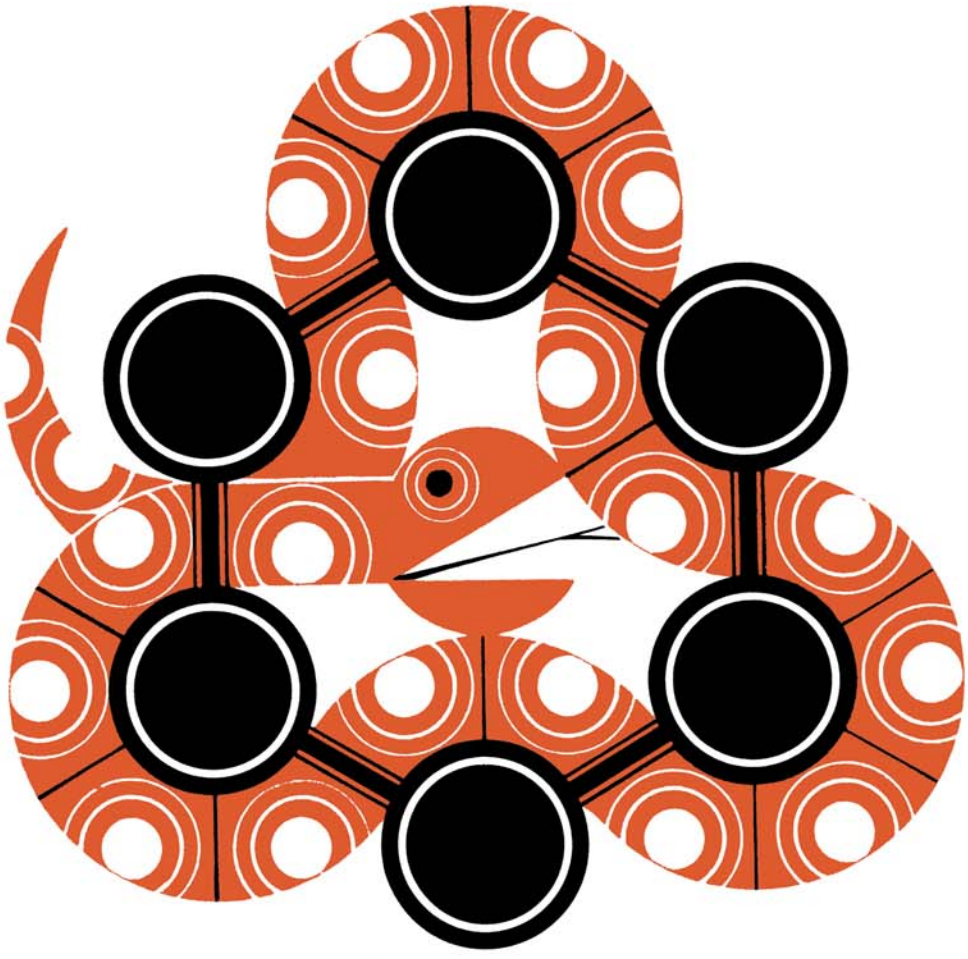

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RECEPTOR CHEMISTRY TOWARDS THE THIRD MILLENNIUM

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Volume 31

RECEPTOR CHEMISTRY TOWARDS THE THIRD MILLENNIUM

Proceedings of the 12th Camerino-Noordwijkerhout Symposium
Camerino, Italy, 5-9 September 1999

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2000

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Amsterdam - Lausanne - New York - Oxford - Shannon - Singapore - Tokyo

ELSEVIER SCIENCE B.V.
Sara Burgerhartstraat 25
P.O. Box 211, 1000 AE Amsterdam, The Netherlands

This volume is reprinted from: Pharnaceutica Acta Helvetiae, 74/2-3

Library of Congress Cataloging in Publication Data
A catalog record from the Library of Congress has been applied for.

ISBN: 0-444-50424-9

♾ The paper used in this publication meets the requirements of ANSI/NISO Z39.48-1992 (Permanence of Paper).
Printed in The Netherlands.

ELSEVIER

Pharmaceutica Acta Helvetiae 74 (2000) v

PHARMACEUTICA ACTA HELVETIAE

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Preface

Despite Langley's brilliant intuition way back in 1878, it was still a long time before the receptor could be called a reality; in fact, even in the early 1960s De Jongh described this molecule as a woman whose lovely seductive image could be deduced only from the type of answer received to the many ''messages'' sent to her. Thus, when we met together for the first time in Camerino back in 1978, we were rightly inspired by that enthusiasm typical of pioneers attracted by the fascination of a discipline still all to be discovered.

Over the last twenty years our joints may well have begun to creak due to increasing age, but our enthusiasm has by no whit been dulled; indeed, even if we now know almost all there is to be known about the way ligands ''mate'' with receptors, which have by now been isolated, characterized, and cloned, many other secrets still remain to tease our curiosity. In particular, differentiation into distinct subpopulations and the multiplicity of transduction processes seem to offer us unhoped for, and even more specific targets in our search for new drugs. And so, that dream of Ehrlich in 1908 to design for each individual pathology a highly selective ''charmed bullet'', and thus one with reduced toxicity, now appears increasingly less utopistic.

It is with these ideas that the 12th Camerino-Noordwijkerhout Symposium has seen us into the third millennium with an awareness that the only success for converting our hopes into reality is a multidisciplinary study based on a wakeful and critical comparison between experiences that have been reaching maturity through different approaches to the problematic - as has always been the case in our Symposia.

Ugo Gulini
Mario Giannella
Gabriella Marucci
Wilma Quaglia

Guest Editors

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Vol. 74 Nos. 2–3 PHARMACEUTICA ACTA HELVETIAE March 2000

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Contents list/Abstracts published in: Chemical Abstracts, Excerpta Medica, International Pharmaceutical Abstracts

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Pharmacological receptors: a century of discovery — and more

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Abstract

A brief survey of the history of the development of the concept of the pharmacological receptor is presented. From the pioneering concepts of Paul Ehrlich, John Langley and others, receptors are described in terms of their recognition properties, their structures, transducing abilities and the impact of genomics and their role in contributing to genetic diseases. The receptor concept has firmly underpinned our advances in drug development and molecular medicine of the latter half of this century and it is clear that it will continue to drive pharmaceutical developments in the 21st century. © 2000 Elsevier Science B.V. All rights reserved.

Keywords: Receptors; Receptor history; Paul Ehrlich; John Newton Langley; Emil Fischer; Louis Pasteur; Drug–receptor interactions; Stereoselectivity; Receptor diseases; Receptor regulation; Receptor structure

1. Introduction

Separate, but complementary, lines of evidence led in the late 19th century to the establishment of the concept of "the receptor" as the cellular site at which drugs, toxins and antibodies mediated their physiological or pathological effects. These lines of evidence are particularly associated with Paul Ehrlich in Germany and John Newton Langley in England. However, their work built upon many centuries of work that attempted to define the action of naturally occurring materials on the body. These earlier contributions have been expertly summarized in the books, "*Murder, Magic and Medicine*" by John Mann (1992) and "*In Search of a Cure*" by M. Weatherall (1990).

From his extensive work on immunology and the chemotherapy of parasitic infections, Ehrlich argued that cells must possess specific and defined protoplasmic side chains that, because of their unique chemistry and steric architecture, could interact specifically with the complementary groups of a chemotherapeutic agent, toxin or antibody (Parascondola, 1981; Ehrlich, 1900):

> "*For the sake of brevity in what follows we shall in general designate as receptor that binding group of the protoplasmic molecule to which a foreign, newly introduced group binds.*" *P. Ehrlich, 1900*

Even prior to these speculations, Langley (1878) had observed:

> "*We may, I think, without much rashness assume that there is some substance or substances in the nerve endings or gland cells with which both atropine and pilocarpine are capable of forming compounds. On this assumption, then, the atropine or pilocarpine compounds are formed according to some law of which their relative mass and chemical affinity for the substance are factors.*" *J.N. Langley, 1878*

But Langley (1906) also recognized the receptor as a transducing engine that:

> "*... receives the stimulus and, by transmitting it causes contraction.*" *J.N. Langley, 1906*

Langley, contemporaneously with the work of Ehrlich, used the term "*receptive substance*" for these specific entities and speculated that specific receptors must exist for curare, atropine, pilocarpine and the other autonomic drugs with which his research had been principally concerned. Certainly, the specificity of such drug–receptor interactions had been anticipated by Emil Fischer who wrote:

> "*... I will say that enzyme and glucoside must fit together like lock and key in order to be able to exercise a chemical action on each other.*" *Emil Fischer, 1894*

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PII: S0031-6865(99)00046-1

Thus, by the beginning of this century, the conceptual foundation had been laid for the existence of pharmacological receptors, albeit as "*black boxes*", that received input and translated it into a physiological, pharmacological or pathological output. The present century has been largely devoted to opening this box and defining its contents. It has been a spectacularly successful century that has culminated with the classification, isolation, characterization and cloning of pharmacological receptors, with the identification of receptors — "orphan receptors" — for which ligands may not have been identified and with the determination of the detailed three-dimensional structure of a membrane receptor — a bacterial potassium channel.

2. Receptors as recognition entities

The specificity of the drug–receptor recognition process has long been regarded as a critical feature of the receptor concept, even when the nature of receptors was entirely unknown. Indeed, the absence of such specificity, including stereoselectivity, is often a component of arguments that a receptor event is *not* involved in the action of a particular drug. These structure–activity relationships were originally qualitative in character, but were transformed first by the application of regression techniques that permitted the elucidation of one-dimensional quantitative structure–activity relationships (QSARs) and then by protein sequence determination and the determination of three-dimensional protein structures and the mapping of receptor sites (Greer et al., 1994).

With these approaches, it is increasingly possible to interpret the actions of drugs at their receptors and to facilitate the design of drugs for new receptor sites. Thus, the design of the HIV-protease inhibitors, a critically available class of drugs for the treatment of this lethal disease, was greatly facilitated by the resolution of the structure of the enzyme. The dimeric, essentially symmetric, structure composed of two identical aspartate protease-like domains, was critical to the development of the first protease inhibitors.

Stereochemistry of interaction has long been recognized in drug–receptor interactions and Pasteur very explicitly recognized that different stereoisomers could have very different physiological properties:

> "*There cannot be the slightest doubt that the only and exclusive cause of this difference in the fermentation of the two tartaric acids is caused by the opposite molecular arrangements of the tartaric acids. In this way, the idea of the influence of the molecular asymmetry of natural organic products is introduced into physiological studies, this important characteristic being perhaps the only distinct line or demarcation which we can draw today between dead and living matter. I have in fact set up a theory of molecular asymmetry, one of the most important and wholly surprising chapters of the science, which opens up a new, distant but definite horizon for physiology.*" *Louis Pasteur, 1860*

The stereochemical basis of drug actions was early investigated by Arthur Cushny at the beginning of this century (Cushny, 1926). These pioneering investigations on atropine and related compounds revealed the quantitative differences that can occur between drug enantiomers. Today, the issue of the chirality of drug–receptor interactions has assumed both scientific *and* regulatory significance. Scientific and clinical significance derives from consideration of the efficacy of a single enantiomer versus a racemate, from considerations of stereoselective metabolism and disposition, and from the impact of the route of administration and patient variability. Regulatory issues derive from considerations that racemic drugs may represent separate agents in fixed combinations: development issues derive from considerations of the costs, including those for chemical synthesis, of pursuing a single enantiomer or a racemic mixture.

Recent developments in stereochemistry have focused upon the gaseous general anesthetics, long a topic of discussion concerning their potential interactions with receptors. The long-standing assumption that these agents interacted non-specifically through partitioning into lipid membranes has been challenged from a variety of sources, including the stereospecificity of interaction of isoflurane and halothane (Moody et al., 1994; Sedensky et al., 1994). The differences, though small, are potentially significant from the perspective of clinical safety.

3. Receptors as transduction machines

As realized by Langley, recognition is a necessary, but not sufficient, characteristic of a receptor. Receptors are also biological machines translating the information of the interaction with the ligand into the cellular response. And with this translation comes the necessary amplification of the input information by several orders of magnitude. Several types of physical and biochemical process are involved in this biological transduction. Of particular significance, because of their widespread occurrence are the transduction events mediated by G protein-coupled receptors and by ion channels. The G proteins are a large group or superfamily of GTP hydrolases and the interaction of an activated receptor with the heteromeric G protein releases bound GDP and replaces it with GTP with concomitant liberation of the activated GTP-associated Gα subunit. This activated subunit then interacts with a number of effector systems, including phospholipase C, adenylyl cyclase and ion channels.

Similar amplification events occur during the opening or closing of ion channels mediated by chemical (ligand-gated channels) or physical (potential-dependent channels).

To a first approximation, ion channel opening is an all-or-none stochastic event and the effect of a stimulus, chemical or physical, is to alter the probability of channel opening. Patch clamp techniques make it possible to observe the opening or closing of single channels and thus to measure single molecular events. This ability, coupled with recent structural information on the K^+ channel, makes possible to a first approximation a molecular description of ion channel function.

4. Receptor classification

Receptor structure, the linear and ultimately the three-dimensional representation of the sequence, provides a definitive classification and basis for the classification of receptors. This permits the identification of "families" and of "super-families" of receptors and, in recent years, has made possible the isolation and characterization of so-called "orphan receptors", for which physiological ligands or physiological function may not have been identified. Earlier classification schemes that used the identity of the physiological ligand that interacts with the receptor, the nature of the physiological or pharmacological response induced by receptor activation or the nature of the antagonist drug all have significant limitations. Multiple systems and receptors control blood pressure and similarly many receptors share a common biochemical cascade — adenylyl cyclase or phospholipase C activation or the opening and closing of K^+ channels. To further complicate matters, many receptors are pleiotropic, initiating multiple consequences that may differ according to cell type and even agonist quality. Similarly, many physiological ligands may interact with multiple receptors that are of fundamentally different classes: acetylcholine interacts with both muscarinic and nicotinic receptors, the former being members of the G protein-coupled family (Bikker et al., 1998) and the latter a member of the ligand-gated ion channel family (Holladay et al., 1997). Finally, many receptors are heteromeric assemblies of multiple types of subunits: the pharmacological specificity and the actions induced can be very dependent upon subunit composition. Despite this complexity, it is convenient to recognize four principal families of chemically sensitive pharmacological receptors (Table 1).

Table 1
Classification of pharmacological receptors

Class	Type	Characteristics
1	Ion channels	Integral membrane; subunit composition; each subunit has two or more membrane inserts as a pore region and four or more form the central pore of the channel
2	G protein-coupled	Seven-transmembrane integral proteins that couple to the G protein family of proteins
3	Enzyme-associated	One-transmembrane integral proteins that have kinase activity; may dimerize during receptor activation
4	Nuclear receptors	Non-membrane, cytosolic proteins with DNA binding domains; transcriptional regulators

5. Receptor structure

The majority of receptors under discussion are integral membrane proteins and have not, until recently, yielded to three-dimensional structural determination. However, progress is now being made in three principal areas — the structure of rhodopsin as a model for the very large G protein-coupled receptor family, the role of the nicotinic acetylcholine receptor as a model for ligand-gated ion channels and a bacterial K^+ channel from *Streptomyces lividans* that will materially define the ionic conductivity, selectivity and gating processes of ion channels. Additionally, powerful molecular biological approaches including selective mutagenesis and the use of chimeric constructs have served to define the roles of particular sequences or residues in receptor recognition and activation processes.

The very large G protein-coupled receptor family has provided many examples of the definition of residue roles in drug interactions. Thus, for the beta-adrenoceptor, critical interacting residues have been determined to be aspartate-113 on helix III, serine-204 and -207 on helix V and phenylalanine-290 on helix VI. Such studies have defined a "homologous" binding pocket on this receptor family that is shared by the cationic neurotransmitters, acetylcholine, histamine, norepinephrine etc., and related small ligands.

Perhaps the most recent dramatic advance has been the determination of the three-dimensional structure of a bacterial K^+ channel from *S. lividans* (Doyle et al., 1998). This channel is composed of four identical subunits, each with two trans-membrane sequences and a "pore" region, that associate in "tepee" shape to form the functional ion channel containing within it the selectivity filter that discriminates K^+ from other ions. The selectivity filter contains a so-called "signature" sequence, highly conserved residues that characterize K^+ ion channels and the Gly–Tyr–Gly components of this sequence in the four subunits bind K^+ through their carbonyl residues and are responsible for the ionic selectivity of the channel.

These structural studies have also revealed the importance that very minor changes, frequently a single residue, can have on the drug–receptor interaction. Thus, the 5-HT_{1B} receptor in the rodent and man is pharmacologically quite distinct, a differentiation that is provided by residue 355, threonine in the human and asparagine in the rat (Oksenberg et al., 1992). Similarly, the interaction of barbiturates and other anesthetics with the GABA p1 receptor depends upon the presence of a single isoleucine residue: replacement of this residue by serine confers

anesthetic sensitivity (Belelli et al., 1999). Such changes are of extreme importance in the determination of individual human sensitivity to drugs where single nucleotide polymorphisms (SNPs) may determine clinically significant drug responses and interactions (Kleyn and Vessell, 1998). Thus, there are a number of polymorphisms in the human beta-adrenoceptors and these have been associated with bronchodilation in response to $beta_2$-agonists and with the development of hypertension (Buscher et al., 1999). The P450-mediated drug metabolism process is highly polymorphic leading to extensive inter-individual variation in drug metabolism (Ingelman-Sundberg et al., 1999). Exploitation of this knowledge, now possible through gene-array technologies, will increasingly alter both drug development and drug prescribing.

6. Orphan receptors

The classical route to the receptor concept has always been the existence first of a drug and an associated family structure with defined physiological and pharmacological effects. A classic example is morphine and the opiates and the subsequent discovery of the endogenous ligands and subsequently the G protein-coupled opiate receptor. The isolated and expressed receptor could then be used as a screen for novel structures that might have more desirable therapeutic properties. Advances in molecular biology now permit the reverse of this process. DNA sequences are identified that are analogs of known receptors. These sequences can be expressed to yield novel or "orphan" receptors for which the endogenous ligand can now be hunted (Soontjens et al., 1996; Robertson and Willy, 1997; Civelli et al., 1998; Wilson et al., 1998).

Both the G protein family and the steroid hormone family have yielded many orphan receptors. At least 140 G protein receptors have been identified from the human genome and since this class has generated major drugs for many therapeutic targets, the status of orphan receptors here has attracted much attention. The identification of the opiod receptor ORL1 is but one example, interacting with a specific endogenous ligand nociceptin that appears to have widespread roles in the mediation of nociception and stress reduction. Over 70 orphan receptors have thus far been identified in the steroid receptor family and for most of these, neither endogenous ligand nor physiological action has yet been defined.

7. Receptors and genetic diseases

Defects in the structure and expression of receptor proteins are increasingly known to be associated with specific disease states. As one of the largest families of receptors, the G protein-coupled receptors exhibit a variety of mutations and associated functional changes, including both "loss of function" and "gain of function" (Spiegel, 1995; Farfel et al., 1999). These defects can lie in the actual receptors or in the associated G proteins. Similarly, mutations in ion channels are being associated with a variety of diseases from cardiac abnormalities to cystic fibrosis.

Loss-of-function mutations in G protein-coupled receptors are quite common, with approximately 100 having been described, and include nephrogenic diabetes insipidus (V2 vasopressin receptor), familial hypothyroidism (TSH receptor), Hirschprung disease (endothelin B receptor) hypercalcemia and neonatal hyperparathyroidism (Ca^{2+} sensing receptor). These loss of function mutations may prevent protein expression, folding or insertion in the membrane or may impair agonist binding or interaction of the receptor with G proteins. Loss-of-function defects may also arise in the associated G proteins. Pseudohypoparathyroidism, resistance to parathyroid hormone with a subnormal urinary cAMP response to the hormone, provides one example. Individuals with the type 1a form of the disease also show resistance to a variety of other hormones that stimulate cAMP formation and this is associated with a defect in the Gs-α subunit. A number of gain-of-function mutations have also been described in which there is constitutive receptor activation. These include McCune–Albright syndrome characterized by excessive cell proliferation, including hyperpigmented skin, precocious puberty, hyperthyroidism, acromegaly and polyostotic fibrous dysplasia and results from persistent activation of the G protein from an inability to hydrolyze GTP and thus to terminate the receptor-G protein cycle. A defect in the beta-subunit of a G protein has been shown to be associated with an increased incidence of hypertension, being found in 53% of patients with essential hypertension and 44% of normotensive patients (Siffert et al., 1998).

Defects associated with ion channels underlie a variety of diseases, including cystic fibrosis, cardiac arrhythmias, episodic ataxia, heritable myasthenia and nocturnal frontal lobe epilepsy (Keating and Sanguinetti, 1996; Ackerman and Clapham, 1997; Cooper and Jan, 1999). Cystic fibrosis arises from a defect in a chloride channel — the cystic fibrosis transmembrane regulator — that blocks chloride transport in epithelial cells. It is a remarkably common defect amongst Caucasians — some 3.5% of the population carrying a defective gene. The most common defect is the deletion of phenylalanine 508, which results in a protein that does not insert properly into the membrane. In long QT syndrome, there is a lengthening of the QT interval of the electrocardiogram, a delay that may initiate cardiac arrhythmias, fibrillation and death. The defect has several origins associated primarily with K^+ channels: one of these channels associated with the HERG gene and accounting for some 30% of LQTS cases is of particular importance since it is a target for a number of clinically available drugs, including some antibiotics, antihistamines and antifungal agents that increase the chance of arrhythmias and sudden death.