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Chemical names

MK571: 3-([{3-(2-[7-chloro-2-quinolinyl]ethenyl)phenyl}-{(3-dimethyl-amino-3-oxopropyl)-thio}-methyl]thio)propanoic acid

MK886: 3-[1-(p-chlorobenzyl)-5-(isopropyl)-3-tert-butylthioindol-2-yl]-2,2-dimethylpropanoic acid

The 'ABC' of GABA receptors

Joachim Bormann

In the conventional view, GABA acts at either ionotropic $GABA_A$ or metabotropic $GABA_B$ receptors. Recently, novel ionotropic GABA receptors that are composed of ρ-subunits have been identified in the vertebrate retina. These bicuculline- and baclofen-insensitive GABA receptors are frequently called $GABA_C$, following an early suggestion by Graham Johnston and colleagues. An IUPHAR committee has recommended that the term $GABA_C$ be avoided and subclassifies the retinal receptors as $GABA_{A0r}$. However, new evidence regarding the pharmacology, structure, function, genetics and cellular localization of ionotropic GABA receptors strengthens the case for the existence of two major classes of these receptors, $GABA_A$ and $GABA_C$.

In the vertebrate CNS, GABA is the most widely distributed inhibitory neurotransmitter[1]. Initially, GABA was found to activate bicuculline-sensitive Cl^- channels and, subsequently, GABA-mediated activation of cation channels was discovered[2,3]. This finding led to the notion of $GABA_A$- and $GABA_B$-type receptors, which was introduced in 1981 by Hill and Bowery[3]. The $GABA_A$ receptor directly gates a Cl^- ionophore and has modulatory binding sites for benzodiazepines, barbiturates, neurosteroids and ethanol[2,4] (Fig. 1a, b). By contrast, $GABA_B$ receptors couple to Ca^{2+} and K^+ channels via G proteins and second messenger systems; they are activated by baclofen and are resistant to drugs that modulate $GABA_A$ receptors[2,3,5]. Early studies by Johnston and colleagues indicated that the partially folded GABA analogue *cis*-4-aminocrotonic acid (CACA) selectively activates a third class of GABA receptors in the mammalian CNS (Ref. 6). These receptors, which were tentatively designated $GABA_C$ in 1984 (Ref. 6), are Cl^- pores that are insensitive to both bicuculline and baclofen[6–9] (Fig. 1c). Only recently has it been possible to study these novel GABA receptors at the molecular level in clearly defined subpopulations of retinal neurones[7,10]. Several lines of evidence now indicate that $GABA_C$ receptors are composed of ρ-subunits[9–12]. Heterologously expressed, these subunits form homooligomeric channels with the characteristic $GABA_C$ pharmacology[6,7,9,11,13]. In the mammalian retina, ρ-subunits and $GABA_C$ responses colocalize in bipolar cells[7,9,10], and are spacially and functionally distinct from $GABA_A$ or glycine receptors[10,14].

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An IUPHAR committee has provisionally recommended that the term $GABA_C$ be avoided and classifies bicuculline- and baclofen-insensitive GABA receptors as a minor subspecies of $GABA_A$ receptors[15]. This article aims to examine these provisional recommendations. The term '$GABA_C$' not only represents a logical and convenient extension of the $GABA_A$, $GABA_B$ nomenclature[6], but is now strongly supported by accumulating evidence on the distinctive pharmacology, structure, function, genetics and cellular localization of these ionotropic GABA receptors.

IUPHAR classification of $GABA_A$ receptors

In a worthwhile attempt at the initial classification of a ligand-gated ion channel, a 'provisional version' of $GABA_A$ receptor classification was published as an IUPHAR report[15]. On the basis of subunit structure and receptor function, this report, which was written by leading experts in the $GABA_A$ field, has catagorized the αβγ-subunit-containing receptors as $GABA_{A1}$ to $GABA_{A6}$, according to the α-subunit present, and categorized the benzodiazepine-insensitive αβδ or αβε isoforms as $GABA_{A0}$ receptors. Because all these subtypes are bicuculline sensitive, the term $GABA_{A0r}$ was invented to classify the

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ρ-containing GABA receptors as a specialized set of $GABA_A$ receptors that are insensitive to both bicuculline and benzodiazepines.

Although it is reasonable to divide GABA receptors into ionotropic and metabotropic GABA receptor types, it is misleading to call all ionotropic GABA receptors $GABA_A$ receptors. Such nomenclature obscures the sound notion of bicuculline-insensitive GABA receptors with distinct properties that clearly differ from classic bicuculline-sensitive $GABA_A$ receptors. In addition, the IUPHAR classification 'relies on the […] data available on the recombinant GABA receptors'[15] and, therefore, native GABA receptors are not directly addressed.

Two formal arguments against the $GABA_C$ terminology are noted in the IUPHAR report: (1) it would be unsatisfactory to separate two branches of ionotropic GABA receptors ($GABA_A$ and $GABA_C$) with a metabotropic family, $GABA_B$, lying between them; and (2) if the designation of $GABA_C$ were retained, it would be difficult to refuse any extension to $GABA_D$, etc. These arguments are not convincing because IUPHAR accepts that the ionotropic $5\text{-}HT_3$ receptor should be placed within a group of several different metabotropic 5-HT receptors[16]!

Why should one have the 'ABC' of GABA receptors?

Pharmacology

$GABA_C$ receptors are a pharmacologically distinct group. Whereas $GABA_A$ and $GABA_B$ receptors are defined by their respective sensitivities to bicuculline and baclofen[3,6–8], $GABA_C$ receptors do not respond to either drug. The different pharmacological profiles of $GABA_C$ and $GABA_A$ receptors are illustrated in Table 1. Notably, CACA is a selective agonist for $GABA_C$ receptors but inactive at $GABA_A$ receptors, whereas the *trans*-enantiomer TACA shows no such preference[6,7,9,10]. Furthermore, TPMPA [(1,2,5,6-tetrahydropyridine-4-yl) methylphosphinic acid] has been identified as a potent and highly selective antagonist for $GABA_C$ receptors[20,21]. $GABA_C$ receptors are insensitive to $GABA_A$-modulatory drugs such as benzodiazepines, barbiturates and neurosteroids[7,9,10,17]. The Cl^- channel blocker picrotoxinin is a strong antagonist at both $GABA_A$ and ρ1 homomeric $GABA_C$ receptors[2,9–11]; however, ρ2 homooligomers and rat native $GABA_C$ receptors that are composed of ρ1ρ2 are rather insensitive to this compound[7,13].

Function

Electrophysiological responses of native or recombinant $GABA_C$ receptors also differ markedly from those of $GABA_A$ receptors (Table 2). $GABA_C$ receptors are about tenfold more sensitive to the physiological agonist[6,7,9,10,17]; the Hill slopes are steeper for $GABA_C$ receptors, which probably reflects the presence of five ligand binding sites on $GABA_C$ receptors[9,10], whereas only two appear to be present on $GABA_A$ receptors[2,4,9,10]. Both activation and inactivation time constants are very slow, albeit differing among ρ1, ρ2 and ρ1ρ2 $GABA_C$ receptors[13,22,23]. These findings have been taken as further evidence for rat retinal $GABA_C$ receptors being ρ1ρ2 pseudohomooligomers[12,13]. A remarkable and physiologically significant feature of $GABA_C$ receptors is their very weak desensitization, even at very high concentrations of agonist[6,7,9,10,17,24]. The robust and sustained responses and the high agonist sensitivity make $GABA_C$ receptors ideally suited for mediating strong lateral inhibition in the vertebrate retina[10]. At the molecular level, $GABA_C$ receptors display a very low single-channel conductance but rather long mean open times[6,7,9,10]. As expected for ligand-gated Cl^- channels, both ionotropic GABA receptor types have a high Cl^- selectivity and a similar pore size[7,9,10].

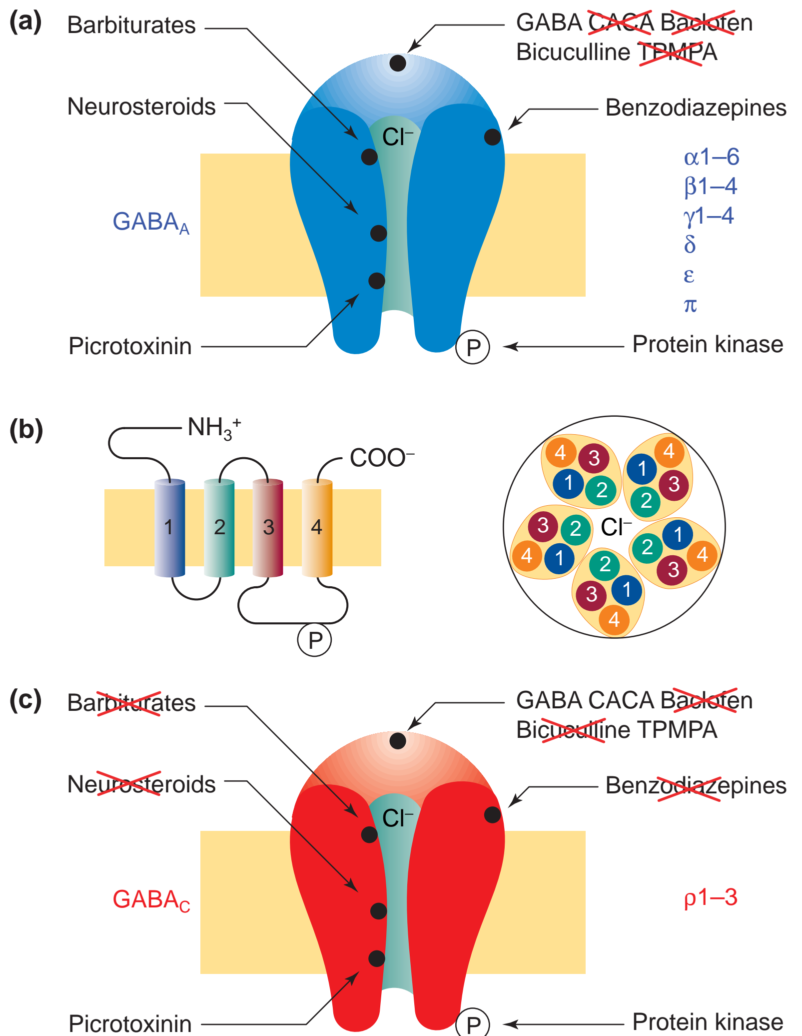

Fig. 1. Multiplicity of ionotropic GABA receptors. **(a)** The $GABA_A$ receptor is a Cl^- pore with ~5 Å diameter and modulatory binding sites for benzodiazepines, barbiturates and neurosteroids. The GABA responses are blocked competitively by bicuculline and noncompetitively by picrotoxinin; they are modulated intracellularly by protein kinases, such as protein kinases A and C. The $GABA_C$ receptor agonist CACA (*cis*-4-aminocrotonic acid) and the $GABA_C$ receptor antagonist TPMPA [1,2,5,6-tetrahydropyridine-4-yl(methylphosphinic acid)] have no effect (red crosses). Likewise, the $GABA_B$ receptor agonist baclofen is inactive. The vertebrate $GABA_A$ receptor complex is built from five subunits belonging to different families (α1–6, β1–4, γ1–4, δ, ϵ and π). **(b)** Each subunit comprises four transmembrane domains (TM1–TM4). The large intracellular loop between TM3 and TM4 contains consensus sites for phosphorylation by protein kinases (P). The amphiphilic TM2 provides the lining of the Cl^- pore intrinsic to the pentameric structure. The most abundant $GABA_A$ receptor in the brain is the α1β2γ2 isoform. **(c)** The membrane topology of $GABA_C$ receptors is assumed to be very similar to that of $GABA_A$ receptors. However, $GABA_C$ receptors are composed exclusively of ρ (ρ1–3) subunits, which can assemble into either homooligomeric or pseudohomooligomeric (e.g. ρ1ρ2) receptors. The $GABA_C$ receptor is also a Cl^- pore and is activated selectively by CACA; it is blocked competitively by TPMPA and noncompetitively by picrotoxinin. Bicuculline, baclofen as well as $GABA_A$-modulatory drugs are all inactive at this GABA receptor (red crosses). $GABA_C$ responses are downregulated upon intracellular phosphorylation by protein kinase C.

Table 1. Differential pharmacology of $GABA_C$ and $GABA_A$ receptors

Ligand	$GABA_C$ receptor	$GABA_A$ receptor	Refs
Bicuculline	Inactive	Antagonist	2, 4, 6, 10, 17–19
Baclofen	Inactive	Inactive	2, 6, 7, 9, 10, 17
Picrotoxinin	Antagonist[a]	Antagonist	2, 6, 7, 9–11, 13, 17, 18
TACA	Agonist	Agonist	6, 7, 9, 10, 19
CACA	Agonist	Inactive	6, 7, 9, 10, 19
TAMP	Agonist	Weak agonist	6, 19
CAMP	Agonist	Inactive	6, 19
Muscimol	Partial agonist	Agonist	2, 4, 6, 7
Isoguvacine	Weak antagonist	Agonist	2, 4, 20
THIP	Weak antagonist	Agonist	2, 6, 19
I4AA	Antagonist	Agonist	19, 20
TPMPA	Antagonist	Inactive	20, 21
1,4-Benzodiazepines	Inactive	Modulators[b]	7, 9, 10, 17, 18
Triazolopyridazines	Inactive	Modulators[b]	9, 10
Imidazopyridines	Inactive	Modulators[b]	9, 10
Barbiturates	Inactive	Modulators	7, 9, 10, 17, 18
Neurosteroids	Inactive	Modulators	7, 9, 10

[a]Strong antagonist only for ρ1 homooligomeric receptors.
[b]Not active at α4βγ, α6βγ, αβδ and αβε $GABA_A$ receptor subtypes[15]. Abbreviations: CACA, *cis*-4-aminocrotonic acid; CAMP, *cis*-2-aminomethyl-cycloproprane carboxylic acid; I4AA, imidazole-4-acetic acid; TACA, *trans*-4-aminocrotonic acid; TAMP, *trans*-2-aminomethyl-cycloproprane carboxylic acid; THIP, 4,5,6,7-tetrahydroisoxazolo[5,4-c]pyridine-3-ol; TPMPA, (1,2,5,6-tetrahydropyridine-4-yl)methylphosphinic acid.

Structure

$GABA_C$ receptors are also structurally distinct from $GABA_A$ receptors. Although fully functional $GABA_A$ receptors require heterooligomeric formation of α-, β- and γ-subunits[4,25], $GABA_C$ receptors can assemble as homooligomers[7,11,13,18,19]. To date, three different ρ-subunits (ρ1–ρ3) have been cloned from several mammalian and vertebrate species[9]. The ρ-subunits share only 30–38% amino acid sequence identity with the $GABA_A$ receptor subunits and they mediate robust bicuculline-insensitive GABA responses in heterologous expression systems[7,11,18,19]. There is no evidence so far from heterologous expression systems that the ρ-subunits coassemble with the $GABA_A$ receptor α-, β- and γ-subunits, or with the glycine receptor β-subunit[18,19].

In the rat retina, $GABA_C$ receptors are probably pseudohomooligomers composed of ρ1- and ρ2-subunits[12,13]; the ρ1ρ2 receptors display characteristic activation and inactivation properties that differ from those of either form of homooligomer[12,13]. Because the ρ1-subunit is expressed predominantly in the retina, $GABA_C$ receptors in other CNS regions are probably ρ2 homooligomers[12,13,26].

Table 2. Functional comparison of $GABA_C$ and $GABA_A$ receptors

Property	$GABA_C$ receptor	$GABA_A$ receptor	Refs
GABA EC_{50}	1–4 μM	5–100 μM	6, 7, 9, 10, 17
Hill slope	3–5	2	6, 7, 9, 10
Activation/inactivation	Slow	Fast	13, 22, 23
Desensitization	Weak	Strong	6, 7, 9, 10, 17, 24
Conductance	7 pS	27–30 pS	2, 6, 7, 9, 10
Open time	150–200 ms	25–30 ms	2, 6, 7, 9, 10
Selectivity	Anions (Cl^-)	Anions (Cl^-)	6, 7, 9, 10, 17
Pore size	5.1 Å	5.6 Å	7, 9, 10

Cellular localization

$GABA_C$ receptors show a distinct cellular and subcellular localization. Although $GABA_A$ receptors are present in all CNS regions, $GABA_C$ receptors are highly enriched in the vertebrate retina[7,27,28]. Using a polyclonal antibody, the ρ-subunits have been localized to the axon terminals and dendrites of bipolar cells[14,27,28]. Synaptic $GABA_C$ receptors comprising ρ-subunits are clustered in hot spots, but $GABA_C$ and $GABA_A$ receptor subunits do not colocalize in the same hot spots to form hybrid receptors[14]. Moreover, ρ-subunits and glycine receptor subunits are also clustered at different synapses[14].

The intracellular anchoring of $GABA_C$ receptors is also distinct from that of $GABA_A$ receptors. Colocalizaton of $GABA_C$ receptors and microtubule-associated protein (MAP-1B) has been shown at postsynaptic sites on bipolar cell terminals[29], which indicates that $GABA_C$ receptors are linked to the cytoskeleton via MAP-1B. This cytoskeletal protein specifically interacts with the ρ1-subunit but not with the $GABA_A$ receptor subunits. For $GABA_A$ receptors, a new cellular protein, $GABA_A$-receptor-associated protein (GABARAP), has been identified, which interacts with the γ2-subunit and colocalizes with $GABA_A$ receptors on cortical neurones[30]. These mechanisms might allow ionotropic $GABA_A$ and $GABA_C$ receptors to be expressed differentially at inhibitory synapses[30,31].

Genetics

The genes for $GABA_C$ receptor subunits are differentially localized within the genome. Inspection of the chromosomal localization of the genes encoding $GABA_A$ and $GABA_C$ receptor subunits reveals the existence of discrete gene clusters. In the case of the $GABA_A$ receptor, each cluster on human chromosomes 4, 5, 15 or X contains the genes for α, β and γ/ε (Ref. 15), a stoichiometry that would account for most of the native $GABA_A$ receptors[25]. Not only do these findings suggest a possible relationship with subunit coexpression[15], but McLean and colleagues have obtained evidence that these clusters derive from an ancestral αβγ gene cluster[32]. The genes for the ρ1- and ρ2-subunits are separated from these clusters and lie together on human chromosome 6 or mouse chromosome 4 (Ref. 15). The distinct chromosomal localizations of the genes for ionotropic GABA receptors further corroborate the clear distinction between $GABA_A$ and $GABA_C$ receptors.

Concluding remarks

Are the ρ-subunit-containing GABA receptors best classified as a specialized set of $GABA_A$ receptors, as provisionally proposed by the IUPHAR (Ref. 15)? Apparently not. Increasing knowledge of the differential pharmacology, structure, function, genetics and cellular localization of ionotropic GABA receptors already justifies the widely used $GABA_C$ terminology. Revising the provisional recommendations of the IUPHAR committee on GABA receptor nomenclature will not only keep track with recent progress but will also clarify the 'ABC' of GABA receptors!

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Sensible use of antisense: how to use oligonucleotides as research tools

Kathleen J. Myers and Nicholas M. Dean

In the past decade, there has been a vast increase in the amount of gene sequence information that has the potential to revolutionize the way diseases are both categorized and treated. Old diagnoses, largely anatomical or descriptive in nature, are likely to be superceded by the molecular characterization of the disease. The recognition that certain genes drive key disease processes will also enable the rational design of gene-specific therapeutics. Antisense oligonucleotides represent a technology that should play multiple roles in this process.

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Antisense oligonucleotides are short stretches of synthetic, chemically modified nucleic acids designed to hybridize to complementary mRNA sequences and block production of proteins encoded by the targeted mRNA transcripts. In principle, the rapid and specific inhibition of gene product expression makes antisense technology conceptually appealing for analysis of the function of newly discovered genes. However, antisense oligonucleotides are also being developed as drugs in their own right, with about a dozen products currently winding their way through various stages of clinical trials.

Although antisense oligonucleotides are being widely used as research tools, there is still considerable confusion about the design and interpretation of experiments using this approach. Antisense technology offers the potential for finely-honed specificity but, as is true for any pharmacological intervention, erroneous conclusions can ensue when experiments are poorly designed or inadequately controlled. The purpose of this article is to provide some guidelines for the use of antisense oligonucleotides in research. Some of the caveats involved in designing oligonucleotides and screening for activity will be

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