Receptors and Recognition

Series B Volume 10

Neurotransmitter Receptors

Part 2
Biogenic Amines

Edited by
H. I. Yamamura
and
S. J. Enna

Receptors and Recognition

Series B Volume 10

Neurotransmitter Receptors

Part 2
Biogenic Amines

Edited by
H. I. Yamamura

Professor of Pharmacology, University of Arizona Health Sciences Center, Tucson, Arizona

and

S. J. Enna

Professor of Pharmacology and Neurobiology, University of Texas Medical School at Houston

LONDON AND NEW YORK

CHAPMAN AND HALL

First published 1981
by Chapman and Hall Ltd
11 New Fetter Lane, London EC4P 4EE

Published in the U.S.A. by
Chapman and Hall
in association with Methuen, Inc.,
733 Third Avenue, New York 10017

Printed in Great Britain at the
University Press, Cambridge

ISBN 0 412 23130 1

British Library Cataloguing in Publication Data

Receptors and recognition.
Series B. Vol.10: Neurotransmitter receptors.
Part 2: Biogenic amines
1. Cell interaction
I. Cuatrecasas, Pedro II. Greaves, Melvyn Francis
III. Enna, S.J. IV. Yamamura, H.I.
V. Neurotransmitter receptors. Part 2:
Biogenic amines
574.8'76 QH604.2 80-40942

ISBN 0-412-23130-1

Receptors and Recognition

General Editors: P. Cuatrecasas and M. F. Greaves

About the series

Cellular recognition – the process by which cells interact with, and respond to, molecular signals in their environment – plays a crucial role in virtually all important biological functions. These encompass fertilization, infectious interactions, embryonic development, the activity of the nervous system, the regulation of growth and metabolism by hormones and the immune response to foreign antigens. Although our knowledge of these systems has grown rapidly in recent years, it is clear that a full understanding of cellular recognition phenomena will require an integrated and multidisciplinary approach.

This series aims to expedite such an understanding by bringing together accounts by leading researchers of all biochemical, cellular and evolutionary aspects of recognition systems. This series will contain volumes of two types. First, there will be volumes containing about five reviews from different areas of the general subject written at a level suitable for all biologically oriented scientists (Receptors and Recognition, series A). Secondly, there will be more specialized volumes (Receptors and Recognition, series B), each of which will be devoted to just one particularly important area.

Advisory Editorial Board

Receptors and Recognition

Series A

Published

Volume 1 (1976)

M.F. Greaves (London), Cell Surface Receptors: A Biological Perspective

F. Macfarlane Burnet (Melbourne), The Evolution of Receptors and Recognition in the Immune System

K. Resch (Heidelberg), Membrane-Associated Events in Lymphocyte Activation

K.N. Brown (London), Specificity in Host-Parasite Interaction

Volume 2 (1976)

D. Givol (Jerusalem), A Structural Basis for Molecular Recognition: The Antibody Case

B.D. Gomperts (London), Calcium and Cell Activation

M.A.B. de Sousa (New York), Cell Traffic

D. Lewis (London), Incompatibility in Flowering Plants

A. Levitski (Jerusalem), Catecholamine Receptors

Volume 3 (1977)

J. Lindstrom (Salk, California), Antibodies to Receptors for Acetylcholine and other Hormones

M. Crandall (Kentucky), Mating-Type Interactions in Micro-organisms

H. Furthmayr (New Haven), Erythrocyte Proteins

M. Silverman (Toronto), Specificity of Membrane Transport

Volume 4 (1977)

M. Sonenberg and A.S. Schneider (New York), Hormone Action at the Plasma Membrane: Biophysical Approaches

H. Metzger (NIH Bethesda), The Cellular Receptor for IgE

T. P. Stossel (Boston), Endocytosis

A. Meager (Warwick) and R.C. Hughes (London), Virus Receptors

M.E. Eldefrawi and A.T. Eldefrawi (Baltimore), Acetylcholine Receptors

Volume 5 (1978)

P.A. Lehmann (Mexico), Stereoselective Molecular Recognition in Biology

A.G. Lee (Southampton, U.K.), Fluorescence and NMR Studies of Membranes

L.D. Kohn (NIH, Bethesda), Relationships in the Structure and Function of Receptors for Glycoprotein Hormones, Bacterial Toxins and Interferon

Volume 6 (1978)

J.N. Fain (Providence, Rhode Island), Cyclic Nucleotides

G.D. Eytan (Haifa) and B.I. Kanner (Jerusalem), Reconstitution of Biological Membranes

P.J. O'Brien (NIH, Bethesda), Rhodopsin: A Light-sensitive Membrane Glycoprotein

Index to Series A, Volumes 1–6

Series B

Published

The Specificity and Action of Animal, Bacterial and Plant Toxins (B1)
edited by P. Cuatrecasas (Burroughs Wellcome, North Carolina)

Intercellular Junctions and Synapses (B2)
edited by J. Feldman (London), N.B. Gilula (Rockefeller University, New York) and J.D. Pitts (University of Glasgow)

Microbial Interactions (B3)
edited by J.L. Reissig (Long Island University, New York)

Specificity of Embryological Interactions (B4)
edited by D.R. Garrod (University of Southampton)

Taxis and Behavior (B5)
edited by G.L. Hazelbauer (University of Uppsala)

Bacterial Adherence (B6)
edited by E.H. Beachey (Veteran's Administration and Hospital and University of Tennessee, Memphis, Tennessee)

Virus Receptors Part 1 Bacterial Viruses (B7)
edited by L.L. Randall and L. Philipson (University of Uppsala)

Virus Receptors Part 2 Animal Viruses (B8)
edited by K. Lonberg-Holm (Du Pont, Delaware) and L. Philipson (University of Uppsala)

Neurotransmitter Receptors Part 1 Amino Acids, Peptides and Benzodiazepines (B9)
edited by S.J. Enna (University of Texas at Houston) and H.I. Yamamura (University of Arizona)

In Preparation

Membrane Receptors: Methods for Purification and Characterization (B11)
edited by S. Jacobs and P. Cuatrecasas (Burroughs Wellcome, North Carolina)

Purinergic Receptors (B12)
edited by G. Burnstock (University College, London)

Receptor Regulation (B13)
edited by R.J. Lefkowitz (Duke University, North Carolina)

Transplantation Antigens (B14)
edited by P. Parham and J. Strominger (Harvard University)

Contributors

Ian Creese, Department of Neurosciences, University of California, San Diego School of Medicine, La Jolla, California, U.S.A.

Henry J. Haigler, Department of Pharmacology, Emory University School of Medicine, Atlanta, Georgia, U.S.A.

Kenneth P. Minneman, Department of Pharmacology, Emory University School of Medicine, Atlanta, Georgia, U.S.A.

Elliott Richelson, Departments of Psychiatry and Pharmacology, Mayo Foundation, Rochester, Minnesota, U.S.A.

John E. Taylor, Departments of Psychiatry and Pharmacology, Mayo Foundation, Rochester, Minnesota, U.S.A.

Gregory J. Wastek, Departments of Pharmacology and Psychiatry, University of Arizona, Health Services Center, Tucson, Arizona, U.S.A.

Henry I. Yamamura, Departments of Pharmacology and Psychiatry, University of Arizona, Health Services Center, Tucson, Arizona, U.S.A.

Contents of *Neurotransmitter Receptors Part 1 Amino Acids, Peptides and Benzodiazepines*

Preface

Since the discovery that neuronal transmission can be chemically mediated, a large number of compounds have been found in the mammalian central nervous system which appear to function as neurotransmitter agents. Recently, electrophysiological and biochemical methods have been developed which have enabled neuroscientists to classify better the myriad of neurotransmitter receptor sites in brain and to study their properties in finer detail. As a result of these investigations, a significant number of new discoveries have been made about the mechanisms involved in neurotransmitter receptor interactions, the role neurotransmitters play in the actions of pharmacological agents and in the pathogenesis of various neuropsychiatric disorders.

The present two volume text was compiled to summarize the information relating to the physiological, biochemical, pharmacological and functional characteristics of neurotransmitter receptor sites. While emphasis is placed on neurotransmitter receptors in the mammalian central nervous system, the characteristics of these receptors in other species, both vertebrate and invertebrate, are also discussed where appropriate. While these books cover the major classes of putative neurotransmitters – amino acids, peptides and biogenic amines – and are therefore broad in scope, each is discussed in a concise fashion to highlight the major points of historical and contemporary interest. In addition to outlining data, each chapter addresses current theories relating to the various aspects of receptor properties and function in an attempt to reveal the directions of future research and as a stimulus for other workers in the field. This work can serve not only as an introductory text for young neuroscientists, but should also be a valuable resource for more senior investigators as both a reference and research guide.

May, 1980

H.I. Yamamura
S.J. Enna

Contents

1 Serotonergic Receptors in the Central Nervous System

HENRY J. HAIGLER

Acknowledgements

Supported in part by National Institute on Drug Abuse grant no. 1-R01-DA01344-04.

Neurotransmitter Receptors Part 2
(*Receptors and Recognition*, Series B, Volume 10)
Edited by H.I. Yamamura and S.J. Enna
Published in 1981 by Chapman and Hall, 11 New Fetter Lane, London EC4P 4EE

1.1 INTRODUCTION

There are numerous definitions of the term receptor. One definition is that a receptor is a specific component of a cell to which a neurotransmitter or hormone binds. The combination of the neurotransmitter or hormone with the receptor produces a chain of events that lead to the expression of a physiological event (Bockaert, 1978; Moran, 1975).

The concept of receptors is based on the following criteria (Moran, 1975). First there should be high degree of specificity in that minor differences of molecular configuration will alter the activity of the drug. Secondly, the interaction of drug with the receptor should produce a biological response. Thirdly, there should be a quantitative relationship between the concentration of drug and biological response that can be expressed using concentration–response curves. Fourthly, there should be complete reversibility of the effect of the drug indicating that the drug combines reversibly with the receptor. Fifthly, there should be selective antagonists that will block the biological response presumably by competing with the drug and blocking the reaction of the drug with the receptor.

These criteria imply that a molecular interaction occurs between the drug and the membrane of the effector cell (Moran, 1975). The effector cells throughout this chapter will be either the serotonin (5-hydroxytryptamine; 5-HT)-containing neurons or those neurons that receive an input from 5-HT neurons.

1.1.1 Historical review

From the above, it is obvious that the definition of a receptor is intimately associated with the neurotransmitter that acts on that receptor. The study of receptors in the brain is complicated because these receptors are not as readily accessible as the receptors are in the periphery. In the periphery a change in a physiological parameter such as heart rate is used to measure the effects of a drug on a receptor. Similarly, in the brain a change in the rate of neuronal firing is used as an indicator that a drug is acting on a receptor. The technique of microiontophoresis has made it possible to study the pharmacology of receptors *in vivo* in the brain because compounds can be administered into the immediate vicinity of a neuron while changes in its activity are monitored (Curtis, 1964). When a drug or putative neurotransmitter alters spontaneous neuronal activity without affecting the amplitude of the action potential, the compound is assumed to be acting on receptors on the neuronal membrane.

A receptor is characterized by the neurotransmitter that acts on it and the physiological effect produced by the combination of the neurotransmitter with the receptor. When 5-HT is administered microiontophoretically, it produces either inhibition or an acceleration of neuronal firing in almost every area of the brain studied, indicating that 5-HT receptors are widespread (Bloom *et al.*, 1972). However, the primary emphasis in this chapter will concern the 5-HT receptors that are associated with the synaptic action of 5-HT. Presumably to have a synaptic action, endogenous 5-HT is released from presynaptic terminals and diffuses across the synapse to act on receptors located on the membrane of the post-synaptic cell. Thus, to study the action of 5-HT on receptors associated with the synaptic action of 5-HT, it is necessary to establish that 5-HT is a neurotransmitter in the CNS.

The various criteria that are used in the identification of neurotransmitters in the autonomic and peripheral nervous systems are stressed by numerous authors (see Hebb, 1970). The criteria that a substance should meet to be considered a neurotransmitter are as follows: (1) the putative transmitter, an inactivating enzyme or re-uptake system, and enzymes associated with the synthesis of the neurotransmitter should all be present; (2) microiontophoretic administration of the transmitter should mimic the action of the endogenous neurotransmitter; and (3) drugs that block the putative neurotransmitter should also block the actions of the endogenously released neurotransmitter (Hebb, 1970; Werman, 1966).

Fluorescence histochemistry has been used to demonstrate that brain 5-HT is primarily a constituent of nerve cells. Prior to histochemical work Heller *et al.* (1962) and Heller and Moore (1965) showed that lesioning the medial forebrain bundle (MFB) produced a depletion of 5-HT in the forebrain. The rate of 5-HT depletion corresponds to the rate of neuronal degeneration implying that 5-HT neuronal pathways are present in the brain. The direct demonstration of the existence and localization of 5-HT neurons was accomplished by means of the formaldehyde-condensation histochemical method of Falck *et al.* (1962) applied to freeze-dried brain tissue (Carlsson *et al.*, 1962). With this method, the location of 5-HT-containing neuronal perikarya (Dahlström and Fuxe, 1965), terminals (Aghajanian *et al.*, 1973; Fuxe, 1965), and pathways (Anden *et al.*, 1968) were mapped. The perikarya of these neurons are almost exclusively located in the brain stem raphe nuclei; fibres from the raphe nuclei project to other portions of the brain stem, to the spinal cord, and to the forebrain. Of special interest is the fact that almost the entire 5-HT input to the forebrain derives from the midbrain raphe nuclei, and the 5-HT input to the spinal cord is primarily derived from the medullary pontine raphe nuclei (MPR; raphe magnus, obscurus, and pallidus).

On the basis of the histochemical mapping of 5-HT (serotonergic)

pathways in the brain, it was possible to test 5-HT against the above criteria important for establishing that a substance is a neurotransmitter. By selectively placing lesions in the raphe nuclei, it has been possible to ascertain whether the biochemical machinery for the synthesis, storage, and metabolism of 5-HT is contained within the raphe projections. Destruction of the midbrain raphe nuclei has the following anatomical and biochemical consequences: (1) a degeneration of histochemically identified 5-HT terminals in the forebrain (Aghajanian *et al.*, 1969; Kuhar *et al.*, 1972a); (2) a selective reduction in forebrain 5-HT content (Heller and Moore, 1965; Jouvet, 1967; Kostowski *et al.*, 1968; Rosecrans and Sheard, 1969); (3) a selective decrease in forebrain tryptophan hydroxylase activity (Kuhar *et al.*, 1971); and (4) a loss of high-affinity 5-HT uptake in synaptosomes from the forebrain (Kuhar *et al.*, 1972b). All of these effects are selective, because other putative transmitters and their associated enzymes are not significantly altered by raphe destruction. Thus raphe projections are distinguished by their content of 5-HT and by the fact that they possess the means for 5-HT synthesis, storage, and high-affinity uptake; the last process could well serve as a means of terminating the synaptic actions of 5-HT in its presumed role as a neurotransmitter.

Knowledge about the location of 5-HT neurons and pathways in brain has served to provide evidence for establishing another neurotransmitter criterion, that of release upon nerve stimulation. Electrical stimulation of the nucleus linearis rostralis in the cat, which is in the vicinity of ascending serotonergic fibers, causes a release of 5-HT as measured in perfusates from the anterior horn of the lateral ventricle (Holman and Vogt, 1972). The release of 5-HT resulting from this stimulation is selective in that there is no concomitant release of acetylcholine (Ashkenazi *et al.*, 1972). These data form an extension of previous experiments showing that selective stimulation of the midbrain raphe nuclei produces an increase in the synthesis and turnover of 5-HT in the forebrain, a region that receives projections from the raphe (Aghajanian *et al.*, 1967; Kostowski *et al.*, 1969; Sheard and Aghajanian, 1968; Shields and Eccleston, 1972). Electrical stimulation of the dorsal raphe nucleus (DR) of the midbrain produces an inhibition of neuronal firing in the amygdala, an effect mimicked by the microiontophoretic administration of 5-HT (Wang and Aghajanian, 1977).

This latter point partially meets Werman's (1966) criterion of identical actions although he advocates intracellular recording to determine if the substance has the same effect on ionic conductance channels as the natural transmitter. However, intracellular recording is difficult to perform in the CNS and extensive experiments comparing the effects of stimulation of the raphe nucleus and the effects of microiontophoretic administration of 5-HT have not yet been carried out.

The above biochemical, histochemical and neurophysiological studies

have set the stage for studying 5-HT neurotransmission from a physiological standpoint. If 5-HT is in fact a neurotransmitter, it should be possible to show that neurons that receive a serotonergic input respond to 5-HT in a fashion that mimics stimulation of the natural pathway (i.e. the raphe system). Furthermore, drugs that might block or mimic the action of 5-HT on postsynaptic receptors (i.e. receptors on cells that receive a serotonergic input) should block or mimic in an identical fashion the response to raphe stimulation and applied 5-HT. All of these approaches simply represent a continuation of the basic theme of attempting to match 5-HT against the classical criteria that should be met by any putative transmitter substance. However, it is obviously much more technically difficult to carry out such an experimental program in the brain than in the periphery, where specific neuroeffector junctional tissue can be isolated and directly visualized. Thus when one is applying 5-HT to a neuron deep in the brain and monitoring its physiological response, how can it be known that the neuron in question does in fact receive a serotonergic input? A further complication is the possibility that there are neurons which may respond to 5-HT but which do not naturally receive a serotonergic input. A high proportion of neurons in many parts of the brain are responsive to the local, microiontophoretic application of 5-HT (see below). Does this mean that all of these cells have 5-HT receptors? If 'receptor' is defined in the broad sense as meaning the presence of 'receptivity' or 'responsivity', then 5-HT receptors are to be found anywhere a response to 5-HT can be made to occur. The narrower definition of '5-HT receptor' would count receptors only at those sites that normally receive a 5-HT input. The latter definition is closely linked with the concept of 5-HT as a neurotransmitter substance. According to this view, one would focus attention on the properties of 5-HT 'receptors' that occur in association with sites of established serotonergic transmission.

1.1.2 Techniques

The characteristics of 5-HT receptors in the CNS vary depending on the technique used to study the receptors. Data from experiments using different techniques will be presented so that the reader can compare the similarities and differences in the characteristics of the 5-HT receptors. Some attempt will be made to resolve the differences in the data derived from *in vitro* and *in vivo* techniques. Data from studies using two neurochemical *in vitro* techniques; two neurophysiological microiontophoretic techniques and techniques involving the behavioral response of an animal to nociceptive stimuli, will be reviewed.

One *in vitro* approach used to study receptors is the technique developed by Pert and Snyder (1973) in which a radioactive form of a drug is incu-

bated with brain homogenates and then rapidly filtered. The radioactive drug is trapped on the filter because it is bound to the receptor. Thus, the amount of radioactivity trapped in the filter is a function of the amount of drug attached to the receptor. When the binding of one drug (e.g. LSD) is measured alone and then in the presence of varying concentrations of another drug (e.g 5-HT) the decrease in binding of the first drug is an indication of the relative binding affinities for both drugs. If at low concentrations, drug A can reduce the binding of drug B, an assumption is that both drugs bind to the same site (receptor) on the membrane.

A second *in vitro* approach uses the activation of a 5-HT-sensitive adenylate cyclase as a measure of the presence of a 5-HT receptor (Bockaert, 1978). The adenylate cyclase has a non-uniform distribution in the CNS indicating that it may be associated with 5-HT synapses. If cAMP is a second messenger mediating the 'machine language' of neurons (Bloom, 1973) then alterations in adenylate cyclase activity may mediate the synaptic actions of 5-HT by altering intracellular levels of cAMP.

One neurophysiological technique that has been used to study receptors in the CNS is the technique of microiontophoresis. This technique involves the recording of neuronal activity from one barrel of a multibarrel micropipette during the ejection of drugs from one of the adjacent barrels. In an operational sense the receptor is the most proximal step or steps of a sequential reaction; where detailed steps are not known, the receptor is then defined as the entire sequence (Moran, 1975). Thus if a drug is ejected microiontophoretically in the brain and there is an alteration in neuronal firing rate, the assumption is that the drug produces this effect by acting on a particular receptor. Most of the current data available are based on extracellular recordings in the brain. Alterations in spontaneous neuronal firing or blockade of an increase in firing that is evoked by a stimulus is accepted as evidence that the drug is acting on a receptor. However, it is possible that drugs produce an inhibition of firing without acting on specific receptors. Instead, at high concentrations they may dissolve in the membrane causing it to swell and block the Na^+ channels and thus produce a local anesthetic effect. Local anesthetic effects are assumed to be present when there is a decrease in action potential size during the microiontophoretic administration of a drug (Fig. 1.1). When a drug is present in the vicinity of the cell in high enough concentration to dissolve in the membrane and produce a local anesthetic effect, this concentration is higher than that necessary for the drug to act on specific receptors. Thus when there is a decrease in action potential size as a consequence of the iontophoretic administration of a drug, the assumption is that specific receptors for that drug are not present on the cell.

A second neurophysiological technique that has been used to study 5-HT receptors is to stimulate an area known to contain 5-HT cell bodies while