmitter was at its height. Several subtypes of serotonin (5-HT) receptors were proposed on the basis of radioligand binding assays. In order to provide further support to the existence of these receptors we performed quantitative autoradiographic mapping of the binding of several ligands for the 5-HT_1 receptor labeling the subtypes 5-HT_{1A}, 5-HT_{1B} and 5-HT_{1C}, and characterized pharmacologically these different receptors. The results demonstrated differential localization of the subtypes of 5-HT_1 receptors indicating that they were expressed by different cell populations, probably neurons, in the brain and further supporting their reality. Shortly afterwards, the cloning of the genes coding for these 5-HT receptors, and many others, ended the dispute by demonstrating that they were different proteins. The advent of Molecular Biology provided new methodologies for the study of the chemical and molecular anatomy of 5-HT receptors in brain, by visualizing cells expressing their mRNA by *in situ* hybridization and showed that the family of mammalian 5-HT receptors has 14 members, a figure much larger than ever suspected at that time.

Original article abstract: Quantitative autoradiographic mapping of serotonin receptors in the rat brain. I. Serotonin-1 receptors: The distribution of serotonin-1 (5-HT_1) receptors in the rat brain was studied by light microscopic quantitative autoradiography. Receptors were labeled with [^{3}H]serotonin (5-[^{3}H]HT), 8-hydroxy-2-[H-dipropylamino-^{3}H]tetralin (8-OH-[^{3}H]DPAT), [^{3}H]LSD and [^{3}H]mesulergine, and the densities quantified by microdensitometry with the aid of a computer-assisted image-analysis system. Competition experiments for 5-[^{3}H]HT binding by several serotonin-1 agonizts led to the identification of brain areas enriched in each one of the three subtypes of 5-HT_1 recognition sites already described (5-HT_{1A}, 5-HT_{1B}, 5-HT_{1C}). The existence of these'selective' areas allowed a detailed pharmacological characterization of these sites to be made in a more precise manner than has been attained in membrane-binding studies. While 5-[^{3}H]HT labeled with nanomolar affinity all the 5-HT_1 subtypes, the other ^{3}H-labeled ligands labeled selectively 5-HT_{1A} (8-OH-[^{3}H]DPAT), 5-HT_{1C} ([^{3}H]mesulergine) and both of them ([^{3}H]LSD). Very high concentrations of 5-HT_1 receptors were localized in the choroid plexus, lateroseptal nucleus, globus pallidus and ventral pallidum, dentate gyrus, dorsal subiculum, olivary pretectal nucleus, substantia nigra, reticular and external layer of the entorhinal cortex. The different fields of the hippocampus (CA_1–CA_4), some nuclei of the amygdaloid complex, the hypothalamic nuclei and the dorsal raphé, among others, also presented high concentrations of sites. Areas containing intermediate densities of 5-HT_1 receptors included the claustrum, olfactory tubercle, accumbens, central gray and lateral cerebellar nucleus. The nucleus caudate-putamen and the cortex, at the different levels studied, presented receptor densities ranging from intermediate to low. Finally, in other brain areas-pons, medulla, and spinal cord-only low or very low concentrations of 5-HT_1 receptors were found. From the areas strongly enriched in 5-HT_1 sites, dentate gyrus and septal nucleus contained 5-HT_{1A} sites, while globus pallidus, dorsal subiculum, substantia nigra and olivary pretectal nucleus were enriched in 5-HT_{1B}. The sites in the choroid plexus, which presented the highest density of receptors in the rat brain, were of the 5-HT_{1C} subtype. The distribution of 5-HT_1 receptors reported here is discussed in correlation with the distribution of serotoninergic neurons and fibers, the related anatomical pathways and the effects which appear to be mediated by these sites. © 1985.*This article is part of a Special Issue entitled SI:50th Anniversary Issue.*

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E-mail address: jmpalacios09@gmail.com

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The work reported in our paper was conducted in Basle (Switzerland) at Sandoz, now Novartis, in a moment of tremendous change in the study of receptors for neurotransmitters. In the late 1970's and early 1980's the dispute between the supporters of a limited number of receptor for a neurotransmitter (one, at the most two), the classical isolated organ, functional pharmacologists and those, the new biochemical "radioactive" pharmacologists, postulating multiplicity of receptors for a neurotransmitter, was at its height (Palacios et al., 2010).

This was driven by, on the one hand, the development of new relatively simple methods, such as radioligand binding techniques, Robert Lefkowitz has written: "if a single technical advance can be said to have opened the door to the molecular era of receptors, it was the development of radioligand binding methods during the 1970's" (Lefkowitz, 2004). On the other hand, the pharmaceutical industry was committed to the discovery and development of new molecules with significant potential for the treatment of diseases of the CNS, and significant economic interest.

I joined Sandoz in August 1981, following a 3 years postdoctoral stay at the Department of Neuroscience, Medical School, Johns Hopkins University, working with Michael Kuhar (Kuhar, 1981), and in the exciting environment created by Sol Snyder. There I took part in the development of the technique of receptor autoradiography, a daughter of the "grind and bind" assays. This relatively simple procedure consisted basically in labeling receptors on microtome sections of brain tissues and generating autoradiographic images of the radiolabeled sites by apposing it to photographic emulsions. The visualization of the binding sites for receptor ligands at the light microscopic level and recently developed digital computerized image analysis systems, allowing the mapping of the brain areas, nuclei and cell layers where the receptors were located and its ligands would exert their actions (Palacios et al., 1981). The methods were quantitative and made it possible to characterize pharmacologically the sites being visualized. Together with the development in the preceding decades of histochemical, and later immunohistochemical, methods for the transmitter and related proteins, our knowledge of the anatomical and cellular geography of neurotransmission in the mammalian brain made a significant leap forward.

The field of serotonin receptors (5-HTRs) was a paradigmatic example of receptor complexities and promises. The existence of multiple 5-HTRs had been postulated already on the 1950's, on the basis of classical pharmacology. Peroutka and Snyder (1979) presented, in 1979, the first evidences for 5-HTRs subtypes from radioligand studies.Their results showed that 5-HTRs could be classified into two classes, 5 HT_1 and 5 HT_2 as differentially labeled by [^{3}H]-5-HT, [^{3}H]-spiperone and [^{3}H]-LSD. Further subdivisions were proposed, based in the properties of new ligand and selective compounds, subdividing 5-HT_1 into 5-HT_{1A} and 5-HT_{1B} (Pedigo et al., 1981). We had proposed 5 HT_{1C} based on the localization and characteristics of the sites labeled by a Sandoz compound mesulergine (Palacios et al., 2010; Pazos et al., 1984).

Obviously not everybody in the neuropharmacological community was happy with the uncontrolled proliferation of 5-HTRs and the polemic took different aspects (see Palacios et al., 2010).

The question Angel Pazos and I put was that if these different proposed 5-HTRs (or "sites", as we were required to call them at the time) were really different molecular entities, they would probably be expressed by different cell populations and show different regional distributions in the brain. To do that properly we should first look at the pharmacological characteristics of the binding sites of different radioligands, using as many unlabeled displacers as necessary and construct saturation and displacement curves, all that at the microscopic level, and then analyze the differential anatomical localization of these sites throughout the brain.

We generated an atlas of the rat brain showing the detailed distribution, density and molecular pharmacological characteristics of 5-HT_{1A}, 5-HT_{1B} and 5-HT_{1C} (later to become 5-HT_{2C}) in the rat brain (Pazos and Palacios, 1985). A companion paper (Pazos et al., 1985) presented similar data for 5-HT_2, then still considered a single population.

The results showed a clear difference in the brain areas labeled or enriched in one or the other population. These differences were not random distributions but rather showed association of receptor subtypes with well-defined anatomical and functional brain areas. For example, we found 5-HT_{1A} enriched in the components of the limbic system, while 5-HT_{1B} where predominant in the basal ganglia and the striato nigral pathway. This suggested different cellular populations expressing these receptors, its involvement in the functions of different brain areas thus adding further support to the actual existence of these receptors, and their interest as new targets for drug development. The concept of target identification did not exist at the time.

Because of the limited cellular resolution of the technique, it was not possible to assign receptors to specific neuronal or glial populations. A way to overcome this limitation was to study the effects of selective lesions in the brain of the experimental animal and examine changes in receptor densities and localizations, and later studying human neuropathologies. Thanks to our collaboration with Alphonse Probst of the Pathology Institute of the University of Basel, and to the fact that receptors could stand the conditions of human postmortem period (Palacios et al., 1986), we went ahead with the characterization of human 5-HTRs (Pazos et al., 1987a, 1987b). These studies provided important information for later imaging studies of 5-HT receptors in the living human brain (Paterson et al., 2013)

It is worth mentioning that all these investigations were always complemented with extensive medicinal chemistry and molecular pharmacology, including detailed membrane binding assays as well as the study signal mechanisms and function of the sites. The expertize of Daniel Hoyer, and that of many other colleagues at Sandoz, was essential for the success of the project (Hoyer et al., 1986a, 1986b). Human studies revealed differences in pharmacology that we extended to other species. In a short time the complexity of the system grew incredibly. Soon important species differences were detected affecting for example drug targets such as the 5-HT_{1B}, not detected in man. This led eventually to the discovery of a new subtype the 5-HT_{1D} (Waeber et al., 1988). Shortly afterwards 5-HT_4 and others will come (see Mengod et al., 2006).

The advent of the molecular age of receptors will change things dramatically. In 1986 Lefkowitz and colleagues (Dixon et al., 1986) reported the cloning of the gene coding for the beta 2 adrenergic receptor, the first G-protein coupled receptor (GPCR) to be cloned.