

Handbook of Experimental Pharmacology

Volume 108/II

Editorial Board

G.V.R. Born, London

P. Cuatrecasas, Ann Arbor, MI

H. Herken, Berlin

K. Melmon, Stanford, CA

GTPases in Biology II

Contributors

M. Biel, L. Birnbaumer, K.J. Blumer, J. Bockaert, E. Bosse,
J.L. Boyer, P. Brabet, M. Camps, M.G. Caron, P.J. Casey, J. Chen,
K. Clark, D. Corda, S. Coulter, P.N. Devreotes, D. Donnelly,
T.D. DuBose, Jr., J.H. Exton, J.B.C. Findlay, R.A. Firtel,
V. Flockerzi, M. Forte, C. Gaskins, P. Gierschik, R. Gundersen,
R.W. Gurich, J.R. Hadcock, J.A. Hadwiger, T.K. Harden,
J.D. Hildebrandt, Y.-K. Ho, F. Hofmann, K.P. Hofmann,
V. Homburger, M.D. Houslay, R. Hullin, D. Hyde, R. Iyengar,
O. Jacobowitz, S. Jahangeer, K.H. Jakobs, G.L. Johnson,
R.L. Johnson, N. Kimura, S.D. Kroll, Y. Kurachi, R.H. Lee,
R.J. Lefkowitz, Y. Li, C. Londos, C.C. Malbon, D.H. Maurice,
K.R. McLeish, G. Milligan, A.J. Morris, E.J. Neer, A.S. Otero,
U. Panten, D. Park, G.S. Pitt, R.T. Premont, F. Quan, M. Rodbell,
W. Rosenthal, M. Russell, P. Ruth, C. Schwanstecher,
M. Schwanstecher, I.A. Simpson, E. Stefani, G. Szabo,
J.A. Thissen, T.D. Ting, L. Toro, G.L. Waldo, A. Welling,
T.G. Wensel, M. Whiteway, W. Wolfgang, L. Wu

Editors

Burton F. Dickey and Lutz Birnbaumer



Springer-Verlag

Berlin Heidelberg New York London Paris
Tokyo Hong Kong Barcelona Budapest

Contents

Section IV: Signal Transduction by Trimeric G Proteins

A. Cellular Architecture and its Role in Signal Transduction

CHAPTER 44

G-Proteins Have Properties of Multimeric Proteins: An Explanation for the Role of GTPases in their Dynamic Behavior

M. RODBELL, S. JAHANGEER, and S. COULTER. With 2 Figures	3
A. Introduction	3
B. Theories	4
I. Shuttle Theory	4
II. Collision-coupling Theory	4
III. Disaggregation Theory	5
C. Evidence for Multimeric Structures of G-Proteins	5
I. Properties in Detergents	5
II. Cross-Linking of G-Proteins in Membranes	6
III. Glucagon Activation of Multimeric G _s in Hepatic Membranes	7
D. Coupling of Receptors to Multimeric G-Proteins	8
E. Hydrolysis of GTP Is Fundamental to Signal Transduction Dynamics	9
F. Conclusions	11
References	12

B. G-Protein Coupled Receptors

CHAPTER 45

The Superfamily: Molecular Modelling

J.B.C. FINDLAY and D. DONNELLY. With 4 Figures	17
A. Introduction	17
B. General Principles – Modelling Integral Membrane Domains	18

CHAPTER 50

The GTPase Cycle: Transducin

T.D. TING, R.H. LEE, and Y.-K. Ho. With 6 Figures	99
A. The Retinal cGMP Cascade and Visual Excitation	99
B. The Coupling Cycle of Transducin	100
C. The Reaction Dynamics of the Transducin Cycle	102
I. Transducin Subunit Interaction	102
II. Pre-Steady-State Kinetic Analysis of the GTP Hydrolysis Reaction	104
III. Quantitative Analysis of the Pre-Steady-State Kinetics	107
D. Relationship of GTP Hydrolysis and PDE Deactivation	110
E. Regulation of the Transducin Coupling Cycle by Phosducin	113
F. Concluding Remarks	115
References	115

CHAPTER 51

Transcriptional, Posttranscriptional, and Posttranslational Regulation of G-Proteins and Adrenergic Receptors

J.R. HADCOCK and C.C. MALBON. With 2 Figures	119
A. Introduction	119
B. Agonist-Induced Regulation of Transmembrane Signaling	120
I. Transcriptional and Posttranscriptional Regulation	120
II. Posttranslational Regulation	123
C. Cross-Regulation in Transmembrane Signaling	123
I. Stimulatory to Inhibitory Adenylyl Cyclase	123
II. Inhibitory to Stimulatory Adenylyl Cyclase	124
III. Stimulatory Adenylyl Cyclase to Phospholipase C	125
IV. Tyrosine Kinase to Stimulatory Adenylyl Cyclase	126
D. Permissive Hormone Regulation of Transmembrane Signaling	126
E. Perspectives	127
References	128

CHAPTER 52

G-Protein Subunit Lipidation in Membrane Association and Signaling

J.A. THISSEN and P.J. CASEY. With 4 Figures	131
A. Introduction	131
B. Myristoylation and Membrane Association of G-Protein α Subunits	133
I. Cotranslational Processing of G-Protein α Subunits	133
II. The Role of Myristoylation in α Subunit–Membrane Association	135

C. Prenylation and Membrane Association of G-Protein γ Subunits	138
I. Posttranslational Processing of G-Protein γ Subunits	138
II. The Role of Prenylation in γ Subunit–Membrane Association	139
1. Geranylgeranyl–Modified γ Subunits	140
2. Farnesyl-Modified γ Subunits	142
D. Future Directions	142
References	143

CHAPTER 53

Phosphorylation of Heterotrimeric G-Protein

M.D. HOUSLAY. With 1 Figure	147
A. Introduction	147
I. Nature of G-Proteins	147
II. Modulation of G-Protein Action	148
1. Phosphorylation	149
B. Phosphorylation of Heterotrimeric G-proteins in Intact Cells	150
I. Hepatocytes	150
II. Promonocytic Cell Line U937	154
III. Platelets	155
1. G_i -2	155
2. G_z	155
IV. Yeast	156
V. Dictyostelium	157
C. In Vitro Phosphorylation of Isolated Heterotrimeric G-Proteins	158
I. Transducin	158
II. G_i and G_o	158
III. G_s	160
IV. Unidentified “G-Proteins”	161
D. Conclusion	162
References	162

CHAPTER 54

**Receptor to Effector Signaling Through G-Proteins:
 $\beta\gamma$ Dimers Join α Subunits in the World of Higher Eukaryotes**

L. BIRNBAUMER. With 6 Figures	167
A. Introduction	167
B. $\beta\gamma$ Dimers and Adenylyl Cyclase	168
I. Hormonal Inhibition of Adenylyl Cyclase and Stimulation of K^+ Channels: Controversies that Settled Mostly in Favor of α Subunits	168

II. Conditional and Subtype-Specific Regulation of Adenylyl Cyclase Activity by $\beta\gamma$ Dimers	169
C. $\beta\gamma$ Dimers and Phospholipase C: Subtype-Specific Stimulation of Type β Phospholipase C by $\beta\gamma$ Dimers	171
D. $\beta\gamma$ Dimers and Receptors: Exquisite Specificity of Receptors for $\beta\gamma$ Subtypes	172
E. Dual Signaling of Single Receptors: Mediation by One or by Two G-Proteins?	174
I. Inhibition of Adenylyl Cyclase and Stimulation of Phospholipase C	174
II. Signaling Quality Through Receptor Quantity?	175
III. Dual Stimulation of Adenylyl Cyclase and Phospholipase C	176
IV. Evidence for Physical Interaction of a Single Receptor with Two Distinct Types of G-Proteins	178
F. The Puzzle of the Up-Shifted Dose-Response Curves for Phospholipase C Elicited by Adenylyl Cyclase Stimulating Agonists	179
G. Concluding Remarks	180
References	181

D. Effectors of G-Proteins

CHAPTER 55

Molecular Diversity of Mammalian Adenylyl Cyclases: Functional Consequences

R.T. PREMONT, J. CHEN, O. JACOBOWITZ, and R. IYENGAR.

With 5 Figures	189
A. Introduction	189
B. Stimulation and Inhibition of Adenylyl Cyclases	190
C. Molecular Diversity of Adenylyl Cyclases	193
I. Multiple Families of Adenylyl Cyclases	193
II. Secondary Structure and Topography	196
III. Putative Catalytic Sites	198
IV. Tissue Distribution of the Various Forms	199
D. G-Protein Regulation of Adenylyl Cyclases	201
I. G_s - α Regulation	201
II. G_i - α Regulation	201
III. $\beta\gamma$ Regulation	201
E. Type-Specific Regulation by Intracellular Ligands	202
I. Ca^{2+} /CaM Regulation	202
II. Inhibition by Low Concentrations of Ca^{2+}	202
III. P-Site Inhibition	204

Contents	XXI
F. Regulation by Protein Phosphorylation	204
I. Regulation by Protein Kinase C	204
II. Protein Kinase A Regulation: A Component of Heterologous Desensitization	205
G. Functional Consequences of Multiple Adenylyl Cyclases	206
I. Integration of Multiple Signals	206
II. Modulation of Signal Transmission	207
References	208

CHAPTER 56

The Light-Regulated cGMP Phosphodiesterase of Vertebrate Photoreceptors: Structure and Mechanism of Activation by G_{ta}

T.G. WENSEL. With 2 Figures	213
A. Physiological Role of cGMP Phosphodiesterase in Visual Signaling	213
B. Structure	213
I. Subunit Composition	213
II. Size and Hydrodynamic Properties	214
III. Primary Structure	214
IV. Posttranslational Modifications	214
V. Domain Structures of Subunits	215
1. Catalytic Subunits	215
2. Inhibitory Subunit	215
C. Functional Properties	216
I. Solubility	216
II. Kinetic Properties	216
III. Noncatalytic cGMP Binding Sites	216
D. Regulation of Catalytic Activity	217
I. Inhibition by PDE_{γ}	217
II. Activation by G-Protein	218
1. Role of G_{ta}	218
2. Role of Membranes in PDE Activation by G_{ta}	218
3. Role of PDE_{γ} in Activation by Transducin	218
4. Is There Cooperativity in the Action of G_{ta} -GTP?	219
5. A Role for Noncatalytic cGMP Binding Sites?	220
References	220

CHAPTER 57

High-Voltage Activated Ca^{2+} Channel

F. HOFMANN, M. BIEL, E. BOSSE, R. HULLIN, P. RUTH, A. WELING, and V. FLOCKERZI. With 1 Figure	225
A. Introduction	225

B. Identified cDNAs of High-Voltage Activated Calcium Channels	225
I. The α_1 Subunit	227
II. The α_2/δ Subunit	228
III. The β Subunit	229
IV. The γ Subunit	229
C. Structure-Function of the Cloned Calcium Channel Proteins	229
I. Expression and Function of the Channel Subunits	229
II. The Binding Sites for Calcium Channel Blockers	232
III. Phosphorylation of the Channel Proteins	233
D. Conclusion	234
References	234

CHAPTER 58

Phospholipase C- β Isozymes Activated by $G\alpha_q$ Members

D. PARK. With 3 Figures	239
References	248

CHAPTER 59

Stimulation of Phospholipase C by G-Protein $\beta\gamma$ Subunits

P. GIERSCHIK and M. CAMPS. With 4 Figures	251
A. Introduction	251
B. Stimulation of Soluble Phospholipase C of HL-60 Granulocytes by G-Protein $\beta\gamma$ Subunits	252
C. Identification of the $\beta\gamma$ -Sensitive Phospholipase C of HL-60 Granulocytes as PLC β_2	254
D. Stimulation of PLC β_2 by G-Protein $\beta\gamma$ Subunits in Intact Cells	256
E. Role of $\beta\gamma$ Subunits in Mediating Receptor Stimulation of Phospholipase C	257
F. Perspectives	259
References	260

E. Specialized Systems

CHAPTER 60

Rhodopsin/G-Protein Interaction

K.P. HOFMANN. With 7 Figures	267
A. Introduction	267
B. Interactions of Rhodopsin in the Visual Cascade	267
C. Biophysical Monitors of G-Protein Activation	270

I. Description of the Monitors	270
II. Instrumentation	271
III. Application to the Analysis of R*-G _t Interaction	273
IV. Preparations	276
D. Interactive States of Rhodopsin	277
I. Molecular Nature of Metarhodopsin II	277
II. Active Forms of Rhodopsin from Alternative Light-Induced Pathways	278
III. Activation of Rhodopsin in the Dark	278
E. Interactive States of Transducin	279
I. Dark Binding	279
II. Stable Light Binding with Empty Nucleotide Site	279
III. Rhodopsin/G-Protein Interaction with Bound Nucleotides ..	280
F. Mechanism of Transducin Activation	280
I. Role of Rhodopsin's Cytoplasmic Loops	280
1. Three Loops Contribute to MII-G _t Interaction	280
2. Loop Mutants: Binding and Activation in MII-G _t Interaction	283
II. Dissection of Reaction Steps	283
1. The GDP/II Switch	283
2. The MII/GTP Switch	284
III. Regulation of the Activation Pathway	286
G. Conclusion	286
References	287

CHAPTER 61

Fast Kinetics of G-Protein Function In Vivo

G. SZABO, Y. LI, and A.S. OTERO. With 6 Figures	291
A. Introduction	291
B. Kinetics of Muscarinic K ⁺ Channel Activation	291
C. Rapid Desensitization	295
D. Kinetics of I _{K(ACh)} Deactivation	298
E. Basic Kinetic Model for Membrane-Delimited Effector Activation by a G-Protein	299
F. Conclusions	300
References	301

CHAPTER 62

The Yeast Pheromone Response G-Protein

K. CLARK and M. WHITEWAY. With 3 Figures	303
A. Introduction	303
B. Overview	304

C. Gpa1, the $G\alpha$ Subunit	305
I. Random Mutagenesis	306
II. Site-Directed Mutagenesis	307
D. Ste4, the $G\beta$ Subunit	309
I. Random Mutagenesis	309
II. Site-Directed Mutagenesis	312
E. Ste18, the $G\gamma$ Subunit	312
I. Random Mutagenesis	313
II. Site-Directed Mutagenesis	314
F. Conclusions	315
References	315

CHAPTER 63

 $G\alpha$ Proteins in *Drosophila*: Structure and Developmental Expression

M. FORTE, F. QUAN, D. HYDE, and W. WOLFGANG. With 3 Figures	319
A. Introduction	319
I. G-Protein-Coupled Signaling in Development	319
II. The <i>Drosophila</i> System	319
B. $G\alpha$ -Proteins in <i>Drosophila</i>	322
I. DG_{sa}	322
1. Gene Structure	322
2. Adult and Embryonic Expression	324
3. Stimulation of Mammalian Adenylyl Cyclase Through DG_{sa}	324
II. DG_{oa}	325
1. Gene Structure	325
2. Adult and Embryonic Expression	325
III. DG_{ia}	327
1. Gene Structure	327
2. Adult and Embryonic Expression	327
IV. DG_{qa}	328
1. Gene Structure	328
2. Adult Expression	329
3. Role in Phototransduction	329
V. <i>concertina</i>	331
1. Mutant Phenotype	331
2. Cloning and Gene Structure	331
3. Expression of <i>cta</i>	332
C. Summary	332
References	333

CHAPTER 64

Signal Transduction by G-Proteins in *Dictyostelium discoideum*

L. WU, C. GASKINS, R. GUNDERSEN, J.A. HADWIGER, R.L. JOHNSON, G.S. PITT, R.A. FIRTEL, and P.N. DEVREOTES. With 8 Figures	335
--	-----

A. Introduction	335
B. Signal Transduction in <i>Dictyostelium</i>	335
C. Diversity of G-Proteins in <i>Dictyostelium</i>	337
D. Roles of G-Proteins in Signal Transduction Processes	340
E. Roles of G-Proteins in Morphogenesis and Differentiation	345
F. Conclusions and Perspectives	347
References	348

CHAPTER 65

Functional Expression of Mammalian Receptors and G-Proteins in Yeast

K.J. BLUMER. With 1 Figure	351
A. Introduction	351
B. Expression of Mammalian G-Protein-Coupled Receptors	352
C. Expression of Mammalian G-Protein Subunits	357
I. Physiological Roles of Yeast G-Protein Subunits	357
II. Mammalian G_{α} Subunits	357
1. Intact G_{α} Subunits	357
2. Chimeric Yeast/Mammalian G_{α} Subunits	358
III. Mammalian G_{β} and G_{γ} Subunits	359
D. Signaling Between Mammalian Receptors and G-Proteins	359
E. Perspectives	359
References	360

CHAPTER 66

G-Proteins in the Signal Transduction of the Neutrophil

K.R. McLEISH and K.H. JAKOBS	363
A. Introduction	363
B. Receptor-Mediated PMN Functions	363
I. Adherence	363
II. Chemotaxis	364
III. Phagocytosis and Bactericidal Activity	364
IV. Regulatory Receptors	364
C. G-Protein-Coupled Receptors	364
I. Chemoattractant Receptors	365
II. Purinergic Receptors	368
III. Other PMN Receptors	369
D. Regulation of Neutrophil Responses	369
I. Priming	369
II. Desensitization	370
References	370

CHAPTER 67

**Hormonal Regulation of Phospholipid Metabolism via G-Proteins:
Phosphoinositide Phospholipase C and Phosphatidylcholine
Phospholipase D**

J.H. EXTON	375
A. Introduction	375
B. Identification of the G-Proteins Regulating PtdInsP ₂ Phospholipase C	375
C. Coupling of G-Proteins to Ca ²⁺ -Mobilizing Receptors	377
D. Specificity of Phosphoinositide Phospholipase C Linked to G _q and G ₁₁	379
E. Mechanisms of Agonist-Stimulated Phosphatidylcholine Breakdown	380
F. Summary	381
References	381

CHAPTER 68

**Hormonal Regulation of Phospholipid Metabolism via G-proteins II:
PLA₂ and Inhibitory Regulation of PLC**

D. CORDA. With 2 Figures	387
A. Introduction	387
B. Modulation of PLA ₂	387
I. Molecular Forms of PLA ₂	388
II. G-Protein-Mediated Activation of PLA ₂	388
III. Molecular Aspects	390
IV. Inhibitory Regulation of PLA ₂	391
C. Activity of PLA ₂ in <i>ras</i> -Transformed Cells	392
D. Inhibitory Regulation of PLC	393
I. Molecular Aspects	395
E. Conclusion	396
References	396

CHAPTER 69

G-Protein Regulation of Phospholipase C in the Turkey Erythrocyte

A.J. MORRIS, D.H. MAURICE, G.L. WALDO, J.L. BOYER, and T.K. HARDEN	401
A. Introduction	401
B. Properties of P _{2Y} Purinergic Receptor and G-Protein-Regulated PLC in Turkey Erythrocytes	403
I. Initial Observations	403
II. Kinetics of Activation of PLC by P _{2Y} Purinergic Receptor Agonists and Guanine Nucleotides	404

C. Identification, Purification, and Primary Structure of the Protein Components of the Turkey Erythrocyte Inositol Lipid-Dependent Signaling System 405

 I. G-Protein-Regulated PLC 405

 1. Purification and Properties of a G-Protein-Regulated PLC from Turkey Erythrocytes 406

 2. Receptor and G-Protein Regulation of the Purified Turkey Erythrocyte PLC 407

 II. G-Protein Activators of PLC 408

 1. Purification and Properties of the Turkey Erythrocyte PLC-Activating G-Protein 410

 2. cDNA Sequence of the Turkey Erythrocyte PLC-Activating G-Protein and its Relationship to Mammalian G-Protein α Subunits 410

D. Concluding Comments 411

References 412

CHAPTER 70

Hormonal Inhibition of Adenylyl Cyclase by α_i and $\beta\gamma$, α_i or $\beta\gamma$, α_i and/or $\beta\gamma$

J.D. HILDEBRANDT. With 2 Figures 417

A. Introduction 417

B. Mechanism(s) Mediating Inhibition of Adenylyl Cyclase 418

 I. Direct Inhibition of Adenylyl Cyclase by α_i 418

 II. Indirect Inhibition of Adenylyl Cyclase by $\beta\gamma$ Suppression of α_s Activation 419

 III. Direct Inhibition of Adenylyl Cyclase by $\beta\gamma$ 420

C. Current View of Inhibition of Adenylyl Cyclase 421

 I. The Mechanism of Inhibition of Adenylyl Cyclase in S49 Cells 422

 II. Significance and Predications of Multiple Mechanism for Inhibition 424

 III. Unresolved Structural and Functional Issues about G-proteins Affecting the Mechanism(s) Mediating Hormone Inhibition of Adenylyl Cyclase 425

D. Conclusion 426

References 426

CHAPTER 71

Neurobiology of G_o

P. BRABET, V. HOMBURGER, and J. BOCKAERT. With 3 Figures 429

A. Introduction 429

B. Gene Structure of $G_o\alpha$ in Vertebrates and Invertebrates	429
I. Gene Structure and Transcription in Vertebrates	429
II. Gene Structure and Transcription in Invertebrates	430
C. Cellular Expression of G_o in Excitable Cells and Its Regulation	432
I. Cellular and Subcellular Distribution	432
1. Neurons	432
2. Nonneuronal Cells	434
II. Control of G_o , G_{o1} , and G_{o2} Expression During Neuronal Differentiation	435
D. Neurotransmitter Receptors Coupled to G_o and Their Inhibitory Effects on Voltage-Sensitive Ca^{2+} Channels	436
I. Nature of Receptors	436
1. Reconstitution of Resolved Receptors and G_o -Proteins	436
2. Reconstitution of Receptor Coupling to VSCC with G_o - Protein in PTX-Treated Cells	436
3. Stimulation of G_o Photolabeling with [α - ^{32}P]GTP Azidoanilide by Neurotransmitters	438
4. Intracellular Injections of G-protein Antibodies and of Antisense Oligonucleotides Complementary to G-Protein or DNA Sequences To Demonstrate the Specificity of the Negative Coupling Between Receptors and VSCC via G_o	438
5. Immunoprecipitation of Receptor- G_o Complexes with Anti- G_o Antibodies and Anti-receptor Antibodies	440
II. Nature of VSCC Inhibited by G_o	440
III. Colocalization of G_o and L-Type VSCC in T-Tubule	440
IV. Conclusions	441
E. General Conclusion	441
References	441

CHAPTER 72

Involvement of Pertussis-Toxin-Sensitive G-Proteins in the Modulation of Ca^{2+} Channels by Hormones and Neurotransmitters

W. ROSENTHAL. With 1 Figure	447
A. Introduction	447
B. Inhibitory Modulation of Voltage-Dependent Ca^{2+} Channels	447
I. Occurrence; Physiological Significance	447
II. Effects of Receptor Agonists, Pertussis Toxin, and Guanine Nucleotides	449
III. Types of Ca^{2+} Channels Affected by Inhibitory Receptor Agonists	450
IV. Mechanistic Aspects	452
1. Cyclic Nucleotides	452

2. Protein Kinase C and Fatty Acids	453
3. Evidence for a Membrane-Delimited Pathway	454
V. Identification of the Involved G-Protein	454
1. Occurrence of G _o	454
2. Reconstitution Experiments with Native and Recombinant G-Proteins; Transfected Cells	455
3. Antibodies	456
4. G _o -Activating Receptors	456
5. Antisense Oligonucleotides	457
C. Stimulatory Modulation of Voltage-Dependent Ca ²⁺ Channels ..	458
I. Occurrence; Physiological Significance	458
II. Effects of Pertussis Toxin and Guanine Nucleotides	459
III. Types of Ca ²⁺ Currents Affected by Stimulatory Receptor Agonists	460
IV. Mechanistic Aspects	460
V. Identity of the G-Protein Involved	461
D. Conclusion	462
References	463

CHAPTER 73

Regulation of Cell Growth and Proliferation by G_o

S.D. KRÖLL and R. IYENGAR. With 3 Figures	471
A. Introduction	471
B. The G _o -Protein	471
C. The G _o -Protein and Cell Cycle Regulation in the <i>Xenopus</i> Oocyte	473
D. Regulation of Oocyte Maturation by Multiple Pathways	477
E. Proliferation of Mammalian Cells by Activated G _o	478
F. Specificity of Transformation by Signaling Through G-Protein Pathways	479
G. Desensitization and Growth Signaling Through G-Protein Pathways	481
References	481

CHAPTER 74

Role of Nucleoside Diphosphate Kinase in G-Protein Action

N. KIMURA. With 6 Figures	485
A. Introduction	485
B. General Model of Membrane Signaling Systems Involving G-Proteins	485
C. Role of NDP Kinase in Membrane Signaling Systems	486
I. Evaluation of the Effect of GDP in Comparison with GP ..	486

II. Role of mNDP Kinase in Signal Transduction	487
III. Comparison Between Hormone and Cholera Toxin Actions	490
IV. Interaction Between mNDP Kinase and G_s , and Its Regulation	490
V. Regulatory Mechanism of G-Protein by NDP Kinase	491
VI. Physiological Relevance of G-Protein Regulation by mNDP Kinase	494
D. Properties of NDP Kinases and Their Structure	495
E. Novel Roles of NDP Kinases in Cellular Functions	495
F. Concluding Remarks	496
References	496

CHAPTER 75

G-Protein Regulation of Cardiac K^+ Channels

Y. KURACHI. With 16 Figures	499
A. Introduction	499
B. Involvement of G-Protein in Muscarinic Activation of the K_{ACh} Channel	499
C. Physiological Mode of G-Protein Activation of the K_{ACh} Channel	502
D. Effects of G-Protein Subunits on the Cardiac K_{ACh} Channel	504
I. Comparison Between the Regulation of Adenylyl Cyclase Activity and the K_{ACh} Channel Activity by Purified G-Protein Subunits	506
II. Effects of $G_{\beta\gamma}$ on the K_{ACh} Channel	507
1. Voltage-Dependent Properties of the $G_{\beta\gamma}$ -Activated K_{ACh} Channel	507
2. Concentration Dependence of $G_{\beta\gamma}$ Activation of the K_{ACh} Channel	509
3. Specificity of $G_{\beta\gamma}$ Activation of the K_{ACh} Channel	510
4. $G_{\beta\gamma}$ Activation of the K_{ACh} Channel Is Not Mediated by Phospholipase A_2	511
5. Antibody 4A Does Not Inhibit the Interaction Between G_K and the K_{ACh} Channel	512
III. Effects of G-Protein on the ATP-Sensitive K Channel	514
E. Stimulatory Modulation of the G_K -Gated Cardiac K_{ACh} Channel	515
I. Arachidonic Acid and Its Metabolites	515
II. Phosphorylation	520
III. NDP-Kinase	521
IV. Intracellular Chloride	522
F. Conclusion	523
References	523

CHAPTER 76

Modulation of K⁺ Channels by G-Proteins

L. BIRNBAUMER. With 9 Figures 527

A. Direct Regulation of Ionic Channels by G-Proteins 527

 I. The Inwardly Rectifying “Muscarinic” K⁺ Channel 527

 1. Experiments Leading to the Discovery of G-Protein Gating 527

 2. Direct Stimulation by hRBC G_i and Its α Subunit 529

 3. Properties of the G_i-stimulated K⁺ Channel 532

 4. Identity of the G_k that Gates the Muscarinic-Type K⁺ Channels 534

 II. The ATP-Sensitive K⁺ Channel: A Second G_i-Gated K⁺ Channel 536

 1. General Properties of the ATP-Sensitive K⁺ Channel/Sulfonylurea Receptor Complex 536

 2. Identity of G-proteins that Regulate the ATP-Sensitive K⁺ Channel 537

 III. G-Protein Gating as a Tool To Discover Novel Ionic Channels: Neuronal G_o-Gated K⁺ Channels 539

B. Effect of $\beta\gamma$ Dimers: Inhibition versus Stimulation of the Muscarinic K⁺ Channel – A Persisting Controversy 541

C. Conclusions 543

References 544

CHAPTER 77

ATP-Sensitive K⁺ Channel: Properties, Occurrence, Role in Regulation of Insulin Secretion

U. PANTEN, C. SCHWANSTECHEER, and M. SCHWANSTECHEER. With 2 Figures 547

A. Introduction 547

B. Biophysical Properties 547

C. Regulation of the K_{ATP} Channel 548

 I. Inhibition by Intracellular Nucleotides 548

 II. Activation by Intracellular Nucleoside Diphosphates 549

 III. Activation by Intracellular MgATP 550

 IV. Activation by G-Proteins 550

 V. Inhibition by G-Proteins 551

 VI. Inhibition by Drugs 551

 VII. Activation by Drugs 552

 VIII. Characteristics of the Sulfonylurea Receptor 552

D. Role of the K_{ATP-} Channel in Regulation of Insulin Secretion . . .	553
References	555

CHAPTER 78

Modulation of Maxi-Calcium-Activated K Channels: Role of Ligands, Phosphorylation, and G-Proteins

L. TORO and E. STEFANI. With 4 Figures	561
A. Introduction	561
B. Mechanisms of Metabolic Regulation of Maxi- K_{Ca} Channels	564
I. Ligand Modulation	564
1. Arachidonic Acid	564
2. Angiotensin II and Thromboxane A_2	565
3. Guanine Nucleotides	565
4. Intracellular pH	566
II. Phosphorylation/Dephosphorylation Cycles	568
1. Pituitary Maxi- K_{Ca} Channels	570
2. Brain Maxi- K_{Ca} Channels	570
3. Colonic Maxi- K_{Ca} Channels	571
4. Myometrial Maxi- K_{Ca} Channels	571
III. G-Protein Gating	572
1. Muscarinic Regulation	573
2. Adrenergic Stimulation	574
C. Conclusions	575
References	576

CHAPTER 79

Regulation of the Endosomal Proton Translocating ATPase (H^+ -ATPase) and Endosomal Acidification by G-Proteins

R.W. GURICH and T.D. DUBOSE, JR. With 6 Figures	581
A. Introduction	581
B. Endocytosis	581
I. General	581
II. The Kidney	582
C. Endosomal Acidification	584
I. Potential Role for G-Proteins in Endosomal Acidification	586
II. Effects of G-Proteins on Endosomal Acidification	588
D. Conclusions	592
References	593

CHAPTER 80

cAMP-Independent Regulation of Adipocyte Glucose Transport Activity and Other Metabolic Processes by a Complex of Receptors and Their Associated G-Proteins

C. LONDOS and I.A. SIMPSON. With 4 Figures	597
A. Introduction	597
B. Lack of a Relationship Between cAMP and Glucose Transporter Activity	598
C. G-Proteins in Glucose Transporter Regulation	600
D. How Do G-Proteins Mediate Glucose Transporter Activity?	602
E. Other R_sG_s - and R_iG_i -Mediated Processes in Adipocytes	605
F. Conclusions and Speculations	606
References	607
Subject Index	611

High-Voltage Activated Ca^{2+} Channel

F. HOFMANN, M. BIEL, E. BOSSE, R. HULLIN, P. RUTH, A. WELLING,
and V. FLOCKERZI

A. Introduction

Calcium channels are part of the signal system which is vital for intercellular communication in higher multicellular organisms. They transduce electrical or hormonal signals into a chemical second messenger, namely calcium. The cytosolic calcium concentration controls numerous cellular functions by binding to distinct calcium receptor binding proteins such as calmodulin, troponin, or calcium-activated potassium channels. Voltage-dependent calcium channels are of particular interest since their opening or closing determinates the cellular calcium concentration of many cells. In the normal heart they are essential to the generation of normal cardiac rhythm, to impulse propagation through the atrioventricular node, and to contraction in atrial and ventricular muscle. In vascular smooth muscle calcium channels provide part of the calcium that controls smooth muscle contraction and vascular tone. In skeletal muscle they are an essential part of the tubular excitation-contraction coupling mechanism. In neuronal and neuroendocrine cells they are essential for neurotransmitter release (for recent reviews see BERTOLINO and LLINAS 1992; BROWN and BIRNBAUMER 1990; MILLER 1992; RIOS et al. 1992; TRAUTWEIN and HESCHELER 1990).

B. Identified cDNAs of High-Voltage Activated Calcium Channels

High-voltage activated calcium channels are present in many tissues and are the major pathway for voltage-dependent calcium entry in excitable cells. They are activated at a high membrane potential, inactivate slowly (long lasting) and are readily blocked by different compounds. L-type calcium channels are blocked by the organic calcium channel blockers (CaCB) such as nifedipine and verapamil, N-type by ω -conotoxin, and P-type by the funnel web spider toxin ω -Age IVA (MINTZ et al. 1992). The principal channel-forming subunit of a high-voltage activated calcium channel is the α_1 subunit. When purified from rabbit skeletal muscle, this protein (apparent molecular mass 165 kDa) is associated with a 55-kDa protein (β), a 32-kDa protein (γ), and a disulfide-linked dimer of 130/28 kDa (α_2/δ) (see HORMANN et al. 1990 and references cited there). The primary structure of

Table 1. Classification of cloned and expressed mammalian calcium channel cDNA's

Gene	Snutch class	Source	Species	Functionally expressed	Sensitive to	Reference
α_1 subunit						
CaCh1	-	Skeletal muscle	Rabbit	Yes	DHP	TANABE et al. 1987
CaCh2a	C	Heart	Rabbit	Yes	DHP	MIKAMI et al. 1989
		Brain	Rat	-		SNUTCH et al. 1991
CaCh2b	C	Lung, smooth muscle	Rabbit	Yes	DHP	BIEL et al. 1990
		Brain	Rat	-		SNUTCH et al. 1991
CaCh3	D	Aorta	Rat	Yes	DHP	KOCH et al. 1990
		Brain	Human	Yes	DHP, ω -conotoxin	WILLIAMS et al. 1992a
CaCh4	A	Brain	Rat	-		HUI et al. 1991
		Pancreatic islet	Human	-		SEINO et al. 1992
		Brain	Rabbit	Yes	Spider venom	MORI et al. 1991
CaCh5	B	Brain	Rat	-		STARR et al. 1991
		Brain	Human	Yes	ω -Conotoxin	WILLIAMS et al. 1992b
		Brain	Rat	-	ω -Conotoxin	BUBEL et al. 1992
α_2/δ subunit						
CaA ₂ 1a	-	Skeletal muscle	Rabbit	Yes	-	ELLIS et al. 1988; MIKAMI et al. 1989
CaA ₂ 1b	-	Brain	Human	Yes		WILLIAMS et al. 1992a
		Brain	Rat	-		KIM et al. 1992
β subunit						
CaB1	-	Skeletal muscle	Rabbit	Yes	-	RUTH et al. 1989
CaB2*	-	Brain	Rat	-		PRAGNELL et al. 1991
		Brain	Human	Yes	-	WILLIAMS et al. 1992a
		Heart	Rabbit	Yes	-	HULLIN et al. 1992
CaB3	-	Brain	Rat	Yes	-	PEREZ-REYES et al. 1992
		Heart	Rabbit	Yes	-	HULLIN et al. 1992
γ subunit						
CaG1	-	Skeletal muscle	Rabbit	Yes	-	JAY et al. 1990; BOSSE et al. 1990

Only full length clones have been included in this table. The nomenclature used for the α_1 subunit is adapted from PEREZ-REYES et al. (1990). For the Snutch classes see SNUTCH et al. (1990). The references in the table refer to the first published sequence. In some cases functional expression of the particular clone has been reported in a different publication.

-, not reported; DHP, dihydropyridine; *, at least three different variants (a-c) of the same gene have been identified.

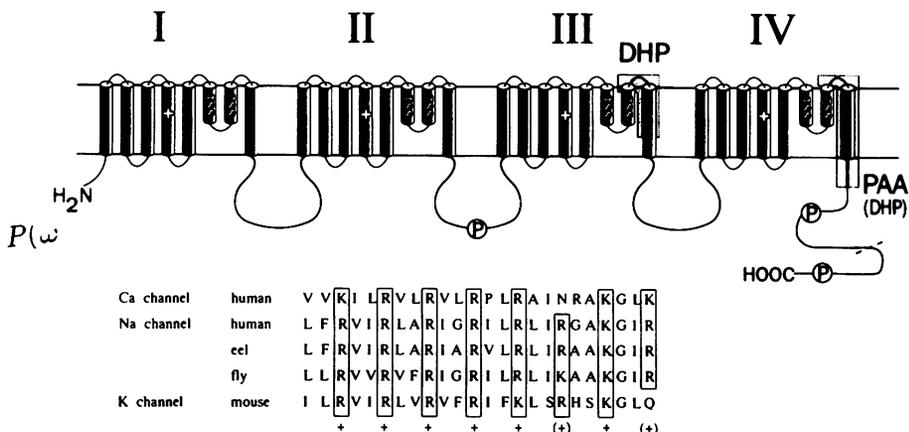


Fig. 1. Proposed topography of the α_1 subunit of the skeletal muscle calcium channel gene CaCh1. *Shaded areas*, proposed binding site for dihydropyridines (*DHP*) and phenylalkylamines (*PAA*). *P*, the in vitro identified cAMP kinase phosphorylation sites, *dashes*, the proposed truncation of the carboxy terminus; +, the amphipathic helix S4. *Below*, the amphipathic helix S4 sequence is compared with the S4 helices of other voltage-dependent ion channels

these proteins has been deduced by cloning their cDNAs (see Table 1 for references).

I. The α_1 Subunit

Complete cDNA clones of four different α_1 subunit gene products (CaCh 1–4) have been sequenced and shown to direct the synthesis of functional calcium channel after expression of their cRNA in *Xenopus oocytes* or cell culture cells (Table 1). The primary sequences of these different gene products are homologous to each other and predict a transmembrane topology which is similar to that of other voltage-dependent ion channels (Fig. 1). The primary sequences of the α_1 subunits predict proteins of 212–273 kDa containing four homologous repeats, each of which is composed of five hydrophobic putative transmembrane α helices and one amphipathic segment (S4) (TANABE et al. 1987; Fig. 1). The “extracellular” loop between the transmembrane helices 5 and 6 (SS1–SS2 region) is predicted to fold into the membrane and to form part of the pore of the channel (GUY and CONTI 1990). The skeletal and the cardiac/smooth muscle calcium channels are encoded by two different genes, CaCh1 and CaCh2. Several splice variants of the CaCh2 gene have been identified (BIEL et al. 1991). One major difference is the presence of two different exons at the transmembrane region IV S3 which alternate between the cardiac (CaCh2a) and smooth muscle (CaCh2b) isochannels. Polymerase chain reaction (PCR) amplification of the sequences around IV S3 suggests that a deletion within

each exon results in two additional splice variants (PEREZ-REYES et al. 1990). The two alternative channels CaCh2a and CaCh2b have been expressed stably in CHO-cells.

No major differences in basic electrophysiological characteristics have been observed, including the amplitude and voltage dependence of inward current and time of activation and inactivation (WELLING et al. 1992b). However, the two splice variants are expressed differentially in heart and smooth muscle (BIEL et al. 1991) and during cardiac development (DIEBOLD et al. 1992). The third gene is expressed in neuroendocrine tissues, whereas the fourth gene appears to be brain specific. The currents induced by the expression of the CaCh1, CaCh2, and CaCh3 genes are inhibited by low concentrations of dihydropyridines and therefore are classified as L-type calcium channels. The neuroendocrine channel CaCh3 is inhibited only at rather high concentration by ω -conotoxin and is not an N-type calcium channel. The current through the CaCh4 gene product is not affected by dihydropyridines but is inhibited by low concentrations of a mixture of the funnel web spider toxins and has been classified as a neuronal P-type channel. The α_1 subunit of CaCh5 has a high-affinity ω -conotoxin binding site (DUBEL et al. 1992; WILLIAMS et al. 1992b) and is expressed as calcium channel only in the presence of the β and α_2 subunits. The current is inhibited at picomolar concentrations of ω -CONOTOXIN (WILLIAMS et al. 1992b), identifying the CaCh5 protein as a neuronal N-type channel.

II. The α_2/δ Subunit

The deduced amino acid sequence of the α_2/δ protein is that of a membrane protein of 125 018 Da (ELLIS et al. 1988; Table 1). It contains three putative transmembrane segments and a large extracellular domain with several consensus sequences for glycosylation. The δ subunit sequence is identical with the carboxy terminal part of the deduced primary structure of the α_2 cDNA starting at amino acid 935 of the predicted sequence (DE JONGH et al. 1990). Presumably, the mature α_2 and δ proteins are the product of the same gene and arise by posttranslational processing. The mature α_2 protein may be located completely extracellularly, linked by a disulfide bridge to the transmembrane δ protein (JAY et al. 1991). Immuno- (NORMAN et al. 1987) and northern blots (ELLIS et al. 1990; BIEL et al. 1991) show that the α_2/δ protein is expressed in skeletal muscle, heart, brain, airway, vascular, and intestinal smooth muscle. Northern blots have identified a predominant 8-kB and a low-abundance 7-kB transcript with a skeletal muscle α_2 probe at high and low stringency (BIEL et al. 1991). Recently an α_2/δ cDNA has been cloned from human brain which is identical to the skeletal muscle α_2/δ cDNA (WILLIAMS et al. 1992a). A splice variant of this α_2/δ gene has been cloned from rat brain, which differs in part from the putative α_2 proteins but contains an identical δ protein (KIM et al. 1992). These results suggest that heart, brain, and smooth muscle express a conserved α_2 protein whereas brain contains an additional α_2/δ protein.

III. The β Subunit

The deduced primary sequence of the skeletal muscle β subunit is compatible with that of a peripheral membrane protein of 57 868 Da (Table 1; RUTH et al. 1989). It contains four α -helical domains, each of which contains a homologous stretch of eight amino acids. Domains II, III, and IV contain a heptad repeat structure. Heptad repeats have been found in cytoskeletal proteins. This suggests that the β subunit may be a cytoskeletal protein which anchors the α_1 subunit to the cytoskeleton. The β -like proteins which are different from the skeletal muscle β subunit exist in heart, aorta, and brain and are derived from two different genes (CaB2 and CaB3; HULLIN et al. 1992). The primary transcript of CaB2 is differentially spliced and leads to the expression of at least three different isoforms (CaB2a, CaB2b, and CaB2c). The overall homology between the novel β subunits found in heart, aorta, and brain and the skeletal muscle β subunit (CaB1) is 71% for CaB2a, 71.5% for CaB2b, and 66.6% for CaB3. Northern blot and PCR analyses show that CaB1 is present in large amounts in skeletal muscle and brain, CaB2 in heart and aorta, and CaB3 in brain and tissues which are rich in smooth muscle such as aorta, lung, and trachea.

IV. The γ Subunit

The deduced primary sequence of the skeletal muscle γ subunit is in agreement with that of an integral membrane protein of 25 058 Da (Table 1; BOSSE et al. 1990; JAY et al. 1990). The deduced sequence contains four putative transmembrane domains and two glycosylation sites which are located extracellularly. A complete cDNA for the γ subunit has been detected only in skeletal muscle. Northern and PCR analyses have not indicated that the same mRNA is present in higher concentrations in other tissues.

C. Structure-Function of the Cloned Calcium Channel Proteins

I. Expression and Function of the Channel Subunits

The cloned cDNA of the four calcium channel genes has been expressed in different cells (Table 2). The skeletal muscle α_1 subunit (CaCh1) has been expressed in L cells (PEREZ-REYES et al. 1989; LACERDA et al. 1991; VARADI et al. 1991) and skeletal muscle myotubes from mice with the muscular dysgenesis mutation (TANABE et al. 1988). Neither cell type has a functional α_1 subunit. The mice myotubes contained the α_2 and the other subunits may also be present. Expression of the skeletal muscle α_1 subunit (CaCh1) in L cells induces a barium current which activates extremely slowly ($\tau_{act.} \approx 665$ ms; PEREZ-REYES et al. 1989). Channel activation is accelerated 75-fold

Table 2. Functional effects of calcium channel subunits on currents induced by different α_1 subunits

α_1 Gene	Subunits expressed	Cell line	DHP sites _a	I_{Ba} ^b	Activation time	Voltage dependence ^c	Reference
Heterologous subunits							
CaCh2a	α_2	Oocyte	-	(↑)	-	-	MIKAMI et al. 1989
CaCh2a	α_2	Oocyte	-	(↑)	↓	(↑)	SINGER et al. 1991
CaCh2a	β	Oocyte	-	(↑)	(↓)	(↑)	SINGER et al. 1991
CaCh2a	γ	Oocyte	-	(↑)	(↓)	↑	SINGER et al. 1991
CaCh2a	$\alpha_2\beta$	Oocyte	-	↑↑	↓	↑	SINGER et al. 1991
CaCh2a	$\alpha_2\beta\gamma$	Oocyte	-	↑↑	↓	↑	SINGER et al. 1991
CaCh2a	β	Oocyte	-	↑	↓	≈	WEI et al. 1991
CaCh2a	$\beta\gamma$	Oocyte	-	↑	(↓)	(↑)	WEI et al. 1991
CaCh2b	β	CHO	↑	↑	(↓)	(↑)	WELLING et al. 1992
CaCh2a	β	Oocyte	-	(↑)	-	-	ITAGAKI et al. 1992
CaCh3	β	Oocyte	-	↑	-	-	WILLIAMS et al. 1992a
CaCh4	$\alpha_2\beta$	Oocyte	-	↑↑	-	-	MORI et al. 1991
Homologous subunits							
CaCh1	β	L cell	↑	≈	↓	-	LACERDA et al. 1991
CaCh1	α_2	L cell	(↑)	≈	≈	-	VARADI et al. 1991
CaCh1	β	L cell	↑	↓*	≈	-	VARADI et al. 1991
CaCh1	γ	L cell	↑↓	↓*	≈	-	VARADI et al. 1991
CaCh1	$\beta\gamma$	L cell	↓	↓*	(↓)	-	VARADI et al. 1991
CaCh1	$\alpha_2\beta\gamma$	L cell	(↑)	(↓)	-	-	VARADI et al. 1991
CaCh2a	β_2	Oocyte	-	↑	↓	-	HULLIN et al. 1992
CaCh2a	β_3	Oocyte	-	↑	↓	-	HULLIN et al. 1992
CaCh2a	β_2	Oocyte	-	↑	↓	(↑)	PEREZ-REYES et al. 1992
CaCh2a	β_2	COS	↑	-	-	-	PEREZ-REYES et al. 1992
CaCh5	$\alpha_2\beta$	HEK293	↑↑§	↑↑	-	-	WILLIAMS et al. 1992b

All effects are compared with that of cells expressing only the α_1 subunit.

^aThe number of dihydropyridine binding sites per mg protein.

^bBarium inward current.

^cA shift in voltage dependence of the I/V curve or steady state activation or inactivation to more negative values.

α_2 , β and γ are identical with CaA1a, CaB1 and CaG1. -, not reported; ≈, similar to cells expressing α_1 alone; *, I_{Ba} not sensitive to BayK 8644. (↑), ↑, and ↑↑ small, moderate and large increase or shift; (↓) and ↓, small and moderate decrease; §, ω -conotoxin binding sites.

($\tau_{\text{act.}} \approx 8 \text{ ms}$) by the coexpression of the skeletal muscle β subunit (CaB1; LACERDA et al. 1991). Expression of the skeletal muscle α_1 subunit in the dysgenic myotubes generates cells with a slowly activating calcium current and normal skeletal muscle excitation-contraction coupling, which does not depend on the influx of calcium (TANABE et al. 1988). Expression of the cardiac muscle α_1 subunit (CaCh2a) produces myotubes with Ca^{2+} currents and excitation contraction coupling as in cardiac muscle (TANABE et al. 1990). TANABE et al. (1990) constructed several chimeras by starting with the cardiac muscle α_1 subunit and introducing skeletal musclelike intracellular loops. Changing the large intracellular loop that connects repeats II and III switched the mode of excitation-contraction coupling to that characteristic of skeletal muscle. Interestingly, however, the Ca^{2+} current produced by this chimera remained characteristic of cardiac muscle, i.e., rapidly activating. Chimeras in which the four homologous repeats of the cardiac muscle protein were each switched to the equivalent skeletal muscle sequence showed that changing merely the first homologous repeat switched the characteristics of the Ca^{2+} current from fast activating (cardiac type) to slowly activating (skeletal muscle type) whereas switching the other three repeats did not have this effect (TANABE et al. 1991).

Stable expression of the α_1 subunits from smooth muscle (CaCh2b) in CHO cells induces dihydropyridine-sensitive barium currents, which have the physiological characteristics as a smooth muscle calcium channel (BOSSE et al. 1992). The single-channel conductance is 26 pSi in the presence of 80 mM Ba^{2+} . The channel has the same voltage dependence of activation and inactivation as reported for the naturally occurring smooth muscle calcium channel. The cardiac α_1 subunit (CaCh2a) cDNA directs the expression of a channel with electrophysiological properties which are indistinguishable from those of the smooth muscle α_1 subunit (WELLING et al. 1992b). Stable coexpression of the CaCh2b protein with the skeletal muscle β gene (CaB1) increases in parallel the number of dihydropyridine binding sites and the amplitude of whole cell barium current, suggesting that the amplitude of inward current is directly related to the number of expressed α_1 subunits of the protein (WELLING et al. 1993). In addition, the coexpression of the β subunit decreases the activation time of the channel by a factor of two and shifts the voltage dependence of steady state inactivation by 18 mV to -13 mV (WELLING et al. 1993). Coexpression of the β subunit does not influence the sensitivity of the expressed channel toward the dihydropyridine agonist Bay K 8644. Similar results were obtained by coexpression of the cardiac (CaCh2a), smooth muscle (CaCh2b), neuroendocrine (CaCh3), and neuronal (CaCh4) α_1 subunit with the skeletal muscle β subunit (CaB1) in *Xenopus* oocytes (Table 2). In each case the current density increased with coexpression of the β subunit (CaB1). Expression of the N type α_1 subunit CaCh5 in HEK239 cells requires the presence of a neuronal β (CaB1) and α_2 subunit to induce ω -conotoxin binding sites and calcium current (WILLIAMS et al. 1992b).

The coexpression of the α_1 (CaCh2a) and β (CaB1–3) subunit together with the α_2 subunit cRNA enhanced also the barium current in *Xenopus* oocytes. In *Xenopus* oocytes the α_2 subunit (SINGER et al. 1991) and the β subunit (WEI et al. 1991) decreased the activation time of the channel (CaCh2a). Identical results were observed when the cardiac α_1 subunit (CaCh2a) was coexpressed with the cardiac (CaB2) or the smooth muscle/neuronal (CaB3) β subunit and the α_2 subunit (HULLIN et al. 1992) in *Xenopus* oocytes. The skeletal muscle γ subunit (CaG1) shifted the voltage dependence of steady-state inactivation of the cardiac α_1 subunit (CaCh2a) by 40 mV to a negative membrane potential as is observed in skeletal muscle. These results suggest that (a) the skeletal muscle β subunit interacts with different α_1 subunits, (b) the β subunits increase barium currents by increasing the number of functional calcium channel proteins, and (c) the β subunits affect the activation time of the channel and the voltage dependence of steady-state inactivation. These conclusions are not supported by the experiments of VARADI et al. (1991), who reported that homologous coexpression of skeletal muscle α_1 and β , α_1 and γ , α_1 , β and γ , α_1 , α_2 , β and γ in L cells decreases the inward current and the stimulatory effect of the calcium channel agonist Bay K 8644. The latter results are difficult to reconcile with those from other laboratories. They could be caused by a nonstoichiometric expression of the channel subunits, i.e., a higher expression of the β subunit than the α_1 subunit (LORY et al. 1992).

II. The Binding Sites for Calcium Channel Blockers

Photoaffinity labeling of the skeletal muscle α_1 subunit and expression of CaCh1 and CaCh2b gene in L cells (KIM et al. 1990) or CHO cells (BOSSE et al. 1992) shows that the α_1 subunit itself contains the binding sites for the known organic calcium channel blockers, the dihydropyridines, phenylalkylamines, and benzothiazepines. Binding of these drugs requires the binding of calcium to a high-affinity binding site (SCHNEIDER et al. 1991; STAUDINGER et al. 1991). The allosteric modulation of the dihydropyridine binding site by phenylalkylamine and benzothiazepine is preserved within each α_1 subunit (KIM et al. 1990; BOSSE et al. 1992). The current induced in cell culture cells or *Xenopus* oocytes by the CaCh1, CaCh2a, and CaCh2b proteins is increased by Bay K 8644, a calcium channel agonist, and is inhibited by the known calcium channel blockers.

Photoaffinity labeling of the purified skeletal muscle α_1 subunit by dihydropyridines and phenylalkylamines suggests that the dihydropyridines bind to the SS1–SS2 region of repeat III (STRIESSNIG et al. 1991; NAKAYAMA et al. 1991) and apparently to a sequence following IVS6 (REGULLA et al. 1991) (Fig. 1). The extracellular location of the binding site at the SS1–SS2 region of repeat III is supported by the finding that dihydropyridines block the calcium channel from the extracellular space (KASS et al. 1991). The

phenylalkylamines label a second putative intracellular site located directly after the IVS6 (STRIESSNIG et al. 1990).

III. Phosphorylation of the Channel Proteins

The L-type current of cardiac, smooth and skeletal muscle, neuroendocrine, and neuronal calcium channels is modulated by hormones through the α subunits of different G-proteins (BROWN and BIRNBAUMER 1990). The open probability of the cardiac and skeletal muscle and of some neuroendocrine cells is increased by cAMP-dependent phosphorylation, suggesting that phosphorylation of the α_1 subunit or a different subunit of the calcium channel is important for its hormonal control. In skeletal muscle, about 90% of the full-length α_1 subunit (CaCh1) is apparently processed to a smaller protein with the carboxy terminus being close to amino acid residue 1690 (DE JONGH et al. 1991). cAMP-kinase phosphorylates in vitro rapidly Ser-687 (RÖHRKASTEN et al. 1988), which is located at the cytosolic loop between repeat II and III, and Ser-1854 (ROTMAN et al. 1992), which is present only in the full-length skeletal muscle α_1 subunit, and slowly Ser-1617 (RÖHRKASTEN et al. 1988). Phosphorylation of these sites may be significant, since the open probability of the reconstituted skeletal muscle CaCB-receptor/calcium channel is increased several-fold by cAMP-dependent phosphorylation (FLOCKERZI et al. 1986; HYMEL et al. 1988; NUNOKI et al. 1989; MUNDINA-WEILENMANN et al. 1991). The α_1 subunit is phosphorylated also in vivo at least at two sites in response to isoproterenol in isolated rat myocytes (LAI et al. 1990; MUNDINA-WEILENMANN et al. 1991).

These in vivo phosphorylation sites may be identical with Ser-687 and Ser-1854. However, it is not clear which of these phosphorylation sites – one of which is present only in the unprocessed α_1 subunit – affect the open probability of the skeletal muscle calcium channel. The in vitro identified phosphorylation sites of the CaCh1 gene are not conserved in the sequences of the other calcium channel genes and therefore are not important for the hormonal regulation of the calcium channel in heart and neuroendocrine cells. The hormonal control of the calcium channels may be exerted by tissue specific β subunits. The deduced amino acid sequence of the skeletal β subunit (CaB1) contains several potential phosphorylation sites. Two of these sites, Ser-182 and Thr-205, are phosphorylated in vitro by cAMP kinase (RUTH et al. 1988; DE JONGH et al. 1989). The products of the CaB2 gene contain a cAMP-kinase phosphorylation site equivalent to Thr-205 of CaB1. This phosphorylation site is not present in the product of CaB3, which is expressed mainly in brain and smooth muscle (HULLIN et al. 1992). This is interesting since in vivo whole cell calcium current is increased in heart (KAMEYAMA et al. 1986) and skeletal muscle (GARCIA et al. 1990) but not smooth muscle (WELLING et al. 1992a) by cAMP-dependent phosphorylation. Expression of the cardiac α_1 subunit with the α_2 and β subunit in *Xenopus* oocytes indicates that cAMP-dependent regulation of

the cardiac calcium channel is mediated by phosphorylation of the β subunit (KLÖCKNER et al. 1992; DASCAL et al. unpublished observation).

D. Conclusion

High-voltage activated calcium channels are encoded by different genes. Their electrophysiological and hormonal regulation may depend on the coexpression of different subunits. The interaction of these channel subunits with additional proteins such as the α subunit of trimeric G-proteins may be required for basic and hormonal regulation of the channel (HAMILTON et al. 1991; CAVALIE et al. 1991; KLEUSS et al. 1991). The availability of the cloned cDNA of several channel proteins and channel regulators will facilitate understanding of the complexities of voltage-gated calcium channels.

Acknowledgments. The experimental work of the authors was supported by grants from DFG, Fond der chemischen Industrie, und Thyssen Stiftung. We thank Mrs B. Schatz for typing part of the manuscript.

References

- Bertolino M, Llinás R (1992) The central role of voltage-activated and receptor operated calcium channels in neuronal cells. *Annu Rev Pharmacol Toxicol* 32:399–421
- Biel M, Hullin R, Freundner S, Singer D, Dascal N, Flockerzi V, Hofmann F (1991) Tissue-specific expression of high-voltage-activated dihydropyridine-sensitive L-type calcium channels. *Eur J Biochem* 200:81–88
- Biel M, Ruth P, Bosse E, Hullin R, Stühmer W, Flockerzi V, Hofmann F (1990) Primary structure and functional expression of a high voltage activated calcium channel from rabbit lung. *FEBS Lett* 269:409–412
- Bosse E, Bottlender R, Kleppisch T, Hescheler J, Welling A, Hofmann F, Flockerzi V (1992) Stable and functional expression of the calcium channel α_1 subunit from smooth muscle in somatic cell lines. *EMBO J* 11 2033–2038
- Bosse E, Regulla S, Biel M, Ruth P, Meyer HE, Flockerzi V, Hofmann F (1990) The cDNA and deduced amino acid sequence of the γ subunit of the L-type calcium channel from rabbit skeletal muscle. *FEBS Lett* 267:153–156
- Brown AM, Birnbaumer L (1990) Ionic channels and their regulation by G protein subunits. *Annu Rev Physiol* 52:197–213
- Cavalié A, Allen TJA, Trautwein W (1991) Role of the GTP-binding protein Gs in the β -adrenergic modulation of cardiac Ca channels. *Pflügers Arch* 419:433–443
- De Jongh KS, Merrick DK, Catterall WA (1989) Subunits of purified calcium channels: a 212-kDa form of α_1 and partial amino acid sequence of a phosphorylation site of an independent β subunit. *Proc Natl Acad Sci USA* 86:8585–8589
- De Jongh KS, Warner C, Catterall WA (1990) Subunits of purified calcium channels; α_2 and δ are encoded by the same gene. *J Biol Chem* 265:14738–14741
- De Jongh KS, Warner C, Colvin AA, Catterall WA (1991) Characterization of the two size forms of the α_1 subunit of skeletal muscle L-type calcium channels. *Proc Natl Acad Sci USA* 88:10778–10782
- Diebold RJ, Koch WJ, Ellinor PT, Wang J-J, Muthuchamy M, Wiczeorek DF, Schwartz A (1992) Mutually exclusive exon splicing of the cardiac calcium channel α_1 subunit gene generates developmentally regulated isoforms in the rat heart. *Proc Natl Acad Sci USA* 89:1497–1501

- Dubel SJ, Starr TVB, Hell J, Ahlijanian MA, Enyeart JJ, Catterall WA, Snutch TP (1992) Molecular cloning of the α -1 subunit of an ω -conotoxin-sensitive calcium channel. *Proc Natl Acad Sci USA* 89:5058–5062
- Ellis SB, Williams ME, Ways NR, Brenner R, Sharp AH, Leung AT, Campbell KP, McKenna E, Koch WJ, Hui A, Schwartz A, Harpold MM (1988) Sequence and expression of mRNAs encoding the α_1 and α_2 subunits of a DHP-sensitive calcium channel. *Science* 241:1661–1664
- Flockerzi V, Oeken HJ, Hofmann F, Pelzer D, Cavalié A, Trautwein W (1986) Purified dihydropyridine-binding site from skeletal muscle t-tubules is a functional calcium channel. *Nature* 323:66–68
- Garcia J, Gamboa-Aldeco R, Stefani E (1990) Charge movement and calcium currents in skeletal muscle fibers are enhanced by GTP γ S. *Pflügers Arch* 417:114–116
- Guy HR, Conti F (1990) Pursuing the structure and function of voltage-gated channels. *TiNS* 13:201–206
- Hamilton S, Codina J, Hawkes MJ, Yatani A, Sawada T, Strickland FM, Froehner SC, Spiegel AM, Toro L, Stefani E, Birnbaumer L, Brown AM (1991) Evidence for direct interaction of G α with the Ca²⁺ channel of skeletal muscle. *J Biol Chem* 266:19528–19535
- Hofmann F, Flockerzi V, Nastainczyk W, Ruth P, Schneider T (1990) The molecular structure and regulation of muscular calcium channels. *Curr Top Cell Regulation* 31:223–239
- Hui A, Ellinor PT, Krizanova O, Wang J-J, Diebold RJ, Schwartz A (1991) Molecular cloning of multiple subtypes of a novel rat brain isoform of the α_1 subunit of the voltage-dependent calcium channel. *Neuron* 7:35–44
- Hullin R, Singer-Lahat D, Freichel M, Biel M, Dascal N, Hofmann F, Flockerzi V (1992) Calcium channel β subunit heterogeneity: functional expression of cloned cDNA from heart, aorta and brain. *EMBO J* 11:885–890
- Hymel L, Striessnig J, Glossmann H, Schindler H (1988) Purified skeletal muscle 1,4-dihydropyridine receptor forms phosphorylation-dependent oligomeric calcium channels in planar bilayers. *Proc Natl Acad Sci USA* 85:4290–4294
- Itagaki K, Koch WJ, Bodi I, Klöckner U, Slish DF, Schwartz A (1992) Native-type DHP-sensitive calcium channel currents are produced by cloned rat aortic smooth muscle and cardiac α_1 subunits expressed in *Xenopus laevis* oocytes and are regulated by α_2 - and β -subunits. *FEBS Lett* 297:221–225
- Jay SD, Ellis SB, McCue AF, Williams ME, Vedvick TS, Harpold MM, Campbell K (1990) Primary structure of the γ subunit of the DHP-sensitive calcium channel from skeletal muscle. *Science* 248:490–492
- Jay SD, Sharp AH, Kahl StD, Vedvick TS, Harpold MM, Campbell KP (1991) Structural characterization of the dihydropyridine-sensitive calcium channel α_2 -subunit and the associated δ peptides. *J Biol Chem* 266:3287–3293
- Kameyama M, Hescheler J, Hofmann F, Trautwein W (1986) Modulation of Ca current during the phosphorylation cycle in the guinea pig heart. *Pflügers Arch* 407:123–128
- Kass RS, Arena JP, Chin S (1991) Block of L-type calcium channels by charged dihydropyridines. *J Gen Physiol* 98:63–75
- Kim HL, Kim H, Lee P, King RG, Chin HR (1992) Rat brain expresses an alternatively spliced form of the dihydropyridine-sensitive L-type calcium channel alpha-2 subunit. *Proc Natl Acad Sci USA* 89:3251–3255
- Kim HS, Wei X, Ruth P, Perez-Reyes E, Flockerzi V, Hofmann F, Birnbaumer L (1990) Studies on the structural requirements for the activity of the skeletal muscle dihydropyridine receptor/slow Ca²⁺ channel. *J Biol Chem* 265:11858–11863
- Kleuss C, Hescheler J, Ewel C, Rosenthal W, Schultz G, Wittig B (1991) Assignment of G-protein subtypes to specific receptors inducing inhibition of calcium currents. *Nature* 353:43–49

- Klößner U, Itagaki K, Bodi I, Schwartz A (1992) β -Subunit expression is required for cAMP-dependent increase of cloned cardiac and vascular calcium channel currents. *Pflügers Arch* 420:413–415
- Koch WJ, Ellinor PT, Schwartz A (1990) cDNA cloning of a dihydropyridine-sensitive calcium channel from rat aorta. *J Biol Chem* 265:17786–17791
- Lacerda AE, Kim HS, Ruth P, Perez-Reyes E, Flockerzi V, Hofmann F, Birnbaumer L, Brown AM (1991) Normalization of current kinetics by interaction between the α_1 and β subunits of the skeletal muscle dihydropyridine-sensitive Ca^{2+} channel. *Nature* 352:527–530
- Lai Y, Seagar MJ, Takahashi M, Catterall W (1990) Cyclic AMP-dependent phosphorylation of two size forms of α_1 subunits of L-type calcium channels in rat skeletal muscle cells. *J Biol Chem* 34:20839–20848
- Lory P, Varadi G, Schultz D, Schwartz A (1992) Subunit composition regulates the skeletal L-type Ca Channel. *FASEB J* 6:A406
- Mikami A, Imoto K, Tanabe T, Niidome T, Mori Y, Takeshima H, Narumiya S, Numa S (1989) Primary structure and functional expression of the cardiac dihydropyridine-sensitive calcium channel. *Nature* 340:230–233
- Miller RJ (1992) Voltage-sensitive Ca^{2+} channels. *J Biol Chem* 267:1403–1406
- Mintz IM, Venema VJ, Swiderek KM, Lee TD, Bean BP, Adams ME (1992) P-type calcium channels blocked by the spider toxin ω -Aga-IVA. *Nature* 355:827–830
- Mori Y, Friedrich T, Kim M-S, Mikami A, Nakai J, Ruth P, Bosse E, Hofmann F, Flockerzi V, Furuichi T, Mikoshiba K, Imoto K, Tanabe T, Numa S (1991) Primary structure and functional expression from complementary DNA of a brain calcium channel. *Nature* 350:398–402
- Mundina-Weilenmann C, Chang CF, Gutierrez LM, Hosey MM (1991) Demonstration of the phosphorylation of dihydropyridine-sensitive calcium channels in chick skeletal muscle and the resultant activation of the channels after reconstitution. *J Biol Chem* 266:4067–4073
- Nakayama H, Taki M, Striessnig J, Glossmann H, Catterall WA, Kanaoka Y (1991) Identification of 1,4-dihydropyridine binding regions within the α_1 subunit of skeletal muscle Ca^{2+} channels by photoaffinity labeling with diazepam. *Proc Natl Acad Sci USA* 88:9203–9207
- Norman RI, Burgess AJ, Allen E, Harrison TM (1987) Monoclonal antibodies against the 1,4-dihydropyridine receptor associated with voltage-sensitive Ca^{2+} channels detect similar polypeptides from a variety of tissues and species. *FEBS Letters* 212:127–132
- Nunoki K, Florio V, Catterall WA (1989) Activation of purified calcium channels by stoichiometric protein phosphorylation. *Proc Natl Acad Sci* 86:6816–6820
- Perez-Reyes E, Castellano A, Kim HS, Bertrand P, Bagstrom E, Lacerda A, Wei X, Birnbaumer L (1992) Cloning and expression of cardiac/brain β subunit of the L-type calcium channel. *J Biol Chem* 267:1792–1797
- Perez-Reyes E, Kim HS, Lacerda AE, Horne W, Wei X, Rampe D, Campbell KP, Brown AM, Birnbaumer L (1989) Induction of calcium currents by the expression of the α_1 subunit of the dihydropyridine receptor from skeletal muscle. *Nature* 340:233–236
- Perez-Reyes E, Wei X, Castellano A, Birnbaumer L (1990) Molecular diversity of L-type calcium channel. Evidence for alternative splicing of the transcripts of three non-allelic genes. *J Biol Chem* 265:20430–20436
- Pragnell M, Sakamoto J, Jay SD, Campbell KP (1991) Cloning and tissue-specific expression of the brain calcium channel β -subunit. *FEBS Lett* 291:253–258
- Regulla S, Schneider T, Nastainczyk W, Meyer HE, Hofmann F (1991) Identification of the site of interaction of the dihydropyridine channel blockers nitrendipine and azidopine with the calcium-channel α_1 subunit. *EMBO J* 10:45–49
- Rios E, Pizarro G, Stefani E (1992) Charge movement and the nature of signal transduction in skeletal muscle excitation-contraction coupling. *Annu Rev Physiol* 54:251–275

- Röhrkasten A, Meyer HE, Nastainczyk W, Sieber M, Hofmann F (1988) cAMP-dependent protein kinase rapidly phosphorylates serine-687 of the skeletal muscle receptor for calcium channel blockers. *J Biol Chem* 263:15325–15329
- Rotman EI, Florio V, Lai Y, De Jongh D, Catterall WA (1992) Specific phosphorylation of a C-terminal site on the 212 kDa form of the α_1 subunit of the skeletal muscle calcium channel by cAMP-dependent protein kinase. *FASEB J* 6:A246
- Ruth P, Röhrkasten A, Biel M, Bosse E, Regulla S, Meyer HE, Flockerzi V, Hofmann F (1989) Primary structure of the β subunit of the DHP-sensitive calcium channel from skeletal muscle. *Science* 245:1115–1118
- Schneider T, Regulla S, Hofmann F (1991) The devapamil-binding site of the purified skeletal muscle receptor for organic-calcium channel blockers is modulated by micromolar and millimolar concentrations of Ca²⁺. *Eur J Biochem* 200:245–253
- Seino S, Chen L, Seino M, Blondel O, Takeda J, Johnson JH, Bell GI (1992) Cloning of the α_1 subunit of a voltage-dependent calcium channel expressed in pancreatic β cells. *Proc Natl Acad Sci USA* 89:584–588
- Singer D, Biel M, Lotan I, Flockerzi V, Hofmann F, Dascal N (1991) The roles of the subunits in the function of the calcium channel. *Science* 253:1553–1557
- Snutch TP, Leonard JP, Gilbert MM, Lester HA, Davidson N (1990) Rat brain expresses a heterogeneous family of calcium channels. *Proc Natl Acad Sci USA* 87:3391–3395
- Snutch TP, Tomlinson WJ, Leonard JP, Gilbert MM (1991) Distinct calcium channels are generated by alternative splicing and are differentially expressed in the mammalian CNS. *Neuron* 7:45–57
- Starr TVB, Prystay W, Snutch TP (1991) Primary structure of a calcium channel that is highly expressed in the rat cerebellum. *Proc Natl Acad Sci USA* 88:5621–5625
- Staudinger R, Knaus H-G, Glossmann H (1991) Positive heterotopic allosteric regulators of dihydropyridine binding increase the Ca²⁺ affinity of the L-type Ca²⁺ channel. *J Biol Chem* 266:10787–10795
- Striessnig J, Glossmann H, Catterall WA (1990) Identification of a phenylalkylamine binding region within the α_1 subunit of skeletal muscle Ca²⁺ channels. *Proc Natl Acad Sci USA* 87:9108–9112
- Striessnig J, Murphy BJ, Catterall WA (1991) Dihydropyridine receptor of L-type Ca²⁺ channels: identification of binding domains for [³H] (+)-PN200-110 and [³H]azidopine within the α_1 subunit. *Proc Natl Acad Sci USA* 88:10769–10773
- Tanabe T, Beam KG, Adams BA, Niidome T, Numa S (1990) Regions of the skeletal muscle dihydropyridine receptor critical for excitation-contraction coupling. *Nature* 346:567–569
- Tanabe T, Beam KG, Powell JA, Numa S (1988) Restoration of excitation-contraction coupling and slow calcium current in dysgenic muscle by dihydropyridine receptor complementary DNA. *Nature* 336:134–139
- Tanabe T, Brett AA, Numa S, Beam KG (1991) Repeat I of the dihydropyridine receptor is critical in determining calcium channel activation kinetics. *Nature* 352:800–803
- Tanabe T, Takeshima H, Mikami A, Flockerzi V, Takahashi H, Kangawa K, Kojima M, Matsuo H, Hirose T, Numa S (1987) Primary structure of the receptor for calcium channel blockers from skeletal muscle. *Nature* 328:313–318
- Trautwein W, Hescheler J (1990) Regulation of cardiac L-type calcium current by phosphorylation and G-proteins. *Annu Rev Physiol* 52:257–274
- Varadi G, Lory P, Schultz D, Varadi M, Schwartz A (1991) Acceleration of activation and inactivation by the β subunit of the skeletal muscle calcium channel. *Nature* 352:159–162
- Wei X, Perez-Reyes E, Lacerda AE, Schuster G, Brown AM, Birnbaumer L (1991) Heterologous regulation of the cardiac Ca²⁺ channel α_1 subunit by skeletal muscle β and γ subunits. *J Biol Chem* 266:21943–21947

- Welling A, Felbel J, Peper K, Hofmann F (1992a) Hormonal regulation of calcium current in freshly isolated airway smooth muscle cells. *Am J Physiol* 262:L351–L359
- Welling A, Bosse E, Ruth P, Bottlender R, Flockerzi V, Hofmann F (1992b) Expression and regulation of cardiac and smooth muscle calcium channels. *J J Pharmacol* 58 Suppl-II 1258p–1262p
- Welling A, Bosse E, Cavalie A, Bottlender R, Ludwig A, Nastainczyk W, Flockerzi V, Hofmann F (1993) Stable co-expression of Calcium Channel α_1 , β and α_2 , δ Subunits in a Somatic Cell Line. *J Physiol*, in Press
- Williams ME, Feldman DH, McCue AF, Brenner R, Velicelebi G, Ellis SB, Harpold MM (1992a) Structure and functional expression of α_1 , α_2 , and β subunits of a novel human neuronal calcium channel subtype. *Neuron* 8:71–84
- Williams ME, Brust PF, Feldman DH, Patti S, Simerson S, Maroufi A, McCue AF, Velicelebi G, Ellis SB, Harpold MM (1992b) Structure and functional expression of an ω -conotoxin-sensitive human N-type calcium channel. *Science* 257:389–395