Different Contributions of GABA<sub>A</sub> and GABA<sub>C</sub> Receptors to Rod and Cone Bipolar Cells in a Rat Retinal Slice Preparation

THOMAS EULER AND HEINZ WÄSSLLE
Max-Planck-Institut für Hirnforschung, D-60528 Frankfurt, Germany

Euler, Thomas and Heinz Wässle. Different contributions of GABA<sub>A</sub> and GABA<sub>C</sub> receptors to rod and cone bipolar cells in a rat retinal slice preparation. J. Neurophysiol. 79: 1384–1395, 1998. Whole cell currents were recorded from rod and cone bipolar cells in a slice preparation of the rat retina. Use of the gramicidin D perforated-patch technique prevented loss of intracellular compounds. The recorded cells were identified morphologically by injection with Lucifer yellow. During the recordings, the cells were isolated synthetically by extracellular cobalt. To distinguish the γ-aminobutyric acid (GABA) receptors pharmacologically, the GABA<sub>A</sub> receptor antagonist, bicuculline, and the GABA<sub>C</sub> receptor antagonist, 3-aminopropyl (methyl) phosphinic acid, were used. In all bipolar cells tested, application of GABA induced postsynaptic chloride currents that hyperpolarized the cells from their resting potential of about −40 mV. GABA was applied at different concentrations to allow for the different affinity of GABA at GABA<sub>A</sub> and GABA<sub>C</sub> receptors. At a GABA concentration of 25 μM, in the case of rod bipolar cells, ~70% of the current was found to be mediated by GABA<sub>C</sub> receptors. In the case of cone bipolar cells, only ~20% of the current was mediated by GABA<sub>C</sub> receptors. Furthermore, this GABA<sub>C</sub>-mediated fraction varied among the different morphological types of cone bipolar cells, supporting the hypothesis of distinct functional roles for the different types of cone bipolar cells. There is evidence that the efficacy of GABA<sub>C</sub> receptors is modulated by glutamate through metabotropic glutamate receptors. We tested this hypothesis by applying agonists of metabotropic glutamate receptors (mGluRs) to rod bipolar cells. The specific agonist (±)-trans-azetidine-2,4-dicarboxylic acid and the potent mGluR agonist quisqualic acid reduced the amplitude of the GABA<sub>C</sub> responses by 10–30%. This suggests a functional role for the modulation of GABA<sub>C</sub> receptors by the metabotropic glutamate receptors mGluR1/5.

INTRODUCTION

In all mammalian retinas investigated to date, 1 type of rod bipolar (RB) cell and between 8 and 11 morphologically distinct types of cone bipolar (CB) cells have been identified (for review, see Euler and Wässle 1995). In the outer plexiform layer (OPL), bipolar cells may receive GABAergic input from horizontal cells or from interplexiform cells (for review, see Djamgoz 1995). However, only very few synapses from horizontal cells have been described (Fisher and Boycott 1974). GABAergic interplexiform cells have been observed in many mammalian retinae (for review, see Marc 1995) and they make conventional synapses onto bipolar cell dendrites (Chun and Wässle 1989; Kolb and West 1977; Pourcho and Owczarzak 1989). Nonetheless, the major GABAergic input bipolar cells receive is on their axons in the inner plexiform layer (IPL) (Hartveit 1996, 1997; Karschin and Wässle 1990; Suzuki et al. 1990), and amacrine cells are the synaptic partners. There are 10–20 different types of GABAergic amacrine cells in any mammalian retina. In addition to γ-aminobutyric acid (GABA), these cells also colocalize many other neuroactive substances (for review, see Masland 1988; Vaney 1990).

GABA receptors can be subdivided into three subclasses: GABA<sub>A</sub> and GABA<sub>C</sub> receptors are chloride channels (ionotropic receptors), whereas GABA<sub>B</sub> receptors (GABA<sub>B</sub>Rs) are metabotropic receptors. GABA<sub>B</sub>Rs of the retina have been reviewed recently by Slaughter (1995) and are not the topic of the present study. GABA<sub>A</sub> receptors (GABA<sub>A</sub>Rs) are protein complexes that consist of several subunits (α1–6, β1–3, γ1–3, δ), which form a pentameric chloride channel (review by MacDonald and Olsen 1994). They can be modulated by, among others, benzodiazepines (Bormann 1988) and can be blocked reversibly by picrotoxin and bicuculline (Feigenspan and Bormann 1994a; Feigenspan et al. 1993). In the retina, nearly all types of neurons including bipolar cells express GABA<sub>A</sub>Rs but with different subunit compositions (Greferath et al. 1993–1995; Grünert and Hughes 1993).

In various parts of the vertebrate brain, the gating of bicuculline-insensitive Cl<sup>−</sup> currents by GABA<sub>A</sub> receptors (GABA<sub>A</sub>Rs) has been described (for reviews, see Bormann and Feigenspan 1995; Johnston 1994, 1996; Lukasiewicz 1996). Like the GABA<sub>A</sub>Rs, they are probably pentameric channels composed of the recently discovered ρ subunits (ρ1−3) (Cutting et al. 1991, 1992; Ogurusu and Shingo 1996). In the mammalian retina, GABA<sub>A</sub>Rs have been described on bipolar cells only (Enz et al. 1996; Euler et al. 1996; Feigenspan and Bormann 1994a; Feigenspan et al. 1993; Pan and Lipton 1995; Yeh et al. 1996); however, in the fish retina, they also have been found on horizontal cells (Dong et al. 1994; Qian and Dowling 1993, 1994). The purpose of our study was to determine the contributions of GABA<sub>A</sub> and GABA<sub>C</sub> receptors to the total GABAergic response of different types of bipolar cells as a further step to elucidating the physiological roles of the various types of bipolar cells. Because the two GABA receptor subtypes have different kinetics, it is possible that, depending on the contributions of GABA<sub>A</sub> and GABA<sub>C</sub>, the output signal of a bipolar cell could have distinct temporal properties.

The cells were studied in a rat retinal slice preparation using the gramicidin perforated-patch clamp technique, which prevents the loss of intracellular compounds (Ebihara et al. 1995; Kyrozis and Reichling 1995). The bipolar cells were filled after the recordings with Lucifer yellow and clas-
sified according to the scheme we proposed recently (Euler and Wässle 1995). For isolating GABA$_C$-mediated currents, we used the GABA$_A$ antagonist bicuculline (Drew et al. 1984; Feigenspan et al. 1993). For isolating GABA$_A$Rs, we used the GABA$_C$ antagonist 3-aminopropyl-1-(methyl)phosphinic acid (3-APMPA) (Woodward et al. 1993). GABA was applied in different concentrations to allow for the different sensitivities of GABA$_A$ and GABA$_C$ receptors (Feigenspan and Bormann 1994a).

It has been shown recently that both GABA$_A$ and GABA$_C$ receptors of the rat retina can be modulated by other neuroactive substances via second messenger systems involving protein kinases (Feigenspan and Bormann 1994b,c; Veruki and Yeh 1992, 1994). The efficacy of GABA$_C$ receptors can be downregulated through metabotropic receptors, and this was tested in the present study by the application of the specific mGluRI/5 agonist (±)-trans-azetidine-2,4-dicarboxylic acid (tADA) and the potent mGluR agonist quisqualic acid (QA).

Parts of the study already have been presented in abstract form (Euler and Wässle 1997).

**Methods**

**Tissue preparation**

Adult albino rats (lab strain) were anesthetized deeply with halothane and decapitated. The eyes were removed quickly and transferred to a plastic Petri dish containing carbogen-buffered Ames medium-like saline (extracellular standard medium, ES, see Solutions and drug application), which was bubbled with 95% O$_2$-5% CO$_2$ continuously. Because the dissection procedure has been described in detail elsewhere (Boos et al. 1993; Euler et al. 1996), only a short description is given here. After opening the eye, the retina was removed carefully from the sclera and cut into quarters. Thin slices of 100–200 μm were cut by hand using the curved edge of a scalpel blade (Boos et al. 1993). The slices were transferred into a recording chamber and placed with the cut side up. The slices were held in place by a grid of parallel nylon strings (Edwards et al. 1989). The remaining pieces of retina could be maintained for 8–12 h in carbogen-bubbled extracellular solution (ES) at room temperature.

**Recording chamber and optical set-up**

The recording chamber was placed on the fixed stage of a microscope (Zeiss ACM, Jena, Germany). The microscope was equipped with epifluorescence illumination and differential interference contrast (Nomarski) optics. A water-immersion objective was used (Zeiss, 40/0.75 W). The recording chamber was perfused continuously (2–3 μl/min) with carbogen-bubbled ES. A valve on the inflow allowed the recording chamber to be perfused with either ES or the cobalt containing solution (ES/Co). The solution in the recording chamber was exchanged completely within ~2 min.

Patch electrodes were pulled from borosilicate glass (1.5 mm OD, wall thickness 0.3 mm; Clark, Redding, UK) on a horizontal electrode puller (DMZ-Universal Puller, Zeitz Instrumente, Augsburg, Germany). The electrodes were heat-polished and their resistance when filled with intracellular solution was 6–12 MΩ.

**Perforated-patch recordings**

For most of the recordings, the perforated-patch technique (Horn and Marty 1988) was used. We used the antibiotic gramicidin D (Sigma Aldrich, St. Louis, MO) for perforating the membrane under the tip of the electrode. Gramicidin D is a commercially available mixture of the related polypeptides gramicidin A, B and C (Kyrozis and Reichling 1995). The advantage over other antibiotics like nystatin (Horn and Marty 1988) is that gramicidin-formed channels are voltage insensitive and only permeable for monovalent cations; their chloride permeability is negligible (Ebihara et al. 1995). The physiological chloride concentration within the cell is stable even with a high chloride-containing electrode solution (Ebihara et al. 1995; Kyrozis and Reichling 1995). Therefore this recording technique is appropriate for examining the function of GABA-induced chloride currents. The electrode tip was filled with intracellular solution (IS) that did not contain gramicidin. The electrode shaft was filled with IS to which gramicidin had been added at a final concentration of 60–100 μg/ml. The membrane under the electrode became electrically permeable within ~1–4 min after seal formation. The experiments were started after the series resistance reached a relatively constant level (10–20 min after gigaseal formation). Because Lucifer yellow (LY, potassium salt, Sigma Aldrich) was included in the IS (0.025% final concentration), we could check whether the patch was intact during the experiments. No filling of the cells with LY was observed. At the end of the experiment, we ruptured the patch to establish a whole cell configuration (Hamill et al. 1981). The cells were filled with LY, and their morphology became visible under the fluorescence light. Within <1 min, fine processes were well filled.

All recordings were made with an EPC-7 patch-clamp amplifier (List, Darmstadt, Germany). The recording electrode was connected via an Ag/AgCl wire to the preamplifier; the reference electrode was an Ag/AgCl pellet in contact with the bath solution. Capacitative currents caused by the electrode were cancelled by the circuit of the EPC-7 amplifier. Additional capacitative currents, which reflected the capacitance of the cell membrane, appeared within a few minutes after sealing onto the cell in perforated-patch mode. Signals were low-pass filtered (Bessel) at 100 Hz or 10 kHz (AP-255-5, A.P. Circuit, Cypress Hills, NY) and digitized at 200 Hz or 40 kHz using the pClamp 6.03 software (Axon Instruments, Foster City, CA) in conjunction with a Labmaster AD/DA board. Additional data analysis was done with ORIGIN for Windows (version 4.1, Microcal Software, Northampton, MA).

Series resistances ($R_S$) were estimated from the slope of the routinely measured capacitative currents. Because the peak amplitudes of the capacitative currents were attenuated by the limited sampling frequency of the recording system, the series resistances are overestimated. In the perforated-patch configuration, $R_S$ can be relatively high and was estimated to range from 40 to 150 MΩ (with seal resistances >1 GΩ). After entering whole cell mode, $R_S$ dropped to 30–70 MΩ. Because we compared the GABA contributions of different cells and not their actual currents, we did not correct for $R_S$ but rather estimated the voltage errors ($\Delta V_{ERR}$) introduced by $R_S$. Within the voltage range of −70 to +20 mV used to determine the slope conductances of the $I-V$ curves, $\Delta V_{ERR}$ was usually <5 mV. At $V_{CONS}$ = +50 mV, the voltage errors were slightly larger. During the recordings, $R_S$ was relatively constant and the voltage errors were estimated to change the GABA$_A$/GABA$_C$ ratios by <10%.

Liquid junction potentials ($V_{IL}$) of the patch electrode were measured as described by Fenwick and coworkers (1982) and calculated using the DOS software JPCALC by Barry (1994). Measurements and calculations showed good agreement. The $V_{IL}$ for the combinations of solutions IS:IS and IS:ES/Co were 4.1 and 4.0 mV, respectively. Because most of our results are concerned with a comparison of amplitudes of ligand-gated currents, these possible errors do not influence the results, except for the accuracy of the absolute value of the reversal potentials. We addressed this problem by correcting all holding potentials for the appropriate liquid junction potentials.
Identification and classification of bipolar cells

Because the identification and classification of the recorded bipolar cells has been described elsewhere (Euler et al. 1996; Hartveit 1997), here we give only a short summary of the method. At the beginning of the experiments, bipolar cells were selected using Nomarski optics according to the position and the shape of their somata within the inner nuclear layer (INL). After the recording, the membrane under the patch electrode was ruptured and the cells were filled with LY. Thus we could verify their identity according to the proposed classification scheme (Fig. 4A) (Euler and Wässle 1995; Euler et al. 1996). Cells with the axon or the dendrites cut were eliminated from the analysis.

Results

GABA responses of bipolar cells

In the present study, we recorded from 72 bipolar cells in total, including 20 rod bipolar and 39 cone bipolar cells. The remaining 13 bipolar cells could not be further identified or were only partially filled.

The resting potential (\(V_R\)) of the bipolar cells was measured twice: at the very beginning of the experiment (\(-42.2 \pm 15.1\, \text{mV; mean ± SD; } n = 72\)) and after the GABA measurements (\(-36.1 \pm 12.0\, \text{mV; } n = 38\)). The increase of 5 mV in the resting potential observed in most cells was probably due to cesium entering the cell from the electrode (see also METHODS). Apart from deviations caused by the use of different extracellular and intracellular solutions, the resting potentials measured were in the range found in the literature (Euler et al. 1996; Karschin and Wässle 1990; Suzuki et al. 1990; Yamashita and Wässle 1991a,b; Yeh et al. 1990). We found no statistically significant differences either between the two bipolar cell classes or among presumed on- and off-bipolar cells.

All recordings were performed with 4 mM cobalt added to the extracellular medium, sufficient to block most of the calcium dependent synaptic transmission within the slice (Boos et al. 1993; Euler et al. 1996; Hartveit 1996). Therefore, the recorded cells presumably were isolated synaptically (for further details about the problems of synaptic isolation in the slice, see DISCUSSION).

GABA-induced currents

We analyzed the GABA responses of 20 RB cells and 39 CB cells in detail. When not mentioned explicitly, GABA was applied at a concentration of 25 \(\mu\text{M}\). At the very beginning of each recording, after the resting potential had stabilized, we switched to current clamp mode. Here, application of GABA hyperpolarized the membrane of all cells tested, confirming the inhibitory action of GABA on bipolar cells in the adult rat retina.

All recorded bipolar cells (\(n = 72\)) responded to GABA at a concentration of 25 \(\mu\text{M}\). Using the perforated-patch configuration, GABA induced net outward currents when the cells were voltage-clamped to a command potential (\(V_C\)) of -24 mV (Fig. 1, A-C, left). Although a high chloride concentration ([Cl\(^-\)] \(\text{symmetric to the ES}\), chloride could not enter the cells through the gramicidin pores, and the low [Cl\(^-\)] found physiologically within the cells showed little change during the experiments. In the perforated-patch mode, the reversal potential (\(V_{\text{REV}}\)) for the GABA-induced currents was -54.0 \(±\) 8.7 mV (\(n = 34\)). In the whole cell mode, cells were dialyzed with the IS, resulting in a high intracellular [Cl\(^-\)], and GABA induced net inward currents at negative command potentials (Fig. 1, D and E, left). The reversal potential (+4.3 \(±\) 7.7 mV; \(n = \))

Solutions and drug application

The standard ES, which was used for the preparation and during the experiment, contained (in mM) 110 NaCl, 5 KCl, 2 CaCl\(_2\), 1 MgCl\(_2\), 1 Na\(_2\)HPO\(_4\), 25 NaHCO\(_3\), 20 choline chloride, 10 N-2-hydroxyethylpiperazine-N’-2-ethanesulfonic acid (HEPES), and 20 glucose. For suppressing synaptic transmission within the slice, an extracellular medium, which contained cobalt instead of calcium ions (ES/C\(_0\)), was used. It also contained (in mM) 112 NaCl, 5 KCl, 4 CaCl\(_2\), 1 MgCl\(_2\), 25 NaHCO\(_3\), 14 choline chloride, 10 HEPES, and 20 glucose. The pH of both solutions was adjusted to 7.4. The standard IS contained (in mM) 10 NaCl, 1 CaCl\(_2\), 1 MgCl\(_2\), 1.5 ethylene glycol-bis-(\(\beta\)-aminoethyl ether)-\(N,N,N',N'\)tetraacetic acid, 135 CsCl, 10 HEPES, 1 Na\(^+\)-ATP(H\(_2\)O), and 1 Na\(^+\)-GTP(H\(_2\)O). The pH was adjusted to 7.2. LY and gramicidin or D (see previous section) were added to the IS. A stock solution of gramicidin D (5 mg/100 \(\mu\text{L}\)) was made up in dimethyl sulfoxide (Sigma Aldrich) and added to the IS, then sonicated for \(≥\) 5 min before use.

Because Cs\(^+\) can enter the cells through the gramicidin D pores, cesium was used instead of K\(^+\) as the internal monovalent cation in the IS. The cesium blocked part of the tonic potassium leak currents, which normally make it more difficult to clamp bipolar cells at positive holding potentials and to measure reversal potentials. The intracellular cesium also may have caused the drop in the resting potential (\(V_R\)) we observed during the experiments (see also RESULTS).

Because the pressure application system used has been described in detail elsewhere (Boos et al. 1993), we will only give a short description here. The drug solutions were prepared from frozen aliquots of stock solution, dissolved in extracellular medium, and applied using multibarreled pneumatic puffer pipettes. When antagonists were used, these were coapplied with the agonists and ejected from the same barrel. These puffer pipettes, with tip diameters of \(~15\ \mu\text{m}\), were placed within \(~30\ \mu\text{m}\) of the recorded cell. Drug solutions were ejected by air pressure. Without pressure applied to the pipette, small amounts of bath solution continuously flowed into the tip of the pipette by capillary attraction and, hence, prevented the drug solutions from leaking out.

The concentrations of the drugs given in the text refers to the concentrations in the barrel of the puffer pipette. These were relatively high; however, the actual concentration at the cell membrane was undoubtedly lower. The concentrations were chosen based on our previous experiments on dissociated rod bipolar cells (Karschin and Wässle 1990; Yamashita and Wässle 1991a,b) and on rat retinal slices (Boos et al. 1993; Euler et al. 1996) in which the same application systems were used.

GABA (5–125 \(\mu\text{M}\)), sodium-ATP and sodium-guanosine 5’-triphosphate, CsOH, gluconic acid, and HEPES were purchased from Sigma Aldrich. The putative mGlur5 agonist TADA (0.25–1 mM) and QA (50 \(\mu\text{M}\)) were obtained from Tocris (Bristol, UK). The GABA\(_2\), antagonist bicuculline (membromethide, 50–200 \(\mu\text{M}\)), the GABA\(_3\) antagonist 3-APMPA (as hydrochloride; SKF 97541, 500 \(\mu\text{M}\)), the N-methyl-d-aspartate (NMDA) antagonist (aminophosphono-heptanoic acid (AP-7, 200 \(\mu\text{M}\)), and the \(\alpha\)-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid/kainate (AMPA/KA) antagonist 6-cyanoquinoxaline-2,3-dione (CNQX, 100 \(\mu\text{M}\)) were obtained from Research Biochemicals (Natick, MA). All other chemicals were obtained from Merck (Darmstadt, Germany).
13) was similar to that predicted by the Nernst equation ($V_{\text{rev}}$ for chloride = +1.4 mV) for the solutions used, confirming that GABA-induced mainly Cl$^-$ currents.

**Pharmacological isolation of GABA receptors**

It is known that in rat retinal rod bipolar cells, only part of the GABA-induced chloride current is sensitive to the GABA$_A$R antagonist, bicuculline (Bic) (Euler et al. 1996; Feigenspan and Bornmann 1994a; Feigenspan et al. 1993; Hartveit 1996; Karschin and Wässle 1990; Pan and Lipton 1995), suggesting that RB cells express both GABA$_A$ and GABA$_C$ receptors. This also was observed in recordings from a few CB cells in the rat retina (Euler et al. 1996). However, which types of CBs express GABA$_C$ and to what extent their GABA response is mediated by this receptor subtype are still unknown. To address these questions, we isolated the current fractions mediated by the two subtypes of GABA receptors using specific antagonists. We used Bic to isolate the GABA$_C$ responses and the competitive GABA$_C$ antagonist 3-APMPA (Hartveit 1996; Pan and Lipton 1995; Woodward et al. 1993) to isolate GABA$_A$Rs.

Figure 1 shows the GABA-induced (25 μM) currents of different cell types of the rat retina. The recordings were performed in voltage-clamp mode using the gramicidin perforated-patch configuration (Fig. 1, A–C); the traces in Fig. 1, D and E, were recorded after entering whole cell mode. In Fig. 1A, the typical GABA responses of a rod bipolar cell are shown. The cell was voltage-clamped to −24 mV. When GABA was applied (Fig. 1A, left), an outward current was observed. Application of GABA together with Bic (100 μM) elicited a comparable outward current (Fig. 1A, middle). Coapplication of GABA and 3-APMPA (500 μM) caused a strong reduction of the outward current (Fig. 1A, right). Similar results were obtained for 19 of 20 RB cells.

For reasons of comparison, the response of an amacrine cell is shown in Fig. 1B: GABA induced an outward current (left), which, in contrast to the RB cell, was blocked nearly completely by Bic (middle) but was not reduced by 3-APMPA (right). In 3 of 13 amacrine cells, the tiny Bic-insensitive GABA currents were measurable; in the other 10, we found no evidence for GABA$_C$Rs (see further in this section).

The responses of the CB cells to the application of GABA and the two antagonists varied greatly; in some cells, Bic completely blocked the GABA response (Fig. 1C, middle), but in most CB cells, both Bic and 3-APMPA reduced the GABA responses by varying amounts (Fig. 1, D and E). When GABA was coapplied with Bic and 3-APMPA, all GABA-induced currents were blocked (not shown). In most RB cells, the Bic- and the APMPA-insensitive currents...
added up to the total GABA current; these fractions were found to be more variable in the case of CB cells. Under the assumption that the Bic-insensitive components (Fig. 1, middle) represent GABA<sub>C</sub>-mediated currents and the 3-APMPA-sensitive GABA responses (Fig. 1, right) represent GABA<sub>S</sub>-mediated currents, the fractions of the GABA responses mediated by these two receptor subtypes could be estimated. The traces in Fig. 1, D and E, suggest that the CB cell in Fig. 1E expresses a higher proportion of GABA<sub>C</sub>Rs than the CB cell in Fig. 1D. In this way, we tested 36 CB cells for GABA<sub>C</sub>-mediated responses and 27 of them displayed Bic-insensitive currents.

**Quantitative analysis of GABA-induced currents in bipolar cells**

For a more quantitative analysis of the fraction of GABA<sub>A</sub>R- and GABA<sub>C</sub>R-mediated responses, one has to consider their different affinities for GABA (Feigenspan and Bormann 1994a): the EC<sub>50</sub> value of the dose response curve is 4 μM for GABA<sub>A</sub>Rs and 27 μM for GABA<sub>C</sub>Rs. To be sure that the "standard" GABA concentration applied in the present study (25 μM in the puffer pipette) was in the dynamic concentration range, within which both receptor subtypes could be activated and no saturation took place, we tested the cells with a GABA concentration of 5, 25, and 125 μM (Fig. 2). Recordings from a rod bipolar cell are shown in Fig. 2A. When GABA was applied at increasing concentrations (Fig. 2A, left), the outward currents increased accordingly. When GABA was applied together with Bic (Fig. 2A, right), that is when mainly GABA<sub>A</sub>-R-mediated responses were measured, the response was nearly saturated at 25 μM GABA and hardly any increase could be observed when the GABA concentration was increased to 125 μM. For a more quantitative estimate of the fractions of GABA<sub>A</sub>R and GABA<sub>C</sub>R contributions, current voltage relations (I-V curves) were measured. Voltage-ramp protocols were applied to the cell (from −70 to +60 mV in 1 s, ΔV = 5 mV). The results in Fig. 2B show the net GABA-induced currents as a function of the command voltage (—). The Bic-resistant GABA<sub>C</sub>R-mediated response also is shown (— — ). The most interesting traces are the two curves where GABA was applied at a concentration of 5 μM; they coincide, indicating that practically all the GABA-induced current is mediated by GABA<sub>C</sub>Rs. Figure 2C shows the GABA<sub>A</sub>R/

**FIG. 2.** GABA-induced currents of bipolar and amacrine cells at different concentrations of GABA. A, left: currents induced in a rod bipolar cell (RB) during the application of GABA at concentrations of 5, 25, and 125 μM. Right: currents elicited by the coapplication of different concentrations of GABA together with Bic (50–200 μM). Cobalt was added to the bathing solution (ES, 4 mM) to block presynaptic GABA effects. B: I-V curve of the GABA responses of the RB cell shown in A. ——, currents induced by the application of GABA (5, 25, or 125 μM); ——, currents elicited by the coapplication of GABA and Bic. C: slope conductances (G<sub>slope</sub>; equal to the slopes of the I-V curves in B) were determined and are plotted as a function of the different GABA concentrations. D and E: comparable diagrams for a CB cell (D) and an amacrine cell (E).
GABA\textsubscript{C}-R contribution quantitatively. The slope conductances ($G_{\text{slope}}$) of the six $I$-$V$ curves in Fig. 2B were calculated by fitting a straight line in the voltage range from $V_C = -70$ to $+20$ mV. The slope conductance is shown as a function of the GABA concentration in Fig. 2C. The total GABA response (Fig. 2C, □) and the Bic-resistant GABA\textsubscript{C}-mediated response (Fig. 2C, ○) are identical at 5 μM and show increasing differences at higher GABA concentrations. When 5 μM GABA is applied, 100% of the response is GABA\textsubscript{C}-R mediated, at 25 μM GABA only 60% and at 125 μM only 50%. In addition the difference between the curves for GABA and for GABA+Bic, most likely representing the GABA\textsubscript{A} mediated response, is inserted (Fig. 2C, △).

In cone bipolar cells, the GABA\textsubscript{A}-mediated responses (Fig. 2D, ○) are smaller. At a concentration of 25 μM GABA, the contribution of GABA\textsubscript{A}-Rs is 27% of the total response. The graph in Fig. 2E shows results from one of the few amacrine cells in which GABA responses could not be completely blocked by the coapplication of Bic. Hence, at first sight this cell could express GABA\textsubscript{A}-Rs. However, the dashed curve in Fig. 2E shows no decrease in slope at increasing concentrations of GABA, which is not typical for GABA\textsubscript{A}-R contributions.

In summary, for all bipolar cell types recorded, application of GABA at a concentration in the puffer pipette of 25 μM seems to be a good choice for measuring the contributions of GABA\textsubscript{A}-Rs and GABA\textsubscript{C}-Rs.

To quantify the current fractions mediated by the GABA receptor subtypes, we measured $I$-$V$ curves for a larger sample of rod and cone bipolar cells during application of 25 μM GABA alone and during the coapplication of GABA and Bic. From each of the $I$-$V$ curves, comparable with those shown in Fig. 2B, two indicators were determined. First, the slope conductance was calculated as described above ($G_{\text{slope}}$). Second, the current amplitude was measured at a command potential of $+50$ mV ($I_{+50}$). The histograms in Fig. 3 show the result: the hatched portion is the fraction of the total GABA response that could not be blocked by coapplication of Bic, in other words the GABA\textsubscript{C}-mediated response. The open portion is the remainder of the response and therefore probably the GABA\textsubscript{A}-mediated response. The results for the slope conductance measurements ($G_{\text{slope}}$) and for the amplitude measurements ($I_{+50}$) are shown separately. The GABA responses of RB cells are mediated by 68 ± 19% (n = 13; $I_{+50}$) or 74 ± 20% (n = 13; $G_{\text{slope}}$) via GABA\textsubscript{C}-Rs. In cone bipolar cells, an average of only 12 ± 16% (n = 10; $I_{+50}$) or 19 ± 22% (n = 10; $G_{\text{slope}}$) is contributed via GABA\textsubscript{C}-Rs. The differences between the two bipolar cell classes are statistically significant at the 0.05 level (Student’s t-test). Relative to the size of the response fractions, the standard deviations (error bars) for the CB cells are much larger than those of the RB cells. The reason for this is that different types of cone bipolar cells with different contribution from GABA\textsubscript{C}-Rs have been pooled for this graph in contrast to RB cells which constitute a single type.

**GABA\textsubscript{A} and GABA\textsubscript{C} receptors of different types of cone bipolar cells**

The different types of bipolar cells according to our classification scheme (Euler and Wässle 1995) are shown in Fig. 4A. The fraction of GABA\textsubscript{A}- and GABA\textsubscript{C}-mediated responses is shown in Fig. 4B. Figure 4B, top row, gives the number of cells (n) that were recorded and identified by subsequent filling with LY. With the exception of type-1 (no cells recorded) and type-2 CB cells (n = 1), at least three cells of every type were analyzed. Similar to Fig. 3, the GABA\textsubscript{A} response was determined by subtracting the Bic-resistant GABA response from the total GABA response. After this analysis, the bipolar cells were divided into four groups: the GABA\textsubscript{A}-R contribution is substantially larger than the GABA\textsubscript{C}-R contribution ($A \ll C$; RB cells); the cells have comparable GABA\textsubscript{A}-R and GABA\textsubscript{C}-R contributions ($A \sim C$; type-6 CB cells); GABA\textsubscript{A}-Rs mediate the major part of the response to GABA ($A > C$; type-2, -4, -7, and -8 CB cells); and very little, if any, of the response seems mediated by GABA\textsubscript{A}-Rs ($A \gg C$; type-3, -5, and -9 CB cells). The boxes in Fig. 4B, bottom, symbolize these four groups by showing the relative proportions of GABA\textsubscript{A} (□) and GABA\textsubscript{C} responses (■).

**GABA responses of amacrine cells**

For reasons of comparison, we also recorded the GABA-induced currents of 13 amacrine cells. Their resting potential was $-51.6 \pm 13.3$ mV; (n = 13) at the beginning of the recordings. In 7 of the 13 amacrine cells, all GABA responses could be blocked by the coapplication of Bic. In three cells, the application of GABA induced a strong outward current—consistent with the Cl$^-$ reversal and holding potential—whereas during coapplication of GABA and Bic a small inward current was observed. It is possible that this is caused by transsynaptic effects of GABA, suggesting that the Co$^{2+}$-block of synaptic transmission was not complete (see DISCUSSION). In three other cells, during coapplication of GABA and Bic, a small outward current was resistant to

---

**Fig. 3.** Comparison of the fractions of GABA\textsubscript{A} receptor (GABA\textsubscript{A}-R) and GABA\textsubscript{C} receptor (GABA\textsubscript{C}-R) mediated currents in rod and cone bipolar cells. GABA was applied at a concentration of 25 μM. □, fraction of GABA\textsubscript{A}-mediated currents that could not be blocked by the coapplication of Bic and represent the contribution of GABA\textsubscript{A}-Rs. ○, contribution of GABA\textsubscript{C}-Rs, that is the fraction of GABA\textsubscript{A}-mediated currents that could be blocked by coapplication of Bic. As explained in the text, the contributions were determined in 2 different ways: by calculating the slope conductances from the $I$-$V$ curves in the voltage range from $V_C = -70$ to $+20$ mV ($G_{\text{slope}}$; see text for further details) and by measuring the amplitudes at $V_C = +50$ mV ($I_{+50}$) of the respective currents. Both results are shown in this bar graph.
Bic, and this could be an indication that these cells might express GABA<sub>B</sub>Rs or low levels of GABA<sub>C</sub>Rs.

**Modulation of GABA<sub>C</sub>Rs in rod bipolar cells**

Feigenspan and Bormann (1994b) have shown that the sensitivity of GABA<sub>C</sub>Rs can be modulated by metabotropic glutamate receptors (mGluR). Recently our lab has shown that bipolar cells express mGluR1 and mGluR5, which are members of the mGluRI family (Koulen et al. 1997b). Here we tested whether these mGluRs modulate GABA<sub>C</sub> receptors of rod bipolar cells in the slice preparation.

This was studied in nine RB cells by applying the specific mGluR1/5 agonist tADA (250 μM to 1 mM) and also the potent mGluR agonist QA (50 μM). Because QA is also an agonist of ionotropic GluRs, the NMDA receptor antagonist AP-7 (200 μM) and the AMPA/K<sub>A</sub> receptor antagonist CNQX (100 μM) were coapplied. The cells were recorded in the voltage-clamp mode. The application of tADA alone had no measurable direct effects on the recorded cells, whereas in a few cases, QA/ AP-7/CNQX evoked tiny responses, which were not further examined (not shown). To inhibit synaptic effects, cobalt was added to the bathing medium. The results are shown in Fig. 5. We coapplied GABA and Bic pulses every 45 s and measured the amplitude of the GABA<sub>C</sub>R-mediated currents. In Fig. 5B, this amplitude is shown as a function of the time. When tADA was applied, a reduction of the amplitude of the GABA responses was observed that reversed within a few minutes after the application of tADA. Although the perforated-patch configuration was used, there was a general “run down” of the GABA responses during the 7-min recording period. Figure 5B shows three selected GABA responses, before application of tADA (left, 2), at maximal reduction (middle, 7), and after recovery (right, 9). In six of the nine RB cells tested, the application of tADA or of QA/ AP-7/CNQX reduced the GABA<sub>C</sub> response. The remaining three cells, in which no modulation of GABA<sub>C</sub>R could be measured, were recorded in whole cell mode. This suggests that indeed a second messenger system is involved with this modulation.

**DISCUSSION**

**Anatomic evidence for the expression of GABA receptors in rod and cone bipolar cells**

The expression of the β-subunits of the GABA<sub>C</sub> receptor in bipolar cells was first demonstrated by in situ hybridization experiments on retinal sections and on dissociated rod
bipolar cells (Enz and Bormann 1995). Application of reverse transcriptase polymerase chain reaction (RT-PCR) to RNA harvested from dissociated bipolar cells confirmed the expression of the $\rho_1$ and $\rho_2$ subunits of the GABA$_C$ receptor (Enz and Bormann 1995; Yeh et al. 1996). When specific antibodies against the $\rho$-subunits became available, strong punctate immunofluorescence was observed in the IPL of all retinae studied to date (Enz et al. 1996; Koulen et al. 1997a). This finding suggests that GABA$_C$ receptors are clustered at postsynaptic sites in the IPL. Weak punctate immunofluorescence was observed in the OPL. Extrasynaptic membranes of bipolar cells also showed weak label.

Application of specific markers for GABA$_A$ receptors to the rat retina showed that different subunits of the GABA$_A$ receptor are expressed in different bipolar cells (Brecha 1992; Brecha and Weigmann 1991; Greferath et al. 1993, 1994; Grigorenko and Yeh 1994; Hughes et al. 1991; Yeh et al. 1996). Rod bipolar cells were found to express the $\alpha_1$, $\alpha_3$, $\beta_2$/$3$, and $\gamma_2$ subunits of the GABA$_A$R (Greferath et al. 1994; Yeh et al. 1996). There is good agreement between the anatomy and the electrophysiology concerning the localization of GABARs along the bipolar cell membrane. GABA responses are most prominent when GABA is applied to the bipolar cell axon terminal (Hartveit 1996, 1997; Karschin and Wässle 1990; Suzuki et al. 1990). Immunoreactivity for GABA$_A$ and GABA$_C$ receptors is strong in the IPL and only weak in the OPL (Enz et al. 1996; Greferath et al. 1994).

Recently GABA$_A$ receptors have been cloned (Kaupmann et al. 1997), and antibodies against GABA$_A$ receptors became available. When these antibodies were applied to the rat retina, labeling was observed in horizontal, amacrine, and ganglion cells but not in bipolar cells (P. Koulen, personal communication). Consistent with these anatomic results, Pan and Lipton (1995) also failed to find GABA$_A$R-mediated effects on isolated RB cells of the rat retina. At least three different GABA transporters (GATs) have been described in the mammalian retina using in situ hybridization (Brecha and Weigmann 1994; Ruiz et al. 1994) and immunocytochemistry (Honda et al. 1995; Johnson et al. 1996). None of the three GABA transporters was located in bipolar cells, suggesting that GABA transporters did not contribute to the measured GABA currents. Additional evidence comes from the physiology: GAT-mediated currents have a characteristic $I$-$V$ relationship—different from the curves we measured—such that the currents decrease asymptotically instead of reversing when the cell membrane depolarizes (Dong et al. 1994).

**Physiological evidence for the expression of GABA$_A$ and GABA$_C$ receptors in rod and cone bipolar cells**

In the adult rat retina, we found that GABA induced mainly chloride currents in every intact bipolar cell, in agreement with earlier studies (Euler et al. 1996; Feigenspan et al. 1993; Hartveit 1996, 1997; Yamashita and Wässle 1991b). The reversal potentials for the GABA-induced chloride currents we measured (mean: $-54$ mV) were more positive than those in previous studies (around $-70$ mV) (Euler et al. 1996; Yamashita and Wässle 1991b). The main difference from those studies is that we used gramicidin instead of nystatin. Although many studies describe the $\text{Cl}^-$ impermeability of the gramicidin pores, even with high $\text{Cl}^-$ in the electrode solution (Ebihara et al. 1995; Kyrozis and Reichling 1995), the possibility of chloride leaking into the cell cannot be excluded. Gramicidin perforated-patch recordings from substantia nigra pars reticulata (SNR) neurons showed an increase in the chloride activity when the cells were clamped at command potentials more positive than $-40$ mV (Ebihara et al. 1995). This higher $\text{Cl}^-$ activity may be due to an increase in $\text{Cl}^-$ permeability and/or to the dysfunction of the $\text{Cl}^-$ pump at higher membrane potentials. However, the actual chloride reversal potentials were not crucial on the study.

Two pharmacological tools were used in the present study to isolate GABA$_A$R- and GABA$_C$R-mediated effects. For antagonizing GABA$_C$Rs, the phosphate analogue of GABA, 3-APMPA, was applied (Woodward et al. 1993). It was first described as a potent agonist for GABA$_A$Rs (Slaughter and Pan 1992) but was later found to competitively antagonize also GABA$_A$R (Hartveit 1996; Pan and Lipton 1995). The action of 3-APMPA was clear cut in the case of RB cells: GABA$_A$Rs mediate most of the GABA effects, which could be suppressed by 3-APMPA (Fig. 1). However, when the drug was applied to CB cells, which show only weak GABA$_C$R-mediated effects, the results were not so convincing. In several recordings, coapplication of GABA and 3-APMPA actually increased the response instead of reducing it. It is, therefore, possible that 3-APMPA acts as a partial agonist at certain isoforms of GABA$_A$Rs. For that reason, 3-APMPA was not used for the quantitative estimate of the GABA$_A$R or GABA$_C$R contributions. All the available evidence shows that Bic does not antagonize the action of GABA-mediated by GABA$_C$ receptors. Therefore, Bic-resistant GABA-gated $\text{Cl}^-$ currents were used in the present study to quantify the relative contributions of GABA$_A$Rs and GABA$_C$Rs.

Feigenspan and Bormann (1994a) found that the GABA$_C$R dose-response curves have an $EC_{50}$ value of 4 $\mu$M GABA, whereas those of GABA$_A$Rs display an $EC_{50}$ value of 27 $\mu$M. We also have found in the present study (Fig. 2) that, at low concentrations of GABA, the GABA$_C$R contribution is more prominent, whereas at high concentrations of GABA, most of the currents are gated by GABA$_A$ receptors. One might argue that GABA$_C$ and GABA$_A$ contributions should be measured at saturating concentrations, a requirement for determining the actual receptor distribution. However, because Bic is a competitive antagonist at the GABA$_A$ receptor, it is very difficult to block saturating responses of GABA at this receptor, and it takes a long period of time to wash out the necessary concentrations of Bic. Instead we estimated the relative contributions of the two receptor subtypes to the GABA response. Because the relative contributions of the two receptor types critically depend on the concentration of GABA applied, the percentages given in Fig. 3 should be interpreted with this proviso in mind. Moreover, the contributions of both GABA$_A$ and GABA$_C$ receptors also depend upon their status of modulation.

**Comparison of rod and cone bipolar cell GABA responses**

Both the physiological results of the present paper and the immunocytochemical results of the $\rho$-subunit localiza-
tion (Enz et al. 1996; P. Koulen, J. H. Brandstätter, H. Wässle, unpublished results) show that RB axon terminals express higher amounts of GABA\(_C\)Rs than CB cells. Concerning the physiological meaning of this bias toward GABA\(_A\)Rs, only speculations are possible. The action of GABA at GABA\(_C\)Rs desensitizes more slowly than the GABA action on GABA\(_A\)Rs. Therefore, inhibition through GABA\(_C\)Rs could be more tonic, whereas GABA\(_A\)Rs could mediate transient inhibitory actions.

The physiological roles of the different types of CB cells are not yet known. Their axons stratify at different levels within the IPL (Fig. 4A). The overall subdivision is into ON and OFF sublayers. Within this ON/OFF dichotomy, further subdivisions occur. However, at present the physiological function of CB cells is not yet known. It is possible, that depending on the contribution of GABA\(_A\) and GABA\(_C\) receptors, the output signal of a bipolar cell could have different temporal properties. Predominantly GABA\(_A\) input would suppress fast changes in the bipolar cell signal, leaving tonic responses unchanged. Predominantly GABA\(_C\) input would make the bipolar cell responses more transient by suppressing the tonic response relatively more than the transient component.

When subunit specific antibodies against GABA\(_A\)Rs were applied to the rat retina, a very precise subdivision of the IPL was observed (Brandstätter et al. 1995; Greferath et al. 1995). It is, therefore, possible that different CB cells not only have different proportions of GABA\(_A\) and GABA\(_C\) receptors but also express specific isoforms of GABA\(_A\) receptors.

There is strong evidence that the different GABA receptors serve the fine tuning of inhibition in the inner retina (reviewed by Johnston 1996; Łukasiewicz 1996). Using imaging techniques, different roles for GABA\(_A\) and GABA\(_C\) receptors on dissociated rat RB cells have been demonstrated by Pan and Lipton (1995). They have shown that GABA receptors on the axon terminals reduce depolarization-induced Ca\(^{2+}\) currents, which have been shown to correlate directly with the release of neurotransmitters (Tachibana et al. 1993; von Gersdorff and Matthews 1994). They demonstrated that GABA\(_A\) receptors mediated fast inhibition but only at high GABA concentrations, whereas GABA\(_C\) receptors mediated inhibition at low GABA concentrations but with a slower onset. Zhang and Slaughter (1995, 1997) have studied GABA effects on light-evoked responses of ganglion cells in the salamander retina. They reported that activation of GABA\(_A\)Rs transiently suppressed all excitatory light responses, whereas activation of GABA\(_C\)Rs mediated a weaker, delayed inhibition of preferentially ON responses. They claimed that the primary site of GABA action was presynaptic to ganglion cells. Bipolar cell terminals could well be this site of GABA action, especially because they receive reciprocal synapses from amacrine cells (Chun and Wässle 1989), which could serve as negative feedback loops, a necessity when modulating the time course of the output signal of bipolar cells.

**GABA responses of amacrine cells**

Feigenspan and coworkers (1993) recorded GABA-induced whole cell currents from bipolar and amacrine cells in an organotypic slice culture of the rat retina and observed GABA\(_C\)-R-mediated responses only in bipolar cells. Qian and Dowling (1993) first observed GABA\(_C\) responses in horizontal cells of the fish retina. Later studies demonstrated GABA\(_C\)Rs also in fish and amphibian bipolar cells (Łukasiewicz et al. 1994; Matthews et al. 1994; Qian and Dowling 1995). In the chick retina, Albrecht and Darlison (1995) found that \(\beta 1\) subunit mRNA is present mainly in bipolar cells and that \(\beta 2\) subunit mRNA is present in both amacrine and horizontal cells. This shows that the expression of GABA\(_C\)Rs in cells other than bipolar cells varies greatly between species.

We tested a small sample of amacrine cells \((n = 13)\) of different morphologies and observed GABA-induced Cl\(^-\) outward currents in all cells tested. In seven amacrine cells, the outward currents were blocked completely by Bic; in three cells, small outward currents persisted, and in three further cells, small inward currents appeared during the Bic blockade. In these latter cells, transynaptic effects of GABA may have caused such inward currents. In the other three cells, the Bic-resistant outward currents could be caused by GABA\(_A\)Rs or possibly by GABA\(_C\)Rs. However, there are two problems with the latter interpretation. The first is that the residual currents did not saturate at higher concentrations of GABA (Fig. 2E), which is normally the case for GABA\(_C\)-R-based currents. The other problem is electrical synapses (gap junctions) in the IPL, which are not blocked by the application of Co\(^{2+}\) in the bathing medium. Such gap junctions also have been found between bipolar and amacrine cells (reviewed by Vaney 1997). It is, therefore, possible that the residual currents are mediated through GABA\(_A\) receptors, which are not completely blocked, or that they represent an action of GABA on bipolar cell GABA\(_C\)Rs that “leaks” into the amacrine cells through gap junctions (Hartveit 1997). Another explanation could be that the amacrine cells have a large dendritic tree where Bic could not block all the GABA\(_C\)Rs-mediated responses. However, clearly more anatomic and physiological information is needed before it can be accepted that some amacrine cells express GABA\(_C\)Rs.

**Modulation of gaba, receptors**

Both subtypes of ionotropic GABA receptors can be modulated by metabotropic receptors via second messenger-induced phosphorylation by protein kinases. GABA\(_A\) receptors on retinal ganglion cells in the rat retina are potentiated by vasoactive intestinal polypeptide involving adenosine 3',5'-cyclic monophosphate and phosphorylation by protein kinase A (Veruki and Yeh 1992, 1994). Activation of D1 dopamine receptors by dopamine and activation of receptors by other neuroactive substances like somatostatin were reported to upregulate GABA\(_A\)Rs via the same intracellular pathway by increasing the open probability (Feigenspan and Bormann 1994c). In contrast to this, GABA\(_C\)Rs are down-regulated by phosphorylation. Studies on RB cells in organotypic slice cultures of the rat retina revealed that activation of protein kinase C (PKC) enhanced the rundown of GABA\(_C\)-mediated currents, whereas the PKC inhibitor tamoxifen decreased the rate of rundown (Feigenspan and Bormann 1994b). Extracellular application of 1-amino-cyclopetanone-
1,3-dicarboxylate, an nonspecific agonist for mGluRs (reviewed by Pin and Duvoisin 1995), stimulated the rundown of the GABA\(_C\) responses via an intracellular pathway involving phospholipase C and a phosphoinositide/Ca\(^{2+}\) cascade. The only mGluRs known to be linked to a phosphoinositide/Ca\(^{2+}\) cascade are the group I metabotropic receptors mGluR1/5 (Aramori and Nakanishi 1992; Masu et al. 1991). Recently, mGluR\(_{1\alpha}\) and mGluR\(_{5a}\) have been localized immunohistochemically on the dendrites of RB cells and also a subset of CB cells (Koulen et al. 1997b; Peng et al. 1995). Hence, an attractive hypothesis is the regulation of GABA\(_C\) input to bipolar cells by mGluR1/5 receptors (P. Koulen, J. H. Brandstatter, and H. Wässle, unpublished data).

We tested this hypothesis on RB cells in the acute slice. The cells were recorded in the perforated-patch configuration, which allows us to keep the second messenger system largely undisturbed. The idea was to modulate the amplitude of repeated GABA+Bic pulses by using the mGluR1/5 specific agonist tADA (Favaron et al. 1993; Thoreson and Ufphani 1995) and QA in combination with antagonists for ionotropic glutamate receptors. Among mGluRs, QA preferentially acts on mGluR1/5 receptors (Abe et al. 1992). In all RB cells tested using the perforated-patch mode, the mGluR1/5 agonists reduced the GABA\(_C\) responses by \(\sim 10-30\%\), suggesting that mGluR1/5 could play in vivo a functional role in modulating GABA\(_C\) Rs in RB cells. We have to note that, although the modulatory effect was consistent, its size was close to the limits of our application system. However, no modulation was observed when the cells were recorded using the whole cell configuration. Feigenspan and Bormann (1994b) reported also that L-2-amino-4-phosphonobutyric acid significantly modulated the GABA\(_C\) currents, indicating that group III mGluRs also may be involved. In the tiger salamander retina, a downregulation of GABA\(_C\) Rs by dopamine was observed (Dong and Werblin 1994; Wells and Werblin 1995). This suggests that a fine tuning of GABA\(_C\) Rs by multiple modulatory pathways exists.

We thank N. Menger, R. Taylor, and J. Bormann for helpful discussion and S. P. Brown for critically reading the manuscript. This work was supported by the Deutsche Forschungsgemeinschaft (SFB269, B4).

We present address of T. Euler: Masland Laboratory, Wellman 429, Massachusetts General Hospital, Fruit St., Boston, MA 02114. Address for reprint requests: H. Wässle, Max-Planck-Institut für Hirnforschung, Deutschordensstr. 46, D-60528 Frankfurt, Germany.

Received 20 June 1997; accepted in final form 4 December 1997.

REFERENCES


Received from http://jn.physiology.org/ by 10.220.33.1 on November 5, 2016

HORN, R. and MARTY, A. Muscarinic activation of ionic currents measured in rod bipolar cells. 


