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Molecular composition of $GABA_C$ receptors

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Abstract

In the central nervous system inhibitory neurotransmission is primarily achieved through activation of receptors for γ-aminobutyric acid (GABA). Three types of GABA receptors have been identified on the basis of their pharmacology and electrophysiology. The predominant type, termed $GABA_A$ and a recently identified type, $GABA_C$, have integral chloride channels, whereas $GABA_B$ receptors couple to separate K^+ or Ca^{2+} channels via G-proteins. By analogy to nicotinic acetylcholine receptors, native $GABA_A$ receptors are believed to be heterooligomers of five subunits, drawn from five classes (α, β, γ, δ, ϵ/χ). An additional class, called ρ, is often categorized with $GABA_A$ receptor subunits due to a high degree of sequence similarity. However, ρ subunits are capable of forming functional homooligomeric and heterooligomeric receptors, whereas $GABA_A$ receptors only express efficiently as heterooligomers. Intriguingly, the pharmacological properties of receptors formed from ρ subunits are very similar to those exhibited by $GABA_C$ receptors and ρ subunits and $GABA_C$ responses have been colocalized to the same retinal cells, indicating that ρ subunits are the sole components of $GABA_C$ receptors. In contrast, the propensity of $GABA_A$ receptor and ρ subunits to form multimeric structures and their coexistence in retinal cells suggests that $GABA_C$ receptors might be heterooligomers of ρ and $GABA_A$ receptor subunits. This review will summarize our current understanding of the molecular composition of $GABA_C$ receptors based upon studies of ρ subunit assembly.

Keywords: $GABA_C$ receptor; ρ Subunits; Ligand-gated ion channel; Receptor assembly; Retina

1. Introduction

GABA is the most important inhibitory neurotransmitter in the mammalian central nervous system [1]. Traditionally, GABA-gated neurotransmitter receptors were classified in two structurally and pharmacologically distinct subclasses. The $GABA_A$ receptor is a ligand-gated ion channel which consists of several subunits: $\alpha 1–6$, $\beta 1–3$, $\gamma 1–3$, ϵ/χ [2] [3]. These subunits containing an extracellular N-terminus and four transmembrane regions (Fig. 1, left side) are thought to assemble into a pentameric Cl^- channel [4]. $GABA_A$ receptors contain modulatory binding sites for benzodiazepines, barbiturates, neurosteroides and ethanol [5–7] and are inhibited by the action of bicuculline and picrotoxin [8]. Because different subunits of the $GABA_A$ receptor are expressed in distinct brain regions and each subunit class determines subtle pharmacological differences, a variety of of $GABA_A$ receptors may be composed of the known subunits [9]. In contrast, $GABA_B$ receptors are members of the seven transmembrane family [10] which are coupled to either K^+ or Ca^{2+} channels via G-proteins and are regulated by intracellular second messenger systems (Fig. 1, right side). They are selectively activated by baclofen and do not respond to the known $GABA_A$ receptor modulators [11].

A third type of GABA receptor, termed $GABA_C$, had been proposed that is distinct from the previously known GABA receptors. $GABA_C$ receptors gate Cl^- currents in various parts of the vertebrate brain and were first described in interneurons of the spinal cord [12–17]. Later, $GABA_C$ receptors in the retina were observed by a group led by Miledi expressing mRNA isolated from the bovine retina in *Xenopus* oocytes [18–20]. $GABA_C$ receptors show distinct electrophysiological properties. Compared with $GABA_A$ receptors, $GABA_C$ receptors have a higher sensitivity for GABA, their currents are smaller and do not desensitize. On the

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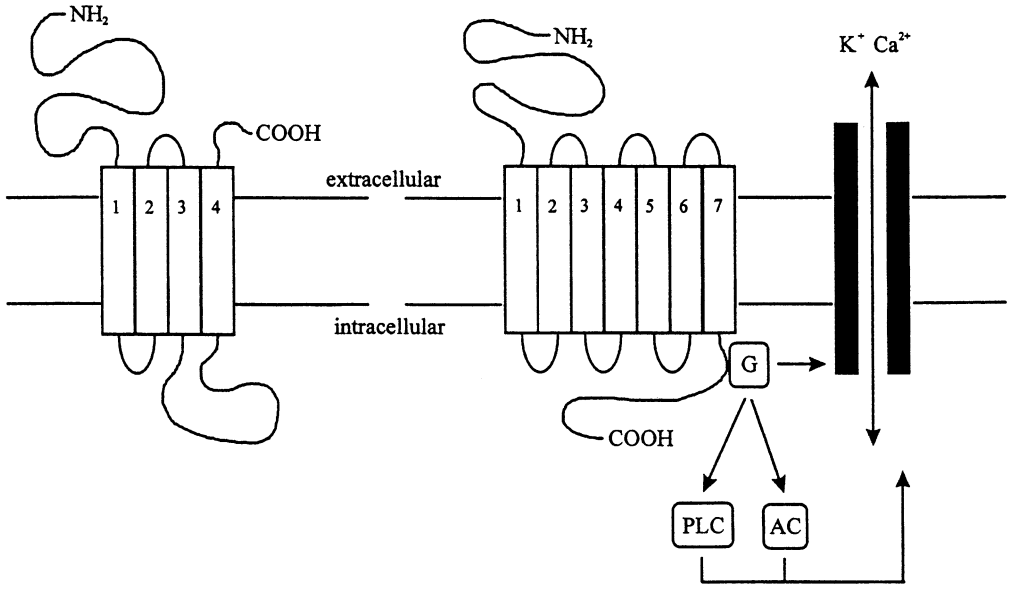

Fig. 1. Schematic representation of individual subunits of $GABA_A$ and $GABA_C$ receptors (left) and $GABA_B$ receptors (right). (Left) By analogy to the nicotinic acetylcholine receptor, subunits of ligand-gated neurotransmitter receptors consist of four transmembrane regions (TM 1–4), a long extracellular N-terminus, a cytoplasmic loop between TM3 and TM4 and a short extracellular C-terminus. The N-terminus contains domains important for ligand binding. The pore of the ion channel is formed by the TM2. (Right) $GABA_B$ receptors are members of the seven transmembrane protein family (TM 1–7). Binding of the agonist causes activation of a G-protein (G), that in turn can either regulate separate ion channels (permeable for K^+ or Ca^{2+}) directly or use second messenger systems such as phospholipase C (PLC) or adenylcyclase (AC).

single-channel level, these receptors are characterized by longer mean-open times and smaller chloride conductance [21,22].

$GABA_C$ receptors also differ in their pharmacological profile compared with $GABA_A$ and $GABA_B$ receptors. They are insensitive to $GABA_A$ receptor modulators, such as barbiturates and benzodiazepines as well as against the typical $GABA_A$ receptor antagonist bicuculline and they are not activated by the $GABA_B$ receptor agonist baclofen (for review see [23]). $GABA_C$ receptors have been characterized most extensively in the retina. In rat, tiger salamander and hybrid bass, $GABA_C$ receptors were found on bipolar cells [21,22,24,91,25,26] and in the white perch on horizontal cells [27,28].

Several lines of evidence indicate that $GABA_C$ receptors are composed of ρ subunits (ρ1,2 in humans: [29,30]; ρ1,2 in rat: [31,32]; ρ2,3 in rat: [33,34]; ρ1,2 in chicken: [35]). When heterologously expressed, ρ subunits show similar electrophysiological and pharmacological properties compared with $GABA_C$ receptors. Furthermore, RT–PCR and in situ hybridization studies showed the expression of ρ subunits in cells with $GABA_C$ receptors: rod bipolar cells of the rat and H4 horizontal cells of the white perch [36,37]. Finally, antibody staining of retinal sections revealed that ρ subunits are present on dendrites and axonterminal systems of rod as well as cone bipolar cells of different species [38,39]. The molecular characteristics of ρ subunits and their relationship to $GABA_C$ receptors will be the focus of this review.

2. Homooligomeric assembly of the ρ1 subunit

A prominent characteristic of ρ subunits is their ability to form functional homooligomeric GABA-gated Cl^- channels (ρ1: [29]; ρ2: [40]). This stands in sharp contrast to $GABA_A$ receptors, where combinations of different subunits are needed for proper function [41]. Therefore ρ subunits provide a useful model system to study the assembly properties of GABA receptor subunits.

Homooligomeric assembly of ρ1 subunits from several species into functional GABA receptors has been reported in different heterologous expression systems. Expression of the human and rat ρ1 subunit in *Xenopus* oocytes [29,42,43,40,31] as well as of the human ρ1 subunit in mammalian HEK 293 cells [44] showed their ability to efficiently form GABA-gated Cl^- channels. Whereas Hill coefficients using the *Xenopus* expression system were around 2, the GABA dose–response curve of the ρ1 subunit expressed in HEK 293 cells is much steeper, with a Hill coefficient between 3 and 4. In contrast, $GABA_A$ receptor subunits expressed in either *Xenopus* oocytes or 293 cells show Hill coefficients of 1–2 [41,45]. The high Hill coefficient of the expressed ρ1 subunit supports the idea of homooligomeric assembly of five ρ1 subunits into a pentameric $GABA_C$ receptor where each subunit has its own GABA binding pocket. Indeed, mathematical calculations predict the existence of five equivalent and independent GABA binding sites in receptors formed of the human ρ1 subunit. It has been proposed that three GABA

molecules are necessary to open the channel, while two additional binding sites increase ligand sensitivity and stabilize the open state of the receptor [46].

By analogy to subunits of the nicotinic acetylcholine receptor [47] it has been suggested that ρ subunits consist of a long extracellular N-terminus, four membrane-spanning regions (TM1 to TM4) and a short extracellular C-terminus (Fig. 1, left side). Between TM3 and TM4 a long intracellular loop may contain consensus sites for intracellular modulation and for localization of receptors to synaptic sites. The standard model predicts that ρ subunits assemble into a pentameric receptor (Fig. 2) with the second transmembrane domain (TM2) of each subunit facing the ion pore.

The topology of the $\rho 1$ subunit relative to membranes has been studied using human $\rho 1$ subunit protein translated in vitro in the presence of microsomal membrane vesicles [48]. While the N-terminus of the $\rho 1$ subunit was protected from cleavage and thus present in the lumen of the membrane vesicles, the C-terminus was degraded. Since the lumen of the vesicle is equivalent to the lumen of the endoplasmic reticulum, this result suggested an extracellular location of the N-terminus of ρ subunits in mature receptors. This orientation is consistent with the N-terminal localization of two GABA binding domains as well as a histidine (His156), which is important for the inhibitory effect of extracellular Zn^{2+} (see Section 6 and [49,50]).

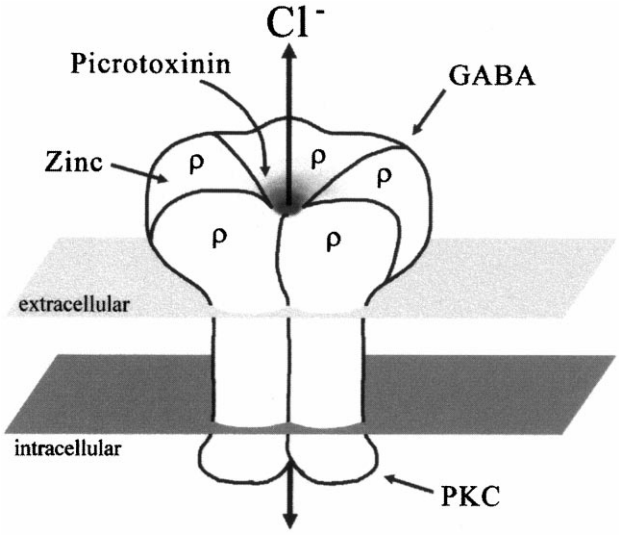

Fig. 2. Proposed structure of a $GABA_C$ receptor *in vivo*. Five ρ subunits assemble into a pentameric protein complex forming the Cl^- channel as a central pore. The binding sites for agonists (e.g. GABA) as well as competitive antagonists and neuromodulators are located in the extracellular part of the protein complex. Picrotoxinin binds in the channel pore but shows also an use-dependent effect, indicating an allosteric mechanism. The intracellular loop between transmembrane regions 3 and 4 may contain consensus sequences for protein kinases such as PKC.

By dividing the $\rho 1$ subunit into the N-terminal part (N-$\rho 1$) which contains the protein up to the first transmembrane domain and the C-terminal part (C-$\rho 1$) which contains all four transmembrane regions and the C-terminus, Hackam and coworkers were able to determine the contribution of each protein fragment in subunit assembly. A mixture of in vitro translated full-length $\rho 1$ (fused to a C-terminal FLAG epitope) was incubated together with N-$\rho 1$ and subsequently immunoprecipitated with an anti-Flag antibody. $\rho 1$ and N-$\rho 1$ were detected in the precipitate, indicating that sufficient information for homooligomeric assembly of the $\rho 1$ subunit is located in the extracellular N-terminus. In a complementary experiment, the presence of receptor assembly domains in N-$\rho 1$ was tested using a heterologous expression system. *Xenopus* oocytes were injected with different ratios of cRNA encoding for N-$\rho 1$ and full-length $\rho 1$. N-$\rho 1$ contained no transmembrane regions and did not form functional Cl^- channels. Moreover, increasing amounts of N-$\rho 1$ reduced GABA activated $\rho 1$ currents in a dose dependent manner. It was proposed that N-$\rho 1$ contained domains that enabled co-assembly between N-$\rho 1$ and full-length $\rho 1$ proteins, resulting in non-functional receptors (Fig. 3). A 5-fold excess of N-$\rho 1$ compared with $\rho 1$ was sufficient to eliminate GABA responses. Finally, Hackam and coworkers demonstrated that a 100 amino acid region in the N-terminus of the $\rho 1$ subunit contains signals that are critical for robust homooligomeric expression [51].

Together, these results suggested that the N-terminal region of the $\rho 1$ subunit is extracellular and that it contains sufficient information for the homooligomeric assembly of $\rho 1$ subunits into functional GABA receptors. However, it cannot be excluded that also the second half of the protein, especially the cytoplasmic loop contains assembly sequences which may only be active in combination with the N-terminus.

3. Homooligomeric assembly of $\rho 2$ and $\rho 3$ subunits

In addition to the $\rho 1$ subunit, other members of this subunit class have the ability to form functional homooligomeric GABA receptors. The human $\rho 2$ subunit forms GABA-gated Cl^- channels with electrophysiological and pharmacological properties that are very similar to those exhibited by $\rho 1$ [52,40]. However, homooligomeric expressed $\rho 2$ subunits generate considerably lower whole-cell currents compared with $\rho 1$. For example, injection of *Xenopus* oocytes with equal amounts of $\rho 1$ and $\rho 2$ cRNA (5 ng) produces currents ranging from 200 to 1000 pA for the $\rho 1$ subunit and from 10 to 100 pA for $\rho 2$ [40]. This difference appears to be independent of the expression method. Nuclear injection of $\rho 2$ DNA into *Xenopus* oocytes resulted in

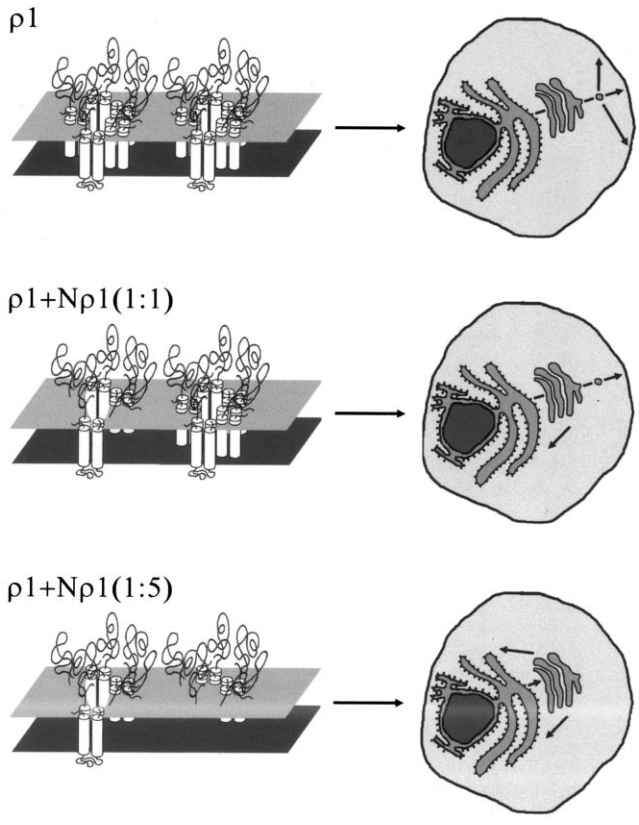

Fig. 3. Assembly of ρ subunits in heterologous expression systems. The left side shows magnified views of ρ subunits assembled in intracellular membranes of *Xenopus* oocytes, that are drawn on the right. (Top) Assembly of five ρ subunits in the rough endoplasmic reticulum is followed by trafficking of the assembled receptor via the golgi network to the cell surface (arrows). (Middle) Interaction of full-length ρ subunits with ρ subunit N-termini interferes with assembly into functional receptors. Consequently fewer functional receptors reach the cell surface while non-functional protein complexes are routed to a degradation pathway. Abnormally assembled receptors may also be trafficked to the cell surface, but are non-functional. The net result is a decrease in GABA-gated whole-cell current. (Bottom) Increasing amounts of ρ subunit N-termini causes a proportionate increase in routing of ρ subunits to the degradation pathway, resulting in a lack of functional GABA receptors at the cell surface.

GABA-gated whole-cell currents that were consistently in the 100–200 pA range, compared with ρ1-generated currents (more than 1000 pA) under the same conditions (Wang and Cutting, unpublished observation). The human ρ2 subunit also forms homooligomeric GABA receptors following transient transfection of HEK 293 cells (Enz and Cutting, unpublished observation). In contrast to the human ρ2 subunit, the rat counterpart does not seem to form functional GABA receptors when ρ2 cRNA was injected into *Xenopus* oocytes [31]. However, the authors did not use nuclear injection to increase the expression level.

It has also been reported that the rat ρ3 subunit as well as a white perch ρ subunit can form functional homooligomeric GABA receptors after cRNA injection in *Xenopus* oocytes [90,37]. In both cases, GABA-gated Cl^- currents were similar to the typical $GABA_C$ receptor characteristics described above. Interestingly, the white perch ρ subunit shares the highest similarity with the human ρ2 subunit (90%). Therefore, the authors suggest that this subunit may be the ρ2 counterpart of the white perch. Indeed, amplitudes of GABA-gated Cl^- currents recorded of the expressed fish subunit are comparable with those obtained from the homooligomeric expressed human ρ2 subunit. These data provide further evidence that functional homooligomeric GABA receptors can not only be formed of the homooligomeric expressed ρ1 subunit but also of other subunits of the ρ family.

4. Heterooligomeric assembly of ρ subunits

At this time, only a few studies have explored the possibility that ρ subunits may heterooligomerize. Coinjection of the rat ρ1 and ρ2 subunits in *Xenopus* oocytes generated functional GABA receptors with distinct pharmacology compared with the ρ1 subunit injected alone [31]. Immunoprecipitation studies using a specific antibody showed that the N-terminal part of human ρ1 is able to interact with the human ρ2 subunit in vitro [48]. Together, these studies suggest that ρ subunits are able to form heterooligomeric GABA receptors. The latter study indicates that information for assembly is located in the N-terminal part of the protein, similar to what was found for homooligomeric assembly of the ρ1 subunit [48].

This result is consistent with studies on $GABA_A$, glycine and nicotinic acetylcholine receptors, where domains important for heterooligomeric subunit assembly have been localized in the N-terminal region [53–56]. However, it it is not known whether different regions of the N-terminus facilitate homooligomeric and heterooligomeric subunit assembly. This information could be important in understanding how the stoichiometry of ρ subunits in a pentameric receptor is controlled. If homooligomeric and heterooligomeric assembly signals are identical, the subunit composition may be determined by the relative abundance of each subunit at the location where assembly occurs. Indeed, different groups have reported transport of mRNA in neurons into dendrites that enable protein biosynthesis and receptor assembly near the synapse (for review see [57,58]). Thus, the presence and number of different ρ subunits in an oligomeric GABA receptor might be controlled by targeting different mRNA types to specific locations within a neuron. In addition, the number of neurotransmitter receptors at specific neuronal sites could be determined by synaptic activity that influences mRNA translation at local areas. For example, it has been shown that the expression of Homer, important for clustering metabotropic glutamate receptors at

synaptic sites, is regulated by neuronal activity [59]. If, in contrast, homo- and heterooligomeric assembly signals are different, then GABA receptors would be formed independent of the local concentrations of each ρ subunit. This was found to be the case for glycine receptor subunits [55].

5. Assembly of ρ subunits with $GABA_A$ and glycine receptor subunits

GABA and glycine receptors are members of the ligand-gated ion channel family. Their subunits are thought to share the membrane topology shown in Fig. 1 (left side). On the amino acid level the similarity between subunit classes (GABA receptor: α, β, γ, δ, ϵ/χ, ρ; glycine receptor: α, β) is 20–30%, whereas within one class (for example $GABA_A$ receptor subunits $\alpha 1$–6) the homology is 70–80%. Furthermore, each of these subunits is capable of oligomerization. This raises the possibility that each of these proteins is capable of forming heterooligomeric GABA receptors. Pharmacological and electrical properties of GABA receptors are determined by their subunit composition. For example, it has been shown that the inclusion of the $GABA_A$ receptor γ subunit confers sensitivity for benzodiazepines [41]. Following the same principle, ρ subunits in heterooligomeric $GABA_A$ receptors might be predicted to alter its sensitivity for GABA, bicuculline and/or modulatory substances. However, co-expression of the human $\rho 1$ subunit with the $GABA_A$ receptor subunits $\alpha 1$, $\beta 1$ and $\gamma 2$ in *Xenopus* oocytes, as well as with a 10-fold excess of the human glycine receptor β subunit in HEK 293 cells showed no detectable alteration in GABA-gated Cl^- currents on the whole-cell level ([42]; Enz and Bormann, unpublished observation). Co-immunoprecipitation of human $\rho 1$, $\rho 2$ subunits or the N-terminus of $\rho 1$ together with the human $\alpha 1$, $\alpha 5$ and $\beta 1$ subunits of the $GABA_A$ receptor showed no physical interaction of $GABA_A$ receptors and ρ subunits in vitro [60]. Furthermore, exchanging the N-terminal domain of the human $\rho 1$ subunit with the corresponding region of the $GABA_A$ receptor $\beta 1$ subunit ($\beta 1\rho 1$) produced a chimeric subunit that could be co-precipitated with the $\alpha 1$ subunit of the $GABA_A$ receptor. Co-expressing the opposite construct (N-terminus of $\rho 1$ and C-terminus of $\beta 1$, $\rho 1\beta 1$) with the wildtype $\rho 1$ subunit in *Xenopus* oocytes eliminated Cl^- currents, whereas co-expression of wildtype $\beta 1$ and $\rho 1$ produced normal GABA-gated currents [60]. Together, these studies suggest that ρ subunits do not assemble with $GABA_A$ or glycine receptor subunits into functional receptors due to different assembly signals in their N-termini.

In contrast to the assembly properties of ρ subunits described above, a mutant rat $\rho 1$ subunit has been reported to assemble with the $GABA_A$ receptor subunit $\gamma 2S$ and the glycine receptor subunit $\alpha 1$ [61]. However, because the $\rho 1$ subunit was mutated in the channel-forming domain (TM2, T314A) and subsequently showed spontaneous channel opening in the absence of agonists as well as mixed inward and outward currents at different GABA concentrations, these experiments do not represent the in vivo situation. Therefore, it is difficult to draw a conclusion from these results regarding the subunit composition of wildtype $GABA_C$ receptors.

In addition to biochemical and electrophysiological data, there is anatomical evidence that ρ subunits do not interact with $GABA_A$ or glycine receptor subunits. Double immunostaining in the rat retina for ρ subunits and for the $\alpha 1,2,3$ and $\beta 2,3$ subunits of the $GABA_A$ receptor as well as for the $\alpha 1,2,3$ and β subunits of the glycine receptor and the glycine receptor associated protein gephyrin showed no colocalization on the light microscopic level [62].

Given evidence that (a) co-expression of a number of $GABA_A$ and glycine receptor subunits with the $\rho 1$ subunit does not alter functional characteristics of the $\rho 1$ subunit in different expression systems; (b) no physical interaction can be detected in immunoprecipitations of in vitro translated subunits; and (c) ρ subunits are not be colocalized anatomically with $GABA_A$ and glycine receptor subunits, it seems unlikely that "mixed" receptor complexes are present in vivo. Therefore, although ρ subunits as well as a number of subunits of the $GABA_A$ and glycine receptor are expressed in bipolar cells of the mammalian retina (ρ subunits: [36,38,63]; $GABA_A$ receptor subunits: [64,65]; glycine receptor subunits: [66,67]), these subunits are likely to assemble in distinct receptor types (Fig. 4). Taken together, these data provide strong evidence that ρ subunits are physically and functionally distinct from subunits of $GABA_A$ and glycine receptors and should therefore be classified as $GABA_C$ receptor subunits.

6. Structure/function studies

Based on the likely composition of $GABA_C$ receptors, identification of functional domains in ρ subunits provides insight into structure/function relationships of $GABA_C$ receptors in vivo. Pharmacological characteristics for ρ subunits that have been investigated include their sensitivity to the Cl^- channel blocker picrotoxinin and to divalent cations such as Zn^{2+}, as well as the structure of the high affinity GABA binding pocket. As noted above homooligomeric GABA receptors composed of the $\rho 1$ or $\rho 2$ subunit differ in the inhibitory effect for picrotoxinin, a drug classically known to block the Cl^- channel of glycine and GABA receptors [8,68]. In addition to the non-competitive inhibition of

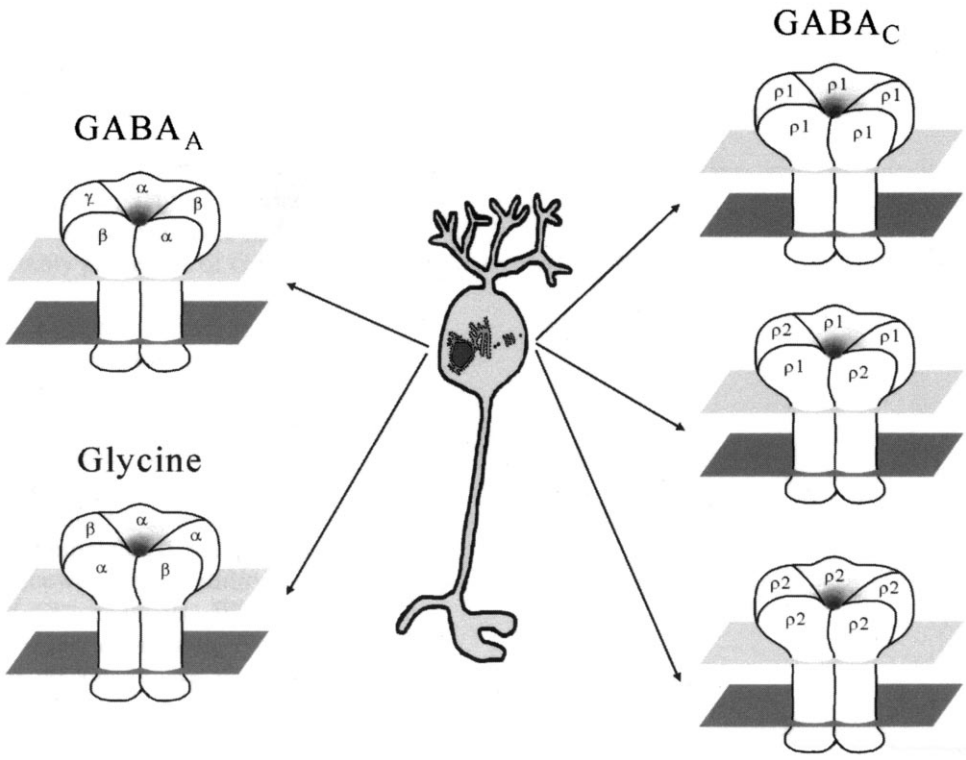

Fig. 4. Proposed heterogeneity of inhibitory ligand-gated ion channels in a mammalian bipolar cell. Various subunits of the $GABA_A$ and glycine receptor as well as ρ subunits are expressed in this cell type. Although all subunits can form multimeric protein complexes, the most likely situation is that subunits of the same class form distinct pentamers. ρ subunits are thought to form heterooligomeric receptors of differing stoichiometry as well as homooligomeric receptors.

$GABA_C$ receptors by channel occlusion, picrotoxinin displays also a degree of competitive inhibition [69]. Therefore, the action of this inhibitor has to be interpreted in the light of both components. Interestingly, native $GABA_C$ receptors of various species have different responses to picrotoxinin (for example: [21,27,24]). For an excellent overview of picrotoxinin inhibition of $GABA_C$-gated currents see refs. Bormann andFeigenspan [23] or Lukasiewicz [70].

Several attempts have been made to determine the molecular domains important for picrotoxinin action in ρ subunits. In the human $\rho 1$ subunit, mutation of a proline in the channel forming transmembrane domain into a serine (P309S) resulted in a ten-fold increase of picrotoxinin sensitivity when expressed in *Xenopus* oocytes and when saturating GABA concentration were used [69]. In the rat $\rho 1$ subunit, a similar mutation (P310S) produced an increase of picrotoxinin sensitivity when expressed in *Xenopus* oocytes [31], consistent with the finding in human $\rho 1$. In contrast, when the human construct was expressed in HEK 293 cells and tested at half-maximal activating GABA concentrations, a four-fold decrease in sensitivity for the blocker was observed compared with the wildtype [44]. Because position 309 in TM2 is also critical for the competitive effect of picrotoxinin [69], the contrary results between *Xenopus* oocytes and HEK 293 cells might be due to different GABA concentrations co-applied with the blocker. Interestingly, a similar residue (V257) was identified to be at or near the binding site for picrotoxinin in the TM2 of the $\alpha 1$ subunit of the $GABA_A$ receptor [71]. The authors used the substituted-cystein-accessibility method, where consecutive residues in the critical protein region are one at a time mutated to cysteine. After expressing the constructs in *Xenopus* oocytes, cysteine residues exposed to the channel lumen can be chemically modified by sulfhydryl-specific reagents applied in the extracellular solution, thus altering the electrical properties of the Cl^- channel. If, however, picrotoxinin binds in the Cl^- channel, the molecule will protect residues in this region in reacting with the applied chemical reagents, resulting in a footprint of the picrotoxinin binding site. In this way, Xu and coworkers demonstrated that C257 (similar to P309 of the $\rho 1$ subunit) was protected from chemical modification by picrotoxinin.

Additional residues critical for picrotoxinin sensitivity have been identified in rat ρ subunits [31]. Mutation of a threonine of the rat $\rho 1$ subunit into a methionine (T314M), the amino acid present in the rat $\rho 2$ subunit at this site, created homooligomeric GABA receptors with only very small Cl^- currents. However, co-expression of the mutant with wildtype rat $\rho 1$ showed a decrease in picrotoxinin sensitivity compared with the

homooligomeric expressed $\rho 1$ subunit. The same effect was found after co-expression of the rat $\rho 2$ subunit with $\rho 1$. In both experiments the reduction of picrotoxinin action was proportional to the amount of mutant $\rho 1$ or $\rho 2$ coinjected with wildtype $\rho 1$. Because the $GABA_C$ receptor in the rat appears to be relatively insensitive against picrotoxinin [rat: 100 μM picrotoxinin at saturating GABA concentrations block 19% of the maximal current [21] versus perch: 500 μM picrotoxinin at saturating GABA concentrations block 100% of the maximal current [27]], the authors concluded that the $GABA_C$ receptor in the rat might be a heterooligomeric protein composed of $\rho 1$ and $\rho 2$ subunits.

The homooligomeric expressed $\rho 1$ subunit also demonstrates distinct sensitivity to several substances such as ethanol [72], protons [32], glycine and β-alanine [73] and halogenated fatty acids [74]. However, only for the action of divalent cations, such as Zn^{2+} has a site of interaction been identified [75]. Zinc, present in synaptic terminals of photoreceptors [76] can act as a modulator for $GABA_C$ receptors and heterologous expressed ρ subunits [81,77,25,75,78–80]. GABA-gated currents of the homooligomeric expressed human $\rho 1$ subunit in *Xenopus* oocytes could be reduced after coapplication of $ZnCl_2$. Change in extracellular pH was found to alter this effect, proposing that histidine residues might play a role in Zn^{2+} binding to ρ subunits. Indeed, mutation of a histidine in the N-terminal extracellular domain of the protein into a tyrosine (H156Y) abolished the Zn^{2+} sensitivity of the expressed construct completely, although other pharmacological and electrical properties did not change. The same residue was also found to be important for the action of other divalent cations such as Ni^{2+}, Cu^{2+} and Cd^{2+} [80,69].

To investigate the molecular basis for the high agonist affinity of $GABA_C$ receptors [23,49] identified five amino acids in the N-terminus of the human $\rho 1$ subunit between the conserved cysteine loop and TM1 that seem to be important for agonist binding. Mutations in any of these residues (Y198, Y200, Y241, T244, Y247) reduced the GABA affinity. The amino acids are grouped in two domains that have a similar position as the recently proposed agonist binding domain of the $GABA_A$ receptor $\beta 2$ subunit [82]. An additional study mutated several residues in the human $\rho 1$ N-terminus as well as in the extracellular loop between TM2 and TM3, that were identical between $\rho 1$ and $\rho 2$ but distinct from other members of the ligand-gated ion channel family [83]. Whereas several mutations resulted in not functional GABA receptors, others decreased the sensitivity for GABA (Q189H, H141A) or increased cooperativity of GABA binding (R316A). However, it remains difficult to draw a picture of the agonist binding pocket of $GABA_C$ receptors from these data.

7. Possible combinations of ρ subunits in vivo

In the retina of different species, ρ subunits are mainly expressed in bipolar cells (rat, cat, rabbit, monkey: [36,38]; cat, goldfish, chicken: [39]; chicken: [35]) and horizontal cells (perch: [37]; chicken: [35]). The most extensive studies performed so far have been in the rat, where $\rho 1$ and $\rho 2$ transcripts were localized in rod bipolar cells by RT–PCR and in-situ hybridization [36]. The corresponding protein could be detected on dendrites and axon terminals of rod and cone bipolar cells using a ρ subunit specific immunserum [38]. In addition, it has been shown by RT–PCR that $\rho 1$ and $\rho 2$ proteins were coexpressed in 11 of 17 rod bipolar cells examined [63]. Message for $\rho 2$ has also been found in 5 of 8 examined ganglion cells by RT–PCR, although no $GABA_C$-like currents could be detected in the same cells, leaving it unclear if $\rho 2$ is expressed at sufficient levels to form functional $GABA_C$ receptors [63].

Outside the retina the $\rho 2$ subunit seems to be more abundant than $\rho 1$ and was detected by RT–PCR in different brain regions [36]. Therefore $GABA_C$ receptors in these regions could be dominantly or perhaps even alone composed of the $\rho 2$ subunit or alternatively of $\rho 2$ and $\rho 3$. For the latter subunit an expression profile has been reported only for the retina [92].

Why might $GABA_C$ receptors be heterooligomers of ρ subunits rather than homooligomers? In $GABA_A$ and glycine receptors, combinations of different subunits into one oligomeric protein complex gives rise to different properties. For example, picrotoxinin sensitivity of the glycine receptor is critically dependent on the presence of the glycine receptor β subunit [68]. Furthermore, targeting of receptors to distinct locations within a neuron can depend on its subunit composition. Whereas $GABA_A$ receptors composed of $\alpha 1\beta 1$ subunits show no special targeting, substitution of $\beta 1$ with $\beta 2$ or $\beta 3$ routes the receptor always to the basolateral site of polarized cells [84]. By analogy, the presence of various ρ subunits could result in a heterogeneous population of $GABA_C$ receptors with biophysical and pharmacological properties as well as location determined by its ρ subunit composition. Indeed $GABA_C$ receptors in the rat retina alter their sensitivity for the Cl^- channel blocker picrotoxinin depending on the assembled ρ subunits [31].

Regarding the assembly properties discussed above, a typical $GABA_C$ receptor could be composed of a mixture of different ρ subunits. Alternatively, homooligomeric $GABA_C$ receptors formed of different ρ subunits could also be present (Fig. 4, right side). Single-channel patch-clamp experiments or subunit-specific histochemical and pharmacological tools must be developed to help solve these issues. To this end, Miledi and coworkers have designed a new $GABA_C$ specific

antagonist [85]. Using this approach it should be possible to design additional subunit specific drugs which help to investigate the molecular composition of $GABA_C$ receptors.

8. Function of $GABA_C$ receptors in the retina

Because the same retinal cells that express $GABA_C$ receptors also express $GABA_A$ receptors, the question arises why one cell should have two GABA-gated receptors that activate intrinsic Cl^- channels. The answer probably lies in the different functional properties of each receptor type. For example, in rod bipolar cells of the rat retina, $GABA_C$ receptors are about 8-times more sensitive to GABA than $GABA_A$ receptors. Whereas $GABA_A$ receptors show a fast peak after agonist application and thus are characterized by transient responses, $GABA_C$ receptors mediate smaller but sustained currents with a slower onset. On the single channel level, $GABA_C$ receptors show a mean open time of 150 ms and a conductance of 8 pS in contrast to $GABA_A$ receptors (25 ms, 27 pS; [21]). Thus, $GABA_C$ receptors would activate at lower GABA concentrations with a prolonged response compared with $GABA_A$ receptors.

How could the different response properties of $GABA_A$ and $GABA_C$ receptors to agonists influence neurotransmission in the retina? It has been shown that bipolar cell terminals receive GABAergic input from amacrine cells [86] and that synaptic transmission from bipolar to ganglion cells is modulated by $GABA_C$ and $GABA_A$ receptors [87,88]. Therefore, the low agonist affinity of $GABA_C$ receptors on bipolar cell axon terminals may enable fine control over the excitability of these cells without interfering with the more transient responding $GABA_A$ receptors on bipolar or ganglion cells. In this way amacrine cells could specifically modulate bipolar cell output onto ganglion cells. This may be important for the receptive field properties of ganglion cells, as, for example, their center–surround organization, that contributes to contrast sensitivity of the retina. $GABA_C$ receptors may have the same effect at bipolar cell dendrites since horizontal cells of many species are reported to be GABAergic. Furthermore, the prolonged response of $GABA_C$ receptors compared with $GABA_A$ receptors might enable amacrine or horizontal cells to have a tonic inhibitory influence in addition to the transient Cl^- currents generated by $GABA_A$ receptors, thus providing an additional effect upon GABA mediated neurotransmission. Interestingly, the non-spiking retinal neurons, such as horizontal and bipolar cells, express $GABA_C$ receptors, whereas so far $GABA_C$ type currents have not been detected in spiking neurons (ganglion and amacrine cells). Thus, $GABA_C$ receptors may play a role in regulating the excitability of retinal neurons transmitting graded potentials.

9. Perspectives

Two main questions will be of specific interest for future investigation. First, the mechanisms that govern ρ subunit assembly into $GABA_C$ receptors should be clarified. Since only two or three ρ subunits appear to exist in the mammalian retina, $GABA_C$ receptors may be less complex than $GABA_A$ receptors, providing a workable system to resolve their molecular structure. A second area will be to test the proposed function of $GABA_C$ receptors. Two strategies can be envisioned. First, the generation of knock-out mice would provide a useful model system to study the role of $GABA_C$ receptors in vivo as well as in slice preparations. However, there exist numerous difficulties with this approach. If the situation in mice is the same as in rat, generating a knock-out mouse for $GABA_C$ receptors may require the elimination of the function of three genes ($\rho 1$, $\rho 2$ and $\rho 3$). Furthermore, Northern blots have revealed expression of the $\rho 1$ subunit outside of the retina [29], leaving it unclear if such a mouse will be viable.

A second strategy could be to suppress $GABA_C$ receptor expression in the retina only. This could be done either by RNA antisense methods or by using a ''dominant-negative'' approach. Specific suppression of neuronal proteins using antisense oligonucleotides against the appropriate mRNA has been achieved (for example, gephyrin: [89]). Alternatively, protein domains that inhibit the assembly of ρ subunits into functional $GABA_C$ receptors could be expressed in retinal cells suppressing $GABA_C$ responses. As described in this review, the N-terminal domains of $\rho 1$ and $\rho 2$ subunits are sufficient for subunit assembly in vitro and are capable of eliminating GABA-gated Cl^- currents when coexpressed with full-length ρ subunits in heterologous expression systems. The same assembly domains are likely to be present in the $\rho 3$ subunit. Therefore, delivery and expression of an N-terminal protein fragment to the appropriate retinal cells should inhibit the formation of $GABA_C$ receptors (Fig. 3) and thus provide a useful model to study the role of $GABA_C$ receptors in vivo.

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References

[1] Sivilotti L, Nistri A. GABA receptor mechanism in the central nervous system. Prog Neurobiol 1991;36:35–92.

[2] Davies PA, Hanna MC, Hales TG, Kirkness EF. Insensitivity of anesthetic agents conferred by a class of $GABA_A$ receptor subunit. Nature 1997;385:820–3.

[3] Garret M, Bascles L, Boue-Grabot E, Sartor P, Charron G, Bloch B, Margolskee RF. An mRNA encoding a putative GABA-gated chloride channel is expressed in the human cardiac conduction system. J Neurochem 1997;68:1382–9.

[4] Bormann J, Hamill OP, Sakmann B. Mechanism of anion permeation through channels gated by glycine and γ-aminobutyric acid in mouse cultured spinal neurons. J Physiol 1987;385:243–86.

[5] Bormann J. Electrophysiology of $GABA_A$ and $GABA_B$ receptor subtypes. Trends Neurosci 1988;11:112–6.

[6] Doble A, Martin IL. Multiple benzodiazepine receptors: no reason for anxiety. Trends Pharmacol Sci 1992;13:76–81.

[7] Macdonald RL, Olsen RW. $GABA_A$ receptor channels. Ann Rev Neurosci 1994;17:569–602.

[8] Akaike N, Hattori K, Oomura Y, Carpenter DO. Bicuculline and picrotoxinin block γ-aminobutyric acid-gated Cl^- conductance by different mechanisms. Experientia 1985;41:70–1.

[9] Wisden W, Seeburg P. $GABA_A$ receptor channels: from subunits to functional entities. Curr Opin Neurobiol 1992;2:263–9.

[10] Kaupmann K, Huggel K, Heid J, et al. Expression cloning of $GABA_B$ receptors uncovers similarity to metabotropic glutamate receptors. Nature 1997;386:239–46.

[11] Bowery N. $GABA_B$ receptors and their significance in mammalian pharmacology. Trends Pharmacol Sci 1989;10:401–7.

[12] Johnston GAR, Curtis DR, Beart PM, Game CJA, McCulloch RM, Twitchin B. *Cis* and *trans*-4-aminocrotonic acid as GABA agonist of restricted conformation. J Neurochem 1975;24:157–60.

[13] Nistri A, Sivilotti L. An unusual effect of γ-aminobutyric acid on synaptic transmission of frog tectal neurons in vitro. Br J Pharmacol 1985;85:917–22.

[14] Drew CA, Johnston G, Weatherby RP. Bicuculline-insensitive GABA receptors: studies on the binding of (−)-baclofen to rat cerebellar membrane. Neurosci Lett 1984;52:317–21.

[15] Sivilotti L, Nistri A. Pharmacology of a novel effect of γ-aminobutyric acid on the frog optic tectum in vitro. Eur J Pharmacol 1989;164:205–12.

[16] Drew CA, Johnston G. Bicuculline- and baclofen-insensitive γ-aminobutyric acid binding to rat cerebellar membranes. J Neurochem 1992;58:1087–92.

[17] Strata F, Cherubini E. Transient expression of a novel type of GABA response in rat CA3 hippocampal neurons during development. J Physiol 1994;480:493–503.

[18] Polenzani L, Woodward RM, Miledi R. Expression of mammalian γ-aminobutyric acid receptors with distinct pharmacology in *Xenopus* oocytes. Proc Natl Acad Sci USA 1991;88:4318–22.

[19] Woodward RM, Polenzani L, Miledi R. Characterization of bicuculline/baclofen-insensitive γ-aminobutyric acid receptors expressed in *Xenopus* oocytes I. Effect of Cl^- channel inhibitors. Mol Pharmacol 1992;42:165–73.

[20] Woodward RM, Polenzani L, Miledi R. Characterization of bicuculline/baclofen-insensitive (ρ-like) γ-aminobutyric acid receptors expressed in *Xenopus* oocytes. II. Pharmacology of γ-aminobutyric $acid_A$ and γ-aminobutyric $acid_B$ receptor agonists and antagonists. Mol Pharmacol 1993;43:609–25.

[21] Feigenspan A, Wässle H, Bormann J. Pharmacology of GABA receptor Cl^- channels in rat retinal bipolar cells. Nature 1993;361:159–62.

[22] Feigenspan A, Bormann J. Differential pharmacology of $GABA_A$ and $GABA_C$ receptors on rat retinal bipolar cells. Eur J Pharmacol 1994;288:97–104.

[23] Bormann J, Feigenspan A. $GABA_C$ receptors. Trends Neurosci 1995;11:112–6.

[24] Lukasiewicz PD, Maple BR, Werblin FS. A novel GABA receptor on bipolar cell terminals in the tiger salamander retina. J Neurosci 1994;14:1202–12.

[25] Qian H, Dowling JE. $GABA_A$ and $GABA_C$ receptors on hybrid bass retinal bipolar cells. J Neurophysiol 1995;74:1920–8.

[26] Euler T, Schneider H, Wässle H. Glutamate responses of bipolar cells in a slice preparation of the rat retina. J Neurosci 1996;16(9):2934–44.

[27] Qian H, Dowling JE. Novel GABA responses from rod-driven retinal horizontal cells. Nature 1993;361:162–4.

[28] Qian H, Dowling JE. Pharmacology of novel GABA receptors found on rod horizontal cells of the white pearch retina. J Neurosci 1994;14:4299–307.

[29] Cutting GR, Lu L, O'Hara BF, et al. Cloning of the γ-aminobutyric acid (GABA) ρ_1 cDNA: a GABA receptor subunit highly expressed in the retina. Proc Natl Acad Sci USA 1991;88:2673–7.

[30] Cutting GR, Curristin S, Zoghbi H, O'Hara BF, Seldin MF, Uhl GR. Identification of a putative γ-aminobutyric acid (GABA) receptor subunit ρ_2 cDNA and colocalization of the genes encoding rho_2 (GABRR2) and rho_1 (GABRR1) to human chromosome 6q14-q21 and mouse chromosome 4. Genomics 1992;12:801–6.

[31] Zhang D, Pan Z-H, Zhang X, Brideau AD, Lipton S. Cloning of a γ-aminobutyric acid type C receptor subunit in rat retina with a methionine residue critical for picrotoxinin channel block. Proc Natl Acad Sci USA 1995;92(11):756–60.

[32] Wegelius K, Reeben M, Rivera C, Kaila K, Saarma M, Pasternack M. The ρ1 GABA receptor cloned from the rat retina is down-modulated by protons. Neuroreport 1996;7:2005–9.

[33] Ogurusu T, Taira H, Shingai R. Identification of $GABA_A$ receptor subunits in the rat retina: cloning of the rat $GABA_A$ receptor ρ2 subunit cDNA. J Neurochem 1995;65:964–8.

[34] Ogurusu T, Shingai R. Cloning of a putative γ-aminobutyric acid (GABA) receptor subunit ρ3 cDNA. Biochim Biophys Acta 1996;1305:15–8.

[35] Albrecht BE, Darlison MG. Localization of the rho1- and rho2 subunit messenger RNAs in chick retina by in situ hybridization predicts the existence of gamma-aminobutyric acid type C receptor subtypes. Neurosci Lett 1995;189:155–8.

[36] Enz R, Brandstätter H, Hartveit E, Wässle H, Bormann J. Expression of GABA receptor subunits ρ1 and ρ2 in the retina and brain of the rat. Eur J Neurosci 1995;7:1495–501.

[37] Qian H, Hyatt G, Schanzer A, Hazra R, Hackam AS, Cutting GR, Dowling JE. A comparison of $GABA_C$ and ρ subunit receptors from the white perch retina. Vis Neurosci 1997;14:843–851.

[38] Enz R, Brandstätter JH, Wässle H, Bormann J. Immunocytochemical localization of $GABA_C$ receptor ρ subunits in the mammalian retina. J Neurosci 1996;16(14):4479–90.

[39] Koulen P, Brandstätter JH, Kröger S, Enz R, Bormann J, Wässle H. Immunocytochemical localization of the $GABA_C$ receptor ρ subunits in the cat, goldfish and chicken retina. J Comp Neurol 1997;380:520–32.

[40] Wang T, Guggino WB, Cutting GR. A novel gamma-aminobutyric acid receptor subunit (rho2) cloned from human retina forms bicuculline-insensitive homooligomeric receptors in *Xenopus* oocytes. J Neurosci 1994;14:6524–31.

[41] Sigel E, Baur R, Trube G, Mohler H, Malherbe P. The effect of subunit composition of rat brain $GABA_A$ receptors on channel function. Neuron 1990;5:703–11.

[42] Shimada S, Cutting G, Uhl GR. γ-Aminobutyric acid A or C receptor? γ-Aminobutyric acid ρ1 receptor RNA induces bicuculline-, barbiturate- and benzodiazepine-insensitive γ-aminobutyric acid responses in *Xenopus* oocytes. Mol Pharmacol 1992;41:683–7.
[43] Kusama T, Spivak CE, Whiting P, Dawson VL, Schaeffer JC, Uhl GR. Pharmacology of GABA ρ1 and GABA α/β receptors expressed in *Xenopus* oocytes and COS cells. Br J Pharmacol 1993;109:200–6.
[44] Enz R, Bormann J. A single point mutation decreases picrotoxinin sensitivity of the human GABA receptor ρ1 subunit. Neuroreport 1995;6(11):45–8.
[45] Seeburg PH, Wisden W, Verdoorn TA, et al. The $GABA_A$ receptor family: molecular and functional diversity. Cold Spring Harbor Symposia on Quantitative Biology, vol. LV. Cold Spring Harbor Laboratory Press, 1990:29–39.
[46] Amin J, Weiss DS. Insights into the activation mechanism of ρ1 GABA receptor obtained by coexpression of wild type and activation-impaired subunits. Proc R Soc London 1996;263:273–82.
[47] Unwin N. Acetylcholine receptor channel imaged in the open state. Nature 1995;373:37–43.
[48] Hackam AS, Wang T-L, Guggino WB, Cutting GR. The N-terminal domain of human GABA receptor ρ1 subunits contains signal for homooligomeric and heterooligomeric interaction. J Biol Chem, 1997;272:13750–13757.
[49] Amin J, Weiss DS. Homomeric ρ1 GABA channels: activation properties and domains. Recept Channels 1994;2:227–36.
[50] Wang T, Hackam AS, Guggino WB, Cutting GR. A single histidine residue is essential for zinc inhibition of GABA ρ1 receptors. J Neurosci 1995;15:7684–91.
[51] Hackam, AS, Wang, T-L, Guggino, WB, Cutting GR. A 100 amino acid region of the GABA ρ1 subunit confers robust homo-oligomeric expression. Neuroreport 1997;8:1425–1430.
[52] Kusama T, Wang T, Guggino WB, Cutting GR, Uhl GR. GABA ρ2 receptor pharmacological profile: GABA recognition site similarities to ρ1. Eur J Pharmacol 1993;245:83–4.
[53] Gu Y, Camacho P, Gardner P, Hall ZW. Identification of two amino acid residues in the ϵ subunit that promote mammalian muscle acetylcholine receptor assembly in COS cells. Neuron 1991;6:879–87.
[54] Verrall S, Hall ZW. The N-terminal domains of acetylcholine receptor subunits contain recognition signals for the initial steps of receptor assembly. Cell 1992;68:23–31.
[55] Kuhse J, Laube B, Magalei D, Betz H. Assembly of the inhibitory glycine receptor: identification of amino acid sequence motives governing subunit stoichiometry. Neuron 1993;11:1049–56.
[56] Korpi ER, Tuner T, Kristo P, Koehler M, Herb A, Lueddens H, Seeburg PH. Small N-terminal deletion by splicing in cerebellar α6 subunit abolishes $GABA_A$ receptor function. J Neurochem 1994;63:1167–70.
[57] Kirsch J, Meyer G, Betz H. Synaptic targeting of ionotropic neurotransmitter receptors. Mol Cell Neurosci 1996;8:93–8.
[58] Steward O. mRNA localization in neurons: a multipurpose mechanism? Neuron 1997;18:9–12.
[59] Brakeman PR, Lanahan AA, O'Brian R, Roche K, Barnes CA, Huganir RL, Worley PF. Homer: a protein that selectively binds metabotropic glutamate receptors. Nature 1997;386:284–8.
[60] Hackam AS, Wang T-L, Guggino, WB, Cutting GR. Sequences in the amino termini of GABA ρ and $GABA_A$ subunits specify their selective interaction in vitro. J Neurochem 1998;70:40–6.
[61] Pan Z-H, Zhang D, Zhang X, Lipton S. GABA ρ subunits mutated in the channel domain display spontaneous, GABA-suppressible currents and co-assemble with $GABA_A$ and glycine receptor subunits. Invest Ophthalmol Vis Sci Abstr Book, Vol. 37/3, No. 1941, 1996.
[62] Koulen P, Brandstätter JH, Enz R, Bormann J, Wässle H. Synaptic clustering of $GABA_C$ receptor ρ subunits in the rat retina. Eur J Neurosci 1998;10:115–27.
[63] Yeh HH, Grigorenko EV, Veruki ML. Correlation between a bicuculline-resistant response to GABA and $GABA_A$ receptor ρ subunit expression in single rat bipolar cells. Vis Neurosci 1996;13:283–92.
[64] Grigorenko EV, Yeh HH. Expression profiling of $GABA_A$ receptor β-subunits in the rat retina. Vis Neurosci 1994;11:379–87.
[65] Greferath U, Grunert U, Fritschy JM, Stephenson A, Mohler H, Wässle H. $GABA_A$ receptor subunits have differential distributions in the rat retina: in situ hybridization and immunohistochemistry. J Comp Neurol 1995;353:553–71.
[66] Greferath U, Brandstätter JH, Wässle H, Kirsch J, Kuhse J, Grunert U. Differential expression of glycine receptor subunits in the retina of the rat: a study using immunohistochemistry and in situ hybridization. Vis Neurosci 1994;11:721–9.
[67] Enz R, Bormann J. Expression of glycine receptor subunits and gephyrin in single bipolar cells of the rat retina. Vis Neurosci 1995;12:501–7.
[68] Pribilla I, Takagi T, Langosch D, Bormann J, Betz H. The atypical M2 segment of the β subunit confers picrotoxinin resistance to inhibitory glycine receptor channels. EMBO J 1992;11:4305–11.
[69] Wang T, Hackam AS, Guggino WB, Cutting GR. A single amino acid in gamma-aminobutyric acid ρ1 receptor affects competitive and noncompetitive components of picrotoxinin inhibition. Proc Natl Acad Sci USA 1995;92:11751–5.
[70] Lukasiewicz PD. $GABA_C$ receptors in the vertebrate retina. Mol Neurobiol 1996;12:181–94.
[71] Xu M, Covey DF, Akabas MH. Interaction of picrotoxinin with $GABA_A$ receptor channel-lining residues probed by cysteine mutants. Biophys J 1995;69:1858–67.
[72] Mikic SJ, Harris RA. Inhibition of ρ1 receptor GABAergic currents by alcohols and volatile anesthetics. J Pharmacol Exp Ther 1996;277:411–6.
[73] Calvo DJ, Miledi R. Activation of $GABA_{\rho 1}$ receptors by glycine and β-alanine. Neuroreport 1995;6:1118–20.
[74] DelRaso NJ, Huang Y, Lu L. Effects of chlorotriflouroethylene oligomer fatty acids on recombinant GABA receptors expressed in *Xenopus* oocytes. J Membr Biol 1996;149:33–40.
[75] Wang T, Hackam AS, Guggino WB, Cutting GR. A single Histidine residue is essential for zinc inhibition of GABA ρ1 receptors. J Neurosci 1995b;15:7684–91.
[76] Wu SM, Qiao X, Noebels JL, Yang XL. Localization and modulation action of zinc in the vertebrate retina. Vis Res 1993;33:2611–6.
[77] Chang Y, Amin J, Weiss DS. Zinc is a mixed antagonist of homomeric ρ1 γ-aminobutyric acid-activated channels. Mol Pharmacol 1995;47:595–602.
[78] Dong C-J, Werblin FS. Zinc downmodulates the $GABA_C$ receptor current in cone horizontal cells acutely isolated from the catfish retina. J Neurophysiol 1995;73:916–9.
[79] Dong C-J, Werblin FS. Use-dependent and use-independent blocking actions of picrotoxinin and zinc at the $GABA_C$ receptor in the retinal horizontal cell. Vis Res 1996;36:3997–4005.
[80] Martina M, Mozrzymas JW, Strata F, Cherubini E. Zinc modulation of bicuculline-sensitive and -insensitive GABA receptors in the developing rat hippocampus. Eur J Neurosci 1996;8:2168–76.
[81] Calvo DJ, Vaquez AE, Miledi R. Cationic modulation of ρ1-type γ-aminobutyrate receptors expressed in *Xenopus* oocytes. Proc Natl Acad Sci USA 1994;91:725–9.
[82] Amin J, Weiss DS. $GABA_A$ receptor need two homologous domains of the β subunit for activation by GABA but not by pentobarbital. Nature 1993;366:565–9.

[83] Kusama T, Wang J-B, Spivak CE, Uhl GR. Mutagenesis of the GABA ρ1 receptor alters agonist affinity and channel gating. Neuroreport 1994;5:1209–12.
[84] Conolly CN, Wooltorton JRA, Smart TG, Moss SJ. Subcellular localization of γ-aminobutyric acid type A receptors is determined by receptor β subunits. Proc Natl Acad Sci USA 1996;93:9899–904.
[85] Ragozzino D, Woodward RM, Murata Y, Eusebi F, Overman LE, Miledi R. Design and in vitro pharmacology of a selective γ-aminobutyric acid$_C$ receptor antagonist. Mol Pharmacol 1996;50:1024–30.
[86] Vaney DI. The mosaic of amacrine cells in the mammalian retina. In: Osborne N, Chader J, editors. Progress in Retinal Research. Oxford: Pergamon, 1990:49–100.
[87] Lukasiewicz PD, Werblin FS. A novel GABA receptor modulates synaptic transmission from bipolar to ganglion and amacrine cells in the tiger salamander retina. J Neurosci 1994;14:1213–23.
[88] Wellis PD, Werblin FS. Dopamine modulates GABA$_C$ receptors mediating inhibition of calcium entry into and transmitter release from bipolar cell terminals in tiger salamanders. J Neurosci 1995;15:4748–61.
[89] Kirsch J, Wolters I, Triller A, Betz H. Gephyrin antisense nucleotides prevent glycine receptor clustering in spinal neurons. Nature 1993;366:745–8.
[90] Shingai R, Yanagi K, Fukushima T, Sakata K, Ogurusu T. Functional expression of GABA rho 3 receptors in *Xenopus* oocytes. Neurosci Res 1996;26:387–90.
[91] Lukasiewicz PD, Wong ROL. GABA$_C$ receptors on ferret retinal bipolar cells: a diversity of subtypes in mammals? Vis Neurosci 1997;14:989–94.
[92] Ogurusu T, Eguchi G, Shingai R. Localization of γ-aminobutyric acid (GABA) receptor ρ3 subunit in the rat retina. Neuroreport 1997;8:925–27.