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COMMENTARY

THE $GABA_A$ RECEPTOR-GATED ION CHANNEL: BIOCHEMICAL AND PHARMACOLOGICAL STUDIES OF STRUCTURE AND FUNCTION

ROCHELLE D. SCHWARTZ*
Department of Pharmacology, Duke University Medical Center, Durham, NC 27710, U.S.A.

Over the past several years neurobiologists have engaged in intense study of neurotransmitter receptor-gated ion channels. Their presence in neuronal and non-neuronal membranes of both invertebrates and vertebrates indicates their importance in maintaining and regulating a variety of cellular functions. One of the most thoroughly studied neurotransmitter receptor-gated ion channels is the nicotinic acetylcholine receptor (nACHR). Because of the abundant supply of nACHR provided by the *Torpedo* electric fish, this receptor has been purified, and its subunits have been sequenced (amino acid), cloned and functionally reconstituted [1–5]. These molecular biological approaches, when combined with classical pharmacologic studies using receptor binding, ion flux and electrophysiologic techniques, have given us a detailed picture of the structure and function of the nACHR. It is not surprising, therefore, that similar approaches are now being used to study the structural and functional characteristics of other ion channels activated by neurotransmitters such as γ-aminobutyric acid (GABA), glycine and glutamate. One of the most interesting aspects of these studies is that some structural and functional properties are shared among the nicotinic, GABA and glycine receptor-gated ion channels (regardless of their ion selectivities) [6–8]. The evidence is particularly striking for structural similarities between the nicotinic and GABA [6] and nicotinic and glycine [7] receptor-gated ion channels, leading researchers to propose that neurotransmitter receptor-gated ion channels may have evolved from a common gene pool [6, 7]. This article presents recent data from biochemical and pharmacological studies of GABA receptor-gated chloride (Cl^-) channel structure and function which contribute to the concept of super families of ligand-gated ion channels [6, 7, 9].

Structural properties of the $GABA_A$ receptor complex

Successful identification of the structural properties of the $GABA_A$ (muscimol- and bicuculline-sensitive, baclofen-insensitive) receptor complex in brain has been largely dependent on the availability of pharmacologic data from functional and receptor binding studies. Initially, electrophysiologists demonstrated that the inhibition of neuronal activity by GABA could be modulated by various depressant/anticonvulsant and convulsant agents (e.g. benzodiazepines, barbiturates and picrotoxin) [see Refs. 10–13 for review]. Subsequent receptor binding studies indicated that these agents interact allosterically with GABA recognition sites in a Cl^--dependent manner [see Refs. 13–15 for review]. Such studies provided a starting point for structural analyses of the $GABA_A$ receptor protein from mammalian brain. For instance, investigators have taken advantage of the association between benzodiazepine recognition sites with the $GABA_A$ receptor complex and used benzodiazepine affinity chromatography to purify $GABA_A$ receptors to homogeneity [16–23]. The binding characteristics of purified $GABA_A$ receptors are modulated by benzodiazepines, barbiturates and convulsants [e.g. *t*-butylbicyclophosphorothionate (TBPS)] in a Cl^--dependent manner, indicating that GABA, benzodiazepine and TBPS recognition sites are copurified with a Cl^- channel [20, 22]. Thus, the $GABA_A$ receptor, comprising several recognition sites and an ion channel, has been purified in sufficient quantities so that its molecular and structural properties can be studied in more detail. (See Fig. 1 for a schematic representation.)

Subunit composition of the $GABA_A$ receptor complex. The subunit composition of the $GABA_A$ receptor complex in brain has been studied with a variety of techniques. Target size analysis (irradiation inactivation) has been used to determine the apparent molecular weight (M_r) of $GABA_A$ and benzodiazepine binding proteins *in situ* [24–28]. Subunit target sizes of 45,000–57,000 daltons have been reported for GABA and benzodiazepine binding sites in frozen membranes [24–27]. In lyophilized membranes, larger target sizes have been observed (M_r 220,000) [28] which are consistent with the M_r of the purified $GABA_A$ receptor oligomer determined by gel filtration and sucrose density gradient analysis [29]. Target size analysis of GABA and benzodiazepine binding sites performed in the same membrane preparation reveals that these recognition sites reside on subunits of the same size (but see below) [25–27]. However, such studies cannot ascertain whether these two recognition sites reside on the same subunit. Target size analysis also indicates that the cage convulsant, TBPS, which labels the GABA receptor Cl^- ionophore [30, 31], binds to a subunit(s) of approximately 140,000 daltons [25–27]. This entity could consist of subunits containing recognition sites for GABA and benzodiazepines, as

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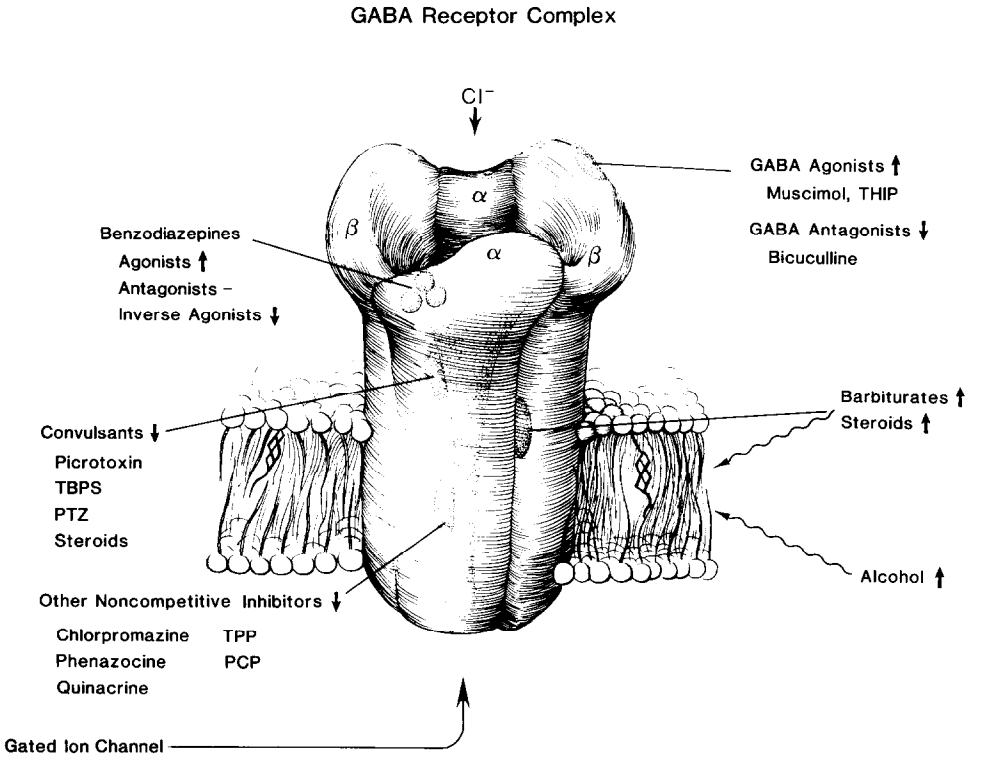

Fig. 1. Schematic model of the $GABA_A$ receptor complex. This model is not meant to indicate the subunit assembly or the location and stoichiometry of the various recognition sites associated with the subunits. Arrows indicate the enhancement (↑) or inhibition (↓) of GABAergic function by various agents. Abbreviations: GABA, γ-aminobutyric acid; THIP, 4,5,6,7-tetrahydroisoxazolo[5,4-c]pyridin-3-ol; TBPS, *t*-butylbicyclophosphorothionate; PTZ, pentylene tetrazole; TPP, tetraphenylphosphonium; and PCP, phencyclidine.

well as another unidentified subunit [25–27]. The irradiation inactivation studies suggest that a fully functional protein species of approximately 548,000 daltons is necessary to observe modulation of TBPS binding by benzodiazepines and pyrazolopyridazines [27].

More specific information on GABA receptor subunit M_r has been obtained from biochemical studies of purified GABA receptors. When purified $GABA_A$ receptor preparations are subjected to sodium dodecyl sulfate (SDS) polyacrylamide gel electrophoresis, two major protein subunits of molecular weights (M_r) 50,000–53,000 daltons (α) and 55,000–58,000 daltons (β) are resolved [16–23]. Incubation of either brain membranes or a purified receptor preparation with low concentrations of [^{3}H]flunitrazepam in the presence of UV light results in apparently selective photoaffinity labeling of the α subunit [23, 32–36]. Minimal photolabeling of the β subunit has been detected under other conditions [16, 35, 36], although Sweetnam and Tallman [37] have suggested that this could represent an artifact of the photolabeling process.

The M_r of the [^{3}H]flunitrazepam photolabeled subunit (50,000–53,000) is decreased after incubation of membranes or purified receptors with glycosidases [33, 37], indicating that the subunits carrying benzodiazepine recognition sites are glycosylated. Sweetnam and Tallman [37] have proposed that differing degrees of α subunit glycosylation in different brain regions may underlie benzodiazepine receptor heterogeneity. In other studies, a small degree of glycosylation of the β subunit has also been detected [33].

Techniques have been developed recently to photolabel the $GABA_A$ receptor complex with [^{3}H]muscimol. In rat cerebellar membranes, the $GABA_A$ agonist, [^{3}H]muscimol, photolabels presumably the α subunit (M_r 50,000–52,000) [38, 39], while in purified receptors from bovine cortex only the β subunit (M_r 57,000) is photolabeled by [^{3}H]muscimol [33, 36, 40]. These studies suggest that the benzodiazepine and GABA recognition sites exist on separate subunits (α and β respectively), although coexistence (perhaps region-specific) of these two sites on the β subunit remains a possibility.

The subunit composition of the $GABA_A$ receptor has been analyzed further using polyclonal or monoclonal antibodies which recognize the purified receptor protein [33, 41–43]. Haring *et al.* [41] have identified monoclonal antibodies to bovine $GABA_A$ receptors which exclusively recognize either the α or the β subunits in rat, bovine and human brain. Since antibodies specific for either the α or β subunit immunoprecipitated both GABA and benzodiazepine recognition sites, the authors concluded

that the $GABA_A$ receptor complex comprises both the α and β subunits. In other studies, Mamalaki *et al.* [33] have identified a monoclonal antibody which recognizes both the α and β subunits and concluded that there must be some degree of structural homology between the two subunits. In addition, both groups of investigators [33, 41] have deduced the subunit stoichiometry to be a tetramer of $\alpha 2\beta 2$, consistent with previous determinations of the native protein M_r (approximately 230,000) [29].

Direct evidence for structural homology between subunits has been provided recently by Schofield *et al.* [6]. Using recombinant DNA techniques to clone the α and β subunits of the $GABA_A$ receptor complex from bovine brain, the authors demonstrated a 35% sequence (amino acid) homology between the two subunits [6]. The deduced subunit sizes (M_r α, 48,000; β, 51,400) are consistent with the molecular sizes of the deglycosylated subunits observed on SDS gels [33, 37].

The proposed subunit composition of the $GABA_A$ receptor complex is also supported by functional reconstitution studies [6]. Schofield *et al.* [6] have elegantly shown that insertion of α and β specific mRNA derived from the cDNA clones into *Xenopus* oocytes results in the expression of functional $GABA_A$ receptors. The reconstituted receptors possess all of the pharmacological properties of native $GABA_A$ receptors. These experiments indicate that both the α and β subunits are required for a functional $GABA_A$ receptor, although the subunit stoichiometry for functional reconstitution cannot be assessed. Since target size analysis suggests that the size of a fully functional protein (at least 400,000 daltons) is larger than an $\alpha 2\beta 2$ tetramer [27], it is possible that additional copies of the α or β subunit, or an as yet unidentified subunit, are necessary to form the complete, functional oligomer as it exists *in situ*.

After sequencing the amino acid structure of both $GABA_A$ receptor subunits, Schofield *et al.* [6] observed a high degree of homology between the subunit amino acid sequences of the $GABA_A$ receptor from bovine brain and the nACHR from *Torpedo* electroplax. The nACHR of the electroplax is a pentameric protein, with a subunit composition of $\alpha 2\beta\alpha\delta$ [1–3]. (Although the structural properties of the brain nACHR have not been fully described, both $\alpha 2\beta 2$ and $\alpha 3\beta 2$ structures have been proposed [5].) The α subunits contain recognition sites for the agonist acetylcholine [1–3, 44], whereas noncompetitive inhibitors of the receptor are thought to act at sites associated with the cation channel [44, 45]. As discussed below, the structural homologies between the subunits of these two receptor-gated ion channels [6] may explain some of the non-specific interactions of several structurally diverse pharmacologic agents with the nACHR and GABA receptor-gated ion channels [46].

Functional properties of the GABA receptor complex

As with the structural studies, various techniques have been used to examine the functional properties of the GABA receptor at the cellular level. Twenty years ago, Krnjevic and Schwartz [47] used electrophysiologic techniques to show that GABA was the neurotransmitter mediating inhibitory postsynaptic potentials in cerebral cortical neurons. Since intracellular injection of Cl^- reversed the GABA-induced membrane hyperpolarization, they concluded that GABA increased membrane Cl^- permeability [47]. Subsequent electrophysiologic studies have indicated that benzodiazepines and barbiturates act either indirectly or directly at the $GABA_A$ receptor to enhance GABA actions [10–13]. The mechanisms for this modulation have been examined using fluctuation analysis in cultured spinal cord neurons [48]. Study and Barker [48] have reported that benzodiazepines increase the frequency of channel opening induced by GABA, while barbiturates prolong the open channel time. The basis for this differential modulation is not yet clear, although one could speculate that benzodiazepines and barbiturates, acting at different sites on the $GABA_A$ receptor oligomer, induce different conformational (allosteric) changes in the receptor oligomer.

Although electrophysiologic studies are functional in nature, they often provide information on the structural properties of ion channels. Hamill *et al.* [49] have used patch clamp analysis to study both GABA- and glycine-induced Cl^- conductance in spinal cord neurons. They concluded that the structure of the two receptor-gated Cl^- channels is similar based on similar anion selectivities and conductance states (a measure of the amount of current flowing across the membrane during the time the ion channel is open) [49]. In related experiments, Bormann *et al.* [8] estimated that the pore diameters of the open GABA and glycine receptor anion channels are similar (5.6 and 5.2 Å respectively). They calculated that, in order to form a pore of about 5.8 Å, the channel walls consist of five transmembrane α-helical segments of the subunits [8]. However, they also suggested that the proposed GABA receptor subunit size of 50,000 daltons is larger than would be required to account for the five membrane spanning helices [8].

Electrophysiologic studies of the functional properties of the GABA receptor-gated ion channel have been complemented by recent efforts using biochemical assays of GABA receptor-mediated ion channel activity in brain. Both GABA- and barbiturate-induced radioactive Cl^- flux (uptake and efflux) have been measured in cultured neurons [50–52], brain slices [53, 54] and brain vesicles (synaptoneurosomes) [55–59]. As demonstrated in electrophysiologic studies, concentrations of barbiturates and benzodiazepine agonists that do not directly affect $^{36}Cl^-$ flux enhance the actions of GABA [53, 54, 57, 60–63], whereas benzodiazepine inverse agonists inhibit GABA-mediated $^{36}Cl^-$ flux [61, 63].

In addition to barbiturates and benzodiazepines, a number of other compounds with diverse chemical structures has been shown to modulate $GABA_A$ receptor function. For example, alcohols [51, 57, 64–66] and volatile anesthetics [66, 67] stimulate $^{36}Cl^-$ flux directly and/or enhance GABA-mediated $^{36}Cl^-$ flux in brain synaptoneurosomes or cultured neurons. A number of synthetic and natural steroids also affect $GABA_A$ receptor function. The anesthetic steroid, alphaxalone, has been reported to enhance

the inhibitory actions of GABA in cultured spinal cord neurons [68, 69]. Similarly, the naturally occurring metabolites of the steroids deoxycorticosterone and progesterone have been shown to stimulate $^{36}Cl^-$ flux directly [70], enhance GABA-mediated $^{36}Cl^-$ flux [71] and enhance GABA inhibition of spinal cord neurons [72].

Conversely, Majewska and Schwartz [73] have demonstrated that the steroid pregnenolone-sulfate inhibits GABA-mediated $^{36}Cl^-$ flux. Both the functional assays and receptor binding assays indicate that these steroids interact with the $GABA_A$ receptor in a manner similar to barbiturates and picrotoxin [70, 73] (see Fig. 1).

Other compounds that have been reported to inhibit GABA-mediated Cl^- transport in brain include various neurotoxic insecticides [74, 75] and inhibitors of Cl^- transporter proteins in non-neuronal cells [50, 56, 58, 76]. In light of these findings, it is tempting to speculate that the GABA receptor-gated Cl^- channel shares some structural properties with Cl^- transporter proteins in red blood cells or intestinal epithelia.

Most recently, functional studies of the GABA receptor [46] have provided additional support for the structural homologies between the GABA and nicotinic receptor-gated ion channels [6]. Schwartz and Mindlin [46] have demonstrated that several compounds that are classical noncompetitive blockers of the nACHR-gated cation channel [45] also noncompetitively inhibit the GABA receptor-gated Cl^- channel. These structurally unrelated compounds include phencyclidine (PCP) and its thienyl derivative, TCP, quinacrine, chlorpromazine, and tetraphenylphosphonium (TPP) ion. In addition, we have observed inhibition of GABA receptor-mediated $^{36}Cl^-$ flux by the benzomorphan opiate, phenazocine, and the dioxalane isomers, levoxadrol and dexoxadrol. Interestingly, the latter compounds as well as PCP also noncompetitively block the *N*-methyl-D-aspartate (NMDA) sensitive glutamate receptor-gated cation channel [for review see Refs. 77 and 78]. Furthermore, barbiturates, which have agonist activity at the GABA receptor-gated Cl^- channel, block both the nACHR [79] and non-NMDA-sensitive glutamate receptor-gated cation channels [80–82]. These findings lend further support to the conservation of structure across ion channels regulated by distinct receptor populations. The interaction of structurally diverse compounds at the different neurotransmitter-gated ion channels is summarized in Table 1.

It is readily apparent that the $GABA_A$, nicotinic, and glutamate receptor-gated ion channels maintain different selectivities for compounds with diverse chemical structures. Whether this is due to small differences in the amino acid sequences of the structurally homologous regions of receptor subunits remains to be established. After photolabeling the nACHR with various noncompetitive inhibitors [45], the noncompetitive antagonist binding sites have been located at specific positions on the ion channel domain of the α, β, and δ subunits [44]. If the noncompetitive sites on the $GABA_A$ and glutamate receptors can be successfully photolabeled, then it is possible that their locations on one or more subunits of the receptors can also be determined.

The interactions of noncompetitive inhibitors with

Table 1. Interaction of structurally diverse compounds with GABA, nicotinic and glutamate receptor-gated ion channels

	Receptor			
			Glutamate	
Compound	$GABA_A$	Nicotinic	NMDA	non-NMDA
Pentobarbital	+,++	−−	−	−,−−
Picrotoxin	−−	−−*†		
Tetraphenylphosphonium	−−			
Triphenylmethylphosphonium	−−	−−		
Quinacrine	−−	−−		
D-Tubocurarine	−−	−−	−	
Chlorpromazine	−	−−−		
PCP	−	−−−	−−−	
TCP	−		−−−	
Ketamine	0	−−*‡		
Phenazocine	−	−−−*‡		
Cyclazocine	−	−−−*‡	−−−	
Pentazocine		−−−*‡	−−−	
(−)*n*-Allylnormetazocine	−			
(+)*n*-Allylnormetazocine	−	−−−*‡	−−	
Dexoxadrol	−		−−−	
Levoxadrol	−		−−	

Inhibition (−) or stimulation (+) of receptor function is indicated in the following concentration range: (−−−) <10 μM; (−−) 10–100 μM; (−) 100–1000 μM; (0) >1000 μM.

* Inhibition of receptor binding.

† Ref. 83.

‡ Ref. 84.

various neurotransmitter receptor-gated ion channels may explain the multiple pharmacologic effects of some of these compounds. Many of these inhibitors produce seizure activity at concentrations greater than those that produce the primary effect. In some cases this has clinical importance. For example, in persons intoxicated (overdose) with PCP, both psychotomimetic effects and epileptiform seizure activity are produced [85], at plasma PCP concentrations similar to those that inhibit GABA mediated $^{35}Cl^-$ flux [46, 86].

Summary

In the past few years, substantial advances have been made in analyzing the structure and function of the GABA receptor-gated Cl^- channel. A major goal is to identify the molecular characteristics of the $GABA_A$ receptor that are necessary for maintaining normal GABAergic neurotransmission. Future studies will undoubtedly include techniques that have been used successfully to construct a detailed structural and dynamic model of the nACHR-gated ion channel [44]. These include X-ray scattering, single group rotation theory, and genetic homology, deletion and site-directed mutation studies [see Ref. 44 for review]. Such techniques will make it possible to identify the structural defects that give rise to abnormal GABA receptor function and possibly to sleep, anxiety and seizure disorders.

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