Molecular Neurobiology

ISSN0893-7648/96/12(3): 181–194/$8.50

$GABA_C$ Receptors in the Vertebrate Retina

Peter D. Lukasiewicz

Departments of Ophthalmology & Visual Sciences and of Anatomy & Neurobiology, Washington University School of Medicine, Box 8096, St. Louis, MO 63110-1093

Abstract

In the central nervous system (CNS), the inhibitory transmitter GABA interacts with three subtypes of GABA receptors, type A, type B, and type C. Historically, GABA receptors have been classified as either the inotropic $GABA_A$ receptors or the metabotropic $GABA_B$ receptors. Over the past 10 yr, studies have shown that a third class, called the $GABA_C$ receptor, also exists. $GABA_C$ receptors are found primarily in the vertebrate retina and to some extent in other parts of the CNS. Although $GABA_A$ and $GABA_C$ receptors both gate chloride channels, they are pharmacologically, molecularly, and functionally distinct. The ρ subunit of the $GABA_C$ receptor, which has about 35% amino acid homology to $GABA_A$ receptor subunits, was cloned from the retina and, when expressed in *Xenopus* oocytes, has properties similar to retinal $GABA_C$ receptors. There are probably distinct roles for $GABA_C$ receptors in the retina, because they are found on only a subset of neurons, whereas $GABA_A$ receptors are ubiquitous. This article reviews recent electrophysiological and molecular studies that have characterized the unique properties of $GABA_C$ receptors and describes the roles that these receptors may play in visual information processing in the retina.

Index Entries: GABA; $GABA_C$ receptor; retina; ρ1 receptor; ρ2 receptor; horizontal cells; bipolar cells; bicuculline-insensitive.

Introduction

GABA is the major inhibitory transmitter in the central nervous system (CNS). It is present in high abundance in the brain and in the retina. GABA receptors have been traditionally classified as either $GABA_A$ or $GABA_B$ receptors *(1)*. $GABA_A$ receptors are heteromeric ligand-gated ion channels composed of different polypeptide subunits (e.g., α, β, γ, δ, with several isoforms in each class). Like other members of ligand-gated ion-channel superfamily, GABA receptors probably exist as pentomers *(2)*. The ionotropic $GABA_A$ receptors are antagonized by bicuculline and picrotoxin and their responses are potentiated by barbiturates and benzodiazepines. In different parts of the CNS, $GABA_A$ receptors exhibit different pharmacological sensitivities, suggesting that there are different subtypes of $GABA_A$ receptors *(3)*. Molecular cloning experiments have revealed many polypeptide subunits for $GABA_A$ receptors, also indicating that there may be a variety of $GABA_A$ receptors *(3,4)*. *In situ* hybridization

Table 1
GABA Receptor Selectivity

	$GABA_A$	$GABA_B$	$GABA_C$
Agonists			
GABA	X	X	X
Muscimol	X	X	
Baclofen		X	
TACA	X	X	X
CACA	X (weak)		X
Antagonists			
Bicuculline	X		
SR95531	X		
Picrotoxin	X		X
I4AA	Weak agonist		X
3-APA		Agonist	X
3-APMPA		Agonist	X

experiments demonstrate that the $GABA_A$ receptor subunits are differentially distributed in various brain regions *(3,4)*, reinforcing the notion that there are regional $GABA_A$ receptor differences. The metabotropic $GABA_B$ receptors are pharmacologically, molecularly, and functionally distinct from $GABA_A$ receptors. The $GABA_B$ receptors activate G-proteins, are sensitive to the agonist baclofen, but are insensitive to bicuculline blockade. Additional studies have shown that a third class of GABA receptors exists that is insensitive to both the $GABA_A$ antagonist bicuculline and the $GABA_B$ agonist baclofen *(5)*. This third class of GABA receptor, the subject of this article, was termed the $GABA_C$ receptor *(6)*. The selectivity of various ligands for the different GABA receptor types is summarized in Table 1.

$GABA_C$ Receptors

The first suggestion of $GABA_C$ receptors comes from binding studies that identified a GABA binding site on rat cerebellum synaptic membranes that was insensitive to both baclofen and bicuculline *(5)*. However, earlier indications of $GABA_C$ receptors come from studies with cis- (CACA) and trans-4-aminocrotonic acid (TACA), folded and extended analogs of GABA, respectively. TACA, which was equal in potency to GABA, inhibited spinal cord firing in a bicuculline-sensitive manner, but CACA, which was about a quarter of the potency of GABA, depressed firing in a bicuculline-insensitive manner *(7)*. CACA probably did not act at a $GABA_B$ receptor to reduce firing, because unlike TACA, it did not inhibit the binding of [^{3}H]baclofen *(5)*. Subsequent work in other parts of the CNS showed that the bicuculline-insensitive action of CACA was mediated by $GABA_C$ receptors *(8–10)*. $GABA_C$ receptor-mediated responses have been described in the retina *(9,10)*, the frog tectum *(8,11)*, the guinea pig superior colliculus *(12)*, and the developing rat hippocampus *(13)*. These studies showed that $GABA_C$ receptors gate chloride channels and are insensitive to bicuculline antagonism or baclofen activation.

Another CACA-sensitive, GABA receptor subtype has been described that modulates calcium currents *(14)*. This receptor is also insensitive to both bicuculline and baclofen, but it is functionally distinct from the $GABA_C$ receptor because it does not activate a chloride channel. Thus, there are multiple CACA-sensitive receptors, making it difficult to characterize $GABA_C$ receptors on the basis of binding studies *(5)* or extracellular recording studies *(7)*. The receptors characterized by Johnston et al. *(15)* may be different from retinal $GABA_C$ receptors in the retina, because unlike retinal $GABA_C$ receptors, they are more sensitive to CACA than TACA.

In vertebrates, $GABA_C$ receptors are found primarily in the retina and, to a lesser extent, in other parts of the brain. The first indication of that $GABA_C$ receptors were abundant in the retina was revealed by molecular biological studies. A $GABA_C$-like receptor subunit primarily expressed in the retina was discovered by Cutting and colleagues *(16)* and was called the ρ1 subunit. The ρ1 subunit displays 30–38% amino acid similarity to the α, β, γ, and δ $GABA_A$ receptor subunits. Comparison of amino add *(16)* or DNA information *(2)* indicates that the ρ1 subunit is most closely related to the β $GABA_A$ receptor subunits. Using

Northern hybridization, Cutting et al. *(16)* showed that ρ1 subunit expression was higher in the retina than in the cerebellum, and there was no expression in other regions of brain and spinal cord.

When mRNA from mammalian retina or cerebral cortex was expressed in *Xenopus* oocytes, only oocytes injected with retinal mRNA produced GABA currents with a unique $GABA_C$-like pharmacology *(17)*. Oocytes injected with cortical mRNA produced currents with a $GABA_A$-like pharmacology. The GABA currents measured in the oocytes injected with retinal mRNA exhibited two components: a major $GABA_C$- or ρ-like component and a minor $GABA_A$-like component. Oocytes injected with ρ1 mRNA also produced $GABA_C$-like currents *(18)*. The GABA responses in both retinal mRNA- and ρ1 mRNA-injected oocytes were insensitive to bicuculline blockade and to barbiturate and benzodiazepine enhancement, but were blocked by picrotoxin. Interestingly, picrotoxin was about 30 times more effective in reducing GABA-evoked currents in oocytes injected with cortical mRNA compared to those injected with retinal mRNA *(19)*. Unlike $GABA_A$ receptor subunits, which function optimally when they form heteromeric channels *(20,21)*, the ρ1 subunits form functional homomeric receptors when expressed in *Xenopus* oocytes *(18)* or COS cells (mor'key kidney cells, transformed by SV40) *(22)* (*see* Fig. 1A). Coexpression of the ρ1 subunit with either the α1 or β1 $GABA_A$ receptor subunits did not change the ρ1 bicuculline or barbiturate insensitivity *(18)*, suggesting that either these $GABA_A$ receptor subunits do not form heteromeric assemblies with the ρ1 subunit or that the ρ1 phenotype is dominant.

GABA receptors are found on almost all retinal neurons. There are five fundamental classes of retinal neurons: photoreceptors, horizontal cells, bipolar cells, amacrine cells, and ganglion cells, as shown in Fig. 2. The retina is a highly organized, layered structure that is part of the CNS and is an outpost of the brain. It has similar embryological origins to the brain, being derived from neuroectoderm. The somas of these neurons are located in three different nuclear layers: photoreceptors in the outer nuclear layer (ONL), amacrine, bipolar, and horizontal cells in the inner nuclear layer (INL), and ganglion and amacrine cells in the ganglion cell layer (GCL). All synaptic interactions occur in two layers, the outer plexiform layer (OPL) between the ONL and INL and the inner plexiform layer (IPL) between the INL and GCL.

Although $GABA_A$ receptors are located on all types of retinal neurons, electrophysiological results show that in many species, $GABA_C$ receptors are located primarily on horizontal and bipolar cells in many species. In the turtle *(24)* and salamander *(25)* ganglion cells, the small component of their GABA-evoked responses that remained in the presence of competitive $GABA_A$ receptor antagonists may be mediated by $GABA_C$ receptors. However, others *(26,27)* do not see evidence for $GABA_C$ receptors on salamander ganglion cells, suggesting that the putative $GABA_C$ component might be the result of incomplete blockade of $GABA_A$ receptors. $GABA_B$ receptors are also found on subsets of retinal neurons. Their function has been studied primarily in salamander retina, in which they are located on some amacrine and ganglion cells *(28)*, as well as a subtype of bipolar cell *(29)*.

Qian and Dowling *(10)* recorded GABA receptor-mediated currents in cultured teleost fish horizontal cells with properties similar to the currents measured in retinal mRNA- and ρ1 mRNA-injected oocytes. The horizontal cell GABA currents are mediated by chloride, are insensitive to bicuculline blockade, are not enhanced by barbiturates or benzodiazpine, but are antagonized by picrotoxin. Baclofen, the $GABA_B$ agonist, did not elicit currents in these horizontal cells. GABA receptors with similar properties were also found on goldfish *(30)*, rat *(9)*, and salamander bipolar cells *(27)*. The properties of $GABA_C$ and $GABA_A$ receptors are summarized in Table 2. However, the $GABA_C$ receptors on rat bipolar cells were found to have a different pharmacological property compared to those described in other species *(9,31)*. The rat $GABA_C$ receptor was relatively insensitive to

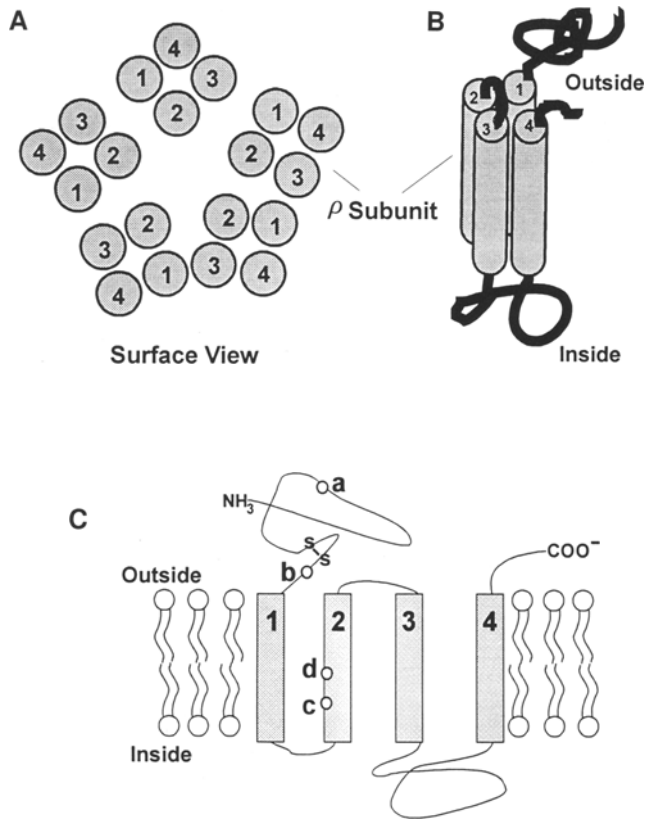

Fig. 1. Different views of the putative structure of the $GABA_C$ receptor. **(A)** View of the presumed pentomeric structure or $GABA_C$ receptor looking down onto the cell surface. The receptor is composed of five subunits; an example of the subunit structure is shown in (B). The opening within the five subunits is thought to be the channel of the receptor. The $GABA_C$ receptor may be a homomeric receptor with five identical ρ1 subunits *(22)*. However, heteromeric $GABA_C$ receptors may also exist, as suggested by the expression of rat heteromeric ρ1/ρ2 receptors in *Xenopus* oocytes *(23)*. **(B)** Each subunit is composed of four membrane-spanning domains. A large extracellular N-terminal domain is attached to the first membrane-spanning domain. The second membrane-spanning domains line the channel of the receptor. A large intracellular domain exists between the third and fourth transmembrane domains. **(C)** Crosssectional view of single subunit in the plane of the membrane. The subunits are separated for clarity. The labeled circles indicate relative positions on the subunit structure where point mutations were made as described in the text. The cysteine loop formed by the disulfide bond **S—S** is indicated as a marker for the relative positions of **(a)** and **(b)**. Mutations at position 156 (a) affect zinc inhibition of ρ1 receptors. Mutations at positions 198, 200,241,244, and 247 (b) affect agonist binding to ρ1 receptors. Mutations at positions 309 **(c)** and 314 **(d)** affect picrotoxin inhibition (*see text* for details).

antagonism by picrotoxin, suggesting that there are multiple forms of $GABA_C$ receptors.

Invertebrate $GABA_C$-Like Receptors

Bicuculline- and baclofen-insensitive GABA receptors are also present on nerves, muscles, and cardiovascular tissue of invertebrates *(32–34)*. The pharmacology of these receptors is similar to the homo-oligomeric ρ receptor expressed in *Xenopus* oocytes and to the retinal $GABA_C$ receptor. For example, a GABA receptor in *Limulus* heart muscle is not antagonized by bicuculline or picrotoxin, nor was its response enhanced by barbiturates or benzodiazepines *(33)*. Bicuculline-insensitive GABA receptor subunits with properties similar to $GABA_C$ receptors were cloned from *Drosophila (35)* and the mollusk, *Lymnea (36,37)*. The *Lymnea* subunit was somewhat similar to vertebrate $GABA_A$ receptor β subunits and $GABA_C$ receptor ρ subunits, exhibiting approx 50 and 45% amino acid identity, respectively. The *Drosophila* GABA receptor subunit, *Rdl*, was found to have no greater amino acid sequence similarity to ρ1 subunits than to any of the other $GABA_A$ receptor subunits, suggesting that despite many similar properties, the vertebrate ρ subunits and the *Drosophila Rdl* subunit are not closely related.

$GABA_C$ Receptor Agonists and Antagonists

Although highly selective $GABA_C$ receptor agonists and antagonists are not currently available, a number of $GABA_A$ and $GABA_B$ receptor ligands do interact with these receptors (*see* Table 1). The $GABA_C$ receptors on retinal

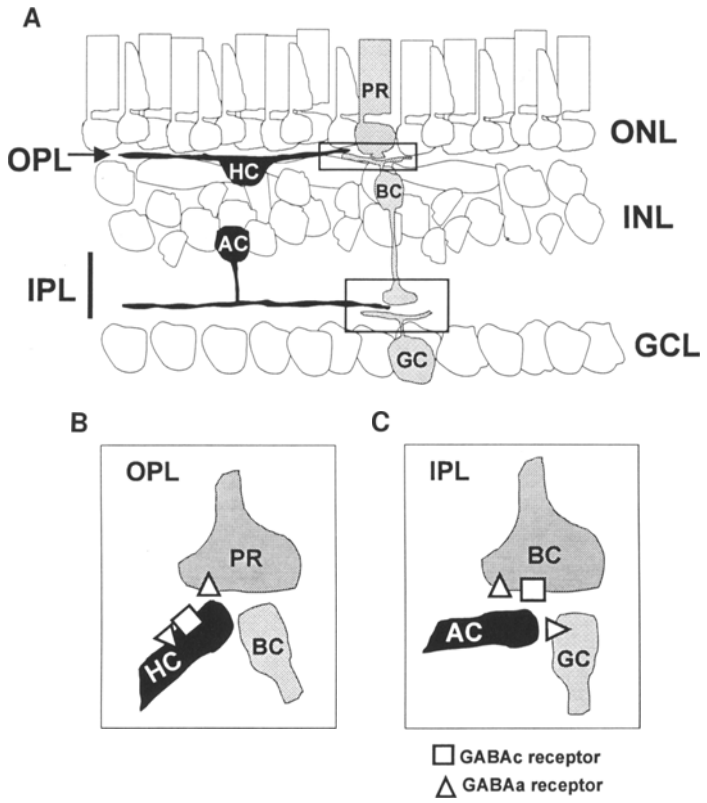

Fig. 2. Cross-section view of layers and cell types in the vertebrate retina illustrating GABAergic synapses. **(A)** Schematic view of retinal nuclear and plexiform layers. Photoreceptor (rods and cones) somata are located in the ONL; bipolar, amacrine, and horizontal cell somata are located in the INL; and ganglion and amacrine cells are located in the GCL. GABAergic neurons are indicated in black. At the OPL, synaptic interactions occur among photoreceptors (PR), bipolar cells (BC), and horizontal cells (HC). The synapses within the box in the OPL are detailed in (B). At the IPL, synaptic interactions occur among bipolar, amacrine, and ganglion cells. The synapses within the box in the IPL are detailed in (C). *See text* for more details. **(B)** Diagram showing a highly magnified view of PR, BC, and HC processes at the OPL. The black, HC process is GABAergic. The squares represent $GABA_C$ receptors and the triangles represent $GABA_A$ receptors. Photoreceptors have $GABA_A$ receptors on their terminals, whereas horizontal cells have both $GABA_C$ and $GABA_A$ receptors. GABA receptors are not shown on bipolar cell dendrites because in many species they have no (or weak) GABA sensitivity. **(C)** Diagram showing a highly magnified view of AC, BC, and GC processes at the IPL. The black, AC process is GABAergic. Bipolar cell terminals have both $GABA_C$ and $GABA_A$ receptors, whereas ganglion cell dendrites have just $GABA_A$ receptors.

neurons are activated by some $GABA_A$ agonists. The rank order of potency at both mammalian and cold-blooded vertebrate types of retinal $GABA_C$ receptor was GABA ≥ TACA > muscimol > CACA *(9,10,26)*. When ρ1 or retinal mRNA was expressed in *Xenopus* oocytes, the order of potency was somewhat different, TACA > GABA > muscimol > CACA. In contrast, for the similarly expressed $GABA_A$ subunits α5β1 *(22)* or cortical mRNA *(38)*, the order of potency was muscimol > GABA > TACA >> CACA.

Picrotoxin, as stated above, is an effective antagonist of $GABA_C$ receptor-mediated responses of retinal neurons in cold-blooded vertebrates, but not in rat. Extensive pharmacological studies of $GABA_C$ receptors on fish horizontal cells were made by Qian and Dowling *(39)*

Table 2
Properties of $GABA_C$ and $GABA_A$ Receptors

Property	$GABA_C$	$GABA_A$
Relative GABA affinity	High affinity	Low affinity
Gate chloride channels	Yes	Yes
Response time-course	Sustained	Transient
Response onset	Slow	Fast
Enhancement by barbiturates or benzodiazepines	No	Yes
Antagonism by bicuculline	No	Yes
Retinal location	Slow potential neurons	Slow potential and spiking neurons

to identify additional antagonists. They showed that imidazole-4-acetic acid I4AA), a low-affinity $GABA_A$ receptor agonist, potently and competitively inhibited $GABA_C$ receptor-mediated GABA responses. Studies with ρ1 receptors expressed in *Xenopus* oocytes show that I4AA is a partial agonist that effectively acts as a competitive inhibitor of GABA-elicited currents, because its maximum agonist response is only 3% GABA's maximum *(22)*. The $GABA_B$ receptor agonists 3-aminopropylphosphonic acid (3-APA) and 3-aminopropyl[methyl]phosphonic acid (3-APMPA) have been shown to act as a competitive antagonists at ρ-like receptors expressed in oocytes injected with retinal mRNA, with 3-APMPA being more potent *(38)*. At both fish horizontal cells *(39)* and rat bipolar cells *(31)*, these $GABA_B$ receptor agonists also acted as selective $GABA_C$ receptor antagonists. Hence, in neurons that lack $GABA_B$ receptors (e.g., fish horizontal cells and rat bipolar cells) 3-APA and 3-APMPA can be used as $GABA_C$ receptor antagonists. However, for I4AA to be employed as a $GABA_C$ receptor antagonist, it must be used in combination with bicuculline to counter its $GABA_A$ receptor agonist actions.

Molecular Studies of ρ Subunits from Retina

Molecular cloning experiments have shown that there are several types of ρ subunits. Two types of human ρ subunits have been cloned, the ρ1 and ρ2 subunits. The human ρ2 subunit is very similar to the ρ1 subunit. They share 74% amino acid sequence identity and form homo-oligomeric GABA receptors when individually expressed in *Xenopus* oocytes that are insensitive to bicuculline, barbiturates, and benzodiazepines, but are sensitive to blockade by picrotoxin and zinc *(40)*. However, the magnitude of currents generated by ρ2 subunits was always smaller than that generated by ρ1 subunits. The genes encoding the two ρ subunits map to the same region of chromosome 6, suggesting that one ρ gene arose from duplication of the other *(41)*.

Human ρ1 and ρ2 subunits expressed in *Xenopus* oocytes, as well as many native $GABA_C$ receptors, are very sensitive to antagonism by picrotoxin. The human ρ2 subunit is approx 10 times more sensitive to picrotoxin than its ρ1 counterpart *(42)*. However, the $GABA_C$ receptor on rat bipolar cells is relatively insensitive to picrotoxin blockade *(9,31)*. Zhang and colleagues *(23)* showed that the cloned rat ρ2 subunit, which has a 91% shared amino acid identity with the same length of human ρ2, was responsible for picrotoxin resistance in rat $GABA_C$ receptors. When ρ2 mRNA was injected into *Xenopus* oocytes, functional channels were not formed. However, heteromeric rat ρ1/ρ2 subunits were expressed in *Xenopus* oocytes and were picrotoxin-resistant, whereas similarly expressed homomeric rat ρ1 subunits were picrotoxin-sensitive. The degree of picrotoxin resistance was shown to be proportional to the amount of ρ2 mRNA coinjected with ρ1 mRNA. Site-directed mutagenesis

demonstrated that a single amino-acid residue in the second membrane-spanning region (position 314; **d** in Fig. 1C), the putative channel-spanning zone, is critical for picrotoxin sensitivity of rat ρ subunits *(23)*. A methionine that is present in the native ρ2 subunit confers picrotoxin insensitivity; when threonine was substituted for methionine, the ρ2 subunit exhibited picrotoxin sensitivity.

Picrotoxin shows both competitive and noncompetitive antagonism of retinal $GABA_C$ receptors *(39)*. Mutagenesis studies of the human ρ1 subunit revealed the molecular basis of picrotoxin inhibition. Substitution of serine for the proline at position 309 in the second transmembrane region (**c** in Fig. 1C) was found to increase the sensitivity to picrotoxin of ρ1 subunits expressed in *Xenopus* oocytes *(42)*. However, when the same point mutation was made in ρ1 subunits transfected into human embryonic kidney cells, the picrotoxin sensitivity was decreased *(43)*. The ρ2 subunit has a serine residue at position 309 and, as stated above, the ρ2 subunits are more sensitive to picrotoxin than the ρ1 subunits when expressed in *Xenopus* oocytes *(42)*. In addition, the competitive component of picrotoxin inhibition of the ρ1 subunit was eliminated by the substitution of serine for proline at position 309 *(42)*.

Both native $GABA_C$ receptors *(44)* and expressed ρ subunits *(40,45,46)* are sensitive to zinc inhibition. Some expressed $GABA_A$ receptors are sensitive to zinc, but the zinc sensitivity depends on their subunit composition. Heteromeric α/β subunits are sensitive to zinc, but inclusion of γ subunits produces receptors that are insensitive to zinc *(47)*. The zinc inhibition of ρ1 subunit-mediated responses was susceptible to changes in external pH over a range to which only histidine (and not other amino acids) was sensitive, indicating that histidine residues might be important for zinc interactions *(42)*. Site-directed mutagenesis of a histidine to a tyrosine in a putative extracellular domain (position 156, **a** in Fig. 1C) abolished zinc sensitivity without affecting any of the other properties of the expressed subunit *(42)*.

What is the molecular basis for the unique pharmacological properties of ρ subunits compared to $GABA_A$ receptor subunits? Amin and Weiss *(48)* set out to determine if these differences were the result of distinct putative agonist binding domains on the ρ subunits. They identified five amino acid residues between the N-terminal, extracellular, cysteine loop and the first transmembrane domain that are critical for GABA activation of the ρ subunits (**b** in Fig. 1C). A site-directed mutation at any of these residues significantly reduced GABA sensitivity. Three of these residues correspond to amino acids important for GABA binding on the β2 subunit of $GABA_A$ receptors, but the other two did not. It has been suggested by Amin and Weiss *(48)* that the differences in GABA binding domains between ρ1 and β2 subunits could account for the distinct pharmacological properties of expressed ρ1 channels.

In situ hybridization studies showed that both ρ1 and ρ2 mRNA transcripts are present in the inner nuclear layer of rat retina *(49)*. The labeling was strongest in the outer half of the inner nuclear layer, suggesting that bipolar and horizontal cells express ρ1 and ρ2 mRNA. The patterns of labeling of ρ1 and ρ2 mRNA were not significantly different, suggesting that both ρ subunits may be expressed in the same cells. It was also demonstrated that both ρ1 and ρ2 subunits were present in isolated rat bipolar cells *(49)*. These data suggest that the picrotoxin insensitivity observed in rat bipolar cells is probably the result of the presence of both ρ1 and ρ2 subunits, since in *Xenopus* oocytes picrotoxin insensitivity was only seen when both subunits were expressed *(23)*. There was no localization of either ρ subunit mRNA in photoreceptors, amacrine cells, and ganglion cells, consistent with electrophysiological findings described below. In chicken retina, both ρ1 and ρ2 transcripts were found almost exclusively in the inner nuclear layer, but the two transcripts were differentially distributed *(50)*. The ρ1 RNA transcript was found predominantly in bipolar cells, whereas the ρ2 RNA transcript was found in amacrine and horizontal cells. The presence of the ρ transcript in

amacrine cells is inconsistent with the physiological findings in other species that indicate a lack of $GABA_C$ receptors on these retinal neurons *(9,26,27)*. Additional studies on chicken amacrine cells are needed to determine whether the ρ2 transcripts are translated into protein and whether ρ2 subunits have a functional role.

$GABA_C$ Receptors in Retinal Circuits

As shown in Fig. 2, the most direct pathway for visual information to pass from the environment to the brain consists of the vertical, three neuron pathway (indicated in gray) composed of photoreceptors, bipolar cells, and ganglion cells. Horizontal cells (indicated in black) mediate lateral signals at the outer plexiform layer and modulate the synaptic interactions between photoreceptors and bipolar cells. Amacrine cells (indicated in black) mediate lateral signals at the inner plexiform layer and modulate signals between bipolar and ganglion cells.

Many horizontal cells in lower vertebrates and amacrine cells in all vertebrates are known to be GABAergic *(51)*. Horizontal cells make synaptic contacts onto photoreceptor terminals, bipolar dendrites, and other horizontal cells, whereas amacrine cells make synaptic contacts onto bipolar cell terminals, other amacrine cells, and ganglion cells (Figs. 2B,C). The characterization of the GABA receptor subtypes that mediate inputs from horizontal and amacrine cells has been the subject of intense investigation. GABA-evoked currents *(52,53)* and inhibitory synaptic inputs *(54)* at photoreceptor terminals were blocked by bicuculline, indicating that they are mediated primarily by $GABA_A$ receptors. $GABA_A$ receptors also appear to mediate most, if not all, of the GABA-evoked chloride currents in amacrine *(9)* and ganglion cells *(26,27)*. In contrast to other types of neurons in the retina, a major fraction of the GABA-evoked chloride currents in both horizontal cells *(10,26,39)* and bipolar cells *(9,27,30,55)* is mediated by $GABA_C$ receptors. In many cases, $GABA_C$ receptors coexist with $GABA_A$ receptors on bipolar and horizontal cells *(9,26,27)*.

Why do both $GABA_A$ and $GABA_C$ receptors exist on the same bipolar or horizontal cell if they both gate chloride channels? The characterization of native $GABA_C$ receptors and ρ-receptors expressed in *Xenopus* oocytes has shown that $GABA_A$ and $GABA_C$ receptors have different functional properties. $GABA_C$ and ρ receptors are more sensitive than $GABA_A$ receptors to GABA. When expressed in *Xenopus* oocytes or COS cells, ρ subunits are 30–60 times more sensitive to GABA than heteromeric $GABA_A$ receptor subunit combinations *(17,22,48)*. In rat bipolar cells, $GABA_C$ receptors are about eight times more sensitive than the $GABA_A$ receptors to GABA when characterized on the same bipolar cell *(56)*. $GABA_C$ receptors on fish horizontal cells were also found to be more sensitive than $GABA_A$ receptors *(10)*.

Additionally, the time-courses of $GABA_C$ receptor-mediated responses are distinct from those for $GABA_A$ receptor-mediated responses. $GABA_A$ receptor-mediated currents are transient; they rapidly reach a peak and then decay to a lower level in the maintained presence of GABA *(10)*. In contrast, $GABA_C$ receptor-mediated currents are sustained, showing minimal desensitization in response to maintained GABA applications *(10,55)*. Similarly, heteromeric $GABA_A$ receptor subunits expressed in *Xenopus* oocytes mediated transient responses, whereas similarly expressed ρ1 receptors mediated sustained responses to maintained GABA applications *(48)*. In addition, the ρ1 receptors activated and closed (deactivated) about eight times more slowly than their $GABA_A$ counterparts composed of α1β2γ2 subunits. Similarly, native $GABA_C$ receptors also deactivate more slowly than native $GABA_A$ receptors (*see* ref. *10*). This suggests that for a given synaptic input, the component mediated by type C receptors may be more prolonged than the component mediated by type A receptors. It should be noted that even though many $GABA_A$ receptor-mediated responses to applied GABA are transient, some $GABA_A$ receptor-

mediated synaptic inputs onto ganglion cells are sustained *(57)*.

In the retina, $GABA_C$ receptors are not as ubiquitous as $GABA_A$ receptors are. To date, $GABA_C$ receptor-mediated currents have been characterized only on horizontal and bipolar cells ($GABA_C$ receptors may play a minor role on some ganglion cells in certain species; *see* refs. *24,25*). Both of these cells are slow potential neurons that do not fire action potentials, and transmitter release from these cells is continuous and graded with membrane potential *(51)*. Spiking-generating retinal neurons, such as amacrine or ganglion cells, do not utilize $GABA_C$ receptors *(9,26,27)*. Nondesensitizing, high-sensitivity GABA receptors may enable slow potential neurons to respond to tonic and very low synaptic levels of GABA. This suggests that the membrane potentials of these neurons will be precisely modulated by levels of GABA that would not activate $GABA_A$ receptors on either themselves or on other types of retinal neurons (e.g., amacrine and ganglion cells). If bipolar and horizontal cells only possessed lower-affinity $GABA_A$ receptors, then they would not be sensitive to low synaptic concentrations of GABA. Thus, the presence of the higher-affinity $GABA_C$ receptors expands the cell's dynamic range, allowing it to respond to a broader range of synaptic GABA concentrations.

Function of $GABA_C$ Receptors on Bipolar Cells

Bipolar cells receive inputs from photoreceptors and make outputs onto amacrine cells and ganglion cells. Bipolar cells are extremely important because all of the information that passes through the retina must go through them. Consequently, any modulation of bipolar cell activity will have profound actions on information flow in the visual system. GABA receptors are found primarily on the axon terminal of bipolar cells *(14,27,31,52,58)*. In most species, both $GABA_A$ and $GABA_C$ receptors on bipolar cell synaptic terminals mediate GABA responses (*see* Fig. 2C) *(9,27,55)*. GABA receptors on the bipolar terminals have been shown to reduce the amplitude of voltage-gated calcium currents by shunting the terminals and preventing the activation of these channels *(27,30,31,59)*. Reduction of the calcium current results in decreased transmitter release from isolated bipolar cells *(60)*.

GABAergic amacrine cells have been shown to make synaptic contacts with bipolar cell terminals *(61–64)*, suggesting that bipolar transmitter release may be modulated by synaptically released GABA. Synaptic transmission between bipolar and ganglion cells has been shown to be modulated by $GABA_C$ receptors in salamander retina *(59,65)*. Excitatory transmitter release from bipolar cells was suppressed by GABA. The suppression was insensitive to the $GABA_A$ receptor antagonists bicuculline or SR95531, indicating that $GABA_A$ receptors did not play a major role. However, the GABA suppression was blocked by picrotoxin, demonstrating that $GABA_C$ receptors were primarily responsible for modulating transmitter release from bipolar cells in these experiments. When $GABA_A$ and $GABA_C$ receptors were blocked by picrotoxin, no additional actions of GABA were observed, indicating that $GABA_B$ receptors did not play a significant role in modulating synaptic transmission between bipolar and ganglion cells.

$GABA_C$ receptor properties may be well suited for the proposed roles of GABA inputs to bipolar terminals. Their high sensitivity to GABA, plus their relatively sustained response properties, indicate that they can exert fine control of the bipolar cell output. GABA feedback inhibition from amacrine cells to bipolar terminals might be involved in control of the bipolar output. GABA feed-forward inhibition from amacrine cells to ganglion cells is mediated by $GABA_A$ receptors (and also $GABA_B$ receptors in some species). Low synaptic concentrations of GABA that would not activate $GABA_A$ receptors could affect the bipolar cell output via higher-affinity $GABA_C$ receptors. Thus, feedback inhibition may be more effective than feed-forward inhibition when synaptic levels

of GABA are low. Some experiments suggest that $GABA_A$ receptors may activate more rapidly than $GABA_C$ receptors at rat bipolar cell terminals, because bicuculline eliminates the early phase of the GABA effect mediated by $GABA_A$ receptors, but not late phase mediated by $GABA_C$ receptors *(31)*. These findings predict that the complement of $GABA_A$ and $GABA_C$ receptors may affect the time-course of GABAergic inhibition at bipolar cell terminals.

Contrast sensitivity in the visual system is owing, in part, to the center-surround receptive field organization of ganglion cells. Bipolar cells also have a center-surround receptive field organization. GABA feedback from horizontal cells to cone photoreceptor terminals is responsible, in part, for the receptive field surround in bipolar cells *(54,66,67)* and is mediated by $GABA_A$ receptors (*see* Fig. 2B) *(52,53)*. However, synaptic interactions at the bipolar terminal may also contribute to bipolar cell and ganglion cell receptive field properties. Since many bipolar terminals have both $GABA_C$ and $GABA_A$ receptors, they should respond to weak as well as strong synaptic GABA signals. Small, sustained inhibitory surround inputs from GABAergic amacrine cells that may not activate the low-affinity $GABA_A$ receptors may still affect bipolar cell outputs by acting at $GABA_C$ receptors. This suggests that high-gain components of the bipolar cell receptive field surround may be mediated by $GABA_C$ receptors on bipolar cell terminals.

Function of $GABA_C$ Receptors on Horizontal Cells

GABA-evoked chloride currents, measured in isolated teleost horizontal cells, are mediated, in large part, by $GABA_C$ receptors *(10,26,68)*. In white perch retina, $GABA_C$ receptors are found only on rod-driven horizontal cells *(10)*, whereas in catfish, they are found (with $GABA_A$ receptors) only on cone-driven horizontal cells (*see* Fig. 2B). Horizontal cell responses to visual stimuli are sustained and show very little desensitization *(51)*. Nondesensitizing $GABA_C$ receptors may be useful in mediating sustained signals to these cells. $GABA_C$ receptors may mediate regenerative responses in horizontal cells, since many horizontal cells that are GABAergic are also sensitive to GABA. Because the chloride equilibrium potential is more positive than the normal resting potential of these cells, GABA depolarizes horizontal cells. The GABA-elicited depolarization causes more GABA release, resulting in positive feedback. The GABA-mediated positive-feedback loop has been proposed to enhance the gain of GABA release as well as regulate the time-course of horizontal cell light responses *(69)*. Horizontal cells have receptive fields that are much larger than their dendritic extents, because gap junctional connections form a syncytium of electrically coupled horizontal cells. GABA acting through high-affinity $GABA_C$ receptors could chemically couple horizontal cells, complementing the electrical coupling. Excitation of a horizontal cell would elicit GABA release that would excite neighboring horizontal cells, whereas inhibition would decrease the rate of GABA release, hyperpolarizing neighboring cells as the result of decreased excitation.

Modulation of Retinal $GABA_C$ Receptors

Several substances that are found in the retina have been shown to modulate $GABA_C$ receptors on retinal neurons. Zinc is found in synaptic terminals of photoreceptors that provide synaptic inputs to horizontal cells *(70)*. Horizontal cell $GABA_C$ receptor-mediated currents have been shown to be inhibited by zinc in a competitive and noncompetitive manner *(44)*. Zinc inhibition is relatively potent (IC_{50} of 8 μM), and it is not voltage-dependent, suggesting that its binding site is not within the channel's electric field. These results suggest that horizontal cell $GABA_C$ receptor function can be downmodulated by zinc that may be released from photoreceptors. Bipolar cell $GABA_C$ receptors have also been shown to be modulated by zinc *(55)*. Most studies have

shown that bipolar $GABA_C$ receptors are concentrated at the axon terminals located in the inner plexiform layer *(14,27,31,58,71)*. The cellular sources of zinc in the inner plexiform layer have not yet been identified.

Recent work has shown that $GABA_C$ receptor function is modulated by dopamine *(59,72)*. Dopamine is a versatile neuromodulator that acts at many cellular sites in the vertebrate retina. In the outer retina, it has been shown to affect photoreceptor, horizontal cell, and bipolar cell function. Additional effects also occur in the inner retina at amacrine and ganglion cells. Horizontal cell $GABA_C$ receptor-mediated responses are reduced by dopamine acting at a D1 receptor *(72)*. These actions of dopamine were mimicked by SKF38393, a D1 receptor agonist, and forskolin, an activator of adenyl cyclase. Dopamine's suppression of the GABA current was blocked by the dopamine antagonist haloperidol. Dopaminergic interplexiform cells make synaptic contacts with horizontal cells *(51)* and are likely sources for dopamine's modulatory actions on horizontal cell $GABA_C$ receptors.

The $GABA_C$ receptor-mediated suppression of transmitter release from bipolar cell terminals is modulated by dopamine *(59)*. Dopamine relieved the GABA inhibition of bipolar cell excitatory transmission to third-order retinal neurons. These results suggest that dopamine acts to fine-tune the level of GABAergic inhibition, which in turn could affect gain control and/or the effectiveness of receptive field surround inputs at the bipolar cell axon terminal.

The effects of synaptically released dopamine on $GABA_C$ receptors have not been determined in an intact retinal circuit. In many species, it has been shown that dopamine is released during daylight when the retina is light-adapted. During the night, when the retina is dark-adapted, dopamine release is low. These findings predict that $GABA_C$ receptors will be downmodulated during daylight, but upregulated during the night.

$GABA_C$ receptor activity may be influenced by its level of phosphorylation. Consensus sequences for protein kinase C (PKC) phosphorylation have been identified on rat ρ1 and ρ2 subunits, suggesting that PKC may phosphorylate $GABA_C$ receptors *(49)*. Feigenspan and Bormann *(73)* found that activators of PKC enhanced the rundown of $GABA_C$ receptor-mediated currents in rat bipolar cells, whereas the PKC inhibitor tamoxifen reduced the rate of rundown, suggesting that rat bipolar cell $GABA_C$ receptor function may be downmodulated by PKC-dependent phosphorylation. Further experiments are needed to demonstrate that this is a physiologically relevant modulatory pathway of $GABA_C$ receptor function.

Summary

$GABA_C$ receptors are present on both bipolar cells and horizontal cells in the vertebrate retina. Both of these cell types are slow potential neurons, responding to synaptic inputs with graded potentials. Interestingly, the spiking retinal neurons, amacrine cells, and ganglion cells possess few, if any, $GABA_C$ receptors. The high-sensitivity, nondesensitizing, slow-activating $GABA_C$ receptors are well suited for modulating the outputs of slow potential neurons. Excitatory synaptic transmission between bipolar cells and ganglion cells can be modulated by $GABA_C$ receptor activation *(59,65)*. The physiological roles of $GABA_C$ receptors on horizontal cells have yet to be determined. The demonstration that $GABA_C$ receptors can be modulated by endogenous retinal substances suggests that the level of activation of these receptors can be finely tuned. Future work is needed to determine the precise roles that these receptors play in the two synaptic layers in the retina.

Acknowledgments

I thank S. Bissen, C. Romano, and R. Wong for their helpful comments on the manuscript. This work was supported by NIH grants EY08922, Core Grant EY02687, and Research to Prevent Blindness.

References

1. Hill D. R. and Bowery N. G. (1981) ^{3}H-Baclofen and ^{3}H-GABA bind to bicuculline-insensitive $GABA_B$ sites in rat brain. *Nature* **290,** 149–152.
2. Ortells M. O. and Lunt G. G. (1995) Evolutionary history of the ligand-gated ion-channel superfamily of receptors. *Trends Neurosci.* **18,** 121–127.
3. Olsen R. W. and Tobin A. J. (1990) Molecular biology of $GABA_A$ receptors. *FASEB J.* **4,** 1469–1480.
4. Wisden W. and Seeburg P. H. (1992) $GABA_A$ receptor channels: from subunits to functional entities. *Curr. Opin. Neurobiol.* **2,** 263–269.
5. Drew C. A., Johnston G. A. R., and Weatherby R. P. (1984) Bicuculline-insensitive GABA receptors: studies on the binding of (–)-baclofen to rat cerebellar membranes. *Neurosci. Lett.* **52,** 317–321.
6. Johnston G. A. R. (1986) Multiplicity of GABA receptors. *Rec. Biochem. Methodol.* **5,** 57–71.
7. Johnston G. A. R., Curtis D. R., Beart P. M., Game C. J. A., McCulloch R. M., and Twitchin B. (1975) *Cis*-and *Trans*-4-aminocrotonic acid as GABA analogues of restricted conformation. *J. Neurochem.* **24,** 157–160.
8. Sivilotti L. and Nistri A. (1989) Pharmacology of a novel effect of γ-aminobutyric acid on the frog optic tectum in vitro. *Eur. J. Pharmacol.* **164,** 205–212.
9. Feigenspan A., Wassle H., and Bormann J. (1993) Pharmacology of GABA receptor Cl-channels in rat retinal bipolar cells. *Nature* **361,** 159–162.
10. Qian H. and Dowling J. E. (1993) Novel GABA responses from rod-driven retinal horizontal cells. *Nature* **361,** 162–164.
11. Nistri A. and Sivilotti L. (1985) An unusual effect of γ-aminobutyric acid on synaptic transmission of frog tectal neurones in vitro. *Br. J. Pharmacol.* **85,** 917–921.
12. Arakawa T. and Okada Y. (1988) Excitatory and inhibitory action of GABA on synaptic transmission in slices of guinea pig superior colliculus. *Eur. J. Pharmacol.* **158,** 217–224.
13. Strata F. and Cherubini E. (1994) Transient expression of a novel type of GABA response in rat CA3 hippocampal neurones during development. *J. Physiol. (Lond.)* **480,** 493–503.
14. Heidelberger R. and Matthews G. (1991) Inhibition of calcium influx and calcium current by γ-aminobutyric acid in single synaptic terminals. *Proc. Natl. Acad. Sci. USA* **88,** 7135–7139.
15. Johnston G. A. R., Allan R. D., Benton A. D., Chen Chow S., Drew C. A., Hiern B. P., Holan G., Kazlauskas R., Rzezniczak H., and Weatherby R. P. (1984) GABA receptors and related binding sites. *IUPHAR 9th Int. Congr. Pharmacol. Proc.* **3,** 179–183.
16. Cutting G. R., Lu L., O'Hara B. F., and Kasch L. M. (1991) Cloning of the γ-aminobutyric acid (GABA) ρ1 cDNA: a GABA receptor subunit highly expressed in the retina. *Proc. Natl. Acad. Sci. USA* **88,** 2673–2677.
17. Polenzani L., Woodward R. M., and Miledi R. (1991) Expression of mammalian γ-aminobutyric acid receptors with distinct pharmacology in *Xenopus* oocytes. *Proc. Natl. Acad. Sci. USA* **88,** 4318–4322.
18. Shimada S., Cutting G., and Uhl G. R. (1992) γ-Aminobutyric acid a or c receptor? γ-Aminobutyric acid ρ1 receptor RNA induces bicuculline-, barbiturate-, and benzodiazepine-insensitive γ-aminobutyric acid responses in *Xenopus* oocytes. *Mol. Pharmacol.* **41,** 683–687.
19. Woodward R. M., Polenzani L., and Miledi R. (1992) Characterization of bicuculline/baclofen-insensitive γ-aminobutyric acid receptors expressed in *Xenopus* oocytes. I. Effects of Cl^- channel inhibitors. *Mol. Pharmacol.* **42,** 165–173.
20. Sigel E., Baur R., Trube G., Mohler H., and Malherbe P. (1990) The effect of subunit composition of rat brain $GABA_A$ receptors on channel function. *Neuron* **5,** 703–711.
21. Verdoorn T. A., Draguhn A., Ymer S., Seeburg P. H., and Sakmann B. (1990) Functional properties of recombinant rat $GABA_A$ receptors depend upon subunit composition. *Neuron* **4,** 919–928.
22. Kusama T., Spivak C. E., Whiting P., Dawson V. L., Schaeffer J. C., and Uhl G. R. (1993) Pharmacology of GABA ρ1 and GABA α/β receptors expressed in *Xenopus* oocytes and Cos cells. *Br. J. Pharmacol.* **109,** 200–206.
23. Zhang D., Pan Z.-H., Zhang X., Brideau A. D., and Lipton S. A. (1995) Cloning of a γ-aminobutyric acid type C receptor subunit in rat retina with a methionine residue critical for picrotoxin channel block. *Proc. Natl. Acad. Sci. USA* **92,** 11,756–11,760.
24. Liu Y. and Lasater E. M. (1994) Multiple GABA receptors of ganglion cells in the turtle retina. *Invest. Ophthalmol. Visual Sci. Suppl.* **35,** 2154.

25. Zhang J. and Slaughter M. M. (1995) Preferential suppression of ON pathway by $GABA_C$ receptors in the amphibian retina. *J. Neurophysiol.* **74,** 1583–1592.
26. Dong C.-J., Picaud S. A., and Werblin F. S. (1994) GABA transporters and $GABA_C$-like receptors on catfish cone—but not rod-driven horizontal cells. *J. Neurosci.* **14,** 2648–2658.
27. Lukasiewicz P. D., Maple B. R., and Werblin F. S. (1994) A novel GABA receptor on bipolar cell terminals in the tiger salamander retina. *J. Neurosci.* **14,** 1201–1212.
28. Slaughter M. M. and Bai S.-H. (1989) Differential effects of baclofen on sustained and transient cells in the mudpuppy retina. *J. Neurophysiol.* **61,** 374–381.
29. Maguire G., Lukasiewicz P., and Werblin F. (1989) Amacrine cell interactions underlying the response to change in the tiger salamander retina. *J. Neurosci.* **9,** 726–735.
30. Matthews G., Ayoub G. S., and Heidelburger R. (1994) Presynaptic inhibition by GABA is mediated via two distinct GABA receptors with novel pharmacology. *J. Neurosci.* **14,** 1079–1090.
31. Pan Z.-H. and Lipton S. A. (1995) Multiple GABA receptor subtypes mediate inhibition of calcium influx at rat retinal bipolar cell terminals. *J. Neurosci.* **15,** 2668–2679.
32. Benson J. A. (1988) Bicuculline blocks the response to acetylcholine and nicotine but not muscarine or GABA in isolated insect neuronal somata. *Brain Res.* **458,** 65–71.
33. Benson J. A. (1989) A novel GABA receptor in the heart of a primitive arthropod, *Limulus polyphemus*. *J. Exp. Biol.* **147,** 421–438.
34. Darlison M. G. (1992) Invertebrate GABA and glutamate receptors: molecular biology reveals predictable structures but some unusual pharmacologies. *TINS* **15,** 469–474.
35. ffrench-Constant R. H., Rocheleau T. A., Steichen J. C., and Chalmers A. E. (1993) A point mutation in *Drosophila* GABA receptors confers insecticide resistance. *Nature* **363,** 449–451.
36. Harvey R. J., Vreugdenhil E., Zaman S. H., Bhandal N. S., Usherwood P. N., Barnard E. A., and Darlison M. G. (1991) Sequence of a functional invertebrate $GABA_A$ receptor subunit which can form a chimeric receptor with a vertebrate alpha subunit. *EMBO J.* **10,** 3239–3245.
37. Zaman S. H., Harvey R. J., Barnard E. A., and Darlison M. G. (1992) Unusual effects of benzodiazepines and cyclodiene insecticides on an expressed invertebrate $GABA_A$ receptor. *FEBS Lett.* **307,** 351–354.
38. Woodward R. M., Polenzani L., and Miledi R. (1993) Characterization of bicuculline/baclofen-insensitive (ρ-like) γ-aminobutyric acid receptors expressed in *Xenopus* oocytes. II. Pharmacology of γ-aminobutyric $acid_A$ and γ-aminobutyric $acid_B$ receptor agonists and antagonists. *Mol. Pharmacol.* **43,** 609–625.
39. Qian H. and Dowling J. E. (1994) Pharmacology of novel GABA receptors found on rod horizontal cells of the white perch retina. *J. Neurosci.* **14,** 4299–4307.
40. Wang Tian-L., Guggino W. B., and Cutting G. R. (1994) A novel γ-aminobutyric acid receptor subunit (p2) cloned from human retina forms bicuculline-insensitive homo-oligomeric receptors in *Xenopus* oocytes. *J. Neurosci.* **14,** 6524–6531.
41. Cutting G. R., Curristin S., Zoghbi H., O'Hara B., Seldin M. F., and Uhl G. R. (1992) Identification of a putative γ-aminobutyric acid (GABA) receptor subunit rho2 cDNA and localization of the genes encoding rho2 (GABRR2) and rho1 (GABRR1) to human chromosome 6q14-q21 and mouse chromosome 4. *Genomics* **12,** 801–806.
42. Wang Tian-L., Hackam A. S., Guggino W. B., and Cutting G. R. (1995) A single amino acid in γ-aminobutyric acid p1 receptors affects competitive and noncompetitive components of picrotoxin inhibition. *Proc. Natl. Acad. Sci. USA* **92,** 11,751–11,755.
43. Enz R. and Bormann J. (1995) A single point mutation decreases picrotoxinin sensitivity of the human GABA receptor p1 subunit. *NeuroReport* **6,** 1569–1572.
44. Dong C.-J. and Werblin F. S. (1995) Zinc downmodulates the $GABA_C$ receptor current in cone horizontal cells acutely isolated from the catfish retina. *J. Neurophysiol.* **73,** 916–919.
45. Calvo D. J., Vaquez A. E., and Miledi R. (1994) Cationic modulation of ρ1-type γ-aminobutyrate receptors expressed in *Xenopus* oocytes. *Proc. Natl. Acad. Sci. USA* **91,** 12,725–12,729.
46. Chang Y., Amin J., and Weiss D. S. (1995) Zinc is a mixed antagonist of homomeric ρ1 γ-aminobutyric acid-activated channels. *Mol. Pharmacol.* **47,** 595–402.
47. Draguhn A., Verdorn T. A., Ewert M., Seeburg P. H., and Sakmann B. (1990) Functional and molecular distinction between recombinant rat $GABA_A$ receptor subtypes by Zn^{++}. *Neuron* **5,** 781–788.

48. Amin J. and Weiss D. S. (1994) Homomeric ρ1 GABA channels: activation properties and domains. *Rec. Chann.* **2,** 227–236.
49. Enz R., Brandstatter J. H., Hartviet E., Wassle H., and Bormann J. (1995) Expression of GABA receptors p1 and p2 subunits in the retina and brain of the rat. *Eur. J. Neurosci.* **7,** 1495–1501.
50. Albrecht B. E. and Darlison M. G. (1995) Localization of the p1- and p2-subunit messenger RNAs in chick retina by in situ hybridization predicts the existence of gamma amino butyric acid type C receptor subtypes. *Neurosci. Lett.* **189,** 155–158.
51. Dowling J. E. (1987) *The Retina: An Approachable Part of the Brain.* The Belknap Press of Harvard University Press, Cambridge, MA.
52. Tachibana M. and Kaneko A. (1984) γ-Aminobutyric acid acts at axon terminals of turtle photoreceptors: difference in sensitivity among cell types. *Proc. Natl. Acad. Sci. USA* **81,** 7961–7964.
53. Kaneko A. and Tachibana M. (1986) Effects of gamma-aminobutyric acid on isolated cone photoreceptors of the turtle retina. *J. Physiol. (Lond.)* **373,** 443–461.
54. Wu S. M. (1991) Input-output relations of the feedback synapse between horizontal cells and cones in the tiger salamander retina. *J. Neurophysiol.* **65,** 1197–1206.
55. Qian H. and Dowling J. E. (1995) GABAA and GABAC receptors on hybrid bass retinal bipolar cells. *J. Neurophysiol.* **74,** 1920–1928.
56. Feigenspan A. and Bormann J. (1994) Differential pharmacology of $GABA_A$ and $GABA_C$ receptors on rat retinal bipolar cells. *Eur. J. Pharmacol.* **288,** 97–104.
57. Belgium J. H., Dvorak D. R., and McReynolds J. S. (1984) Strychnine blocks transient but not sustained inhibition in mudpuppy retinal ganglion cells. *J. Physiol. (Lond.)* **354,** 273–286.
58. Karschin A. and Wassle H. (1990) Voltage- and transmitter-gated currents in isolated rod bipolar cells of rat retina. *J. Neurophys.* **63,** 860–876.
59. Wellis D. P. and Werblin F. S. (1995) Dopamine modulates $GABA_C$ receptors mediating inhibition of calcium entry into and transmitter release from bipolar cell terminals in tiger salamanders. *J. Neurosci.* **15,** 4748–4761.
60. Tachibana M., Okada T., Kobayashi K., and Picolino M. (1993) Dihydropyridine-sensitive calcium current mediate neurotransmitter release from bipolar cells of the goldfish retina. *J. Neurosci.* **13,** 2898–2909.
61. Marc R. E., Stell W. K., Bok D., and Lam D. M. K. (1978) GABA-ergic pathways in the goldfish retina. *J. Comp. Neurol.* **182,** 221–246.
62. Yazulla S., Studholme K. M., and Wu J.-Y. (1987) GABAergic input to the synaptic terminals of mb1 bipolar cells in the goldfish retina. *Brain Res.* **411,** 400–405.
63. Chun M. and Wassle H. (1989) GABA-like immunoreactivity in the cat retina: electron microscopy. *J. Comp. Neural.* **279,** 55–67.
64. Pourcho R. and Owczarzak M. (1989) Distribution of GABA immunoreactivity in the cat retina: a light- and electron-microscopic study. *Vis. Neurosci.* **2,** 425–435.
65. Lukasiewicz P. D. and Werblin F. S. (1994) A novel GABA receptor modulates synaptic transmission from bipolar to ganglion and amacrine cells in the tiger salamander retina. *J. Neurosci.* **14,** 1213–1223.
66. Murakami M., Shimoda Y., Kakatani K., Miyachi E.-I., and Wantanbe S.-I. (1982) GABA mediated negative feedback from horizontal cells to cones in the carp retina. *Jpn. J. Physiol.* **32,** 911–926.
67. Toyoda J.-I. and Fujimoto M. (1983) Analyses of neural mechanisms mediating the effect of horizontal cell polarization. *Vision Res.* **23,** 1143–1150.
68. Takahashi K., Miyoshi S., and Kaneko A. (1994) Two components of GABA-induced currents in catfish retinal horizontal cells. *Jpn. J. Physiol.* **44(Suppl. 2),** s141–144.
69. Kammermans M. and Werblin F. (1992) GABA-mediated positive autofeedback loop controls horizontal cell kinetics in tiger salamander retina. *J. Neurosci.* **12,** 2451–2463.
70. Wu S. M., Qiao X., Noebels J. L., and Yang X. L. (1993) Localization and modulatory actions of zinc in vertebrate retina. *Vision Res.* **33,** 2611–2616.
71. Tachibana M. and Kaneko A. (1987) γ-Aminobutyric acid exerts a local inhibitory action on the axon terminal of bipolar cells: evidence for negative feedback from amacrine cells. *Proc. Natl. Acad. Sci. USA* **84,** 3501–3505.
72. Dong C.-J. and Werblin F. S. (1994) Dopamine modulation of $GABA_C$ receptor function in an isolated retinal neuron. *J. Neurophysiol.* **71,** 1258–1260.
73. Feigenspan A. and Bormann J. (1994) Modulation of $GABA_C$ receptors in rat retinal bipolar cells by protein kinase C. *J. Physiol. (Lond.)* **481,** 325–330.