ION CHANNELS DOWNUNDER

EDITED BY

DOMINIC GERAGHTY AND LACHLAN D. RASH

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PREFACE

Ion channels are the fastest cellular signaling system, underlying rapid processes such as axon conduction and synaptic transmission. However, ion channels are also found in nonexcitable cells and are indispensable for processes such as secretion, gene expression, and cell division. With over 140 members, ion channels are the second largest family of signaling molecules in the body and are activated by a diverse range of stimuli such as ligands, membrane voltage changes, temperature, stretch, and changes in pH. As "first responders," a detailed understanding of ion channels is crucial to understanding how cells initially respond to changes in their environment.

There have been spectacular advances in this area in the past two decades, highlighted by the award of the 2003 Nobel Prize in Chemistry to Roderick MacKinnon for his determination of the 3D structure of a voltage-gated potassium channel. Despite the recent leaps and bounds of progress made in the area of ion channel structural biology (e.g., cryo-EM), it is our ability to selectively modulate ion channel function in vitro and in vivo that holds the key to unlocking the physiological and pathological roles of ion channels. To this end, high-quality ion channel pharmacology will provide the tools and therapeutic leads to address many unmet medical needs. The chapters in this volume demonstrate that the momentum has not changed and, indeed, has increased. Whether dissecting the activation of ryanodine receptors, describing the development of subunit-selective ligands for glycine and GABA receptors, or the contribution of calcium imaging in high-throughput identification of drug leads, the contributors have used state-of-the-art techniques and provided narratives and insights that will generate new ideas for years to come.

We wish to thank the many contributors to this volume. They have covered the pharmacology and role of a large number of channels in health and disease, and included some uniquely Australian research, such as employing peptides from our (many) venomous animals. You will no doubt

agree that *Ion Channels DownUnder* demonstrates the depth and breadth of excellent research being undertaken on the pharmacology ion channels around Australia.

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CONTENTS

CHAPTER ONE

$GABA_A$ Receptors and the Diversity in their Structure and Pharmacology

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Contents

Abstract

$GABA_A$ receptors ($GABA_A$Rs) are a class of ligand-gated ion channels with high physiological and therapeutic significance. In the brain, these pentameric receptors occur with diverse subunit composition, which confers highly complex pharmacology to this receptor class. An impressive range of clinically used therapeutics are known to bind to distinct sites found on $GABA_A$Rs to modulate receptor function. Numerous experimental approaches have been used over the years to elucidate the binding sites of these drugs, but unequivocal identification is challenging due to subtype- and ligand-dependent pharmacology. Here, we review the current structural and pharmacological understanding of $GABA_A$Rs, besides highlighting recent evidence which has revealed greater complexity than previously anticipated.

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ABBREVIATIONS

CS read counter-clockwise when viewed from the extracellular side

1. INTRODUCTION

γ-Aminobutyric acid (GABA), the major inhibitory neurotransmitter in the mammalian central nervous system (CNS), is the conductor of an intricate inhibitory orchestra fundamental to the harmonious coordination of brain function. The inhibitory effects of GABA rely on two types of receptors—the fast-acting, Cl^--conducting ionotropic $GABA_A$ receptors ($GABA_ARs$) and the slower-acting, G protein-coupled metabotropic $GABA_B$ receptors ($GABA_BRs$) (Note: GABA- and GABAR-mediated actions may be excitatory under certain circumstances, but these actions are not discussed in this chapter). $GABA_ARs$ are ubiquitously expressed throughout the mammalian CNS and have indispensable physiological roles emphasized by a few lines of evidence. First, the mutation or deletion of various genes encoding for $GABA_AR$ subunits in mice is highly disruptive to the normal phenotype, causing developmental defects, sensorimotor dysfunction, hypersensitive behavior, anxiety, epilepsy, and/or reduced lifespan (DeLorey et al., 1998; Gunther et al., 1995; Homanics et al., 1997; Vien et al., 2015). Second, aberrant $GABA_AR$ trafficking, expression and/or gating effects have been implicated in autism (Fatemi, Reutiman, Folsom, & Thuras, 2009), schizophrenia (Mueller, Haroutunian, & Meador-Woodruff, 2014), and a range of idiopathic epileptic syndromes (Hirose, 2014) in humans. Furthermore, genetic association studies have also linked $GABA_AR$ subunit genes with alcohol dependence (Li et al., 2014), eating disorder outcomes (Bloss et al., 2011), autism (Collins et al., 2006; Ma et al., 2005), and bipolar disorders (Ament et al., 2015; Craddock et al., 2010).

$GABA_ARs$ are also important drug targets, as evidenced by the successful clinical utilization of $GABA_AR$ modulators in the treatment of CNS-related disorders such as insomnia, anxiety, and epilepsy, as well as in the induction of anesthesia in surgical patients (Sieghart, 2015). While clinically useful, the misuse of these drugs poses risks of dependence, addiction, abuse, and life-threatening conditions associated with overdose or withdrawal. Hence, understanding the functions of $GABA_ARs$ and the underlying mechanisms of the clinical and undesirable effects of $GABA_AR$-targeting drugs to

improve the selectivity and safety of these therapeutics are topics of intensive research (Atack, 2010; Rudolph & Knoflach, 2011). However, the striking structural and functional heterogeneity of these channels pose major challenges in the study of $GABA_A$Rs. In this chapter, we provide an overview of the structure and pharmacology of $GABA_A$Rs. We also discuss recent evidence which highlights the potential for greater diversity in $GABA_A$R pharmacology due to subunit stoichiometric and arrangement differences.

2. ARCHITECTURE OF $GABA_A$Rs

$GABA_A$Rs are members of the pentameric ligand-gated ion channel (pLGIC) superfamily, which includes the nicotinic acetylcholine receptors (nAChRs), 5-hydroxytryptamine type 3 receptors (5-HT_3Rs), and glycine receptors. These receptors are made up of five homologous subunits that surround a central ion-conducting pore, a structure which is often likened to a barrel with five staves. Each receptor subunit has an extracellular domain (ECD), a transmembrane domain (TMD), and an intracellular domain (ICD). The ECDs are mostly made up of β-sheets and contribute to agonist binding sites, whereas the TMDs consist of the pore-forming α-helices and the structurally variable ICDs are involved in receptor assembly, trafficking and clustering.

The recent determination of a three-dimensional crystal structure of the β3 homomeric $GABA_A$R captures structural details at 3 Å resolution (Miller & Aricescu, 2014). This receptor subtype is unlikely to be physiologically relevant, but its functional expression in heterologous systems is well known, and has been used as a model to study heteromeric $GABA_A$Rs (Taylor et al., 1999; Yip et al., 2013). The receptor stands approximately 110 Å in height when viewed parallel to the membrane (Fig. 1A). The five subunits assemble in a doughnut-like shape with a diameter around 80 Å, when viewed from the extracellular space, down the channel pore (Fig. 1B). The large ECD (~65 Å in height) of each subunit is made up of an N-terminal α-helix followed by a β-sandwich core with 10 antiparallel β-sheets (Fig. 1C). The TMD consists of four membrane-spanning α-helices (M1–M4), with the M2 helices of all five subunits arranging themselves to form a tapered ion-conducting pore (Fig. 1B and C). The outermost M4 helix harbors the C-terminus on the extracellular end. The ICD contains a small M1–M2 loop and a much larger M3–M4 loop (G333–N446; residue numbering follows sequence of P28472 in UniProt) which was replaced with a 7-amino acid linker for the crystallization of this structure (Fig. 1C).

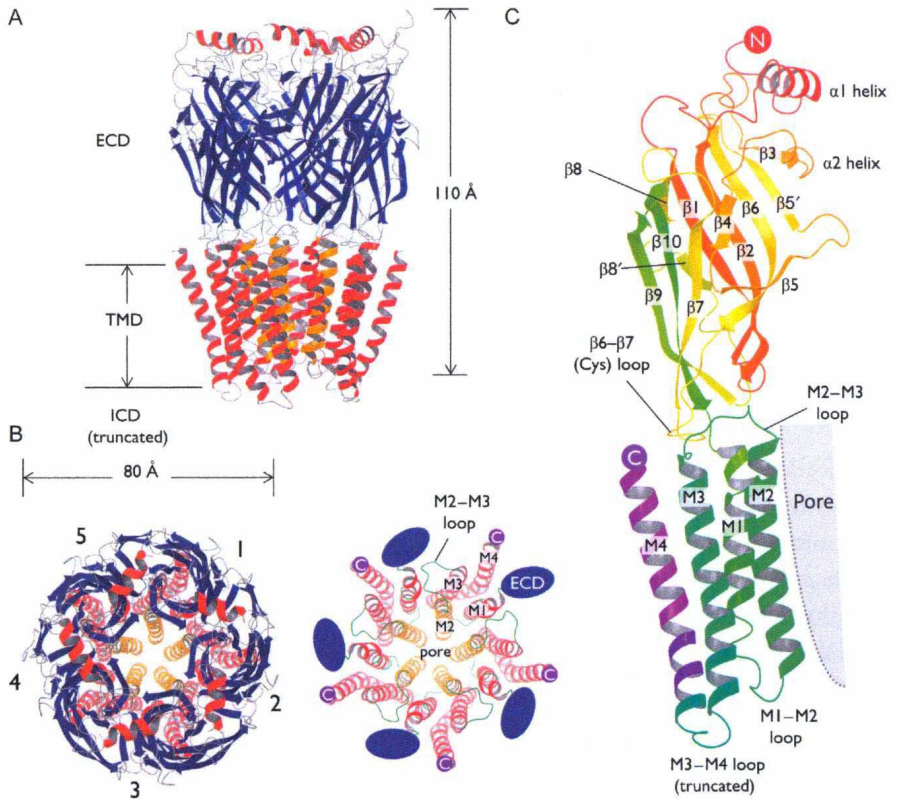

Fig. 1 The crystal structure of a human β3 homomeric $GABA_AR$ (PDB: 4COF). (A) Cartoon representation of $GABA_AR$ viewed parallel to the membrane, colored according to secondary structures (α-helices are in *red* except for the pore-forming helices (*orange*), β-sheets are in *blue*). (B) *Left*, $GABA_AR$ viewed from the extracellular space, with the five subunits labeled 1–5. *Right*, Transmembrane region of $GABA_AR$, with the ECDs simplified as *blue ovals* for clarity. The arrangement of the four TMDs (M1–M4), the C-terminus (*purple circles*), and the M2–M3 loop (*green*) are illustrated. (C) Topology of a single subunit of $GABA_AR$, *rainbow* colored from the N-terminus (*red*) to the C-terminus (*purple*). The β-sheets of the ECD, the α-helices of the TMDs, the characteristic Cys-loop, and other relevant loops are indicated. *Note*: the intracellular M3–M4 loop (G333–N446) was replaced with a 7-amino acid linker for crystallization. Figures were prepared using Maestro, v. 9.5.014, Schrödinger, LLC.

3. $GABA_AR$ ASSEMBLY: SELECTIVE SUBUNIT OLIGOMERIZATION

Human genome sequencing has identified at least 19 $GABA_AR$ subunit genes (α1–6, β1–3, γ1–3, δ, ε, θ, π, and ρ1–3). Given the heteromeric nature of $GABA_ARs$ in vivo, this long list of subunits, together with the

splice variants of some of the subunits allow for an enormous range of theoretically possible subunit combinations. Yet, experimental evidence suggests that only a few dozen combinations exist in vivo (see Section 4), indicating that $GABA_AR$ assembly is a selective, and not a random process. A hierarchical assembly mechanism has been proposed, in which certain subunits are preferred over others to form dimeric intermediates that ultimately assemble into pentameric complexes (Sarto-Jackson & Sieghart, 2008).

Different methods have been used to define the rules underlying receptor assembly. In concert, data obtained using functional, immunoimaging, and sucrose gradient centrifugation techniques suggest that both α and β subunits are obligatory for the surface expression of fully functional pentameric receptors in heterologous cell systems (Angelotti, Uhler, & Macdonald, 1993; Connor, Boileau, & Czajkowski, 1998; Gorrie et al., 1997). The additional third γ subunit has been shown to enhance the efficiency of receptor assembly (Tretter, Ehya, Fuchs, & Sieghart, 1997). In contrast, the recombinant expression of individual α, β, and γ subunits, and the αγ and βγ combinations mainly yielded di-, tri-, and tetrameric oligomers which were retained in the endoplasmic reticulum (Connolly, Krishek, McDonald, Smart, & Moss, 1996; Gorrie et al., 1997). There are a few notable exceptions, however, with the homomeric β1 and β3 and the heteromeric β3γ2 receptors expressing readily in heterologous systems (Chua, Absalom, Hanrahan, Viswas, & Chebib, 2015; Sanna et al., 1999; Taylor et al., 1999; Wooltorton, Moss, & Smart, 1997).

Amino acid residues important for assembly have been identified in several studies using the chimeric receptor and site-directed mutagenesis approaches (Sarto-Jackson & Sieghart, 2008). These residues are found mainly in the ECD, and to a lesser extent in the intracellular M3–M4 loop. In accordance with these data, the β3 $GABA_AR$ crystal structure revealed extensive energetically favorable interactions such as hydrogen bonds, salt bridges, and van der Waals forces along the interfaces between subunit ECDs (Miller & Aricescu, 2014). Disruption to these interactions could affect receptor assembly, and may be the reason for impaired $GABA_AR$ surface expression with epilepsy-associated mutations found in the N-terminal regions such as β3G32R and γ2R43Q (Frugier et al., 2007; Gurba, Hernandez, Hu, & Macdonald, 2012; Sancar & Czajkowski, 2004).

While considerable insights have been provided by these studies, the molecular determinants could not be firmly established for several reasons. First, these determinants differ depending on the partner subunits.

For example, four residues in the ECD of the β3 subunit (G171, K173, E179, and R180) have been identified to be critical for the assembly of β3 and β3γ2 receptors, but they are not compulsory for the assembly of αβ receptors (Taylor et al., 1999). In another study that investigated the role of the N-terminal regions in the expression of α1β2γ2 $GABA_A$Rs, subunit-specific contributions to receptor assembly were found (Wong, Tae, & Cromer, 2015). When deleted, the N-terminus of the α1 subunit had the most prominent effect on the expression of α1β2γ2 receptors, whereas deletion in similar regions of the β2 and γ2 subunits had minimal effect on surface expression. Second, multiple residues may be involved in oligomerization with the same neighboring subunit (Sarto et al., 2002). As such, when a putative binding residue is mutated and has no effect on receptor expression, it does not necessarily indicate no participation in intersubunit linking. Conversely, an expression-impairing mutation does not validate its significance in subunit oligomerization, as the residue may indirectly contribute to this process (e.g., by stabilizing interacting regions). All in all, $GABA_A$R assembly is a highly complex, multistep process which involves subunit-specific determinants that govern the subunit composition of $GABA_A$Rs found natively.

4. MULTIPLE SUBTYPES, LOCATIONS, AND ACTIONS OF $GABA_A$Rs

In recent years, it has become evident that the multiplicity in $GABA_A$R subunit composition (or subtypes) is one of the main reasons for the heterogeneity observed in their cellular and subcellular distributions, biophysical characteristics, pharmacological properties, in addition to physiological functions (Farrant & Nusser, 2005; Jacob, Moss, & Jurd, 2008; Rudolph, Crestani, & Möhler, 2001). Furthermore, the subunit composition of $GABA_A$Rs is plastic. Alterations in brain $GABA_A$R subtypes have been reported under various developmental and pathological conditions (Brooks-Kayal, Shumate, Jin, Rikhter, & Coulter, 1998; Fritschy, Paysan, Enna, & Mohler, 1994; Steiger & Russek, 2004). As such, answering the question "which $GABA_A$R subtypes actually occur in vivo?" is essential to understanding the diverse roles played by GABAergic inhibition.

Currently, existing experimental techniques are unable to unequivocally identify $GABA_A$R subunit composition in neurons. To help determine the likelihood of a receptor subtype being expressed physiologically, the IUPHAR committee has introduced five classification criteria (Olsen &