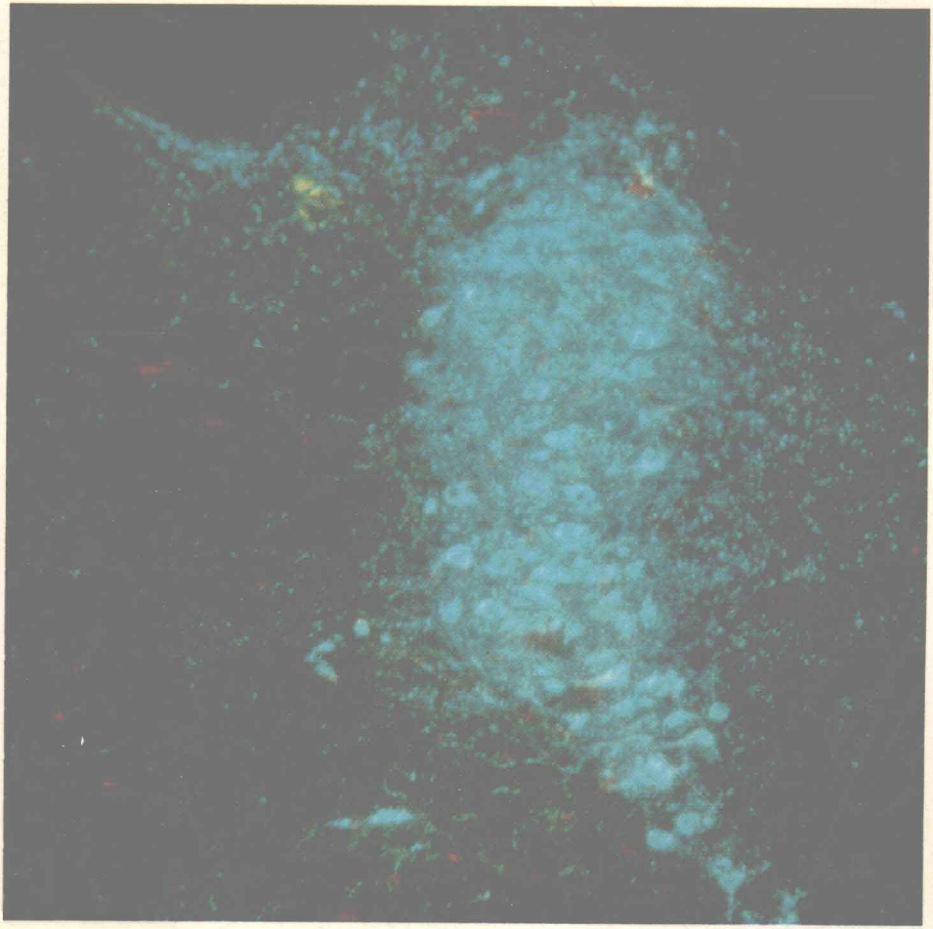
The Biochemical Basis of Neuropharmacology



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Fourth Edition

The Biochemical Basis of Neuropharmacology

FOURTH EDITION

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New York Oxford
OXFORD UNIVERSITY PRESS
1982

Library of Congress Cataloging in Publication Data

Cooper, Jack R., 1924–
The biochemical basis of neuropharmacology.

Bibliography: p.
Includes index.
1. Neurochemistry. 2. Neuropharmacology.
I. Bloom, Floyd E., 1936– . II. Roth,
Robert H., 1939– . III. Title. [DNLM:
1. Autonomic agents. 2. Central nervous system—
Drug effects. 3. Nerve tissue—Analysis. 4. Nerve
tissue—Physiology. 5. Psychopharmacology. QV
77 C777b]
QP356.3.C66 1982 615'.78 82-3475
ISBN 0-19-502093-1 AACR2
ISBN 0-19-503094-X (pbk.)

Cover: Fluorescence photomicrograph of rat locus ceruleus. Norepinephrine-containing neurons and their processes fluoresce a bright green-blue owing to condensation with glyoxylic acid. Blood vessels perfused with pontamine sky-blue fluoresce red. The high degree of vascularity surrounding the noradrenergic neurons can be seen. (Unpublished micrograph from Dr. Leonard Koda, Salk Institute) × 200

Printing (last digit): 9 8 7 6 5 4 3 2 1

Printed in the United States of America

The Biochemical Basis of Neuropharmacology

This book is dedicated to the memory
of Nicholas J. Giarman, colleague and dear friend

Preface to the Fourth Edition

With this fourth edition it is time to redress in the preface a long-standing omission: to thank Jeffrey House of Oxford University Press for his continued support and encouragement. Even though he pressures us to meet publication deadlines, and even though he deletes from our manuscript what we think are wonderfully clever witticisms and what he thinks are asides that are a bit harsh, we are very fond of him. His integrity, efficiency, and kindness make it a pleasure to work with him.

In this edition the chapter on memory and learning has been eliminated for a number of reasons. It did not relate well to the rest of the book, advances in this area have been minimal in the last five years, and we have included behavioral changes related to transmitters or drugs where appropriate in the other chapters. Another major change in this edition, prompted by the explosion of research on neuroactive peptides, is the addition of a separate chapter on opioid peptides. Finally, the remaining chapters have been updated in the light of recent findings.

September 1981

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Contents

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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 7.

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.

Whether a neuron is depolarized or hyperpolarized will depend largely on its excitatory and inhibitory synaptic inputs, and these inputs must obviously involve neurotransmitters or neurohormones. 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.

By 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. As will be evident in later chapters, drugs can act postsynaptically by either blocking or regulating receptor activity and presynaptically by altering the synthesis, storage, release, re-uptake, or metabolism of the transmitter.

The gap between our descriptive knowledge of neurotropic agents and our knowledge of molecular mechanisms of action of these drugs, though narrowing, is still large, and it is pertinent to examine the reasons for the discrepancy. First and foremost, we were unable, until quite recently, to locate, isolate, and characterize receptors for these various drugs. For example, although we can state that a 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 barbiturate 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 one reason for this deficiency is that biophysical research techniques and approaches of the requisite sophistication have emerged only recently.

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.

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, immunocyto-

chemistry, 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 measurement of receptors is also a key problem (see Chapter 5).

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, namely, the one that a team has just learned how to measure. Current trends in the neural sciences include 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 isolating 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