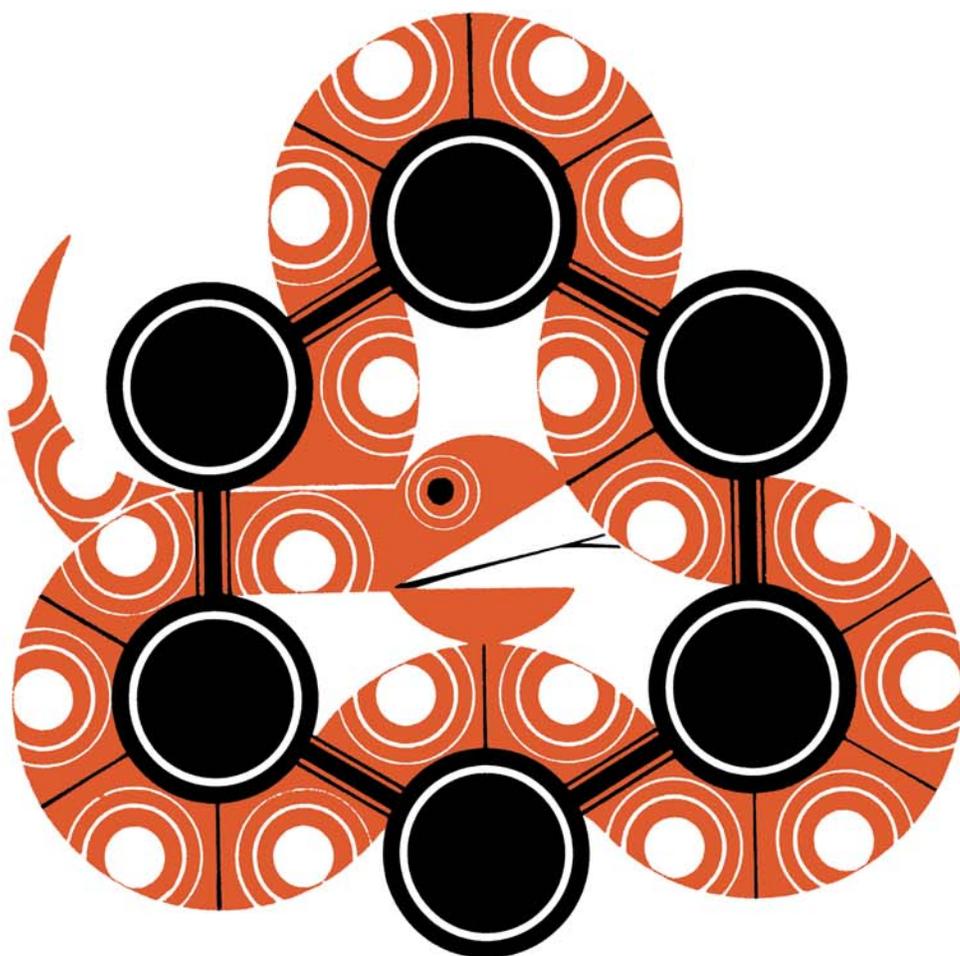


PHARMACO  
CHEMISTRY  
LIBRARY

**31**



# RECEPTOR CHEMISTRY TOWARDS THE THIRD MILLENNIUM

EDITED BY

UGO GULINI, MARIO GIANELLI, WILMA QUAGLIA  
AND GABRIELLA MARRUCCI

ELSEVIER

PHARMACOCHEMISTRY LIBRARY - VOLUME 31

**RECEPTOR CHEMISTRY  
TOWARDS THE THIRD MILLENNIUM**

PHARMACOCHEMISTRY LIBRARY, edited by H. Timmerman

Other titles in this series

- Volume 18 Trends in Receptor Research. Proceedings of the 8th Noordwijkerhout-Camerino Symposium, Camerino, Italy, 8-12 September, 1991  
*edited by P. Angeli, U. Gulini and W. Quaglia*
- Volume 19 Small Peptides. Chemistry, Biology and Clinical Studies  
*edited by A.S. Dutta*
- Volume 20 Trends in Drug Research. Proceedings of the 9th Noordwijkerhout-Camerino Symposium, Noordwijkerhout (The Netherlands), 23-27 May, 1993  
*edited by V. Claassen*
- Volume 21 Medicinal Chemistry of the Renin-Angiotensin System  
*edited by P.B.M.W.M. Timmermans and R.R. Wexler*
- Volume 22 The Chemistry and Pharmacology of Taxol® and its Derivatives  
*edited by V. Farina*
- Volume 23 Qsar and Drug Design: New Developments and Applications  
*edited by T. Fujita*
- Volume 24 Perspectives in Receptor Research  
*edited by D. Giardinà, A. Piergentili and M. Pignini*
- Volume 25 Approaches to Design and Synthesis of Antiparasitic Drugs  
*edited by Nitya Anand*
- Volume 26 Stable Isotopes in Pharmaceutical Research  
*edited by Thomas R. Browne*
- Volume 27 Serotonin Receptors and their Ligands  
*edited by B. Olivier et al.*
- Volume 28 Proceedings XIVth International Symposium on Medicinal Chemistry  
*edited by F. Awouters*
- Volume 29 Trends in Drug Research II. Proceedings of the 11th Noordwijkerhout-Camerino Symposium, Noordwijkerhout (The Netherlands), 11-15 May, 1997  
*edited by H. Van der Goot*
- Volume 30 The Histamine H<sub>3</sub> Receptor. A Target for New Drugs  
*edited by Rob Leurs and Henk Timmerman*

PHARMACOCHEMISTRY LIBRARY  
Editor: H. Timmerman



Volume 31

# RECEPTOR CHEMISTRY TOWARDS THE THIRD MILLENNIUM

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

Edited by

Ugo Gulini, Mario Gianella, Wilma Quaglia  
and Gabriella Marucci

Department of Chemical Sciences, University of Camerino  
1 Via San Agostino, 62032 Camerino (MC), Italy



2000

ELSEVIER

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

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

© 2000 Elsevier Science B.V. All rights reserved.

This work is protected under copyright by Elsevier Science, and the following terms and conditions apply to its use:

#### Photocopying

Single photocopies of single chapters may be made for personal use as allowed by national copyright laws. Permission of the Publisher and payment of a fee is required for all other photocopying, including multiple or systematic copying, copying for advertising or promotional purposes, resale, and all forms of document delivery. Special rates are available for educational institutions that wish to make photocopies for non-profit educational classroom use.

Permissions may be sought directly from Elsevier Science Rights & Permissions Department, PO Box 800, Oxford OX5 1DX, UK; phone: (+44) 1865 843830, fax: (+44) 1865 853333, e-mail: [permissions@elsevier.co.uk](mailto:permissions@elsevier.co.uk). You may also contact Rights & Permissions directly through Elsevier's home page (<http://www.elsevier.nl>), selecting first >Customer Support=, then >General Information=, then >Permissions Query Form=.

In the USA, users may clear permissions and make payments through the Copyright Clearance Center, Inc., 222 Rosewood Drive, Danvers, MA 01923, USA; phone: (978) 7508400, fax: (978) 7504744, and in the UK through the Copyright Licensing Agency Rapid Clearance Service (CLARCS), 90 Tottenham Court Road, London W1P 0LP, UK; phone: (+44) 171 631 5555; fax: (+44) 171 631 5500. Other countries may have a local reprographic rights agency for payments.

#### Derivative Works

Tables of contents may be reproduced for internal circulation, but permission of Elsevier Science is required for external resale or distribution of such material.

Permission of the Publisher is required for all other derivative works, including compilations and translations.

#### Electronic Storage or Usage

Permission of the Publisher is required to store or use electronically any material contained in this work, including any chapter or part of a chapter.

Except as outlined above, no part of this work may be reproduced, stored in a retrieval system or transmitted in any form or by any means, electronic, mechanical, photocopying, recording or otherwise, without prior written permission of the Publisher.

Address permissions requests to: Elsevier Science Rights & Permissions Department, at the mail, fax and e-mail addresses noted above.

#### Notice

No responsibility is assumed by the Publisher for any injury and/or damage to persons or property as a matter of products liability, negligence or otherwise, or from any use or operation of any methods, products, instructions or ideas contained in the material herein. Because of rapid advances in the medical sciences, in particular, independent verification of diagnoses and drug dosages should be made.

This volume is reprinted from: *Pharmaceutica 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.



## 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 un hoped 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  
Gabiella Marucci  
Wilma Quaglia

*Guest Editors*

## PHARMACOCHEMISTRY LIBRARY

### ADVISORY BOARD

<b>T. Fujita</b>	Department of Agricultural Chemistry, Kyoto University, Kyoto, Japan
<b>E. Mutschler</b>	Department of Pharmacology, University of Frankfurt, Frankfurt, Germany
<b>N.J. de Souza</b>	Research Centre, Wookhardt Centre, Bombay, India
<b>F.J. Zeelen</b>	Heesch, The Netherlands

## CONTENTS

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

**Special Issue: Receptor Chemistry Towards the Third Millennium, Proceedings of the 12<sup>th</sup> Camerino-Noordwijkerhout Symposium, Camerino, Italy, 5–9 September 1999**

<i>Preface</i>	v
<i>Acknowledgements</i>	vi
Pharmacological receptors: a century of discovery — and more	
<i>D.J. Triggle</i>	79
Cholinergic receptors and neurodegenerative diseases	
<i>F. Gualtieri</i>	85
Nicotinic systems in central nervous systems disease: degenerative disorders and beyond	
<i>P.A. Newhouse and M. Kelton</i>	91
Central nicotinic receptor ligands and pharmacophores	
<i>R.A. Glennon and M. Dukat</i>	103
Structural aspects of high affinity ligands for the $\alpha 4\beta 2$ neuronal nicotinic receptor	
<i>M.J. Dart, J.T. Wasicak, K.B. Ryther, M.R. Schrimpf, K.H. Kim, D.J. Anderson, J.P. Sullivan, M.D. Meyer</i>	115
Recombinant human receptors and functional assays in the discovery of altinicline (SIB-1508Y), a novel acetylcholine-gated ion channel (nAChR) agonist	
<i>N.D.P. Cosford, L. Bleicher, J.-M. Vernier, L. Chavez-Noriega, T.S. Rao, R.S. Siegel, C. Suto, M. Washburn, G.K. Lloyd, I.A. McDonald</i>	125
Receptors in neurodegenerative diseases, muscarinic cholinergic receptors	
<i>P. Angeli</i>	131
Design and development of selective muscarinic agonists for the treatment of Alzheimer's disease: characterization of tetrahydropyrimidine derivatives and development of new approaches for improved affinity and selectivity for $M_1$ receptors	
<i>W.S. Messer Jr., W.G. Rajeswaran, Y. Cao, H.-J. Zhang, A.A. El-Assadi, C. Dockery, J. Liske, J. O'Brien, F.E. Williams, X.-P. Huang, M.E. Wroblewski, P.I. Nagy, S.M. Peseckis</i>	135
CI-1017, a functionally $M_1$ -selective muscarinic agonist: design, synthesis, and preclinical pharmacology	
<i>H. Teclé, R.D. Schwarz, S.D. Barrett, M.J. Callahan, B.W. Caprathe, R.E. Davis, P. Doyle, M. Emmerling, D.J. Lauffer, T. Mirzadegan, D.W. Moreland, W. Lipiniski, C. Nelson, C. Raby, C. Spencer, K. Spiegel, A.J. Thomas, J.C. Jaen</i>	141
Ligands for the common allosteric site of acetylcholine $M_2$ -receptors: development and application	
<i>U. Holzgrabe, W. Bender, H.M. Botero Cid, M. Staudt, R. Pick, C. Pflutschinger, E. Balatková, C. Tränkle, K. Mohr</i>	149
Receptors in cardiovascular disease: review and introduction	
<i>A. Leonardi, G. Sironi, G. Motta</i>	157
Adrenoceptor subclassification: an approach to improved cardiovascular therapeutics	
<i>J.P. Hieble</i>	163
The $\alpha_{1a}$ and $\alpha_{1b}$ -adrenergic receptor subtypes: molecular mechanisms of receptor activation and of drug action	
<i>S. Cotecchia, O. Rossier, F. Fanelli, A. Leonardi, P.G. De Benedetti</i>	173
$\alpha_1$ -Adrenoreceptor antagonists bearing a quinazoline or a benzodioxane moiety	
<i>C. Melchiorre, P. Angeli, M.L. Bolognesi, A. Chiarini, D. Giardinà, U. Gulini, A. Leonardi, G. Marucci, A. Minarini, M. Pignini, W. Quaglia, M. Rosini, V. Tumiatto</i>	181
Selection, design and evaluation of new radioligands for PET studies of cardiac adrenoceptors	
<i>V.W. Pike, M.P. Law, S. Osman, R.J. Davenport, O. Rimoldi, D. Giardinà, P.G. Camici</i>	191
Enigmatic receptors	
<i>L. Brasili</i>	201
Imidazoline receptors: a challenge	
<i>P. Bousquet, V. Bruban, S. Schann, J. Feldman</i>	205

Sigma receptors: recent advances and new clinical potentials	
<i>W.D. Bowen</i>	211
Excitatory amino acid receptors	
<i>G. Gaviraghi</i>	219
Excitatory amino acid agonists and antagonists: pharmacology and therapeutic applications	
<i>D.G. Trist</i>	221
Metabotropic glutamate receptors: a structural view point	
<i>R. Pellicciari, G. Costantino, A. Macchiarulo</i>	231
Synthesis and pharmacological properties of novel glycine antagonists	
<i>D. Donati and R. Di Fabio</i>	239
Receptors in neurodegenerative diseases	
<i>W. Froestl</i>	247
Neurotrophin receptor structure and interactions	
<i>H. Yano and M.V. Chao</i>	253
The RET receptor tyrosine kinase: activation, signalling and significance in neural development and disease	
<i>I. Mason</i>	261
The ciliary neurotrophic factor and its receptor, CNTFR $\alpha$	
<i>M.W. Sleeman, K.D. Anderson, P.D. Lambert, G.D. Yancopoulos, S.J. Wiegand</i>	265
Rediscovering good old friend IGF-1 in the new millenium: possible usefulness in Alzheimer's disease and stroke	
<i>S. Doré, S. Kar, W.-H. Zheng, R. Quirion</i>	273
Apoptosis induced by death receptors	
<i>P. Schneider and J. Tschopp</i>	281
Hijacked receptors	
<i>D.J. Triggle</i>	287
ICAM-1 receptors and cold viruses	
<i>J. Bella and M.G. Rossmann</i>	291
Viral-encoded G-protein coupled receptors: new targets for drug research?	
<i>M.J. Smit, H. Timmerman, D. Verzijl, R. Leurs</i>	299
Chemokine receptors: interaction with HIV-1 and viral-encoded chemokines	
<i>S. Sozzani, P. Allavena, A. Vecchi, J. Van Damme, A. Mantovani</i>	305
General topics and perspectives	
<i>H. Timmerman</i>	313
Pharmacological evidence of muscarinic receptor heterodimerization	
<i>S. Chiacchio, M. Scarselli, M. Armogida, R. Maggio</i>	315
Constitutive activity of G protein coupled receptors and drug action	
<i>R. Leurs, M.S.R. Pena, R.A. Bakker, A.E. Alewijnse, H. Timmerman</i>	327
New dimensions in G protein signalling: G $\beta$ 5 and the RGS proteins	
<i>W.F. Simonds and J.-H. Zhang</i>	333
Kappa opioid agonists as targets for pharmacotherapies in cocaine abuse	
<i>J.L. Neumeyer, N.K. Mello, S. Stevens Negus, J.M. Bidlack</i>	337
<i>Author Index</i>	345
<i>Keyword Index</i>	347

# Pharmacological receptors: a century of discovery — and more

David J. Triggle \*

*The Graduate School, 562 Capen Hall, State University of New York, Buffalo, NY 14260, USA*

## 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*

\* Tel.: +1-716-645-7315; fax: +1-716-645-2941; e-mail: triggle@buffalo.edu

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 G $\alpha$  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<sup>+</sup> 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<sup>+</sup> 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).

#### 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<sup>+</sup> 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<sup>+</sup> 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<sup>+</sup> from other ions. The selectivity filter contains a so-called “signature” sequence, highly conserved residues that characterize K<sup>+</sup> ion channels and the Gly–Tyr–Gly components of this sequence in the four subunits bind K<sup>+</sup> 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<sub>1B</sub> 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

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

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<sub>2</sub>-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 opioid 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<sup>2+</sup> 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- $\alpha$  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<sup>+</sup> 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.