The Biochemical Basis of Neuropharmacology

Jack R. Cooper Floyd E. Bloom
Robert H. Roth

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JACK R. COOPER, Ph.D.

PROFESSOR OF PHARMACOLOGY

YALE UNIVERSITY SCHOOL OF MEDICINE

FLOYD E. BLOOM, M.D.

CHIEF, LABORATORY OF NEUROPHARMACOLOGY

NATIONAL INSTITUTE OF MENTAL HEALTH

WASHINGTON, D. C.

ROBERT H. ROTH, Ph.D.

ASSOCIATE PROFESSOR OF PHARMACOLOGY (PSYCHIATRY)

YALE UNIVERSITY SCHOOL OF MEDICINE

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1 | Introduction

WE MIGHT BROADLY DEFINE neuropharmacology 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 which affect mood and behavior, anesthetics, sedatives, hypnotics, narcotics, anticonvulsants, analeptics, analgetics, and a variety of drugs that affect the autonomic nervous system. Unfortunately, these drugs which are used therapeutically are not curative. They do not eliminate the cause of the disease state but instead merely contain or control the disorder. Thus, for example, convulsive disorders for many individuals represent a life-long problem; the anticonvulsant drugs, when effective, will prevent seizures but will not alter the idiopathic seizure mechanism.

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 (5-hydroxytryptamine [5-HT]) and a suggested mechanism of action of α-methyldopa in the chapter on the catecholamines.

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 appear to 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. Whether a neuron is depolarized or hyperpolarized will depend largely on its excitatory and inhibitory synaptic inputs, and these inputs will in most cases involve neurotransmitters. It can therefore be appreciated that most neuropharmacological agents will exert their action at axo-axonic, axo-dendritic, or axo-somatic junctions. What is enormously difficult to comprehend is the contrast between the action of a drug on a simple neuron causing 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.

Studying the molecular mechanisms of action of drugs affecting the nervous system, we can reason that the ultimate effect of these agents must be on ion movements, since the function of the brain is to transmit and store information, its functional unit is the neuron, and neuronal activity is expressed by ion movements across nerve membranes. It should be kept in mind, however, that the psychotropic agents, as well as drugs that affect the autonomic nervous system, appear to exert their primary effect at synapses.

The gap between our descriptive knowledge of neurotropic agents and our knowledge of molecular mechanisms of action of these drugs is wide, and it is pertinent to examine the reasons for the discrepancy. First and foremost is that to date we have been unable to locate, isolate, and characterize receptors for these various drugs. For example, although we can state that the primary

site of action of barbiturates is the reticular activating system, and that structure-activity experimentation has given us some idea of the requirements, including spatial configuration, of an active barbiturate, we know very little about the physiology of the reticular activating system and nothing about the presumed attachment of a barbiturate molecule to a synapse in this system. Even more intriguing is the question why such a dramatic specificity exists in neuropharmacology, where, for example, the addition of an extra methyl group on the side chain of pentobarbital changes the compound from a hypnotic drug to a powerful convulsant drug. Even assuming that by some ingenious technique we could isolate the receptor (presumably a protein), how would we know that its properties have not changed because of its isolation from the cell? How would we prove that it was indeed the specific receptor for barbiturates? Finally, and the most difficult question, how would we relate this bit of protein to sedation and hypnosis?

Another reason we cannot explain the action of neuropharmacologic agents is that normal and abnormal neural activity at a molecular level have not been explained. And the reason for this deficiency is that biophysical research techniques and approaches of the requisite sophistication have only recently emerged.

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. More recently, an investigation of the action of pyrithiamine, an antimetabolite of thiamine, on isolated nerve preparations led to the discovery of the probable cause of the fatal genetic disease, sub-

acute necrotizing encephalomyelopathy. Patients with this disease produce a compound which inhibits the synthesis in the brain of thiamine triphosphate.

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 techniques such as electron microscopy, freeze-etching, circular dichroism, and birefringence, 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, knowing what to measure is of obvious importance, but the importance of knowing how to measure it cannot be overestimated. 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 of neuropharmacology learn not to accept data without a severe appraisal of the procedures that were employed to obtain the results.

As already stated, our approach to neuropharmacology is by way of the basic physiology and biochemistry of nervous tissue with particular reference to neurotransmitters. The book is based on a course at Yale given to graduate and medical students. We do not intend each chapter to be a review article but rather a presentation of what we think are the important aspects of the topic. In addition, we have tried to point out serious gaps in our knowledge and to suggest possibly profitable lines of future research. Selected references, mainly recent review articles rather than original papers, are given at the end of each chapter.

2 | Cellular Foundations of Neuropharmacology

NERVE CELLS have two special properties which make them distinctive from all other cells of the body. The first is their ability to conduct bioelectric impulses over long distances without any loss of signal strength. The second, directly related to the first, is their specific input and output connections, both with other nerve cells and with innervated tissues such as muscles and glands. These connections dictate what types of information each nerve cell is to receive and what types of response it can give.

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. One of the first observations made with empirical silver impregnation stains such as the Golgi or Cajal stain was that nerve cells are heterogeneous with respect to both size and shape. (One of the confusing features of the nervous system is that each specific region of the brain and each part of each nerve cell not only has its own particular name but often has several synonymous names. So, for example, we find that the nerve cell body is also called the soma and the perikaryon—literally, the part that surrounds the nucleus).

One of the many ways of classifying nerve cells is in terms of 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: 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 certain 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 which 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, on which we will later dwell in depth.

When we examine the nerve cell with the electron microscope (Fig. 2-1) and compare it with other cell types whose biology has been well characterized, we find that in general, most nerve cells have very large nuclei in which can be seen one or more nucleoli, believed to be the sites for DNA to RNA transcription. In the cytoplasm of the perikaryon we find both free ribosomes (ribonucleoprotein sites for protein synthesis) as well as multiple cisternae of rough endoplasmic reticulum in which secretory proteins are believed to be manufactured. In addition to the free ribosomes and rough endoplasmic reticulum, we also find a specialized type of smooth endoplasmic reticulum known as

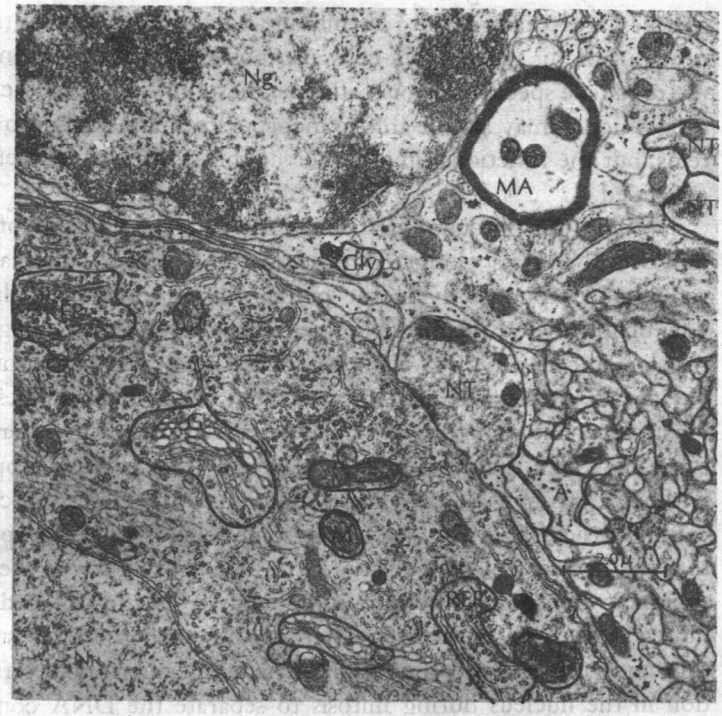

FIGURE 2-1. Low-power electron micrograph of rat cerebellar cortex. At the lower left, a large neuron can be seen. Its nucleus (Nn), two sets of Golgi apparatus (G), and areas of rough endoplasmic reticulum (RER) can be seen as well as numerous mitochondria (M) and free ribosomes (*). At upper left is the nucleus of an oligodendroglia (Ng); its cytoplasm is scant but numerous free ribosomes can be seen. One prominent nerve terminal (NT) is making specialized contact with the nerve cell. Within the nerve terminals numerous synaptic vesicles and mitochondria can be seen. One myelinated axon is shown in cross section (MA). Also visible in the neuropil are the processes of astrocytes (A) filled with glycogen granules (Gly). Two other nerve terminals (NT) are shown making specialized contacts at upper right with a dendritic spine.

the Golgi zone. In the classical secretory cycle of the pancreatic acinar cell, the Golgi zone is the site at which the secretory organelles are packaged into membrane-bound particles for transport out of the cell. Nerve cells also possess many mitochondria, the organelle specialized for oxidative phosphorylation. Mitochondria of nerves may also be able to incorporate amino acids into proteins, but the functional importance of this synthesis is as yet unexplained.

While the mitochondria occur throughout all parts of the nerve cell and its elongated processes, the rough endoplasmic reticulum is found only in the perikaryon and in the dendrites. The dendrites and the axons both exhibit microtubules, elongated tubular objects 240 Å in diameter whose long axis runs throughout the extent of the axon or dendrite. These microtubules are identical in fine structure to similar organelles found in other cell types in which their function is believed to be cellular support. Although this may be their main function in the elongated cytoplasmic processes of the nerve cell, some scientists have proposed that the microtubules may serve as directional guides for the cytoplasmic transport of cellular ingredients from the cell body to more distal parts of the cell. Microtubules with the same structure and amino acid composition as neuronal microtubules function in the nucleus during mitosis to separate the DNA components of the chromosomes. This process can be blocked by the drug colchicine, which breaks up the subunits of the microtubules. Radioactive colchicine can be shown to bind specifically to the microtubules of the nerve cell and to block transport of many neuronal constituents down the axon.

In addition to mitochondria and microtubules, axons also exhibit structures called neurofilaments. Biochemically, the neurofilaments appear to be composed of the same sub-units as the microtubules although they are organized into a much smaller (100 Å) fibrous structure. Generally, axons which have large numbers of microtubules have very few filaments, and vice versa. Small (i.e. narrow) axons tend to have microtubules but few neurofilaments. Large axons may have many neurofilaments but few mi-

crotubules. In some cases the neurofilaments may extend into the nerve ending, where they tend to form a ring-like configuration around the external perimeter of the nerve-ending axoplasm. Except for the fact that the neurofilaments can be stained by silver and probably account for the so-called neurofibrils of classical light microscopy, no specific functions for neurofilaments have yet been uncovered.

The Synapse

The last specialized structural features of the neuron we shall discuss are the contents of the nerve ending and the characteristics of the specialized contact which has been identified as the site of functional interneuronal communication. As the axon approaches the site of its termination it exhibits structural features not found more proximally (Fig. 2-2). Most striking is the occurrence of large numbers of microvesicles, which have been dubbed synaptic vesicles. These structures tend to be spherical in shape, with diameters varying between 200 and 1200 Å. Depending upon the type of fixation used, the vesicles may exhibit one or more types of internal electron-opaque granularities; this cytochemical characteristic is related to certain endogenous small molecules considered to be potential synaptic transmitting substances (see Chapters 4 and 5). The nerve endings also exhibit mitochondria, but never exhibit microtubules unless the nerve ending belongs to the class of axons possessing several accumulations of synaptic vesicles along their terminal passage. Each of these endings forms a specialized contact with one or more dendritic branches before the ultimate termination. Such endings are known as *en passant* terminals. In this sense, the term "nerve terminal" or "nerve ending" connotes more a functional transmitting site than a structural blind alley.

Electron micrographs of synaptic regions in the central nervous system reveal a specialized contact zone between the axonal nerve ending and the postsynaptic structure. This specialized contact zone is composed of presumed proteinaceous material lin-

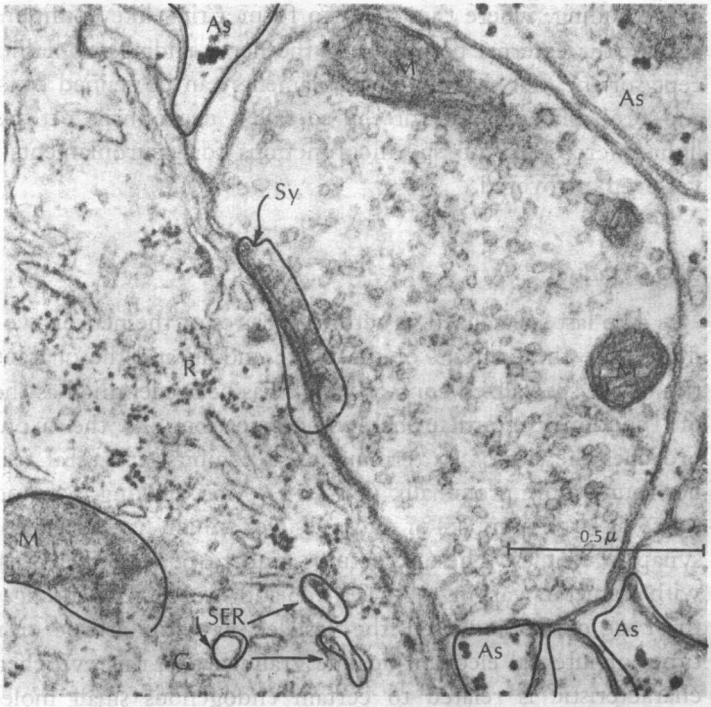

FIGURE 2-2. High-power view of the same nerve ending seen in Fig. 1. At this magnification the synaptic vesicles can be seen more clearly, as can the zone of specialized contact (Sy). Astrocyte processes containing glycogen (As) can be seen as well as smooth endoplasmic reticulum (SER) and free ribosomes (R) within the cytoplasm of the nerve cell. Note that only about half of the contact between the nerve terminal and the nerve cell membrane exhibits the specialized zone of contact.

ing the intracellular portions of the pre- and postsynaptic membranes and filling the synaptic cleft between the apposed cell surfaces. Such types of specialized contacts are a general form of the specialized cell contacts seen between many types of cell derived from the embryonic ectoderm, of which the nerve cell is but

one. However, the specialized contact between neurons is polarized: that is, the presynaptic terminal intracellular material is composed of interrupted presynaptic dense projections measuring about 500-700 Å in diameter and separated from each other by distances of 300-400 Å. This material may be present only to bind specific presynaptic nerve endings permanently to specific postsynaptic cell sites. Alternatively, the specialized contact zone could assist in the efficiency of transmission and could constitute one potential method for modulating synaptic transmission in terms of discharge frequency. This is a subject upon which much future research can be expected.

Functional Interpretations from Cytology

If we were to try to infer the functions of a neuron from the foregoing catalogue of its cellular machinery, we would be entitled to the following assumptions. Despite wide variations in cell shape, size, and volume, most nerve cells possess large amounts of unattached ribosomes, presumably for the synthesis of intracellular materials such as enzymes and structural macromolecules. However, the cell body is also filled with varying quantities of rough endoplasmic reticulum and smooth endoplasmic reticulum indicative of cells that package synthetic material for transcellular secretion. Thus, we anticipate the neuron to be a dynamic secretory cell with broad synthetic capabilities.

While organelles with such synthesizing capabilities characterize both the dendrite and cell body cytoplasm, they are never found within the axon. Since the axon possesses no apparent synthetic capacity of its own, other mechanisms have had to be developed to explain the way in which the axon can maintain its vitality. One such proposal is that the cell body synthesizes all the required macromolecules for the axon and that a process of cytoplasmic flow carries them from the cell body down the axon. This theory of axoplasmic flow has been substantiated by studies involving actual physical barriers to axonal flow and by labeling

proteins in the cell body to time their rates of migration toward the periphery. Such studies have indicated that the flow rates may vary from as little as one-tenth of a millimeter per day to as much as many centimeters in an hour, depending upon the organelle or metabolite used as a tracer.

Glia

A second element in the maintenance of the axon's integrity depends on a type of cell known as neuroglia, which we have not yet discussed. There are two main types of neuroglia. The first is called the fibrous astrocyte, a descriptive term based on its star-like shape in the light microscope and on the fibrous nature of its cytoplasmic organelles, which can be seen in both light and electron microscopy. The astrocyte is found mainly in regions of axons and dendrites and tends to surround or contact the adventitial surface of blood vessels. Functions such as insulation (between conducting surfaces) and organization (to surround and separate functional units of nerve endings and dendrites) have been empirically attributed to the astrocyte, mainly on the basis of its structural characteristics.

The second type of neuroglia is known as the oligodendrocyte. It is called the satellite cell when it occurs close to nerve cell bodies, and the Schwann cell when it occurs in the peripheral nervous system. The cytoplasm of the oligodendrocyte is characterized by rough endoplasmic reticulum but its most prominent characteristic is the enclosure of concentric layers of its own surface membrane around the axon. These concentric layers come together so closely that the oligodendrocyte cytoplasm is completely squeezed out and the original internal surfaces of the membrane become fused, presenting the ring-like appearance of the myelin sheath in cross section (Fig. 2-1). Along the course of an axon, which may be many centimeters in length, many oligodendrocytes are required to constitute its myelin sheath. At the boundary between adjacent portions of the axon covered by sep-

arate oligodendrocytes, there is an uncovered axonal portion known as the node of Ranvier.

Many central axons and certain elements of the peripheral autonomic nervous system do not possess myelin sheaths. Even these axons, however, are not bare and exposed to the extracellular fluid, but rather they are enclosed within single invaginations of the oligodendrocyte surface membrane. Because of this close relationship between the conducting portions of the nerve cell, its axon, and the oligodendrocyte, it is easy to see the origin of the proposition that the oligodendrocyte may contribute to the nurture of the nerve cell. While this idea may be correct, no evidence is yet available. Clearly, the glia are incapable of supporting the axon when it has been severed from the cell body, for example, by trauma or by surgically induced lesioning. This incapacity is fortuitous for neuroscientists, since one of the chief methods of defining nerve circuits within the brain has been the staining of degenerating axons following brain lesions.

Brain Permeability Barriers

While the unique cytological characteristics of neurons and glia are sufficient to establish the complex intercellular relationships of the brain, there is yet another histophysiologic concept to consider. Numerous chemical substances pass from the bloodstream into the brain at rates which are far slower than for entry into all other organs in the body. There are similar slow rates of transport between the cerebrospinal fluid and the brain, although there is no good standard in other organs against which to compare this latter movement.

These permeability barriers appear to be the end result of numerous contributing factors which present diffusional obstacles to chemicals on the basis of molecular size, charge, solubility, and specific carrier systems. The difficulty has not been in establishing the existence of these barriers, but rather in determining their mechanisms. When the relatively small protein (MW = 43,000) horseradish peroxidase is injected intravenously into mice, its lo-