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COMMENTARY

NEUROTRANSMITTER AND DRUG RECEPTORS IN THE BRAIN*

SOLOMON H. SNYDER
Departments of Pharmacology and Experimental Therapeutics, and Psychiatry and the Behavioral Sciences, Johns Hopkins University School of Medicine, Baltimore, Md. 21205, U.S.A.

During the past 2 yr, receptor sites in the brain for several neurotransmitters and for opiates have been characterized biochemically in our laboratory. The nicotinic acetylcholine receptor in the electric organ of electric eels and torpedo previously had been labeled by several groups with potent, virtually irreversibly binding polypeptide toxins. Students of other neurotransmitters were disheartened at the lack of a magic toxin to label their pet transmitter. Our success in labeling the opiate receptor with the opiates themselves, small molecules which bind reversibly, prompted an interest in identifying neurotransmitter receptors by studying the binding of antagonists of small molecular weight or the transmitters themselves. We focus here on the opiate, muscarinic acetylcholine, glycine, GABA and serotonin receptors, which have been best characterized. Their identification has been far from a trivial task. Techniques which worked for one receptor did not work for another, and for every success we have endured nine or more failures. From our accumulated experience, general principles appear applicable to the technology of receptor identification as well as to the functional properties of neurotransmitter and drug receptors in the brain, which may have implications for general mechanisms of synaptic neurotransmission.

TECHNICAL CONSIDERATIONS

First and most fundamental in receptor identification is ensuring specificity. Just because a ligand binds in a saturable fashion with considerable affinity to brain tissue by no means establishes that the binding substance is a specific receptor for the ligand. Binding which is biologically irrelevant can often be extraordinarily deceptive. Thus, Cuatrecasas and Hollenberg [1] noted that insulin can bind to glass or talcum powder with affinity in the nanomolar range and with the capacity to discriminate among several insulin analogues according to their biological potency. Opiate pharmacologic effects are stereospecific with the (−)-isomer usually possessing all the activity. Certain glass filters in our laboratory bind opiates stereospecifically with a preference for the (−)-isomer [2]. Cerebrosides, ubiquitous lipids, can also bind opiates stereospecifically [3], conceivably accounting for the properties of a soluble opiate-binding substance isolated from brain tissue [4].

Because of these concerns, in our receptor studies, we always compare the relative binding affinity of a wide range of analogues with their biological potency and, for the receptors discussed in this review, we have noted impressive correlations. Since neurotransmitters are often distributed heterogeneously throughout the brain, the regional localization of receptor binding often is another means of ensuring specificity. There is a close correlation between the endogenous levels of glycine, high affinity uptake into unique populations of synaptosomes, the capacity of glycine to mimic the natural inhibitory transmitter and the density of glycine receptor binding sites [5, 6].

However, one need not anticipate a perfect correlation between levels of a transmitter and concentration of receptor sites in various brain areas. For instance, while there is a general correlation between muscarinic cholinergic receptor binding and endogenous levels of acetylcholine, there are some interesting discrepancies. Receptor binding is similar in all areas of the monkey cerebral cortex but the pyriform cortex has three to four times higher levels of choline acetyltransferase and high affinity choline uptake than other cerebral cortical areas, though its concentration of muscarinic receptors is no greater than any other region [7, 8]. The pyriform cortex may be the "exception that proves the rule." We suspect that the high levels of pre-synaptic cholinergic markers in the pyriform cortex in the absence of uniquely elevated muscarinic receptor binding means that the pyriform cortex may contain a preponderance of nicotinic receptors whereas, in most of the brain, cholinergic receptors are primarily muscarinic. In the monkey, GABA receptor binding in various regions parallels levels of endogenous GABA, while, for reasons which are not apparent, the correlation is much poorer in rat brain regions [9] (S. Enna and S. H. Snyder, manuscript in preparation).

Stereospecific LSD and serotonin binding [10–12] display many properties consistent with that of the post-synaptic serotonin receptor. For many regions of the monkey brain there is a good correlation between high affinity nerve terminal uptake of serotonin and specific LSD binding. However, there are major discrepancies such as the cerebral cortex, whose LSD binding is greater than that of hypothalamus despite the far lower levels of endogenous serotonin in the cerebral cortex than in the hypothalamus. This discrepancy may have bearing upon factors which determine the density of post-synaptic receptor

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sites. Histochemical staining for serotonin is distributed diffusely throughout the cerebral cortex in a fashion suggesting that almost all cerebral cortical cells receive serotonin terminals, but that no cells receive a large number of them [13]. By contrast, in the hypothalamus, the proportion of cells receiving serotonin terminal input may be considerably less than in the cerebral cortex, but many cells receive an extremely large number of serotonin boutons. If the density of receptors on a cell is determined in an all-or-none fashion by the presence or absence of nerve terminals rather than by the number of nerve terminals, than one might predict that the cerebral cortex should have a greater concentration of serotonin receptors than the hypothalamus. A lack of correlation between receptor density and level of endogenous neurotransmitter might also occur if the amount of neurotransmitter in each nerve terminal varies considerably in different brain regions or if the relative surface area of post-synaptic membrane associated with a terminal varies at synapses throughout the brain.

In developing techniques for biochemical identification of receptors by binding assays, one wishes to reduce nonspecific binding to a minimum but at the same time avoid dissociation of one's ligand from specific receptor sites. If a ligand has high affinity for the receptor being studied, the ratio of specific to nonspecific binding should be augmented if ligand of high specific activity is available, permitting assays with extremely low ligand concentrations.

While it is usually desirable to use a ligand with the highest affinity possible for the receptor, this is not always the case. Thus, we have experienced difficulties in studies of the muscarinic receptor using [^{3}H]quinuclidinyl benzilate (QNB) whose dissociation constant for the receptor is about 0·2 nM. The highest specific activity for [^{3}H]QNB available to us is about 1 Ci/m-mole, so that to conduct our assays with QNB concentrations below the dissociation constant, incubations must contain only about 2000 cpm of [^{3}H]QNB and specific receptor binding is only 200 cpm [8].

Filtration is an efficient technique for trapping bound ligand. To minimize nonspecific binding, it is desirable to wash the filters several times with large volumes of buffer solutions. During the trapping and washing procedure, one might anticipate that a ligand which dissociates rapidly from the receptor would "wash off." Since slower dissociation rates are usually associated with higher affinities for binding sites, we tended to feel, from early studies, that receptor binding can only be studied with ligands that have affinities in the nanomolar range. However, we have found more ligands that break than that conform to this rule. Thus strychnine binding to the glycine receptor is half saturated at about 4 nM concentration. We assumed that it would dissociate slowly from the receptor. However, even at 4° it dissociates with a half-life of about 30 sec. The nanomolar dissociation constant for strychnine results because the rapid dissociation is compensated for by a very rapid association rate. Because of rapid dissociation, it is quite difficult to measure strychnine binding by filtration. Our routine assays employ centrifugation, since during centrifugation the free and bound [^{3}H]strychnine remain in equilibrium.

Because the neurotransmitters themselves seem to have relatively low affinities for their receptor sites in the micromolar range, we had initially assumed that it would be impossible to label receptors with their own transmitters, since nonspecific binding would be anticipated to have as much affinity as specific receptor binding. However, by avoiding major contaminating features, such as using sodium-free medium to prevent transmitter binding to sodium-dependent uptake sites, we have successfully identified direct binding of GABA and serotonin to their receptors [9, 12].

GENERAL PROPERTIES OF RECEPTORS

From studies of the influence of enzymes and detergents, we have observed strikingly similar chemical features of several different neurotransmitter and drug receptors. All those studied are exquisitely sensitive to degradation by the proteolytic enzymes trypsin and chymotrypsin as well as phospholipase A. Phospholipase C and D appear to be much weaker and neuraminidase is virtually inactive. These seem to be general properties of brain receptors, since hormone and neurotransmitter receptors in other tissues are considerably less sensitive to these proteolytic enzymes and do not display such a selective sensitivity to phospholipase A.

All the receptors we have examined in the brain are also readily degraded by numerous detergents with prominent effects at concentrations as low as 0·01%. Since these concentrations of detergents are much lower than those used to solubilize membrane proteins, it has been quite difficult to obtain neurotransmitter or opiate receptors in a soluble state capable of binding ligand. The detergents do not destroy the receptors themselves since it has been possible to solubilize the the glycine receptor and regain strychnine-binding capacity after dialyzing away the detergent and precipitating the receptor, even though while in a soluble state strychnine binding cannot be demonstrated (A. Young, personal communication). Moreover, after binding a [^{3}H]opiate of high specific activity, we have been able to solubilize the opiate receptor and examine its properties (G. W. Pasternak, C. B. Pert and S. H. Snyder, manuscript in preparation).

Receptor binding assays permit estimation of the number of receptor sites in the brain. For all those we have studied, receptor density in whole brain is remarkably similar (about 20–80 pmoles/g wet weight). This is surprising, since the number of synapses utilizing each of the transmitters varies markedly. It is generally thought that about 30 per cent of brain synapses are GABAnergic, 25–40 per cent of brainstem and spinal cord synapses are glycinergic, while only 5–10 per cent of synapses in the brain are cholinergic and considerably less than 1 per cent are serotonergic. The simplest explanation for these findings derives from the notions discussed above regarding factors which determine receptor development. Thus, according to this conceptual model, if every cerebral cortical cell received 1 serotonin terminal and 100 GABA terminals, one would anticipate the same concentration of GABA and serotonin receptors in the cerebral cortex.

A MODEL OF RECEPTOR FUNCTION

Potentially the most important concept to develop from our receptor investigations relates to interconvertible receptor conformations determining agonist and antagonist activity of ligands. Sodium markedly increases receptor binding of opiate antagonists and decreases to the same extent the binding of opiate agonists [14, 15]. This effect is highly specific for sodium, being elicited to a lesser extent by lithium, whose atomic radius resembles sodium but not by numerous other monovalent or divalent cations. The differential affinity of drugs for the opiate receptor in the presence or absence of sodium predicts with considerable precision their pharmacologic properties as agonists, antagonists or mixed agonists–antagonists.

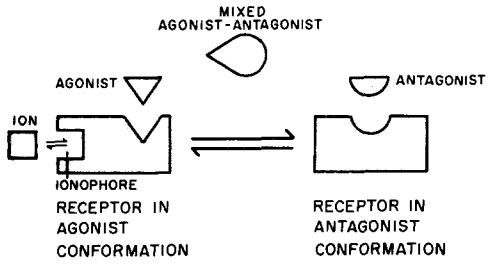

Fig. 1. A model of neurotransmitter receptor function.

We have postulated that the opiate receptor can exist in two interconverible forms, an "antagonist" conformation which binds sodium, and an "agonist" or no-sodium conformation [15]. Antagonists have a high affinity for the sodium and a low affinity for the no-sodium conformation, while the reverse situation occurs for agonists. Typical analgesic effects of opiates occur only if a drug binds to the agonist form of the receptor. This affords an explanation of the difference between the pharmacologic actions of antagonists and agonists. Conventional pharmacologic theory holds that both agonists and antagonists bind to receptors, but antagonists cannot elicit pharmacologic effects (lack "intrinsic activity") and simply sit on the receptor, preventing the access of agonists. According to our model, which has previously been postulated without direct biochemical evidence for the nicotinic cholinergic receptor [16, 17], antagonists bind to the sodium form of the receptor, shifting the equilibrium between sodium and no-sodium states and making fewer no-sodium receptors available for interaction with agonists. Sodium has a crucial role in effecting the transition between the two states of the receptor.

For the muscarinic receptor, we have observed that the potencies of pure antagonists in competing with [^{3}H]QNB for receptor binding in the guinea pig ileum correspond closely to their biological potency. Pure agonists, on the other hand, are up to two or three orders of magnitude more potent biologically than they are in competing for QNB binding sites, while mixed agonist–antagonists display an intermediate behavior (H. Yamamura, K. Chang and S. H. Snyder, manuscript in preparation). Similarly, Burgen and Hiley [18] have observed that acetylcholine is much less potent in inhibiting receptor binding of a radioactive antagonist than in binding directly to the receptor itself. These observations are consistent with a model for the muscarinic receptor similar to that described for the opiate receptor. Antagonists have high and low affinities, respectively, for the antagonist and agonist forms of the receptor and the situation is reversed for agonists. This would readily explain the lesser potency of agonists in inhibiting the binding of QNB to the antagonist form of the receptor than in eliciting biological responses by binding directly to the agonist form of the receptor. As observed for the opiate receptor and postulated for the nicotinic cholinergic receptor, one might expect the ion whose altered conductance is responsible for muscarinic neurotransmission to effect the transition between the two states of the muscarinic receptor. Neurophysiologic evidence suggests that potassium is the most relevant ion. We have not yet been able to demonstrate direct effects of potassium on muscarinic receptor binding.

We do have direct evidence for an ion-modulated alteration of the glycine receptor. Chloride is the ion whose conductance is enhanced by the inhibitory synaptic actions of glycine. Glycine and strychnine bind to distinct conformations or sites of the glycine receptor [19]. The binding of strychnine is inhibited by physiological concentrations of chloride and by other anions in proportion to their ability to mimic the post-synaptic potential alterations induced by chloride [20]. Thus, strychnine binding seems to be associated with the chloride ion channel of the glycine receptor; this is consistent with a model in which chloride ions regulate an interconversion of the glycine receptor between a glycine "agonist" and a strychnine "antagonist" state. We have preliminary data supporting two forms of the post-synaptic serotonin receptor in the brain, one with preferential affinity for serotonin and one for which LSD has selective affinity (J. P. Bennett and S. H. Snyder, manuscript in preparation). These data suggest that LSD may act as a mixed agonist–antagonist at post-synaptic serotonin receptors in the brain.

It would seem reasonable to extrapolate from our data with several brain receptors to suggest that all neurotransmitter receptors can exist in two forms which are interconvertible. The teleologic requirement for two receptor conformations would not be to deal with antagonists, which generally do not occur within the organism endogenously. Instead, the transition between receptor states, mediated by ionic or other conductance modulators, constitutes the fundamental mechanism translating neurotransmitter recognition into changes in the firing or other functions of the post-synaptic cell.

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