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Review

Insights into the biophysical properties of $GABA_A$ ion channels: Modulation of ion permeation by drugs and protein interactions

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ABSTRACT

The fundamental properties of ion channels assure their selectivity for a particular ion, its rapid permeation through a central pore and that such electrical activity is modulated by factors that control the opening and closing (gating) of the channel. All cell types possess ion channels and their regulated flux of ions across the membrane play critical roles in all steps of life. An ion channel does not act alone to control cell excitability but rather forms part of larger protein complexes. The identification of protein interaction partners of ion channels and their influence on both the fundamental biophysical properties of the channel and its expression in the membrane are revealing the many ways in which electrical activity may be regulated. Highlighted here is the novel use of the patch clamp method to dissect out the influence of protein interactions on the activity of individual $GABA_A$ receptors. The studies demonstrate that ion conduction is a dynamic property of a channel and that protein interactions in a cytoplasmic domain underlie the channel's ability to alter ion permeation. A structural model describing a reorganisation of the conserved cytoplasmic gondola domain and the influence of drugs on this process are presented.

Contents

1. Introduction

Ion channels provide a unique opportunity to observe protein function in real time. The current passing through an individual ion channel in a cell membrane may be recorded with continuous sub-microsecond resolution, in situ by virtue of the patch clamp technique [1]. Through the meticulous variation of experimental conditions such recordings enable the fundamental properties of an ion channel to be elucidated. To date this technique has provided copious information about the properties of ion selectivity, gating (the opening and closing of an ion channel) and single-channel conductance (the ease with which ions flow) in numerous channel types. Most recently this technique of patch clamping has been applied to study the dynamic nature of protein–protein interactions that affect the activity of the ligand-gated $GABA_A$ receptor [2]. This work is interpreted in light of the structure and function of ligand-gated ion channels.

2. The $GABA_A$ ion channel

The $GABA_A$ receptor is a simple multi-subunit chloride-permeable ionophore whose activity is controlled by the binding of two GABA

Abbreviations: $GABA_A$ receptor, γ-amino butyric acid type A receptor; MA helix, amphipathic helix

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molecules at extracellular inter-subunit sites [3]. However, the receptor displays immense diversity in its biological function and that diversity begins with the 19 different genes encoding an array of subunits (α1–6, β1–3, γ1–3, δ, ε, π, θ, ρ1–3) that are potential components of this heteromeric pentamer. As a general rule the regulated assembly of two α isoforms, two β isoforms and a single γ or δ subunit combine in the endoplasmic reticulum, with each combination conferring some unique property on the physiology and pharmacology of the ion channel [4]. Of the remaining subunits the ρ isoforms appear unique in that they can form homo-pentamers and are expressed predominantly in the retina [5] while little is known about the functional role of neuronal $GABA_A$ receptors containing ε, π or θ subunits. It is interesting to note, however, that π for example, appears to be expressed in a number of peripheral tissues associated with reproduction such as breast epithelial cells [6] and endometrium [7]. This diversity imparted by receptor subtypes provides enormous variety yet, interestingly, little is known about the regulatory signals that drive their generation. Individual subunits share a common architecture beginning with a large extracellular ligand domain, followed by 4 transmembrane (TM) α-helices, which fold to form the pore. Located between transmembrane domain 3 and 4 each subunit contains a large intracellular loop (IL2 loop), which provides numerous regulatory motifs and auxiliary protein binding sites that are different in every subunit isoform (Fig. 1). Sequence comparison reveals little homology and this region is also the target of splice variants.

GABA is the only endogenous ligand for the $GABA_A$ receptor but many endogenous (neurosteroids) and exogenous potentiators exist including some major drug classes such as benzodiazepines, barbiturates, general anaesthetics and alcohol. It has been proposed that the major site of action for many of these drugs is the extrasynaptic $GABA_A$ receptor populations (e.g., on the cell soma), and not those found at synapses [8,9]. At the level of an individual channel such potentiators increase the open probability of the channel through changes in the open and closed time distributions (e.g., [10–13]). Such drug-induced kinetic changes are not discussed further. In addition to these kinetic changes, an increase in the single-channel conductance is sometimes observed upon drug potentiation [2,14–19]. The molecular mechanism by which dynamic variations in the $GABA_A$ channel alter its permissiveness to Cl^- ions, the structural rearrangements in the channel that modulate the ion pathway and the way in which drugs influence these processes are discussed in this review. Understanding these molecular processes is fundamental if the cellular signaling events that regulate and respond to this form of relatively intense inhibitory signaling in the brain are to be elucidated.

2.1. All major drug classes increase the conductance of $GABA_A$ receptors

Traditionally, conductance has been viewed as an immutable property of an ion channel and indeed, was used as one of the distinguishing functional characteristics in single-channel recordings. The observation that the benzodiazepine drug, diazepam, was able to increase the single-channel conductance of native extrasynaptic $GABA_A$ receptors implied otherwise, indeed that conductance was in fact a dynamic property of the ion channel [14]. Subsequent studies have revealed that all of the major drug classes known to potentiate the GABA current are indeed able to increase the conductance of native extrasynaptic receptors, thereby implying that this may be a common mode of action of drugs and presumably, a general property of all $GABA_A$ channel subtypes. For example, in the presence of subthreshold concentrations of GABA, general anaesthetics (e.g., etomidate [19], propofol [16]) barbiturates [15], neurosteroids (allopregnanolone and alphaxalone) [20], and alcohol (ethanol, Fig. 2E and F (Tierney, unpublished)) increase the Cl^- current through $GABA_A$ receptors by increasing both the frequency of channel openings (of an individual channel as well as the number of channels opening) and the single-channel conductance. The relatively long mean open time of these larger conductance channels, the increased ion permeation though an individual channel (>40 pS) and the increased likelihood of multiple channels opening would ensure a sustained inhibitory current as is necessary, for example, to maintain the tone and regulate the excitability of the network [21–23]. The fact that all these drug-binding events have a common molecular outcome, increased conductance levels coupled with long mean open times, suggests that despite binding at different sites on the receptor, the drugs affect a common mechanism that underlies the increased permissiveness to ion flow.

2.2. Protein interactions alter the conductance through $GABA_A$ ion channels

Numerous proteins are known to bind to the different subunits of pentameric $GABA_A$ receptors, thereby dictating their temporal and spatial distribution in the membrane [24,25] as well as regulating the movement of receptor subtypes into and out of their synaptic and extrasynaptic neuronal locations under various physiological stimuli [26–28]. Once in the membrane it appears that receptors are constrained, i.e., their free diffusion in the lipid bilayer is prevented. The $GABA_A$ receptor associated protein, GABARAP, is one protein that facilitates this latter process. GABARAP traffics γ-containing $GABA_A$ receptors from the endoplasmic reticulum to the plasma membrane where such receptors are then able to form clusters both in neurons

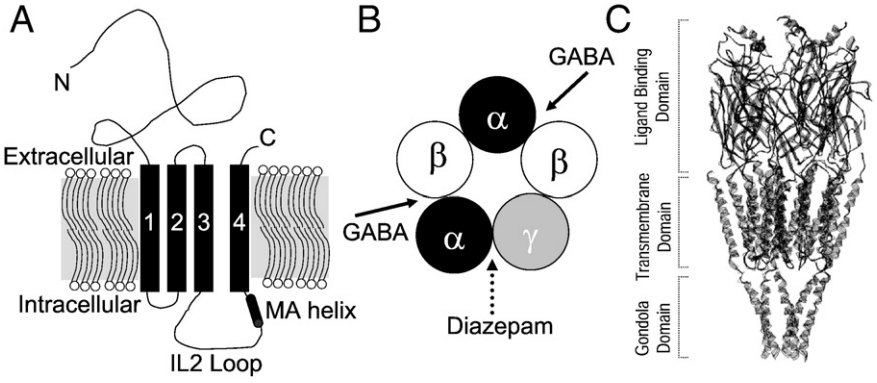

Fig. 1. Molecular architecture of $GABA_A$ receptors. A. Each subunit consists of a large extracellular N-terminal domain that contains ligand and well as some drug-binding sites. Situated within the lipid bilayer 4 transmembrane α-helices fold to form part of the ion conduction pathway with transmembrane domain 2 lining the pore. The large intracellular loop between the 3rd and 4th transmembrane domains contains binding sites for auxiliary proteins as well as numerous post-translational modification sites. The amphipathic (MA) helix at the C-terminal end of this loop is conserved in all subunits of ligand-gated ion channels and it participates in protein binding events. B. The $GABA_A$ receptor is a pentamer with the most common subunit combination found in the brain consisting of 2α, 2β and a single γ subunit. C. 3-Dimensional model of the $GABA_A$ receptor based on the cryo-em structure of the nicotinic acetylcholine receptor [32]. The 5 MA helices (one per subunit) form a 'gondola' structure directly beneath the permeation pore that spans the bilayer.

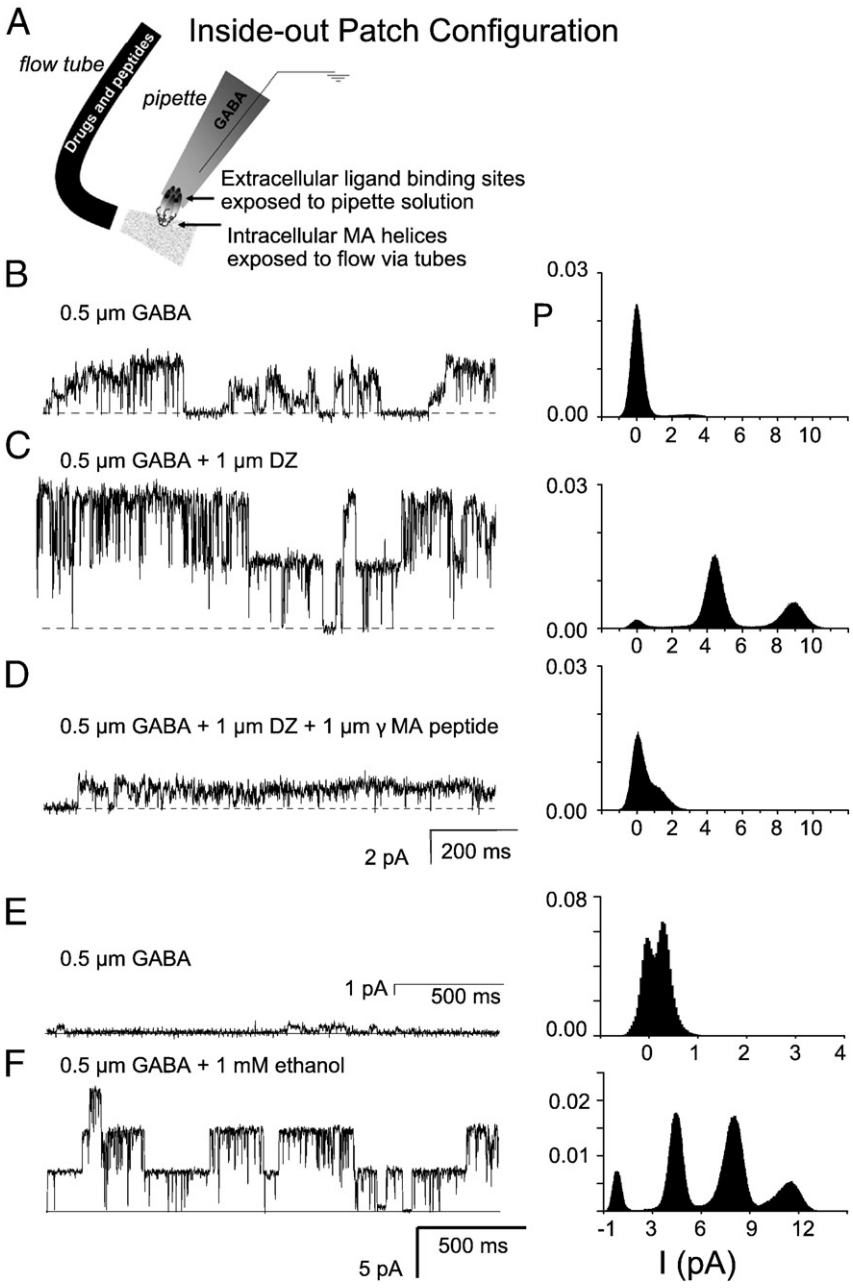

Fig. 2. Drug modulation of native $GABA_A$ channels. A. Single-channel recordings were performed in the inside-out patch configuration thereby exposing the receptor's intracellular MA helices to a stream of solution via gravity fed flow tubes. Drugs and peptides were applied by the lateral movement of a flow tube positioned to within 100 μm of the membrane patch. No difference is observed when drugs are applied to the intracellular membrane as opposed to the external membrane (e.g., [14,53]). Currents were recorded from newborn rat hippocampal neurons (Div8-15) at a membrane potential of −Vp = −60 mV. B. The current activated by 0.5 μM GABA shows a number of amplitudes, predominantly 0.85, 1.48 and 2.16 pA. C. Addition of 1 μM diazepam caused an increase in both the open channel probability as illustrated by the presence of 2 channels (2 equally spaced peaks in the current amplitude probability histogram) and in the average amplitude of the current to 4.5 pA (75 pS). D. In the continued presence of GABA and diazepam addition of the γ MA peptide (1 μM) caused a reduction in the current amplitude to ~1.2 pS and a reduced open probability (only one peak in the current amplitude probability histogram). Current amplitude probability histograms on the right depict activity recorded over a longer period of 30 s. E. In another experiment exposure to 0.5 μM GABA produced a small amplitude current (0.5 pA; −Vp = −60 mV). F. Addition of 1 mM ethanol to the patch caused an increase in both the open channel probability as shown by the presence of 3 channels (3 equally spaced peaks in the current amplitude probability histogram) and in the average amplitude of the current to 4.25 pA (70 pS).

[29] and in recombinant expression systems [17,30]. As a consequence of GABARAP co-expression, clustered recombinant $GABA_A$ channels acquire additional biophysical properties. Significantly, such receptors are able to undergo transitions to high conductance states upon diazepam potentiation [17,18], similar to that of native extrasynaptic $GABA_A$ channels [14]. Identifying this correlation between receptor clustering and enhanced ion permeation was pivotal as it provided not only an important clue about the molecular mechanism underlying conductance changes in $GABA_A$ channels but also a means by which to test its existence. For example, for receptors to remain clustered in the membrane they must be participating in protein interactions that oppose their separation by diffusion. Therefore if such protein interactions underlie the formation of high conductance channels then it should be possible to reverse their formation by disrupting the protein interactions that create them. A commonly employed experimental approach aimed at disrupting protein interactions is the use of competitor peptides. Such molecules have been extensively used to characterize the function of the targeted protein interactions at both a cellular and whole animal level [31,32]. In contrast to all such earlier studies however, we have

defined a mechanism at the single molecule level by combining the application of competitor peptides with single-channel recordings [2]. Uniquely, this experimental approach provides a remarkable visual insight into the dynamic nature of protein–protein interactions that affect the activity of a single ion channel as it occurs in real time.

Receptor–protein interactions have been identified in members of the ligand-gated ion channel family. Many of them contribute to receptor trafficking and involve the conserved amphipathic intracellular helix (MA helix) [30,33]. MA helices share no sequence homology; however, the helical and chemical nature is conserved. In $GABA_A$ receptors this MA helix motif in the single γ-subunit per receptor interacts with either the trafficking protein GABARAP resulting in clustering of receptors, or with itself with unknown physiological consequences. Because of the MA motif's predisposition to form protein interactions we examined the ability of subunit-specific competitor peptides, corresponding to each subunit's intracellular amphipathic (MA) helix, to disrupt high conductance $GABA_A$ channels. Currents were potentiated by the γ-subunit-specific drug diazepam thereby defining the receptor subtype responding and peptides were applied directly onto the cytoplasmic side of membrane patches while recording single-channel currents using the inside-out patch configuration (Fig. 2A).

Only the application of a peptide mimicking the MA helix of the γ2 subunit ($\gamma_{381–403}$) of the $GABA_A$ receptor, and not that of the $\alpha_{(371–393)}$ or $\beta_{(403–425)}$ subunits, attenuated the potentiating effect of diazepam on native receptors by substantially reducing their conductance (Fig. 2B–D) [2]. Similarly, a reduction in conductance was observed when diazepam was used to potentiate the GABA current of a defined receptor combination (α1β2γ2S) co-expressed with GABARAP in L929 cells. The effect of the γ2 MA peptide, however, was specific for clustered $GABA_A$ receptors able to undergo transitions to high conductance states as it was ineffectual on diffusely expressed recombinant αβγ receptors (i.e., expressed in the absence of GABARAP) whose conductance never exceeds 40 pS [34,35]. Together, these data imply that the protein–protein interaction competed by the γ MA peptide involved an intermolecular interaction mediated by the single γ-subunit per pentamer. Interestingly, in patches displaying high conductance channels the action of the peptide reduced conductance levels to unitary values (20–30 pS), that is, those levels seen in diffusely expressed recombinant $GABA_A$ receptors. One would predict the appearance of such levels if the peptide was indeed preventing channels from interacting with each other.

While the competitive action of the 23 residue $\gamma2_{(381–403)}$ peptide clearly implicated the γ MA helix as participating in an inter-protein interaction it did not identify its interaction partner. Shorter peptides capable of distinguishing between a γ2-GABARAP interaction and a γ2–γ2 self interaction were therefore synthesized. A γ2 MA peptide lacking the N-terminal 5 amino acids ($\gamma_{386–403}$) defines the minimum sequence required to interact with GABARAP and is effective in this capacity both in recombinant expression systems [36] and in neurons [37,38]. Application of this γ2-C18 MA peptide did not significantly affect the conductance of recombinant αβγ receptors co-expressed with GABARAP (Fig. 5A and B; [2]). To verify that GABARAP was not the interaction partner, a peptide was tested in which the last 5 amino acids were excluded ($\gamma_{381–398}$; N18), thereby removing the critical residues needed for a γ2-GABARAP interaction. Application of this γ2 N18 MA peptide was still able to reduce the conductance to unitary levels, albeit less effectively than the full 23 residue peptide (Fig. 5C and D; [2]). Collectively, the data support a mechanism whereby adjacent $GABA_A$ receptors interact via their solitary γ2 subunit MA helices, altering ion permeation through each channel (Fig. 4). The role of GABARAP in the process is most likely as a trafficking protein, targeting γ-containing receptors to the membrane and facilitating (perhaps even regulating) their inter-$GABA_A$ receptor interactions.

2.3. Structural basis underlying conductance changes in the $GABA_A$ ion channel

It has been proposed that all ligand-gated ion channels will share a similar 3-dimensional structure and gating mechanism based on sequence homology. The images derived from cryo-electron microscopy of tubular arrays of the *Torpedo marmorata* acetylcholine receptor reveal what an individual ligand-gated ion channel looks like in the closed state [39]. The only structure visible on the cytoplasmic side of the receptor is that formed by the conserved MA helices, one per subunit, which appear as a 'hanging gondola' beneath the pore. The remaining >80% of this loop is unstructured under these conditions [39]. Theoretically, the cytoplasmic gondola structure that is positioned directly beneath the transmembrane pore-forming domain (Fig. 3A, star) could physically impede ion flow through the channel and indeed it has been proposed that gaps visualised between adjacent MA helices within an individual acetylcholine receptor, may influence ion flow in the acetylcholine receptor [39]. Consistent with such a role, mutations within the MA helices of diffusely expressed recombinant ligand-gated ion channels activated by acetylcholine, 5-hydroxytryptamine and glycine have all been shown to affect single-channel conductance [40–42]. These mutational data indicate that the MA helices are in a position to influence ion permeation where it has been suggested that steric constraints imposed by intra-receptor MA helices are responsible for conductance variability [42].

An extension of the concept that the gondola domain beneath the pore modulates ion permeation is that any structural perturbation that alters this domain has the potential to also affect conductance. Furthermore, if changes in channel conductance are achieved through the regulated movement of the cytoplasmic gondola domain then one might expect this domain to exhibit some modular flexibility. Indeed, such flexibility has been demonstrated. Early electron microscopic images of negatively stained tubular arrays of the acetylcholine receptor revealed the distribution of the protein components that comprise the ion channel that spans both sides of the lipid bilayer [43]. When viewed in transverse sections what is now known to be the MA helix-forming gondola domain was seen to overlie the axial path of the channel beneath the lipid bilayer (Fig. 3A). Equivalent images of tubes frozen in alkaline solution caused the gondola domain to redistribute in an irregular way over the cytoplasmic surface while the transmembrane and extracellular ligand-binding domain retained their position in the crystal lattice (Fig. 3B).

Based on the predicted structural homology of ligand-gated ion channels and the propensity of the 23 residue γ2 MA peptide from the $GABA_A$ receptor to form a helix (Fig. 3C) it seems reasonable to suggest that the MA helices of $GABA_A$ receptors will form a similar gondola structure as exists in the acetylcholine receptor. Thus protein interactions involving the M3–M4 loop have the potential to alter the disposition of the MA helices and therefore alter the gondola structure beneath each pore. One possible explanation for the increase in conductance observed in native $GABA_A$ receptors and clustered recombinant receptors could be therefore, that physical interactions between the γ2 subunits (involving residues 381–403) of adjacent receptors provide the potential to alter the structural organization beneath the pore (Fig. 4). Such an interpretation does not require the 'co-ordinated opening' of adjacent channel gates to account for high conductance channels as suggested previously [14,17]. However, changes in the gating kinetics of an individual channel could be expected to accompany transitions to high conductance states if such a domain acts as an interdependent gate similar to that described for KirBac channels [44]. While such kinetic analysis have yet to be completed it is interesting to note that the gating behaviour of recombinant αβγ $GABA_A$ channels expressed with GABARAP is strikingly distinct in the absence and presence of the $\gamma2_{(381–403)}$ competitor peptide (Fig. 4, [2]).

Sequence alignments of the MA helices of the various ligand-gated ion channels reveal little (cation channels [42]) or no homology

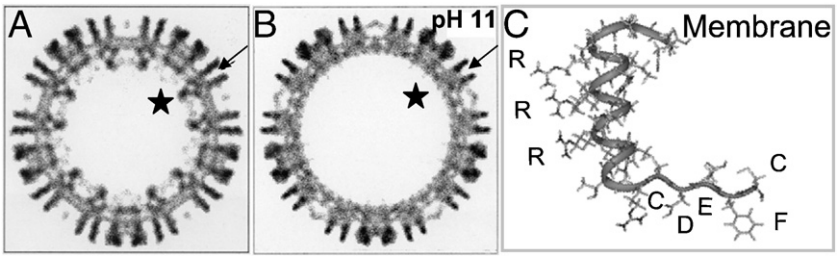

Fig. 3. Structural insights into the MA helical domain. A and B: Electron microscopic images of negatively stained tubular arrays of the nicotinic acetylcholine receptor viewed in transverse sections. A. The distribution of the protein components that comprise the ion channel spans both sides of the lipid bilayer. The arrow indicates the extracellular mouth of the channel through which ions permeate. The star indicates the intracellular gondola domain formed by the five MA helices, which overlies the axial path of the channel beneath the lipid bilayer. Its position implies that ions must also permeate this structure. B. Equivalent images of tubes frozen in alkaline solution (pH 11) cause the gondola domain to redistribute in an irregular way over the cytoplasmic surface while the transmembrane and extracellular ligand-binding domain retained their position in the crystal lattice (images courtesy of N Unwin). C. NMR structure of the $GABA_A$ $\gamma_{381-403}$ MA peptide. In solution the peptide shows a propensity to adopt an α-helical structure. The structure reveals a positively charged helical face dominated by 3 arginine residues (R) and a relatively more extended structure where the critical 5N-terminal residues lie (CFEDC).

(anion channels) at the amino acid level. At a chemical level, such regions share an amphipathic nature and at a structural level they are predicted to form an α-helix. What is not clear at this point in time is whether the $GABA_A$ receptor is the only member of this family able to undergo transitions to high conductance states via the formation of intermolecular protein–protein interactions involving the conserved MA helices.

Conductance levels of ligand-gated ion channels do vary, which are influenced in part by the expression system (e.g., oocytes vs. mammalian vs. native receptors (e.g., nAChR [45], glycine receptor [46,47]). The maximum single-channel conductance level recorded for recombinant, clustered $GABA_A$ channels achieved by drug potentiation is approximately 70 pS (c/a patch αβγ2S + GABARAP; [17]) whereas native $GABA_A$ channels have been recorded in excess of 90 pS [16]. Interestingly, some members of the ligand-gated ion channel family are able to achieve similar high conductance levels when expressed in heterologous systems and do not require any exogenous facilitator protein. For example, the recombinantly expressed heteromeric *Torpedo californica* acetylcholine receptor (α2βγδ) [48] and homomeric α1 glycine receptors [40] exhibit conductances as high as 87 and 92 pS, respectively. On the basis of structural and functional conservation within the ligand-gated ion channel family it is possible therefore, that all members have the potential to conduct ions in this highly permissive fashion but some need to be potentiated in order to do so. Hence the MA helices may function as regulatory domains in the various members that, by participating in protein–protein, or protein–lipid interactions and/ or by undergoing post-translational modifications, have the potential to make conductance sensitive to intracellular regulation.

2.4. Drug-induced conformational states in clustered $GABA_A$ receptors

Drugs acting at $GABA_A$ receptors increase the inhibitory current by increasing the open probability of the channel. Classically the biophysical properties of the ion channel reflecting this change included increases in channel open times, decreases in closed times and an increase in the frequency of opening (e.g., prolonged bursting and the opening of additional channels) [10–13,34,35,49]. In clustered $GABA_A$ receptors the single-channel conductance is also increased and there is a strong correlation between higher conductances and longer mean open times [18,19]. Little is known of the mechanism through which drug potentiation confers any of these properties on the ion channel. In high conductance $GABA_A$ channels where the GABA current was potentiated by diazepam, sub-maximal conductance states (substates) appear following the application of the γ2 MA peptide (in the continued presence of drug) (Fig. 2B–D). This observation suggests that substate activity of the channel arises from conformational changes in the conserved MA helices that comprise the gondola structure. By extension, it also implies that drugs must facilitate the movement of such a structure in the $GABA_A$

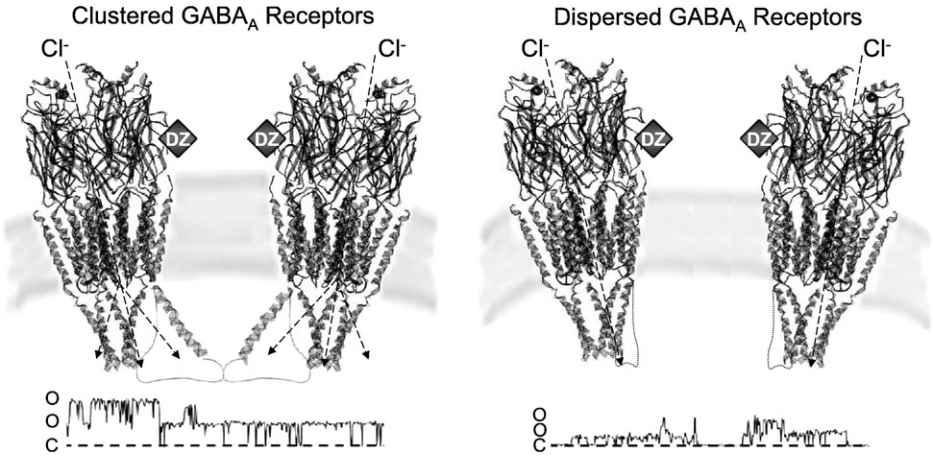

Fig. 4. Model depicting protein–protein interactions between adjacent $GABA_A$ receptors that enable receptor cross-talk, facilitating the enhanced biophysical properties of the ion channels. It is postulated that trafficking of $GABA_A$ receptors by GABARAP facilitates interactions between γ-subunit intracellular loops of adjacent receptors. Drug binding provides the potential to alter the disposition of the gondola structure beneath the pore, which, in these clustered receptors increases ion permeation through each channel because of the reduced steric hindrance. In contrast, the unregulated expression of $GABA_A$ receptors produces dispersed receptors in the plasma membrane, which lack the inter-receptor interactions. These channels exhibit unitary conductance levels and drugs increase only the open probability of the channel(s). Single-channel traces depicted beneath each receptor model illustrate the large difference in current size between clustered and non-clustered $GABA_A$ receptors, which is likely to impose functional differences in cellular signaling and variations in physiological responses. C = closed, O = open.

receptor. Since drug binding does not occur at a common site on $GABA_A$ receptors such a model requires that all potentiator binding events effect a common conformational change in the channel structure, which we suggest is the movement of the gondola domain. How this movement of an intracellular domain is co-ordinated with gating conformational changes occurring in the pore domain have yet to be examined.

A study involving mutations in a motif identified within the conserved extracellular cystine loop (Cys loop) of subunits in the $GABA_A$ receptor (aromatic, proline, aliphatic sequence, ArProAl motif) provides some clues as to how the binding of potentiators could provide the additional free energy to drive such a process in the gondola domain [50]. The conserved extracellular Cys loop of the different subunits in the αβγ2S $GABA_A$ receptor was seen to participate asymmetrically in agonist activation (α and β Cys loops) and drug modulation (γ and α Cys loops). While all acted equivalently to resist gating-dependant conformational changes it was the potentiation by drugs (and presumably endogenous potentiators) that recruited the single γ-subunit into the activation process thereby channelling additional ligand-binding energy into driving the gating-dependant conformational changes and rendering the activation process pseudo-symmetrical [50]. Interestingly, all the different classes of drugs tested in the study acted similarly to affect potentiation (benzodiazepines, barbiturates and general anaesthetics). A common theme linking drug potentiation in the mutational study of the conserved extracellular Cys loop and that describing the existence of protein–protein interactions involving the conserved cytoplasmic MA helices is the critical involvement of the γ-subunit in both these molecular events. Thus drug potentiation could be viewed as arising from the additional energy required to shift the impediment to ion flow provided by the gondola structure.

Drugs do not significantly increase the conductance level of dispersely expressed recombinant $GABA_A$ receptors [2,17]. However, irrespective of composition, recombinant receptors do exhibit multiple conductance states, a main state and a substate, approximately 30 and 22 pS, respectively [34,35,49]. What drugs do influence under these conditions is the proportion of time that the channel spends in these states, shifting the equilibrium significantly towards the main, longer-lived conductance state [34,35]. How an individual $GABA_A$ channel achieves this subtle variation in conductance is not known. In light of the results with high conductance $GABA_A$ channels and competitive MA peptides, it is possible that drugs similarly facilitate and stabilise movements in the gondola domain of these unclustered receptors; however, the structural perturbations are smaller and consequently the increased permissiveness to ions is less. Alternatively, the conformation of the gondola domain that gives rise to high conductance states is less stable in unclustered receptors and simply not occupied with any great probability.

3. Conductance properties of ion channels in general

Ligand-gated ion channels are but one class of ion channel. That its members show a wide range of conductance levels is not unique to this class. Potassium channels form a large diverse family of channels and its members also show variation in their conductance properties. A recent X-ray crystallographic study of inward rectifier channels from bacteria (KirBac channels) has provided the first structural evidence for conformational changes in an intracellular domain correlating with ion conduction at the selectivity filter, some 20 Å away in the membrane [44]. Such a study highlights the existence of multiple 'gates,' all of which have the potential to influence ion permeation and that such gates can be remote from the constriction. It is interesting to note that the single-channel records of wild-type KirBac channels share a remarkable similarity to those of $GABA_A$ channels. For example, both the potassium and the $GABA_A$ channels exhibit high conductance states (>40 pS) as well as multiple lower conductance states. It will be of interest to see whether the conductance state transitions of the KirBac channel correlate with conformational changes in the cytoplasmic domain and indeed how the binding of modulators to this domain, regulate ion permeation.

4. Concluding remarks

It is generally accepted that the considerable potential of the brain to diversify its inhibitory response is endowed through the activation of an array of $GABA_A$ receptors, distinct in their subunit composition, subcellular location and brain region distribution [22,51,52]. The discovery that intermolecular protein interactions dramatically alter $GABA_A$ ion channel properties suggests that the interaction status of the receptor may represent another level of intracellular regulation which, potentially, could be effective on a much faster time scale than changes effected by differential expression of receptor subunits. As the field of ligand-gated ion channels emerges from studying an individual ion channel to that of its signaling complexes one can begin to appreciate how the ability of such macromolecular complexes to respond is dependent upon the compliment of proteins associated in the complex. The dynamic exchange of protein interaction partners would provide the brain with an enormous capacity to adapt and respond to environmental stimuli.

Acknowledgements

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