Functional domains of $GABA_A$ receptors

Geoffrey B. Smith and Richard W. Olsen

The transmitter-gated ion channels mediate rapid synaptic transmission, for example, at the neuromuscular junction using acetylcholine and in the CNS using primarily the amino acids glutamate and GABA. $GABA_A$-receptor Cl^- channels share sequence homology with a superfamily of these channels including nicotinic acetylcholine receptor and inhibitory glycine receptor. In this article, **Geoffrey Smith and Richard Olsen** discuss recent affinity labelling and site-directed mutagenesis studies on $GABA_A$ receptors that have identified amino acid residues essential for binding of agonists and allosteric modulators as well as the ion channel wall formation. The structural domains identified are consistent with results obtained with other members of the transmitter-gated ion channel superfamily and suggest that structural models for one member of the family may apply to the others as well.

$GABA_A$ receptors mediate most of the inhibitory synaptic transmission in the CNS and serve as the target for many important neuroactive drugs, including benzodiazepines, barbiturates, steroids, general anaesthetics and possibly alcohol[1].

Molecular structure

$GABA_A$ receptors share, with other members of the transmitter-gated ion channel superfamily, certain structural and functional similarities. These include a heteromeric pseudo-symmetric transmembrane structure[2–6], which, like the nicotinic acetycholine receptor[6], appears to be in pentamers[7]. The cDNAs for all of the subunits for the various members of the superfamily are evolutionarily related, sharing a common sequence homology and predicted topological structure for the polypeptides. In particular, they are composed of a putative large N-terminal extracellular domain, thought to be responsible for ligand–channel interactions, and four putative transmembrane domains (TM) with a large intracellular loop containing sites for regulation, for example, phosphorylation, between TM3 and TM4 (Refs 2–5, 8–10) (Fig. 1).

The $GABA_A$ receptor can be considered a family of receptors because there are numerous genes coding for distinct, but related, subunits. These are named according to the degree of sequence homology, for example, α subunits have about 70% homology, but only about 25% homology with β subunits (and 15–20% with other members of the transmitter-gated ion channel superfamily). The existence of six α, four β, four γ, one δ, and two ρ subunits and splicing variants of many of them suggests that a large number of heteropentameric isoforms might occur. Available evidence suggests that 12–24 isoforms are sufficiently abundant to play significant physiological roles. This diversity presumably serves a number of biological roles, including tissue- and age-dependent transcription control and tissue-dependent functional control at the protein level[1,3,11]. The existence of receptor subtypes raises the possibility, not yet realized, of selective drugs of improved clinical profile, for example, in the treatment of anxiety, epilepsy, and insomnia[11].

Studies on $GABA_A$ receptor subunit composition have included attempts to reconstitute pharmacological profiles using various combinations of recombinant receptor subunits expressed in heterologous cells[1,11–16]. Protein chemistry and antibody separations to determine which polypeptides are associated with native pharmacological receptor subtypes suggest that many naturally occurring isoforms contain at least two copies of the α and β subunits, one or more copies of the γ subunit, with the δ subunit sometimes replacing the γ subunit[17–21]. Exact native subunit combinations and stoichiometries remain speculative.

Although none of these transmitter-gated ion channel proteins has been amenable to X-ray crystallography studies so far, some structural information has been deduced with affinity labelling/microsequencing, site-directed mutagenesis and domain-specific antibody localization, especially with the nicotinic acetylcholine receptor (for reviews see Refs 9, 10). A 9 Å structure of this receptor in the closed- and open-channel conformations, obtained by computer-enhanced electron microscopy[6], may serve as a model for all members of the superfamily. The few studies performed on the other members of the superfamily are consistent with conclusions suggested from work on the nicotinic acetylcholine receptor. Recently, results on the $GABA_A$ receptor have contributed additional information to understanding the structure and function of this important class of proteins.

GABA binding site

A single point mutation of the rat α1 subunit at Phe64, in the putative N-terminal extracellular domain (Fig. 1), produced a marked decrease in agonist and antagonist affinities when coexpressed with β2 and γ2 subunits[14]. In addition, we have recently shown that this position (bovine equivalent is Phe65) is also covalently modified by the GABA site photoaffinity agonist [^{3}H]muscimol[22]. The same, or similar labelled, sequence is present in all α subunits, in the γ2 and δ subunits, but not in β subunits. Affinity labelling suggests that rat Phe64 is probably positioned near a GABA binding site, although this conclusion is limited by the lack of knowledge of the actual chemical reaction involved in the photoaffinity-labelling with muscimol.

Two other regions in the β2 subunit were also shown, by mutagenesis, to affect functional activation by GABA

G. B. Smith, Postdoctoral Fellow Center for Molecular Recognition, Columbia University College of Physicians and Surgeons 630 W 168th Street, New York, NY 10032, USA and **R. W. Olsen**, Professor, Department of Molecular and Medical Pharmacology, UCLA School of Medicine, Center for Health Sciences Room 23-120, 10833 LeConte Ave, Los Angeles, CA 90024, USA.

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(Ref. 16). These homologous domains, with the second domain being in the reverse orientation of the first, are Tyr157-Gly158-Tyr159-Thr160 and Thr202-Gly203-Ser204-Tyr205 and are situated between the disulphide loop and TM1 (Fig. 1). Mutation of Tyr157 or Tyr205 to Phe, or Thr160 or Thr202 to Ser in the β2 subunit significantly reduced binding affinity for both agonists and antagonists at the GABA site. Mutations in the corresponding residues in α and γ subunits had little or no effect. The mutated receptors were still able to be activated by pentobarbital, indicating that there was no difference in the channel structure[16].

The ρ subunit, expressed in retina, forms homomeric channels (when expressed in host cells) that show unusual pharmacological properties, including insensitivity to several modulatory drugs and the GABA receptor antagonist bicuculline and the GABA receptor agonist 4,5,6,7-tetrahydroisoxazolo(5,4c)pyridin-3-ol (THIP), but a 40-fold higher affinity for GABA compared to the receptor consisting of the α1, ρ2 and γ2 subunits. Mutation of several residues changing in the ρ subunit to ones not found in the subunit resulted in reduced GABA affinity but unaltered THIP affinity for the channel. These residues identified another important domain at human ρ subunit His141/Arg144 (Ref. 23). The ρ subunit His141 aligns with, or is adjacent to, the human α1 subunit His102, the equivalent of the rat α1 subunit His101 (Fig. 1) implicated in benzodiazepine binding. A possible additional region in the disulphide loop at Gln189 was also found to affect GABA binding[23]. In another study of the ρ subunit, three of the same residues implicated in β2 subunit: Tyr198, Thr244 and Tyr247, as well as two others nearby (Tyr200 and Tyr241) were critical for the unique GABA affinity as well as different channel opening, closing and desensitization kinetics[24].

A model for agonist binding to transmitter-gated ion channel receptors has been suggested[25] that postulates the involvement of the extracellular disulphide loop which is conserved in all members of this family[2] (Fig.1). Recent mutagenesis studies have cast doubt upon such a role for this structure in $GABA_A$, as well as nicotinic acetylcholine and glycine receptors[26–28].

Three domains of agonist binding have been defined on the α subunit of *Torpedo* nicotinic acetylcholine receptors, around Tyr93, Trp149 and Tyr190/Cys192/Tyr197 (Ref. 9). Similar approaches have identified similar regions important to agonist binding in glycine receptors: Tyr161 and Tyr202 (Ref. 29) and Phe159 and Tyr161 (Ref. 30); modification of the latter produced a glycine receptor that could bind GABA. In nicotinic acetylcholine receptors Trp149 and in rat glycine receptors Phe159 and Tyr161 are homologous to the rat β2 subunit (amino acid residues 157–160) domain on $GABA_A$ receptors, while Tyr197 in nicotinic acetylcholine receptors and Tyr202 in glycine receptors are in the vicinity of the β2 subunits (residues 202 to 205) implicated in GABA binding in $GABA_A$ receptors[16].

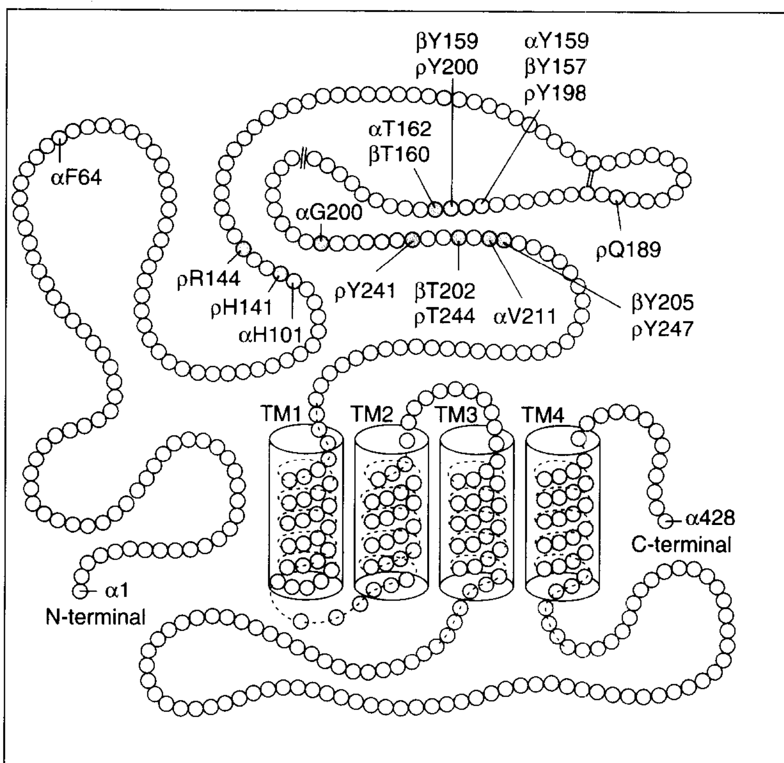

Fig. 1. Structure of the rat $GABA_A$ receptor α1 subunit with deduced amino acid sequence indicating amino acid residues implicated in ligand binding domains. GABA receptor α subunit residues implicated in ligand binding are indicated (orange). Residues implicated in ligand binding at both GABA receptor α subunit and other subunits are shown (pink); residues implicated in ligand binding at other receptors are indicated (blue). The greek letter indicates the subunit(s) involved, followed by the amino acid (single letter code) and its sequence number. The break in the sequence at amino acid 171 indicates an additional 20 amino acids not shown. Modified from Ref. 8 with permission.

The residue Arg55 in the α subunit of *Torpedo* nicotinic acetylcholine receptors, which is homologous to the rat Phe64 in $GABA_A$ receptor α subunits (important in GABA binding), is essential for α-bungarotoxin activity, as shown by mutagenesis. In addition, a synthetic peptide encompassing Arg55 is able to inhibit binding of this toxin and mutation of this residue or other nearby residues prevents its activity[31].

The agonist binding site in transmitter-gated ion channel receptors may occur at an interface between two dissimilar subunits. In nicotinic acetylcholine receptors, residues at the α subunit–δ subunit and α subunit–γ subunit interfaces were identified by photoaffinity labelling with [^{3}H]D-tubocurarine[32]. In the δ and γ subunits these residues are located in the region homologous with Phe64 in the $GABA_A$ receptor α1 subunit. Also, mutation of the γ subunit Tyr117 dramatically reduced curare binding[33]. Finally, a subsite of negatively charged residues on the δ subunit (Glu189 and Asp180) were shown, by crosslinking reagents and site-directed mutagenesis, to affect acetylcholine binding affinity[10,34]. In recombinant $GABA_A$ receptors, although channels can be expressed from many combinations of subunits, a minimal composition of α and β subunits is thought to be required for reconstruction of agonist-dependent gating[1,3,11,13–16].

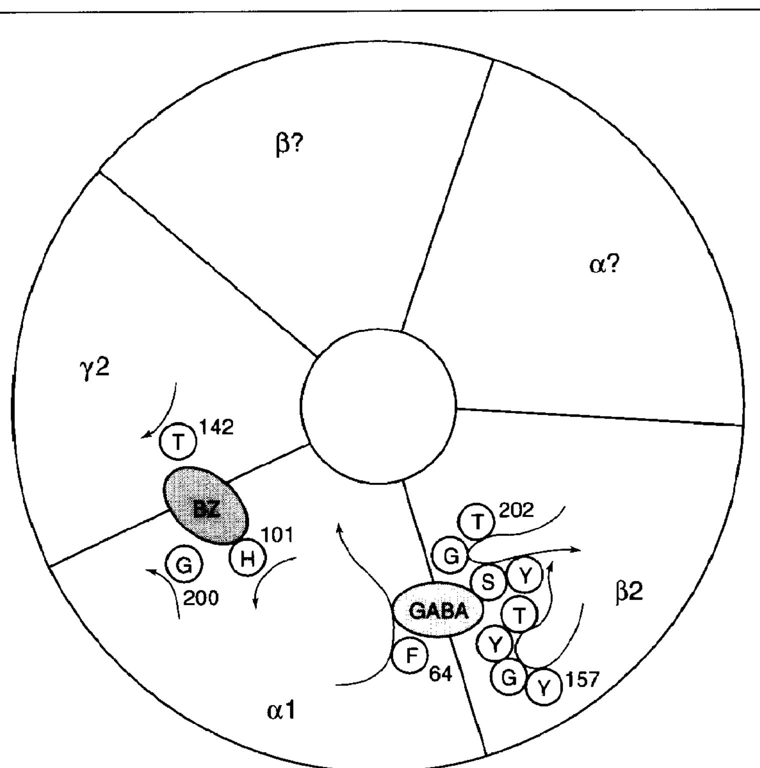

Fig. 2. Model of $GABA_A$ receptor protein with five subunits, indicating amino acid residues implicated in the binding of GABA and benzodiazepines (BZ). The subunit stoichiometry is tentative, as indicated by the question mark. Single letters and numbers indicate the amino acids (and their sequence positions) thought to be involved in GABA and benzodiazepine binding. Arrows indicate the direction in which the sequence runs.

Benzodiazepine binding site

Benzodiazepines are a clinically important class of compounds which allosterically modulate the actions of agonists on $GABA_A$ receptor function. Benzodiazepine agonists act by increasing the probability of Cl^- channel opening in response to GABA; they do not open channels in the absence of GABA. The benzodiazepine site is pharmacologically unique in that a range of efficacies is observed. In addition to full and partial agonists, compounds that act as full and partial inverse agonists decrease channel function, and antagonists block the effects of both agonists and inverse agonists[1,11]. Benzodiazepines do not bind to the same site as GABA, and the two sites allosterically modulate each other[1,2,11]. In addition, the domains for agonist and inverse agonist binding may only partially overlap and not totally coincide[11,35,36], in which case the amino acids involved in the binding will depend on the ligand used.

Some residues involved in the binding of benzodiazepine ligands have been tentatively identified. The site of [^{3}H]flunitrazepam photoincorporation probably lies between residues 59–148 of the α1 subunit[37]. Within this domain, residue His102 in bovine α1 subunit appears to be a major site of photoincorporation of [^{3}H]flunitrazepam[38]. The same single residue markedly changed benzodiazepine agonist affinity and efficacy as demonstrated by mutagenesis: the His101 residue of the rat α1 subunit was shown[39] to confer diazepam-sensitive benzodiazepine binding while mutation of this residue to Arg produced a subunit which, when coexpressed with β2 and γ2 subunits, was insensitive to benzodiazepine agonists. The Arg-containing mutant α1 subunits produced oligomers that bind the inverse agonist Ro154513 with no change in affinity; however, the benzodiazepine antagonist flumazenil has reduced affinity. Binding of [^{3}H]Ro154513 was diazepam-insensitive, as observed in the naturally occurring α4 and α6 subunits, which contain Arg in residue 101. Diazepam-insensitive binding of [^{3}H]Ro154513 is also observed in the cerebellum, where the α6 subunit is exclusively expressed[11,40]. In addition, a naturally occurring variant of the α6 subunit with Gln101 was isolated from a strain of rats showing high susceptibility to motor impairment by benzodiazepines and alcohol; the allelic variant of the α6 subunit produced intermediate affinity for agonists when expressed with β and γ subunits [40]. Considering the photolabelling data, and that these single amino acid mutations produced such a large change in efficacy and selectivity, even though inverse agonist affinity is less affected, residue His101 in the α1 subunit is likely to be closely associated with the binding site for some of these ligands (Fig. 2).

The α subunit residue Tyr93 of the nicotinic acetylcholine receptor, implicated in acetylcholine binding[9], is equivalent to the $GABA_A$ receptor α subunit His101. While the benzodiazepines are allosteric modulators rather than agonists or antagonists for the GABA site, it is interesting to note that the binding domain may be equivalent to one for nicotinic acetylcholine receptor agonists and antagonists. This suggests that part of the benzodiazepine binding site of the $GABA_A$ receptor α subunit may be a modified form of an agonist site, and that some subunits, such as the ρ subunit, do not contain the modification that produces affinity for benzodiazepines. The idea that the benzodiazepine binding site might involve the same domain, in part, as GABA sites on other subunits, and, like GABA sites, occur at subunit interfaces, was proposed recently by Galzi and Changeux[9].

Additionally, a residue some distance away, between the disulphide loop and TM1, Gly200 of the α1 subunit (Figs 1 and 2), was shown to affect binding affinity for benzodiazepine ligands[41]. This residue is Glu in all other α subunits and conversion to Gly, for example, in the α3 subunit, produces high affinity for ligands such as CL218872, β-carbolines and zolpidem (which have some selectivity for the BZ_1 site); this characteristic is normally associated with the presence of the α1 subunit, while α2 and α3 subunits account for BZ_2 site binding (lower affinity for the same ligands) and the α5 subunit produces zolpidem-insensitive benzodiazepine binding[11,12,35]. Additional modest changes in benzodiazepine ligand affinity were achieved[35] by conversion of other α subunit residues to the corresponding α1 subunit residues at Thr162 and Val211 (Fig. 1). Because the changes produced were ones of relative binding affinity

for agonist binding subtypes, these regions may or may not actually participate in the binding pocket itself.

Finally, an amino acid in the γ2 subunit has been implicated in benzodiazepine efficacy and possibly affinity. The γ2 subunit Thr142, when conservatively mutated to Ser, improved the efficacy of some benzodiazepine agonists and reduced that of others; it also changed the action of antagonists to partial agonists[15]. Inclusion of a γ2 subunit appears to be required for benzodiazepine binding and modulation of $GABA_A$ receptor function[11–15,35] (although the nature of the γ and α subunits can affect benzodiazepine sensitivity[1,11,18–21,35]). However, the photolabelling with [³H]flunitrazepam is present on α subunits, or perhaps at α subunit–γ subunit interfaces[17] (Fig. 2).

Taken together, the domains implicated for GABA and benzodiazepine binding in the $GABA_A$ receptor appear to be quite homologous to those on the other transmitter-gated ion channel receptors, and the GABA receptor model can be compared to those more complete models proposed for the nicotinic acetylcholine receptor (for example, Refs 6, 9, 10).

The channel

As in the nicotinic acetylcholine receptors[9,10], TM2 of the $GABA_A$ receptor has been implicated in formation of the channel wall. In *Drosophila* GABA receptors, field-isolated variant organisms resistant to the GABA receptor antagonist picrotoxin and the insecticide dieldrin had a different residue in TM2 (Ser replacing Ala); further, mutation of the wild type Ala to Ser reproduced this insecticide resistance[42]. Mutations of the corresponding TM2 residues in mammalian α, β, or γ subunits resulted in picrotoxin-resistant $GABA_A$ receptor channels[43]. Modification of these TM2 residues to Cys and subsequent block of channel function with membrane-impermeant sulphydryl reagents revealed that the TM2 probably forms an α-helix with one surface exposed to solvent, presumably in the channel lumen[44]. This result is similar to those obtained with the nicotinic acetylcholine receptor[10,45].

Site-directed mutagenesis of residues just prior to, or just after, TM2 in $GABA_A$ receptor α, β, or γ subunits resulted in altered rectification in current–voltage plots, suggesting altered channel properties. This study, mixing normal and mutated recombinant subunits, also suggested a subunit stoichiometry of 2α, 1β and 2γ subunits for the GABA receptors consisting of α3, β2 and γ2 subunits[13]. Likewise, residues in TM2 of glycine receptors differ between α and β subunits and in picrotoxin sensitivity: homomeric α channels show, unlike native receptors, sensitivity to the GABA receptor antagonist picrotoxin, while αβ heteromers are resistant, as expected. Mutation of TM2 residues in the α subunit to conform with the β subunit sequence removes the picrotoxin sensitivity[46]. Minor mutations of the nicotinic acetylcholine receptor in the TM2 channel wall, substituting with $GABA_A$ or glycine receptor residues, convert the normal cation channel into an anion channel[47], supplying further evidence for structural similarities among members of the superfamily.

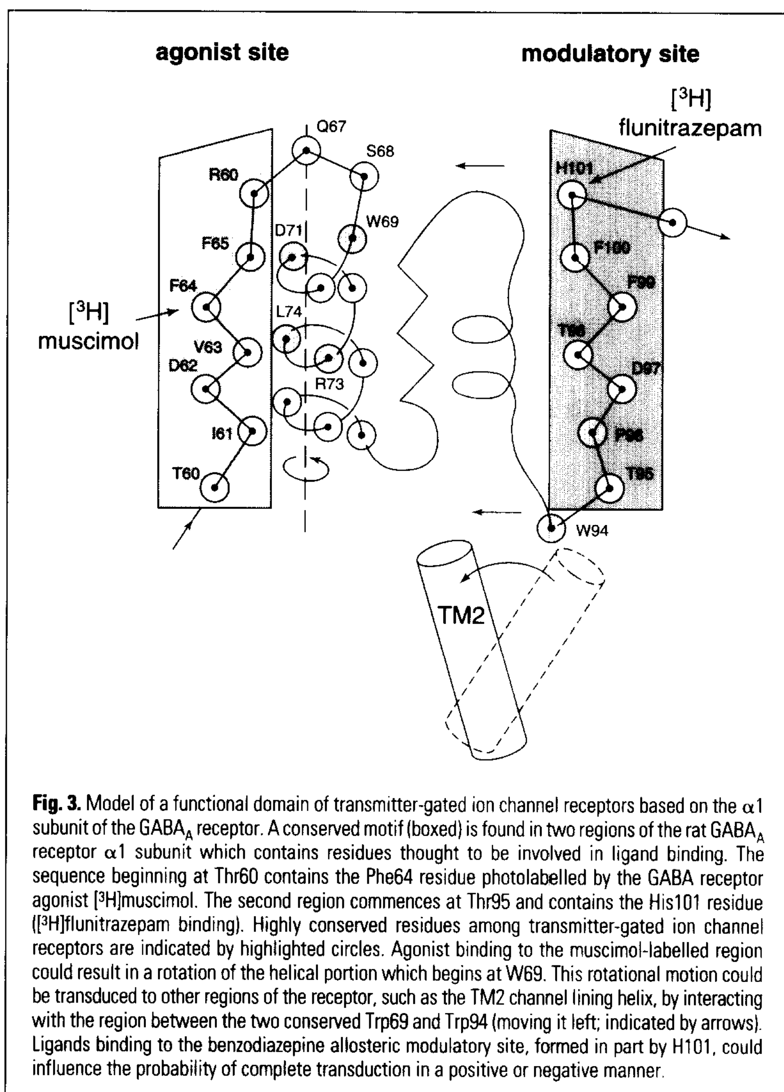

Fig. 3. Model of a functional domain of transmitter-gated ion channel receptors based on the α1 subunit of the $GABA_A$ receptor. A conserved motif (boxed) is found in two regions of the rat $GABA_A$ receptor α1 subunit which contains residues thought to be involved in ligand binding. The sequence beginning at Thr60 contains the Phe64 residue photolabelled by the GABA receptor agonist [³H]muscimol. The second region commences at Thr95 and contains the His101 residue ([³H]flunitrazepam binding). Highly conserved residues among transmitter-gated ion channel receptors are indicated by highlighted circles. Agonist binding to the muscimol-labelled region could result in a rotation of the helical portion which begins at W69. This rotational motion could be transduced to other regions of the receptor, such as the TM2 channel lining helix, by interacting with the region between the two conserved Trp69 and Trp94 (moving it left; indicated by arrows). Ligands binding to the benzodiazepine allosteric modulatory site, formed in part by H101, could influence the probability of complete transduction in a positive or negative manner.

A model of the agonist binding site in transmitter-gated ion channels

Sequences for GABA and benzodiazepine binding domains

Two α subunit regions, beginning at Thr60 and Thr95, are likely to be involved in the GABA and benzodiazepine binding sites, respectively (Fig. 3). These regions share a common motif: TXDXFF, with this being conserved in both regions of all α subunits. The Thr residue near the N-terminal of these two regions also shows conservation, both in $GABA_A$ receptor subunits and those of other transmitter-gated ion channel receptor subunits (Fig. 4a). The His101 residue in the benzodiazepine-binding region (Figs 3, 4) confers benzodiazepine agonist binding[35,39,40]. Mutation of this residue to Arg produces a subunit that is insensitive to agonists. It is notable that the Arg66 residue in the GABA-binding domain aligns with the His101 residue and suggests that this portion of the benzodiazepine site may be a modified form of an equivalent domain in the GABA site.

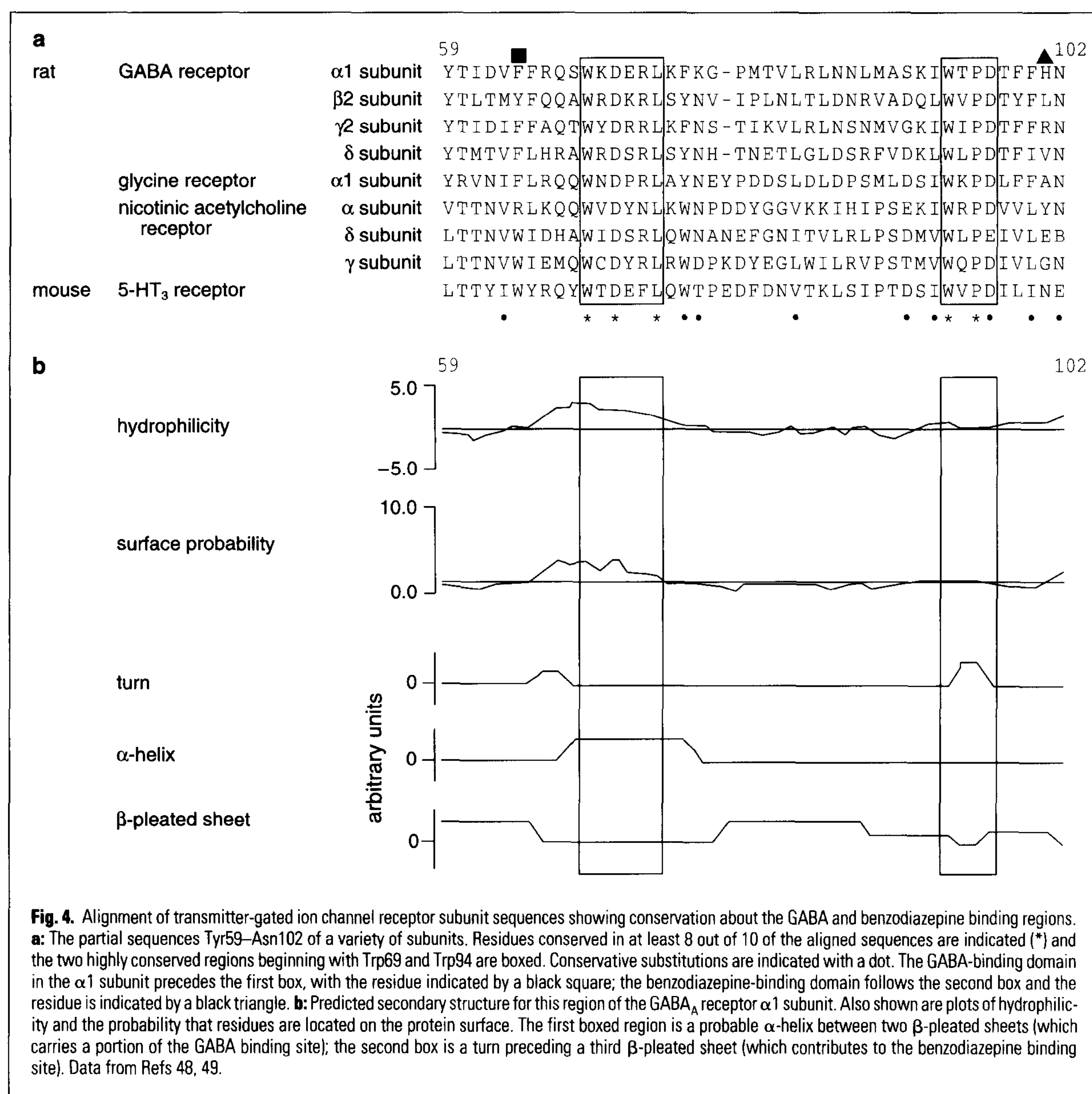

Fig. 4. Alignment of transmitter-gated ion channel receptor subunit sequences showing conservation about the GABA and benzodiazepine binding regions. **a:** The partial sequences Tyr59–Asn102 of a variety of subunits. Residues conserved in at least 8 out of 10 of the aligned sequences are indicated (*) and the two highly conserved regions beginning with Trp69 and Trp94 are boxed. Conservative substitutions are indicated with a dot. The GABA-binding domain in the α1 subunit precedes the first box, with the residue indicated by a black square; the benzodiazepine-binding domain follows the second box and the residue is indicated by a black triangle. **b:** Predicted secondary structure for this region of the $GABA_A$ receptor α1 subunit. Also shown are plots of hydrophilicity and the probability that residues are located on the protein surface. The first boxed region is a probable α-helix between two β-pleated sheets (which carries a portion of the GABA binding site); the second box is a turn preceding a third β-pleated sheet (which contributes to the benzodiazepine binding site). Data from Refs 48, 49.

Secondary structure motifs for partial ligand binding sites

Examination of the secondary structural predictions[48,49] for these regions shows (Fig. 4b) that the predicted conformation for the two TXDXFF motif segments is a β-pleated sheet. The prediction is supported by the alternation of polar and nonpolar residues for both regions suggesting hydrophilic and hydrophobic faces to the strands. The residues in the alternating hydrophilic positions, which could be exposed at the protein surface, are also the residues that are conserved in both segments and apparently modified by photoaffinity labelling[22,38], or those that produced large changes in binding[14,39]. Both segments show a tendency to form β-pleated sheets in all transmitter-gated ion channel subunit subtypes and the conserved motif suggests that they may be oriented in parallel to one another.

A sequence of approximately 25 residues between these two regions also contains highly conserved amino acids (Fig. 4a). This intervening region is demarcated in the $GABA_A$ α subunit by Trp69 and Trp94. These residues are conserved in all superfamily subunits. In addition, residues corresponding to Asp71 and Leu74 of the α1 subunit are also conserved throughout the superfamily. The residue corresponding to Arg73 is also conserved in transmitter-gated ion channels with the exceptions being nicotinic acetylcholine receptor α subunits and $5HT_3$ receptors. These latter three conserved residues (Asp71, Arg73 and Leu74) are found in the N-terminal of the Trp-demarcated segment, following the first of the two β-pleated sheets. This region may adopt a turn followed by a short helix of about three turns (Fig. 4b). Following this putative helix is a region predicted by Chou and Fasman[48] to be β-pleated sheet (Fig. 4b) in the centre of the Trp-bounded domain.

Similar βαβ motifs form structural units commonly found in proteins of diverse function and cellular environments and are made up of two parallel β-pleated

sheets connected by an intervening α-helix. In particular, it has been observed that residues which contribute to ligand binding or enzyme active-sites are often found within a hairpin turn connecting the C-terminal of the first β-pleated sheet and the N-terminal of the α-helix[50]. The Phe64 residue photolabelled with [^{3}H]muscimol (Fig. 3) could possibly lead into a binding loop with the helix that begins at Trp69. The segment between the second β-pleated sheet of the βαβ motif and the conserved Trp94 is less well predicted by this technique, although another helical element is weakly implied by a variation of this programme[48].

Such a helix-based binding motif in transmitter-gated ion channel receptors would agree with recent structural findings for the nicotinic acetylcholine receptor reported by Unwin[6].

Conserved structural and functional domains

This region formed by residues between the conserved Trp69 and Trp94 could correspond to a conserved structural unit and possibly to a functional domain which performs a similar role among members of this superfamily. If residues in the flanking β-pleated sheet regions contribute to ligand binding in $GABA_A$ receptors, then they would also be expected to serve similar functions in other receptor types. The homology observed between the two ligand-binding segments for GABA and benzodiazepines suggests that gene duplication may have resulted in a modified agonist site which now functions as an allosteric modulatory site in the $GABA_A$ receptor.

Conformational coupling of ligand binding structural unit to ion channel gating

The entire binding structural unit may form a single functional domain and function in the complex transduction of ligand binding to channel activation. Although the tertiary structure is not known, one potential mechanism involves the 'agonist' labelled segment (Fig. 3) causing a rotation of the α-helix. This rotation and associated movement of this structural unit could be transmitted to other domains of the receptor resulting in conformational changes associated with channel gating in TM2 and re-orientation of residues providing steric impediment to ion flow. Since the energy of ligand binding alone probably would not be sufficient to cause a conformational shift resulting in channel opening, this concerted movement of the larger motif may be a necessary process for channel function. Within this functional domain, the absolutely conserved Trp residues could participate in translation of this essential motion because of their size and hydrophobicity, causing them to be concentrated together in the interior of the protein. In this way, they could act as a structural anchor, or as a mechanical fulcrum, for transmission of this energy to other parts of the receptor protein.

Concluding remarks

The partial colocalization of GABA and benzodiazepine-binding domains to a single structural unit could also provide an explanation for the observed allosteric interactions between these two binding sites. The unique positive and negative modulatory actions upon agonist-dependent channel activity might result if compounds acting at the modulatory site could influence the extent or probability of ligand-induced rotation, with positive modulators increasing and negative modulators decreasing either the degree, or the likelihood, of helical rotation, conformational change of the structural unit and channel gating.

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Acknowledgements
This work was supported by NIH grant NS28772. The authors would like to thank S. Srinivasan for helpful discussions.

Chemical names

CL218872: 3-methyl-6-[(3-trifluoromethyl)phenyl]-1,2,4-triazolo(4,3β)pyridiazine

Ro154513: ethyl-8-azido-5,6-dihydro-5-methyl-6-oxo-4*H*-imidazo(1,5α)(1,4)benzodiazepine-3-carboxylate

How should P_{2X} purinoceptors be classified pharmacologically?

Charles Kennedy and Paul Leff

When ATP is released as a neurotransmitter from central and peripheral nerves it acts at P_{2X} purinoceptors to produce postsynaptic depolarization and excitation. The P_{2X} purinoceptor was originally classified on the basis of the relative agonist potencies of ATP and a number of its structural analogues. However, it is now clear that the potency of some agonists is greatly decreased by breakdown by ectonucleotidase enzymes, leading to an incorrect determination of agonist potency order. In this article, **Charles Kennedy and Paul Leff** discuss recent results that indicate that the established classification of P_{2X} purinoceptors is no longer valid and needs redefinition.

C. Kennedy, Lecturer, Department of Physiology and Pharmacology, University of Strathclyde, Royal College, 204 George St, Glasgow, UK G1 1XW, and **P. Leff,** Head, Department of Pharmacology, Fisons Research and Development Laboratories, Bakewell Road, Loughborough, UK LE11 0RH.

The physiological and pharmacological actions of ATP are mediated through activation of P_2 purinoceptors, of which at least five subtypes (P_{2X}, P_{2Y}, P_{2U}, P_{2T} and P_{2Z} purinoceptors) have been identified[1–5]. In the absence of selective, potent, antagonists, the receptors were classified largely on the basis of the relative agonist potencies of ATP and a number of structural analogues, in particular α,β-methyleneATP and 2-methylthioATP. Subsequent support for the existence of these subtypes came with the cloning of the P_{2Y} and P_{2U} purinoceptors, which belong to the G protein-coupled receptor superfamily[6,7] and, more recently, genes for the P_{2X} purinoceptor were identified and found to encode transmitter-gated ion channels[8,9].

The P_{2X} purinoceptor was originally defined by a rank order of agonist potency of α,β-methyleneATP >> 2-methylthioATP ≥ ATP, with α,β-methyleneATP being approximately three orders of magnitude more potent than ATP (Ref. 1). Comparative studies of agonist potency should ideally be performed in the absence of metabolism of the agonists, but until recently this was not possible, as selective, potent, inhibitors of the ectonucleotidase enzymes that degrade ATP were not available. It is now clear that the influence of ectonucleotidases on agonist potency is much greater than anticipated, resulting in a possible erroneous characterization of the P_{2X} purinoceptor. Recent studies show that the potencies of ATP and 2-methylthioATP are decreased by 100–1000-fold by breakdown and, that when this is prevented, ATP and 2-methylthioATP are more potent than α,β-methyleneATP as agonists at the P_{2X} purinoceptor.

The original definition of P_{2X} purinoceptors

Burnstock and Kennedy[1] proposed a subdivision of P_2 purinoceptors into P_{2X} and P_{2Y} purinoceptor subtypes on the basis of two broad patterns of agonist activity. In the guinea-pig vas deferens and urinary bladder, α,β-methyleneATP was more potent than ATP, which was equipotent with 2-methylthioATP, at evoking contraction. In contrast, in the guinea-pig taenia coli and rabbit portal vein, 2-methylthioATP was more potent than ATP, which was more potent than α,β-methyleneATP, at causing relaxation. The former profile was defined as characterizing P_{2X} purinoceptors and the latter profile P_{2Y} purinoceptors. Also, repeated administration of α,β-methyleneATP selectively desensitized the P_{2X} but not the P_{2Y} purinoceptor.

One limitation of these studies was that the agonist concentration–response curves were often non-sigmoidal, not parallel and, in many cases, did not reach a clearly defined maximum. Thus, the curves could not be analysed quantitatively according to the Hill equation and EC_{50} values could not be calculated. Nevertheless, over the next few years numerous reports, in a wide range of tissues, added support to the proposed subdivision (for review see Ref. 10).

The first quantitative analysis of P_{2X} purinoceptor agonist action was performed in the rabbit isolated ear artery[11]. α,β-MethyleneATP, 2-methylthioATP and ATP all appeared to be full agonists and their

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