An Update on GABA\textsubscript{p} Receptors

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Abstract: The present review discusses the functional and molecular diversity of GABA\textsubscript{p} receptors. These receptors were originally described in the mammalian retina, and their functional role in the visual pathway has been recently elucidated; however, new studies on their distribution in the brain and spinal cord have revealed that they are more spread than originally thought, and thus it will be important to determine their physiological contribution to the GABAergic transmission in other areas of the central nervous system. In addition, molecular modeling has revealed peculiar traits of these receptors that have impacted on the interpretations of the latest pharmacological and biophysical findings. Finally, sequencing of several vertebrate genomes has permitted a comparative analysis of the organization of the GABA\textsubscript{p} genes.

Keywords: GABA receptor gene organization, Ligand Gated Ion Channel, Receptor structure, TPMPA.

DIVERSITY OF GABA RECEPTORS

GABA is one of the most important inhibitory neurotransmitters in the vertebrate central nervous system (CNS) and is involved in manifold physiological and pathological processes. As a molecule, it was first described in the early 1900\textquotesingle s, whereas its presence in the CNS and its role as a neurotransmitter were recognized only in the 1950\textquotesingle s [1-4]. During the following two decades, numerous studies were done to determine the specific mechanism of action of GABA, and in the 1960\textquotesingle s firm evidence established its inhibitory role in the cerebral cortex [5]. It is estimated that GABA is the most abundant inhibitory neurotransmitter in the CNS; it is widely distributed in all major areas of the brain and participates in about 40\% of the inhibitory synapses in adult vertebrates [6, 7].

GABA exerts its actions through two different types of plasma membrane receptors: GABA\textsubscript{A} and GABA\textsubscript{B}, which have different pharmacological, structural, and molecular characteristics [8-10]. In the 1990\textquotesingle s the existence of a new class of GABA\textsubscript{A} receptor was reported: the GABA\textsubscript{p} receptor, and in this review we will discuss the molecular properties, tissue distribution and the structural genes coding for these GABA\textsubscript{p} receptors. We will also present the evidence for their expression in the brain, a comparative analysis of their genomic structures, data derived from molecular modeling, and insight on their intracellular trafficking.

During the mid 1970\textquotesingle s several isolated reports described the existence of a type of GABA receptor that was insensitive to bicuculline, which contrasted with the antagonistic effect of this alkaloid on classical GABA\textsubscript{A} receptors. This peculiar component was also found in several areas of the CNS, including the cat spinal cord [11], frog optic tectum [12, 13], guinea pig superior colliculus [14], cerebellum, and rat cerebral cortex [15]. However, it was not until 1991 when expression of retinal mRNA in X. laevis oocytes clearly demonstrated the presence of an ionotropic GABA receptor, insensitive to bicuculline and resistant to baclofen [16]. This new receptor was named GABA\textsubscript{p} to denote its retinal origin.

The first subunit of this receptor family was cloned from a human retina cDNA library. The gene was named GABA\textsubscript{p1} [17], and it codes for a receptor that is abundant in the retina; when expressed in X. laevis oocytes it exhibited the same characteristics as the receptor found after injection of retinal mRNA. To denote the clear pharmacological and molecular properties of this receptor, a new family name was coined: the GABA\textsubscript{C} receptor, which comprised at that point the GABA\textsubscript{p} subunit but eventually grew to a total of three subunits: GABA\textsubscript{p}1, GABA\textsubscript{p}2, and GABA\textsubscript{p}3. Currently, the name GABA\textsubscript{C} is in disuse, and the three GABA\textsubscript{p} genes are included in the GABA\textsubscript{A} receptor family (GABA\textsubscript{A} receptor, \(\beta\) subunits); they are part of the Cys-loop superfamily of neurotransmitter receptors, also called the ligand-gated ion-channel (LGIC), which includes the GABA\textsubscript{A} receptors, nicotinic acetylcholine receptors (nAChR), glycine receptors (GlyR), ionotropic 5-HT receptors (5HT\textsubscript{3}), and a Zn\textsuperscript{2+}-activated ion channel [18].

BASIC STRUCTURE, BIOPHYSICS, AND PHARMACOLOGY

Based on studies performed with other ionotropic receptors and on electrophysiological evidence, it is thought that GABA\textsubscript{p} subunits assemble into a pentamer that forms a Cl\textsuperscript{-} channel in its center. The general structure of every subunit consists of an extracellular amino terminal domain, four transmembrane domains, and an extracellular carboxy terminus. The binding site of GABA is located in the extracellular amino-terminal domain that, upon activation, leads to the
opening of the ion channel with the subsequent flux of Cl⁻ through it.

There are three very distinctive functional characteristics that are unique to the GABA<sub>p</sub> receptor: long mean opening time of the channel, low conductance, and low rate of desensitization and the mean open time of the channel ranges from 150 to 200 ms, which is more than five-fold longer than that of other GABA<sub>A</sub> subunits [19-23].

The conductance of the human GABA<sub>p1</sub> channel ranges between 0.6 and 1.6 pS, and is considerably smaller than other GABA<sub>A</sub> subunits [24]. Membrane noise analysis of the cloned GABA<sub>p</sub> receptors of the white perch showed that the conductance of GABA<sub>p1</sub>A is 0.2 pS, that of GABA<sub>p2</sub>A is 3.2 pS, and that of GABA<sub>p2</sub>B is 3.5 pS [25]. It has been shown in X. laevis oocytes that after activation, GABA<sub>p1</sub> keeps the channel conducting even after ten minutes or more of exposure to the agonist, and the magnitude of the response diminishes only 8 to 10% during this time. Furthermore, repetitive applications of the agonist do not lead to a diminished response to subsequent applications, in clear contrast with other types of ionotropic receptors [16, 26]. In the axon terminal of the retinal bipolar neurons of the goldfish, the conductance of the GABA<sub>p1</sub> receptor is 4 pS [27], and it is 8 pS in the bipolar neurons of the rat [20]. In summary, the channel of the homomeric GABA<sub>p</sub> receptors conducts for a longer time with respect to different heteromeric combinations of the GABA<sub>A</sub> receptors, the main-state conductances are smaller, and have a lower rate of desensitization; however, we still have to characterize these properties for GABA<sub>p3</sub> receptors in the ganglion neurons of the retina and neurons in several other areas of the brain that express them.

One of the most relevant characteristics is the high sensitivity to GABA, with an EC₅₀ in the range of 0.8 to 2.2 μM for homomeric GABA<sub>p1</sub> and GABA<sub>p2</sub> recombinant receptors [16, 21, 22, 28-30] and around 7.5 μM for homomeric GABA<sub>p3</sub> receptors [22], which is over five-fold more sensitive than other GABA<sub>A</sub> subunits. In addition, GABA<sub>A</sub> currents on bipolar cells are rapid and transient, whereas GABA<sub>p</sub> currents are slower and desensitize very little; furthermore, GABA<sub>p</sub> receptors also have much higher sensitivity to GABA than typical GABA<sub>A</sub> receptors in retinal bipolar cells [31].

Four agents have been of great use for the study of GABA<sub>p</sub> receptors due to their high selectivity. Cis-4-aminoacetoic acid (CACA) and cis-2-aminoethyl cyclopropanoic acid (CAMP) are the most selective agonists for GABA<sub>p</sub> receptors [32], whereas the 1, 2, 5, 6-tetrahydropyridine-4 methylphosphonic acid (TPMPA) and 3-aminocyclopentyl methylphosphinic acid [(±)-cis-3-ACPMPA] are selective antagonists [33, 34]. Other antagonists reported to be highly specific for GABA<sub>p1</sub> are guanidine-acetic acid, amino-cyclopent-1-enyl phosphonic acid, and 3-aminocyclobutane phosphonic acid [35, 33, 5], whereas cyclothiazide blocks GABA<sub>p2</sub> [36]. Cis-and trans-(3-aminocyclopropenyl) butylphosphonic acid are a new generation of conformationally restricted analogues that competitively block GABA<sub>p</sub> receptors and prevent the development of experimental myopia [37, 38]. In addition, these compounds enhance learning and memory in rats, as assessed by the reduced time that the animals take to find the platform in the Morris water maze, which suggests a role for the receptor in cognitive processes.

The action of GABA on the GABA<sub>p</sub> receptors is allosterically modulated by a wide variety of chemical entities which interact with distinct binding sites at the GABA receptor complex [39, 40], either acting as inhibitors (Zn<sup>2+</sup>, Ni<sup>2+</sup>, Cd<sup>2+</sup>) or potentiators (Ba<sup>2+</sup>, Sr<sup>2+</sup>) [41, 42]. The lanthanides potentiate the GABA receptors in the cloned GABA<sub>p1</sub> receptor [41, 43].

Finally, the insensitivity to the GABA<sub>_A</sub> receptor antagonist bicuculline, its resistance to the GABA<sub>_A</sub>-receptor agonist baclofen [32, 35, 44, 45], and the lack of response to the GABA<sub>_A</sub>-receptor modulators, such as benzodiazepines [32, 46], barbiturates, and neurosteroids [32, 47] set apart this class of receptor.

**MOLECULAR MODELING**

The Cys-loop receptors, a gene superfamily to which the ionotropic GABA<sub>p</sub> receptors belong, have two conserved cysteines that form a disulfide bond [48]. Current understanding of the structural basis of how ionotropic Cys-loop receptors work is relatively advanced compared to other areas of ion channel biophysics [48-55]. Nevertheless, many questions remain, not only about biophysical properties but also about the structural basis for how these receptors affect cellular and systems physiology.

The best information about the molecular structure of the Cys-loop family comes from the nicotinic acetylcholine receptor (nAChR). This receptor forms heteropentameric complexes [14, 56-60] that have dextrohelicoidal symmetry with respect to an axis at the midpoint of the ion pathway and a tilt angle of 10° [61]. The GABA<sub>p</sub> receptors share three main domains with all the family members. The first one is the N-terminal extracellular domain, which consists of 10 β-sheets, 2 α-helices, and one 3<sub>10</sub>-helix; it is very similar to the conformation of the immunoglobulin β-sandwich domain and contains the agonist binding site and the Cys-loop. This domain also forms an extracellular ionic-preseletion vestibule of the electrostatic potential conferred by the receptor surface that is accessible to the solvent [61].

The second main domain is the pore itself, composed of the second (M2) of four transmembrane α-helices (M1-M4), whereas the remaining three (M1, M3, M4) form a “hydrophobic shelter”, protecting and incorporating the pore into the plasma membrane. The pore contains subdomains of functional importance, such as the “gate” associated with hydrophobic residues in the middle of an α-helix which is structurally distorted when the receptor is activated, leading to global conformational changes, and the “selectivity filter” which is conferred by the electrostatic restriction of charged residues in the narrowest and most intracellular region of the channel [49, 61-63].

Each transmembrane α-helix is connected with the next one through a loop; the third loop has an intracellular disposition and a variable length according to the subunit that forms receptor. Functionally, the second loop is very impor-
tant and confers several structural properties, such as key interactions with the cytoskeleton, anchoring and stabilizing the receptor, and determining its cellular location; it probably also affects the ion conductance. Finally, this loop forms another α-helix just before the M4 helix; this continued loop is called the MA helix and it forms an intracellular funnel-like structure which gives rise to a vestibule through which the ions are forced to enter the cell by way of the intersubunit window-like spaces [64].

In contrast, the GABA<sub>P</sub> receptor has proven to be difficult to overexpress and purify, and its structure has not been solved by high-resolution methods such as X-ray crystallography or nuclear magnetic resonance (NMR) [65, 66]. Thus, the homology-based modeling currently gives the most accurate and reliable structural model. It is based on the general observation that evolutionarily related (homologous) proteins are likely to have similar structures [67, 68]. We developed a model for the GABA<sub>P</sub> receptor (Fig. 1), using the well-known structure of the close homologue, nAChR from the Torpedo marmorata, and prokaryotic homologues such as the Erwinia chrysanthemi and Gleobacter violaceus [69, 70]. These models has helped us to design experiments to determine the functional role of amino acid residues of the M4 domain [71], and it will be useful to understand the structural rearrangements generated by ligand binding which lead to the channel activation.

Binding and gating are mutually coupled in an allosteric way in the GABA<sub>P</sub>1 receptor, in such a way that the binding for GABA appears strongly potentiating its affinity becoming virtually infinite, locking the agonist in the binding pocket. Channel gating thus produces broad conformational changes from the agonist binding pocket to the gating of the channel [30, 72]. A recent study has pointed out that the increase in agonist binding affinity and the channel opening do not occur simultaneously [73], but instead in two sequential steps: 1) the distortion in the binding domain, and 2) the channel opening. There is an intermediate state, termed the “flip state”, with the receptor binding domain switching to a high affinity state before channel opening. This event is well described by the Monod-Wyman-Changeux model of allosteric activation (MWC), suggesting strong similarity to the process of enzymatic catalysis [74-76].

The structural model of the agonist binding pocket was further validated and extended when the high resolution structure of the acetylcholine binding protein (AchBP) was determined [77-81]. In addition, the functional connection between the agonist binding pocket and channel opening was defined by using as template the structures of bacterial homologues [69, 70, 82]. Thus, it has been determined that tilting of M2, dilation of the pore, and a quaternary twist of the five subunits, are the key events of channel gating [83]. There are five potential binding pockets on each of the five subunit interfaces of Cys-loop receptors, and the five tyrosine residues (Tyr102, Tyr198, Tyr200, Tyr241, and Tyr247) that play a central role in the binding site are conserved in GABA<sub>P</sub>1 [84, 85]. It is stated that the GABA carboxylate

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**Fig. (1). Structural model of the GABA<sub>P</sub> receptors.** A) Ribbon diagram of a single GABA<sub>P</sub>1 subunit viewed in the plane of the transmembrane domains, beginning at the N-terminus of the ribbon and finishing at the C-terminus. The extracellular α-helix is labeled α-1. The β-strands are labeled 1-10; the four transmembrane α-helices (M1-M4), MA domains, and the cys-loop are highlighted. Dashed lines indicate the position of the plasma membrane. B) Transverse, and C) longitudinal representations of the GABA<sub>P</sub>1 homopentameric complex, with respect to the plane of the plasma membrane, highlighting the main domains: extracellular domain (ECD), transmembrane domain (MD), intracellular domain (MA), and the ion pathway.
group forms a salt bridge with Arg104, Ser168, Ser243, whereas its ammonium moiety is engaged in cation–π interactions or forms a hydrogen bond with Tyr198 in the so-called “loop C” that connects β-sheets 9 and 10 [44, 86, 87].

To achieve the long-range communication that is fundamental to the Cys-loop receptor function, other extracellular domain residues must be involved in communicating the binding event to the channel gate, playing a crucial step in the gating pathway [88]. Different regions and residues of the Cys-loop receptor subunits are essential in channel gating: loops β1–β2, β6–β7, and β8–β9 at the interface between the extracellular domain and transmembrane domains have been identified as constitutive transducers of the opening signal from the agonist binding site to the transmembrane domain [55].

However, in spite of these advances and understanding progressively more about the structural characteristics of the receptor, further experiments will be necessary to determine the precise, detailed mechanism of allosteric regulation, which may enable the design of drugs to palliate diseases involving GABA\textsubscript{A} receptors.

**DISTRIBUTION IN THE CNS AND BEYOND**

GABA\textsubscript{A} receptors have been extensively studied in the retina of several species, where they are mainly expressed in bipolar and horizontal cells [25, 38, 89-92, 95]; at least one report shows their expression in photoreceptors [96], and for many years it was believed that their expression was limited to this structure of the nervous system. However, over the last several years evidence has accumulated showing that GABA\textsubscript{A} receptors are, in fact, widely expressed, not only in the retina or areas related to the visual system but also in the peripheral nervous systems and even in the gastrointestinal, where the motor function within the myenteric plexus could be influenced by GABA\textsubscript{A} activation of the inhibitory motor neurons. In the cardiovascular system GABA acts directly on cardiac function through GABA receptors located within the electrical conduction system cells [97], and in sperm cells are involved in the acrosome reaction [98].

It is now known that GABA\textsubscript{A} subunits are expressed in the brain cortex, thalamus, hippocampus, superior colliculus, cerebellum, and spinal cord [99-104], ventral and dorsal lateral geniculate nuclei [105, 106], thyrotropin secreting cells of the pituitary [99, 107], the dorsolateral geniculate gyrus of the thalamus [108], and pretectal nucleus of the optic tract [109]. However, there are other areas of the CNS where their expression of GABA\textsubscript{A} subunits remains to be determined or is puzzling.

**Retina**

Numerous studies showed that GABA\textsubscript{A}1 and GABA\textsubscript{A}2 were specifically expressed in the bipolar and horizontal cells of the retina, whereas GABA\textsubscript{A}3 was found in the ganglion neurons [38, 89, 90, 92, 93, 95, 103, 110-114]. Further insight into the physiological role of these receptors in the retina was gained through the study of knock out mice for the GABA\textsubscript{A}1 subunit [115, 116]. This subunit plays no role in retinal development since there was no evidence of ana-

**Midbrain and Superior Colliculus**

The superficial layers of the mammalian superior colliculus (SC) are involved in processing the visual information, and it is precisely in this area that GABA\textsubscript{A} receptors are expressed [14, 120, 121]. GABAergic interneurons in the stratum gray superficialis (SGS) and pretectal nucleus complex (PNC) have been found to express the receptor and GABA transporter 1 (GAT 1) [102, 122-124]. In situ hybridization revealed GABA\textsubscript{A}1 and GABA\textsubscript{A}2 in the superficial gray layer of the rat SC [125, 126]. These findings have been confirmed by immunohistochemistry and electrophysiology [102, 109, 125, 127, 128]. Furthermore, a punctate pattern of expression was found in the superficial gray layer, which suggests the synaptic localization of these receptors. Interestingly, an increase in neuronal excitability was observed after the activation of GABA\textsubscript{A} receptors in this structure [128], in contrast to their effects in the retinal bipolar neurons [112]. Finally, GABA\textsubscript{A}2 seems to be of particular importance in the SC, because the knockout of GABA\textsubscript{A}1 does not eliminate GABA\textsubscript{A} receptor responses there [129], whereas GABA\textsubscript{A} responses are completely abolished in the retina [38]. By comparison to previously published data, this could indicate a fundamental difference between the retina and the SC in the rules that govern the expression and subunit composition of the GABA\textsubscript{A} receptors. In the visual cortex, only the GABA\textsubscript{A}2 subunit mRNA is expressed [102]. Finally, it has also been shown by in situ hybridization that GABA\textsubscript{A}1 and GABA\textsubscript{A}2 are expressed in the Stratum opticum [121], optic nerve tract [99], and superficial gray layer [130].

**Thalamus**

Also considered to be related to the visual system [131], the thalamus expresses the GABA\textsubscript{A}1 receptor [100], and they have been found in several nuclei. They were located in a subpopulation of cells in the dorsolateral geniculate nucleus; the GABA\textsubscript{A} subtype mediate 25% or more of the total current of the GABA\textsubscript{A}ergic ionotropic receptors predominantly, if not exclusively, expressed by local GABA\textsubscript{A}ergic interneurons [132]. GABA\textsubscript{A} receptors are also expressed in neurons of the tectothalamic projection [121] and nucleus habenularis medialis [127].

**Brain Cortex**

There is very little information about GABA\textsubscript{A} receptors in this structure. While the expression of GABA\textsubscript{A} receptors
is well known and in general, the GABAergic system plays a major role in the inhibitory functions of the cerebral cortex, the expression of GABAP receptors in this vast area of intricate neuronal networks is not well understood. By RT-PCR it has been possible to identify the GABAP2 subunit in the frontal, temporal, occipital, and parietal areas of the human cortex, whereas GABAP1 was found only in the frontal and temporal cortex [100]. It has also been demonstrated by in situ hybridization in the rat brain cortex that GABAP subunits are localized in the visual area of the occipital cortex [100]. It has also been demonstrated by RT-PCR that GABA receptors are localized in the visual area of the occipital cortex, whereas GABA receptors are localized in the visual area of the occipital cortex [100].

Evidence about the expression of GABAP receptors in this structure is still limited. So far, the expression of GABAP3 mRNA has been shown in rat [94] and of GABAP1 and GABAP2 in bovine striatum, and potential alternatively spliced variants of the GABAP1 subunit were identified in this species [133, 134]. The presence of GABAP in the basal ganglia is an interesting result, since the GABAergic system in this region is well known to be involved in movement control, and GABAP receptors were found in spiny pyramidal neurons of the dorsal caudate putamen [134]. However, the electrophysiological responses to GABA in this structure are consistent with the more common GABA receptors [58]. Further research will be necessary to clarify the expression and precise cellular localization of GABAP subunits and in addition, it would be necessary to explain the lack of GABAP receptor-like currents. One possibility could be a co-assembly of GABAP with GABAP subunits [8, 95, 135, 136], which would mask the typical GABAP pharmacology, but such complexes have not yet been reported in this area of the brain.

**Basal Ganglia**

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**Pituitary**

Evidence of the expression of GABAP1, GABAP2, and GABAP3 has been found in the pituitary of rat and guinea pig, including localization in somatrophs, gonadropins, and in an adenoma cell line (GH3) that was derived from this gland [99, 107, 133]. Interestingly, the GABA currents found in TSH cells are insensitive to bicuculline and resistant to baclofen, but they desensitize rapidly like a classic GABA receptor [99]. Other evidence suggests a specific role of GABAP receptors in the facilitation of prolactin secretion from anterior pituitary cells [137].

**Cerebellum**

One of the first clues to the existence of GABAP receptors was obtained from binding studies with tritiated baclofen and GABA, which showed the presence of GABA and CACA sites that are insensitive to bicuculline and resistant to baclofen [138]. Later on, GABAP1 and GABAP2 were detected in human cerebellum with higher expression of GABAP2 [127, 100]. Finally, GABAP1–3 mRNAs subunits and GABAP-like currents were reported in X. laevis oocytes injected with mRNA of cerebellar cortex [139], in agreement with previous evidence for the presence of mRNA in the Purkinje cell line and basket cells [103, 133], where a role for GABAP receptors in the phasic inhibition at interneuron-Purkinje cell synapses has been suggested [140].

**Brain Stem and Spinal Cord**

There is clear evidence for the expression of GABAP receptors in the spinal cord and brain stem as well as their co-assembly with other GABA subunits [45, 99, 120, 133, 141, 142], which suggests the formation by GABA and GABAP of functional, heteromeric receptors [120, 135, 134]. These studies also indicate that the mRNA of GABAP receptors is more abundant in the spinal cord than in the brainstem.

**Corpus Callosum**

There is little evidence supporting the expression of GABAP receptors in the commissures, where the population of neurons is very low and the main cell types are neuroglia. The expression of GABAP1 mRNA was demonstrated [133]; however, its function has not been proved, and GABAP currents reported in several studies show only the classic GABA component [143].

**Hippocampus**

Initially, the expression of GABAP receptors in this area was suggested by the transient expression of a bicuculline-insensitive, GABA-gated CI channel during early development [144]. Later on, several studies reported the localization and electrophysiological expression of GABAP receptors from both neurons in culture and slices of pyramidal and granule cells of hippocampus [99, 101, 106, 145-148]. Recent reports have suggested that activated GABAP receptors help to protect against neurotoxicity in hippocampal cultures [149].

**Amygdala**

GABAP receptors have been detected in several areas of the amygdala, including the lateral, basolateral, and central nuclei [150, 151]. Likewise, it has been reported postsynaptic GABAP receptors in this area [152]. Neurons in the lateral division of the central amygdala have two types of fast inhibitory synapses with either GABA or GABAP receptors but targeted to spatially and functionally distinct synapses [152]. Recordings in the lateral division of central amygdala showed miniature inhibitory postsynaptic currents (mIPSC) with small amplitude and slow rise-time whose frequency indicated the presence of GABAP receptors [153].

**Invertebrates**

Ionotropic GABA receptors described in invertebrates have characteristics that do not fit completely with those of vertebrate GABA receptors. The GABA-gated cation channel EXP-1, cloned from C. elegans [154], belongs to the LGIC superfamily and exhibits sequence homology to the Drosophila LCCH3 and GRD subunits. Interestingly, the Rdl (resistant to dieldrin) subunit of Drosophila displays 43% homology at the amino acid level to the rat GABAP1 subunit [155], but the functional and pharmacological characteristics are quite different. Analysis of the Drosophila gene sequences predicts one additional candidate, AAF48539 [156], and one more was predicted in the genome of one cnidarian Hydra magnipapillata, Gene ID 100201086 [157]. However,
to date, no invertebrate receptor subunit has been characterized as a clear homolog of vertebrate GABA\(\alpha\).

**GENOMIC STRUCTURE OF THE GABA\(p\) GENES AND INSIGHT INTO THEIR EVOLUTION**

Completion of the sequence of several vertebrate genomes, including human and chimpanzee as well as other organisms such as the tunicate *Ciona intestinalis*, allowed defining the distribution and the intron-exon structure of the genes coding for ionotropic GABA-receptor subunits. Mapping and sequencing of GABA\(\alpha\) subunit genes showed that they are organized in gene clusters composed of \(\alpha\), \(\beta\), and \(\gamma\) subunit genes \([68, 158-161]\). It is generally considered that the evolution of the myriad of genes encoding for GABA\(\alpha\) receptors comprised a number of events in the early evolution of the chordate lineage: first, a whole-genome duplication gave rise to the loci in at least two chromosomes; this was followed by a tandem duplication of an \(\alpha\)-subunit gene, after which a second whole-genome duplication occurred \([162]\). These events could account both for the multiple isoforms of \(\alpha\), \(\beta\), and \(\gamma\) subunits as well as for their localization in different chromosomes \([158, 161]\).

The GABA\(p\) coding-genes are considered to be very closely related to the GABA\(\alpha\)- and Glycine-receptor genes (about 40% nucleotide sequence homology), and it has been proposed that the two families share a common ancestor \([162]\). However, the evolutionary history and structural organization of the genes encoding the three GABA\(p\) subunits has been little explored \([159, 163]\).

Two of the three genes encoding for GABA\(p\) subunits (GABA\(p1\) and GABA\(p2\)) are located on chromosome 6q14-q21 of the human genome \([163]\), whereas GABA\(p3\) is found on chromosome 3q11-q13 \([159]\). DNA sequence comparisons among several completed genome sequences allowed us to determine the localization of GABA\(p\) genes and several other GABA\(p\)-like loci. The exon-intron composition of these genes, as predicted by computer analysis of intron-exon boundaries, is shown in Fig. (2). The DNA coding-sequence homology between human GABA\(p\) subunits and other vertebrates ranges from 82% for the fish *Morone americana* to 99% for chimpanzee *Pan troglodytes*.

The chromosome localization of the GABA\(p\) genes within the genomes of higher vertebrates is clearly syntenic and matches their human and murine distribution: GABA\(p1\) and GABA\(p2\) are found as a single cluster, whereas GABA\(p3\) is located on a different chromosome.

Analysis of the genomic sequence of the tunicate *C. intestinalis* \([164]\), revealed a GABA\(p\)-like gene on chromosome 4. Although a full functional analysis of the structural gene is necessary, as well as of the receptor itself, it is clear that this gene is closely related to the GABA\(p\) subunits.

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**Fig. (2).** Comparison of the exon and intron structure of GABA\(p\) genes. Relative positions of the coding and non-coding sequences are shown to scale. Exons are illustrated in red for all the genes. Notice the compact structure of the *C. intestinalis* GABA\(p\)-like gene.
GABA

Another interesting feature about the comparison of the timing of the first vertebrate genome duplication is in order to reconcile this observation with the hypothesis of an exhaustive search is needed in this and other tunicates prior to the appearance of the jawed fish [166, 167].

Another interesting feature about the comparison of GABA genes is their structural variation among different phylogenetic lineages. The number of exons for the three genes is generally between 8 and 10; however, two interesting exceptions occur: 1) the predicted number of exons for GABA2 of Pan troglodytes is 16, whereas the human gene has only 8. Nevertheless, the nucleotide sequences of the coding regions are 99% identical for the two species. The unique distribution and number of exons in the chimpanzee GABA2 raises the possible existence of splicing isoforms of its mRNAs. 2) An extreme example of a radically different gene structure is that of Gavus gallus. In this species, the number of exons is 21 for GABA1, whereas GABA2 and GABA3 have 12 and 7, respectively.

Despite all the differences in the gene structure of GABA receptors, it is worth mentioning that the amino acid sequence of the three receptors is highly conserved among the different taxa evaluated here. With its ancient origin, C. intestinalis represents the most divergent amino acid sequence of all chordates, about 40% homologous to the human GABA1.

The cluster-organization of genes may be of developmental and physiological relevance, contributing to their time- and cell-specific expression. Support for this idea is provided by the coordinated expression of GABA1 and GABA2 during early retinal development, where both subunit genes are transcriptionally active in the bipolar neurons at postnatal day 9, whereas GABA3 is expressed in a population of ganglion neurons [111, 141]. Based on the genomic arrangement of GABA genes, future experimental approaches will define the role of the cluster organization within the transcription regulatory context as well as the possible implications of the exon/intron composition of the genes in generating mRNA variants. Furthermore, sequence analysis of the upstream regions of each gene could suggest conserved motifs important for their expression in the retina.

SUBCELLULAR DISTRIBUTION

The precise location of the receptor channels in the synaptic cleft is a determinant for them to play their essential role in transmitting the synaptic impulse. The GABA receptors have been detected in the synaptic axon terminal of the bipolar neuron of the retina [90, 31, 93, 27, 114, 168]. Here, they modulate the surround and lateral inhibition [169]. We do not yet know how the receptor reaches this terminal end, and we have ignored the cellular and molecular components that dictate the trafficking of the receptor from its point of translation and towards the axon terminal.

Numerous interactions within the synapse organization regulate protein localization [170, 171], mediating the proper clustering and anchoring of receptors to the cytoskeleton. For some years, it has been proposed that GABA receptors associate with the Microtubule-Associated Protein 1B (MAP1B) through the interaction with the second intracellular loop of the GABA1 subunit [172]. In contrast, Meixner [173] showed that disruption of MAP1B is not a crucial element in the expression and localization of the receptor. Controversy emerged once again when Billups [174] demonstrated the association of MAP-1B with two key residues (KY) in the large intracellular loop of GABA1, and showed that this interaction modulates the mean open-time and kinetics of the channel.

Those contrasting observations are not definitive and the saga will continue until precise experiments define the association between GABA receptors and cytoskeleton. On the other hand, an interaction proposed between GABA1 and the cellular retinoic acid receptor could serve as a link between the GABA signaling pathway and the control of gene expression in neurons [175]; however, this has not been proven experimentally.

Receptors tagged with fluorescent labels will certainly be major tools to determine associations between the GABA and molecular and cellular components and to follow the trafficking of receptors in the neuron [176-178]. Likewise, the fact that the trafficking of the receptor may be actually visualized by using real time fluorescence microscopy from the site of protein translation to its final destination at the plasma membrane could shed some light on the dynamics and mechanisms that control receptor recycling. Many of these processes are currently being explored for GABA receptors [179, 180].

Since GABA receptor trafficking may be an important control point for regulating cell and network activity in the SNC and retina, new experimental approaches will help to answer emerging questions such as: What are the mechanisms involved in the liberation and/or selective retention that contribute to maintaining the dynamics and polarized distribution of the GABA receptors in neurons? Is the endocytic system a station of direct distribution versus liberation and/or is there a necessary step for the accumulation of the receptors, as well as of a new population of receptors expressed de novo? Is the distribution of receptors mediated by the differential distribution of proteins of membrane and/or cytoskeleton in the different neuronal compartments?

PERSPECTIVES

More studies are necessary to determine the precise distribution of GABA receptors in the central and peripheral nervous systems as well as in other organs and systems where they are expressed, such as the heart, the liver and the gastrointestinal tract [52, 99, 181]. We already know the location of the receptor in bipolar neurons of the retina, but future studies will disclose the cellular localization and function of the GABA receptors in other areas of the CNS such as the caudate nucleus, bulb, cerebellum, pons, and corpus callosum. The possible expression and role of alternatively spliced variants of these receptors in different areas of the CNS must also be examined [26, 99, 109, 133].

All of the evidence regarding the widespread distribution of GABA receptors suggests that they may be involved in more functions than previously thought, and we still have to
determine their physiological relevance. For example, it has been shown that TPMPA, the selective antagonist of GABAp receptors, increases both quiet and active waking, decreases total slow-wave sleep essentially by decreasing the slow-wave stage, and also decreases paradoxical sleep [9]. In the hippocampus, these receptors could also have a role in memory processes, as already suggested by some experiments [107]. According to the findings of several studies that located GABAp receptors in areas such as spinal cord motor neurons, Purkinje cells of the cerebellar cortex, bulb, pons and caudate nucleus, they could also play an important role in movement control [103, 133]. Furthermore, the expression of GABAp receptors in the amygdala [73, 182] and its possible correlation with anxiety should be considered. Alterations of GABA-signaling have been associated with alcohol dependence, and a recent study revealed single nucleotide polymorphisms in GABAp2 that is associated with this dependence [183].

One of the most interesting developments on GABAp receptors research is the possible therapeutic applications that these receptors may have. In a model of hepatic encephalopathy that affects hippocampal neurons, the stimulation of GABAp receptors reduces ammonia-induced accumulation of Cl−, leading to decreased hyperexcitability [147]. More recently, it has been described an association of polymorphisms in the GABAp2 gene with serum creatinine levels, an important biomarker for assessment of kidney function [184], thus proposing a potential role of GABAp receptors in the regulation of renal function. These are only a few of many potential regulatory roles of GABAp receptors and its participation in different physiological processes [10, 58, 83, 97, 98, 132, 152, 181, 183-188]. On the other hand, expression mediated by adenovirus of GABAp receptors suppressed neuronal hyperexcitability and the associated neuronal death [189] and therefore, virus-mediated neuronal GABAp receptor expression offers a potential therapeutic tool for directly inhibiting hyperexcited neurons responsible for clinical problems, thereby avoiding the generalized nervous system depression associated with pharmacological therapy [189]. Thus, it will be important to know the precise regulatory mechanism that activates the expression of the receptor in the proper cell types. Furthermore, the potential of adenovirus to express tagged receptors in living cells may allow the tracking of the localization and dynamics [189, 190].

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An Update on GABA<sub>p</sub> Receptors

Current Neuropharmacology. 2010, Vol. 8, No. 4 433

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