G Protein-Coupled Receptors as Drug Targets

Analysis of Activation and Constitutive Activity

Edited by
Roland Seifert and Thomas Wieland

WILEY-VCH Verlag GmbH & Co. KGaA

G Protein-Coupled Receptors as Drug Targets

Edited by
Roland Seifert, Thomas Wieland

Methods and Principles in Medicinal Chemistry

Previous Volumes of this Series:

D. Smith, D. Walker,
H. van de Waterbeemd

Pharmacokinetics and Metabolism in Drug Design

Vol. 13

2001, ISBN 3-527-30197-6

T. Lenaguer (ed.)

Bioinformatics – From Genomes to Drugs

Vol. 14

2002, ISBN 3-527-29988-2

J. K. Seydel, M. Wiese

Drug-Membrane Interactions

Vol. 15

2002, ISBN 3-527-30427-4

O. Zerbe (ed.)

BioNMR in Drug Research

Vol. 16

2002, ISBN 3-527-30465-7

P. Arloni, F. Alber (eds.)

Quantum Medicinal Chemistry

Vol. 17

2003, ISBN 3-527-30456-8

H. van de Waterbeemd, H. Lennernäs,
P. Artursson (eds.)

Drug Bioavailability

Vol. 18

2003, ISBN 3-527-30438-X

H.-J. Böhm, S. S. Abdel-Meguid (eds.)

Protein Crystallography in Drug Discovery

Vol. 20

2004, ISBN 3-527-30678-1

Th. Dingermann, D. Steinhilber,
G. Folkers (eds.)

Molecular Biology in Medicinal Chemistry

Vol. 21

2004, ISBN 3-527-30431-2

H. Kubinyi, G. Müller (eds.)

Chemogenomics in Drug Discovery

Vol. 22

2004, ISBN 3-527-30987-X

T. I. Oprea (ed.)

Chemoinformatics in Drug Discovery

Vol. 23

2005, ISBN 3-527-30753-2

G Protein-Coupled Receptors as Drug Targets

Analysis of Activation and Constitutive Activity

Edited by
Roland Seifert and Thomas Wieland

WILEY-VCH Verlag GmbH & Co. KGaA

Series Editors:

Prof. Dr. Raimund Mannhold
Biomedical Research Center
Molecular Drug Research Group
Heinrich-Heine-Universität
Universtätsstrasse 1
40225 Düsseldorf
Germany
Raimund.mannhold@uni-duesseldorf.de

Prof. Dr. Hugo Kubinyi
Donnersbergstrasse 9
67256 Weisenheim am Sand
Germany
kubinyi@t-online.de

Prof. Dr. Gerd Folkers
Collegium Helveticum
STW/ETH Zentrum
8092 Zürich
Switzerland
folkers@collegium.ethz.ch

Volume Editors:

Prof. Thomas Wieland
Institut für Pharmakologie und Toxikologie
Fakultät für Klinische Medizin Mannheim
Der Universität Heidelberg
Maybachstraße 14-16
68169 Mannheim
Germany
thomas.wieland@urz.uni-heidelberg.de

Prof. Roland Seifert
Department of Pharmacology and Toxicology
University of Regensburg
Universitätsstraße 31
D-93053 Regensburg
Germany
roland.seifert@chemie.uni-regensburg.de

Library of Congress Card No.: Applied for.

British Library Cataloguing-in-Publication Data:
A catalogue record for this book is available from the British Library.

Bibliographic information published by Die Deutsche Bibliothek
Die Deutsche Bibliothek lists this publication in the Deutsche Nationalbibliografie; detailed bibliographic data is available in the Internet at http://dnb.ddb.de

Typesetting Mitterweger & Partner, Kommunikationsgesellschaft mbH, Plankstadt
Printing Strauss GmbH, Mörlenbach
Bookbinding J. Schäffer GmbH, Grünstadt

Printed in the Federal Republic of Germany

ISBN-13: 978-3-527-30819-4
ISBN-10: 3-527-30819-9

Table of Contents

Preface *XI*

A Personal Foreword *XIII*

List of Contributors *XV*

Abbreviations and Terminology *XX*

I General Concepts *1*

1 Historical Background and Introduction *3*

2 The Nature of Constitutive Activity and Inverse Agonism *11*
2.1 Historical Perspective *11*
2.2 Theoretical Basis of Inverse Agonism: Relevance of Receptor Type *13*
2.3 The Interaction of Systems with Ligands *18*
2.4 Inverse Agonism as a Phenotypic Behavior *23*
2.5 Conclusion *25*

3 Molecular Mechanisms of GPCR Activation *27*
3.1 Introduction *27*
3.2 GPCR Structure and Ligand Recognition *28*
3.3 Conformational Changes in the GPCR Activation Process *29*
3.4 Conversion to the Active Receptor State Involves Release of Stabilizing Intramolecular Interactions *35*
3.5 Kinetics of Agonist Binding and Receptor Activation *37*
3.6 GPCR Activation in an Oligomeric Context *38*

4 Molecular and Cellular Determinants of GPCR Splice Variant Constitutive Activity *43*
4.1 Introduction *43*

4.2 Constitutive Activation of Second Messenger Production by C-Terminal Splice Variants of GPCRs *45*
4.2.1 The Constitutive Activities of C-Terminal 5-HT_4 Receptor Splice Variants: the Shortest, the Strongest *45*
4.2.2 The Constitutive Activities of $mGlu_1R$ and $mGlu_5R$ C-t Splice Variants: a Case for which a Physiological Control does exist *48*
4.2.3 Other Examples of GPCR C-t Splice Variants with Different Constitutive Activities *50*
4.3 Differential Constitutive Internalization of C-t GPCR Splice Variants *50*
4.3.1 The Thromboxane A2 Receptor $TP_\beta R$, but not the $TP_\alpha R$ Splice Variant, is Constitutively Internalized by Clathrin-dependent, GRK- and Arrestin-independent Mechanisms *51*
4.3.2 The Prostaglandin $F_{2\alpha}$ Receptor FP_BR, but not the FP_AR C-Terminal Splice Variant, is Constitutively Internalized by a Clathrin-independent, PI3-Kinase-dependent Mechanism *52*
4.4 Conclusion *53*

5 Naturally Occurring Constitutively Active Receptors: Physiological and Pharmacological Implications *55*
5.1 Introduction *55*
5.2 Wild-type Interspecies Homologues *56*
5.3 Wild-type Receptor Subtypes within a Given Species *57*
5.4 Wild-type Alternatively Spliced Receptors *57*
5.5 Polymorphisms in GPCRs *57*
5.6 GPCR Mutation-induced Disease *59*
5.7 Future Challenges *60*

6 The Impact of G Proteins on Constitutive GPCR Activity *63*
6.1 Introduction *63*
6.2 The Contribution of G proteins to Constitutive Activity *64*
6.2.1 Basic Features *64*
6.2.2 The Distribution of G Proteins in the Plasma Membrane *65*
6.3 GPCR–G Protein Fusion Proteins *66*
6.3.1 Basic Features *66*
6.3.2 Modulation of the GPCR–G Protein Interface Alters Constitutive Activity *66*
6.3.3 Use of G Protein Variation to Detect Ligand Efficacy *68*
6.4 Conclusions *69*

7 (Patho)physiological and Therapeutic Relevance of Constitutive Activity and Inverse Agonism at G Protein-Coupled Receptors *71*
7.1 Introduction *71*
7.2 Physiological Relevance of Constitutive Activity of GPCRs *72*
7.3 Constitutive Activity of GPCRs and Pathophysiology of Disease *73*
7.4 Physiological Relevance of Inverse Agonists *76*

7.5 Inverse Agonists as Drugs *77*
7.6 Conclusions *79*

8 Methodological Approaches *81*
8.1 Introduction *81*
8.2 Analysis of Constitutive GPCR Activity in Membranes and Intact Cells *82*
8.2.1 Procedure for Sf9 Cell Culture and Membrane Preparation *84*
8.2.2 GPCR Radioligand Binding Studies *86*
8.2.3 GTPase Assay *90*
8.2.4 [^{35}S]GTPγS Binding Assay *96*
8.2.5 Adenylyl Cyclase Assay *101*
8.3 Measurement of Constitutive Activity of GPCRs in Intact Cells *106*
8.3.1 Quantitative Determination of cAMP Concentrations in Cell Culture Lysates *109*
8.3.2 Determination of Inositol Phosphate Formation in Living Cells *110*
8.3.3 Determination of G Protein Activation by SRF-mediated Gene Transcription *113*
8.3.4 Deorphanization and Constitutive Activity of GPCRs by Aequorin-based Ca^{2+} Determinations *115*

II Constitutive Activity of Selected GPCR Systems *121*

9 Constitutive Activity of β-Adrenoceptors: Analysis in Membrane Systems *123*
9.1 Introduction *123*
9.2 Analysis of $\beta AR/G_s$ Protein Coupling in Membranes *124*
9.3 Development of the Concept that βARs are Constitutively Active *127*
9.4 Probing Models of GPCR Activation with β_2AR_{wt} and β_2AR_{CAM} with Inverse Agonists *128*
9.5 Probing Models of GPCR Activation with β_2AR_{wt} and β_2AR_{CAM} and with Partial and Full Agonists *130*
9.6 Probing Models of GPCR Activation with β_2AR_{wt} and Purine Nucleotides *131*
9.7 Constitutive Activity of the β_2AR Coupled to Various $G\alpha_s$ Proteins *133*
9.8 Probing Models of GPCR Activation with β_2AR Coupled to Various Classes of G proteins *135*
9.9 Comparison of the Constitutive Activities of the β_1AR and the β_2AR *135*
9.10 Conclusions *136*

10 Constitutive Activity of β-Adrenoceptors: Analysis by Physiological Methods *141*
10.1 Introduction *141*
10.2 Constitutive Activity and Inverse Agonism: Definition and Detection *142*
10.3 β_1-Adrenoceptors *143*
10.3.1 Constitutive Activity of Overexpressed β_1ARs *143*

10.3.2 Is there any Evidence for a Physiological Effect of Constitutively Active Receptors in Normal Cardiomyocytes? *145*
10.3.3 Substates of the β_1AR: the Putative β_4AR *147*
10.4 β_2-Adrenoceptors *148*
10.4.1 Constitutive Activity of Overexpressed β_2ARs *148*
10.4.2 Inverse Agonism at the β_2AR *150*
10.4.3 βAR Antagonists: Inverse Agonists at β_2AR-G_s or Full Agonists at β_2AR-G_i? *152*
10.4.4 Involvement of the β_2AR in the "Putative β_4AR" Effect *153*
10.5 Homo- and Heterodimerization of β_1- and β_2ARs *154*
10.6 Conclusions *154*

11 Constitutive Activity at the α_1-Adrenoceptors: Past and Future Implications *159*
11.1 Introduction *159*
11.1.1 The α_1-Adrenoceptors: Main Structure–Functional Features *159*
11.1.2 The Discovery of Constitutively Activating Mutations and its Implications *161*
11.2 Theoretical and Experimental Approaches for Study of Constitutive GPCR Activity *162*
11.2.1 Theoretical Analysis of CAM GPCR Pharmacology *162*
11.2.2 Computational Modeling of the α_{1B}AR *163*
11.2.3 Measuring Constitutive Activity of the α_1AR Subtypes *165*
11.3 Constitutively Activating Mutations of the α_1AR Subtypes *166*
11.3.1 Where the Mutations have been Found *166*
11.3.2 Constitutive Activation of Multiple Signaling Pathways *169*
11.4 A Putative Model of Receptor Activation for the α_{1B}AR *169*
11.5 Constitutive Activity of Wild-type α_1ARs and Inverse Agonism *171*
11.5.1 Constitutive Activity of Wild-type α_1AR Subtypes *171*
11.5.2 Inverse Agonism at the α_1ARs *172*
11.6 Receptor Regulation and Constitutive Activity of the α_1ARs *173*
11.7 Conclusions *174*

12 Constitutive Activity of Muscarinic Acetylcholine Receptors: Implications for Receptor Activation and Physiological Relevance *177*
12.1 Introduction *177*
12.2 Constitutive Activity – Native Systems *178*
12.3 Constitutive Activity – Recombinant Systems *178*
12.4 Constitutive Activation by G Proteins *179*
12.5 Structure–Function Analysis of Receptor Activation *180*
12.5.1 Transmembrane Domain 3 *182*
12.5.2 Transmembrane Domain 6 *183*
12.5.3 Transmembrane Domain 7 *185*
12.5.4 Other Transmembrane Domains and Extracellular Domains *186*
12.5.5 Cytoplasmic Domains *186*
12.5.6 i3 Loop *187*

12.5.7 i2 Loop *188*
12.6 Structure–Function Model for Activation *189*
12.7 Conclusions *189*

13 Constitutively Active Histamine Receptors *195*
13.1 Introduction *195*
13.2 The Histamine Receptors *196*
13.2.1 The H_1R *197*
13.2.2 The H_2R *201*
13.2.3 The H_3R *202*
13.2.4 The H_4R *206*
13.3 Assay Systems for Detection of Constitutive Activity of Histamine Receptors *209*
13.3.1 Histamine Receptor Expression and the Detection of Constitutive Activity *209*
13.3.2 Changes in Intracellular Ca^{2+} *210*
13.3.3 [^{35}S]GTPγS Binding Assays (see also Chapter 8) *211*
13.3.4 IP_3 Formation (see also Chapter 8) *212*
13.3.5 cAMP Assays (see also Chapter 8) *212*
13.3.6 Measurements of Arachidonic Acid (AA) Release *213*
13.3.7 Reporter Gene Assays (see also Chapter 8) *213*
13.3.8 Activation of Kinases *214*
13.3.9 Effects of the Cellular Environment on Histamine Receptor Activity *214*
13.3.10 Construction and Expression of Constitutively Active Mutant Receptors *215*
13.3.11 Contamination with Endogenous Histamine *216*
13.4 Conclusions *216*

14 Constitutively Active Serotonin Receptors *223*
14.1 Introduction *223*
14.2 5-HT_{1A} Receptor (5-HT_{1A}R) *224*
14.3 5-HT_{1B} and 5-HT_{1D} Receptors (5-HT_{1B}R and 5-HT_{1D}R) *226*
14.4 5-HT_{2A} Receptor (5-HT_{2A}R) *228*
14.5 5-HT_{2C} Receptor (5-HT_{2C}R) *231*
14.6 Conclusion *237*

15 Virally Encoded Constitutively Active Chemokine Receptors *243*
15.1 Introduction *243*
15.1.1 Viral Strategies to Evade the Host Immune System *243*
15.1.2 Chemokines and Chemokine Receptors *243*
15.1.3 Viral Homologues of Chemokines and Chemokine Receptors and Viral Chemokine-binding Proteins *246*
15.2 The Human Cytomegalovirus-encoded Chemokine Receptor Homologue pUS28 *248*
15.2.1 Characteristics of Human Cytomegalovirus Infection *248*

15.2.2 Functional Characteristics of pUS28 *249*
15.2.3 Signaling Pathways Regulated by pUS28 *249*
15.2.4 Regulation of Transcriptional Activity by pUS28 *250*
15.2.5 Regulation of Constitutively Active pUS28 *252*
15.2.6 Cellular Functions of pUS28 *253*
15.3 The Human Kaposi's Sarcoma Virus-encoded Chemokine Receptor KSHV-GPCR *255*
15.3.1 Characteristics of Human Kaposi's Sarcoma Virus Infection *255*
15.3.2 Functional Characteristics of KSHV-GPCR *255*
15.3.3 Signaling Pathways Regulated by KSHV-GPCR *256*
15.3.4 Regulation of Transcriptional Activity by KHSV-GPCR *256*
15.3.5 Regulation of KSHV Activity by Chemokines *258*
15.3.6 Structure–Function Relationships of KHSV-GPCR *258*
15.3.7 Cellular Functions of KHSV-GPCR in vivo *259*
15.4 Conclusions *260*

Index *265*

Preface

Why do we address G protein coupled receptors (GPCRs) as drug targets and emphasize their constitutive activity in our series on „Methods and Principles in Medicinal Chemistry"? Taking into account that a large variety of currently used drugs are either agonists or antagonists at GPCRs, the broad interest of medicinal chemists in GPCRs is obvious. But why do we highlight the constitutive activity of GPCRs? When this phenomen was described first in the 1980's it was regarded with high scepticism and often attributed to experimental artefacts. Nevertheless, during the last two decades the phenomenon has gained more and more scientific attention and thus constitutive GPCR activity has been included in the theoretical and molecular models of GPCR activation. Consequently, GPCR ligands are today subgrouped into full agonists, partial agonists, neutral antagonist and inverse agonists. Interestingly, many of the clinically used „GPCR-blockers" turned out to be not neutral antagonists but inverse agonists at their respective receptors.

Therefore, the present book comprehensively discusses an important biological process that has not yet been covered in such depth in any other existing books on GPCRs. In the first part the international team of authors addresses in detail current models and concepts to introduce medicinal chemists, physiologists, pharmacologists and medical researchers into the advances in the understanding of GPCR activation and constitutive activity. In addition, the book provides a chapter with an overview on methods of investigating constitutive GPCR activity on a cellular and subcellular level. In the second part of the book, the knowledge on constitutive activity of selected important GPCR systems is described in more detail. This includes consequences of constitutive activity for drug action and side effects. Most important, one chapter of the book is attributed to the major unresolved issue of constitutive GPCR activity, i.e. its physiological, pathophysiological and therapeutic relevance.

The series editors believe that this book adds a fascinating facet to the series which is unique in its topic and presentation. We are indebted to the international consortium of highly distinguished authors for their contributions which reflect today's situation in biosciences, i.e., that scientists from many disciplines have to work together closely to advance our knowledge on such important but complex issues. We would like to thank Roland Seifert and Thomas Wieland for their enthusiasm to organize this volume. We also want to express our gratitude to Frank Weinreich from Wiley-VCH for his valuable contributions to this project.

May 2005

Raimund Mannhold, Düsseldorf
Hugo Kubinyi, Weisenheim am Sand
Gerd Folkers, Zürich

A Personal Foreword

When the first observations of constitutive (i.e., agonist-independent) activity of G protein-coupled receptors (GPCRs) were made in the mid-to-late 1980s, probably nobody expected that 15 years later this would be a central theme in the biomedical sciences. Indeed, it is now clear that a large fraction of wild-type GPCRs exhibit different degrees of constitutive activity. In addition, most GPCR antagonists known so far have actually turned out to be inverse agonists, and furthermore, mutations in GPCRs can result in exaggerated constitutive activity and severe human diseases. Analysis of constitutive GPCR activity has also given rise to profound insights into the molecular mechanisms of GPCR activation and is now even exploited for drug development, including ligand identification for orphan GPCRs. During the past 15 years, sophisticated models of constitutive GPCR activity have been developed, and are being continuously refined. We now have in hand a broad spectrum of sensitive experimental methods to study constitutive activity, and many of them can be implemented in most research laboratories. Despite all the progress in the field, a major unresolved question remains. What is the (patho)physiological and therapeutic relevance of constitutive GPCR activity?

Given the complexity of the field, it is not surprising that scientists from many disciplines – classic and molecular pharmacologists, molecular biologists, theoretical biochemists, physiologists, biophysicist, immunologists, neuroscientists, medicinal chemists, and clinical scientists – have made important contributions to the field. Several review articles on different aspects of constitutive GPCR activity are available, but given the multitude of aspects of the field, it is impossible for an individual scientist to write "the" ultimate in-depth review on this topic.

Bearing those thoughts in mind and considering the broad relevance of constitutive GPCR activity to many biomedical disciplines, we developed the idea of putting together a book that covers important aspects of the field. We are very happy that we were successful in motivating many key investigators in the field to contribute to this project. Intentionally, we do not seek to be comprehensive but rather to cover seminal aspects of the field without duplicating existing reviews.

While, of course, each author has her or his own point of view and interpretation of data, there is now general consensus in the scientific community that GPCRs exist in at least one inactive (R) and one active (R*) state. Throughout the book we have tried our best to ensure that consistent IUPHAR nomenclature of GPCRs, pharmacological

terms, and designation of amino acid mutations and the positions of amino acids in transmembrane domains are used in order to avoid confusion, and have also integrated cross-references between chapters to connect different aspects. You can start reading the book wherever you want. Each chapter has its own introduction and stands by itself as an entity. If you are interested in a particular GPCR, we refer you to Table 1.1 in Chapter 1, which will then guide you to the chapter(s) in which your GPCR of interest is discussed.

We are grateful to the authors of this book for their dedication, time, and willingness to consider our critique, suggestions, and formal requests. We are also thankful to editors of the book series and Dr. Frank Weinreich from WILEY-VCH for their advice in the planning stage of the book and to Irene Rupprecht and the staff of WILEY-VCH for bringing all the contributions into a suitable form. We hope that the book will be of use for basic and clinical scientists, experts and non-experts, seasoned scientists, undergraduate students, and graduate students and will serve as a starting point for solving the remaining problems in the field of constitutive GPCR activity.

Mannheim and Lawrence
May 2005 *Roland Seifert and Thomas Wieland*

List of Contributors

Remko A. Bakker
Leiden/Amsterdam Center
for Drug Research
Division of Medicinal Chemistry
Faculty of Chemistry
De Boelelaan 1083
1081 HV Amsterdam
The Netherlands

Martin Beinborn
Molecular Pharmacology
Research Center
Molecular Cardiology Research Institute
Tufts-New England Medical Center
Box 7703
750 Washington Street
Boston, MA 02111
USA

Joël Bockaert
CNRS UPR9023, CCIPE
141 rue de la Cardonille
34094 Montpellier Cedex 05
France

Richard A. Bond
Dept. of Pharmacological and
Pharmaceutical Sciences
University of Houston
521 Science and Research Bldg., 2
Houston, TX 77204-5037
USA

Ethan S. Burstein
ACADIA Pharmaceuticals Inc
3911 Sorrento Valley Boulevard
San Diego, CA 92121
USA

Sylvie Claeysen
CNRS UPR9023, CCIPE
141 rue de la Cardonille
34094 Montpellier Cedex 05
France

Tommaso Costa
Istituto Superiore di Sanit
Viale Regina Elena 299
00161 Rome
Italy

Susanna Cotecchia
Institut de Pharmacologie et de
Toxicologie
Faculté de Médecine
27 Rue du Bugnon
1005 Lausanne
Switzerland

Aline Dumuis
CNRS UPR9023, CCIPE
141 rue de la Cardonille
34094 Montpellier Cedex 05
France

Laurent Fagni
CNRS UPR9023, CCIPE
141 rue de la Cardonille
34094 Montpellier Cedex 05
France

Francesca Fanelli
Dulbecco Telethon Institute
and Department of Chemistry
University of Modena and Reggio Emilia
Via Campi 183
41100 Modena
Italy

Ulrik Gether
Molecular Neuropharmacology Group
Dept. of Pharmacology 16–18
Panum Inst.
University of Copenhagen
2200 Copenhagen N
Denmark

Peter Gierschik
Abteilung Pharmakologie
und Toxikologie
Universität Ulm
Albert-Einstein-Allee 11
89081 Ulm
Germany

Sian E. Harding
National Heart and Lung Institute
Imperial College School of Medicine
Dovehouse Street
London SW3 6LY
United Kingdom

Lutz Hein
Institut für Experimentelle und Klinische
Pharmakologie und Toxikologie
Universität Freiburg
Albertstraße 25
79104 Freiburg
Germany

Katharine Herrick-Davis
Center for Neuropharmacology
and Neuroscience, MC-136
Albany Medical College
47 New Scotland Ave.
Albany, NY 12208
USA

Lara Joubert
CNRS UPR9023, CCIPE
141 rue de la Cardonille
34094 Montpellier Cedex 05
France

Terry Kenakin
GlaxoSmithKline Research
and Development
5 Moore Drive
Research Triangle Park, NC 27709
USA

Alan S. Kopin
Molecular Pharmacology
Research Center
Molecular Cardiology Research Institute
Tufts-New England Medical Center
Box 7703
750 Washington Street
Boston, MA 02111
USA

Robert J. Lefkowitz
Dept. of Medicine
Duke University Medical Center
Box 3821, Rm. 467, CARL Bldg.
Durham, NC 27710
USA

Rob Leurs
Leiden/Amsterdam Center for
Drug Research
Division of Medicinal Chemistry
Faculty of Chemistry
De Boelelaan 1083
1081 HV Amsterdam
The Netherlands

Clive J. Lewis
St Georges Hospital
Blackshaw Road
Tooting
London, SW17 OQT
United Kingdom

Graeme Milligan
Molecular Pharmacology Group
Division of Biochemistry
and Molecular Biology
Institute of Biomedical and Life Sciences
Davidson Building
University of Glasgow
Glasgow G12 8QQ
Scotland, United Kingdom

Barbara Mӧpps
Abteilung Pharmakologie
und Toxikologie
Universität Ulm
Albert-Einstein-Allee 11
89081 Ulm
Germany

Søren G. F. Rasmussen
Molecular Neuropharmacology Group
Dept. of Pharmacology 16–18
Panum Inst.
University of Copenhagen
2200 Copenhagen N
Denmark

Ursula Ravens
Institut für Pharmakologie
und Toxikologie
Technische Universität Dresden
Fetscherstr. 74
01307 Dresden
Germany

Alexander Scheer
Serono International S.A.
15 bis, chemin des Mines
Case postale 54
1211 Geneva 20
Switzerland

Roland Seifert
Lehrstuhl für Pharmakologie
und Toxikologie
Universität Regensburg
Universitätsstraße 31
93053 Regensburg
Germany

Tracy A. Spalding
Genomics Institute of the
Novartis Research Foundation
10675 John Jay HopkinsDrive
San Diego CA 92121
USA

Thomas Wieland
Institut für Pharmakologie
und Toxikologie
Fakultät für Klinische Medizin
Mannheim der Universität Heidelberg
Maybachstrasse 14
68169 Mannheim
Germany

Abbreviations and Terminology

α-adrenoceptor	αAR
α-adrenoceptor, subtype 1	α_1AR, α_{1A}AR, α_{1B}AR, α_{1D}AR
α-adrenoceptor, subtype 2	α_2AR, α_{2A}AR, α_{2B}AR, α_{2D}AR
(*S*)-(+)-α-fluoromethylhistidine	α-FMH
β-adrenoceptor	βAR
β-adrenoceptor, subtype 1	β_1AR
wild-type β_1AR	β_1AR_{wt}
β-adrenoceptor, subtype 2	β_2AR
constitutively active β_2AR mutant	β_2AR_{CAM}
wild-type β_2AR	β_2AR_{wt}
β_3-adrenoceptor	β_3AR
κB *cis*-enhancer element	κB
arachidonic acid	AA
(4'-[3-((3*R*)-3-dimethylaminopyrrolidin-1-yl) propoxy]biphenyl-4-carbonitrile	A-331440
adenylyl cyclase	AC
AC isoforms I to IX	AC I to IX
adenosine deaminases	ADAR1 and ADAR2
attention-deficit hyperactivity disorder	ADHD
4-*n*-butyl-1-[4-(2-methylphenyl)-4-oxo-1-butyl]-piperidine hydrogen chloride	AC-42
agouti-related protein	AgRP
(–)-alprenolol	ALP
adapter protein	AP
activator protein 1	AP1
activator protein 1/tetradecanoyl phorbol acetate-response element	AP-1/TRE
adrenoceptor	AR
arginine	Arg
aspartate	Asp

angiotensin II type 1 receptor	AT_1R
angiotensin II subtype 1A receptor	$AT_{1A}R$
leukotriene B_4-receptor	BLTR
2-bromolysergic acid diethylamide	BOL
bradykinin receptor	BR
bradykinin B2-receptor	B_2R
bioluminescence resonance energy transfer	BRET
complement C5a receptor	C5aR
constitutively active mutant	CAM
cyclic AMP	cAMP
cAMP enzyme immunoassay	cAMP-EIA
Ca^{2+}/calmodulin-dependent protein kinases	CaMKs
(*Rp*)-adenosine-3':5'-cyclic monophosphothioate triethylamine	(*Rp*)-cAMPS
capri pox virus	CaPV
calcium-sensing receptor	CaSR
calcium-permeable voltage-sensitive channel subunit 2.1	Cav2.1
cannabinoid receptor	CBR
cholecystokinin receptor	CCKR
cholecystokinin receptor subtype 2	CCK_2R
[(±)-4-(3-*tert*-butylamino-2-hydroxypropoxy) benzimidazol-2-one]	CGP 12177A
(±)-2-hydroxy-5-[2-({2-hydroxy-3-[4-(1-methoxy-4-trifluoromethyl-1*H*-imidazol-2-yl)phenoxy]propyl}amino) ethoxy]-benzamide	CGP 20712
(±)-2-hydroxy-5-[2-({2-hydroxy-3-[4-(1-methyl-4-trifluoromethyl-1*H*-imidazol-2-yl)phenoxy]propyl}amino) ethoxy]-benzamide monomethanesulfonate	CGP 20712A
nicotinoyl-Tyr-Lys(Z-Arg)-His-Pro-Ile-OH	CGP42112A
Chinese hamster ovary	CHO
Chinese hamster fibroblast	CHW
C terminus of the i3 loop	Ci3
casein kinase 2	CK2
chemokine-binding protein	CKBP
cytomegalovirus	CMV
cyclic nucleotide-gated	CNG
African green monkey kidney cells	COS-7
counts per minute	cpm
1-(3-chlorophenyl)piperazine	*m*-CPP
cAMP response element	CRE
cow pox virus	CPV
cAMP-response element binding protein	CREB
cAMP-response element binding protein/cAMP response element	CREB/CRE

cyclosporin H	CsH
carboxy-terminal	C-t
cubic ternary complex model	CTC model
cytotoxic T lymphocyte antigen 4	CTLA-4
chemokine receptors	CXCR, CCR
cysteine	Cys
DADLE	([D-Ala^2, D-Leu]enkephalin)
1,2-diacylglycerol	DAG
4-diphenylacetoxy-*N*-methylperidine.	4-DAMP
dihydroalprenolol	DHA
Dulbecco's modified Eagle medium	DMEM
4-iodo-2,5-dimethoxyphenylisopropylamine	DOI
δ-opioid receptor	DOP(δ)R
dopamine receptor	DR
dopamine receptor, subtype 1	D_1R, $D_{1A}R$
dopamine receptor, subtypes 2 and 3	D_2R, D_3R
Asp-Arg-Tyr motif	DRY
Epstein Barr virus	EBV
effective concentration 50%	EC_{50}
extracellular domain	ECD
extracellular loop	ECL
extracellular loop 2	ECL2
enhanced green fluorescent protein and the pleckstrin homology domain of the PLCδ1	EGFP-$PH_{PLC\delta}$
epidermal growth factor receptor	EGFR
equine herpes virus type 2	EHV-2
nitric oxide synthase type 3	eNOS
prostaglandin E_2 receptor	EPR
electron paramagnetic resonance spectroscopy	EPR
prostaglandin E_2 receptor, subtype 3	EP_3R or $EP_{3\gamma}R$
endoplasmic reticulum	ER
extracellular signal-related kinase	ERK
extracellular signal-regulated protein kinase 1/2	ERK1/2
Glu-Arg-Leu motif	ERL motif
extended ternary complex model	ETC model
enabled Vasp homology	EVH
Ena/VASP homology 1/Wiskott–Aldrich syndrome protein homology 1	EVH1/WH1
focal adhesion kinase	FAK
Federal Drug Administration	FDA
N-formyl-L-methionyl-L-leucyl-L-phenylalanine	fMLP

prostaglandin $F_{2\alpha}$ receptor	FPR
formyl peptide receptor	FPR1
fluorescence resonance energy transfer	FRET
follicle stimulating hormone receptor	FSHR
Fourier transform infrared	FTIR
γ-aminobutyric acid	GABA
γ-aminobutyric acid receptor, subtype B	$GABA_BR$: $GABA_{B1}R$ (GBR1) and $GABA_{B2}R$ (GBR2)
glycosaminoglycan	GAG
guanosine 5'-diphosphate	GDP
guanine nucleotide exchange factor	GEF
green fluorescent protein	GFP
G protein-coupled receptor interacting proteins (or GPCR interacting proteins)	GIPs
glutamate	Glu
gonadotropin-releasing hormone receptor	GnRHR
guinea pig	gp
G protein-coupled receptor	GPCR
guanosine 5'-[β,γ-imido]diphosphate	GppNHp
guanine nucleotide binding protein	G protein
3-[3-(dimethylamino)propyl]-4-hydroxy-*N*-[4-(4-pyridinyl)phenyl]benzamide dihydrochloride	GR-55562
G protein-coupled receptor kinase	GRK
glycogen synthase kinase 3β	GSK3β
guanosine 5'-triphosphate	GTP
guanosine 5'-[γ-thio]triphosphate	GTPγS
Hank's buffered saline solution	HBSS
human cytomegalovirus	HCMV
heptahelical domain	HD
histidine decarboxylase	HDC
human embryonic kidney	HEK
human herpes virus *n*	HHV*n*
5-hydroxyindoleacetic acid	5-HIAA
hypoxia-inducible factor 1α	HIF-1α
human immunodeficiency virus	HIV
histamine receptor	HR
histamine receptors, subtypes 1 to 4	H_1R, H_2R, H_3R, H_4R
5-hydroxytryptamine or serotonin	5-HT
5-hydroxytryptamine receptor	5-HTR
5-hydroxytryptamine receptor, subtype 1	5-HT_1R, 5-$HT_{1A}R$
5-hydroxytryptamine receptor, subtype 2	5-HT_2R, 5-$HT_{2C}R$

5-hydroxytryptamine receptor, subtypes 3 to 7	5-HT_3R, 5-HT_4R, 5-HT_5R, 5-HT_6R, 5-HT_7R
herpes virus saimiri	HVS
intracellular loop 2	i2
intracellular loop 3	i3
N,N'-dimethyl-*N*-(iodoacetyl)-*N'*-(7-nitrobenz-2-oxa-1,3-diazol-4-yl)-ethylenediamide	IANBD
isobutylmethylxanthine	IBMX
inhibitor concentration 50 %	IC_{50}
peak L-type Ca^{2+} current	I_{Ca}
intercellular adhesion molecule-1	I-CAM-1
((±)-1-[(7-methyl-2,3-dihydro-1*H*-inden-4-yl)oxy]-3-[(1-methylethyl)amino]-2-butanol) or *erythro*-DL-1-(7-methylindan-4-yloxy)-3-isopropylaminobutan-2-ol	ICI 118 551
[*N,N'*-diallyl-Tyr^1,$Aib^{2,3}$]Leu^5-enkephalin	ICI 174 864
immediate-early	IE
hyperpolarization-activated current	I_f
muscarinic acetylcholine receptor-gated atrial potassium channel	I_{KACh}
"unedited" ($Ile^{156(3.54)}$, $Asn^{158(3.56)}$, $Ile^{160(3.58)}$) isoform of human brain 5-HT_{2C}R	INI
inositol phosphate	IP
inositol bisphosphate	IP_2
inositol 1,4,5-trisphosphate	IP_3 or InsP_3
(–)-isoproterenol	ISO
inosine 5'-triphosphate	ITP
International Union of Pharmacology	IUPHAR
Janus kinase/signaling transducer and activator of transcription	Jak/STAT
1-[(5-chloro-1*H*-indol-2-yl)carbonyl]-4-methylpiperazine	JNJ7777120
c-Jun amino-terminal kinase	JNK
dissociation constant	K_d
knock-out	KO
Kaposi's sarcoma	KS
Kaposi's sarcoma herpes (or "sarcoma-associated") virus	KSHV
KS-derived KSHV-negative endothelial cell line	KSIMM
luteinizing hormone	LH
luteinizing hormone receptor	LHR
littermate	LM
lysergic acid diethylamide	LSD

lumpin skin disease virus	LSDV
leukotriene B_4	LTB_4
mitogen-activated protein	MAP
mitogen-activated protein kinase	MAPK
[INLKALAALAKALL-NH_2]	Mas-7
mouse cytomegalovirus	MCMV
melanocortin receptor	MCR
melanocortin receptor, subtypes 1, 3 and 4	MC_1R, MC_3R, MC_4R
molluscum contagiosum virus	MCV
molecular dynamics	MD
1-[2-(4-fluorophenyl)ethyl]-4-piperidinemethanol	MDL100907
Marek's disease virus	MDV
metabotropic glutamate receptor	mGluR
metabotropic glutamate receptor subtype 1	$mGlu_1R$
metabotropic glutamate receptor subtype 5	$mGlu_5R$
major histocompatibility class	MHC
murine γ-herpes virus 68	MHV68
μ-opioid receptor	MOP(μ)R
muscarinic acetylcholine receptor	MR
muscarinic acetylcholine receptor, subtypes 1 to 5	M_1R, M_2R, M_3R, M_4R, M_5R
(–)-5,9α-diethyl-2-(3-furyl-methyl)-2'-hydroxy-6,7-benzomorphan	MR 2266
ethylammonium methanethiosulfonate	MTSEA
myxoma virus	MV
naloxone benzoylhydrazone	NalBzOH
Na^+/Ca^{2+}-exchanger	NCX
nuclear factor κB	NF-κB
nuclear factor κB/NF-κB cis-enhancer element	NF-κB/κB
nuclear factor of activated T cells	NFAT
N terminus of the i3 loop	Ni3
N-methyl scopolamine	NMS
nitric oxide/cGMP-dependent protein kinase	NO PKG
former acronym for nucleotide-binding protein (= G protein)	N protein
nucleoside 5'-triphosphate	NTP
8-hydroxy-2-(di-*n*-propylamino)tetralin	8-OH-DPAT
opoid receptor	OPR
"open reading frame"	ORF
((–)-2-cyano-1-methyl-3-[(2*R*,5*R*)-5-(1*H*-imidazol-4(5)-yl)tetrahydrofuran-2-ylmethyl]guanidine	OUP-16

p38-mitogen activated kinase	p38 MAPK
platelet-activating factor receptor	PAFR
1-(1-phenylcyclohexyl)piperidine	PCP
phosphodiesterase	PDE
PSD95/DLG/ZO-1	PDZ
prostaglandin $F_{2\alpha}$	$PGF_{2\alpha}$
pleckstrin homology domain of PLCδ1	$PH_{PLC\delta}$
post infection	p.i.
phosphatidylinositol 3'-kinase	PI3-Kinase, PI3K
phosphatidylinositol 4,5-bisphosphate	PIP_2
protein kinase A	PKA
protein kinase B	PKB
protein kinase C	PKC
cGMP-dependent protein kinase	PKG
phospholipase A_2	PLA_2
phospholipase C	PLC
phospholipase C-β isozymes	PLCβs
phospholipase D	PLD
proline	Pro
parathyroid hormone	PTH
parathyroid hormone receptor subtype 1	PTH1R
pertussis toxin	PTX
proline-rich kinase 2	Pyk2
quinuclidinyl benzilate	QNB
inactive form of a GPCR	R
active form of a GPCR	R*
related adhesion focal tyrosine kinase	RAFTK
ground state form of a GPCR	Rg
regulator of G protein signaling	RGS
rho guanine nucleotide exchange factor	RhoGEF
(*S*)-2-(6-chloro-5-fluoroindol-1-yl)-1-methylethylamine hydrochloride	Ro 60-0175
receptor selection and amplification technology	R-SAT
respiratory syncytial virus	RSV
ryanodine receptors	RyRs
(*N*-[1-(2,3-dihydro[1,4]dioxin-5-yl)piperidin-4-yl] indan-2-ylamine)	S18127
5-methyl-1-(3-pyridylcarbamoyl)-1,2,3,5-tetrahydropyrrolo [2,3-f]indole hydrochloride	SB-206553
1'-methyl-5-{[2'-methyl-4'-(5-methyl-1,2,4-oxadiazol-3-yl) biphenyl-4-yl]carbonyl}-2,3,6,7-tetrahydrospiro[furo [2,3-f]indole-3,4'-piperidine] oxalate	SB-224289

1'-ethyl-5-[2'-methyl-4'-(5-methyl-1,3,4-oxadiazol-2-yl) biphenyl- 4-carbonyl]-2,3,6,7-tetrahydrospiro[furo [2,3-f]indole-3,4'-piperidine]	SB-224289
6-chloro-5-methyl-1-[6-(2-methylpyridin-3-yloxy) pyridin-3-ylcarbamoyl]indoline	SB-242084
5-methyl-1-{2-[(2-methyl-3-pyridyl)oxy]-5-pyridyl]carbamoyl}- 6-trifluoromethylindoline hydrochloride	SB-243213
substituted cysteine accessibility method	SCAM
sodium dodecylsulfate	SDS
Spodoptera frugiperda	Sf9
smooth muscle cell	SMC
single nucleotide polymorphism	SNP
surface plasmon resonance	SPR
swine pox virus	SPV
N-(piperidin-1-yl)-5-(4-chlorophenyl)-1-(2,4-dichlorophenyl)- 4-methyl-pyrazole-3-carboxamide	SR 141716A
serum response element	SRE
serum response factor	SRF
somatostatin 2-receptor	$SRIF_2R$
simian virus 40	SV40
trichloroacetic acid	TCA
T cell factor	TCF
transmembrane (domain)	TM
*n*th transmembrane domain	TM*n*
receptor for thromboxane A_2	TPR
thyroid stimulating hormone	TSH
tetradecanoyl phorbol acetate-response element	TRE
thyroid stimulating hormone receptor	TSHR
thyrotropin-releasing hormone receptor	TRHR
transient receptor channels 1 and 4	TRPC1, TRPC4
5-bromo-*N*-(4,5-dihydro-1*H*-imidazol-2-yl)-6-quinoxalinamine	UK14304
vasopressin receptor	VR
vasodilator-stimulated phosphoprotein	VASP
viral Bcl-2	vBCL-2
vascular cell adhesion molecule-1	VCAM-1
vascular endothelial growth factor	VEGF
"fully edited" ($Val^{156(3.54)}$, $Gly^{158(3.56)}$, $Val^{160(3.58)}$) $5\text{-}HT_{2C}R$ isoform identified in human brain.	VGV
viral interferon factor 1	vIRF-1
1-[5-(imidazol-4-yl)pentyl)-3-(4-chlorophenylmethyl)thiourea	VUF 4742
[5-(1*H*-imidazol-4-yl)-pentyl]-isopropyl-amine	VUF 4904
4-[3-(1*H*-imidazol-4-yl)propyl]piperidine	VUF 5681

(5-chloro-1*H*-benzo[d]imidazol-2-yl)-(4-methylpiperazin-1-yl)methanone	VUF 6002
vaccinia virus	VV
N-{2-[4-(2-methoxyphenyl)piperazin-1-yl]ethyl}-N-(2-pyridyl)-cyclohexanecarboxamide	WAY 100635
xanthosine 5'-triphosphate	XTP
neuropeptide Y receptor	YR
neuropeptide Y receptor, subtype 1, 2, and 4	Y_1R, Y_2R, Y_4R
Yaba-like disease virus	YLDV