C. MELCHIORRE and M. GIANNELLA
Editors

HIGHLIGHTS IN RECEPTOR CHEMISTRY

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Proceedings of the 2nd Camerino Symposium on Recent Advances in Receptor Chemistry held in Camerino, Italy, 5-8 September, 1983

Editors

C. Melchiorre
and
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PREFACE

This volume is a collection of papers contributed by participants in the 2nd Camerino Symposium on Recent Advances in Receptor Chemistry held in Camerino (MC), Italy, September 5 - 8, 1983.

Receptor research today constitutes a wide area, with exponentially growing interest from a variety of different disciplines, including chemistry, biochemistry, pharmacology, biophysics and physiology. The multidisciplinary approach is certainly contributing to a rapid understanding of receptor structure and function. However, too often the two major groups of scientists active in this field, that is chemists and pharmacologists, are unable to communicate to each other due to the inherited barrier of the scientific language. The 2nd Camerino Symposium was designed to provide a favourable situation for the meeting of chemists and biologists in the receptor field by deliberately putting the emphasis on both chemical and pharmacological aspects of receptor research. This book includes the plenary lectures of the meeting presenting up-to-date surveys of the most promising areas in the field including receptor theory, receptor for acetylcholine, dopamine, histamine, aminoacids, the α and β-adrenergic receptors, opiates, the coupling of receptors to adenylcyclase, biochemical aspects of ion channels, quantitative structure-activity relationships and computational procedures for rationalizing drug-receptor interactions. It will be of interest to pharmacologists, biochemists, molecular biologists and medicinal chemists.

Camerino, Italy
September, 1983

Carlo Melchiorre
Mario Giannella

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PHARMACOLOGICAL RECEPTORS: A CENTURY OF PROGRESS

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Elucidation of the mechanisms underlying the function of pharmacological receptors is inevitably a problem of the nature and transmission of information in cellular systems. At the most basic level biological information is encoded within the genetic machinery, the self-replicating custodian of all cellular potential. A particularly fundamental exchange of biological information is thus demonstrated during sexual encounters. However, optimal response of a cell or organism demands that it both be sensitive to and responsive to a variety of signals of intracellular and extracellular origin. Specific cellular mechanisms must therefore exist to ensure such environmental sensitivity. This signal responding ability is a function of receptors designed to respond specifically to the coding sequences of a wide variety of chemical and physical stimuli. The world of the receptor, the ways in which receptors form dynamic components of the cell, the ways in which receptors are complementary to cell signals and the ways in which the signal-activated receptor is able to initiate biological response, is therefore a fundamental topic of discussion at this Symposium.

Much of our current level of understanding of cellular receptors and their function has accrued within the past one hundred years. It seems appropriate therefore to review some of the developments of this period and to outline some of the contributions that have brought us to the contemporary level of understanding of receptor phenomena expressed at this Symposium. Of necessity this cannot be a complete review and the contributions of many distinguished authorities are not cited specifically.

The study of cellular receptors appears to have had several origins expressed, like many other developments in science, almost simultaneously. The relationship between chemical structure and biological activity was early noted by Crum-Brown and Fraser (1) who concluded that:

> "...there can be no reasonable doubt but that a relation exists between the physiologic action of a substance and its chemical composition and constitution".

Subsequent developments of critical importance were initiated by J.N. Langley (1852-1925) who, studying the antagonism between pilocarpine and atropine on salivary flow, concluded in 1878 (2):

"...we may, I think, without much rashness assume that there is a substance or substances in the nerve endings or gland cells with which both atropine and pilocarpine compounds are formed according to some law of which their relative mass and chemical affinity are factors".

This conclusion contained very clearly the essence of the receptor concept, namely the recognition specificity that exists between a drug and its receptor.

In subsequent work, some quarter of a century later, Langley, studying the effects of nicotine and curare on skeletal muscle responses, observed that neither curare nor nicotine prevented direct stimulation of the muscle and suggested that they must therefore combine with some other excitable substance on the muscle cell (3). He wrote:

"It is convenient to have a term for the specially excitable constituent and I have called it the receptive substance. It receives the stimulus and by transmitting it, causes contraction".

Thus, by the beginning of this century Langley had directed attention towards two fundamental features of the drug-receptor interaction: specificity, the basis of cellular recognition, and activation, the ability of the drug-receptor complex to initiate response. These features are cardinal components of the contemporary definition of receptor function.

During the quarter century encompassed by Langley's work the receptor concept was being developed simultaneously by Paul Erlich (1854-1915) whose work in this area undoubtedly owed much to his early interest in the staining of tissues by dyestuffs. Erlich's concepts of cellular receptors were originally focussed on the idea of specific protoplasmic side chains with which toxins interacted and were thus neutralized. Subsequently Ehrlich extended this idea to drug molecules and in 1907 concluded (4):

"I have now formed the opinion that some of the chemically defined sustances are attached to the cell by groupings that are analogues of toxin receptors; these I will distinguish from the toxin-receptors by the name of chemo-receptors".

Thus, by the very early years of this century these major contributions had already led to the first tentative formulations of the receptor concept. Less than one hundred years later our understanding of pharmacologic receptors has achieved the sophistication of definition of the gene and amino acid sequences for the several subunits of the nicotinic acetylcholine receptor, a level of

achievement surely not envisaged either by Langley or by Ehrlich. It is therefore of interest to consider some of the events that occured during the intervening years. It seems clear that these events were several in number and included the quantitative analyses of structure-activity and dose-response relationships, the development of the concept of receptors as information processing and transmitting systems, the determination of receptor localization, the elucidation of receptor-effector coupling mechanisms and the discovery of receptor and receptor-ligand traffic at both plasma membrane and intracellular loci.

The question of receptor classification has always been of considerable importance to both the chemist and pharmacologist and thus has paralleled the development of structure-activity relationships, initially at a qualitative level but increasingly at more quantitative levels including sophisticated molecular modelling strategies. Of necessity the pursuit of increasingly more quantitative structure-activity relationships has placed increasing demands for quantitative analyses of dose-response relationships. Here much is owed to the efforts of A.J. Clark, J.H. Gaddum and H.O. Schild. The work of Clark (5) was instrumental in focussing attention on both the significance, in terms of surface reactions, and the ambiguities of dose-response relationships while the work of Gaddum (6) and Schild (7) demonstrated that by avoiding the definition of the relationship between response and receptor occupancy by agonist quantitative determinations could be made of the affinities of competitive antagonists. In turn such determinations made possible a more precise pharmacologic classification of both drug receptors and of their activating and inhibitory ligands.

Clark's original and simple ideas have, of course, been subject to substantial and necessary modification as have the concepts of drug antagonism. Of particular importance has been the development of the concept of allosterism whereby ligand action at a site topographically distinct from the agonist binding site may also serve to regulate receptor function (8).

Implicit in the development of the receptor concept was the thesis that an initially formed drug-receptor complex was a necessary intermediate for generation of the biological signal. In terms of receptors as information processing systems it is of interest to consider whether the information flow to be activated exists within the drug or within the receptor or whether both drug and receptor contribute. In most classical receptor systems, notably those for neurotransmitters, the function of the drug molecule is to activate a program within the receptor itself. For other systems, however, the function of the

receptor is to translocate the ligand to the cell interior for processing and signal generation. In these cases, including a number of toxins, low density lipoprotein etc., the information may reside within the ligand. It is increasingly likely, however, that a number of ligand-receptor systems may contain information both within the ligand and within the receptor. This is particularly true for polypeptide hormones, notably insulin and growth factors where initial cellular responses may be due to activation of receptor based information and where later and more global responses may be due to intracellular processing of information within the translocated hormone-receptor complex.

The extent to which receptor-based information processing is shared amongst common or diverse pathways has not been resolved. However, it is increasingly clear that at least two systems are capable of accommodating a large number of receptor activation processes. These processes, which are not mutually exclusive, are the cyclase coupled and ion channel coupled systems, both of which may mediate excitatory and inhibitory signals. At least conceptually, such information processing systems may be viewed as composed of the recognition and activation or catalytic components of Langley and Ehrlich linked by a coupling component. The separate character of these components has been very clearly demonstrated in the case of the cyclase linked systems.

The majority of pharmacologic receptors are membrane species and our understanding of the dynamics of receptors has therefore reflected our understanding of membrane structure and function. Just as our view of cell membranes has progressed from a static to a dynamic picture so too has our view of pharmacologic receptors. No longer is it appropriate to consider membrane receptors as static entities unresponsive, like the dinosaurs, to a changing environment. It is now clear that receptors, like other membrane components, are constantly being incorporated into and removed from the membrane and that this traffic is regulated by the very ligands which serve to activate.

Receptor traffic is a component of the normal physiology of cellular systems and changes in receptor density are a component of circadian rhythms within excitable cells. However, in an increasingly large number of instances it is recognized that defects at one or more stages of receptor mobilization or receptor coupling processes exist within pathological states and there exists an increasing list of receptor-related diseases, including myasthenia gravis, familial hypercholesteremia, Graves disease etc., whose pathology is directly related to such defects.

The events of a hundred years have, therefore, brought dramatic progress to

the development of the concept of pharmacologic receptors. From cloudy and uncertain beginnings we now with confidence can discuss receptor structures, coupling, diseases, defects and can use this knowledge to design new pharmacologic and therapeutic tools. As the drama of the second century of pharmacologic receptors unfolds we can be confident that it will be as exciting and as productive as the first.

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PRESYNAPTIC RECEPTORS REGULATING NEUROTRANSMITTER RELEASE IN THE CENTRAL NERVOUS SYSTEM

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INTRODUCTION

Receptors mediating regulation of neurotransmitter release exist on presynaptic nerve terminals of the central nervous system. Due to their localization, these receptors have been termed *presynaptic receptors* (for reviews see 1-3). Although the ever increasing number of presynaptic receptors reported in the literature may require in the near future the utilization of sophisticated nomenclatures, in the present chapter we found it convenient to subdivide presynaptic receptors into *autoreceptors* and *heteroreceptors*. Autoreceptors are presynaptic receptors on which a neurotransmitter acts to regulate (in general to inhibit) its own release. Heteroreceptors are presynaptic receptors which mediate regulation of the release of a given transmitter by other transmitters or modulatory agents.

A number of presynaptic auto- and heteroreceptors have been characterized in our laboratory using nerve endings isolated from various areas of the mammalian brain. Some of the recent results have been summarized in this chapter, in which particular emphasis has been given to the pharmacological aspects of presynaptic receptors.

FEEDBACK INHIBITION OF TRANSMITTER RELEASE MEDIATED BY PRESYNAPTIC AUTORECEPTORS

A number of criteria should be met before assuming the existence of autoreceptors (or heteroreceptors) mediating regulation of transmitter release on the membrane surface of presynaptic nerve terminals: a) the release of a given transmitter should be regulated by the transmitter itself (or by other transmitters) acting at the *external* presynaptic membrane; b) the regulation of release should be detectable in isolated nerve terminals; c) the regulatory action of the transmitter should be counteracted by selective receptor antagonists.

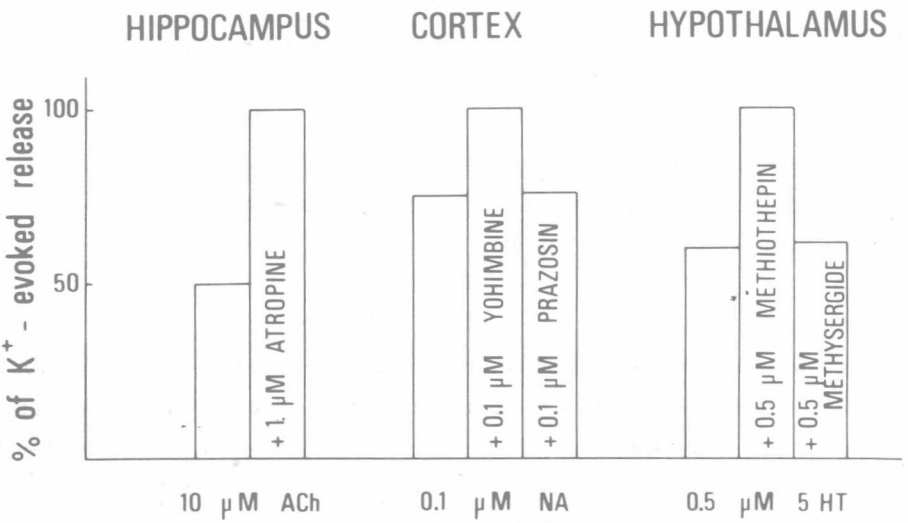

Fig. 1. Muscarinic, alpha$_2$-adrenergic and serotoninergic autoreceptors on nerve endings of rat brain. Synaptosomes from hippocampus, cortex or hypothalamus were labeled respectively with ^{3}H-choline (0.1 µM; 4 min), ^{3}H-noradrenaline (0.04 µM; 10 min) or ^{3}H-5-hydroxytryptamine (0.04 µM; 10 min) and superfused as previously described (6). Depolarization was obtained with 15 mM KCl. The fractions collected during superfusion were analyzed for their content of ^{3}H-amines or ^{3}H-acetylcholine and fractional release curves obtained as described previously (12, 16). The data reported represent the percent of the K^+-evoked release at the peak point of the release curves.

Direct evidence that acetylcholine (ACh) can inhibit its own release in cholinergic nerve terminals was obtained using synaptosomes prepared from rat hippocampus (4) or cerebral cortex (5), prelabeled with ^{3}H-choline and depolarized with 15 mM KCl in a superfusion system (6). As shown in Figure 1, 10 µM ACh depressed by 50% the depolarization-evoked Ca^{2+}-dependent release of ^{3}H-ACh from rat hippocampal synaptosomes. Atropine (1 µM) completely counteracted the action of ACh. Various muscarinic agonists mimicked ACh as inhibitors of ^{3}H-ACh release, either in brain slices (7,8) or in synaptosomes (4,5,9) and their action was

antagonized by atropine or scopolamine. Striatal nerve endings seem to differ from hippocampal or cortical nerve endings; in fact, muscarinic autoreceptors inhibiting the K^+-evoked ^{3}H-ACh release were much less effective in rat striatal synaptosomes (5).

Presynaptic autoreceptors of the alpha$_2$-adrenergic type have been reported to exist both in the peripheral and in the central noradrenergic system (1, 2). Release studies carried out with isolated brain nerve terminals (10, 11) have yielded results supporting the existence of alpha$_2$-adrenergic autoreceptors on the external membrane of central noradrenergic nerve endings. Exogenous noradrenaline (NA), in the presence of desipramine added to prevent NA intraterminal penetration through the NA uptake carrier, inhibited in a concentration-dependent way the release of ^{3}H-NA evoked by 15 mM KCl from rat cortex synaptosomes (12; Figure 6). The inhibitory action of NA (0.1 μM) was antagonized by yohimbine (0.1 μM), a selective alpha$_2$-adrenoceptor blocker, but not by the alpha$_1$-antagonist prazosin (Figure 1).

Also the depolarization-evoked release of tritiated 5-hydroxytryptamine (5HT), previously taken up into hypothalamic synaptosomes (13) or cerebral cortex slices (14), was inhibited by exogenous 5HT, in the presence of chlorimipramine added to prevent the intraterminal penetration of 5HT through its uptake carrier. The action of 5HT was antagonized by methiothepin, but not by other know 5HT receptor antagonists, like cyproheptadine, methysergide or mianserin (13; Fig. 1), suggesting that 5HT presynaptic autoreceptors differ pharmacologically from 5HT postsynaptic receptors.

In conclusion, the data obtained with isolated nerve endings and summarized in Figure 1 indicate that muscarinic cholinergic, alpha$_2$-adrenergic and serotonin autoreceptors are located on presynaptic nerve endings in various areas of the rat brain.

REGULATION OF RELEASE BY PRESYNAPTIC HETERORECEPTORS

Cholinergic regulation of dopamine release through muscarinic heteroreceptors

It was found that the basal release of dopamine (DA), newly synthesized from tyrosine in striatal slices, was potentiated by ACh through the activation of

muscarinic receptors (15). We reported recently (16) that muscarinic receptors located on striatal DA terminals (muscarinic heteroreceptors) can mediate potentiation of DA release not only under resting conditions, but also when "quasi-physiological" depolarizing stimuli (i.e. 15 mM KCl) are used. Figure 2 shows that the release of ^{3}H-DA induced by 15 mM KCl from superfused rat striatal synaptosomes, previously labelled with the radioactive amine, was increased by exogenous ACh. Atropine (0.1 μM) totally antagonized the stimulatory action of 10 μM ACh. The potentiation by ACh of the K^{+}-evoked ^{3}H-DA release decreased with increasing K^{+} concentrations and totally disappeared when synaptosomes were depolarized with 55 mM KCl (not shown). Muscarinic receptors mediating potentiation of electrically evoked ^{3}H-DA release were reported to exist also in cat caudate slices (17).

Regulation of serotonin release through alpha$_2$-adrenergic heteroreceptors

As reported above, noradrenergic axon terminals are endowed with autoreceptors of the alpha$_2$-adrenergic type. Studies with rat hippocampal (18) and occipital cortex (19) slices indicate that also the depolarization-evoked release of ^{3}H-5HT can be inhibited by exogenous NA through the activation of alpha$_2$-adrenoceptors. Experiments carried out with cerebral cortex synaptosomes (20) have provided direct evidence for the existence of presynaptic alpha$_2$-adrenergic heteroreceptors on the external membrane of serotonin nerve endings. The results of these experiments are summarized in Figure 3 showing that the inhibitory action of exogenous NA on the K^{+}-evoked ^{3}H-5HT release from superfused cortical synaptosomes was counteracted by the mixed type alpha$_1$-alpha$_2$ adrenergic antagonist phentolamine and by yohimbine, but not by prazosin. Also mianserin, an antidepressant drug endowed with alpha$_2$-adrenergic antagonistic activity (21) could reverse the inhibition by NA of ^{3}H-5HT release.

HETEROGENEITY OF PRESYNAPTIC MUSCARINIC AND ALPHA$_2$-ADRENERGIC RECEPTORS

Heterogeneity of presynaptic muscarinic receptors

The possible existence of subtypes of muscarinic receptors has been the object of several investigations (for reviews see 22-24). Apart from theoretical, physiological and pathological considerations, the presence of subclasses of