Cell Membrane Receptors for Drugs and Hormones:
A Multidisciplinary Approach

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Ralph W. Straub, M.D. Liana Bolis, M.D.

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Ralph W. Straub, M.D.
Professor and Chairman
Department of Pharmacology
University of Geneva
Geneva, Switzerland

Liana Bolis, M.D.
Professor and Chairman
Department of General Physiology
University of Messina
Messina, Italy

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Preface

The concept of receptors has gained increasing importance in pharmacology during the past few years, and numerous important studies have been designed to isolate and purify cell membrane receptors for neurotransmitters, drugs, and hormones. This book provides a multidisciplinary approach to the problem of cell membrane receptors. Studies on the morphology and chemical composition of the cell membrane are described, followed by chapters on the morphology, biochemistry, and mode of action of receptors.

A great variety of methods used in these studies are discussed, including immunological identification of receptors, light microscopy and ultrastructural techniques, measurements of the kinetics of drug binding by observation of membrane noise, measurements of transmembrane potentials and currents, transmembranal tracer fluxes, as well as methods of classic physiology.

Other contributions discuss biochemical techniques for the purification and characterization of receptors and for studying the binding of drugs and hormones. The detailed mode of action of receptors is described mainly in relation to ion transport and fluxes. Here again, various approaches are considered, including the use of specific inhibitors, altering the composition of natural membranes, optical studies of the mobility of membrane proteins, and the reconstitution of transport systems in an artificial environment.

It is hoped that this volume will stimulate collaboration among specialists in a spectrum of disciplines, including pharmacologists, biochemists, physiologists, and molecular biologists, who are engaged in the study of cell membrane receptors.

Ralph W. Straub
Geneva, Switzerland

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Contributors

J. H. Abel, Jr.
Department of Physiology and Biophysics
Colorado State University
Fort Collins, Colorado 80523

Beatrice M. Anner
Department of Cell Biophysics
Baylor College of Medicine and The Fondren-Brown Cardiovascular Research and Training Center of the Methodist Hospital
Houston, Texas 77030

Brian H. Barber
National Institute for Medical Research
Mill Hill
London NW7 1AA, England

Etienne-Emile Baulieu
Unité de Recherches sur le Métabolisme Moléculaire et la Physio-Pathologie des Stéroides
Institut National de la Santé et de la Recherche Médicale
Département de Chimie Biologique
Faculté de Médecine de Bicêtre
94270 Bicêtre, France

Bernhard F. Becker
Physiologisches Institut der Universität München
D-8000 München 2, Germany

Konrad Bloch
Department of Chemistry
Harvard University
Cambridge, Massachusetts 02138

V. L. Borovjagin
Institute of Biophysics
USSR Academy of Sciences
Moscow State University
Moscow, USSR

Emily T. Brake
Biology Division
Oak Ridge National Laboratory
Oak Ridge, Tennessee 37830

Geoffrey Burnstock
Department of Anatomy and Embryology
University College London
Gower Street
London WC1E 6BT, England

Mitzy Canessa
Departments of Medicine and Physiology
Massachusetts General Hospital and Harvard Medical School
Boston, Massachusetts

T. T. Chen
Department of Physiology and Biophysics
Colorado State University
Fort Collins, Colorado 80523

R. J. Cherry
Eidgenössische Technische Hochschule
Laboratorium für Biochemie
ETH-Zentrum
CH-8092 Zürich, Switzerland

D. Colquhoun
Department of Pharmacology
St. George's Hospital Medical School
London SW 17 ORE, England

John S. Cook
Biology Division
Oak Ridge National Laboratory
Oak Ridge, Tennessee 37830

J. L. Cousin
Laboratoire de Physiologie Cellulaire
Faculté des Sciences
Parc Valrose
06034 Nice Cedex, France

R. K. Crane
Department of Physiology
College of Medicine and Dentistry of New Jersey
Rutgers Medical School
Piscataway, New Jersey 08854

Michael J. Crumpton
National Institute for Medical Research
Mill Hill
London NW7 1AA, England

M. A. Diekman
Department of Physiology and Biophysics
Colorado State University
Fort Collins, Colorado 80523

Jochen Duhm
Physiologisches Institut der Universität München
D-8000 München 2, Germany

Mark H. Ellisman
Department of Neurosciences
School of Medicine
University of California, San Diego
La Jolla, California 92093

D. B. Endres
Department of Physiology and Biophysics
Colorado State University
Fort Collins, Colorado 80523

P. Fairclough
Department of Physiology
College of Medicine and Dentistry of New Jersey
Rutgers Medical School
Piscataway, New Jersey 08854

J. Ferrero
Département de Pharmacologie
École de Médecine
1211 Genève 4, Switzerland

Jorgen Funder
Department of Medical Physiology A and Department of Biophysics
The Panum Institute
University of Copenhagen
DK-2200 Copenhagen N, Denmark

Peter Geck
Abteilung für Physikalische Biochemie
Gustav-Embden-Zentrum der Biologischen Chemie
J. W. Goethe Universität
6000 Frankfurt am Main 70, Germany

G. Giebisch
Department of Physiology
Yale University School of Medicine
New Haven, Connecticut 06510

D. G. Haylett
Department of Pharmacology
University College London
Gower St.
London WC1E 6BT, England

Erich Heinz
Abteilung für Physikalische Biochemie
Gustav-Embden-Zentrum der Biologischen Chemie
J. W. Goethe Universität
6000 Frankfurt am Main 70, Germany

Ernst J. M. Helmreich
Department of Physiological Chemistry
University of Würzburg
8700 Würzburg, Germany

C. Sue Hudson
Department of Pharmacology and Experimental Therapeutics
University of Maryland School of Medicine
Baltimore, Maryland 21201

D. H. Jenkinson
Department of Pharmacology
University College London
Gower St.
WC 1E 6BT London, England

P. Jirounek
Département de Pharmacologie
École de Médecine
1211 Genève 4, Switzerland

K. Koller
Department of Pharmacology
University College London
Gower St.
London WC1E 6BT, England

Pedro Labarca
Departments of Medicine and Physiology
Massachusetts General Hospital and Harvard Medical School
Boston, Massachusetts

Alexander Leaf
Departments of Medicine and Physiology
Massachusetts General Hospital and Harvard Medical School
Boston, Massachusetts

P. Malathi
Department of Physiology
College of Medicine and Dentistry of New Jersey
Rutgers Medical School
Piscataway, New Jersey 08854

M. C. McClellan
Department of Physiology and Biophysics
Colorado State University
Fort Collins, Colorado 80523

Nicola Medici
Institute of General Pathology
School of Medicine and Surgery
University of Naples
Naples, Italy

Maria Molinari
Institute of General Pathology
School of Medicine and Surgery
University of Naples
Naples, Italy

Bruno Moncharmont
Institute of General Pathology
School of Medicine and Surgery
University of Naples
Naples, Italy

R. Motais
Laboratoire de Physiologie Cellulaire
Faculté de Sciences
Parc Valrose
06034 Nice Cedex, France

U. Müller
Eidgenössische Technische Hochschule
Laboratorium für Biochemie
ETH-Zentrum
CH-8092 Zürich, Switzerland

G. D. Niswender
Department of Physiology and Biophysics
Colorado State University
Fort Collins, Colorado 80523

G. J. V. Nossal
Walter & Eliza Hall Institute of Medical Research
Royal Melbourne Hospital
Victoria 3050, Australia

H. Passow
Max Planck Institute for Biophysics
6000 Frankfurt/Main, Germany

Peter Petrusz
Departments of Anatomy and Pharmacology and
Laboratory for Reproductive Biology
University of North Carolina
Chapel Hill, North Carolina 27514

Barbara Pfeiffer
Abteilung für Physikalische Biochemie
Gustav-Embden-Zentrum der Biologischen Chemie
J. W. Goethe Universität
6000 Frankfurt am Main 70, Germany

Thomas Pfeuffer
Department of Physiological Chemistry
University of Würzburg
8700 Würzburg, Germany

Christian Pietrzyk
Abteilung für Physikalische Biochemie
Gustav-Embden-Zentrum der Biologischen Chemie
J. W. Goethe Universität
6000 Frankfurt am Main 70, Germany

M. P. Pineda
Department of Physiology and Biophysics
Colorado State University
Fort Collins, Colorado 80523

V. G. Plakunova
Institute of Biophysics
USSR Academy of Sciences
Moscow State University
Moscow, USSR

H. Preiser
Department of Physiology
College of Medicine and Dentistry of New Jersey
Rutgers Medical School
Piscataway, New Jersey 08854

Giovanni Alfredo Puca
Institute of General Pathology
School of Medicine and Surgery
University of Naples
Naples, Italy

Robert D. Purves
Department of Anatomy and Embryology
University College London
Gower Street
London WC1E 6BT, England

John E. Rash
Department of Pharmacology and Experimental Therapeutics
University of Maryland School of Medicine
Baltimore, Maryland 21201

J. M. Ritchie
Department of Pharmacology
Yale University School of Medicine
New Haven, Connecticut 06510

M. Rouiller
Département de Pharmacologie
École de Médecine
1211 Genève 4, Switzerland

A. Salamin
Département de Pharmacologie
École de Médecine
1211 Genève 4, Switzerland

Madhabananda Sar
Departments of Anatomy and Pharmacology and
Laboratories for Reproductive Biology
University of North Carolina
Chapel Hill, North Carolina 27514

H. R. Sawyer
Department of Physiology and Biophysics
Colorado State University
Fort Collins, Colorado 80523

M. H. J. Schmitz
Department of Physiology and Biophysics
Colorado State University
Fort Collins, Colorado 80523

G. Schneider
Eidgenössische Technische Hochschule
Laboratorium für Biochemie
ETH-Zentrum
CH-8092 Zürich, Switzerland

R. W. Straub
Département de Pharmacologie
École de Médecine
1211 Genève 4, Switzerland

Walter E. Stumpf
Departments of Anatomy and Pharmacology and
Laboratories for Reproductive Biology
University of North Carolina
Chapel Hill, North Carolina 27514

Frank S. Walsh
National Institute for Medical Research
Mill Hill
London NW7 1AA, England

Jens Otto Wieth
Department of Medical Physiology A and
Department of Biophysics
The Panum Institute
University of Copenhagen
DK-2200 Copenhagen N, Denmark

W. Wilbrandt
Pharmakologisches Institut
3000 Berne, Switzerland

Contents

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Some Speculations on the Structure and Function of the Sterol Molecule

Konrad Bloch

Department of Chemistry, Harvard University, Cambridge, Massachusetts 02138

This chapter considers certain unique structural features of the sterol molecule and argues that cholesterol and the closely related fungal and plant sterols were shaped to functional perfection by selection pressures. In eukaryotic membranes the sterol molecule is an invariant component of the plasma membrane along with phospholipids. What makes it inviting to rationalize the sterol architecture are the complementary interactions of these two lipids in cell membranes. Only in this context but not as an isolated molecule can the structure of cholesterol be understood.

Oparin and Haldane were the first to speculate on the ultimate origin of biological molecules. According to their hypotheses the major structural types that compose contemporary cells were created chemically before life began. This process of prebiotic evolution is believed to have taken place in an oxygen-free terrestrial atmosphere, the absence of oxygen preserving organic molecules from destruction. In recent years chemically reasonable schemes for the prebiotic synthesis of sugars, amino acids, and nucleic acid precursors have been proposed, and some of these syntheses have been achieved experimentally under anaerobic "primitive earth" conditions. Moreover, the very existence of strictly anaerobic cells lends credence to the hypothesis that life began in an oxygen-free environment, the rich prebiotic soup providing a reservoir of organic molecules for sustaining the predominantly heterotrophic life mode of ancestral cells. However, if we accept the notion of an anaerobic atmosphere on the primitive earth, then the potentialities for chemical creation must have been severely restricted. It is this limitation that forces one to conclude that cholesterol or related prototypes could not have been produced by chemical evolution unless we invent pathways that are without chemical or biochemical precedent. Sterols are exceedingly rare in prokaryotes and apparently missing entirely in anaerobic bacteria. We conclude that the advent of aerobic cells was a prerequisite for the creation of the sterol structure (2,4).

There is sufficient chemical precedent for visualizing how isoprene units, isoprene polymers, and even squalene might have been formed abiotically. Yet there is no chemically plausible way for transforming the acyclic squalene to the hydroxyl-bearing sterol ring system. In the contemporary pathway the prod-

uct of oxidative cyclization is lanosterol, or 4,4',14-trimethylcholestadienol (3). *Per se* lanosterol does not seem to have any metabolic function; it does not accumulate intracellularly as an end product in any cell. Rather, it is an intermediate that rapidly loses the three extra methyl groups to yield the cholesterol structure. This demethylation is an oxidative process requiring oxygen as the compulsory electron acceptor (18). Thus, unless one postulates some arbitrary pathway of which no vestiges have remained, the conclusion seems inescapable that the postsqualene steps of cholesterol biosynthesis evolved during the aerobic phase of biological evolution.

The next and major point to be raised is to inquire why the sterol pathway proceeded beyond lanosterol. Stereochemically cholesterol and its trimethyl derivative lanosterol are identical. If evolutionary pressure caused these "extra" methyl groups to be removed, the demethylated structure must be functionally more competent for essential cellular events.

The competence of cholesterol and the inability of lanosterol to replace it in biological systems has been observed in several instances, three of which will be cited. In anaerobic yeast, an artificial sterol auxotroph, lanosterol, supports growth but only poorly compared to cholesterol or ergosterol (14). Second, for insects that are nutritionally dependent on an exogenous sterol, lanosterol and also 4,4'-dimethylcholesterol are totally ineffective in supporting larval development, nor do they have a cholesterol-sparing effect (8). Finally, Vagelos and co-workers have recently described a sterol-requiring mutant of Chinese hamster ovary cells in which the sterol pathway is blocked at the lanosterol stage (7). Such cells grow normally on cholesterol, whereas in the absence of sterol supplement lanosterol accumulates intracellularly. Such cells rapidly die and lyse. Clearly, the trimethyl cholestane derivative cannot replace cholesterol in sustaining normal intracellular function in representative eukaryotic systems.

Experiments with vesicles prepared from sonicated phosphatidylcholine have revealed some of the structural features required for productive interaction between sterols and phospholipids in membranes as measured by solute permeability (9). For example, incorporation of cholesterol into such vesicles will drastically reduce the exit of trapped glucose in parallel with lowering of membrane fluidity. Only sterols having a free equatorial β-hydroxy group, the ring stereochemistry of the cholestane system, and an aliphatic side chain will modify membrane properties in the same direction as cholesterol, that is, restrain glucose transport (9). We have recently carried out similar experiments with lecithin vesicles containing lanosterol or several of the alkylated derivatives that normally arise in the cholesterol pathway (Lin, Lala, and Bloch, *unpublished*) (Fig. 1). In this model system the effect of lanosterol is marginal, glucose exit occurring at nearly the same rate as in sterol-free membranes. On the other hand, 4,4'-dimethylcholesterol, 4α-methylcholesterol, and 4β-methylcholestenol show nearly the same retardation on glucose exit as cholesterol itself. Two synthetic, not naturally occurring sterols were included in this series, 3α-methylcholesterol and 4,4'-diethylcholestanol. The former compound has no effect on glucose transport, whereas the latter reduces this rate moderately.

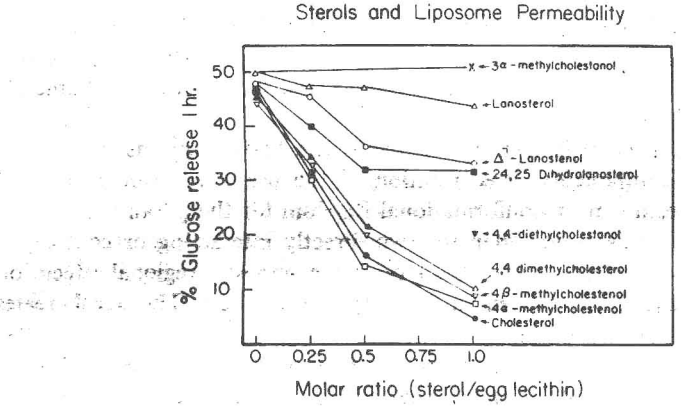

FIG. 1. Liposomes containing sterols were prepared from egg lecithin and 5 mole % of dicetylphosphate and assayed for release of trapped glucose by the method of Demel et al. (9). After dialysis, aliquots of the liposomes were assayed enzymatically before and after incubation for 1 hr at 40°C.

The powerful tool of NMR spectroscopy has recently been applied to a study of the behavior of sterol in membranes (20), and here again the results obtained with cholesterol and lanosterol differ strikingly. In lecithin vesicles containing cholesterol and phospholipid in a ratio of 2:1, no ^{13}C resonances are seen. The molecule is strongly immobilized by interaction with phospholipid. By contrast, when lanosterol replaces cholesterol, the ^{13}C-NMR spectrum displays several resonances not arising from phospholipid. These have been assigned to the ring carbons C_{14} and C_{13}, to C_{21} or C_{19}, and to C_{18} or C_{30} of lanosterol. With 4,4-dimethylcholesterol-containing membranes the ^{13}C-NMR spectrum is not significantly different from that of cholesterol-containing membranes. In summary, the results obtained by ^{13}C-NMR spectroscopy and by measuring glucose transport are complementary by showing that lanosterol is markedly more mobile in model phospholipid membranes than partially (4,4-dimethyl-) or fully demethylated lanosterol metabolites. We are therefore led to conclude that the 14α-CH_3 group of lanosterol critically interferes with the productive incorporation of this sterol into membranes.

According to current views on the arrangement of sterol in membranes, certain complementary regions of cholesterol and phospholipids favor the packing between the two ligands such as to produce stoichiometric complexes (6,16). Cholesterol can be stably accommodated between the long phospholipid acyl chains owing to the conformationally restrained and therefore immobile tetracyclic ring system. Moreover, the α face or underside of cholestane is flat because five axial hydrogen atoms (at C_1,C_3,C_7,C_9, and C_{14}) lie in a single plane. If we adopt the assumption (6,12,20) that the sterol OH group engages in essential hydrogen bonding with one of the carbonyl ester oxygens at the polar head group of phospholipid, then the planar sterol α face (the ring region) will be positioned suitably for van der Waals contacts with the C_2–C_8 or C_9 segment

of a fatty acyl chain in the all-*trans* conformation. The immobilizing sterol effect on the conformationally less restrained fatty acids will therefore be exerted on the bilayer region near the water interface and extend to approximately the midpoint of a half-bilayer.

Little interaction is to be expected between the "tail" sections of the two lipid membrane components. Free rotation of the branched (isooctyl) sterol side chain will permit more conformational freedom for the paraffinic carbons (9 to 16 or 18) in this region than for two directly interacting or contiguous fatty acyl chains. Cholesterol will therefore have opposite regional effects on the bilayer, solidifying one segment and liquefying the other: "The sterol creates an intermediate fluid condition" (16).

Experiments cited above have emphasized the fitness of the cholesterol molecule in biological and artificial membranes and the incompetence of lanosterol to serve in an analogous membrane role. In light of the membrane model just discussed, we can now offer an explanation of why lanosterol modification led to functional improvement. Space-filling models show the 14α-CH_3 group (C_{30}) of lanosterol protruding from the otherwise planar α-sterol face. Because of the bulk of this substituent the interactions between the α-sterol face and the proximal segment of the fatty acyl chain will be drastically weakened. It is noteworthy that in the contemporary pathway for sterol biosynthesis the obstructing 14-methyl group is the first to be eliminated (10).[1]

As already mentioned, the effects of the 4,4-dimethyl and 4-monomethyl derivatives on artificial membranes are not substantially different from those of cholesterol (Fig. 1). In accord with these observations, these substituents do not interfere sterically with packing, judging from space-filling models. On the other hand, the unnatural 4,4-diethyl cholesterol retards glucose exit from liposomes significantly less than cholesterol, suggesting that the C_4 region may be critical for the α-face interactions. Perhaps the response of artificial membranes is too insensitive for detecting disturbing steric effects by C_4 substituents less bulky than ethyl groups. There may be other explanations why the vast majority of membrane sterols no longer carry methyl substituents at α-face positions. Methyl groups vicinal to C_3 may affect hydrogen bonding between the sterol hydroxyl group and the polar phospholipid head group. In turn, the strength and the site of the hydrogen bond may determine how far the sterol molecule penetrates into the bilayer. Finally, the reasons for the elimination of the C_4 methyl groups may be chemical rather than steric. To mention one, Δ^4-3-keto systems are a common structural feature of all steroid hormones, a system that cannot be formed unless at least one of the C_4—CH_3 groups is removed. However, this explanation is less satisfactory because fully demethylated sterols are found not only in the membranes of animal (steroid hormone producing) cells but in membranes of plants and fungi as well.

[1] The occurrence of a 14-methyl cholestenol in small amounts has been reported (13) and 14α-methyl fecosterol accumulates in certain yeast mutants (19). The effect of these compounds on membranes remains to be investigated.