Temporal Modulation of Scotopic Visual Signals by A17 Amacrine Cells in Mammalian Retina In Vivo

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Submitted 6 November 2002; accepted in final form 3 December 2002

INTRODUCTION

The retinas of the majority of mammalian species contain rod and cone photoreceptor systems that are responsible for night and day vision, respectively. In the classic mammalian rod pathway, rod bipolar cells do not communicate directly with retinal ganglion cells. Instead, they give their synaptic output to two types of amacrine cells, glycinergic AII and GABAergic A17 cells (Freed et al. 1987; Kolb and Famiglietti 1974; Massey et al. 1992; Pourcho and Goebel 1985; Raviola and Dacheux 1987; Strettoi et al. 1990). While AII cells are mandatory interneurons in the throughput pathway that convey scotopic postreceptoral responses (assessed with the scotopic electroretinogram b-wave). Application of a GABA antagonist prolonged preferentially the decay of the scotopic b-wave. The degree of prolongation increased as the light intensity decreased. Application of selective GABA antagonists accelerated the kinetics of the scotopic b-wave. This effect was abolished when the GABA antagonist was coapplied. Selective ablation of A17 cells mimicked the action of the GABA antagonist. In A17 cell–ablated retinas, the GABA antagonist was no longer very effective to slow the decay of the scotopic b-wave. Thus the A17 feedback, activated by light stimulation and mediated mainly by the GABA receptors, makes the scotopic b-wave more transient by accelerating preferentially its decay. The strength of the feedback can be modulated by GABA receptor–mediated inhibition and by light intensity. Our results also suggest that in the mammalian retina the feedback may be a novel mechanism that contributes postphotoreceptorally to the termination of rod signals, especially those elicited by very dim light stimuli.

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We examined function of the feedback pathway from A17 GABAergic amacrine cells to rod bipolar cells (A17 feedback), a critically located inhibitory circuit in the classic rod pathway of the mammalian retina whose role in processing of scotopic visual information is still poorly understood. We show evidence that this A17 feedback has a profound influence on the temporal properties of rod-driven photoreceptoral responses (assessed with the scotopic electroretinogram b-wave). Application of a GABA antagonist prolonged preferentially the decay of the scotopic b-wave. The degree of prolongation increased as the light intensity decreased. Application of selective GABA antagonists accelerated the kinetics of the scotopic b-wave. This effect was abolished when the GABA antagonist was coapplied. Selective ablation of A17 cells mimicked the action of the GABA antagonist. In A17 cell–ablated retinas, the GABA antagonist was no longer very effective to slow the decay of the scotopic b-wave. Thus the A17 feedback, activated by light stimulation and mediated mainly by the GABA receptors, makes the scotopic b-wave more transient by accelerating preferentially its decay. The strength of the feedback can be modulated by GABA receptor–mediated inhibition and by light intensity. Our results also suggest that in the mammalian retina the feedback may be a novel mechanism that contributes postphotoreceptorally to the termination of rod signals, especially those elicited by very dim light stimuli.

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The molecular biology and biophysics of the GABA receptor have been well characterized over the last decade. Compared with the more widely distributed GABA receptor, the GABA subtype is more sensitive to GABA and shows little or no desensitization to the sustained presence of GABA (for recent reviews see Chebib and Johnston 1999; Lukasiewicz and Shields 1998; Zhang et al. 2001). The unusual cellular distribution and biophysical properties of the GABA receptor suggest a unique role for the receptor in scotopic signal transfer that remains to be elucidated.

We determined the function of the A17 feedback in temporal tuning of the rod-driven response in vivo in the rabbit by examining the effects of GABAergic agents and selective ablation of A17 cells on the kinetics of the ensemble light response of the rod bipolar cell (assessed with the scotopic electroretinogram b-wave, see DISCUSSION for details). Our re-
sults suggest that A17 feedback, mediated mainly by the GABA<sub>a</sub> receptor, exerts a profound influence on the temporal properties of the rod-driven postphotoreceptorial response by accelerating preferentially its decay. Our results also suggest that the feedback may be a novel mechanism that contributes postphotoreceptorially to the termination of rod signals in the mammalian retina.

METH O DS

Adult pigmented (Dutch belted) rabbits were used in this study. Animals were placed in a holder following general anesthesia with ketamine (30 mg/kg/h) and xylazine (15 mg/kg/h). The pupil of the rabbit eyes were dilated with topical 1% tropicamide. Corneal electrotoretinographic (ERG) responses from both left and right eyes were recorded simultaneously using two Burian–Allen bipolar contact-lens electrodes (Hansen Ophthalmic Development Lab, Iowa City, IA). A subcutaneous needle electrode connected to the ground of the amplifier was placed on the back of the animal. Light stimulation (5–8 ms flashes) for both eyes was provided by two white LEDs (6,500 K color temperature). The intensity of the LED stimulators was calibrated using a photometer (International Light IL-1700). Near full-field stimulation was achieved by illuminating custom-made diffusing screens placed very close to the contact-lens electrodes. Stimulus intensity and duration were controlled by a Pentium PC. All animals were dark adapted for 40 min prior to recording. ERG signals were preamplified and band-pass filtered (0.1–1,000 Hz) before being digitized at 4 kHz. To record slow ERG signals, such as those observed in the presence of GABA<sub>a</sub> antagonists, it is critical that the cutoff frequency of the high-pass filter be set to 0.1 Hz or lower. We found that a setting of 0.3 Hz or higher attenuated significantly the effects of some of these GABA<sub>a</sub> receptors (Fletcher and Wassle 1999).

To avoid any adapting effect of the previous flash, the interflash intervals were 2 to 20 s depending on stimulus intensity. All drugs were dissolved in PBS and sterile filtered. Drugs were administered by intravitreal injection of a 50-μl volume through a 30-gauge needle inserted at the pars plana region. Control injections of 50 or 100 μl of PBS had no effect on ERG responses. Drug effects were evaluated 80–100 min following injections. Control experiments showed that steady-state drug effects are reached by that time and maintained for several hours thereafter. Final vitreal concentrations, given in the text and figure captions, were estimated by assuming 1.2 ml vitreous volume and full equilibration.

Ablation of A17 cells was achieved by injections of 50 μl PBS that contained 50 μg 5,7-dihydroxytryptamine (DHT), 1.25 μg GB-R-12909, and 50 μg ascorbic acid on 2 successive days. ERG recording and optical imaging were conducted 5 wk after the second injection. For optical imaging of A17 cells in control and DHT-treated eyes, the same DHT cocktail (50 μl) was injected intravitreally. Eighty minutes after the injection the animals were killed and the eyes were removed. A round piece of retina (8 mm diam) was cut from immediately below the optic nerve head and flat-mounted in a plastic chamber filled with HEPES-buffered Ames medium. The retina was imaged at 25 sites in an Olympus microscope (BX50WI) equipped with an epifluorescence unit. The images were taken with a Hamamatsu C4742–95 digital camera controlled by Image-Pro Plus software (V4.5).

R E S U L T S

Blocking GABA<sub>a</sub> receptors prolongs preferentially the decay of the scotopic b-waves

We used the scotopic b-wave to examine in vivo the role of A17 feedback in temporal modulation of the ensemble activity of rod bipolar cells. The b-wave is a light-elicited transretinal field potential reflecting activity of postphotoreceptor neurons, especially on bipolar cells (including rod bipolar cells and cone bipolar cells, Massey et al. 1983; Masu et al. 1995). It can be recorded noninvasively and thus is a unique tool to study the function of retinal neural pathways in vivo, especially when used in combination with selective pharmacological agents. Under scotopic conditions, the b-wave is a sensitive and reliable measure of the gross rod-driven postphotoreceptorial response (RDR) (Dong et al. 1988; Xu et al. 2000; see Discussion for details). We used four dim light flashes that stimulated only or predominantly rod photoreceptors. They were 0.66, 1.37, 2.15, and 2.77 log units above an arbitrarily defined threshold intensity (0.016 Cd/m<sup>2</sup>, 5-ms flash) that, on average, elicited a b-wave of 16.4 ± 3.6 μV (mean ± SE) in 10 dark-adapted control eyes. To quantify the effect of A17 feedback, we determined the half-width of the rising (T<sub>1/2R</sub>) and decay (T<sub>1/2D</sub>) phases (see Fig. 1A) of the scotopic b-wave from the right and left eyes of dark-adapted control animals. T<sub>1/2R</sub> and T<sub>1/2D</sub> values measured from two eyes of control animals at these four intensities were essentially identical (Fig. 1, A and C). Therefore, in subsequent experiments, one eye (chosen randomly) of an animal was used as a control and injected intravitreally with vehicle while the other was injected with various pharmacological agents. In the rod pathway, GABA<sub>c</sub> receptors are expressed predominantly at rod bipolar cell axon terminals (Euler and Wasse 1998; Shields et al. 2000). It has been shown that A17 amacrine cells are at least presynaptic to some of these GABA<sub>c</sub> receptors (Fletcher and Wasse 1999). To investigate modulation of scotopic responses by A17 feedback, we first applied a specific GABA<sub>c</sub> receptor antagonist, (1,2,5,6-tetrahydropyridine-4-yl)methylphosphinic acid (TPMPA). TPMPA prolonged preferentially the decay phase of the scotopic b-waves (Fig. 1, B and D). Interestingly, this effect was intensity dependent; that is, dimmer stimuli were associated with a greater slowing of the response decay. The percent change for the T<sub>1/2D</sub> ratio increased from 140 ± 7% of control (n = 4) with the brightest stimuli to 274 ± 46% with the dimmest flashes. Similar results were obtained with picrotoxin, which blocks the Cl<sup>−</sup> channels associated with both GABA<sub>a</sub> and GABA<sub>c</sub> receptors (data not shown). These results suggest that the GABA<sub>a</sub> input to rod bipolar cells from amacrine cells, including A17 cells, normally makes the scotopic b-waves more transient, especially those elicited by dim stimuli.

Blocking GABA<sub>a</sub> receptors accelerates the kinetics of the scotopic b-waves

In addition to a major excitatory synaptic input from rod bipolar cells, A17 amacrine cells receive GABA<sub>a</sub>-mediated inputs from other GABAergic amacrine cells as well (Grunert and Hughes 1993; Menger and Wasse 2000). Also, rod bipolar cell terminals express GABA<sub>a</sub> and glycine receptors, in addition to GABA<sub>c</sub> receptors (Karschin and Wasse 1990; Shields et al. 2000; Suzuki et al. 1990). To gain some insights into how activity of A17 cells is regulated by other inhibitory pathways and how GABA<sub>a</sub> and/or glycine receptors contribute to temporal modulation of the scotopic b-waves, we applied selective GABA<sub>a</sub> (SR95531) and glycine (strychnine) receptor antagonists, either alone or in combination. Application of the glycine
antagonist had no significant effect (data not shown). But surprisingly, injection of the GABA\textsubscript{a} antagonist was associated with an acceleration of the scotopic b-wave (Fig. 2). Both rising and decay phases were affected. The effect of a combination of SR95531 and strychnine was similar to that of SR95531 alone (data not shown), suggesting that inhibition mediated by the strychnine-sensitive glycine receptor does not contribute significantly to the kinetics of the scotopic b-wave. The acceleration of the kinetics of the scotopic b-wave following application of SR95531 is somewhat surprising, since application of a GABA\textsubscript{a} antagonist (TPMPA) slowed the kinetics (Fig. 1, B and D). If the effect of TPMPA is produced by blocking GABA\textsubscript{c} receptors at rod bipolar cell terminals, the effect of SR95531 (Fig. 2) is most likely caused by blocking GABA\textsubscript{a} receptors at a different location since both GABA\textsubscript{a} and GABA\textsubscript{c} receptors are coupled to a Cl\textsuperscript{−}/H\textsuperscript{+} channel and it is very unlikely that blocking selectively these two types of ionotropic receptors on rod bipolar cell terminals produces opposite ef-

FIG. 1. GABA\textsubscript{c} antagonist prolongs preferentially the decay phase of rod-driven responses (RDRs). A: comparison of the b-waves from the right (blue traces) and left (green traces) eyes of a control rabbit to 4 dim flashes of increasing intensity. Both original (top row) and normalized (bottom row) responses are shown. Calibration bars: 50 μV (vertical, for the top row only) and 50 ms (horizontal, for all responses in A and B). Half-width of the rising (\(T_{1/2R}\)) and decay (\(T_{1/2D}\)) phases of RDRs are defined as the time it takes for the b-wave to rise from 50 to 100% level and to decay from 100 to 50% level, respectively. B: normalized b-waves elicited by the same 4 dim flashes in the presence (red traces) and absence (black traces) of a selective GABA\textsubscript{c} receptor antagonist (1,2,5,6-tetrahydropyridine-4-yl)methylphosphinic acid, TPMPA). The traces were from a representative rabbit. One of the eyes (chosen randomly) of a rabbit was injected with TPMPA (200 μM, estimated vitreal concentration; see MATERIALS AND METHODS) while the other was injected with vehicle and served as control. C and D: mean ratios of \(T_{1/2R}\) (light gray) and \(T_{1/2D}\) (dark gray) measured from control (n = 3) and TPMPA-treated (n = 4) animals. In this and all other figures, the error bars indicate SE. * Statistically significant at \(P < 0.05\), t-test. ** Statistically significant at \(P < 0.01\), t-test.

FIG. 2. GABA\textsubscript{a} antagonist accelerates RDR kinetics. A: comparison of the b-waves elicited by 4 dim flashes in the presence and absence of a selective GABA\textsubscript{a} receptor antagonist, SR95531 (100 μM). Traces are from a representative rabbit. Horizontal calibration bar: 50 ms. B: mean ratios of \(T_{1/2R}\) (light gray) and \(T_{1/2D}\) (dark gray) measured from SR95531-treated animals (n = 4).
fects on the kinetics of the scotopic b-wave. One mechanism that could account, at least partially, for the observed effect of SR95531 is that it causes an enhancement of the GABA<sub>c</sub> feedback from A17 cells by eliminating GABA<sub>a</sub> inhibition onto A17 cells.

**Coapplication of GABA<sub>c</sub> antagonist eliminates GABA<sub>a</sub> antagonist–induced acceleration of b-wave kinetics**

To test whether an enhancement of GABA<sub>a</sub> feedback is responsible for the GABA<sub>a</sub> antagonist–induced acceleration of the kinetics (Fig. 2), we coapplied TPMPA in combination with SR95531 (TPMPA + SR). In the presence of TPMPA, the SR95531-induced kinetics acceleration was completely eliminated (Fig. 3). The effect of the combination was similar to that of TPMPA alone (Fig. 1D). Similar results were also observed with picrotoxin (data not shown), which blocks both GABA<sub>a</sub> and GABA<sub>c</sub> receptor–coupled chloride channels in the rabbit (McGillem et al. 2000) and thus is functionally equivalent to the combination of TPMPA and SR95531. Taken together, these data suggest that the SR95531-induced kinetics acceleration results from an enhanced GABA<sub>c</sub> feedback inhibition. Thus, under physiological conditions, the strength of the GABA<sub>c</sub> feedback in the rod pathway can be modulated not only by light intensity (Fig. 1, B and D) but by GABA<sub>a</sub> inhibition as well.

**Coapplication of GABA<sub>c</sub> and GABA<sub>a</sub> antagonists unmasks a second effect of the GABA<sub>a</sub> receptor on the kinetics**

Application of a combination of TPMPA and SR95531 unmasked a second role of the GABA<sub>c</sub> receptor in temporal tuning of the scotopic b-wave. The difference caused by addition of TPMPA was rather striking: blocking the GABA<sub>a</sub> receptor with SR95531 now induced a significant slowing (Fig. 3, A and B), rather than acceleration (Figs. 2 and 3C), of the rising phase of those responses elicited by very dim flashes. This was a very consistent finding in all four animals treated with TPMPA in combination with SR95531. This kinetics slowdown is probably caused by blocking direct GABA<sub>a</sub> inputs to rod bipolar cells, since blocking the GABA<sub>c</sub> gate Cl<sup>-</sup> conductance on rod bipolar cells also causes a slowing of the scotopic b-wave (albeit affecting the decay rather than rising phase of the response). Our results suggest that at least two GABA<sub>a</sub> pathways affect the kinetics: an indirect pathway that normally slows the kinetics through inhibition of GABA<sub>c</sub> feedback, and a probably direct pathway that normally accelerates the kinetics by acting on rod bipolar cells themselves (see DISCUSSION for more details).

**Ablation of A17 cells mimics the effect of GABA<sub>c</sub> antagonism on the kinetics**

Immunocytochemical studies (Enz et al. 1996; Shields et al. 2000) show a low-level expression of the ρ subunit at rod bipolar cell dendrites in addition to a strong expression of the subunit at their terminals. To test whether the observed effect of TPMPA on the kinetics of the scotopic b-wave is produced by blocking the GABA<sub>c</sub> receptors expressed at the rod bipolar cell terminals and whether the GABA<sub>c</sub>-mediated temporal modulation is indeed associated with A17 amacrine cells, we ablated the A17 cells and examined the effect on the scotopic b-wave. In the rabbit, A17-like amacrine cells selectively take up serotonin and can be further divided into two main subtypes (S1 and S2 cells) that show similar morphology and synaptic connectivity (Sandell and Masland 1986; Vaney 1986), but different coupling patterns (Li et al. 2002). The functional difference between these two subtypes is unknown. S1 and S2 cells can be selectively labeled and ablated by serotonin analogs, such as DHT (Ehinger and Floren 1978; Sandell and Masland 1986; Vaney 1986). In one group of rabbits (n = 7), we ablated A17 cells with DHT. We recorded the scotopic b-wave at 5 wk following the second DHT injection (see MATERIALS AND METHODS). Figure 4 shows that the effect of the DHT treatment on the scotopic b-wave was remarkably similar to that produced by TPMPA (Fig. 1); it prolonged preferentially the decay of the response and this effect was also intensity dependent. Again, dimmer stimuli were associated with a greater slowing of the response decay.

**FIG. 3. Coapplication of a selective GABA<sub>c</sub> antagonist abolishes the kinetic acceleration of RDRs by a selective GABA<sub>a</sub> antagonist. A: comparison of the b-waves elicited by 4 dim flashes in the presence and absence of a combination of GABA<sub>a</sub> (SR95531, 100 μM) and GABA<sub>c</sub> (TPMPA, 200 μM) receptor antagonists. Traces are from a representative rabbit. Horizontal calibration bar: 50 ms. B: mean ratios of T<sub>1/2R</sub> (light gray) and T<sub>1/2D</sub> (dark gray) measured from the animals (n = 4) treated with SR95531 in combination with TPMPA.**
Ineffectiveness of the GABA\textsubscript{c} antagonist in prolonging the decay phase of the scotopic b-wave after ablation of A17 cells

If the observed temporal modulation of the scotopic b-wave by TPMPA (Fig. 1) is mediated mainly by the GABA\textsubscript{c} receptors expressed at rod bipolar cell terminals and this modulation is associated with A17 cells, as suggested by the results of Fig. 4, TPMPA should not be very effective in the DHT-treated eyes. Indeed, in the DHT-treated eyes (n = 4), TPMPA was no longer very effective in slowing the decay of the scotopic b-wave: only a small effect was observed at the lowest stimulus intensity (Fig. 5A, blue column). Optical imaging experiments confirmed ablation of A17 cells at 5 wk following the DHT treatment. In control eyes, the total number of DHT-labeled cells in all 25 fields imaged (see MATERIALS AND METHODS) was 663 ± 36 (n = 7). In the DHT-treated eyes there were no labeled cells in four of seven eyes. In the remaining three eyes a few labeled cells were seen in some fields while no cells were detected in the majority of fields. Representative fields from control and DHT-treated eyes are shown in Fig. 5B. Taken together, our results indicate that the observed temporal modulation of the scotopic b-wave is produced predominantly by A17 cells via GABA\textsubscript{c} receptors on rod bipolar cell axon terminals and that contribution from the dendritic GABA\textsubscript{c} receptors, if any, is negligible.

**DISCUSSION**

Our findings provide strong evidence that A17 feedback in the classic rod pathway has a profound influence on the temporal properties of the rod-driven response. Selective ablation of A17 cells produced a marked slowing of the kinetics of the scotopic b-waves, especially those elicited by very dim flashes (Fig. 4). This indicates that at least one physiological role of this feedback mechanism is to make the visual response more transient, similar to the role of the GABA\textsubscript{c} feedback in the lower vertebrate retinas (Dong and Werblin 1998; Zhang et al. 1997) in which bipolar cells receive mixed rod and cone inputs. A17 cell ablation affected preferentially the decay of the scotopic b-wave (Fig. 4), suggesting that the tonic activity of the A17 feedback pathway is low in the dark and that light stimulation activates the feedback that in turn accelerates the decay of the rod-driven response.

The scotopic b-wave as a good indicator of the ensemble activity of rod bipolar cells

We used the ERG b-wave to examine in vivo the role of A17 feedback in temporal modulation of the rod-driven response. Under scotopic conditions, the b-wave provides a sensitive and reliable measure of the rod-driven postphotoreceptor response (Dong et al. 1988; Xu et al. 2000). The b-wave is generated by activity of postphotoreceptor neurons, particularly on bipolar cells (including both rod bipolar cells and cone bipolar cells), since functional disruption of these cells, produced either pharmacologically with a mGluR6 agonist (Knapp and Schiller 1984; Massey et al. 1983; Slaughter and Miller 1981) or genetically by knocking out mGluR6 (Masu et al. 1995), eliminates the b-wave. Rod bipolar cells are on
bipolar cells and are the major source for generation of the scotopic b-wave (Green and Kapousta-Bruneau 1999; Robson and Frishman 1996). Also, since all rod bipolar cells share the same pattern of synaptic connectivity and are functionally homogeneous (Grunert and Martin 1991; Strettoi et al. 1990), the scotopic b-wave complements other recording techniques, such as intracellular and whole-cell recordings, to provide a unique tool for assessing the ensemble activity of rod bipolar cells in vivo. The A17 cell ablation experiments (Fig. 4) provide further direct evidence that synaptic inputs, such as the feedback inhibition from A17 amacrine cells, expected to influence rod bipolar cells activity (Euler and Masland 2000) do affect the scotopic b-wave.

**A17 feedback is mediated mainly by the GABA$_c$ receptor**

We show that the effect of ablation of A17 cells on the kinetics of the scotopic b-wave (Fig. 2) was very similar to that of the GABA$_c$ antagonist TPMPA (Fig. 1) and that TPMPA was not very effective in the DHT-treated eyes (Fig. 5). This indicates that A17 feedback is mediated mainly by the GABA$_c$ receptor expressed on rod bipolar cell axon terminals. Since A17 cells do not make synapses onto rod bipolar cell dendrites (Nelson and Kolb 1985; Sandell et al. 1989), our results also suggest that contribution from the dendritic GABA$_c$ receptor (its presence is suggested mainly by a weak staining for the $\alpha_1$ subunit, see Enz et al. 1996; Shields et al. 2000) to the kinetics, if any, is insignificant. This conclusion is consistent with previous electrophysiological results that showed that dendritic GABA responses in bipolar cells are mediated predominantly by GABA$_c$ receptors (Du and Yang 2000; Shields et al. 2000; Vaquero and de la Villa 1999).

Rod bipolar cell terminals express both GABA$_a$ and GABA$_c$ receptors, but the GABA$_a$ receptor appears to be the dominant subtype (Euler and Wasse 1998; McCall et al. 2002; McGillem et al. 2000). The great similarity between the effects of TPMPA and ablation of A17 cells on the kinetics of the scotopic b-wave (Figs. 1, 4, and 5) indicates that contribution of the GABA$_a$ receptor on rod bipolar cell terminals to A17 feedback is insignificant. Furthermore, the effect of a combination of the GABA$_a$ and GABA$_c$ antagonists on the kinetics of the scotopic b-wave elicited by very dim flashes (Fig. 3) was rather different from that produced by ablation of A17 cells (Fig. 4). Blocking the GABA$_a$ receptor in the presence of TPMPA slowed significantly the rising phase of the scotopic b-wave elicited by very dim flashes in all four eyes tested. This slowing in the rising phase was never observed in the A17 cell–ablated eyes (n = 7). This raises the possibility that these GABA$_a$ receptors expressed on rod bipolar cell axon terminals may mediate inputs predominantly from GABAergic amacrine cells other than A17 cells. If these GABA$_a$ receptors were also postsynaptic to A17 cells, a similar slowing in the rising phase of the scotopic b-wave seen in Fig. 3 should be observed after ablation of A17 cells. Alternatively, the slowing of the rising phase of the scotopic b-wave by the GABA$_a$ antagonist (Fig. 3) may be produced by the GABA$_a$ receptors on the dendrites of rod bipolar cells (and/or on some other sites in the rod pathway) since the GABA$_a$ receptors are widely distributed and our results do not suggest to us a particular localization. Immunocytochemical studies (Fletcher and Wasse 1999) have provided compelling evidence that A17 cells are presynaptic to GABA$_c$ receptors on rod bipolar cell terminals. However, whether A17 cells are also presynaptic to GABA$_a$ receptors on rod bipolar cell terminals is less clear. More work is needed to clarify this important issue of receptor specificity.

**Using TPMPA as a GABA$_a$ receptor antagonist**

We used TPMPA as a GABA$_a$ receptor antagonist. In the mammalian retina, the effectiveness of TPMPA as a GABA$_c$ receptor antagonist has been well documented (McCall et al. 2002; McGillem et al. 2000). TPMPA is also a weak GABA$_b$ receptor agonist (Ragozzino et al. 1996). This does not, however, affect our conclusion that A17 feedback modulates temporal properties of rod-driven responses mainly through the GABA$_c$ receptor for two reasons: first, rod bipolar cells, the postsynaptic neurons of A17 cells, do not express GABA$_b$ receptors (Gillette and Dacheux 1995; Karschin and Wasse 1990; Yeh et al. 1990). The great similarity between effects of TPMPA and A17 cell ablation on the kinetics of the scotopic b-wave indicates that the observed TPMPA effects are produced by its GABA$_c$ blocking action. Second, the effects of TPMPA on the kinetics were similar to those produced by picrotoxin (data not shown), a ligand-gated Cl$^-$ channel blocker that blocks both GABA$_c$- and GABA$_b$ currents in the rabbit retina (McGillem et al. 2000).

**Multiple GABA$_a$ pathways modulate the kinetics**

Our results suggest that there are at least two separate GABA$_a$ pathways that are involved in temporal modulation of scotopic responses. One works indirectly and normally slows the kinetics of the scotopic b-wave by inhibiting the GABA$_c$ feedback. Blocking this GABA$_c$ pathway led to an acceleration of the kinetics through disinhibiting the GABA$_c$ feedback (Figs. 2 and 3). A second GABA$_a$ pathway should normally accelerate the kinetics since blocking the pathway slowed down the kinetics (Fig. 3). We speculate that this second pathway acts directly through GABA$_a$ receptors on rod bipolar cell terminals for two reasons. First, blocking this pathway slowed the kinetics similar to blocking the GABA$_c$ feedback. This is consistent with the fact that both the GABA$_a$ and GABA$_c$ receptors are associated with a Cl$^-$ channel and activation of these receptors is expected to generate similar effects. Second, the GABA$_a$ mechanism acted more rapidly and affected mainly the rising phase of the scotopic b-wave while the GABA$_c$ mechanism acted more slowly and affected the decay phase of the response. This is consistent with the results of previous patch-clamping studies on retinal bipolar cells, which showed that GABA$_c$ current has a faster kinetics than GABA$_c$ current (Lukasiewicz and Shields 1998; Shields et al. 2000). However, we cannot rule out the possibility that the GABA$_a$-induced kinetics acceleration is produced at sites other than rod bipolar cell terminals.

**Comparison of results from the rabbit and the p1 knockout mouse**

Our results show that selective ablation of A17 cells or acute block of GABA$_c$ receptors slowed the kinetics of the scotopic b-wave in adult rabbits. This is somewhat different from the results obtained from the p1 knockout mouse in which the
rising phase of the scotopic b-wave is slightly accelerated (Fig. 6 of McCall et al. 2002). While we do not know the precise reasons for the discrepancy, differences in experimental conditions seem to be a contributing factor. We blocked acutely the GABA<sub>A</sub> receptor in adult rabbits. This is different from disabling the receptor using the knockout approach, in which developmental changes and/or remodeling of other inhibitory transmitter systems may occur. For example, in the p1 knockout mouse, GABA<sub>A</sub> inhibition to rod bipolar cells is enhanced by a few hundred percent to approximately 60 pA compared with 10 pA in the wild-type (see Fig. 5, A and B of McCall et al. 2002). This would compensate at least partially for the loss of GABA<sub>A</sub> inhibition at rod bipolar cell terminals since both the GABA<sub>A</sub> and GABA<sub>C</sub> receptors are coupled with a Cl<sup>-</sup> channel. Our results (Fig. 3) show that blocking GABA<sub>A</sub> receptors in the presence of the GABA<sub>C</sub> antagonist caused a slowing of the kinetics, particularly the rising phase. This suggests that the normal role of this GABA<sub>A</sub> pathway (not the indirect GABA<sub>A</sub> pathway that acts by inhibiting the GABA<sub>A</sub> feedback) is to accelerate the kinetics. It is likely therefore that an enhancement of GABA<sub>A</sub> inhibition to rod bipolar cells in the absence of functional GABA<sub>C</sub> receptors could cause kinetics acceleration in the p1 knockout mouse. Other factors, such as species difference and differences in settings of the high-pass filter for the recording of the b-wave (see MATERIALS AND METHODS), are also likely to contribute to the discrepancy. Examination of the kinetics of the scotopic b-wave after blocking acutely the GABA<sub>A</sub> receptor in the adult wild-type mouse may help to determine whether the role of the GABA<sub>C</sub> feedback in temporal tuning of scotopic responses in the mouse is fundamentally different from that in the rabbit.

A17 feedback may be a novel postphotoreceptor mechanism that contributes to termination of rod signals

In both TPMPA-treated and A17 cell-ablated rabbits, the degree of prolongation of the decay of the scotopic b-wave increased as the intensity of light stimulus decreased. This suggests that the strength of A17 feedback increases as the stimulus intensity decreases and that, under the physiological conditions, the feedback plays a substantial role in accelerating the decay of rod-driven responses after light flash. In the rod pathway, elements in the phototransduction cascade of rod photoreceptors, such as rhodopsin kinase and arrestin, play a critical role in termination of rod signals (Kuhn and Wilden 1987; Stryer 1986). The marked prolongation of the decay of the scotopic response after the A17 feedback was blocked (Fig. 1) or eliminated (Fig. 4), suggests that this feedback represents a novel postphotoreceptor mechanism that contributes to termination of rod signals, especially those elicited by very dim stimuli.

We thank Dr. John McReynolds for helpful comments on the manuscript and P. Agey for excellent assistance in imaging experiments.

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