

CONTENTS

Contributors

xi

Preface

xvii

1. Mouse Neuroblastoma CB₁ Cannabinoid Receptor-Stimulated [³⁵S] GTPγS Binding: Total and Antibody-Targeted Gα Protein-Specific Scintillation Proximity Assays

1

Khalil Eldeeb, Sandra Leone-Kabler, and Allyn C. Howlett

1. Introduction

2

2. Membrane Preparations for Cultured Neuronal Cells

7

3. Methods of Determining [³⁵S]GTPγS Binding to Total Gα in Membrane Preparations

9

4. Antibody-Targeted Scintillation Proximity Assay for [³⁵S]GTPγS Binding to Specific Gα Proteins in Membrane Preparations

12

5. Summary and Conclusion

15

Acknowledgments

16

References

17

2. Protocols and Good Operating Practices in the Study of Cannabinoid Receptors

23

Linda M. Console-Bram, Pingwei Zhao, and Mary E. Abood

1. Introduction

24

2. Receptor Assays

25

3. Summary and Conclusions

39

References

41

3. Real-Time Measurement of Cannabinoid Receptor-Mediated cAMP Signaling

43

Morag R. Hunter, David B. Finlay, Christa E. Macdonald, Erin E. Cawston,

Natasha L. Grimsey, and Michelle Glass

1. Introduction

44

2. Detecting Cytoplasmic cAMP Using CAMYEL

48

3. Design and Validation of V8-CAMYEL

52

4. Summary

56

References

56

v |

4. Techniques for the Cellular and Subcellular Localization of Endocannabinoid Receptors and Enzymes in the Mammalian Brain 61

Luigia Cristino, Roberta Imperatore, and Vincenzo Di Marzo

1. Introduction	63
2. Use of Conventional Fluorescence Microscopy to Study the ECS	64
3. Localization-Based Microscopy	68
4. Transmission Electron Microscopy	73
5. Confocal, TEM, and STORM Microscopy Applied to Study the ECS: A Technical Comparison	77
6. FRET	83
7. Conclusions	87
References	88
Further Reading	97

5. Endocannabinoid Transport Proteins: Discovery of Tools to Study Sterol Carrier Protein-2 99

Cecilia J. Hillard, Huan Huang, Caleb D. Vogt, Beatriz E. Rodrigues, Terrence S. Neumann, Daniel S. Sem, Friedhelm Schroeder, and Christopher W. Cunningham

1. Introduction	100
2. Materials and Methods	102
3. Results and Discussion	105
4. Conclusions	118
References	118
Further Reading	121

6. Lipidomics: A Corrective Lens for Enzyme Myopia 123

Heather B. Bradshaw and Emma Leishman

1. Introduction	124
2. Molecule Specifist vs Generalist	126
3. Lipoamines (aka Fatty Acid Amides; Lipo Amino Acids; N-Acyl Amides; N-Acyl Amino Acids)	128
4. 2-Acyl Glycerols Beyond 2-AG	132
5. Prostaglandins Create Additional Complexity to the AA-Derived Signaling System	133
6. What Do Enzymes Tell Us About Lipoamines and What Do Lipoamines Tell Us about Enzymes?	134
7. Seeing Our Way Forward	138
References	138

7. Functional Analysis of Mitochondrial CB1 Cannabinoid Receptors (mtCB1) in the Brain 143

Su Melser, Antonio C. Pagano Zottola, Roman Serrat, Nagore Puente,
Pedro Grandes, Giovanni Marsicano, and Etienne Hebert-Chatelain

1. Introduction 145
2. Detection of mtCB1 148
3. Impact of mtCB1 on Mitochondrial Respiration 157
4. Impact of mtCB1 on Complex I Activity 163
5. Impact of mtCB1 on ATP Levels 164
6. Impact of mtCB1 on Mitochondrial Mobility in Neurons 166
7. Conclusions 170
- Acknowledgments 171
- References 171

8. Modeling Neurodegenerative Disorders for Developing Cannabinoid-Based Neuroprotective Therapies 175

Javier Fernández-Ruiz, María Gómez-Ruiz, Concepción García,
Mariluz Hernández, and José A. Ramos

1. Introduction 177
2. The Biomedical Challenge of Neurodegenerative Disorders 177
3. Improving the Modeling of Neurodegenerative Disorders in Cells
and Laboratory Animal Species 181
4. Cannabinoids as Neuroprotectant Agents 183
5. Concluding Remarks and Future Perspectives 193
- Acknowledgments 194
- References 194

9. Metabolic Profiling of CB1 Neutral Antagonists 199

Herbert H. Seltzman, Rangan Maitra, Katharine Bortoff, Jay Henson,
Patricia H. Reggio, Daniel Wesley, and Joseph Tam

1. Introduction 200
2. Background 202
3. Computational Modeling 202
4. Chemistry and Structure Proof and Stability Ki CB1 CB2 204
5. In Vivo Metabolic Effects 205
6. PIMSR and Binge Alcoholic Hepatic Steatosis 209
7. Conclusions 213
- Acknowledgments 213
- References 213

10. Ligand-Assisted Protein Structure (LAPS): An Experimental Paradigm for Characterizing Cannabinoid-Receptor Ligand-Binding Domains	217
David R. Janero, Anisha Korde, and Alexandros Makriyannis	
1. Introduction	218
2. Ligand-Assisted Protein Structure	219
3. Application of LAPS to Endocannabinoid-System GPCRs	221
4. Conclusions	228
Acknowledgments	231
References	232
11. New Methods for the Synthesis of Cannabidiol Derivatives	237
Ana Lago-Fernandez, Vanessa Redondo, Laura Hernandez-Folgado, Laura Figuerola-Asencio, and Nadine Jagerovic	
1. Introduction	238
2. Synthesis of CBD Derivatives	239
3. Experimental Details for Schemes 13 and 14	250
4. Conclusions	254
Acknowledgments	255
References	255
12. Approaches to Assess Biased Signaling at the CB1R Receptor	259
Robert B. Laprairie, Edward L. Stahl, and Laura M. Bohn	
1. Introduction	260
2. Quantifying CB1R Ligand Bias	263
3. Assays Used to Examine CB1R Ligand Bias	265
4. Important Considerations and Caveats	271
5. Conclusions	275
Acknowledgment	275
References	275
13. Design and Synthesis of Cannabinoid 1 Receptor (CB1R) Allosteric Modulators: Drug Discovery Applications	281
Abhijit R. Kulkarni, Sumanta Garai, David R. Janero, and Ganesh A. Thakur	
1. Introduction	283
2. CB1R Allosteric Modulators	288
3. Designer Probes for Characterizing CB1R Allosteric Site(s)	306
4. Summary and Future Directions	308
Acknowledgment	310
References	310

14. Assessing Allosteric Modulation of CB₁ at the Receptor and Cellular Levels	317
Caitlin E. Scott and Debra A. Kendall	
1. Introduction	318
2. Methods of Allosteric Ligand Binding Analysis	321
3. G-Protein Coupling	329
4. Cellular Internalization of CB ₁	330
5. Analysis of Kinase Phosphorylation	332
6. Perspectives	336
Acknowledgments	337
References	337
15. Purification of Functional CB₁ and Analysis by Site-Directed Fluorescence Labeling Methods	343
Jonathan F. Fay and David L. Farrens	
1. Introduction	344
2. Expression and Purification of Functional CB ₁	345
3. Site-Directed Fluorescence Labeling Studies of CB ₁	356
4. Summary and Conclusion	363
References	368
16. Mass Spectrometry Analysis of Human CB₂ Cannabinoid Receptor and Its Associated Proteins	371
Zhao-Hui Song, Alyssa S. Laun, and Jian Cai	
1. Introduction	372
2. Mass Spectrometry Analysis of Human CB ₂ Cannabinoid Receptor	374
3. Functional Proteomic Analysis of CB ₂ -Associated Proteins	378
4. Chemical Cross-linking and Mass Spectrometry Analysis of CB ₂ -G Protein Complex	381
5. Conclusion	384
Acknowledgment	385
References	385
17. Expression and NMR Structural Studies of Isotopically Labeled Cannabinoid Receptor Type II	387
Alexei Yeliseev and Klaus Gawrisch	
1. Introduction	388
2. Preparation of Stable Isotope-Labeled Cannabinoid Receptor—An Overview of the Method	389
3. Materials	391

4. Expression of CB ₂ in a Fermenter	391
5. Purification of Recombinant CB ₂ Receptor	394
6. Reconstitution of CB ₂ Into Lipid Bilayers	396
7. Acquisition of NMR Spectra	397
8. Protocol Notes	399
9. Conclusions	401
Acknowledgments	401
References	401
18. Methods for the Development of In Silico GPCR Models	405
Paula Morales, Dow P. Hurst, and Patricia H. Reggio	
1. Introduction	406
2. Sequence Alignment	407
3. Choosing the Appropriate Template for Model Construction	413
4. Model Development	418
5. Docking Studies	437
6. Model Refinement	441
7. Summary	441
References	442
Further Reading	448
19. Molecular Dynamics Methodologies for Probing Cannabinoid Ligand/Receptor Interaction	449
Diane L. Lynch, Dow P. Hurst, Derek M. Shore, Mike C. Pitman, and Patricia H. Reggio	
1. Introduction	451
2. General Methods	455
3. Methods for Each System	460
4. Results and Discussion	466
5. Conclusions	483
References	485
Author Index	491
Subject Index	525