PREFACE

I would like to start this Preface by giving the reader a very short synopsis of what I call the history of the modern era of cannabinoid research. Although cannabinoids have been shown to act at other sites such as TRP channels (Di Marzo & De Petrocellis, 2010), this summary focuses on cannabinoid action at the cannabinoid CB1 and CB2 receptors. Because of its brevity, this synopsis leaves out the work of many important cannabinoid researchers, and for this I apologize.

The cannabinoid field entered its modern era in 1964 when Raphael Mechoulam reported that the principal psychoactive compound in hashish was (−)-trans-Δ^9-tetrahydrocannabinol ((−)-Δ^9-THC) (Gaoni & Mechoulam, 1964; Mechoulam, Braun, & Gaoni, 1967; Mechoulam & Gaoni, 1967). This identification ushered in two important lines of research: (1) the isolation and synthesis of phytocannabinoid compounds such as cannabidiol (CBD) and their metabolites (Lander et al., 1976) and (2) the synthetic elaboration of new cannabinoid compounds based initially on the (−)-Δ^9-THC structure. This led to the identification of nonclassical cannabinoids such as the potent CB1 ligand, CP55940 (Melvin, Milne, Johnson, Wilken, & Howlett, 1995), and the identification of aminoalkylindoles as CB1 ligands (D'Ambra et al., 1992). Animal models were developed for testing compounds. The most used in vivo assay over the years has been the mouse tetrad assay which includes measurements of rectal temperature, catalepsy, locomotor behavior, and the tail flick assay. When I entered the field in 1985, hundreds of cannabinoid compounds had been synthesized and evaluated in vivo. So, I began my work by developing a structure–activity relationship for psychoactive cannabinoids. At this point, no cannabinoid receptor had been identified and many thought that cannabinoid effects were not receptor mediated, but rather were the result of changes in membrane fluidity produced by highly lipophilic cannabinoids.

Allyn Howlett began to build a case for the existence of a cannabinoid receptor. She first demonstrated that psychoactive cannabinoids produced an inhibition of adenylate cyclase in neuroblastoma cell membranes (Howlett & Fleming, 1984). Then in 1988, Allyn's lab proved that a cannabinoid receptor was present in rat brain and that this receptor was a G-protein-coupled receptor (Devane, Dysarz, Johnson, Melvin, & Howlett, 1988). This permitted for the first time the development of an SAR based on receptor affinity.

Two important pieces of the cannabinoid receptor story were still missing. No antagonist of the CB1 receptor had been discovered and the endogenous ligand of the cannabinoid receptor was yet unknown. In 1994, Sanofi Recherche published their development of SR141716A, the first cannabinoid receptor antagonist (Rinaldi-Carmona et al., 1994). Then in 1992, the Mechoulam lab reported that anandamide (*N*-arachidonoylethanolamine; AEA), an arachidonic acid derivative, was the endogenous ligand of the cannabinoid receptor in brain (Devane et al., 1992). The DNA sequence of the CB1 receptor was published by Tom Bonner's group in 1990 (Matsuda, Lolait, Brownstein, Young, & Bonner, 1990). This discovery permitted the creation of the first CB1 computer model (Bramblett, Panu, Ballesteros, & Reggio, 1995).

Given the wide-ranging effects of THC that included many non-neuronal effects, such as immune system modulation, the existence of at least one other cannabinoid receptor seemed likely. In 1993, Munro and coworkers cloned a peripheral cannabinoid receptor, CB_2, from a human promyelocytic leukemia cell HL60 DNA library (Munro, Thomas, & Abu-Shaar, 1993). Then in 1995, two groups identified the endogenous ligand of the CB2 receptor to be 2-arachidonoylglycerol (2-AG) (Mechoulam et al., 1995; Sugiura et al., 1995). 2-AG was also found to bind to CB1. Many cannabinoid compounds had similar affinities at both CB_1 and CB_2, so the search was on for CB_2 selective compounds, including a CB_2 antagonist. In 1998, Sanofi Recherche reported the development of the first CB2 antagonist, SR144528 (Rinaldi-Carmona et al., 1998).

For many years, the cannabinoid receptor was closely associated only with receptors for drugs of abuse. However, in 2001, Wilson and Nicoll (Wilson & Nicoll, 2001) identified the central role of CB1 receptors and the endocannabinoid system in brain to be a mechanism by which neurons can communicate backward across GABAergic synapses to modulate their inputs (i.e., retrograde signaling) using 2-AG as the messenger. The biosynthetic enzymes for 2-AG were discovered to be localized on postsynaptic neurons in dendritic spines and somatodendritic compartments. Released 2-AG was found to control the activity of the complementary presynaptic neuron by binding to the CB1 receptor which is often expressed there (Di Marzo, 2009). Degradation of 2-AG was found to be accomplished presynaptically, principally by a membrane-associated enzyme, monoacylglycerol lipase (Dinh et al., 2002).

AEA synthetic pathways are still the subject of active research. One synthetic pathway that has been reported by Nyilas and coauthors involves

N-acylphosphatidylethanolamine-hydrolyzing phospholipase D, a biosynthetic enzyme (Okamoto, Wang, Morishita, & Ueda, 2007) that is concentrated presynaptically and is associated with intracellular calcium stores. This indicates that, in contrast to 2-AG, endocannabinoids like AEA may have a presynaptic origin and their production may reflect the status of axon terminal [Ca^{2+}] (in part following release from intracellular stores) (Nyilas et al., 2008). After acting at presynaptic CB1 receptors, AEA was found to be taken up by postsynaptic cells. Ben Cravatt's lab reported that AEA degradation was produced by a membrane-bound enzyme, fatty acid amide hydrolase (FAAH) (Bracey, Hanson, Masuda, Stevens, & Cravatt, 2002). This enzyme was shown by immunohistochemistry to be localized to the endoplasmic reticulum (Arreaza & Deutsch, 1999; Giang & Cravatt, 1997). One of the lingering questions in the cannabinoid field is how does AEA reach FAAH for degradation? Are there proteins that act as carriers for the endocannabinoids either in membranes or aqueous compartments, including the cytosol and extracellular space, or does AEA move via simple diffusion? A specific anandamide transporter and carriers that transport multiple classes of lipid-related ligands, such as fatty acid-binding protein and sterol-carrier protein-2, have been proposed, but the field has not reached consensus on this yet (Di Marzo et al., 1994; Hillard & Jarrahian, 2000; Kaczocha, Glaser, & Deutsch, 2009; Liedhegner, Vogt, Sem, Cunningham, & Hillard, 2014; Piomelli, Beltramo, Giuffrida, & Stella, 1998). It may be that more than one carrier protein is involved in this process.

Since 2005, new classes of cannabinoid ligands have emerged. The first allosteric modulator of the CB1 receptor, ORG27569, was reported by Ruth Ross and coauthors (Price et al., 2005). This compound is a positive allosteric modulator for CP55940 affinity, but a negative allosteric modulator of CP55940 signaling. In 2014, Pier-Vincenzo Piazza and coauthors reported the first endogenous allosteric modulator of the CB1 receptor, pregnenolone (Vallee et al., 2014). Deborah Kendall's lab discovered that ORG27569 can act as a biased agonist of the CB1 receptor, signaling via beta-arrestin-1 (Ahn, Mahmoud, Shim, & Kendall, 2013). This was the first demonstration of a purely biased cannabinoid ligand and the first demonstration that a CB1 compound can signal via a G-protein-independent beta-arrestin signaling pathway.

Finally, in 2016, the first crystal structures of the cannabinoid CB1 receptor were published (Hua et al., 2016; Shao et al., 2016). Both structures were crystallized with CB1 antagonists/inverse agonists and therefore represent

the inactive state of the receptor. Each has a portal formed by the extracellular portions of TMH1/TMH7 that can serve as an entry point for antagonists/inverse agonists from the lipid bilayer. These results are consistent with earlier molecular dynamics studies which suggested that 2-AG can access the CB2 receptor via the lipid bilayer (Hurst et al., 2010). As this chapter went to press, the first agonist-bound structure of CB1 was published (Hua et al., 2017).

The chapters in this volume describe methods that can be used to take the cannabinoid field in new, interesting directions. Chapters 1–3 describe new, more efficient ways to assay for G-protein-dependent signaling of the cannabinoid receptors. These include Allyn Howlett and Khalil Eldeeb's chapter entitled "Mouse Neuroblastoma CB1 Cannabinoid Receptor-Stimulated [^{35}S]GTPγS Binding: Total and Antibody-Targeted Gα Protein-Specific Scintillation Proximity Assays" (Chapter 1); Mary Abood's chapter entitled "Protocols and Good Operating Practices in the Study of Cannabinoid Receptors" (Chapter 2); and Michelle Glass' chapter entitled "Real-Time Measurement of Cannabinoid Receptor-Mediated cAMP Signaling" (Chapter 3).

Chapters 4–6 focus on assays used to study the endocannabinoid system. Vincenzo Di Marzo presents "Techniques for the Cellular and Subcellular Localization of Endocannabinoid Receptors and Enzymes in the Mammalian Brain" (Chapter 4). Cecilia Hillard's chapter entitled "Endocannabinoid Transport Proteins: Discovery of Tools to Study Sterol Carrier Protein-2" looks at assays for studying endocannabinoid transport and methods for identifying new candidate transport inhibitors (Chapter 5). Heather Bradshaw's chapter entitled "Lipidomics: A Corrective Lens for Enzyme Myopia" (Chapter 6) makes the argument that using lipidomics as a tool for characterizing enzymatic function over more traditional toolkit options will allow investigators to see the consequences of a selective knockout of an endocannabinoid system enzyme for the entire brain lipidome.

Giovanni Marsicano's chapter, entitled "Functional Analysis of Mitochondrial CB1 Cannabinoid Receptors (mtCB1) in the Brain" (Chapter 7), presents some key experimental approaches established in the Marsicano lab to identify anatomical, biochemical, and functional features of mtCB1 receptors in the brain. This includes procedures to obtain reliable and controlled detection of mtCB1 receptors by immunogold electron microscopy and by immunoblotting methods; and methods for studying direct cannabinoid effects on the electron transport system and oxidative phosphorylation.

Javier Fernández-Ruiz's chapter is entitled "Modeling Neurodegenerative Disorders for Developing Cannabinoid-Based Neuroprotective Therapies" (Chapter 8). The chapter proposes that the cannabinoids and their classic (e.g., endocannabinoid receptors and enzymes) and nonclassic (e.g., PPARs, transcription factors) targets can be a useful system for developing novel therapies for neurological disorders.

Chapters 9–11 describe the development of new cannabinoid ligands. Herb Seltzman's chapter, entitled "Metabolic Profiling of CB1 Neutral Antagonists," explores the therapeutic potential of the neutral antagonist, PIMSR (Chapter 9). Alexandros Makriyannis' chapter, entitled "Ligand-Assisted Protein Structure (LAPS): An Experimental Paradigm for Characterizing Cannabinoid-Receptor Ligand-Binding Domains" (Chapter 10), discusses an experimental paradigm employed in the Makriyannis lab termed "ligand-assisted protein structure" (LAPS) that affords a means of characterizing, at the amino-acid level, CB1R and CB2R structural features key to ligand engagement and receptor-dependent information transmission. The nonpsychoactive phytocannabinoid, CBD, has been shown to have neuroprotective effects. In Nadine Jagerovic's chapter entitled "New Methods for the Synthesis of Cannabidiol Derivatives" (Chapter 11), Nadine illustrates new synthetic routes for CBD derivatives.

There is growing interest in using biased signaling of ligands to direct signaling downstream of CB1R toward desirable therapeutic outcomes and to avoid adverse side effects. In Laura Bohn's chapter entitled "Approaches to Assess Biased Signaling at the CB1R Receptor" (Chapter 12), Laura shares the methodology employed in her lab to characterize biased ligands.

Chapters 13 and 14 focus on the synthesis and identification of CB1 allosteric modulators. In Ganesh Thakur's chapter entitled "Design and Synthesis of Cannabinoid 1 Receptor (CB1R) Allosteric Modulators: Drug Discovery Applications" (Chapter 13), Ganesh shows new synthetic routes to CB1 allosteric ligands, while in Chapter 14, entitled "Assessing Allosteric Modulation of CB1 at the Receptor and Cellular Levels," Debra Kendall describes methodology used in her lab to characterize such allosteric modulators.

Chapters 15–17 are devoted to the use of biophysical/analytical methods to study the structure/function of CB1 and CB2. In Chapter 15, entitled "Purification of Functional CB1 and Analysis by Site-Directed Fluorescence Labeling Methods," David Farrens describes methodology used in his lab to characterize CB1 receptor conformational changes. Chapter 16, by

Zhao-Hui Song, is entitled "Mass Spectrometry Analysis of Human CB2 Cannabinoid Receptor and Its Associated Proteins." Here, Zhao-Hui describes the use of cross-linking followed by mass spectroscopic analysis to identify interaction sites between CB2 and its associated proteins. In Chapter 17, entitled "Expression and NMR Structural Studies of Isotopically Labeled Cannabinoid Receptor Type II," Klaus Gawrisch presents methodology for studying NMR structural studies of CB2.

The final two chapters here describe methods to construct and study the dynamical behavior of computer models of Class A G-protein-coupled receptors such as CB1 and CB2. Paula Morales' chapter (Chapter 18), entitled "Methods for the Development of In Silico GPCR Models," describes methodology to construct these models, and Diane Lynch's chapter (Chapter 19), entitled "Molecular Dynamics Methodologies for Probing Cannabinoid Ligand/Receptor Interaction," describes molecular dynamics methodology for studying the behavior of such models in fully hydrated lipid bilayers.

As you can see, the cannabinoid field has progressed since 1964. It has been exciting to be part of this story. It is my hope that this volume will help new scientists entering the cannabinoid field. I wish you the best of luck.

Video file is available on http://dx.doi.10.1016/S0076-6879(17)30257-4.

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