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Positive Allosteric Modulators of Glycine Receptors and Their Potential Use in Pain Therapies

Casey I. Gallagher, Damien A. Ha, Robert J. Harvey, and Robert J. Vandenberg

Molecular Biomedicine, School of Medical Sciences, University of Sydney, Sydney, Australia (C.I.G., D.A.H., R.J.V.) and Biomedical Science, School of Health and Behavioural Sciences and Sunshine Coast Health Institute, University of the Sunshine Coast, Maroochydore, Australia (R.J.H.)

***Abstract*——Glycine receptors are ligand-gated ion channels that mediate synaptic inhibition throughout the mammalian spinal cord, brainstem, and higher brain regions. They have recently emerged as promising targets for novel pain therapies due to their ability to produce antinociception by inhibiting nociceptive signals within the dorsal horn of the spinal cord. This has greatly enhanced the interest in developing positive allosteric modulators of glycine receptors. Several pharmaceutical companies and research facilities have attempted to identify new therapeutic leads by conducting large-scale screens of compound libraries, screening new derivatives from natural sources, or synthesizing novel compounds that mimic endogenous compounds with antinociceptive activity. Advances in structural techniques have also led to the publication of multiple high-resolution structures of the receptor, highlighting novel allosteric binding sites and providing additional information for previously identified binding sites. This has greatly enhanced our understanding of the functional properties of glycine receptors and expanded the structure activity relationships of novel pharmacophores. Despite this, glycine receptors are yet to be used as drug targets due to the difficulties in obtaining potent, selective modulators with favorable pharmacokinetic profiles that are devoid of side effects. This review presents a summary of the structural basis for how current compounds cause positive allosteric modulation of glycine receptors and discusses their therapeutic potential as analgesics.**

Address correspondence to: Robert J. Vandenberg, Room 510, Molecular Bioscience Building G08, University of Sydney, 2 Butlin Ave, Darlington NSW 2008, Sydney, Australia. E-mail: robert.vandenberg@sydney.edu.au

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***Significance Statement*——Chronic pain is a major cause of disability, and in Western societies, this will only increase as the population ages. Despite the high level of prevalence and enormous socioeconomic burden incurred, treatment of chronic pain remains limited as it is often refractory to current analgesics, such as opioids. The National Institute for Drug Abuse has set finding effective, safe, nonaddictive strategies to manage chronic pain as their top priority. Positive allosteric modulators of glycine receptors may provide a therapeutic option.**

I. Introduction

Glycine receptors (GlyRs) are anion channels that mediate inhibitory neurotransmission within the spinal cord and brain stem (Baer et al., 2009; Lynch, 2009). They belong to a class of structurally related pentameric ligand-gated ion channels (pLGICs) commonly known as Cys-loop receptors, which also includes the mammalian γ-aminobutyric subtype A receptors ($GABA_A$Rs), nicotinic acetylcholine receptors (nAChRs), and serotonin type 3 receptors. It also includes nonmammalian channels, such as the bacterial *Gleobacter* (GLIC) and *Erwinia* (ELIC) channels and the invertebrate glutamate-gated Cl^- channel (GluCl) (Jaiteh et al., 2016). The Zn^{2+}-activated chloride channel is also a Cys-loop receptor; however, it shares <15% amino acid sequence identity with other Cys-loop receptors and is the least understood receptor of this class (Trattnig et al., 2016).

Within the mammalian genome, four genes have been identified that encode GlyR α-subunits (α_1–α_4, ~48 kDa) and a single gene encoding the β-subunit (58 kDa) (Grudzinska et al., 2005). The α-subunits have high sequence homology (>90%), with variances occurring mainly within the large intracellular domain (ICD) and transmembrane domain 4 (TM4). These subunits combine to form pentameric homomers comprised of only α-subunits or heteromers, which also incorporate the β-subunit; however, the stoichiometry of heteromeric receptors remains a contested topic. Previously, a 2α:3β stoichiometry had been accepted (Grudzinska et al., 2005; Burgos et al., 2016); however, recent structural analysis supports a 4α:1β stoichiometry and suggests the incorporation of more than one β-subunit would occlude the Cl^- channel (Yu et al., 2021; Zhu and Gouaux, 2021). Although it is possible for β-subunits to form homomeric receptors (5β), they are considered nonfunctional due to their extremely low conductance and agonist affinity (Grenningloh et al., 1990; Handford et al., 1996), which is consistent with the recent structural findings. The β-subunit also contains a binding motif that allows it to anchor to the scaffolding protein gephyrin (Meyer et al., 1995). This causes heteromeric receptors to form synaptic clusters, which are responsible for fast synaptic neurotransmission (Baer et al., 2003), whereas homomeric receptors diffuse into the extrasynaptic space to mediate tonic-inhibitory activity (McCracken et al., 2017) or may be expressed on presynaptic terminals to regulate neurotransmitter release (Jeong et al., 2003).

Each subunit has a distinct expression pattern throughout the nervous system, which governs their physiologic functions. The β-subunit is required for synaptic clustering and is widely expressed throughout the central nervous system (Malosio et al., 1991a; Weltzien et al., 2012), whereas the expression of the α-subunits is more limited. GlyRα_2 are highly expressed during embryonic stages and play a key role in development (Avila et al., 2013); however, theirexpression levels decline sharply after birth and are replaced with GlyRα_1 (Becker et al., 1988; Lynch, 2009). Receptors containing the α_1-subunit are the predominant adult form, which are expressed widely throughout the nervous system and have critical roles in motor control, somatosensation, and respiration (Baer et al., 2003; Baer et al., 2009; Lynch, 2009; Liu and Wong-Riley, 2013). Alternative splicing of the α_1 subunit has been shown to occur, which results in the insertion of 8-amino acid residues into the ICD (α_1^{ins}). This variant has a more restricted expression within the spinal cord, where it constitutes a large portion of the GlyRs present (Malosio et al., 1991b). GlyRα_3 also have a reduced distribution and are primarily located in the retina, auditory brainstem, and the dorsal horn of the spinal cord (Baer et al., 2009; Burgos et al., 2016). Alternative splicing of the α_3 subunit also occurs, which inserts 15-amino acid residues into the ICD (α_{3L}) and greatly impacts receptor kinetics (Nikolic et al., 1998; Breitinger et al., 2009). The GlyRα_4 is a pseudogene that is not expressed in humans due to a premature stop codon; however, it is suggested to occur in other vertebrates (Harvey et al., 2000; Aguayo et al., 2004; Leacock et al., 2018).

A. Glycine Receptors and Pain

GlyRs containing the α_1 and α_3 subunits are highly expressed within the dorsal horn of the spinal cord

ABBREVIATIONS: 5-HT_3R, serotonin type 3 receptor; CB1, cannabinoid receptor type 1; CB2, cannabinoid receptor type 2; CBD, cannabidiol; CNS, central nervous system; cryoEM, cryogenic electron microscopy; DD-THC, Δ9-tetrahydrocannabinol analogue with both oxygen groups removed; DH-CBD, cannabidiol with 3′ hydroxyl group removed; DSPC, 1, 2-distearoyl-sn-glycero-3-phosphocholine; ECD, extracellular domain; ELIC, Erwinia ion channel; GABAAR, γ-aminobutyric subtype A receptors; GLIC, Gleobacter ion channel; GluCl, glutamate-gated chloride channel; GlyR, glycine receptor; GlyT2, glycine transporter type 2; ICD, intracellular domain; MBN, N-methylated ICS-205, 930; MD, molecular dynamic; nAChR, nicotinic acetylcholine receptor; NAGly, N-arachidonoyl glycine; PAM, positive allosteric modulator; PGE_2, prostaglandin E2; PKC, protein kinase C; pLGIC, pentameter ligand gated ion channel; POPC, palmitoyl-2-oleoyl-sn-glycero-3-phosphocholine; THC, Δ9-tetrahydrocannabinol; TM4, transmembrane domain 4; Tropisetron, ICS-205, 930; TRPV1, transient receptor potentiation cation channel subfamily V member 1.

and have been implicated in nociceptive signaling (San Martín et al., 2022). They are expressed on inhibitory interneurons, where they mediate somatosensory signals and prevent overactivation of the nociceptive pathway (Cronin et al., 2004; Baer et al., 2009). However, injury and prolonged inflammation can sensitize these neurons by reducing glycinergic neurotransmission (Zeilhofer and Zeilhofer, 2008) and therefore allow the activation of nociceptive protein kinase C (PKC)-γ neurons, which causes allodynia (Miraucourt et al., 2007). Allodynia and other nociceptive pain-related symptoms can also be evoked experimentally by the GlyR antagonist strychnine (Yaksh, 1989), whereas intrathecal injection of glycine reduces nociceptive sensitivity in neuropathic pain models (Simpson et al., 1996). This links the degree of nociceptive sensitivity to GlyR activity within the spinal cord.

Chronic pain symptoms have been associated with reduced GlyR expression within the spinal cord (Simpson and Huang, 1998), which is suggested to occur through inflammatory-mediated mechanisms (Harvey et al., 2004; Chen et al., 2012). Several studies have demonstrated a persistent upregulation of cyclooxygenase enzymes, prostaglandin E2 (PGE_2), and PGE receptors in response to nociceptive pain and during chronic pain conditions (Ma and Eisenach, 2003; Ma and Quirion, 2008; Ma et al., 2010). In rodent models, intrathecal injection of PGE_2 produces hypersensitivity in a timeframe that directly correlates to reduced expression of GlyRα_1 and GlyRα_3 in spinal slices (Wang et al., 2018). This is due to the upregulation of PGE_2 causing excess activation of PGE receptors on postsynaptic lamina II neurons, which induces GlyRα_3 phosphorylation, internalization, and degradation (Ahmadi et al., 2002; Harvey et al., 2004; Zeilhofer, 2005; Velázquez-Flores and Salceda, 2011). Intracellular phosphorylation of GlyRα_3 also elicits global structural changes, including conformational changes within the agonist binding site (Han et al., 2013), which may be responsible for their decreased conductance (Moraga-Cid et al., 2020). Similarly, GlyRα_1^{ins} expressed within the spinal cord have been shown to undergo inflammatory-mediated phosphorylation, resulting in endocytosis of the receptor and reduced glycinergic neurotransmission (Huang et al., 2007; Zhang et al., 2019a). Additionally, reversing this process through dephosphorylation has been shown to provide antinociception in inflammatory pain conditions (Diao et al., 2020). Intracellular phosphorylation of GlyRs can also disrupt gephyrin binding (Specht et al., 2011). This prevents GlyRs from forming synaptic clusters and instead causes them to diffuse into the extracellular space (Specht et al., 2011; Cantaut-Belarif et al., 2017), which may contribute to the reduced synaptic GlyR expression and overall reductions in glycinergic neurotransmission.

GlyRα_2 are considered to be an embryonic form of GlyR as they are predominantly expressed during prenatal stages, and their expression levels decline sharply after birth (Becker et al., 1988; Malosio et al., 1991a; Lynch, 2009). They are not usually expressed with the adult spinal cord; however, expression within the dorsal horn of the spinal cord has been observed in rat models of chronic pain (Imlach et al., 2016). GlyRα_2 have slower activation kinetics and cannot be activated by rapid neurotransmitter release (Mangin et al., 2003). This makes them unable to participate in nociceptive signaling, and thus, their expression reduces glycinergic input onto lamina II neurons (Imlach et al., 2016). Overall, these findings suggest that a reduction of glycinergic neurotransmission within the spinal cord is a causative factor in chronic pain symptoms. Re-establishing the balance between excitatory and inhibitory signaling within the spinal cord by strengthening neural inhibition may alleviate chronic pain symptoms (Tanabe et al., 2008). One way of achieving this is through positive modulation of GlyRs.

B. Structure and Function of Glycine Receptors

Cys-loop receptors are composed of five pseudo-symmetrically arranged subunits that enclose an aqueous channel pore. Each subunit consists of a large extracellular domain containing a series of flexible loops and twisted β-sheets, followed by four TM domains (TM1–TM4). The TM domains are aliphatic α-helices that form concentric rings around the channel pore to isolate it from the lipid bilayer (Burgos et al., 2016). The inner ring is lined by TM2 domains that form the pore of the ion channel and determine its ion selectivity (Breitinger and Becker, 2002; Keramidas et al., 2002; Harpole and Grosman, 2019). The TM2 domains are enclosed by a ring of alternating TM1 and TM3 domains, which make minor contacts with the lipid membrane. This is followed by the hydrophobic TM4 domains, which extend outward from the channel and form the predominant interface between the protein and lipid environment (Henault et al., 2015). The TM domains are linked by short, flexible loops, with the exception of the TM3-TM4 loop, which is a large, dynamic intracellular loop that forms the ICD.

The orthosteric binding pocket is located within the center of the extracellular domain (ECD) at the interface between adjacent subunits. The binding cavity is shaped by a series of bulky and aromatic residues from loop B (F159, Y161) and loop C (Y202, F207) of the primary subunit (+) and from loop D (F63) and loop E (L117) of the complementary subunit (-). Functional and structural studies have also highlighted important residues S129(-), R65(-), and T204(+) within these loops that directly bind with the carboxyl group of the glycine molecule (Vandenberg et al., 1992; Breitinger and Becker, 2002; Kumar et al., 2020). This forms five distinct agonist binding pockets on each receptor, although occupation of two or more of these sites is sufficient for receptor activation (Grewer, 1999; Gentet and Clements, 2002). This pocket is able to accommodate the primary agonist glycine and partial agonists

β-alanine, taurine, and GABA (Horikoshi et al., 1988; Yu et al., 2021a,b), as well as the GlyR antagonist strychnine (Du et al., 2015). Agonist binding causes a large inward "capping" movement of loop C toward the binding pocket, which induces a reorientation of many ECD loops. In particular, the conserved β6-β7 (cys-loop) and β1-β2 loop at the lower portion of the ECD, which interact with the TM2-TM3 linker to signal agonist binding through to the TM domains (Soh et al., 2017; Yu et al., 2021). This causes TM2 to rotate clockwise about the channel's axis, which moves the pore lining L261 residues away from the center of the channel and produces an overall expansion of the TM domains (Kumar et al., 2020). This increases the channel radius from 1.4 Å to 4-5 Å, which is sufficient for hydrated and dehydrated Cl^- ions (3.3 Å and 1.8 Å, respectively) to pass through the channel (Du et al., 2015). Activation of GlyRs cause an influx of Cl^- that hyperpolarizes the postsynaptic neuron and elicits neural inhibition (Dutertre et al., 2012). After prolonged exposure to high concentrations of agonist, GlyRs will undergo desensitization, where the degree of ionic flux will decay despite the continued presence of the agonist to prevent overactivation (Wang and Lynch, 2011; Kumar et al., 2020). Desensitization of other inhibitory pLGICs has been shown to impact receptor phosphorylation, expression, and the overall efficacy of inhibitory synapses through the induction of long-term potentiation (Field et al., 2021).

Molecular dynamic (MD) simulations, cryogenic electron microscopy (cryoEM), and crystallography techniques have also identified allosteric binding sites within the ECD, as well as intra- and intersubunit cavities within the TM domains (Fig. 1) (Cerdan et al., 2020; Thompson and Baenziger, 2020). Many analogous studies were originally conducted using the invertebrate GluCl (Hibbs and Gouaux, 2011; Althoff et al., 2014) or the bacterial GLIC (Bocquet et al., 2009; Nury et al., 2011) and ELIC (Henault et al., 2019; Tong et al., 2019) channels; however, recent advances have allowed the use of mammalian channels. These structures have indicated a high degree of conservation of these allosteric binding cavities among cys-loop receptors. In particular, structures of GlyRα_1 (Du et al., 2015) and GlyRα_3 (Huang et al., 2017) have highlighted the conservation of an intersubunit cavity toward the intracellular portion of the TM domains, which was previously identified in GluCl (Hibbs and Gouaux, 2011; Althoff et al., 2014). Despite the high level of conservation and common binding mechanisms, many of these sites produce diverse effects due to subtle differences in subunit composition.

II. Positive Allosteric Modulators of Glycine Receptors

Despite GlyRs being highlighted as a promising target for novel nociceptive pain therapeutics, there are no currently approved positive allosteric modulators (PAMs) for this purpose. Chronic pain continues to be treated with medications such as opioids, antidepressants, anticonvulsants, and nonsteroidal anti-inflammatory drugs (Moulin et al., 2014; Cioffi, 2018; Ho et al., 2018; Shaheed et al.,

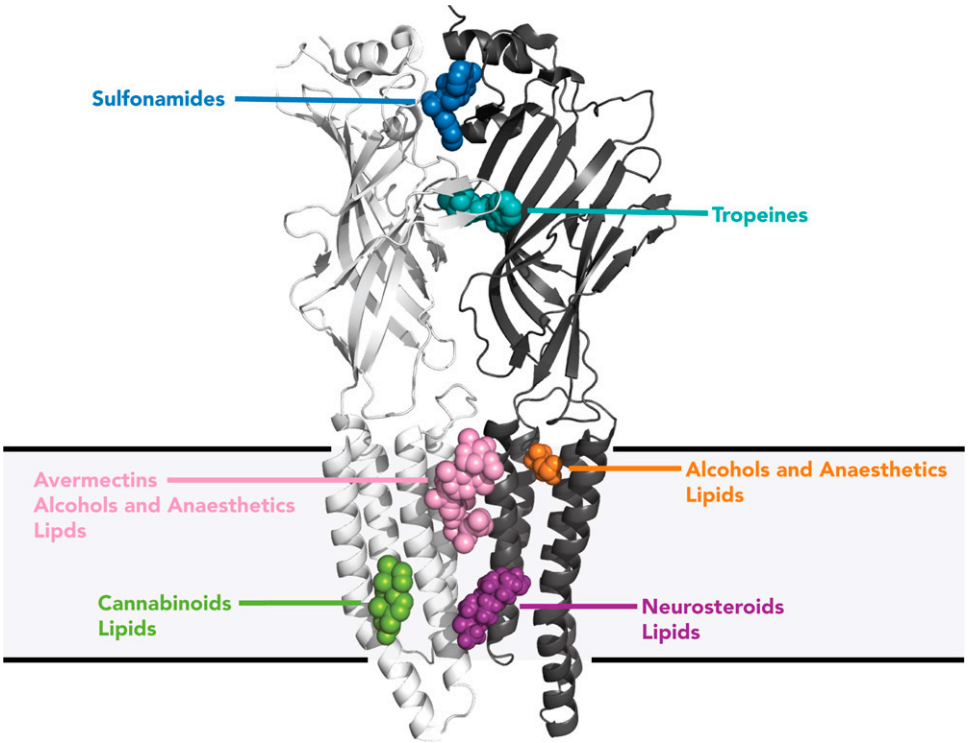

Fig. 1. Binding sites of positive allosteric modulators on the glycine receptor. Sulfonamides (blue) and tropeines (teal) bind within extracellular domain of the glycine receptor between adjacent subunits. The primary subunit is shown in white, and the complementary subunit is shown in black. Within the transmembrane domains there are four cavities that have been shown to bind lipids and lipophilic modulators, including neurosteroids (purple), cannabinoids (green), avermectins (pink), alcohols, and anesthetics (pink and orange). Black lines indicate the lipid membrane.

2020), which are often inadequate for providing pain relief (Dworkin et al., 2010) or have significant side effects and potential for abuse (Hojsted et al., 2010; https://www2.deloitte.com/au/en/pages/economics/articles/cost-pain-australia.html). This has enhanced the interest of pharmaceutical companies to identify novel PAMs of GlyRs (Stead et al., 2016; Bregman et al., 2017) and the development of "The Glycine Receptor Allosteric Ligands Library" (Cerdan et al., 2020) to aid in this process.

There are several known classes of modulators for GlyRs; however, many of these are not efficacious in vivo due to their nonselective nature (Xiong et al., 2012a; Yang, 2012; Chandler, 2018). It is particularly difficult to obtain selectivity over other cys-loop receptors due to their high degree of structural similarity (Chesnoy-Marchais, 1996; Chesnoy-Marchais et al., 2000). On-target side effects are also predicted to arise due to the widespread distribution of GlyRs throughout the nervous system (Schmid et al., 1991; Baer et al., 2009), requiring further selectivity for specific GlyR subunits, which is difficult to achieve. Additionally, many of these modulators tend to have a poor pharmacokinetic profile and are highly lipophilic (Yang et al., 2008; Gallagher et al., 2020), thus making them unsuitable for pharmaceutical application.

However, the recent advances in structural biology techniques have helped elucidate novel allosteric binding sites and provided additional information for sites previously identified (Huang et al., 2017a,b; Yu et al., 2021a). This has greatly enhanced our understanding of receptor functionality and has opened up new avenues of research, which allow for structure-based optimization of known pharmacophores or the design of new PAMs based on novel binding sites. This review will assess the structure activity relationships developed for known PAMs of GlyRs and evaluate their therapeutic potential as analgesics in light of the new structural and functional information available.

A. Avermectins

Avermectins are a family of 16-membered macrocyclic lactones synthesized by the soil bacterium *Streptomyces avermitilis*. They were originally isolated and characterized by Merck, leading to the development of several avermectin compounds, which became commercially available due to their anthelmintic, insecticidal, and acaricidal activity (Jansson and Dybas, 1998). Avermectins were found to enhance Cl^- conductance and were sensitive to picrotoxin, which suggested they act at $GABA_A$Rs (Fritz et al., 1979). This was later expanded to other Cl^--conducting pLGICs including the GlyR and GluCl (Vassilatis et al., 1997b), which are also sensitive to avermectins (Vassilatis et al., 1997a; Dawson et al., 2000; Shan et al., 2001). Despite their low selectivity, avermectins are relatively safe for commercial use as they have lower potencies at mammalian channels and are unable to readily cross the blood-brain barrier (Yang, 2012; Chandler, 2018). They are also used therapeutically to treat inflammatory rosacea and neglected topical diseases (Omura and Crump, 2014; Kircik et al., 2016).

One of the most well studied of the family is ivermectin, which is an anthelmintic compound that acts as a potent, allosteric agonist at the GluCl channel (Huang et al., 2017) and is a dual partial allosteric agonist and PAM of GlyRs. At concentrations as low as 30 nM, ivermectin selectively potentiates glycinergic currents evoked by low glycine concentrations (Shan et al., 2001; Lynagh et al., 2011). However, between 0.3 and 30 μM, it exhibits dual functions and additionally acts as a pseudo-irreversible partial allosteric agonist. Ivermectin induces a slow activation of the channel and achieves maximal currents that are approximately 78% of a maximal glycine current (Shan et al., 2001; Lynagh and Lynch, 2010; Lynagh et al., 2011). Ivermectin was suggested to work through a novel mechanism distinct from the glycine binding site as ivermectin-activated currents were not impacted by mutations within the orthosteric binding pocket and could not be inhibited by high levels of zinc or picrotoxin (Shan et al., 2001).

Mutagenesis studies predicted ivermectin binding at the interface between the ECD and the upper region of TM domains, involving residues from the TM2-TM3 loop, the conserved cys-loop (β6-β7 loop), and the β1-β2 loop (Lynagh and Lynch, 2010; Lynagh et al., 2011). Ivermectin's agonist activity correlated to the side-chain volume at position 288 within TM3 (Gly$R\alpha_1$ numbering). This is an alanine in GlyRs and some $GABA_A$Rs, which are less sensitive to ivermectin activity, and a glycine residue in GluCl and other highly sensitive channels within parasites and nematodes (Lynagh and Lynch, 2010). Making the reverse mutation in Gly$R\alpha_1$ to reduce steric bulk (A288G) significantly enhances ivermectin sensitivity, whereas mutation to a bulkier phenylalanine (A288F) abolishes ivermectin's agonist activity (Lynagh et al., 2011). The 288 position was predicted to occur at the mouth of the binding cavity, and thus, larger residues would occlude binding. These findings were later confirmed by crystal structures of ivermectin bound to the GluCl channel (Hibbs and Gouaux, 2011) and the human Gly$R\alpha_3$ (Huang et al., 2017) and cryoEM structures of ivermectin bound to the zebrafish Gly$R\alpha_1$ (Du et al., 2015; Kumar et al., 2020). In all three structures, ivermectin binds to an intersubunit cavity in the upper portion of the TM domains (Fig. 2), which has also been shown to bind lipids (Althoff et al., 2014). The benzofuran group of ivermectin is responsible for the majority of binding interactions as it interchelates between TM3(+) and TM1(-) of adjacent subunits and inserts deeply into the cavity, making contacts with the pore lining TM2(+). The spiroketal and the disaccharide groups make some hydrophobic interactions with the TM domains; however, they primarily

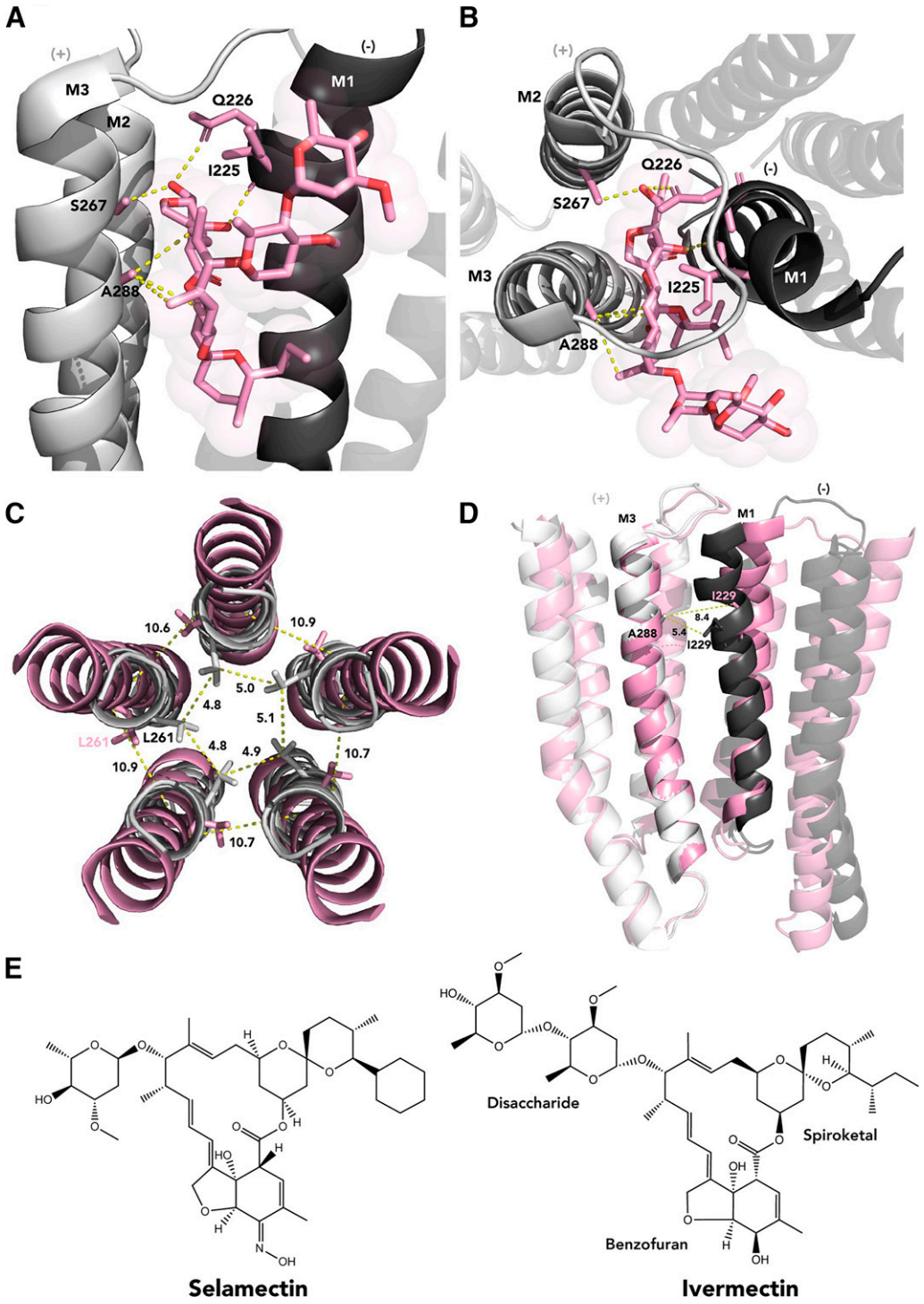

Fig. 2. Avermectin binding to the glycine receptor. Ivermectin (pink) binding within the upper intersubunit cavity of glycine receptors viewed from (A) the membrane and (B) the top down. The hydroxyl group of the benzofuran moiety hydrogen bonds with S267 from the primary subunit (white) and Q226 and I225 from the complementary subunit (black). A228 makes hydrophobic interactions with the disaccharide and spiroketal groups. (C) Ivermectin binding (pink) rotates the pore-lining L261 residue away from the channel and widens the upper channel pore. (D) Ivermectin binding (pink) wedges apart the upper portions of adjacent TM1(-) and TM3(+) domains by 3Å. (E) Chemical structures of selamectin and ivermectin. Accession codes for PBD files used: 5VDH and 5CFB.

protrude away from the pore and interact with the lipid membrane.

The agonist activity of ivermectin has been attributed to the C5-hydroxyl moiety within the benzofuran group (Michael et al., 2001). It forms hydrogen bonds with S267 TM2(+) and Q226 TM1(-), which causes TM2 to tilt away from the channel axis and widens the channel pore at 9' L261 (Fig. 2C) (Huang et al., 2017). Altering

the structure of ivermectin to remove this interaction abolishes agonist activity while retaining potentiating capabilities, as seen with the structurally related selamectin (Fig. 2E) (Michael et al., 2001; Lynagh et al., 2011). When comparing the structures of GluCl and GlyRα_3, ivermectin inserts 1 Å deeper into the cavity of GluCl due to the smaller side chain volume of glycine at the 288 TM3(+) position, accounting for its greater sensitivity (Huang et al., 2017). A288 also forms several hydrophobic interactions with both the benzofuran and spiroketal groups, whereas I225 TM1(-) from the neighboring subunit forms a hydrogen bond with the C7-hydroxyl group. Within GlyRα_3, this causes an overall expansion of the upper TM domains, separating TM3(+) and TM1(1) by approximately 3Å (Fig. 2D) (Huang et al., 2017). Ivermectin binding also elicits conformational changes within the extracellular domains, in particular loops C, D, and F (Wang and Lynch, 2012). These changes may facilitate agonist binding or elicit conformational changes that favor the activated state. This not only highlights the importance of these extracellular loops for receptor activation but provides a mechanistic basis for how avermectins potentiate GlyRs (Wang and Lynch, 2012).

Ivermectin was one of the first PAMs bound to the crystallized GlyR, and its binding cavity remains one of the most well defined across cys-loop receptors. The crystallography and cryoEM structures of ivermectin greatly helped define the intersubunit binding cavity and important binding residues, which have also been shown to interact with lipids (Althoff et al., 2014), steroids (Henin et al., 2014; Alvarez and Pecci, 2019), alcohols, and anesthetics (Crawford et al., 2007; Howard et al., 2011; McCracken et al., 2016), making it a promising cavity to target for further PAM development. However, ivermectin itself produces toxic GABA-mimetic side effects at concentrations that would be required to observe possible antinociceptive activity. These side effects include dizziness, muscle pain, nausea, and hypotension; and can lead to respiratory failure, coma and death at high concentrations (Yang, 2012; Chandler, 2018). Greater optimization that focuses on enhancing selectivity and dampening agonist activity would be required to further the therapeutic potential of avermectin compounds to treat chronic pain conditions. This has caused researchers to instead explore chemogenetic approaches. Two chemogenetic receptors based on the GluCl and GlyR have been developed, which are highly sensitive to ivermectin and silence nociceptive signaling when virally infected into spinal neurons (Islam et al., 2017; Weir et al., 2017). The chemogenetic GluCl channel has been successfully expressed in the spinal sensory neurons of mice and caused ivermectin to produce long-lasting antinociceptive activity in acute and neuropathic pain models, without incurring any motor defects (Weir et al., 2017). This study not only reinforces that inhibition of nociceptive signaling is a viable approach to treat chronic pain conditions but highlights a novel way to therapeutically use ivermectin's modulatory activity at cys-loop receptors. Chemogenetics remain a controversial topic and undoubtedly requires extensive research before being implemented as a therapeutic approach (Campbell and Marchant, 2018). However, it poses an innovative way to address intractable health issues such as chronic pain and is also being explored for the treatment of epilepsy (Avaliani et al., 2016; Lieb et al., 2019) and Parkinson disease (Pienaar et al., 2015).

B. Alcohol and Anesthetics

One of the first drug classes found to modulate GlyRs was alcohols. Ethanol is the prototypic alcohol studied and was initially shown in 1988 to increase glycine sensitivity in cultured chick spinal neurons (Celentano et al., 1988), which was suggested to aid in alcohol's sedative effects. This idea continues to be explored, with recent rodent studies showing that mutagenic mouse strains expressing ethanol-insensitive GlyRs recover significantly quicker from the sedative effects of ethanol (Aguayo et al., 2014). They also have modified ethanol consumption levels and altered place preference behaviors in response to ethanol (Munoz et al., 2020). This links the modulatory effect of alcohols on GlyR to sedation and addiction; however, GlyR modulation may also contribute to the high level of antinociception associated with alcohol use (Thompson et al., 2017; Capito et al., 2020).

Primary studies found that ethanol enhances GlyR activity (Celentano et al., 1988), which was more pronounced in GlyRα_1 than GlyRα_2 due to nonconserved A52 residue in the extracellular β1-β2 loop (Mascia et al., 1996a). However, longer chain alcohols were found to have similar potencies between GlyRs, which increased with chain length up to tridecanol (12-carbon length), which was inactive (Mascia et al., 1996b). This "cut-off" length was similar to that observed for $GABA_AR$, which suggested a long, conserved hydrophobic binding pocket. This pocket was also predicted to bind propofol and volatile general anesthetics, which were similarly found to potentiate GlyRs (Hales and Lambert, 1991; Downie et al., 1996; Mascia et al., 1996a,b). A chimeric approach was then used to narrow down possible residues within this binding pocket. Chimeras of the GlyRα_1 and $GABA_AR\rho_1$ identified the TM1–TM3 regions, in particular residues S267 (TM2), T264 (TM2), and A288 (TM3) (Mihic et al., 1997). Inverse mutations made in GlyRα_1 (S267I and A288W) abolished potentiation by general anesthetics, ethanol, and other primary and tertiary alcohols (Mihic et al., 1997; Krasowski and Harrison, 2000; Ahrens et al., 2008). Mutations at the S267 position also exhibited a strong correlation between residue volume and potentiation effects, with bulky mutations such as S267W

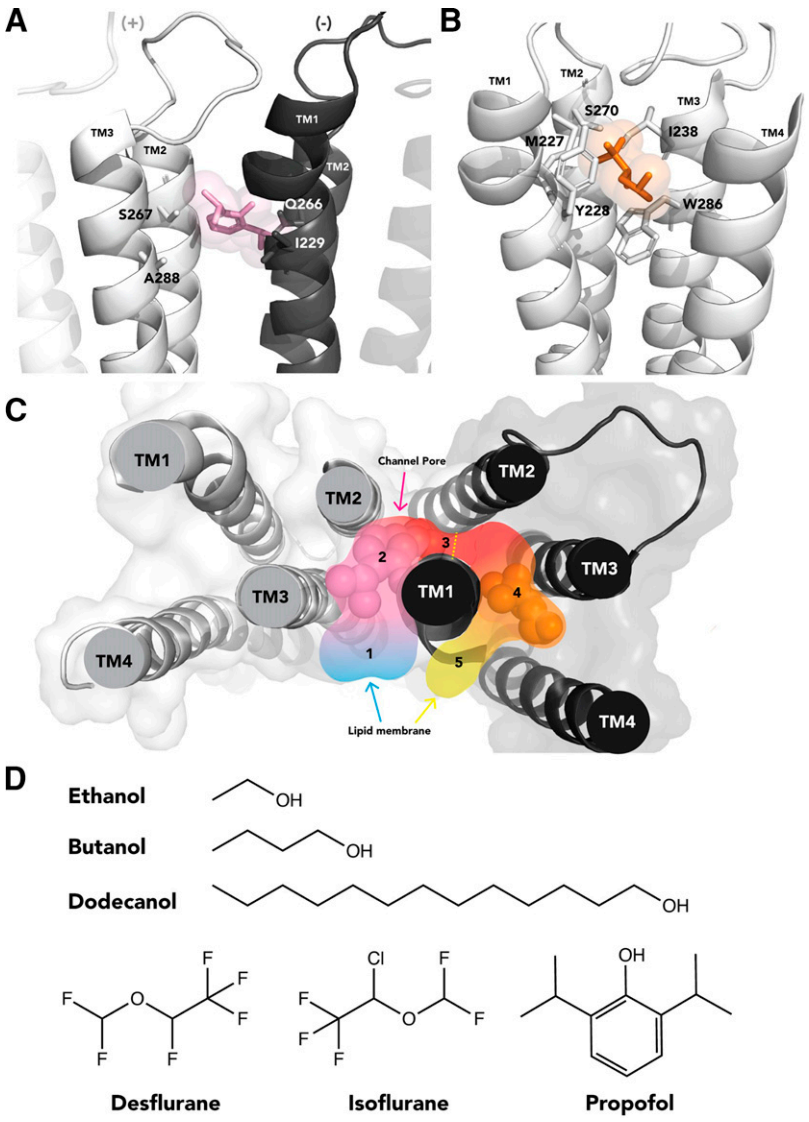

Fig. 3. Alcohol and anesthetic binding to the glycine receptor. Structures of anesthetics bound to GLIC were aligned to the glycine receptor. (A) Propofol (pink) binds to the upper intersubunit cavity of glycine receptors. (B) Desflurane (orange) binds to the upper intrasubunit cavity of glycine receptors. (C) Alcohols and anesthetics can enter the intersubunit binding cavity site 2 (pink) from the lipid membrane via site 1 (blue) or from the channel pore between TM2 domains. Alcohols and anesthetics can enter the intrasubunit binding cavity site 4 (orange) from the lipid membrane via site 5 (yellow). Site 2 and site 4 are linked by a small hydrophobic tunnel, site 3 (red), which has a restriction point of approximately 3Å, indicated by a yellow dashed line, allowing the passage of smaller alcohols and anesthetics. (D) Chemical structures of alcohol and anesthetic modulators of glycine receptors. Accession codes for PBD files used: 5MVM, 3P4W, and 5TIO.

and S267Y converting ethanol into an inhibitor (Ye et al., 1998), and abolished the effects of larger general anesthetics such as enflurane (Mihic et al., 1997). Based on the predicted binding site at A52, it was originally suggested that S267 may act as a transduction pathway that stabilizes different conformations of the receptor depending on the volume of the side chain at this position. However, mutations at this site were found to impact the alcohol "cut-off" length (Wick et al., 1998) and the modulation of larger general anesthetics (Ye et al., 1998), suggesting that S267 occurs within the binding site or in close proximity to a binding site whose dimensions are perturbed by S267. This highlighted the possibility of two binding sites—one within the ECD lined by A52 that directly modulates agonist affinity and one within the TM domains that contains S267 and enhances agonist affinity by signaling via the extracellular TM2-TM3 (Dupre et al., 2007) and β1-β2 loops (Davies et al., 2004; Perkins et al., 2008).

This prompted several studies that aimed to characterize the two sites using both structural and functional techniques. However, collectively, these studies found the "two" sites actually constitute the upper and lower regions of a singular large, intersubunit cavity constituting the primary binding site and additionally identified a secondary intrasubunit cavity, which is linked to the primary cavity by a hydrophobic tunnel (Crawford et al., 2007; Howard et al., 2011; McCracken et al., 2016).

The primary binding site is an intersubunit cavity that is formed predominantly by TM2(+) and TM3(+) from the principal subunit and TM1(-) from the complimentary subunit (Fig. 3A). This cavity can be accessed from the Cl^- channel via a narrow pore-facing tunnel (Fig. 3C) (Howard et al., 2011), which is the dominant access pathway for smaller hydrophilic molecules such as ethanol (Murail et al., 2011). However, this cavity can also be accessed from the lipid membrane, which was identified using a cysteine crosslinking method. These studies found that when A288 from TM3(+) and I229 from TM1(-) were both mutated to cysteine (A288C/I229C), disulfide bonds were able to form between

adjacent subunits (Lobo et al., 2008) and, when cross-linked, significantly reduced the potentiation of butanol and isoflurane (McCracken et al., 2016). This not only confirmed that these residues act as an entrance gate that permits the access of larger and hydrophobic modulators from the membrane (Murail et al., 2011) but also confirmed that ligands bound to this cavity are responsible for receptor potentiation. The volume of residues lining this access pathway and key residues within the cavity, including S267, correlate well with alcohol cut-off lengths (Murail et al., 2011) and the ability for longer-chain alcohols to produce potentiation (Howard et al., 2011). CryoEM structures of a GLIC-GlyRα_1 chimera highlighted conformational changes that occur within the primary binding cavity upon receptor activation, which result in a narrowing of the pocket and a reduction of the cavity's volume (Moraga-Cid et al., 2015). Further analysis of this site using MD simulations found that ethanol binding increased the cavity volume by 50Å, which corresponds to an approximate 10% increase. The expansion of this binding cavity by alcohols and general anesthetics may therefore produce potentiation by facilitating interactions between the TM domains (McCracken et al., 2016) and stabilizes the open conformation by preventing the TM movements required to transition into the closed state (Murail et al., 2011).

Crystal structures of general anesthetics and alcohols bound to the bacterial homolog GLIC (Howard et al., 2011; Nury et al., 2011) further identified an intrasubunit cavity (Nury et al., 2011; Fourati et al., 2018). In structures of GLIC bound to desflurane and propofol (Nury et al., 2011), the anesthetics occupy an intrasubunit cavity that occurs at the upper portion of the TM domains (Fig. 3B) and is accessible from the lipid membrane between TM1 and TM4 (Fig. 3C). Within these structures, propofol sandwiches between TM1 and TM3, forming a hydrogen bond with Y254 (Q227), whereas desflurane buries deeper into the cavity to form hydrophobic interactions with I201, I202 from TM1 (M227, Y228), T255 and I258 from TM3 (I238, W286), and V242 from TM2 (S270). Interestingly, this intrasubunit cavity connects to the primary intersubunit cavity via a narrow $<$3Å pore that forms between TM1 and TM2 (Fig. 3C). In GLIC, the primary cavity is not accessible from the lipid membrane due to bulky residues obstructing access, whereas GlyRs and $GABA_AR$ natively have smaller residues, which allows the primary cavity to be membrane accessible (Nury et al., 2011; Fourati et al., 2018). This is responsible for the predominantly potentiating activity of anesthetics in GlyRs compared with their inhibitory activity at GLIC. The acyl chains of membrane lipids have also been shown to enter and obstruct the entrance of the intrasubunit cavity, suggesting that anesthetics may modulate pLGICs by competing with endogenous lipid mediators (Nury et al., 2011).

Fourati et al. (2018) further explored the inter- and intrasubunit binding cavities through extensive mutagenesis, producing 10 structures of GLIC variants in the presence and absence of various general anesthetics. Firstly, when the channel is closed, anesthetics were found to bind within the pore and produce inhibition through allosteric closure and stabilizing the closed conformation. Secondly, bulky mutations made at the bottom of the intrasubunit cavity caused anesthetics to incur bimodal effects and produce potentiation at lower concentrations (Heusser et al., 2013). These mutations reduced the volume of the intrasubunit cavity by up to 40% and produced crystal structures that were in an apparent open conformation, suggesting the intrasubunit cavity may not be wholly inhibitory (Fourati et al., 2018). Lastly, they assessed the primary intersubunit cavity. A single phenylalanine-to-alanine mutation (F238A GLIC numbering, Q266 GlyR numbering) within the primary cavity reduced steric bulk and turned bromoform from an inhibitor to a potent potentiator (Sauguet et al., 2013). Bromoform is a halogenated alkane that acts as a general anesthetic and is often used to explore anesthetic binding cavities in structural studies (Kash et al., 2003; Laurent et al., 2016). An additional N239A mutation, which corresponds to residue S267 in GlyRs, reduced bulk further and allowed propofol to bind within the primary site, enabling GLIC to be potently potentiated by propofol. Within structures of the F238A/N239A GLIC variant, general anesthetics only occupied the intersubunit cavity and were in an open conformation (Fourati et al., 2018). Overall, these findings suggested the pore region and intrasubunit cavity are predominantly inhibitory, whereas the primary intersubunit cavity is responsible for potentiation.

Phosphorylation within the ICD was also found to impact alcohol modulation in GlyRs. Initial studies found that PKC and protein kinase A inhibitors significantly reduce ethanol potentiation of GlyRs, as did the GlyRα_1 S391A mutation, which removes a PKC phosphorylation site (Mascia et al., 1998). This was found to impact the effects of ethanol in vivo and was linked to ethanol's antinociceptive activity. Rodents that were acutely or chronically exposed to ethanol and then underwent withdrawal would elicit nociceptive pain responses, including mechanical allodynia and thermal hypersensitivity (Gatch, 1999; Gatch and Lal, 1999). This was attributed to alterations in PKC signaling in the spinal cord, identified through immunohistochemical analysis of spinal cord sections (Shumilla et al., 2005), which altered the response of GlyRs to ethanol (Mascia et al., 1998). Further mutagenesis studies of the ICD identified residues K385 and K386 as important for ethanol modulation; however, mutation of these residues was selective for ethanol and did not impact the activity of long-chain alcohols, anesthetics, or other PAMs such as neurosteroids and zinc (Yevenes et al., 2008; Castro et al., 2012). These lysine residues had previously been shown to

interact with the G$\beta\gamma$ intracellular protein, which potentiates GlyRs (Yevenes et al., 2003; Yevenes et al., 2006). This suggested that low concentrations of ethanol may indirectly potentiate GlyRs by inducing G$\beta\gamma$-GlyR interactions. Using protein scavengers or peptides that inhibit G$\beta\gamma$ indeed attenuated ethanol potentiation at low concentrations (Yevenes et al., 2008; San Martin et al., 2012). However, GlyRα_2 and GlyRα_3 are not potentiated by similar ethanol concentrations despite containing the identified lysine residues (Yevenes et al., 2010; Sanchez et al., 2015). A chimeric approach was taken to further elucidate this interaction and suggested that regions other than the ICD and TM4 domains were important for ethanol-G$\beta\gamma$ modulation, in particular, residues A52, G254, and S286, which occur in the ECD, TM2, and TM3, respectively (Yevenes et al., 2010). These results suggest that G$\beta\gamma$ binding is dependent on residues within the ICD; however, the modulatory effects arise from conformational changes along a complex transduction pathway that includes all receptor domains. A knock-in mouse model containing the double K385A/K386A mutation in GlyRα_1 was also developed to further explore the impact of G$\beta\gamma$ modulation in vivo. Spinal cord slices from the mutant mouse strain had normal GlyR expression within the spinal cord and glycinergic neurotransmission indistinguishable from wild-type mice. However, mutant GlyRs were functionally insensitive to ethanol potentiation, and behavioral studies found that mutant mice were significantly less impacted by the sedative effects of ethanol (Aguayo et al., 2014).

Although alcohols and anesthetics are promiscuous compounds that are unlikely to be therapeutically viable due to significant off-target side effects, they remain important tools for exploring the functional properties of GlyRs. The study of ethanol and propofol modulation in particular has given insight into the multiple hydrophobic binding cavities, identified important intracellular interactions, and linked GlyR activity to nociceptive pathways. Ethanol is still commonly used to compare the activity of novel PAMs or to assess the impact of mutations within GlyRs (Chesnoy-Marchais, 1999; Xiong et al., 2011; Yao et al., 2020). This has also led to the development of GlyRs that are ultrasensitive to ethanol, which contain mutations in the β1-β2 loop and are potentiated by ethanol in the low nanomolar range (Naito et al., 2014; Naito et al., 2015). These receptors are not only being used to enhance our understanding of GlyR modulation but are also used as a tool to better understand the neurologic impacts of alcohol in brain research (Naito et al., 2014).

C. Cannabinoids

Cannabinoids are a group of compounds that are produced by the cannabis plant and synthetic analogs or derivates. Cannabis has been used to treat nociceptive pain and inflammation since ~1500 BC, originating in ancient China (Maione et al., 2013); however, the active constituents responsible for its antinociceptive effects were not identified until the 20th century. The first cannabinoids isolated from the cannabis plant were a series of lipophilic terpenophenolic compounds, including cannabinol, which was isolated in 1940 (Adams et al., 2002), followed by cannabidiol (CBD) and Δ9-tetrahydrocannabinol (THC) in 1964 (Gaoni and Mechoulam, 1964). Their general structure contains a series of oxygenated cyclic moieties conjugated to a short acyl tail (Fig. 4B). These cannabinoids were found to act directly through 2 orphan G protein-coupled receptors, which were later termed the cannabinoid receptor type 1 (CB1) (Matsuda et al., 1990) and type 2 (CB2) (Munro et al., 1993; Piscitelli and Di Marzo, 2021). Activation of these receptors within the medulla modulates descending nociceptive pathways and produces antinociception in inflammatory pain conditions (Bouchet and Ingram, 2020). Their discovery also led to the identification of several endogenous ligands for CB1 and CB2 receptors, which were termed endocannabinoids (Devane et al., 1992; Di Marzo and Fontana, 1995). These

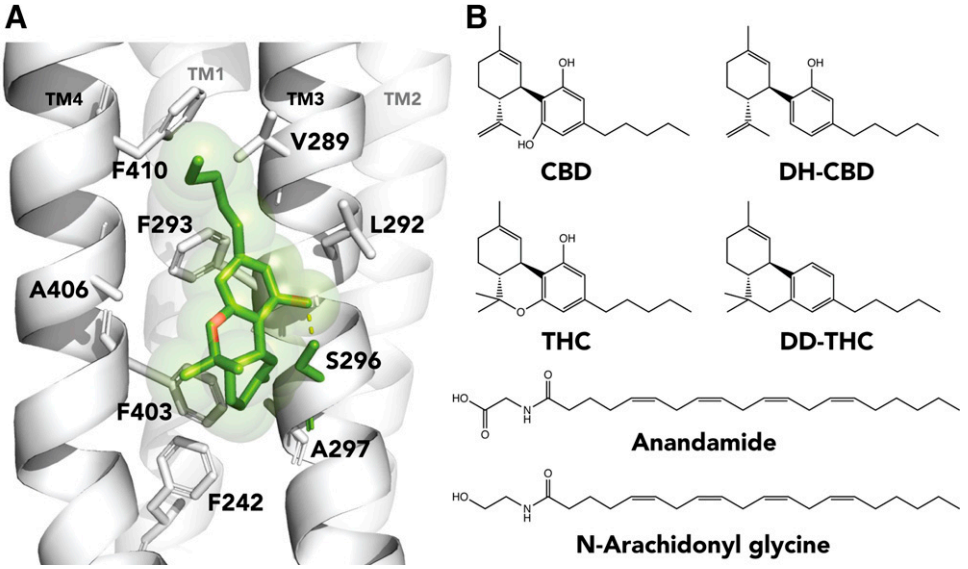

Fig. 4. Cannabinoid binding to glycine receptors. (A) THC (green) docked within the lower intra-subunit binding cavity of the glycine receptor. The hydroxyl-moiety of THC forms a hydrogen bond with S296 from TM3. (B) Chemical structures of exogenous cannabinoids from the cannabis plant and their dehydroxyl and deoxygenated analogs, and endogenous endocannabinoids. Accession code of PBD file used: 5TIO. DD-CBD, cannabidiol with both hydroxyl groups removed.

compounds did not contain cyclic moieties but were instead comprised of simplified amide or amino acid groups conjugated to long, unsaturated acyl tails (Fig. 4B).

Endocannabinoids are lipophilic eicosanoids that are synthesized de novo from membrane phospholipids (Di Marzo, 2008). Their synthesis is significantly enhanced in chronic pain states (Petrosino et al., 2007) and can be triggered by stress and nociceptive stimuli (Hohmann et al., 2005; Drew et al., 2009). This led to the hypothesis that the endocannabinoid system has evolved specifically to modulate nociceptive pain and inflammation, making it an ideal target for pain therapies. The first endocannabinoids identified were arachidonoyl-ethanolamide (anadamide) (Devane et al., 1992) and 2-arachidonyl glycerol (Mechoulam et al., 1995), which were found to produce antinociceptive and psychotropic effects similar to THC and CBD (Fride and Mechoulam, 1993; Smith et al., 1994; Di Marzo and Fontana, 1995). Several arachidonyl amino acids were later identified, including *N*-arachidonoyl glycine (NAGly), which is synthesized in high concentrations within the spinal cord and also produces antinociceptive activity in neuropathic and inflammatory pain models, without incurring motor deficits (Burstein et al., 2000; Huang et al., 2001; Succar et al., 2007; Vuong et al., 2008). However, the antinociceptive activity of these endocannabinoids are not completely reversed by CB1 and CB2 antagonists (Adams et al., 1998; Succar et al., 2007; Vuong et al., 2008), suggesting their antinociceptive activity is partly mediated through alternate pathways. This was initially suggested to be the GlyR, due to its high degree of homology with CB1 and CB2 receptors in regions where cannabinoids were predicted to bind (Lozovaya et al., 2005). Anandamide was found to inhibit glycinergic currents in hippocampal pyramidal neurons (Lozovaya et al., 2005) but caused potentiation of GlyRα_1 expressed in HEK293 cells (Yang et al., 2008) and *Xenopus laevis* oocytes (Hejazi et al., 2006). Anandamide also potentiates glycine responses mediated by GlyRs in rodent midbrain neurons (Hejazi et al., 2006) and cultured spinal neurons (Xiong et al., 2012b). Similarly, NAGly was found to modulate glycinergic activity within the dorsal horn of the spinal cord (Jeong et al., 2010) and cause subunit-specific modulatory effects at GlyRs expressed in *X. laevis* oocytes (Gallagher et al., 2020) and HEK293 cells (Yang et al., 2008; Yevenes and Zeilhofer, 2011). Endocannabinoids were predominantly found to potentiate GlyRα_1 and were mostly inactive or modestly inhibitory at GlyRα_2 and GlyRα_3 (Yang et al., 2008; Yevenes and Zeilhofer, 2011; Xiong et al., 2012b; Gallagher et al., 2020).

Exogenous cannabinoids have also been shown to significantly modulate GlyRs, which likely contributes to the antinociception induced by the cannabis plant. THC significantly potentiates GlyRα_1 and GlyRα_3 when expressed in *X. laevis* oocytes (Hejazi et al., 2006), HEK293 cells, and cultured spinal neurons (Xiong et al., 2011; Xiong et al., 2012b). Immunoblotting assays confirmed these effects are not due to alterations in GlyR expression or receptor trafficking but instead through direct binding interactions (Xiong et al., 2011). In *X. laevis* oocytes, THC was found to have an EC_{50} in the nanomolar range and was highly selective for GlyRs over $GABA_A$Rs (Hejazi et al., 2006). CBD also potentiates homomeric and heteromeric GlyRs in HEK293 cells with an EC_{50} in the low micromolar range and produces modest agonist activity at high micromolar concentrations (Ahrens et al., 2009). To further explore the structure activity relationships of cannabinoids and aid in identifying their binding sites on GlyRs, several synthetic cannabinoid analogs have also been synthesized. Two examples of this are ajulemic acid and HU-210, which also significantly modulate GlyRs (Yang et al., 2008; Foadi et al., 2010).

Cannabinoids were initially predicted to bind within the alcohol binding site proximal to S267; however, S267 mutations that abolish ethanol potentiation had no effect on THC modulation (Hejazi et al., 2006). One exception to this was S267I, which was found to reduce the potentiating effects of CBD and HU-210; however, this mutation also disrupted normal GlyR functionality (Foadi et al., 2010). When looking for other possible binding sites, Xiong et al. (2011) noted the S296 residue that was conserved between the α_1 and α_3 subunits, whereas the corresponding residue in α_2 is an alanine (A303). The S296A mutation significantly reduced THC potentiation of GlyRα_1 and GlyRα_3 without impacting glycine sensitivity or the modulatory activity of other PAMs such as alcohol and anesthetics (Xiong et al., 2011). The S296A mutation also significantly reduced anandamide, CBD, and HU-210 potentiation in GlyRα_1 and GlyRα_3, whereas the reverse A303S mutation in GlyRα_2 enhanced all cannabinoid potentiation (Xiong et al., 2012b), suggesting a novel intrasubunit cannabinoid binding site. MD simulations of anandamide and THC predicted cannabinoid binding between TM4 and TM3 of a single subunit and proposed hydrogen bond formation with S296 via their oxygen or hydroxyl groups (Fig. 4A) (Xiong et al., 2012b). Docking studies conducted with a homology model of $GABA_A$R also predicted 2-arachidonyl glycerol to bind within this cavity (Baur et al., 2013). Removing the oxygen and hydroxyl groups from anandamide (Xiong et al., 2012b), THC (Xiong et al., 2011), and CBD (Xiong et al., 2012b) significantly reduces their potentiating activity, supporting the initial MD findings. A THC analog with both oxygen groups removed (DD-THC) is inactive at GlyRs but prevents the potentiating effects of THC when coapplied. This suggests that deoxygenated cannabinoids are still able to bind within the cavity but cannot elicit potentiation. Yet, this response does not occur when DD-THC is coapplied with propofol, suggesting the binding cavity is cannabinoid specific. Similarly, CBD with both hydroxyl

groups removed has significantly reduced activity at GlyRα_1 but competes with CBD for the cannabinoid binding site (Xiong et al., 2012b). However, removing only one hydroxyl group from the 3′ position of CBD while retaining the 1' hydroxyl (DH-CBD) significantly enhances potentiation compared with CBD. Removal of the 3′ hydroxyl group likely reduces steric bulk and allows the remining hydroxyl to better interact with the S296 residue. Potentiation of GlyRs by endocannabinoids such as NAGly are also impacted by the S296A mutation. However, endocannabinoids are also affected by a range of mutations within the ECD, TM2, and the ICD, depending on polarity of the endocannabinoid head group (Yevenes and Zeilhofer, 2011). This indicates that the modulatory effects of endocannabinoids at GlyRs are more complex and involve multiple regions of the receptor.

THC, CBD, and other cannabinoid analogs induce strong antinociception in rodent models of nociceptive pain, which are not significantly reduced by CB1 antagonists. However, their effects can be significantly reduced by strychnine or coinjection with inactive deoxygenated analogs (Hejazi et al., 2006; Xiong et al., 2011; Xiong et al., 2012a). THC-induced antinociception is almost completely abolished in GlyRα_3 knockout mice but remains in GlyRα_2, CB1, and CB2 knockout mice (Xiong et al., 2012a). The small retention in antinociceptive activity in GlyRα_3 knockout mice is likely due to GlyRα_1 in the spinal cord, which is also potentiated by THC. Mice carrying the GlyRα_1 S296A mutation are also significantly less sensitive to cannabinoid-induced antinociception, highlighting that both GlyRα_1 and GlyRα_3 mediate cannabinoid induced antinociception (Lu et al., 2018).

Cannabinoid modulation of GlyRs was also shown to be sensitive to cholesterol concentrations. Lowering the cholesterol content of HEK293 cells with methyl-β-cyclodextrin was found to significantly reduce the potentiating effects of THC, anandamide, and CBD in cells expressing GlyRs, without impacting the activity of other modulators such as alcohols and anesthetics (Yao et al., 2020). To help elucidate these cannabinoid-specific effects, MD simulations of THC binding to GlyRα_1 were conducted in a model membrane with or without cholesterol. Cholesterol was found to lower the free energy for S296-THC binding and cause a lateral and radial tilt of the TM4 domain toward the pore, which alters the proposed THC binding pocket (Yao et al., 2020). Furthermore, this interaction was found to impact cannabinoid antinociception in vivo. Simvastatin is a lipid-lowering drug that is often prescribed to those suffering with high cholesterol (Pedersen and Tobert, 2004). Mice injected with simvastatin were found to have an approximate 20% reduction in spinal cord cholesterol, which directly correlated to a reduction of GlyR potentiation by THC and DH-CBD when recorded from spinal cord slices (Yao et al., 2020a). The antinociceptive activity of DH-CBD in rodent models of acute and inflammatory pain is also significantly reduced in simvastatin-treated mice (Yao et al., 2020b).

Despite the significant antinociception achieved by cannabinoids, their psychotropic side effects and propensity for recreational use have previously hindered their legality and therapeutic applications. Only recently have cannabinoid-based therapies been legalized in countries such as Canada, America, and New Zealand. As cannabinoids are generally lipophilic and undergo significant hepatic metabolism, their most effective, noninvasive route of administration is sublingual (Xiong et al., 2012a; Urits et al., 2020). The most prevalent cannabis-based therapy is nabiximol, sold under the name Sativex, which is a 1:1 THC:CBD oromucosal spray that is used to treat multiple sclerosis (Giacoppo et al., 2017; D'hooghe et al., 2021), chronic pain conditions (Nurmikko et al., 2007; Johnson et al., 2013), and mental illnesses including depression and anxiety (Gulbransen et al., 2020). Many clinical trials have highlighted the effectiveness of nabiximol and other cannabinoid-based therapies to improve a variety of nociceptive pain symptoms. They are generally well tolerated and have minimal adverse effects—the most common being dizziness, nausea, dry mouth, and sedation (Johnson et al., 2013; Gulbransen et al., 2020). Notably, these therapeutics may be suitable for chronic use as clinical trials found several patients continued treatment of extended periods of time without requiring increased dosing or additional pain relief (Nurmikko et al., 2007; Gulbransen et al., 2020). Despite this, some patients remain hesitant to use cannabinoids or reduce their use of other pain medications in fear of losing opioid prescriptions and the general uncertainty concerning the noninsured costs and lack of regulation of cannabinoid products (Urits et al., 2020). The stigma associated with cannabis use plagues therapeutic cannabinoids and has prompted studies that aim to target the cannabinoid binding site within GlyRs with noncannabis related compounds. A virtual screen of over 1500 Food and Drug Administration–approved drugs was conducted targeting the S296 binding cavity highlighted by cannabinoid research and identified 7 hits that potentiate GlyRα_1 to a greater extent than THC when tested at equivalent concentrations (Wells et al., 2015). Several of these hits are medications that are already used for pain relief despite their mechanisms of antinociception not being fully understood, such as pimozide and sulindac. This not only suggests their antinociceptive activity is in part due to GlyR potentiation but also highlights the ability to target the cannabinoid biding cavity within GlyRs with alternate therapeutically viable compounds.

D. Lipid Modulators

Lipids are known to indirectly modulate pLGICs by permeating into the membrane and altering the

physiochemical properties of the surrounding lipid environment (Sooksawate and Simmonds, 2001; daCosta et al., 2009; Antollini and Barrantes, 2016). This idea was initially based on the observation that nAChRs from the *Torpedo californica* must be purified in the presence of specific lipids to retain functionality (Criado et al., 1982). Extensive research conducted by McNamee and colleagues found that nAChRs conferred a degree of lipid specificity (Andreasen and McNamee, 1977) and that lipid composition impacts the transitional kinetics of these receptors and resulting ion flux, without impacting agonist binding (Ochoa et al., 1983; Criado et al., 1984; Fong and McNamee, 1986). Lipids that optimally match the membrane-exposed regions of the receptor were proposed to enhance functionality by specifically associating within the annular belt. This is the first shell of membrane lipids that occur at the lipid-protein interface and make direct contact with the receptor (Lee, 2011). These lipids can form domains with different fluidity, phase, and composition to the rest of the bulk membrane, which does not directly interact with the receptor (Sunshine and McNamee, 1994; Antollini et al., 2005).

However, additional studies have highlighted specific lipid effects that cannot be accounted for by membrane properties alone. Sunshine and McNamee (1994) found that lipids that incur similar effects on membrane properties produced different modulatory effects. One example of this was squalene, which was able to enhance the functionality of reconstituted nAChRs despite producing membranes with similar fluidity to lipids such as dipalmitoylphosphatidylcholine, which produced low-functioning receptors. This has also been shown recently with vaccenic acid, cis-13-octadecenoic acid, and petroselenic acid, which all incur similar effects on membrane fluidity; however, only petroselenic acid modulates nAChRs (Perillo et al., 2012). These studies suggest that lipids not only interact with annular sites at the lipid-protein interface but can also participate in specific binding interactions at nonannular sites, which occur between the α-helices of the TM domains (Lee, 2011).

Several studies have explored these specific lipid interactions across pLGICs and identified common structure activity relationships that apply to all cys-loop receptors. Overall, these studies conclude that lipid modulators require a double bond in the *cis* conformation and tail lengths between ~10–22 carbon atoms to be active (Witt et al., 1996; Zhang and Xiong, 2009; Antollini and Barrantes, 2016; Perillo et al., 2016). The double bonds induce angularity within the lipid tail that is proposed to perturb the TM at regions, which can disrupt receptor function (Perillo et al., 2012), whereas the increased length of the acyl-chain likely participates in hydrophobic interactions that stabilize lipid binding. Recently, a study that further explored these relationships within GlyRs confirmed that lipids require a *cis* double bond in the center of the acyl tail to be active at GlyRs and produced the greatest potentiation when occurring in the ω7–ω9 positions (Gallagher et al., 2020). This study also found that lipids with small and aliphatic head groups produced greater potentiation than bulkier, aromatic head groups and suggests this may be due to conformationally restrictive binding cavities within the TM domains. Linoleoyl- and oleoyl-glycine (Fig. 5B) were found to significantly potentiate homomeric and heteromeric receptors and were more selective for receptors containing the α_1 and α_2 subunits (Gallagher et al., 2020). Moreover, MD simulations conducted with oleoyl-glycine and structurally related lipids in a model membrane suggest these lipids do not alter the physiochemical properties of the bilayer or general protein structures (Mostyn et al., 2019b; Schumann-Gillett and O'Mara, 2019) and must therefore be potentiating GlyRs through direct binding interactions.

Lipids have also been shown to tightly bind to pLGICs during purification, which further suggests their importance in receptor function and their ability to specifically bind to membrane-exposed regions. Structures of GLIC have shown lipids such as 1,2-distearoyl-sn-glycero-3-phosphocholine (DSPC) and diundecyl phosphatidyl choline can bind in the upper and lower portions of the TM domains (Fig. 5A) (Bocquet et al., 2009; Hu et al., 2018), overlapping with several known binding cavities. DSPC binds to the extracellular portion of the TM domains between TM1 and TM4 of the same subunit. The acyl tails are found to protrude into an intrasubunit cavity shown to bind anesthetics and obstruct the cavities entrance, which may functionally compete for this binding site (Nury et al., 2011). The phosphate backbone of the phosphocholine head group also bridges interactions between F315 (K419) within the post TM4 region and F121 (M147) in the extracellular β6-β7 loop (Bocquet et al., 2009). Recently, a similar phosphocholine lipid was shown to bridge this interaction in a cryoEM structure of a human GABAR (Laverty et al., 2019). This interaction is important for receptor coupling and communicating agonist binding to the TM domains (Baenziger et al., 2000; daCosta and Baenziger, 2009; daCosta et al., 2009; Dacosta et al., 2013). Although this has not been found in structures of GlyRs, recent MD simulations have shown that numerous phospholipids interact with the GlyR at this region, which occurs more prominently in the active state versus the inactive state (Damgen and Biggin, 2021). The zwitterionic lipid head groups interact with charged restudies within the β6-β7 loop of the principal subunit and the β8-β9 loop and pre-TM1 region of the complementary subunit. Lipids can also bind in the intersubunit cavity, which binds ivermectin, most notably 1-palmitoyl-2-oleoyl-*sn*-glycero-3-phosphocholine (POPC), which is a lipid potentiator of the GluCl (Althoff et al., 2014). Similar to the benzofuran group of ivermectin, the polar phosphocholine head group of POPC wedges between

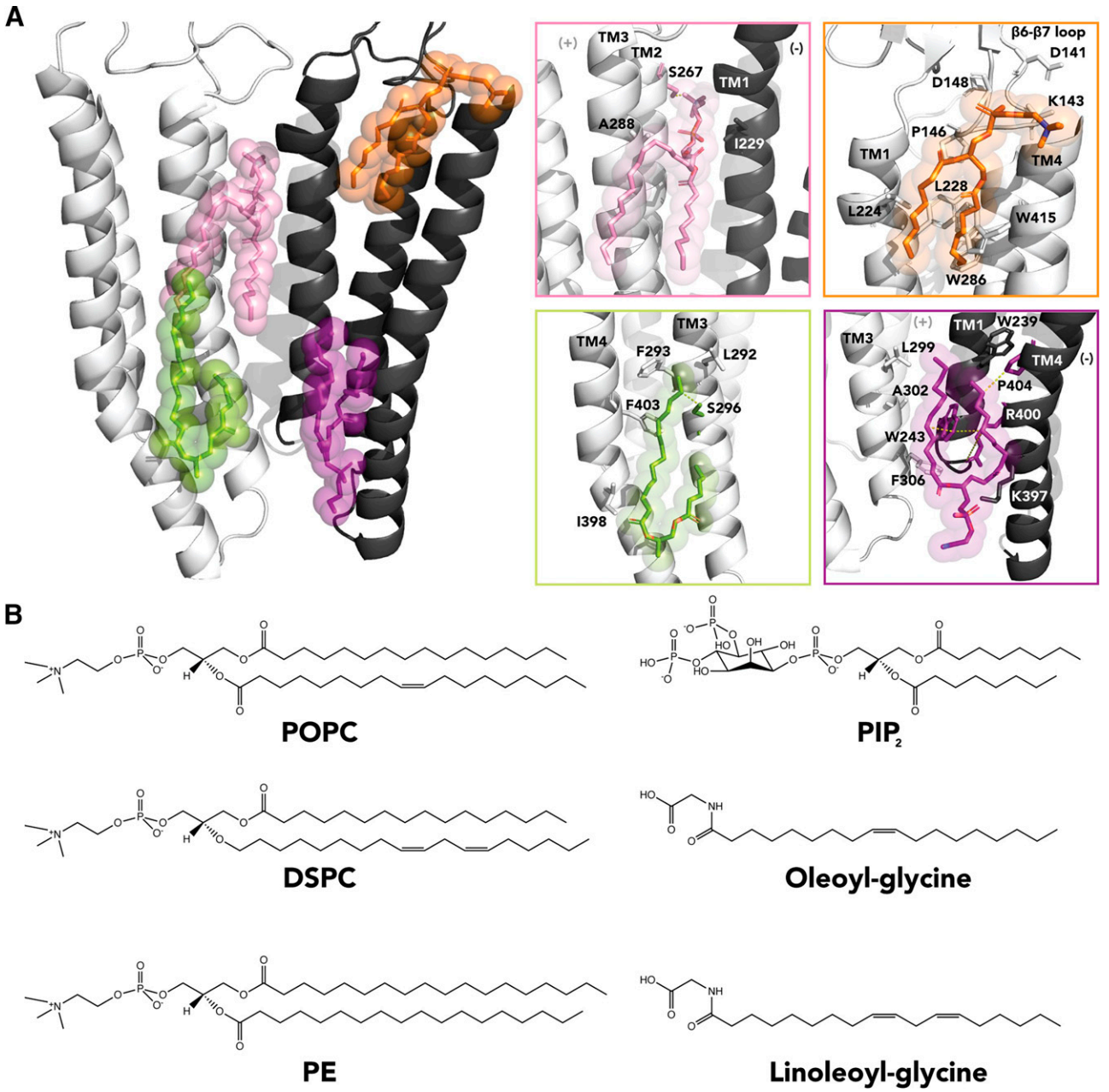

Fig. 5. Lipid binding cavities in the glycine receptor. Structures of lipids bound to GLIC, GluCl, and ELIC were aligned to the glycine receptor. (A) POPC (pink) binds within the upper intersubunit cavity of glycine receptors between adjacent subunits, and the phosphocholine head group interchelates between TM2 domains and interacts with S267. POPC (orange) binds within the upper intrasubunit cavity, and the phosphocholine head group bridges interactions between the post-TM4 region and the β6-β7 loop in the ECD. DSPC (green) binds within the lower intrasubunit cavity with the lipid tails coming within 3.2Å of S296. Phosphatidylethanolamine (PE) (purple) binds within the lower intersubunit cavity and interacts with the conserved P404, W243, and R400 triad. (B) Chemical structures of lipids shown to bind to pLGICs and potentiate glycine receptors. Accession codes for PBD files used: 4TNW, 3EAM, 6HJX, and 5TIO. PIP_2, Phosphatidylinositol 4,5-bisphosphate.

TM1(-) and TM3(+) and protrudes toward TM2(+) (Fig. 5A). This expands the distance between adjacent TM2 domains and increases the channel radius from 1.4Å to 2.4Å in the POPC-bound structure, thus potentiating the receptor by stabilizing it in a conformation that requires less energy for channel opening. Additional conformational changes in the TM2-TM3 and β6-β7 loops transduce signals to the extracellular domain, which is hypothesized to further strengthen ligand binding (Althoff et al., 2014). The acyl tails of POPC remain interchelated between TM1(-) and TM3(+) and extend outward, toward the lipid membrane. Although the structures of lipids bound to GlyRs have not been determined, MD simulations have shown a high stability of membrane lipids in close proximity to residues I229 (TM1-) and A288 (TM3+), which are located at the entrance to this cavity (Murail et al., 2011).

Lipids have also been shown to bind within the intracellular portions of the TM domains, overlapping with the proposed cannabinoid and neurosteroid binding site

(Xiong et al., 2012b; Laverty et al., 2017; Miller et al., 2017; Chen et al., 2018). In structures of GLIC, lipids are shown to bind between TM3 and TM4 of the same subunit, within <4Å of V268 (Fig. 5A) (Bocquet et al., 2009), which corresponds to S296 in GlyRs, a key residue for cannabinoid potentiation (Xiong et al., 2011; Xiong et al., 2012b). Phosphatidylinositol 4,5-bisphosphate has also been shown to occupy this site in GABARs (Laverty et al., 2019). Additionally, lipids have been shown to bind within the intracellular intersubunit binding cavity of ELIC (Henault et al., 2019) and GLIC (Bocquet et al., 2009; Hu et al., 2018). Phosphatidylethanolamine binds to ELIC and interacts with a tryptophan-proline-arginine triad that is conserved among anion-selective pLGICs, corresponding to W243 (TM1), R400 (TM4), and P404 (TM4) in GlyRs (Fig. 5A). This triad creates a characteristic kink at the intracellular end of the TM4 and, when mutated, significantly enhances desensitization kinetics without impacting receptor activation (Henault et al., 2019). This is because the extracellular region of TM4 that proceeds the proline kink is highly flexible and dynamic. When the proline kink is removed, the α-helix straightens and causes TM4 to extend toward the adjacent subunit. This disrupts intrasubunit interactions that are important for receptor activation. When lipids bind within this region, they stabilize TM4 in a kinked conformation, which allows for more efficient receptor activation and slower desensitization rates (Henault et al., 2019), thus potentiating the receptor.

The tryptophan-proline-arginine triad is one of the key components that highlight evolutionary differences between cationic and anionic pLGICs, which may have evolved to modify lipid sensitivity. The proline kink allows TM4 to angle back toward TM1 and TM3, allowing the post-TM4 region to effectively interact with the β6-β7 loop, which communicates agonist binding to the TM domains (Henault et al., 2019). Without this, TM4 would extend outward from the channel pore to compensate for the hydrophobic mismatch and become uncoupled, as seen with nAChRs (Antollini et al., 2005; daCosta and Baenziger, 2009; Dacosta et al., 2013). The second component is a phylogenetically conserved network of aromatic residues that line TM1, TM3, and TM4 in eukaryotic anionic channels such as GABA and GlyRs, whereas cationic channels like nAChRs have much lower aromaticity within this region (Haeger et al., 2010; Carswell et al., 2015). Within the GlyR, there are 16 aromatic residues within the TM1, TM3, and TM4 domains. Homology modeling of the GlyR revealed that three aromatic residues on TM4 (Y406, W407, Y410) face into the membrane and interact with phospholipids to stabilize the receptor (Haeger et al., 2010). The remaining form two clusters of aromatic residues, which form π-π interactions. One in the upper portion of the TM domains includes F402 and F405 from TM4, Y228 from TM1, and W286 and F293 from TM3 and another in the lower portion of the TM domains between F399 and F395 from TM4 and W293 and F242 from TM1 (Haeger et al., 2010; Tang and Lummis, 2018). In combination, these two components make GlyRs less sensitive to the lipid membrane and less dependent on the membrane thickness for receptor activation. However, the residues involved in these processes occur within annular and nonannular sites of receptor and line lipophilic cavities that participate in specific lipid interactions. Although lipids are yet to be resolved in structures of GlyRs, functional studies (Gallagher et al., 2020) and MD simulations (Murail et al., 2011; Damgen and Biggin, 2021) show specific lipid interactions that could be further enhanced through lipid drug design. Greater optimization of lipid PAMs for GlyRs and further structural analysis may allow for lipophilic-based drugs that target allosteric sites within the TM domains of GlyR to treat pain conditions. The main limitation for these compounds, in terms of potential therapeutic applications, are their poor pharmacokinetic profiles, which will be discussed further in section III.C.

E. Tropeines

Tropeines are alkaloids derived from atropine that act as potent serotonin type 3 receptor (5-HT_3R) antagonists. They have commonly been used for their antiemetic effects, in particular ICS-205,930 (tropisetron), which is a well tolerated first-line therapy for acute nausea (Simpson et al., 2000) and is also being explored as an analgesic for fibromyalgia (Haus et al., 2000; Papadopoulos et al., 2000). Tropeines have also been shown to positively modulate GlyRs in the femtomolar range (Yang et al., 2007), and novel tropeine analogs have been synthesized in an attempt to achieve GlyR-specific PAMs.

5-Hydroxytryptamine is a primary agonist for serotonin receptors but was serendipitously found to also act as an inhibitor of glycinergic currents in rat spinal neurons (Chesnoy-Marchais and Barthe, 1996). This prompted studies that assessed the activity of various 5-hydroxytryptamine analogs and 5-HT_3R antagonists at the GlyR. Many had only modest activity, except for the antagonists LY-278,584, MDL-72222, and tropisetron, which incurred biphasic activity, potentiating GlyRs at low concentrations and causing inhibition at higher concentrations (Chesnoy-Marchais, 1996). All potentiating tropeines contained a tropanyl group which was linked to a phenyl or indole group via an ester bond and could be converted to inhibitors by extending either the tropane ring or ester bond (Chesnoy-Marchais, 1996; Maksay, 1998; Chesnoy-Marchais et al., 2000). Around the same time as these discoveries, several tropeines were found to produce antinociceptive effects, including tropisetron and MDL-72222, which elicited antinociceptive effects in rats at concentrations sufficient to saturate the potentiated effects on GlyRs (Glaum et al., 1990; Maksay, 1998; Scapecchi et al., 1998).

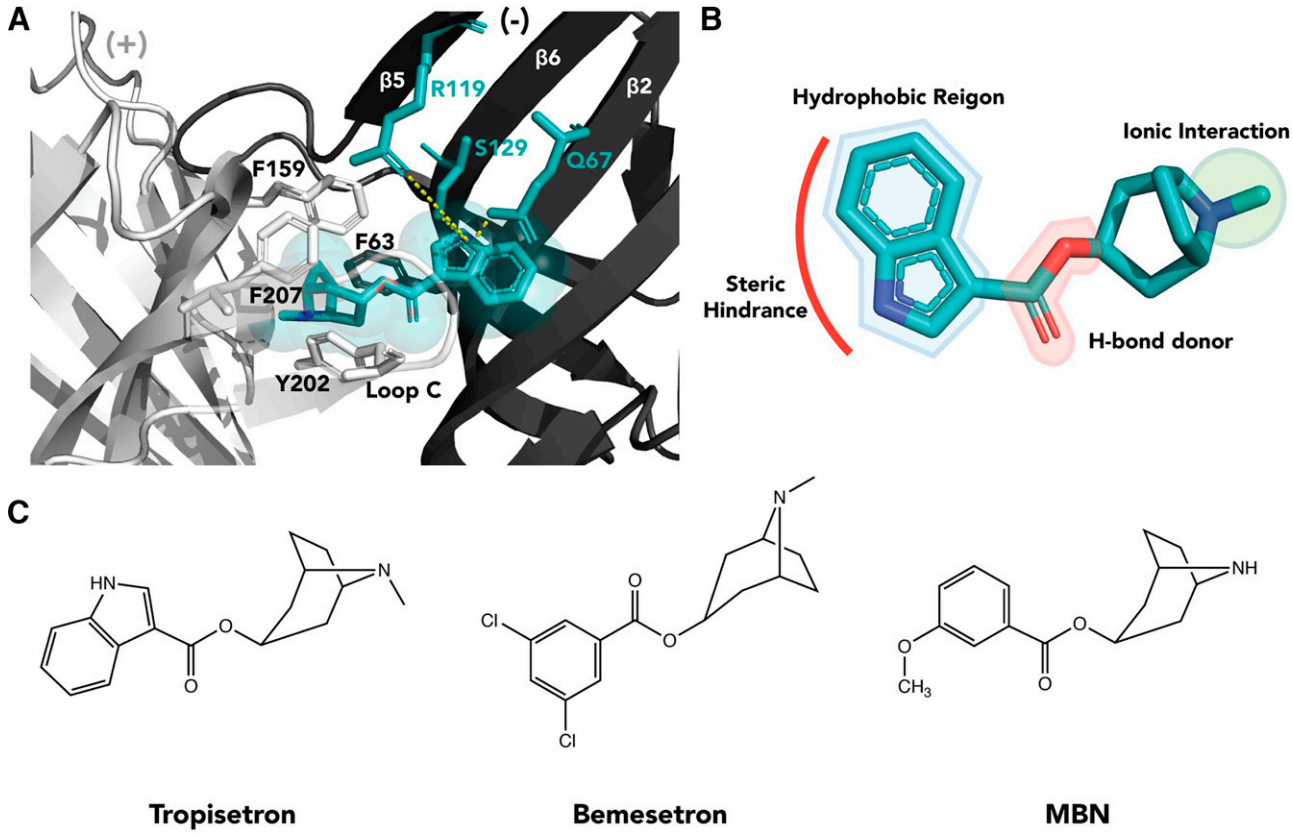

Fig. 6. Tropeine binding in glycine receptors. A structure of tropisetron bound to the mouse serotonin type 3 receptor was aligned to the glycine receptor. (A) Tropisetron (teal) binds within an intersubunit cavity of the ECD, closely aligning with the agonist binding cavity. The tropane moiety sits within an aromatic vestibule formed predominantly by the primary subunit (white), whereas the amide group extends toward the complementary subunit (black) to interact with R119m S129 and Q67. Loop C from the primary subunit closes in on tropisetron, a movement required for receptor activation. (B) Schematic view of the structure activity relationships developed for tropeine activity at glycine receptors. (C) Chemical structures of tropeines with modulatory activity at glycine receptors. Accession codes for PBD files used: 6HIS and 5TIO.

This suggests that tropeines may be eliciting antinociceptive effects through potentiation of GlyRs.

The potentiating effects of tropisetron occurred in the presence of both glycine and taurine (Chesnoy-Marchais, 1996) and were not impacted by coapplication with zinc, ethanol, or propofol, which suggested a novel binding site and modulatory mechanism (Chesnoy-Marchais, 1999). Heteromeric GlyR$\alpha_1\beta$ was more sensitive to potentiation than GlyRα_1, and inclusion of the β-subunit induced potentiation in GlyR$\alpha_2\beta$, compared with GlyRα_2, which is insensitive (Supplisson and Chesnoy-Marchais, 2000). Conversely, GlyRα_2 was found to be more sensitive to the inhibitory effects of tropisetron and atropine compared with GlyRα_1, and inhibition was more prominent in homomeric receptors compared with heteromeric GlyRs (Maksay et al., 1999). Structure activity relationships were further developed by screening a range of tropeine analogs. The results from these studies predicted that the phenyl ring bound within a hydrophobic pocket, which is sterically hindered toward the paraposition, whereas the ester group is likely to interact with a hydrogen-bond donor (Maksay et al., 2004). The tropane ring has the most structurally distinctive interactions, with most substitutions significantly impacting activity. Significantly, demethylation of the nitrogen within the tropane group significantly enhanced potency at GlyRs while reducing activity at 5-HT$_3$R, opening an avenue for selective GlyR drug development. *n*-Methylation of the indole ring (MBN) was also found to attenuate inhibitory effects without impacting GlyR potentiation and produced a greater selectivity for GlyRs over 5-HT$_3$R (Maksay et al., 2009).

Early mutagenesis studies suggested that tropeines bind within the ECD, involving residues T112 (Maksay et al., 1999) and E103 (Chesnoy-Marchais et al., 2000), and were unlikely to interact with the TM2 domain (Supplisson and Chesnoy-Marchais, 2000). Several mutations around the orthosteric binding pocket were found to abolish or reduce inhibitory effects (Maksay et al., 2009), including the N102A mutation, which completely abolishes inhibitory activity without impacting potentiation (Yang et al., 2007). The asparagine residue was predicted to make hydrogen bonds with the nitrogen group within the tropane ring, similar to its docking position in 5-HT$_3$R. Homology modeling and docking studies using MBN also suggested distinct binding interactions for inhibitory

and potentiating tropeines that occur at adjacent binding sites in the ECD, proximal to the orthosteric binding pocket (Maksay et al., 2009). Within the inhibitory site, MBN penetrates deeply below loop C and stabilizes it in an outwardly, half-open conformation, thus hindering the full closure required to elicit channel gating. The tropane moiety binds within a hydrophobic vestibule, with its aromatic region interchelated between F63(-) and F159(+) and the secondary amino group forming hydrogen bonds with N102(+) and N46(-), whereas the carbonyloxy moiety bonds with R131(-) (Maksay et al., 2009). The potentiating binding pocket is more superficial. The tropane ring remains buried within a hydrophobic cleft formed by F63(-) and F159(+); however, it is rotated and extends upwards to include Y202(+) and F207(+) (Fig. 6A). The amide group now forms interactions with Q67(-), R119(-), and S129(-). In this conformation, MBN stabilizes loop C in a closed conformation that mimics that of the agonist-bound receptor. This likely produces potentiation by facilitating channel opening (Maksay et al., 2009).

An interesting aspect of tropeine modulation is the ability to achieve such high affinity at the GlyRα_1 (Yang et al., 2007) and GlyR$\alpha_1\beta$ (Supplisson and Chesnoy-Marchais, 2000) with compounds that are already clinically approved, orally available, and shown to have antinociceptive effects in fibromyalgia (Haus et al., 2000; Papadopoulos et al., 2000) and inflammatory and neuropathic pain (Stratz and Müller, 2000; Stratz and Muller, 2004; Nasirinezhad et al., 2016). Many of the antinociceptive effects have been attributed to serotonergic modulation; however, it is likely that positive modulation of GlyRs may also play a role in their antinociceptive activity. Further optimization of lead tropeine compounds that enhance their potentiating activity at GlyRs may therefore produce compounds with superior antinociceptive effects. As tropeines have desirable pharmacokinetic profiles and several are already clinically approved, this makes tropeines an advantageous stepping stone for further GlyR PAM development as potential pain therapies.

However, one major drawback is the lack of potentiation at GlyRα_3, despite the putative binding site being conserved between GlyRs (San Martín et al., 2019). It was suggested that the allosteric transduction pathway linking the ECD to channel gating may differ for GlyRα_3 and thus makes GlyRα_3-selective tropeines unobtainable. Nevertheless, with the increasing number of structures refined for mammalian GlyRs and enhanced MD capabilities, further development of the tropeine pharmacophore could be developed to confer greater selectivity for the potentiating effects and attempt to resolve the lack of GlyRα_3 potentiation. It also remains questionable whether GlyRα_3 potentiation is required for antinociceptive activity, and the ability for tropeines to produce antinociceptive effects, which are likely mediated by GlyRα_1, supports this argument.

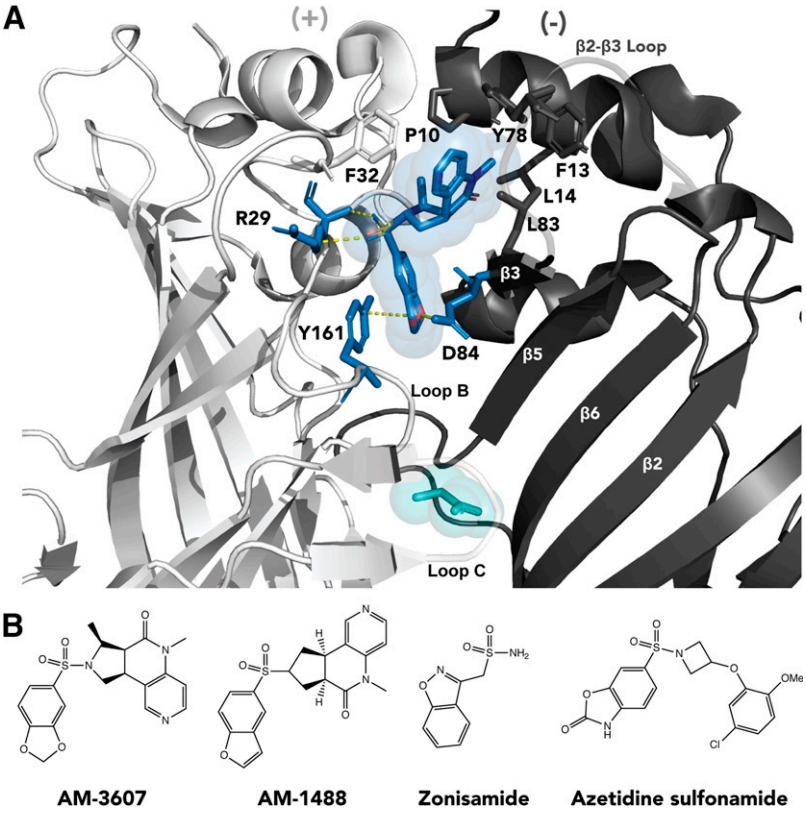

Fig. 7. Sulfonamide binding in glycine receptors. (A) AM-3607 (blue) binds within an intersubunit cavity of the ECD approximately 10Å above the agonist binding cavity (teal). The benzodioxole moiety interacts with Y161 from the primary subunit (white) and D84 from the complementary subunit (black). The sulfonamide group is coordinated by R29 from the α1-β1 loop, and the pyrrolidine moiety buries within a hydrophobic cavity formed by the α1-helix (+) and the β2-β3 loop (-). (B) Chemical structures of sulfonamides with modulatory activity at glycine receptors. Accession code of PBD file used: 5VDH.

F. Sulfonamides

The pharmaceutical companies Pfizer and Amgen have both conducted large-scale screens of compound libraries in an attempt to identify novel PAMs of GlyRs. The screens from both companies identified a range of sulfonamides, which are a class of compounds that contain a common SO_2NH_2 moiety. They are commonly known as bacteriostatic antibiotics that have been used to treat bacterial infections and allergies; however, the sulfonamide moiety has previously been incorporated into therapeutics to treat epilepsy, hypertension, and glaucoma (Mujumdar and Poulsen, 2015; Davies et al., 2020).

The initial screen was conducted by Pfizer and used a fluorescence-based membrane potential assay in CHO cells expressing GlyRα_1, GlyRα_3, and GlyR$\alpha_3\beta$ (Stead et al., 2016). Of the 56,558 compounds tested, 147 were primary hits. These were then screened using an automated electrophysiology IonFlux HT assay yielding seven true hits—five benzoic acids and two novel benzene-sulfonamides. The most efficacious of the sulfonamides was 4-Fluoro-N-(2-(quinolin-8-yloxy)ethyl)-benzenesulfonamide, which potentiated both GlyRα_1 and GlyRα_3 and showed no activity at $GABA_A$Rs (Stead et al., 2016). Soon after, Amgen published a similar screen that used a membrane potential dye assay in HEK293 cells expressing GlyR$\alpha_3\beta$ to identify hits, which were further characterized using Schild shift assays (Bregman et al., 2017). The four hits from this screen were all tricyclic sulfonamides with a benzodioxole moiety. The major metabolic pathways of these hits were through oxidation of the quinolinone core, pyrrolidine ring, and benzodioxole moiety, and thus, modifications were made to reduce oxidation. Incorporating a nitrogen into the quinolinone ring enhanced the sulfonamides potency and achieved high passive permeability, good solubility, and good oral absorption. Removing an oxygen from the sulfonamide moiety to form a benzofuran group (AM-1488) was found to achieve good potency across homomeric and heteromeric α_1 and α_3 GlyRs (Huang et al., 2017). Furthermore, AM-1488 possesses good selectivity over other cys-loop receptors, GCPRs, human *ether-a-go-go*-related gene K^+ channels, the bile-salt export pump, cytochrome P450s, and the purinergic receptor PXR (Bregman et al., 2017). When tested in a neuropathic pain model in mice, AM-1488 reversed tactile allodynia to levels similar to gabapentin and could be administered orally without incurring any motor side effects (Bregman et al., 2017; Huang et al., 2017).

Modification of the pyrrolidine moiety to incorporate a methyl group at the 2 position (AM-3607) further enhanced potency, achieving a potency two orders of magnitude greater than the initial hits (Bregman et al., 2017; Huang et al., 2017). AM-3607 progressed onto structural studies and was cocrystallized with glycine using a modified human GlyRα_3, in which the ICD was replaced by a tripeptide linker and the ECD glycosylation site removed (Huang et al., 2017). AM-3607 is bound to a novel intersubunit binding site within the ECD (Fig. 7A). The benzodioxole moiety sits approximately 10Å above the glycine biding site and stacks between Y161(+) from loop B and D84(-) from the β3 strand. The oxygens from the sulfonamide group are coordinated by R29(+), whereas the pyrrolidine moiety buries within a hydrophobic cavity formed by F32(+) and the α1 α-helix (P10, F13, L14) and the β2-β3 loop (Y78, L83) of the neighboring subunit. Sulfonamide binding is hypothesized to enhance agonist affinity by stabilizing the orthosteric binding pocket. This likely occurs through loop B, which lines both cavities and participates in binding interactions with glycine and AM-3607 simultaneously.

Amgen also conducted further screens of commercial compounds based on the primary leads. These screens used known parameters of the developing pharmacophore and strict pharmacokinetic properties. This yielded compounds with azetidine and methoxy-phenyl groups replacing the tricyclic moiety and compounds containing multiple trifluoro-functional groups (Chakka et al., 2017). These compounds were classed as aminothiazole sulfonamides, which contained a lipophilic core and polar termini and adopt similar conformations to AM-3607 when docked into the identified binding site. Other groups have also identified PAMs of GlyRs that contain sulfonamide moieties, such as zonisamide, which is a therapeutically available anticonvulsant. Zonisamide potentiates GlyRα_1 and, to a lesser extent, GlyRα_3 (Devenish et al., 2021). It potentiates currents elicited by low glycine and taurine concentrations by over 10-fold, likely due to the benzodioxole group interacting with loop B to enhance agonist affinity, similar to AM-3607. However, it has a significantly lower potency due to the simplified amide group replacing the tricyclic moiety, therefore preventing interactions with the adjacent hydrophobic pocket, that stabilizes binding.

The development of sulfonamide PAMs has focused heavily on metabolism and solubility, ensuring the lead compounds remained drug-like and would be orally available. This makes them one of the most likely candidates to be followed through to clinical trials. However, much like other GlyR PAMs, sulfonamides are unable to achieve a greater selectivity for receptors containing the α_3 subunit over the α_1 subunit (Chakka et al., 2017; Huang et al., 2017). Although compounds that bind in the ECD are more likely to achieve greater solubility, this may limit their ability to target GlyRα_3, which is suggested to have a poor ECD-TMD allosteric transduction pathway (San Martin et al., 2019). The inability to selectively target GlyRα_3 does not appear to incur motor deficits or other significant side effects at antinociceptive concentrations in rodent neuropathic models (Chakka et al., 2017; Huang et al., 2017); however, this may produce hesitancy in moving forward to

clinical trials. It is unlikely that further pharmacophore development will achieve GlyRα_3 selectivity, and thus, further toxicity screening may be required to determine if sulfonamides will be therapeutically viable.

G. Sestertepene Glycinyl-Lactams

Sesterterpene glycinyl-lactams are a class of marine metabolites isolated from sea sponges. They were originally identified in a screen of Australian and Arctic marine invertebrates and algae in an attempt to identify novel PAMs of GlyRs (Balansa et al., 2010). Many of the primary hits from this screen were sesterterpene tetronic acids, which were later found to have no activity at GlyRs or potentiate GlyRα_1 but significantly inhibit GlyRα_3. However, the screen also highlighted glycinyl lactam sesterterpenes, which were a rare class of metabolites that had only recently been identified in literature at the time from a Korean sea sponge (Shin et al., 2001; Liu et al., 2003; Wang et al., 2008).

Conjugated tetronic acid metabolites from the *Irciniidae* sponge family were found to have significant potentiating activity at GlyRα_1 if they contained a hydroxyl group at the 8 position (Fig. 8), with all 8-hydroxyl metabolites potentiating glycine currents by over 200% (Balansa et al., 2010). 8-Hydroxyircinialactam B was the first glycinyl-lactam to be identified that also potentiated GlyRα_3 and was the only metabolite where the oxygen within the glycinyl-lactam moiety was attached in the 1 position. All other metabolites that were inactive or had inhibitory activity at GlyRα_3 were not oxygenated or contained an oxygen attached at the 4 position. This suggested that the 8-hydroxy of the tetronic acid was important for potentiating activity, whereas the degree of oxygenation and isomerization of the glycinyl-lactam group conferred subunit selectivity (Balansa et al., 2010). Further screening of metabolites from the *Ianthella* sponge family supported this idea. Structurally similar sesquiterpene metabolites that do not contain a hydroxyl group are devoid of any activity at GlyR, even if they contain an oxygenated glycinyl-lactam moiety (Balansa et al., 2013b). Several indole alkyloids were also identified and significantly inhibited GlyR activity, broadening the scope of this pharmacophore. Ircinianin glycinyl-lactams were later isolated from *Psammocinia* sponges and also cause modulatory effects. (-)-Ircinianin lactam A contains the 1-oxygenated glycinyl-lactam moiety, which is now numbered as position 4 (Fig. 8), and selectively potentiates GlyRα_3 by 260% (Balansa et al., 2013a). Its sulfonated derivate also potentiates GlyRs, but it is nonselective and less potent, potentiating both GlyRα_1 and GlyRα_3 by approximately 70%. Several other ircinianin metabolites without the oxygenated glycinyl-lactam moiety incur modest potentiation of GlyRα_1 but were inactive or inhibitory at GlyRα_3 (Balansa et al., 2013a), supporting the original hypothesis that the oxygenation and isomerization of the glycinyl-lactam group confers subunit selectivity (Balansa et al., 2010).

Compounds of this class are promising for therapeutic development due to their ability to selectively potentiate GlyRα_3, which has proven not to be the

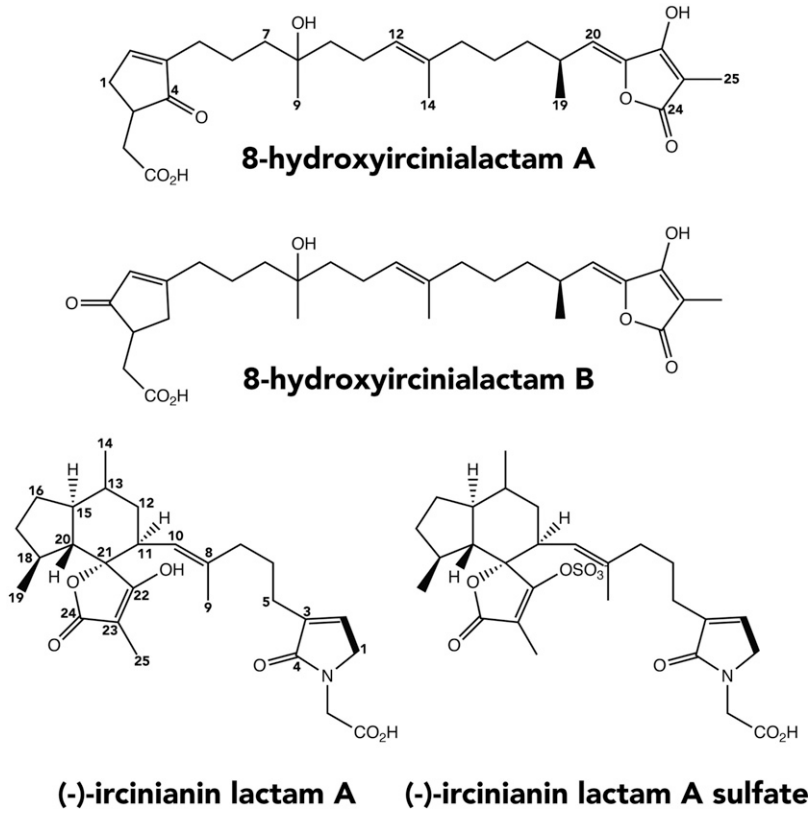

Fig. 8. Chemical structures of sestertepene glycinyl-lactams. Chemical structure of natural sestertepene glycinyl-lactams isolated from *Irciniidae* and *Psammocinia* sponge families, which have been shown to modulate glycine receptors. For hydroxyircinialactams, a hydroxyl in the 8 position is important for potentiating activity and an oxygen in the 1 position is required for activity at GlyRα_3. (-)-Ircinian lactam A derivatives also require a 4-oxygen (corresponding to position 1 in hydroxyircinialactams) for activity at GlyRα_3.

case for other classes of PAM. However, the limited supply of raw material has restricted the ability to study these compounds further. The high number of chiral centers within these compounds makes them difficult to synthesize or modify, therefore relying solely on isolation from natural sources to obtain yields sufficient for functional analysis. This has also prevented the ability to elucidate binding interactions or study their mechanism of action. For these compounds to be considered for therapeutic application, a greater understanding of the pharmacophore would need to be developed by broadening the chemistry of the analogs screened. It has recently been suggested that compounds based on the glycinyl-lactam pharmacophore have been synthesized; however, these data are currently unpublished. The newly synthesized derivates are proposed to maintain the high GlyR selectivity and have favorable physiochemical properties and antinociceptive potency (Lynch et al., 2017). These synthetic analogs may open a new avenue of research for this class of compounds. Importantly, it may allow for production methods that produce sufficient yields for in vivo testing to confirm its potential antinociceptive activity.

III. Limitations

Despite many known PAMs of GlyRs showing promising antinociceptive activity, there is a hesitancy in developing them for therapeutic application due to their nonselective nature. Additionally, several of the primary candidates are highly lipophilic and bind within the TM domains of the receptor, which can be significantly impacted in vivo by endogenous modulators. Overall, these limitations raise concerns regarding potential on- and off-target side effects and whether these modulators can achieve in vivo efficacy.

A. Side Effects and Subunit Selectivity

Glycine neurotransmission is not only involved in nociceptive signaling but also aids in motor and respiratory control, the reward system, and sensory perception (reviewed by Zeilhofer et al., 2018). Therefore, potential target-related adverse effects must be considered when developing PAMs for therapeutic application. A primary concern is GlyRα_1 expression throughout the Bötzinger complex, which exerts inhibitory control on the respiratory system (Schmid et al., 1991; Zeilhofer et al., 2018). Mice containing hyperekplexic mutations within the α_1 subunit and GlyRα_1 knockout mice incur severe motor deficits and respiratory abnormalities, which often results in premature death (Buckwalter et al., 1994; Harvey et al., 2004; Hirzel et al., 2006; McCracken et al., 2013). This has likely deterred pharmaceutical companies from advancing modulators that significantly potentiate receptors containing the α_1 subunit, despite promising antinociceptive activity (Bregman et al., 2017; Huang et al., 2017). GlyRα_3 has also been implicated in respiration; however, GlyRα_3 knockout mice survive with no obvious respiratory deficits or only minor abnormalities (Harvey et al., 2004; Manzke et al., 2010). The α_3 subunit also has a more restricted expression within the central nervous system (CNS) (Baer et al., 2009; Burgos et al., 2016). This suggests that PAMs that selectively target GlyRα_3 may reduce the potential for on-target adverse effects and be more selective for nociceptive neurotransmission. However, obtaining GlyRα_3-selective potentiators has proven to be difficult. Only the sponge derivative (-)-ircinianin lactam A has been shown to selectively potentiate GlyRα_3 (Balansa et al., 2013a); however, its mechanism of action, pharmacokinetic profile, off-target effects, and in vivo activity are yet to be determined. An alternate avenue may be to develop modulators that selectively potentiate the alternatively spliced GlyRα_1^{ins} and GlyRα_3^{ins} expressed within the spinal cord (Malosio et al., 1991b). However, this would likely prove difficult due to the high sequence similarity and due to the insert occurring in the ICD, which is the only region of the GlyR yet to be structurally determined.

Despite GlyR involvement in respiration, PAMs that significantly modulate the α_1 subunit have been used without incurring respiratory depression or significant motor deficits. Primary examples of this are tropisetron and Sativex (THC plus CBD). Tropisetron, THC, and CBD have all been shown to significantly potentiate GlyRα_1 (Chesnoy-Marchais, 1999; Hejazi et al., 2006; Yang et al., 2007; Ahrens et al., 2009) and are currently used as therapeutics in many countries without incurring severe or intolerable side-effect profiles (Gulbransen et al., 2020; Johnson et al., 2013). Interestingly, both of these drugs are used to treat neuropathic pain or pain-related conditions (Haus et al., 2000; Stratz and Müller, 2000; Stratz and Muller, 2004; Nurmikko et al., 2007; Johnson et al., 2013); however, their nociceptive activities are largely attributed to other proteins. This suggests that the adverse on-target side effects from GlyRα_1 may be overstated, and prior suggestions to avoid the receptor for analgesic drug development may be unnecessarily cautious. Further research into the potential toxic side effects and the therapeutic window of GlyR PAMs must be conducted to shed light on this discrepancy and progress this area of research.

B. Protein Selectivity

Due to the high degree of sequence and structural similarity between pLGICs, many GlyR modulators also incur modulatory activity at other receptors. This greatly enhances the possibility of off-target side effects and likely reduces the therapeutic window of these compounds. Examples of this include tropeines, which significantly modulate 5-HT$_3$Rs (Simpsonet al., 2000) that are expressed through the CNS and digestive system (Thompson and Lummis, 2006); avermectins, which produce neurotoxic

activity via GABARs (Krůsek and Zemková, 1994; Ménez et al., 2012); and cannabinoids that target the CB1 and CB2 receptors, which causes psychotropic effects (Hejazi et al., 2006; Xiong et al., 2011; Xiong et al., 2012a). Although this makes the current candidates unsuitable for therapeutic application, further insight into their structure-activity relationships could identify distinctive interactions that could be used to enhance GlyR selectivity. Studies that explore alterations within the tropane and indole rings of tropeines have already identified differences between the GlyR and 5-HT_3Rs and have been used to develop compounds with greater GlyR selectivity (Maksay et al., 2009). Similarly, removing hydroxyl groups from CBD enhances GlyR selectivity over CB1 receptors, which abolishes the psychotropic side effects (Xiong et al., 2012a). Further development of these distinctive interactions could aid in the development of more GlyR-selective compounds and widen the therapeutic window for these drug classes.

Even if complete GlyR selectivity is not obtainable, many of the "off-target" proteins have also been implicated in nociceptive signaling and could be beneficial in achieving multitargeted analgesic compounds. Ongoing research into nociceptive signaling has led to a greater understanding of the complex network involved in nociceptive neurotransmission and has highlighted several targets that may be useful in treating nociceptive pain. This includes GlyRs (Harvey et al., 2004; Miraucourt et al., 2007; Imlach et al., 2016), glycine transporter type 2 (GlyT2) (Dohi et al., 2009; Vandenberg et al., 2014; Zeilhofer et al., 2018), NaV1.7 channels (Dib-Hajj et al., 2017; Hoffmann et al., 2018; Hameed, 2019; Chen et al., 2020), transient receptor potential cation channel subfamily V member 1 (TRPV1) channels (Huang et al., 2006), 5-HT_3R (Kukushkin and Igon'kina, 2003; Nasirinezhad et al., 2016), and $GABA_A$Rs (Enna and McCarson, 2006; Zeilhofer and Zeilhofer, 2008). Traditional drug development has aimed to produce compounds selective for a single nociceptive target; however, this may be insufficient for attenuating sensitization across all signaling pathways and likely activates complex cellular mechanisms in vivo that can circumvent pharmacological effects (Csermely et al., 2005). Primary examples of this are AZD1386 and AMG517, which are both selective TRPV1 antagonists that failed in clinical trials due to lack of efficacy and toxic side effects occurring at concentrations required to produce antinociception (Pang et al., 2012). This indicates that selectively targeting TRPV1 is not a viable way to produce antinociception (Gavva et al., 2008), despite this channel being a prominent protein within the nociceptive signaling pathway and showing promising results in vitro (Huang et al., 2006; Szallasi et al., 2006). Developing multitargeted therapies may achieve greater efficacy in vivo as they are more likely to cause synergetic mechanisms that result in whole-system changes and are less likely to be impacted by compensatory mechanisms or cause toxicity (Csermely et al., 2005; Pang et al., 2012).

This approach is already being explored with lipid PAMs of GlyRs (Sheipouri et al., 2020). A series of *N*-acyl amino acids that potently inhibit GlyT2 (Mostyn et al., 2017), the transporter responsible for removing glycine from nociceptive synapses (Vandenberg et al., 2014), has recently been screened on GlyRs and found to produce modulatory activity (Gallagher et al., 2020). One of the most efficacious GlyT2 inhibitors has been shown to cause antinociceptive activity in a rodent model of neuropathic pain (Mostyn et al., 2019a); however, a dual-acting lipid has yet to be tested in vivo. This would be expected to achieve even greater antinociceptive activity by synergistically enhancing glycine concentrations and potentiating GlyRs at nociceptive synapses, thus requiring a lower concentration to achieve antinociceptive activity and lowering the chance of adverse side effects. Furthermore, this multitarget mechanism may be responsible for the antinociceptive activity that occurs with tropisetron and cannabinoids that are already clinically available (Haus et al., 2000; Stratz and Müller, 2000; Stratz and Muller, 2004; Nurmikko et al., 2007; Johnson et al., 2013). Despite cannabinoids not currently being considered a first-line pain medication, cannabinoid-containing products have been prescribed to over 84,000 individuals in Australia to treat chronic pain (Henderson et al., 2021). They have also been shown to elicit superior antinociceptive effects and have better long-term outcomes in patients that are refractory to other first-line medications such as opioids (Johnson et al., 2013). This likely occurs due to their multitargeted effects, including their potentiation of GlyRs, Future research into multitargeted antinociceptive drug development may shed a light on why many promising compounds fail during or prior to reaching clinical trials and may aid in the development of analgesics that achieve therapeutic effects in vivo.

C. Poor Pharmacokinetic Profiles

Many of the current drug leads for GlyR PAMs are lipophilic compounds that bind within the TM domains. Lipophilic compounds are often overlooked for drug development as they do not fit Lipinski's rules for drug likeness (Lipinski et al., 2001) and have poor bioavailability when utilizing desirable administration routes, such as oral administration (Porter et al., 2007). However, even within Lipinski's rules, there are several caveats that open the possibility of lipophilic drug discovery, including lipophilicity rules not holding true for lipids that are substrates for active transporters (Lipinski et al., 2001; Benet et al., 2016). There are several transporters present within the blood-brain barrier that transport lipophilic compounds into the CNS, including the fatty acid transporter protein and fatty acid binding proteins (Mitchell and Hatch, 2011; Mitchell et al., 2011). There is also evidence that

suggests the existence of an endocannabinoid transporter responsible for accumulating lipophilic endocannabinoids such as anandamide (Fowler, 2013). A primary candidate is the sterol carrier protein-2, which is expressed in the brain and has been shown to mediate anandamide translocation (Liedhegner et al., 2014; Hillard et al., 2017). Lipophilic drugs may therefore attain sufficient concentrations within the CNS if they act as substrates at one of the active lipophilic transporters.

Lipid-based drugs indeed already exists. Most notably fingolimod, which is a lipophilic, orally available multiple sclerosis treatment that transverses the blood-brain barrier to act on sphingosine-1-phosphate receptors within the CNS (Brinkmann et al., 2010; Hunter et al., 2016). Alternate pathways of administration are also being explored, including sublingual administration, which is used for lipophilic cannabinoid-based therapies (Xiong et al., 2012a; Urits et al., 2020). This helps bypass first-pass hepatic metabolism and avoid lipid metabolism—another issue for lipid-based drug discovery (Markovic et al., 2020; Pifferi et al., 2021). However, intranasal, transdermal, and nanotechnology-based administration methods are also being explored to enhance systemic availability (Bruni et al., 2018).

Many of the lipophilic PAMs of GlyRs are in their initial stages of drug discovery and require further development before they can be considered for therapeutic application. As a greater understanding of their structure-activity relationships are developed, optimization of drug leads can be conducted to enhance drug likeness, reduce metabolic functional groups, or enhance their activity at lipophilic transporters. Despite the traditional understanding of small-molecule drug design, it may be possible to design lipophilic drugs that are still highly effective and bind within the TM domains.

D. Impact of Endogenous Mediators

In addition to PAMs that have been developed through drug design, there are several endogenous compounds that can modulate GlyR function in vivo. Many of these have important physiologic functions or are implicated in disease states such as hyperekplexia (Al-Futaisi et al., 2012; Zhou et al., 2013) and inflammatory pain (Harvey et al., 2004). In particular, the ICD is susceptible to many intracellular processes such as phosphorylation (Ahmadi et al., 2002; Han et al., 2013; Moraga-Cid et al., 2020), ubiquitination (Buttner et al., 2001; Zhang et al., 2019b), and protein binding (Kirsch et al., 1991; Yevenes et al., 2003; Yevenes et al., 2006; Breitinger et al., 2020). These processes can alter the expression and functional properties of the receptor, which can be difficult to mimic in vitro. Furthermore, endogenous modulators that produce significant functional and structural changes may disrupt how exogenous PAMs designed for drug development interact with the receptor in vivo.

The most well studied endogenous mediator of GlyRs is zinc, which is a potent biphasic modulator that potentiates receptor activity between 10 nM and 10 μM and inhibits activity at concentration above 100 μM (Bloomenthal et al., 1994; Lynch et al., 1998; Harvey et al., 1999; Miller et al., 2005). Zinc is present throughout the nervous system and colocalizes within glycinergic vesicles at nociceptive synapses (Birinyi et al., 2001), where it tonically mediates neuronal excitability (Zhang and Thio, 2007) and nociceptive sensitivity (Jo et al., 2008). The tonic concentration of zinc in the extracellular space is estimated to be in the low nanomolar range (Frederickson et al., 2006); however, it can reach synaptic concentrations of at least 10 μM during activation (Zhang et al., 2016). Within this physiologic concentration range, zinc can endogenously modulate both synaptic and nonsynaptic GlyRs and has been shown to act presynaptically to facilitate glycine release (Birinyi et al., 2001).

Zinc has also been shown to synergistically enhance the activity of other PAMs—in particular, enhancing ethanol modulation of homomeric GlyRs (McCracken et al., 2010; McCracken et al., 2013a,b). When tested on GlyRs expressed in *X. laevis* oocytes, the potentiation achieved by the coapplication of zinc and ethanol was greater than the sum of their individual effects, which suggests a synergistic mechanism (McCracken et al., 2010). As zinc is a ubiquitous contaminant in many buffers, purified products, and on common laboratory ware (Kay, 2004; Cornelison and Mihic, 2014), it has been suggested to impact previous functional analyses of GlyRs and has caused overestimations of PAM effectiveness. It is suggested that studies assessing PAMs of GlyR take additional steps to assess their activity in the absence of zinc. Several studies have used metal chelators, including tricine (Kirson et al., 2013; McCracken et al., 2013; Cornelison et al., 2017; Gallagher et al., 2020), EDTA (Kay, 2004; Trombley et al., 2011), and ZX1 (Perez-Rosello et al., 2015; Zhang et al., 2016) in an attempt to remove contaminating zinc from recording solutions; however, these chelators are not completely selective and thus can disrupt functional analysis by chelating other divalent ions in recording solutions such as calcium and magnesium (Ramos Silva et al., 2001; Radford and Lippard, 2013). Some studies have suggested these chelators have minimal impact on baseline controls; however, this does not negate the possibility that chelators may interact with PAMs in solution or with the GlyR itself. Other groups have used zinc-insensitive mutants such as GlyRα_1 W170S (Zhou et al., 2013) to screen PAMs (Cornelison et al., 2017). It is important to determine the effectiveness of novel PAMs in the presence and absence of zinc to ensure they are likely

to be effective in vivo and to determine possible synergistic effects that may enhance their activity at nociceptive synapses where zinc colocalizes with GlyRs (Birinyi et al., 2001).

Cholesterol has also been shown to modulate GlyR function. Cholesterol is an integral part of cell membranes that can indirectly modulate membrane proteins by altering the physiochemical properties of the lipid bilayer (Cathcart et al., 2015; Subczynski et al., 2017). However, cholesterol can also directly modulate membrane proteins by binding deeply within nonannular sites, thus acting as a PAM (Henin et al., 2014; Damgen and Biggin, 2021). In addition to its direct effects, cholesterol is also a precursor in the synthesis of endogenous neurosteroids (Reddy, 2010), including pregnenolone and dehydroepiandrosterone, which have both been shown to modulate GlyRs (Maksay et al., 2001; Weir et al., 2004). Both cholesterol and neurosteroids have been shown to bind within the TM domains within cavities that overlap with ivermectin, alcohols, anesthetics, and cannabinoids (Henin et al., 2014; Laverty et al., 2017; Miller et al., 2017; Chen et al., 2018; Damgen and Biggin, 2021).

Similar to zinc, cholesterol has also been shown to impact the activity of other PAMs, in particular the cannabinoids THC, anandamide, and CBD (Yao et al., 2020). Cholesterol has been shown to alter the suspected cannabinoid binding cavity within the TM domains, which impacts cannabinoid antinociception in vivo (Yao et al., 2020). This could also impact the activity of other PAMs in vivo and should be taken into consideration when determining PAM activity. The membrane cholesterol content used for MD simulations can vary greatly and thus impact their results (Yao et al., 2020). Furthermore, the cholesterol content varies between commonly used cells lines such as *X. laevis* oocytes and HEK293 cells (Brannigan, 2017), which both contain less cholesterol than neuronal membranes (Ingólfsson et al., 2017). Cholesterol levels also vary greatly between individuals (Tharu and Tsokos, 2017), and this may be something to consider when developing GlyR mediated analgesics—in particular their use with commonly prescribed cholesterol-lowering drugs like simvastatin.

Recently, glucose has also been shown to modulate glycine receptors at physiologically relevant concentrations (Breitinger et al., 2015; Breitinger and Breitinger, 2016; Breitinger et al., 2016) and produce GlyR-mediated antinociception in rodent models of pain (Yamamoto et al., 2014; Hussein et al., 2019). As glucose is a required supplement for many mammalian-derived cell lines and is often present within cell media, it may be an underlying factor that causes discrepancies between functional studies of GlyRs. Studies conducted using HEK293 cells found that glucose was able to significantly shift the glycine dose response of both homomeric and heteromeric GlyRs and significantly reduced the variability between experiments (Breitinger et al., 2015; Breitinger and Breitinger, 2016). This is suggested to occur through irreversible glycation of GlyRs within the ECD and TM domains, proximal to the ivermectin binding cavity (Hussein et al., 2020).

Blood glucose levels in healthy individuals vary between 2.2 and 8 mM; however, they can increase to over 15 mM in diabetic patients (Oyibo et al., 2002; Petersmann et al., 2019). As there is a linear correlation between blood glucose levels and glucose concentration in the cerebrospinal fluid, even during hyperglycemia, this permits spinal concentrations to exceed 8 mM (Leen et al., 2012). Within these ranges, glucose can significantly modulate GlyRs (Breitinger et al., 2015; Breitinger and Breitinger, 2016) and likely impacts nociceptive signaling, especially for those with diabetic pain. Indeed, diabetic patients with painful neuropathy were found to have greater glucose flux compared with those without pain (Oyibo et al., 2002). Glucose has also been shown to modify descending inhibitory modulation of the spinal cord to tonically mediate nociceptive signaling (Terry et al., 2016) and can be triggered by nociceptive stimuli (Sim et al., 2012). This not only suggests that glucose can function as an endogenous mediator of nociception but may also be a confounding factor in several in vivo studies. If glucose concentrations are not maintained across test groups, this may have significant impacts on baseline nociception levels and on the ability to modulate glycine neurotransmission. Additionally, PAMs that are anticipated to produce antinociception by binding within the same cavity as glucose may be unable to bind and could explain the poor in vivo efficacy of many PAMs of GlyRs. Future research may benefit from testing PAMs in the presence of physiologically relevant glucose concentrations to ensure the likelihood of compounds retaining efficacy in endogenous conditions.

IV. Conclusion

Positive modulation of GlyRs has been shown to produce antinociceptive effects and may provide a promising avenue for the development of novel pain therapies. The findings summarized in this review outline the development of currently known PAMs of GlyRs and their ability to influence nociceptive signaling. Recent advances in structural techniques have also improved our understanding of the molecular mechanisms underpinning their modulation and have facilitated structure-based design of novel therapeutics. Future studies that explore the limitations of receptor selectivity and adverse effects associated with this approach are also required to ensure these novel therapeutics provide a safer and more effective solution compared with the current first-line pain medications.

Authorship Contributions

Wrote or contributed to the writing of the manuscript: Gallagher, Ha, Harvey, Vandenberg.

References

Adams IB, Compton DR, and Martin BR (1998) Assessment of anandamide interaction with the cannabinoid brain receptor: SR 141716A antagonism studies in mice and autoradiographic analysis of receptor binding in rat brain. *J Pharmacol Exp Ther* **284**:1209–1217.

Adams R, Pease DC, and Clark JH (1940) Isolation of Cannabinol, Cannabidiol and Quebrachitol from Red Oil of Minnesota Wild Hemp. *J Am Chem Soc* **62**:2194–2196.

Aguayo LG, Castro P, Mariqueo T, Muñoz B, Xiong W, Zhang L, Lovinger DM, and Homanics GE (2014) Altered sedative effects of ethanol in mice with α1 glycine receptor subunits that are insensitive to G$\beta\gamma$ modulation. *Neuropsychopharmacology* **39**:2538–2548.

Aguayo LG, van Zundert B, Tapia JC, Carrasco MA, and Alvarez FJ (2004) Changes on the properties of glycine receptors during neuronal development. *Brain Res Brain Res Rev* **47**:33–45.

Ahmadi S, Lippross S, Neuhuber WL, and Zeilhofer HU (2002) PGE(2) selectively blocks inhibitory glycinergic neurotransmission onto rat superficial dorsal horn neurons. *Nat Neurosci* **5**:34–40.

Ahrens J, Demir R, Leuwer M, de la Roche J, Krampfl K, Foadi N, Karst M, and Haeseler G (2009) The nonpsychotropic cannabinoid cannabidiol modulates and directly activates alpha-1 and alpha-1-Beta glycine receptor function. *Pharmacology* **83**:217–222.

Ahrens J, Leuwer M, Stachura S, Krampfl K, Belelli D, Lambert JJ, and Haeseler G (2008) A transmembrane residue influences the interaction of propofol with the strychnine-sensitive glycine alpha1 and alpha1beta receptor. *Anesth Analg* **107**:1875–1883.

Al-Futaisi AM, Al-Kindi MN, Al-Mawali AM, Koul RL, Al-Adawi S, and Al-Yahyaee SA (2012) Novel mutation of GLRA1 in Omani families with hyperekplexia and mild mental retardation. *Pediatr Neurol* **46**:89–93.

Althoff T, Hibbs RE, Banerjee S, and Gouaux E (2014) X-ray structures of GluCl in apo states reveal a gating mechanism of Cys-loop receptors. *Nature* **512**:333–337.

Alvarez LD and Pecci A (2019) Mapping the neurosteroid binding sites on glycine receptors. *J Steroid Biochem Mol Biol* **192**:105388.

Andreasen TJ and McNamee MG (1977) Phospholipase A inhibition of acetylcholine receptor function in Torpedo californica membrane vesicles. *Biochem Biophys Res Commun* **79**:958–965.

Antollini SS and Barrantes FJ (2016) Fatty Acid Regulation of Voltage- and Ligand-Gated Ion Channel Function. *Front Physiol* **7**:573.

Antollini SS, Xu Y, Jiang H, and Barrantes FJ (2005) Fluorescence and molecular dynamics studies of the acetylcholine receptor gammaM4 transmembrane peptide in reconstituted systems. *Mol Membr Biol* **22**:471–483.

Avaliani N, Andersson M, Runegaard AH, Woldbye D, and Kokaia M (2016) DREADDs suppress seizure-like activity in a mouse model of pharmacoresistant epileptic brain tissue. *Gene Ther* **23**:760–766.

Avila A, Nguyen L, and Rigo JM (2013) Glycine receptors and brain development. *Front Cell Neurosci* **7**:184.

Baenziger JE, Morris ML, Darsaut TE, and Ryan SE (2000) Effect of membrane lipid composition on the conformational equilibria of the nicotinic acetylcholine receptor. *J Biol Chem* **275**:777–784.

Baer K, Waldvogel HJ, During MJ, Snell RG, Faull RL, and Rees MI (2003) Association of gephyrin and glycine receptors in the human brainstem and spinal cord: an immunohistochemical analysis. *Neuroscience* **122**:773–784.

Baer K, Waldvogel HJ, Faull RL, and Rees MI (2009) Localization of glycine receptors in the human forebrain, brainstem, and cervical spinal cord: an immunohistochemical review. *Front Mol Neurosci* **2**:25.

Balansa W, Islam R, Fontaine F, Piggott AM, Zhang H, Webb TI, Gilbert DF, Lynch JW, and Capon RJ (2010) Ircinialactams: subunit-selective glycine receptor modulators from Australian sponges of the family Irciniidae. *Bioorg Med Chem* **18**:2912–2919.

Balansa W, Islam R, Fontaine F, Piggott AM, Zhang H, Xiao X, Webb TI, Gilbert DF, Lynch JW, and Capon RJ (2013a) Sesterterpene glycinyl-lactams: a new class of glycine receptor modulator from Australian marine sponges of the genus Psammocinia. *Org Biomol Chem* **11**:4695–4701.

Balansa W, Islam R, Gilbert DF, Fontaine F, Xiao X, Zhang H, Piggott AM, Lynch JW, and Capon RJ (2013b) Australian marine sponge alkaloids as a new class of glycine-gated chloride channel receptor modulator. *Bioorg Med Chem* **21**:4420–4425.

Baur R, Kielar M, Richter L, Ernst M, Ecker GF, and Sigel E (2013) Molecular analysis of the site for 2-arachidonylglycerol (2-AG) on the β_2 subunit of GABA(A) receptors. *J Neurochem* **126**:29–36.

Becker CM, Hoch W, and Betz H (1988) Glycine receptor heterogeneity in rat spinal cord during postnatal development. *EMBO J* **7**:3717–3726.

Benet LZ, Hosey CM, Ursu O, and Oprea TI (2016) BDDCS, the Rule of 5 and drugability. *Adv Drug Deliv Rev* **101**:89–98.

Birinyi A, Parker D, Antal D, and Shupliakov O (2001) Zinc co-localizes with GABA and glycine in synapses in the lamprey spinal cord. *J Comp Neurol* **433**:208–221.

Bloomenthal AB, Goldwater E, Pritchett DB, and Harrison NL (1994) Biphasic modulation of the strychnine-sensitive glycine receptor by Zn2+. *Mol Pharmacol* **46**:1156–1159.

Bocquet N, Nury H, Baaden M, Le Poupon C, Changeux JP, Delarue M, and Corringer PJ (2009) X-ray structure of a pentameric ligand-gated ion channel in an apparently open conformation. *Nature* **457**:111–114.

Bouchet CA and Ingram SL (2020) Cannabinoids in the descending pain modulatory circuit: Role in inflammation. *Pharmacol Ther* **209**:107495.

Brannigan G (2017) Direct Interactions of Cholesterol With Pentameric Ligand-Gated Ion Channels: Testable Hypotheses From Computational Predictions. *Curr Top Membr* **80**:163–186.

Bregman H, Simard JR, Andrews KL, Ayube S, Chen H, Gunaydin H, Guzman-Perez A, Hu J, Huang L, Huang X, et al. (2017) The Discovery and Hit-to-Lead Optimization of Tricyclic Sulfonamides as Potent and Efficacious Potentiators of Glycine Receptors. *J Med Chem* **60**:1105–1125.

Breitinger HG and Becker CM (2002) The inhibitory glycine receptor-simple views of a complicated channel. *ChemBioChem* **3**:1042–1052.

Breitinger HG, Villmann C, Melzer N, Rennert J, Breitinger U, Schwarzinger S, and Becker CM (2009) Novel regulatory site within the TM3-4 loop of human recombinant alpha3 glycine receptors determines channel gating and domain structure. *J Biol Chem* **284**:28624–28633.

Breitinger U and Breitinger HG (2016) Augmentation of glycine receptor alpha3 currents suggests a mechanism for glucose-mediated analgesia. *Neurosci Lett* **612**:110–115.

Breitinger U, Raafat KM, and Breitinger HG (2015) Glucose is a positive modulator for the activation of human recombinant glycine receptors. *J Neurochem* **134**:1055–1066.

Breitinger U, Sticht H, and Breitinger HG (2016) Modulation of Recombinant Human α1 Glycine Receptors by Mono- and Disaccharides: A Kinetic Study. *ACS Chem Neurosci* **7**:1077–1087.

Breitinger U, Weinländer K, Pechmann Y, Langlhofer G, Enz R, Becker CM, Sticht H, Kneussel M, Villmann C, and Breitinger HG (2020) A proline-rich motif in the large intracellular loop of the glycine receptor α1 subunit interacts with the Pleckstrin homology domain of collybistin. *J Adv Res* **29**:95–106.

Brinkmann V, Billich A, Baumruker T, Heining P, Schmouder R, Francis G, Aradhye S, and Burtin P (2010) Fingolimod (FTY720): discovery and development of an oral drug to treat multiple sclerosis. *Nat Rev Drug Discov* **9**:883–897.

Bruni N, Della Pepa C, Oliaro-Bosso S, Pessione E, Gastaldi D, and Dosio F (2018) Cannabinoid Delivery Systems for Pain and Inflammation Treatment. *Molecules* **23**:2478.

Buckwalter MS, Cook SA, Davisson MT, White WF, and Camper SA (1994) A frameshift mutation in the mouse alpha 1 glycine receptor gene (Glra1) results in progressive neurological symptoms and juvenile death. *Hum Mol Genet* **3**:2025–2030.

Burgos CF, Yévenes GE, and Aguayo LG (2016) Structure and Pharmacologic Modulation of Inhibitory Glycine Receptors. *Mol Pharmacol* **90**:318–325.

Burstein SH, Rossetti RG, Yagen B, and Zurier RB (2000) Oxidative metabolism of anandamide. *Prostaglandins Other Lipid Mediat* **61**:29–41.

Büttner C, Sadtler S, Leyendecker A, Laube B, Griffon N, Betz H, and Schmalzing G (2001) Ubiquitination precedes internalization and proteolytic cleavage of plasma membrane-bound glycine receptors. *J Biol Chem* **276**:42978–42985.

Campbell EJ and Marchant NJ (2018) The use of chemogenetics in behavioural neuroscience: receptor variants, targeting approaches and caveats. *Br J Pharmacol* **175**:994–1003.

Cantaut-Belarif Y, Antri M, Pizzarelli R, Colasse S, Vaccari I, Soares S, Renner M, Dallel R, Triller A, and Bessis A (2017) Microglia control the glycinergic but not the GABAergic synapses via prostaglandin E2 in the spinal cord. *J Cell Biol* **216**:2979–2989.

Capito ES, Lautenbacher S, Wolstein J, and Horn-Hofmann C (2020) Effects of oral alcohol administration on heat pain threshold and ratings of supra-threshold stimuli. *Scand J Pain* **20**:623–634.

Carswell CL, Sun J, and Baenziger JE (2015) Intramembrane aromatic interactions influence the lipid sensitivities of pentameric ligand-gated ion channels. *J Biol Chem* **290**:2496–2507.

Castro PA, Figueroa M, Yevenes GE, San Martin LS, and Aguayo LG (2012) The basic property of Lys385 is important for potentiation of the human α1 glycine receptor by ethanol. *J Pharmacol Exp Ther* **340**:339–349.

Cathcart K, Patel A, Dies H, Rheinstädter MC, and Fradin C (2015) Effect of Cholesterol on the Structure of a Five-Component Mitochondria-Like Phospholipid Membrane. *Membranes (Basel)* **5**:664–684.

Celentano JJ, Gibbs TT, and Farb DH (1988) Ethanol potentiates GABA- and glycine-induced chloride currents in chick spinal cord neurons. *Brain Res* **455**:377–380.

Cerdan AH, Sisquellas M, Pereira G, Barreto Gomes DE, Changeux JP, and Cecchini M (2020) The Glycine Receptor Allosteric Ligands Library (GRALL). *Bioinformatics* **36**:3379–3384.

Chakka N, Andrews KL, Berry LM, Bregman H, Gunaydin H, Huang L, Guzman-Perez A, Plant MH, Simard JR, Gingras J, et al. (2017) Applications of parallel synthetic lead hopping and pharmacophore-based virtual screening in the discovery of efficient glycine receptor potentiators. *Eur J Med Chem* **137**:63–75.

Chandler RE (2018) Serious Neurological Adverse Events after Ivermectin-Do They Occur beyond the Indication of Onchocerciasis? *Am J Trop Med Hyg* **98**:382–388.

Chen L, Effraim PR, Carrara J, Zhao P, Dib-Hajj FB, Dib-Hajj SD, and Waxman SG (2020) Pharmacological characterization of a rat Nav1.7 loss-of-function model with insensitivity to pain. *Pain* **161**:1350–1360.

Chen Q, Wells MM, Arjunan P, Tillman TS, Cohen AE, Xu Y, and Tang P (2018) Structural basis of neurosteroid anesthetic action on $GABA_A$ receptors. *Nat Commun* **9**:3972.

Chen SH, Tsai YJ, Wang HY, Lin CT, Li SF, and Lue JH (2012) Decreases of glycine receptor expression induced by median nerve injury in the rat cuneate nucleus contribute to NPY release and c-Fos expression. *Life Sci* **90**:278–288.

Chesnoy-Marchais D (1996) Potentiation of chloride responses to glycine by three 5-HT3 antagonists in rat spinal neurones. *Br J Pharmacol* **118**:2115–2125.

Chesnoy-Marchais D (1999) Mode of action of ICS 205,930, a novel type of potentiator of responses to glycine in rat spinal neurones. *Br J Pharmacol* **126**:801–809.

Chesnoy-Marchais D and Barthe JY (1996) Voltage-dependent block of NMDA responses by 5-HT agonists in ventral spinal cord neurones. *Br J Pharmacol* **117**:133–141.

Chesnoy-Marchais D, Lévi S, and Acher F (2000) Glycinergic potentiation by some 5-HT(3) receptor antagonists: insight into selectivity. *Eur J Pharmacol* **402**:205–213.

Cioffi CL (2018) Modulation of Glycine-Mediated Spinal Neurotransmission for the Treatment of Chronic Pain. *J Med Chem* **61**:2652–2679.
Cornelison GL, Daszkowski AW, Pflanz NC, and Mihic SJ (2017) Interactions between Zinc and Allosteric Modulators of the Glycine Receptor. *J Pharmacol Exp Ther* **361**:1–8.
Cornelison GL and Mihic SJ (2014) Contaminating levels of zinc found in commonly-used labware and buffers affect glycine receptor currents. *Brain Res Bull* **100**:1–5.
Crawford DK, Trudell JR, Bertaccini EJ, Li K, Davies DL, and Alkana RL (2007) Evidence that ethanol acts on a target in Loop 2 of the extracellular domain of alpha1 glycine receptors. *J Neurochem* **102**:2097–2109.
Criado M, Eibl H, and Barrantes FJ (1982) Effects of lipids on acetylcholine receptor. Essential need of cholesterol for maintenance of agonist-induced state transitions in lipid vesicles. *Biochemistry* **21**:3622–3629.
Criado M, Eibl H, and Barrantes FJ (1984) Functional properties of the acetylcholine receptor incorporated in model lipid membranes. Differential effects of chain length and head group of phospholipids on receptor affinity states and receptor-mediated ion translocation. *J Biol Chem* **259**:9188–9198.
Cronin JN, Bradbury EJ, and Lidierth M (2004) Laminar distribution of GABAA- and glycine-receptor mediated tonic inhibition in the dorsal horn of the rat lumbar spinal cord: effects of picrotoxin and strychnine on expression of Fos-like immunoreactivity. *Pain* **112**:156–163.
Csermely P, Agoston V, and Pongor S (2005) The efficiency of multi-target drugs: the network approach might help drug design. *Trends Pharmacol Sci* **26**:178–182.
D'hooghe M, Willekens B, Delvaux V, D'haeseleer M, Guillaume D, Laureys G, Nagels G, Vanderdonckt P, Van Pesch V, and Popescu V (2021) Sativex® (nabiximols) cannabinoid oromucosal spray in patients with resistant multiple sclerosis spasticity: the Belgian experience. *BMC Neurol* **21**:227.
daCosta CJ and Baenziger JE (2009) A lipid-dependent uncoupled conformation of the acetylcholine receptor. *J Biol Chem* **284**:17819–17825.
daCosta CJ, Medaglia SA, Lavigne N, Wang S, Carswell CL, and Baenziger JE (2009) Anionic lipids allosterically modulate multiple nicotinic acetylcholine receptor conformational equilibria. *J Biol Chem* **284**:33841–33849.
daCosta CJB, Dey L, Therien JPD, and Baenziger JE (2013) A distinct mechanism for activating uncoupled nicotinic acetylcholine receptors. *Nat Chem Biol* **9**:701–707.
Dämgen MA and Biggin PC (2021) State-dependent protein-lipid interactions of a pentameric ligand-gated ion channel in a neuronal membrane. *PLOS Comput Biol* **17**:e1007856.
Davies DL, Crawford DK, Trudell JR, Mihic SJ, and Alkana RL (2004) Multiple sites of ethanol action in alpha1 and alpha2 glycine receptors suggested by sensitivity to pressure antagonism. *J Neurochem* **89**:1175–1185.
Davies TQ, Tilby MJ, Skolc D, Hall A, and Willis MC (2020) Primary Sulfonamide Synthesis Using the Sulfinylamine Reagent *N*-Sulfinyl-*O*-(*tert*-butyl)hydroxylamine, *t*-BuONSO. *Org Lett* **22**:9495–9499.
Dawson GR, Wafford KA, Smith A, Marshall GR, Bayley PJ, Schaeffer JM, Meinke PT, and McKernan RM (2000) Anticonvulsant and adverse effects of avermectin analogs in mice are mediated through the gamma-aminobutyric acid(A) receptor. *J Pharmacol Exp Ther* **295**:1051–1060.
Devane WA, Hanuš L, Breuer A, Pertwee RG, Stevenson LA, Griffin G, Gibson D, Mandelbaum A, Etinger A, and Mechoulam R (1992) Isolation and structure of a brain constituent that binds to the cannabinoid receptor. *Science* **258**:1946–1949.
Devenish SO, Winters BL, Anderson LL, Arnold JC, McGregor IS, Vaughan CW, Chebib M, and Absalom NL (2021) The anticonvulsant zonisamide positively modulates recombinant and native glycine receptors at clinically relevant concentrations. *Neuropharmacology* **182**:108371.
Di Marzo V (2008) Endocannabinoids: synthesis and degradation. *Rev Physiol Biochem Pharmacol* **160**:1–24.
Di Marzo V and Fontana A (1995) Anandamide, an endogenous cannabinomimetic eicosanoid: 'killing two birds with one stone'. *Prostaglandins Leukot Essent Fatty Acids* **53**:1–11.
Diao XT, Yao L, Ma JJ, Zhang TY, Bai HH, Suo ZW, Yang X, and Hu XD (2020) Analgesic action of adenosine A1 receptor involves the dephosphorylation of glycine receptor $\alpha1^{ins}$ subunit in spinal dorsal horn of mice. *Neuropharmacology* **176**:108219.
Dib-Hajj SD, Geha P, and Waxman SG (2017) Sodium channels in pain disorders: pathophysiology and prospects for treatment. *Pain* **158** (Suppl 1):S97–S107.
Dohi T, Morita K, Kitayama T, Motoyama N, and Morioka N (2009) Glycine transporter inhibitors as a novel drug discovery strategy for neuropathic pain. *Pharmacol Ther* **123**:54–79.
Downie DL, Hall AC, Lieb WR, and Franks NP (1996) Effects of inhalational general anaesthetics on native glycine receptors in rat medullary neurones and recombinant glycine receptors in Xenopus oocytes. *Br J Pharmacol* **118**:493–502.
Drew GM, Lau BK, and Vaughan CW (2009) Substance P drives endocannabinoid-mediated disinhibition in a midbrain descending analgesic pathway. *J Neurosci* **29**:7220–7229.
Du J, Lü W, Wu S, Cheng Y, and Gouaux E (2015) Glycine receptor mechanism elucidated by electron cryo-microscopy. *Nature* **526**:224–229.
Dupre ML, Broyles JM, and Mihic SJ (2007) Effects of a mutation in the TM2-TM3 linker region of the glycine receptor alpha1 subunit on gating and allosteric modulation. *Brain Res* **1152**:1–9.
Dutertre S, Becker CM, and Betz H (2012) Inhibitory glycine receptors: an update. *J Biol Chem* **287**:40216–40223.
Dworkin RH, O'Connor AB, Audette J, Baron R, Gourlay GK, Haanpää ML, Kent JL, Krane EJ, Lebel AA, Levy RM, et al. (2010) Recommendations for the pharmacological management of neuropathic pain: an overview and literature update. *Mayo Clin Proc* **85**(3, Suppl)S3–S14.
Enna SJ and McCarson KE (2006) The role of GABA in the mediation and perception of pain. *Adv Pharmacol* **54**:1–27.
Field M, Dorovykh V, Thomas P, and Smart TG (2021) Physiological role for $GABA_A$ receptor desensitization in the induction of long-term potentiation at inhibitory synapses. *Nat Commun* **12**:2112.
Foadi N, Leuwer M, Demir R, Dengler R, Buchholz V, de la Roche J, Karst M, Haeseler G, and Ahrens J (2010) Lack of positive allosteric modulation of mutated alpha(1)S267I glycine receptors by cannabinoids. *Naunyn Schmiedebergs Arch Pharmacol* **381**:477–482.
Fong TM and McNamee MG (1986) Correlation between acetylcholine receptor function and structural properties of membranes. *Biochemistry* **25**:830–840.
Fourati Z, Howard RJ, Heusser SA, Hu H, Ruza RR, Sauguet L, Lindahl E, and Delarue M (2018) Structural Basis for a Bimodal Allosteric Mechanism of General Anesthetic Modulation in Pentameric Ligand-Gated Ion Channels. *Cell Rep* **23**:993–1004.
Fowler CJ (2013) Transport of endocannabinoids across the plasma membrane and within the cell. *FEBS J* **280**:1895–1904.
Frederickson CJ, Giblin LJ, Krezel A, McAdoo DJ, Mueller RN, Zeng Y, Balaji RV, Masalha R, Thompson RB, Fierke CA, et al. (2006) Concentrations of extracellular free zinc (pZn)e in the central nervous system during simple anesthetization, ischemia and reperfusion [published correction appears in *Exp Neurol* (2006) **202**:523]. *Exp Neurol* **198**:285–293.
Fride E and Mechoulam R (1993) Pharmacological activity of the cannabinoid receptor agonist, anandamide, a brain constituent. *Eur J Pharmacol* **231**:313–314.
Fritz LC, Wang CC, and Gorio A (1979) Avermectin B1a irreversibly blocks postsynaptic potentials at the lobster neuromuscular junction by reducing muscle membrane resistance. *Proc Natl Acad Sci USA* **76**:2062–2066.
Gallagher CI, Sheipouri D, Shimmon S, Rawling T, and Vandenberg RJ (2020) Identification of N-acyl amino acids that are positive allosteric modulators of glycine receptors. *Biochem Pharmacol* **180**:114117.
Gaoni Y and Mechoulam R (1964) Isolation, Structure, and Partial Synthesis of an Active Constituent of Hashish. *J Am Chem Soc* **86**:1646–1647.
Gatch MB (1999) Effects of benzodiazepines on acute and chronic ethanol-induced nociception in rats. *Alcohol Clin Exp Res* **23**:1736–1743.
Gatch MB and Lal H (1999) Effects of ethanol and ethanol withdrawal on nociception in rats. *Alcohol Clin Exp Res* **23**:328–333.
Gavva NR, Treanor JJ, Garami A, Fang L, Surapaneni S, Akrami A, Alvarez F, Bak A, Darling M, Gore A, et al. (2008) Pharmacological blockade of the vanilloid receptor TRPV1 elicits marked hyperthermia in humans. *Pain* **136**:202–210.
Gentet LJ and Clements JD (2002) Binding site stoichiometry and the effects of phosphorylation on human alpha1 homomeric glycine receptors. *J Physiol* **544**:97–106.
Giacoppo S, Bramanti P, and Mazzon E (2017) Sativex in the management of multiple sclerosis-related spasticity: An overview of the last decade of clinical evaluation. *Mult Scler Relat Disord* **17**:22–31.
Glaum SR, Proudfit HK, and Anderson EG (1990) 5-HT3 receptors modulate spinal nociceptive reflexes. *Brain Res* **510**:12–16.
Grenningloh G, Pribilla I, Prior P, Multhaup G, Beyreuther K, Taleb O, and Betz H (1990) Cloning and expression of the 58 kd beta subunit of the inhibitory glycine receptor. *Neuron* **4**:963–970.
Grewer C (1999) Investigation of the alpha(1)-glycine receptor channel-opening kinetics in the submillisecond time domain. *Biophys J* **77**:727–738.
Grudzinska J, Schemm R, Haeger S, Nicke A, Schmalzing G, Betz H, and Laube B (2005) The beta subunit determines the ligand binding properties of synaptic glycine receptors. *Neuron* **45**:727–739.
Gulbransen G, Xu W, and Arroll B (2020) Cannabidiol prescription in clinical practice: an audit on the first 400 patients in New Zealand. *BJGP Open* **4**:bjgpopen20X101010.
Haeger S, Kuzmin D, Detro-Dassen S, Lang N, Kilb M, Tsetlin V, Betz H, Laube B, and Schmalzing G (2010) An intramembrane aromatic network determines pentameric assembly of Cys-loop receptors. *Nat Struct Mol Biol* **17**:90–98.
Hales TG and Lambert JJ (1991) The actions of propofol on inhibitory amino acid receptors of bovine adrenomedullary chromaffin cells and rodent central neurones. *Br J Pharmacol* **104**:619–628.
Hameed S (2019) $Na_v1.7$ and $Na_v1.8$: Role in the pathophysiology of pain. *Mol Pain* **15**:1744806919858801.
Han L, Talwar S, Wang Q, Shan Q, and Lynch JW (2013) Phosphorylation of $\alpha3$ glycine receptors induces a conformational change in the glycine-binding site. *ACS Chem Neurosci* **4**:1361–1370.
Handford CA, Lynch JW, Baker E, Webb GC, Ford JH, Sutherland GR, and Schofield PR (1996) The human glycine receptor beta subunit: primary structure, functional characterisation and chromosomal localisation of the human and murine genes. *Brain Res Mol Brain Res* **35**:211–219.
Harpole TJ and Grosman C (2019) A Crucial Role for Side-Chain Conformation in the Versatile Charge Selectivity of Cys-Loop Receptors. *Biophys J* **116**:1667–1681.
Harvey RJ, Depner UB, Wässle H, Ahmadi S, Heindl C, Reinold H, Smart TG, Harvey K, Schütz B, Abo-Salem OM, et al. (2004) GlyR alpha3: an essential target for spinal PGE2-mediated inflammatory pain sensitization. *Science* **304**:884–887.
Harvey RJ, Schmieden V, Von Holst A, Laube B, Rohrer H, and Betz H (2000) Glycine receptors containing the alpha4 subunit in the embryonic sympathetic nervous system, spinal cord and male genital ridge. *Eur J Neurosci* **12**:994–1001.
Harvey RJ, Thomas P, James CH, Wilderspin A, and Smart TG (1999) Identification of an inhibitory Zn2+ binding site on the human glycine receptor alpha1 subunit. *J Physiol* **520**:53–64.
Haus U, Varga B, Stratz T, Späth M, and Müller W (2000) Oral treatment of fibromyalgia with tropisetron given over 28 days: influence on functional and vegetative symptoms, psychometric parameters and pain. *Scand J Rheumatol Suppl* **113**:55–58.
Hejazi N, Zhou C, Oz M, Sun H, Ye JH, and Zhang L (2006) Delta9-tetrahydrocannabinol and endogenous cannabinoid anandamide directly potentiate the function of glycine receptors. *Mol Pharmacol* **69**:991–997.
Hénault CM, Govaerts C, Spurny R, Brams M, Estrada-Mondragon A, Lynch J, Bertrand D, Pardon E, Evans GL, Woods K, et al. (2019) A lipid site shapes the agonist response of a pentameric ligand-gated ion channel. *Nat Chem Biol* **15**:1156–1164.
Hénault CM, Sun J, Therien JP, daCosta CJ, Carswell CL, Labriola JM, Juranka PF, and Baenziger JE (2015) The role of the M4 lipid-sensor in the folding,

trafficking, and allosteric modulation of nicotinic acetylcholine receptors. *Neuropharmacology* **96** (Pt B):157–168.
Henderson LA, Kotsirilos V, Cairns EA, Ramachandran A, Peck CC, and McGregor IS (2021) Medicinal cannabis in the treatment of chronic pain. *Aust J Gen Pract* **50**:724–732.
Hénin J, Salari R, Murlidaran S, and Brannigan G (2014) A predicted binding site for cholesterol on the GABAA receptor. *Biophys J* **106**:1938–1949.
Heusser SA, Howard RJ, Borghese CM, Cullins MA, Broemstrup T, Lee US, Lindahl E, Carlsson J, and Harris RA (2013) Functional validation of virtual screening for novel agents with general anesthetic action at ligand-gated ion channels. *Mol Pharmacol* **84**:670–678.
Hibbs RE and Gouaux E (2011) Principles of activation and permeation in an anion-selective Cys-loop receptor. *Nature* **474**:54–60.
Hillard CJ, Huang H, Vogt CD, Rodrigues BE, Neumann TS, Sem DS, Schroeder F, and Cunningham CW (2017) Endocannabinoid Transport Proteins: Discovery of Tools to Study Sterol Carrier Protein-2. *Methods Enzymol* **593**:99–121.
Hirzel K, Müller U, Latal AT, Hülsmann S, Grudzinska J, Seeliger MW, Betz H, and Laube B (2006) Hyperekplexia phenotype of glycine receptor alpha1 subunit mutant mice identifies Zn(2+) as an essential endogenous modulator of glycinergic neurotransmission. *Neuron* **52**:679–690.
Ho KY, Gwee KA, Cheng YK, Yoon KH, Hee HT, and Omar AR (2018) Nonsteroidal anti-inflammatory drugs in chronic pain: implications of new data for clinical practice. *J Pain Res* **11**:1937–1948.
Hoffmann T, Sharon O, Wittmann J, Carr RW, Vyshnevska A, Col R, Nassar MA, Reeh PW, and Weidner C (2018) NaV1.7 and pain: contribution of peripheral nerves. *Pain* **159**:496–506.
Hohmann AG, Suplita RL, Bolton NM, Neely MH, Fegley D, Mangieri R, Krey JF, Walker JM, Holmes PV, Crystal JD, et al. (2005) An endocannabinoid mechanism for stress-induced analgesia. *Nature* **435**:1108–1112.
Højsted J, Nielsen PR, Guldstrand SK, Frich L, and Sjøgren P (2010) Classification and identification of opioid addiction in chronic pain patients. *Eur J Pain* **14**:1014–1020.
Horikoshi T, Asanuma A, Yanagisawa K, Anzai K, and Goto S (1988) Taurine and beta-alanine act on both GABA and glycine receptors in Xenopus oocyte injected with mouse brain messenger RNA. *Brain Res* **464**:97–105.
Howard RJ, Murail S, Ondricek KE, Corringer PJ, Lindahl E, Trudell JR, and Harris RA (2011) Structural basis for alcohol modulation of a pentameric ligand-gated ion channel. *Proc Natl Acad Sci USA* **108**:12149–12154.
Hu H, Ataka K, Menny A, Fourati Z, Sauguet L, Corringer PJ, Koehl P, Heberle J, and Delarue M (2018) Electrostatics, proton sensor, and networks governing the gating transition in GLIC, a proton-gated pentameric ion channel. *Proc Natl Acad Sci USA* **115**:E12172–E12181.
Huang J, Zhang X, and McNaughton PA (2006) Modulation of temperature-sensitive TRP channels. *Semin Cell Dev Biol* **17**:638–645.
Huang R, He S, Chen Z, Dillon GH, and Leidenheimer NJ (2007) Mechanisms of homomeric alpha1 glycine receptor endocytosis. *Biochemistry* **46**:11484–11493.
Huang SM, Bisogno T, Petros TJ, Chang SY, Zavitsanos PA, Zipkin RE, Sivakumar R, Coop A, Maeda DY, De Petrocellis L, et al. (2001) Identification of a new class of molecules, the arachidonyl amino acids, and characterization of one member that inhibits pain. *J Biol Chem* **276**:42639–42644.
Huang X, Chen H, and Shaffer PL (2017a) Crystal Structures of Human GlyRα3 Bound to Ivermectin. *Structure* **25**:945–950.e2.
Huang X, Shaffer PL, Ayube S, Bregman H, Chen H, Lehto SG, Luther JA, Matson DJ, McDonough SI, Michelsen K, et al. (2017b) Crystal structures of human glycine receptor α3 bound to a novel class of analgesic potentiators. *Nat Struct Mol Biol* **24**:108–113.
Hunter SF, Bowen JD, and Reder AT (2016) The Direct Effects of Fingolimod in the Central Nervous System: Implications for Relapsing Multiple Sclerosis. *CNS Drugs* **30**:135–147.
Hussein RA, Ahmed M, Breitinger HG, and Breitinger U (2019) Modulation of Glycine Receptor-Mediated Pain Signaling *in vitro* and *in vivo* by Glucose. *Front Mol Neurosci* **12**:280.
Hussein RA, Ahmed M, Sticht H, Breitinger HG, and Breitinger U (2020) Fine-Tuning of Neuronal Ion Channels-Mapping of Residues Involved in Glucose Sensitivity of Recombinant Human Glycine Receptors. *ACS Chem Neurosci* **11**:3474–3483.
Imlach WL, Bhola RF, Mohammadi SA, and Christie MJ (2016) Glycinergic dysfunction in a subpopulation of dorsal horn interneurons in a rat model of neuropathic pain. *Sci Rep* **6**:37104.
Ingólfsson HI, Carpenter TS, Bhatia H, Bremer P-T, Marrink SJ, and Lightstone FC (2017) Computational Lipidomics of the Neuronal Plasma Membrane. *Biophys J* **113**:2271–2280.
Islam R, Zhang Y, Xu L, Sah P, and Lynch JW (2017) A Chemogenetic Receptor That Enhances the Magnitude and Frequency of Glycinergic Inhibitory Postsynaptic Currents without Inducing a Tonic Chloride Flux. *ACS Chem Neurosci* **8**:460–467.
Jaiteh M, Taly A, and Hénin J (2016) Evolution of Pentameric Ligand-Gated Ion Channels: Pro-Loop Receptors. *PLoS One* **11**:e0151934.
Jansson RK and Dybas RA (1998) Avermectins: Biochemical Mode of Action, Biological Activity and Agricultural Importance, in *Insecticides with Novel Modes of Action* (Ishaaya I and Degheele D, eds) pp 152–170, Springer, Berlin.
Jeong HJ, Jang IS, Moorhouse AJ, and Akaike N (2003) Activation of presynaptic glycine receptors facilitates glycine release from presynaptic terminals synapsing onto rat spinal sacral dorsal commissural nucleus neurons. *J Physiol* **550**:373–383.
Jeong HJ, Vandenberg RJ, and Vaughan CW (2010) N-arachidonyl-glycine modulates synaptic transmission in superficial dorsal horn. *Br J Pharmacol* **161**:925–935.
Jo SM, Danscher G, Schrøder HD, and Suh SW (2008) Depletion of vesicular zinc in dorsal horn of spinal cord causes increased neuropathic pain in mice. *Biometals* **21**:151–158.
Johnson JR, Lossignol D, Burnell-Nugent M, and Fallon MT (2013) An open-label extension study to investigate the long-term safety and tolerability of THC/CBD oromucosal spray and oromucosal THC spray in patients with terminal cancer-related pain refractory to strong opioid analgesics. *J Pain Symptom Manage* **46**:207–218.
Kash TL, Jenkins A, and Harrison NL (2003) Molecular volume determines the activity of the halogenated alkane bromoform at wild-type and mutant GABA(A) receptors. *Brain Res* **960**:36–41.
Kay AR (2004) Detecting and minimizing zinc contamination in physiological solutions. *BMC Physiol* **4**:4.
Keramidas A, Moorhouse AJ, Pierce KD, Schofield PR, and Barry PH (2002) Cation-selective mutations in the M2 domain of the inhibitory glycine receptor channel reveal determinants of ion-charge selectivity. *J Gen Physiol* **119**:393–410.
Kircik LH, Del Rosso JQ, Layton AM, and Schauber J (2016) Over 25 Years of Clinical Experience With Ivermectin: An Overview of Safety for an Increasing Number of Indications. *J Drugs Dermatol* **15**:325–332.
Kirsch J, Langosch D, Prior P, Littauer UZ, Schmitt B, and Betz H (1991) The 93-kDa glycine receptor-associated protein binds to tubulin. *J Biol Chem* **266**:22242–22245.
Kirson D, Cornelison GL, Philpo AE, Todorovic J, and Mihic SJ (2013) Physiological concentrations of zinc reduce taurine-activated GlyR responses to drugs of abuse. *Neuropharmacology* **75**:286–294.
Krasowski MD and Harrison NL (2000) The actions of ether, alcohol and alkane general anaesthetics on GABAA and glycine receptors and the effects of TM2 and TM3 mutations. *Br J Pharmacol* **129**:731–743.
Krůsek J and Zemková H (1994) Effect of ivermectin on gamma-aminobutyric acid-induced chloride currents in mouse hippocampal embryonic neurones. *Eur J Pharmacol* **259**:121–128.
Kukushkin ML and Igon'kina SI (2003) Role of 5-HT3 receptors in the mechanisms of central pain syndrome. *Bull Exp Biol Med* **135**:552–555.
Kumar A, Basak S, Rao S, Gicheru Y, Mayer ML, Sansom MSP, and Chakrapani S (2020) Mechanisms of activation and desensitization of full-length glycine receptor in lipid nanodiscs. *Nat Commun* **11**:3752.
Laurent B, Murail S, Shahsavar A, Sauguet L, Delarue M, and Baaden M (2016) Sites of Anesthetic Inhibitory Action on a Cationic Ligand-Gated Ion Channel. *Structure* **24**:595–605.
Laverty D, Desai R, Uchański T, Masiulis S, Stec WJ, Malinauskas T, Zivanov J, Pardon E, Steyaert J, Miller KW, et al. (2019) Cryo-EM structure of the human $\alpha1\beta3\gamma2$ $GABA_A$ receptor in a lipid bilayer. *Nature* **565**:516–520.
Laverty D, Thomas P, Field M, Andersen OJ, Gold MG, Biggin PC, Gielen M, and Smart TG (2017) Crystal structures of a $GABA_A$-receptor chimera reveal new endogenous neurosteroid-binding sites. *Nat Struct Mol Biol* **24**:977–985.
Leacock S, Syed P, James VM, Bode A, Kawakami K, Keramidas A, Suster M, Lynch JW, and Harvey RJ (2018) Structure/Function Studies of the α4 Subunit Reveal Evolutionary Loss of a GlyR Subtype Involved in Startle and Escape Responses. *Front Mol Neurosci* **11**:23.
Lee AG (2011) Lipid-protein interactions. *Biochem Soc Trans* **39**:761–766.
Leen WG, Willemsen MA, Wevers RA, and Verbeek MM (2012) Cerebrospinal fluid glucose and lactate: age-specific reference values and implications for clinical practice. *PLoS One* **7**:e42745.
Lieb A, Weston M, and Kullmann DM (2019) Designer receptor technology for the treatment of epilepsy. *EBioMedicine* **43**:641–649.
Liedhegner ES, Vogt CD, Sem DS, Cunningham CW, and Hillard CJ (2014) Sterol carrier protein-2: binding protein for endocannabinoids. *Mol Neurobiol* **50**:149–158.
Lipinski CA, Lombardo F, Dominy BW, and Feeney PJ (2001) Experimental and computational approaches to estimate solubility and permeability in drug discovery and development settings. *Adv Drug Deliv Rev* **46**:3–26.
Liu Q and Wong-Riley MT (2013) Postnatal development of glycine receptor subunits α1, α2, α3, and β immunoreactivity in multiple brain stem respiratory-related nuclear groups of the rat. *Brain Res* **1538**:1–16.
Liu Y, Mansoor TA, Hong J, Lee C-O, Sim CJ, Im KS, Kim ND, and Jung JH (2003) New cytotoxic sesterterpenoids and norsesterterpenoids from two sponges of the genus Sarcotragus. *J Nat Prod* **66**:1451–1456.
Lobo IA, Harris RA, and Trudell JR (2008) Cross-linking of sites involved with alcohol action between transmembrane segments 1 and 3 of the glycine receptor following activation. *J Neurochem* **104**:1649–1662.
Lozovaya N, Yatsenko N, Beketov A, Tsintsadze T, and Burnashev N (2005) Glycine receptors in CNS neurons as a target for nonretrograde action of cannabinoids. *J Neurosci* **25**:7499–7506.
Lu J, Fan S, Zou G, Hou Y, Pan T, Guo W, Yao L, Du F, Homanics GE, Liu D, et al. (2018) Involvement of glycine receptor α1 subunits in cannabinoid-induced analgesia. *Neuropharmacology* **133**:224–232.
Lynagh T and Lynch JW (2010) A glycine residue essential for high ivermectin sensitivity in Cys-loop ion channel receptors. *Int J Parasitol* **40**:1477–1481.
Lynagh T, Webb TI, Dixon CL, Cromer BA, and Lynch JW (2011) Molecular determinants of ivermectin sensitivity at the glycine receptor chloride channel. *J Biol Chem* **286**:43913–43924.
Lynch JW (2009) Native glycine receptor subtypes and their physiological roles. *Neuropharmacology* **56**:303–309.
Lynch JW, Jacques P, Pierce KD, and Schofield PR (1998) Zinc potentiation of the glycine receptor chloride channel is mediated by allosteric pathways. *J Neurochem* **71**:2159–2168.
Lynch JW, Zhang Y, Talwar S, and Estrada-Mondragon A (2017) Glycine Receptor Drug Discovery. *Adv Pharmacol* **79**:225–253.
Ma W, Chabot JG, Vercauteren F, and Quirion R (2010) Injured nerve-derived COX2/PGE2 contributes to the maintenance of neuropathic pain in aged rats. *Neurobiol Aging* **31**:1227–1237.
Ma W and Eisenach JC (2003) Four PGE2 EP receptors are up-regulated in injured nerve following partial sciatic nerve ligation. *Exp Neurol* **183**:581–592.
Ma W and Quirion R (2008) Does COX2-dependent PGE2 play a role in neuropathic pain? *Neurosci Lett* **437**:165–169.

Maione S, Costa B, and Di Marzo V (2013) Endocannabinoids: a unique opportunity to develop multitarget analgesics. *Pain* **154** (Suppl 1):S87–S93.
Maksay G (1998) Bidirectional allosteric modulation of strychnine-sensitive glycine receptors by tropeines and 5-HT3 serotonin receptor ligands. *Neuropharmacology* **37**:1633–1641.
Maksay G, Laube B, and Betz H (1999) Selective blocking effects of tropisetron and atropine on recombinant glycine receptors. *J Neurochem* **73**:802–806.
Maksay G, Laube B, and Betz H (2001) Subunit-specific modulation of glycine receptors by neurosteroids. *Neuropharmacology* **41**:369–376.
Maksay G, Laube B, Schemm R, Grudzinska J, Drwal M, and Betz H (2009a) Different binding modes of tropeines mediating inhibition and potentiation of alpha1 glycine receptors. *J Neurochem* **109**:1725–1732.
Maksay G, Nemes P, and Bíró T (2004) Synthesis of tropeines and allosteric modulation of ionotropic glycine receptors. *J Med Chem* **47**:6384–6391.
Maksay G, Vincze Z, and Nemes P (2009b) Synthesis of heteroaromatic tropeines and heterogeneous binding to glycine receptors. *Bioorg Med Chem* **17**:6872–6878.
Malosio ML, Grenningloh G, Kuhse J, Schmieden V, Schmitt B, Prior P, and Betz H (1991b) Alternative splicing generates two variants of the alpha 1 subunit of the inhibitory glycine receptor. *J Biol Chem* **266**:2048–2053.
Malosio ML, Marquèze-Pouey B, Kuhse J, and Betz H (1991a) Widespread expression of glycine receptor subunit mRNAs in the adult and developing rat brain. *EMBO J* **10**:2401–2409.
Mangin JM, Baloul M, Prado De Carvalho L, Rogister B, Rigo JM, and Legendre P (2003) Kinetic properties of the alpha2 homo-oligomeric glycine receptor impairs a proper synaptic functioning. *J Physiol* **553**:369–386.
Manzke T, Niebert M, Koch UR, Caley A, Vogelgesang S, Hülsmann S, Ponimaskin E, Müller U, Smart TG, Harvey RJ, et al. (2010) Serotonin receptor 1A-modulated phosphorylation of glycine receptor α3 controls breathing in mice. *J Clin Invest* **120**:4118–4128.
Markovic M, Ben-Shabat S, Aponick A, Zimmermann EM, and Dahan A (2020) Lipids and Lipid-Processing Pathways in Drug Delivery and Therapeutics. *Int J Mol Sci* **21**:3248.
Mascia MP, Machu TK, and Harris RA (1996b) Enhancement of homomeric glycine receptor function by long-chain alcohols and anaesthetics. *Br J Pharmacol* **119**: 1331–1336.
Mascia MP, Mihic SJ, Valenzuela CF, Schofield PR, and Harris RA (1996a) A single amino acid determines differences in ethanol actions on strychnine-sensitive glycine receptors. *Mol Pharmacol* **50**:402–406.
Mascia MP, Wick MJ, Martinez LD, and Harris RA (1998) Enhancement of glycine receptor function by ethanol: role of phosphorylation. *Br J Pharmacol* **125**:263–270.
Matsuda LA, Lolait SJ, Brownstein MJ, Young AC, and Bonner TI (1990) Structure of a cannabinoid receptor and functional expression of the cloned cDNA. *Nature* **346**:561–564.
McCracken LM, Blednov YA, Trudell JR, Benavidez JM, Betz H, and Harris RA (2013a) Mutation of a zinc-binding residue in the glycine receptor α1 subunit changes ethanol sensitivity in vitro and alcohol consumption in vivo. *J Pharmacol Exp Ther* **344**:489–500.
McCracken LM, Lowes DC, Salling MC, Carreau-Vollmer C, Odean NN, Blednov YA, Betz H, Harris RA, and Harrison NL (2017) Glycine receptor α3 and α2 subunits mediate tonic and exogenous agonist-induced currents in forebrain. *Proc Natl Acad Sci USA* **114**:E7179–E7186.
McCracken LM, Trudell JR, Goldstein BE, Harris RA, and Mihic SJ (2010) Zinc enhances ethanol modulation of the alpha1 glycine receptor. *Neuropharmacology* **58**:676–681.
McCracken LM, Trudell JR, McCracken ML, and Harris RA (2013b) Zinc-dependent modulation of α2- and α3-glycine receptor subunits by ethanol. *Alcohol Clin Exp Res* **37**:2002–2010.
McCracken ML, Gorini G, McCracken LM, Mayfield RD, Harris RA, and Trudell JR (2016) Inter- and Intra-Subunit Butanol/Isoflurane Sites of Action in the Human Glycine Receptor. *Front Mol Neurosci* **9**:45.
Mechoulam R, Ben-Shabat S, Hanus L, Ligumsky M, Kaminski NE, Schatz AR, Gopher A, Almog S, Martin BR, Compton DR, et al. (1995) Identification of an endogenous 2-monoglyceride, present in canine gut, that binds to cannabinoid receptors. *Biochem Pharmacol* **50**:83–90.
Ménez C, Sutra J-F, Prichard R, and Lespine A (2012) Relative neurotoxicity of ivermectin and moxidectin in Mdr1ab (-/-) mice and effects on mammalian GABA(A) channel activity. *PLoS Negl Trop Dis* **6**:e1883.
Meyer G, Kirsch J, Betz H, and Langosch D (1995) Identification of a gephyrin binding motif on the glycine receptor beta subunit. *Neuron* **15**:563–572.
Michael B, Meinke PT, and Shoop W (2001) Comparison of ivermectin, doramectin, selamectin, and eleven intermediates in a nematode larval development assay. *J Parasitol* **87**:692–696.
Mihic SJ, Ye Q, Wick MJ, Koltchine VV, Krasowski MD, Finn SE, Mascia MP, Valenzuela CF, Hanson KK, Greenblatt EP, et al. (1997) Sites of alcohol and volatile anaesthetic action on GABA(A) and glycine receptors. *Nature* **389**:385–389.
Miller PS, Da Silva HM, and Smart TG (2005) Molecular basis for zinc potentiation at strychnine-sensitive glycine receptors. *J Biol Chem* **280**:37877–37884.
Miller PS, Scott S, Masiulis S, De Colibus L, Pardon E, Steyaert J, and Aricescu AR (2017) Structural basis for $GABA_A$ receptor potentiation by neurosteroids. *Nat Struct Mol Biol* **24**:986–992.
Miraucourt LS, Dallel R, and Voisin DL (2007) Glycine inhibitory dysfunction turns touch into pain through PKCgamma interneurons. *PLoS One* **2**:e1116.
Mitchell RW and Hatch GM (2011) Fatty acid transport into the brain: of fatty acid fables and lipid tails. *Prostaglandins Leukot Essent Fatty Acids* **85**:293–302 PLEFA.
Mitchell RW, On NH, Del Bigio MR, Miller DW, and Hatch GM (2011) Fatty acid transport protein expression in human brain and potential role in fatty acid transport across human brain microvessel endothelial cells. *J Neurochem* **117**:735–746.
Moraga-Cid G, San Martín VP, Lara CO, Muñoz B, Marileo AM, Sazo A, Muñoz-Montesino C, Fuentealba J, Castro PA, Guzmán L, et al. (2020) Modulation of glycine receptor single-channel conductance by intracellular phosphorylation. *Sci Rep* **10**:4804.
Moraga-Cid G, Sauguet L, Huon C, Malherbe L, Girard-Blanc C, Petres S, Murail S, Taly A, Baaden M, Delarue M, et al. (2015) Allosteric and hyperekplexic mutant phenotypes investigated on an α1 glycine receptor transmembrane structure. *Proc Natl Acad Sci USA* **112**:2865–2870.
Mostyn SN, Carland JE, Shimmon S, Ryan RM, Rawling T, and Vandenberg RJ (2017) Synthesis and Characterization of Novel Acyl-Glycine Inhibitors of GlyT2. *ACS Chem Neurosci* **8**:1949–1959.
Mostyn SN, Rawling T, Mohammadi S, Shimmon S, Frangos ZJ, Sarker S, Yousuf A, Vetter I, Ryan RM, Christie MJ, et al. (2019a) Development of an N-Acyl Amino Acid That Selectively Inhibits the Glycine Transporter 2 To Produce Analgesia in a Rat Model of Chronic Pain. *J Med Chem* **62**:2466–2484.
Mostyn SN, Wilson KA, Schumann-Gillett A, Frangos ZJ, Shimmon S, Rawling T, Ryan RM, O'Mara ML, and Vandenberg RJ (2019b) Identification of an allosteric binding site on the human glycine transporter, GlyT2, for bioactive lipid analgesics. *eLife* **8**:e47150.
Moulin D, Boulanger A, Clark AJ, Clarke H, Dao T, Finley GA, Furlan A, Gilron I, Gordon A, Morley-Forster PK, et al.; Canadian Pain Society (2014) Pharmacological management of chronic neuropathic pain: revised consensus statement from the Canadian Pain Society. *Pain Res Manag* **19**:328–335.
Mujumdar P and Poulsen SA (2015) Natural Product Primary Sulfonamides and Primary Sulfamates. *J Nat Prod* **78**:1470–1477.
Muñoz B, Gallegos S, Peters C, Murath P, Lovinger DM, Homanics GE, and Aguayo LG (2020) Influence of nonsynaptic α1 glycine receptors on ethanol consumption and place preference. *Addict Biol* **25**:e12726.
Munro S, Thomas KL, and Abu-Shaar M (1993) Molecular characterization of a peripheral receptor for cannabinoids. *Nature* **365**:61–65.
Murail S, Wallner B, Trudell JR, Bertaccini E, and Lindahl E (2011) Microsecond simulations indicate that ethanol binds between subunits and could stabilize an open-state model of a glycine receptor. *Biophys J* **100**:1642–1650.
Naito A, Muchhala KH, Asatryan L, Trudell JR, Homanics GE, Perkins DI, Davies DL, and Alkana RL (2014) Glycine and GABA(A) ultra-sensitive ethanol receptors as novel tools for alcohol and brain research. *Mol Pharmacol* **86**:635–646.
Naito A, Muchhala KH, Trang J, Asatryan L, Trudell JR, Homanics GE, Alkana RL, and Davies DL (2015) Manipulations of extracellular Loop 2 in α1 GlyR ultra-sensitive ethanol receptors (USERs) enhance receptor sensitivity to isoflurane, ethanol, and lidocaine, but not propofol. *Neuroscience* **297**:68–77.
Nasirinezhad F, Hosseini M, Karami Z, Yousefifard M, and Janzadeh A (2016) Spinal 5-HT3 receptor mediates nociceptive effect on central neuropathic pain; possible therapeutic role for tropisetron. *J Spinal Cord Med* **39**:212–219.
Nikolic Z, Laube B, Weber RG, Lichter P, Kioschis P, Poustka A, Mülhardt C, and Becker CM (1998) The human glycine receptor subunit α3. Glra3 gene structure, chromosomal localization, and functional characterization of alternative transcripts. *J Biol Chem* **273**:19708–19714.
Nurmikko TJ, Serpell MG, Hoggart B, Toomey PJ, Morlion BJ, and Haines D (2007) Sativex successfully treats neuropathic pain characterised by allodynia: a randomised, double-blind, placebo-controlled clinical trial. *Pain* **133**:210–220.
Nury H, Van Renterghem C, Weng Y, Tran A, Baaden M, Dufresne V, Changeux JP, Sonner JM, Delarue M, and Corringer PJ (2011) X-ray structures of general anaesthetics bound to a pentameric ligand-gated ion channel. *Nature* **469**:428–431.
Ochoa EL, Dalziel AW, and McNamee MG (1983) Reconstitution of acetylcholine receptor function in lipid vesicles of defined composition. *Biochim Biophys Acta* **727**:151–162.
Omura S and Crump A (2014) Ivermectin: panacea for resource-poor communities? *Trends Parasitol* **30**:445–455.
Oyibo SO, Prasad YDM, Jackson NJ, Jude EB, and Boulton AJM (2002) The relationship between blood glucose excursions and painful diabetic peripheral neuropathy: a pilot study. *Diabet Med* **19**:870–873.
Pang MH, Kim Y, Jung KW, Cho S, and Lee DH (2012) A series of case studies: practical methodology for identifying antinociceptive multi-target drugs. *Drug Discov Today* **17**:425–434.
Papadopoulos IA, Georgiou PE, Katsimbri PP, and Drosos AA (2000) Treatment of fibromyalgia with tropisetron, a 5HT3 serotonin antagonist: a pilot study. *Clin Rheumatol* **19**:6–8.
Pedersen TR and Tobert JA (2004) Simvastatin: a review. *Expert Opin Pharmacother* **5**:2583–2596.
Perez-Rosello T, Anderson CT, Ling C, Lippard SJ, and Tzounopoulos T (2015) Tonic zinc inhibits spontaneous firing in dorsal cochlear nucleus principal neurons by enhancing glycinergic neurotransmission. *Neurobiol Dis* **81**:14–19.
Perillo VL, Fernández-Nievas GA, Vallés AS, Barrantes FJ, and Antollini SS (2012) The position of the double bond in monounsaturated free fatty acids is essential for the inhibition of the nicotinic acetylcholine receptor. *Biochim Biophys Acta* **1818**:2511–2520.
Perillo VL, Peñalva DA, Vitale AJ, Barrantes FJ, and Antollini SS (2016) Transbilayer asymmetry and sphingomyelin composition modulate the preferential membrane partitioning of the nicotinic acetylcholine receptor in Lo domains. *Arch Biochem Biophys* **591**:76–86.
Perkins DI, Trudell JR, Crawford DK, Alkana RL, and Davies DL (2008) Targets for ethanol action and antagonism in loop 2 of the extracellular domain of glycine receptors. *J Neurochem* **106**:1337–1349.
Petersmann A, Müller-Wieland D, Müller UA, Landgraf R, Nauck M, Freckmann G, Heinemann L, and Schleicher E (2019) Definition, Classification and Diagnosis of Diabetes Mellitus. *Exp Clin Endocrinol Diabetes* **127** (S 01):S1–S7.
Petrosino S, Palazzo E, de Novellis V, Bisogno T, Rossi F, Maione S, and Di Marzo V (2007) Changes in spinal and supraspinal endocannabinoid levels in neuropathic rats. *Neuropharmacology* **52**:415–422.

Pienaar IS, Gartside SE, Sharma P, De Paola V, Gretenkord S, Withers D, Elson JL, and Dexter DT (2015) Pharmacogenetic stimulation of cholinergic pedunculopontine neurons reverses motor deficits in a rat model of Parkinson's disease. *Mol Neurodegener* **10**:47.
Pifferi F, Laurent B, and Plourde M (2021) Lipid Transport and Metabolism at the Blood-Brain Interface: Implications in Health and Disease. *Front Physiol* **12**:645646.
Piscitelli F and Di Marzo V (2021) Cannabinoids: a class of unique natural products with unique pharmacology. *Rend Lincei Sci Fis Nat* **32**:5–15.
Porter CJH, Trevaskis NL, and Charman WN (2007) Lipids and lipid-based formulations: optimizing the oral delivery of lipophilic drugs. *Nat Rev Drug Discov* **6**:231–248.
Radford RJ and Lippard SJ (2013) Chelators for investigating zinc metalloneurochemistry. *Curr Opin Chem Biol* **17**:129–136.
Ramos Silva M, Paixão JA, Matos Beja A, and Alte da Veiga L (2001) Conformational flexibility of tricine as a chelating agent in catena-poly-[[(tricinato)copper(II)]-mu-chloro]. *Acta Crystallogr C* **57**:9–11.
Reddy DS (2010) Neurosteroids: endogenous role in the human brain and therapeutic potentials. *Prog Brain Res* **186**:113–137.
San Martin L, Cerda F, Jimenez V, Fuentealba J, Muñoz B, Aguayo LG, and Guzman L (2012) Inhibition of the ethanol-induced potentiation of α1 glycine receptor by a small peptide that interferes with G$\beta\gamma$ binding. *J Biol Chem* **287**:40713–40721.
San Martín VP, Burgos CF, Marileo AM, Lara CO, Sazo A, Fuentealba J, Guzmán L, Castro PA, Aguayo LG, Moraga-Cid G, et al. (2019) Inhibitory Actions of Tropeines on the α3 Glycine Receptor Function. *Front Pharmacol* **10**:331.
San Martín VP, Sazo A, Utreras E, Moraga-Cid G, and Yévenes GE (2022) Glycine Receptor Subtypes and Their Roles in Nociception and Chronic Pain. *Front Mol Neurosci* **15**:848642.
Sánchez A, Yévenes GE, San Martin L, Burgos CF, Moraga-Cid G, Harvey RJ, and Aguayo LG (2015) Control of ethanol sensitivity of the glycine receptor α3 subunit by transmembrane 2, the intracellular splice cassette and C-terminal domains. *J Pharmacol Exp Ther* **353**:80–90.
Sauguet L, Howard RJ, Malherbe L, Lee US, Corringer P-J, Harris RA, and Delarue M (2013) Structural basis for potentiation by alcohols and anaesthetics in a ligand-gated ion channel. *Nat Commun* **4**:1697.
Scapecchi S, Giorgi A, Bellucci C, Dei S, Ghelardini C, Manetti D, Romanelli MN, and Teodori E (1998) Further structure-activity relationships in the series of tropanyl esters endowed with potent antinociceptive activity. *Farmaco* **53**:764–772.
Schmid K, Böhmer G, and Gebauer K (1991) Glycine receptor-mediated fast synaptic inhibition in the brainstem respiratory system. *Respir Physiol* **84**:351–361.
Schumann-Gillett A and O'Mara ML (2019) The effects of oxidised phospholipids and cholesterol on the biophysical properties of POPC bilayers. *Biochim Biophys Acta Biomembr* **1861**:210–219.
Shaheed CA, Machado GC, and Underwood M (2020) Drugs for chronic pain. *Br J Gen Pract* **70**:576–577.
Shan Q, Haddrill JL, and Lynch JW (2001) Ivermectin, an unconventional agonist of the glycine receptor chloride channel. *J Biol Chem* **276**:12556–12564.
Sheipouri D, Gallagher CI, Shimmon S, Rawling T, and Vandenberg RJ (2020) A System for Assessing Dual Action Modulators of Glycine Transporters and Glycine Receptors. *Biomolecules* **10**:1618.
Shin J, Rho JR, Seo Y, Lee H-S, Cho KW, and Sim CJ (2001) Sarcotragins A and B, new sesterterpenoid alkaloids from the sponge Sarcotragus sp. *Tetrahedron Lett* **42**:3005–3007.
Shumilla JA, Liron T, Mochly-Rosen D, Kendig JJ, and Sweitzer SM (2005) Ethanol withdrawal-associated allodynia and hyperalgesia: age-dependent regulation by protein kinase C epsilon and gamma isoenzymes. *J Pain* **6**:535–549.
Sim Y-B, Park S-H, Kang Y-J, Jung J-S, Ryu O-H, Choi M-G, and Suh H-W (2012) Various pain stimulations cause an increase of the blood glucose level. *Anim Cells Syst* **16**:385–390.
Simpson RK and Huang W (1998) Glycine receptor reduction within segmental gray matter in a rat model in neuropathic pain. *Neurol Res* **20**:161–168.
Simpson K, Spencer CM, and McClellan KJ (2000) Tropisetron: an update of its use in the prevention of chemotherapy-induced nausea and vomiting. *Drugs* **59**:1297–1315.
Simpson Jr RK, Gondo M, Robertson CS, and Goodman JC (1996) Reduction in the mechanonociceptive response by intrathecal administration of glycine and related compounds. *Neurochem Res* **21**:1221–1226.
Smith PB, Compton DR, Welch SP, Razdan RK, Mechoulam R, and Martin BR (1994) The pharmacological activity of anandamide, a putative endogenous cannabinoid, in mice. *J Pharmacol Exp Ther* **270**:219–227.
Soh MS, Estrada-Mondragon A, Durisic N, Keramidas A, and Lynch JW (2017) Probing the Structural Mechanism of Partial Agonism in Glycine Receptors Using the Fluorescent Artificial Amino Acid, ANAP. *ACS Chem Biol* **12**:805–813.
Sooksawate T and Simmonds MA (2001) Effects of membrane cholesterol on the sensitivity of the GABA(A) receptor to GABA in acutely dissociated rat hippocampal neurones. *Neuropharmacology* **40**:178–184.
Specht CG, Grünewald N, Pascual O, Rostgaard N, Schwarz G, and Triller A (2011) Regulation of glycine receptor diffusion properties and gephyrin interactions by protein kinase C. *EMBO J* **30**:3842–3853.
Stead C, Brown A, Adams C, Nickolls SJ, Young G, Kammonen J, Pryde D, and Cawkill D (2016) Identification of Positive Allosteric Modulators of Glycine Receptors from a High-Throughput Screen Using a Fluorescent Membrane Potential Assay. *J Biomol Screen* **21**:1042–1053.
Stratz T and Müller W (2004) Treatment of chronic low back pain with tropisetron. *Scand J Rheumatol Suppl* **119**:76–78.
Stratz T and Müller W (2000) The use of 5-HT3 receptor antagonists in various rheumatic diseases–a clue to the mechanism of action of these agents in fibromyalgia? *Scand J Rheumatol Suppl* **113**:66–71.
Subczynski WK, Pasenkiewicz-Gierula M, Widomska J, Mainali L, and Raguz M (2017) High Cholesterol/Low Cholesterol: Effects in Biological Membranes: A Review. *Cell Biochem Biophys* **75**:369–385.
Succar R, Mitchell VA, and Vaughan CW (2007) Actions of N-arachidonyl-glycine in a rat inflammatory pain model. *Mol Pain* **3**:24.
Sunshine C and McNamee MG (1994) Lipid modulation of nicotinic acetylcholine receptor function: the role of membrane lipid composition and fluidity. *Biochim Biophys Acta* **1191**:59–64.
Supplisson S and Chesnoy-Marchais D (2000) Glycine receptor beta subunits play a critical role in potentiation of glycine responses by ICS-205,930. *Mol Pharmacol* **58**:763–770.
Szallasi A, Cruz F, and Geppetti P (2006) TRPV1: a therapeutic target for novel analgesic drugs? *Trends Mol Med* **12**:545–554.
Tanabe M, Takasu K, Yamaguchi S, Kodama D, and Ono H (2008) Glycine transporter inhibitors as a potential therapeutic strategy for chronic pain with memory impairment. *Anesthesiology* **108**:929–937.
Tang B and Lummis SCR (2018) The roles of aromatic residues in the glycine receptor transmembrane domain. *BMC Neurosci* **19**:53.
Terry EL, Güereca YM, Martin SL, and Rhudy JL (2016) Is blood glucose associated with descending modulation of spinal nociception as measured by the nociceptive flexion reflex? *J Pain Res* **9**:187–193.
Tharu BP and Tsokos CP (2017) A Statistical Study of Serum Cholesterol Level by Gender and Race. *J Res Health Sci* **17**:e00386.
Thompson AJ and Lummis SC (2006) 5-HT3 receptors. *Curr Pharm Des* **12**:3615–3630.
Thompson MJ and Baenziger JE (2020) Structural basis for the modulation of pentameric ligand-gated ion channel function by lipids. *Biochim Biophys Acta Biomembr* **1862**:183304.
Thompson T, Oram C, Correll CU, Tsermentseli S, and Stubbs B (2017) Analgesic Effects of Alcohol: A Systematic Review and Meta-Analysis of Controlled Experimental Studies in Healthy Participants. *J Pain* **18**:499–510.
Tong A, Petroff 2nd JT, Hsu FF, Schmidpeter PA, Nimigean CM, Sharp L, Brannigan G, and Cheng WW (2019) Direct binding of phosphatidylglycerol at specific sites modulates desensitization of a ligand-gated ion channel. *eLife* **8**:e50766.
Trattnig SM, Gasiorek A, Deeb TZ, Ortiz EJ, Moss SJ, Jensen AA, and Davies PA (2016) Copper and protons directly activate the zinc-activated channel. *Biochem Pharmacol* **103**:109–117.
Trombley PQ, Blakemore LJ, and Hill BJ (2011) Zinc modulation of glycine receptors. *Neuroscience* **186**:32–38.
Urits I, Gress K, Charipova K, Habib K, Lee D, Lee C, Jung JW, Kassem H, Cornett E, Paladini A, et al. (2020) Use of cannabidiol (CBD) for the treatment of chronic pain. *Best Pract Res Clin Anaesthesiol* **34**:463–477.
Vandenberg RJ, Handford CA, and Schofield PR (1992) Distinct agonist- and antagonist-binding sites on the glycine receptor. *Neuron* **9**:491–496.
Vandenberg RJ, Ryan RM, Carland JE, Imlach WL, and Christie MJ (2014) Glycine transport inhibitors for the treatment of pain. *Trends Pharmacol Sci* **35**:423–430.
Vassilatis DK, Arena JP, Plasterk RH, Wilkinson HA, Schaeffer JM, Cully DF, and Van der Ploeg LH (1997a) Genetic and biochemical evidence for a novel avermectin-sensitive chloride channel in Caenorhabditis elegans. Isolation and characterization. *J Biol Chem* **272**:33167–33174.
Vassilatis DK, Elliston KO, Paress PS, Hamelin M, Arena JP, Schaeffer JM, Van der Ploeg LH, and Cully DF (1997b) Evolutionary relationship of the ligand-gated ion channels and the avermectin-sensitive, glutamate-gated chloride channels. *J Mol Evol* **44**:501–508.
Velázquez-Flores MA and Salceda R (2011) Glycine receptor internalization by protein kinases activation. *Synapse* **65**:1231–1238.
Vuong LA, Mitchell VA, and Vaughan CW (2008) Actions of N-arachidonyl-glycine in a rat neuropathic pain model. *Neuropharmacology* **54**:189–193.
Wang HC, Cheng KI, Chen PR, Tseng KY, Kwan AL, and Chang LL (2018) Glycine receptors expression in rat spinal cord and dorsal root ganglion in prostaglandin E2 intrathecal injection models. *BMC Neurosci* **19**:72.
Wang N, Song J, Jang KH, Lee H-S, Li X, Oh K-B, and Shin J (2008) Sesterterpenoids from the sponge Sarcotragus sp. *J Nat Prod* **71**:551–557.
Wang Q and Lynch JW (2011) Activation and desensitization induce distinct conformational changes at the extracellular-transmembrane domain interface of the glycine receptor. *J Biol Chem* **286**:38814–38824.
Wang Q and Lynch JW (2012) A comparison of glycine- and ivermectin-mediated conformational changes in the glycine receptor ligand-binding domain. *Int J Biochem Cell Biol* **44**:335–340.
Weir CJ, Ling AT, Belelli D, Wildsmith JA, Peters JA, and Lambert JJ (2004) The interaction of anaesthetic steroids with recombinant glycine and GABAA receptors. *Br J Anaesth* **92**:704–711.
Weir GA, Middleton SJ, Clark AJ, Daniel T, Khovanov N, McMahon SB, and Bennett DL (2017) Using an engineered glutamate-gated chloride channel to silence sensory neurons and treat neuropathic pain at the source. *Brain* **140**:2570–2585.
Wells MM, Tillman TS, Mowrey DD, Sun T, Xu Y, and Tang P (2015) Ensemble-based virtual screening for cannabinoid-like potentiators of the human glycine receptor α1 for the treatment of pain. *J Med Chem* **58**:2958–2966.
Weltzien F, Puller C, O'Sullivan GA, Paarmann I, and Betz H (2012) Distribution of the glycine receptor β-subunit in the mouse CNS as revealed by a novel monoclonal antibody. *J Comp Neurol* **520**:3962–3981.
Wick MJ, Mihic SJ, Ueno S, Mascia MP, Trudell JR, Brozowski SJ, Ye Q, Harrison NL, and Harris RA (1998) Mutations of gamma-aminobutyric acid and glycine receptors change alcohol cutoff: evidence for an alcohol receptor? *Proc Natl Acad Sci USA* **95**:6504–6509.
Witt MR, Westh-Hansen SE, Rasmussen PB, Hastrup S, and Nielsen M (1996) Unsaturated free fatty acids increase benzodiazepine receptor agonist binding depending on the subunit composition of the GABAA receptor complex. *J Neurochem* **67**:2141–2145.
Xiong W, Cheng K, Cui T, Godlewski G, Rice KC, Xu Y, and Zhang L (2011) Cannabinoid potentiation of glycine receptors contributes to cannabis-induced analgesia. *Nat Chem Biol* **7**:296–303.

Xiong W, Cui T, Cheng K, Yang F, Chen SR, Willenbring D, Guan Y, Pan HL, Ren K, Xu Y, et al. (2012a) Cannabinoids suppress inflammatory and neuropathic pain by targeting α3 glycine receptors. *J Exp Med* **209**:1121–1134.

Xiong W, Wu X, Li F, Cheng K, Rice KC, Lovinger DM, and Zhang L (2012b) A common molecular basis for exogenous and endogenous cannabinoid potentiation of glycine receptors [published correction appears in *J Neurosci* (2012) **32**:12979]. *J Neurosci* **32**:5200–5208.

Yaksh TL (1989) Behavioral and autonomic correlates of the tactile evoked allodynia produced by spinal glycine inhibition: effects of modulatory receptor systems and excitatory amino acid antagonists. *Pain* **37**:111–123.

Yamamoto RT, Foulds-Mathes W, and Kanarek RB (2014) Antinociceptive actions of peripheral glucose administration. *Pharmacol Biochem Behav* **117**:34–39.

Yang CC (2012) Acute human toxicity of macrocyclic lactones. *Curr Pharm Biotechnol* **13**:999–1003.

Yang Z, Aubrey KR, Alroy I, Harvey RJ, Vandenberg RJ, and Lynch JW (2008) Subunit-specific modulation of glycine receptors by cannabinoids and N-arachidonyl-glycine. *Biochem Pharmacol* **76**:1014–1023.

Yang Z, Ney A, Cromer BA, Ng HL, Parker MW, and Lynch JW (2007) Tropisetron modulation of the glycine receptor: femtomolar potentiation and a molecular determinant of inhibition. *J Neurochem* **100**:758–769.

Yao L, Liu C, Wang N, Du F, Fan S, Guo Y, Zhang L, Pan Y, and Xiong W (2020) Cholesterol regulates cannabinoid analgesia through glycine receptors. *Neuropharmacology* **177**:108242.

Yao L, Wells M, Wu X, Xu Y, Zhang L, and Xiong W (2020) Membrane cholesterol dependence of cannabinoid modulation of glycine receptor. *FASEB J* **34**:10920–10930.

Ye Q, Koltchine VV, Mihic SJ, Mascia MP, Wick MJ, Finn SE, Harrison NL, and Harris RA (1998) Enhancement of glycine receptor function by ethanol is inversely correlated with molecular volume at position alpha267. *J Biol Chem* **273**:3314–3319.

Yevenes GE, Moraga-Cid G, Avila A, Guzmán L, Figueroa M, Peoples RW, and Aguayo LG (2010) Molecular requirements for ethanol differential allosteric modulation of glycine receptors based on selective Gbetagamma modulation. *J Biol Chem* **285**:30203–30213.

Yevenes GE, Moraga-Cid G, Guzmán L, Haeger S, Oliveira L, Olate J, Schmalzing G, and Aguayo LG (2006) Molecular determinants for G protein betagamma modulation of ionotropic glycine receptors. *J Biol Chem* **281**:39300–39307.

Yevenes GE, Moraga-Cid G, Peoples RW, Schmalzing G, and Aguayo LG (2008) A selective G betagamma-linked intracellular mechanism for modulation of a ligand-gated ion channel by ethanol. *Proc Natl Acad Sci USA* **105**:20523–20528.

Yevenes GE, Peoples RW, Tapia JC, Parodi J, Soto X, Olate J, and Aguayo LG (2003) Modulation of glycine-activated ion channel function by G-protein betagamma subunits. *Nat Neurosci* **6**:819–824.

Yévenes GE and Zeilhofer HU (2011) Molecular sites for the positive allosteric modulation of glycine receptors by endocannabinoids. *PLoS One* **6**:e23886.

Yu H, Bai XC, and Wang W (2021b) Characterization of the subunit composition and structure of adult human glycine receptors. *Neuron* **109**:2707–2716.e6.

Yu J, Zhu H, Lape R, Greiner T, Du J, Lü W, Sivilotti L, and Gouaux E (2021a) Mechanism of gating and partial agonist action in the glycine receptor. *Cell* **184**:957–968.e21.

Zeilhofer HU (2005) The glycinergic control of spinal pain processing. *Cell Mol Life Sci* **62**:2027–2035.

Zeilhofer HU, Acuna MA, Gingras J, and Yevenes GE (2018) Glycine receptors and glycine transporters: targets for novel analgesics? *Cell Mol Life Sci* **75**:447–465.

Zeilhofer HU and Zeilhofer UB (2008) Spinal dis-inhibition in inflammatory pain. *Neurosci Lett* **437**:170–174.

Zhang HX and Thio LL (2007) Zinc enhances the inhibitory effects of strychnine-sensitive glycine receptors in mouse hippocampal neurons. *J Neurophysiol* **98**:3666–3676.

Zhang L and Xiong W (2009) Modulation of the Cys-loop ligand-gated ion channels by fatty acid and cannabinoids. *Vitam Horm* **81**:315–335.

Zhang Y, Keramidas A, and Lynch JW (2016) The Free Zinc Concentration in the Synaptic Cleft of Artificial Glycinergic Synapses Rises to At least 1 μM. *Front Mol Neurosci* **9**:88.

Zhang ZY, Bai HH, Guo Z, Li HL, He YT, Duan XL, Suo ZW, Yang X, He YX, and Hu XD (2019a) mGluR5/ERK signaling regulated the phosphorylation and function of glycine receptor α1ins subunit in spinal dorsal horn of mice. *PLoS Biol* **17**:e3000371.

Zhang ZY, Guo Z, Li HL, He YT, Duan XL, Suo ZW, Yang X, and Hu XD (2019b) Ubiquitination and inhibition of glycine receptor by HUWE1 in spinal cord dorsal horn. *Neuropharmacology* **148**:358–365.

Zhou N, Wang CH, Zhang S, and Wu DC (2013) The GLRA1 missense mutation W170S associates lack of Zn2+ potentiation with human hyperekplexia. *J Neurosci* **33**:17675–17681.

Zhu H and Gouaux E (2021) Architecture and assembly mechanism of native glycine receptors. *Nature* **599**:513–517.