The Biochemical Basis of Neuropharmacology

FIFTH EDITION

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Cover: Darkfield photomicrograph of a cluster of dopamine neurons in the A8 dopamine cell group of the rat. The dopaminergic neurons and their processes are visualized by immunohistochemically staining for tyrosine hydroxylase (TH), the catecholamine synthetic enzyme. TH is localized to noradrenergic and adrenergic neurons as well as dopamine-containing cells. Identification of these neurons as dopaminergic was made on the basis of lack of staining of A8 neurons for dopamine-beta-hydroxylase, the enzyme which catalyzes the oxidation of dopamine to norepinephrine. Both perikarya and processes of the dopamine neurons can be clearly visualized, and varicosities present in the axons of TH-positive axons can be observed. (Unpublished photomicrograph courtesy of Dr. Ariel Y. Deutch, Yale University School of Medicine) × 400

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Preface to the Fifth Edition

Considerable revision has occurred with this edition. Two new chapters have been added, one on molecular neurobiology, the other on modulation of synaptic transmission. We have also eliminated the separate chapter on cyclic nucleotides, incorporating these agents with other second messenger systems in the modulation chapter. The neuroactive peptides and the endorphins have been combined into one chapter. Other chapters have been updated with new information. Despite some complaints about the paucity of references, we still hold to our original aim of presenting overviews rather than specific articles so that only selected papers, which would contain useful references, are cited along with recent reviews.

We are grateful to those readers who took the time to offer useful suggestions. Finally, we once again with pleasure acknowledge the efficient and tireless (but never tiresome) help of our Oxford University Press editor, Jeffrey House.

July 1985

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1 | Introduction

Neuropharmacology can be defined simply as the study of drugs that affect nervous tissue. This, however, is not a practical definition since a great many drugs whose therapeutic value is extraneural can affect the nervous system. For example, the cardiotonic drug digitalis will not uncommonly produce central nervous system effects ranging from blurred vision to disorientation. For our purposes we must accordingly limit the scope of neuropharmacology to those drugs specifically employed to affect the nervous system. The domain of neuropharmacology would thus include psychotropic drugs that affect mood and behavior, anesthetics, sedatives, hypnotics, narcotics, anticonvulsants, analeptics, analgetics, and a variety of drugs that affect the autonomic nervous system.

Since, with few exceptions, the precise molecular mechanism of action of these drugs is unknown, and since recitations of their absorption, metabolism, therapeutic indications, and toxic liability can be found in most textbooks of pharmacology, we have chosen to take a different approach to the subject. We will concentrate on the biochemistry and physiology of nervous tissue, emphasizing neurotransmitters, and will introduce the neuropharmacologic agents where their action is related to the subject under discussion. Thus a discussion of LSD is included in the chapter on serotonin and a suggested mechanism of action of the antipsychotic drugs in Chapter 10.

It is not difficult to justify this focus on either real or proposed neurotransmitters since they act at junctions rather than on the events that occur with axonal conduction or within the cell body. Except for local anesthetics, which interact with axonal membranes, all neuropharmacological agents whose mechanisms of action are to some extent documented seem to be involved primarily with synaptic events. This finding appears quite logical in view of the regulatory mechanisms in the transmission of nerve impulses. The extent to

which a neuron is depolarized or hyperpolarized will depend largely on its excitatory and inhibitory synaptic inputs, and these inputs must obviously involve neurotransmitters or modulators. What is enormously difficult to comprehend is the contrast between the action of a drug on a simple neuron, which causes it either to fire or not to fire, and the wide diversity of central nervous system effects, including subtle changes in mood and behavior, which that same drug will induce. As will become clearer in subsequent chapters, at the molecular level, an explanation of the action of a drug is often possible, at the cellular level, an explanation is sometimes possible, but at a behavioral level, our ignorance is abysmal. There is no reason to assume, for example, that a drug that inhibits the firing of a particular neuron will therefore produce a depressive state in an animal. There may be hundreds of unknown intermediary reactions involving transmitters and modulators between the demonstration of the action of a drug on a neuronal system and the ultimate effect on behavior.

The fact, however, that one can find compounds with a specific chemical structure to control a given pathological condition is an exciting experimental finding, since it suggests an approach that the neuropharmacologist can take to clarify normal as well as abnormal brain chemistry and physiology. The use of drugs that affect the adrenergic nervous system has, for instance, uncovered basic and hitherto unknown neural properties such as the uptake, storage, and release of the biogenic amines. The recognition of the analogy between curare poisoning in animals and myasthenia gravis in humans led to the understanding of the cholinergic neuromuscular transmission problem in myasthenia gravis and to subsequent treatment with anticholinesterases.

We have already referred to neuroactive agents involved in synaptic transmission as neurotransmitters, neuromodulators, and neurohormones so definitions are now in order. Although we can define these terms in a strict, rigid, fashion, it will be apparent—as noted later—that it is an exercise in futility to apply these definitions to a neuroactive agent as a classification unless one both un-

derstands its activity and specifies its locus. Briefly, the traditional definition of a *neurotransmitter* states that the compound must be synthesized and released presynaptically, it must mimic the action of the endogenous compound that is released on nerve stimulation, and, where possible, a pharmacological identity is required where drugs that either potentiate or block postsynaptic responses to the endogenously released agent also act identically to the administered suspected neurotransmitter. Conventionally, based on the studies of ACh at the neuromuscular junction, transmitter action was thought to be a brief and highly restricted point-to-point process. If one takes the word *modulation* literally, then a *neuromodulator* has no intrinsic activity but is only active in the face of ongoing synaptic activity where it can modulate transmission either pre- or postsynaptically. In many instances, however, a modulating agent does produce changes in conductance or membrane potential. Typically, modulatory effects involve a second messenger system. A *neurohormone* can be released from both neuronal and nonneuronal cells, and, most important to the definition, travels in some circulation to act at a site distant from its release site. Just how far a neurohormone has to travel before it loses its neurotransmitter status and becomes a neurohormone has never been decided.

We stated earlier that while we could define these terms it would be of little use to pigeonhole known neuroactive compounds until the site of action and the activity of the agent was specified. For example, dopamine is a certified neurotransmitter in the striatum, yet it is released from the hypothalamus and travels through the hypophysial-portal circulation to the pituitary where it inhibits the release of prolactin. Here it obviously fits the definition of a neurohormone. Similarly, serotonin is a neurotransmitter in the raphe nuclei, yet at the facial motor nucleus it acts primarily as a neuromodulator and secondarily as a transmitter. Most peptides with their multiple activities in the brain and gut are generally considered to be neuromodulators, yet Substance P fulfills the criteria of a transmitter at sensory afferents to the dorsal horn of the spinal cord. In sum, the plethora of exceptions to the aforementioned definitions

of a transmitter, modulator, or hormone has generated confusion in the literature. Better to describe the activity of a neuroactive agent at a specified site rather than attempt a profitless definition.

The multidisciplinary aspects of pharmacology in general are particularly relevant in the field of neuropharmacology, where a "pure" neurophysiologist or neurochemist would be severely handicapped in elucidating drug action at a molecular level. The neuropharmacologist should be aware of the tools that are available for the total dissection of a biological problem. These would include morphological techniques such as electron microscopy, fluorescence microscopy and freeze-etching, and immunological techniques as a basis for developing radioimmunoassays, immunocytochemistry, and monoclonal antibodies as well as the classical electrophysiological and biochemical procedures. In addition, if the investigator is concerned with certain aspects of the action of psychotropic drugs, he should have some knowledge of the techniques of behavioral testing.

In science, one measures something. One must know what to measure, where to measure it, and how to measure it. This sounds rather obvious, but the student should be aware that, particularly in the neural sciences, these seemingly simple tasks can be enormously difficult. For example, suppose one were interested in elucidating the presumed biochemical aberration in schizophrenia. *What* would one measure? ATP? Glucose? Ascorbic acid? Unfortunately, this problem has been zealously investigated in the last dozen years by people who have measured everything they could think of, generally in the blood, in their search for differences between normal individuals and schizophrenics. As could be predicted, the problem has not been solved. (It may be assumed, however, that these studies have produced a large population of anemic schizophrenics from all the bloodletting.) The situation is the same for a variety of neurological diseases. Even in epilepsy, where there is some evidence that points to a neurochemical lesion, we have no idea what to measure.

Deciding *where* to measure something in neuroscience is complicated by the heterogeneity of nervous tissue: In general, unless one

has a particular axon to grind, it is preferable to use peripheral nerve rather than the CNS. Suburban neurochemists have an easier time than their CNS counterparts, since it is not only a question of which region of the brain to use for the test preparation but which of the multitude of cell types within each area to choose. If a project involved a study of amino-acid transport in nervous tissue, for example, would one use isolated nerve-ending particles (synaptosomes), glial cells, neuronal cell bodies in culture, a myelinated axon, a ganglion cell? Up to the present time most investigators have used cortical brain slices, but the obvious disadvantage of this preparation is that one has no idea which cellular organelle takes up the amino acid.

How to measure something is a surprisingly easy question to answer, at least if one is dealing with simple molecules. With the recent advances in microseparation techniques and in fluorometric, radiometric, and immunological assays, there is virtually nothing that cannot be measured with a high degree of both specificity and sensitivity. In this regard one should be careful not to overlook the classical bioassay, which tends to be scorned by young investigators but in fact is largely responsible for the striking progress in our knowledge of both the prostaglandins and the opiate receptor with its peptide agonists. The major problem is with macromolecules. How can neuronal membranes be quantified, for example, if extraneuronal constituents are an invariable contaminant and markers to identify unequivocally a cellular constituent are often lacking? The quantitative and spatial measurement of receptors utilizing autoradiography is also a key problem. Where labeled ligands are employed to map receptors in brain via light microscopy, a mismatch is often encountered. Reasons offered for this problem are (1) except for autoreceptors, neurotransmitters and receptors are located in different neurons, (2) in addition to the synapse, receptors and transmitters are found throughout the neuron, (3) ligands may label only a subunit of a receptor or only one state of the receptor, and (4) autoradiography is subject to quenching. With immunohistochemical peptide mapping, a possible problem is the recognition by the antibody of a prohormone or, alternatively, a fragment of a

peptide hormone in addition to the well-recognized problem of cross-reactivity of the antibody with a physiologically different peptide.

This harangue about measurement is meant to point out that what would on the surface appear to be the simplest part of research can in fact be very difficult. It is for this reason that in each section of this book a critical assessment of research techniques is made. It is vital that students learn not to accept data without an appraisal of the procedures that were employed to obtain the results.

Finally, although the theme is not explicitly dealt with in this book, students may find it educational and often entertaining to attempt to define patterns of research design in neuropharmacology as well as current trends in research areas. One common pattern is for someone to observe something in brain tissue, trace its regional distribution in the brain, and then perform a developmental study of the phenomenon in laboratory animals from prenatal through adult life. Another common pattern is for someone to develop a technique and then search (sometimes with what appears to be desperation) for projects that will utilize the technique. Yet another is a somewhat simplistic idea of attempting to relate a behavioral effect to a changing level of a single neurotransmitter, invariably the one that a team has just learned how to measure. Current trends in the neural sciences include isolating ion channels, utilizing molecular genetics to uncover new peptides, neural cartography, that is, the mapping of transmitters and neuroactive peptides in the CNS, searching for toxins with specific effects on conduction or transmission, isolating and characterizing receptors for drugs as well as endogenous neuroactive agents, and identifying trophic factors involved in synaptogenesis. It can also easily be predicted that within the next few years an intensive search will be undertaken to explain the function and integration of the approximately three dozen "classical" neurotransmitters, the neuroactive peptides, and the unclassifiable items such as adenosine, in eliciting behavioral changes. Clearly, in this search neuropharmacologic agents will be invaluable probes.

2 | Cellular Foundations of Neuropharmacology

As we begin to consider the particular problems that underlie the analysis of drug actions in the central nervous system, it may be asked, "Just what is so special about nervous tissue?" Nerve cells have two special properties that distinguish them from all other cells in the body. First, they can conduct bioelectric signals for long distances without any loss of signal strength. Second, they possess specific intercellular connections with other nerve cells and with innervated tissues such as muscles and glands. These connections determine the types of information a neuron can receive and the range of responses it can yield in return.

Cytology of the Nerve Cell

We do not need the high resolution of the electron microscope to identify several of the more characteristic structural features of the nerve cell. The classic studies of Cajal (Ramón y Cajal) with metal impregnation stains demonstrated that nerve cells are heterogeneous with respect to both size and shape. An essential structural feature of the nervous system is that each specific region of the brain and each part of each nerve cell often have several synonymous names. So, for example, we find that the body of the nerve cell is also called the soma and the perikaryon—literally, the part that surrounds the nucleus. A fundamental scheme classifies nerve cells by the number of cytoplasmic processes they possess. In the simplest case, the perikaryon has but one process, called an axon; the best examples of this cell type are the sensory fibers whose perikarya occur in groups in the sensory or dorsal root ganglia. In this case, the axon conducts the signal—which was generated by the

sensory receptor in the skin or other viscera—centrally through the dorsal root into the spinal cord or cranial nerve nuclei. At the next step of complexity we find neurons possessing two processes: the bipolar nerve cells. The sensory receptor nerve cells of the retina, the olfactory mucosa, and the auditory nerve are of this form, as is a class of small nerve cells of the brain known as granule cells.

All other nerve cells tend to fall into the class known as multipolar nerve cells. While these cells possess only one axon or efferent-conducting process (which may be short or long, branched or straight, and which may possess a recurrent or collateral branch that feeds back onto the same type of nerve cell from which the axon arises), the main differences are in regard to extent and size of the receptive field of the neuron, termed the dendrites or dendritic tree. In silver-stained preparations for the light microscope, the branches of the dendrites look like trees in winter time, although the branches may be long and smooth, short and complex, or bearing short spines like a cactus. It is on these dendritic branches, as well as on the cell body, where the termination of axons from other neurons makes the specialized interneuronal communication point known as the synapse.

The Synapse

The last specialized structures of the neuron we shall discuss are the contents of the nerve ending and the characteristic specialized contact zone that has been presumptively identified as the site of functional interneuronal communication, that is, the synapse. As the axon approaches the site of its termination, it exhibits structural features not found more proximally. Most striking is the occurrence of dilated regions of the axon (varicosities) within which are clustered large numbers of microvesicles (synaptic vesicles). These synaptic vesicles tend to be spherical in shape, with diameters varying between 400 and 1200 Å. Depending upon the type of fixation used, the shape and staining properties of the vesicles can be related to their neurotransmitter content. The nerve endings also exhibit mitochondria, but do not exhibit microtubules unless the varicosity is