

GABA_C Receptors Control Adaptive Changes in a Glycinergic Inhibitory Pathway in Salamander Retina

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We studied the role of GABA in adaptive changes in a lateral inhibitory system in the tiger salamander retina. In dark-adapted retinal slice preparations picrotoxin caused a slow enhancement of glycine-mediated IPSCs in ganglion cells. The enhancement of glycinergic IPSCs developed slowly over the course of 5–20 min, even though picrotoxin blocked both GABA_A and GABA_C receptors within a few seconds. The slow enhancement of glycinergic IPSCs by picrotoxin was much weaker in light-adapted preparations. The slow enhancement of glycinergic inhibitory inputs was not produced by bicuculline, indicating that it involved GABA_C receptors. The responses of ganglion cells to direct application of glycine were not en-

hanced by picrotoxin, indicating that the enhancement was not caused by an action on glycine receptors. In dark-adapted eyecup preparations picrotoxin caused a slow enhancement of glycinergic IPSPs and transient lateral inhibition produced by a rotating windmill pattern, similar to the effect of light adaptation. The results suggest that the glycinergic inhibitory inputs are modulated by an unknown substance whose synthesis and/or release is inhibited in dark-adapted retinas by GABA acting at GABA_C receptors.

Key words: retina; adaptation; GABA_C receptor; neuromodulator; glycine; salamander

The vertebrate retina undergoes several types of morphological and physiological adaptive changes during light and dark adaptation. Adaptive changes that occur beyond the photoreceptor level are generally termed “network adaptation” and may involve reorganization of synaptic pathways by several mechanisms such as changes in gap junction conductance or changes in gain at specific types of synapses in the retinal network. An understanding of these mechanisms is important because they may also occur in other parts of the nervous system. In the retina, several of these changes are mediated by the neuromodulator substances dopamine or nitric oxide (Dowling, 1991; Witkovsky and Derrary, 1991; Greenstreet and Djamgoz, 1994; Mills and Massey, 1995). However, there are other adaptive changes in the retina and other neural networks for which the mechanism and the modulatory substance(s) that mediate them are still poorly understood.

One such type of network adaptation in the retina is the modulation of change-sensitive or transient lateral inhibition (TLI), in which ganglion cells are inhibited by changing light stimuli. This inner retinal mechanism, which has been described in both amphibian and mammalian retinas, is mediated by transient (on–off) amacrine cells (Werblin, 1972; Werblin and Copenhagen, 1974; Thibos and Werblin, 1978; Enroth-Cugell and Jakiela, 1980). TLI is weak or absent in dark-adapted retinas, but it gradually becomes enabled over a time course of 5–20 min when the retina is exposed to an adapting light (Cook and McReynolds, 1998). Because the amacrine cells that mediate TLI in salamander retina are glycinergic (Cook et al., 1998), we studied glycinergic inhibition of ganglion cells under different

conditions of adaptation. Because salamander ganglion cells receive both GABAergic and glycinergic inputs (Belgium et al., 1984), GABA antagonists were used to isolate the glycinergic inhibition. We found that in dark-adapted retinas picrotoxin, but not bicuculline, caused a slow enhancement of glycinergic inhibition that mimicked the effect of light adaptation. The results suggest that GABA, acting via GABA_C receptors, affects the synthesis or release of an unknown substance that modulates glycinergic inhibition in the inner retina.

MATERIALS AND METHODS

Eyecup preparations were made from larval tiger salamanders (*Ambystoma tigrinum*) obtained from Charles Sullivan, Inc. (Nashville, TN). The care and use of animals were in accordance with the University of Michigan and the Society for Neuroscience policies on the use of animals in research. Details of the preparation, electrical recording, and light stimulation are described in detail elsewhere (Cook et al., 1998). Intracellular voltage recordings were made from on–off ganglion cells using micropipettes filled with 2 M potassium acetate (resistance, 300–500 MΩ) and conventional electronics. Light stimuli were a 400-μm-diameter spot in the receptive field center (determined before the experiment) and concentric annuli [inner diameter (i.d.), 1200 or 500 μm; outer diameter (o.d.), 2600 μm]. All light stimuli were 560 nm, the intensity of which was controlled with calibrated neutral density filters and expressed as log quanta·cm⁻²·sec⁻¹. Retinas were superfused with amphibian Ringer’s solution (in mM): NaCl, 110; KCl, 2.5; CaCl₂, 1.8; MgCl₂, 1.2; glucose, 11; and HEPES buffer, 5, adjusted to pH 7.8 with NaOH; drugs were added by switching to another Ringer’s solution containing either strychnine (2–10 μM), 150 μM picrotoxin, or both.

Retinal slice preparations (350–500 μm thick) were made from larval tiger salamander eyes as described in detail elsewhere (Lukasiewicz et al., 1994; Cook et al., 1998). Whole-cell voltage-clamp recordings were made using patch electrodes containing (in mM): cesium gluconate, 99; tetraethylammonium chloride, 8; NaCl, 3.4; MgCl₂, 0.4; CaCl₂, 0.4; EGTA, 11; and HEPES buffer, 10, adjusted to pH 7.7 with CsOH. The bath solution contained (in mM): NaCl, 112; KCl, 2; CaCl₂, 2; MgCl₂, 1; glucose, 5; and HEPES buffer, 5, adjusted to pH 7.8 with NaOH. Light stimuli were diffuse flashes of white light whose intensity at the retinal surface was equivalent to 3.6 × 10⁸ quanta·cm⁻²·sec⁻¹ at 560 nm. The

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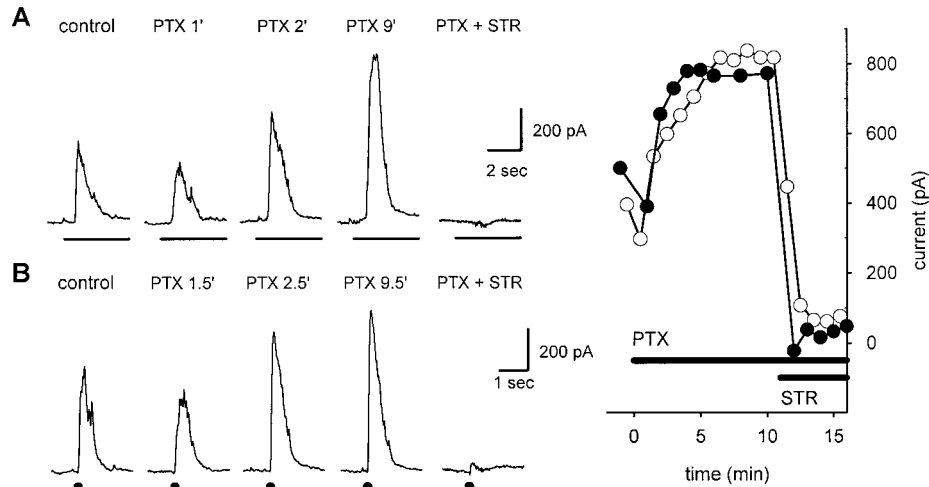


Figure 1. Effects of picrotoxin on inhibitory currents in ganglion cells. Responses are from an on-off ganglion cell in a tiger salamander slice preparation. The cell was voltage clamped at 0 mV to eliminate glutamate-mediated excitatory currents. *A*, IPSCs elicited by 4 sec full-field light stimuli (indicated by the horizontal bar below each trace). Traces show IPSCs recorded before application of picrotoxin (control) and 1, 2, and 9 min after onset of superfusion with picrotoxin (PTX 1', 2', and 9'). Subsequent addition of 2 μM strychnine in the continued presence of picrotoxin (PTX + STR) completely blocked the enhanced IPSCs, indicating that they were mediated by glycine. The holding current was +42 pA. The light flashes also elicited IPSCs at light off (data not shown); the effects on the off responses were similar to those of the on responses. *B*, Same as *A*, except that the IPSCs were evoked by zaps (+0.5 μA; 1 msec) in the

outer plexiform layer directly above the recorded ganglion cell. The time of the zap stimulus is indicated by dot below each response. *Inset*, Right, Peak light-evoked (filled circles) and zap-evoked (open circles) IPSC amplitudes at additional times.

slice was superfused, and drugs were applied through a large diameter pipette connected to a gravity-driven superfusion system, which permitted rapid switching between control and test solutions. Ganglion cells were identified by the location of their somas in the ganglion cell layer and by large (>1000 pA) inward currents elicited by depolarizing voltage steps. Some cells were also visually identified by inclusion of Lucifer yellow in the recording pipette.

Current recordings from slice preparations were digitized at 2 kHz. GABA_A- and GABA_C-mediated synaptic currents were blocked by the presence of 150 μM picrotoxin in the superfusate. In some experiments the retina was electrically stimulated by applying zaps (1 msec positive current pulses; 0.1–2 μA) using a constant-current stimulator (Grass S48 with stimulus isolation unit PSU6) through a Ringer's solution-filled pipette whose tip was located in the outer plexiform layer (OPL) directly over the recording site. The return path for the current was a silver-silver chloride electrode, separate from the recording ground electrode, connected to the bath through an agar bridge filled with 1 mM KCl. The OPL site was used because it produced larger and more consistent responses than when the electrode was located in the inner retina. Focal applications (puffs) of neurotransmitter were made by pressure (5 msec at 5 psi) ejection (Picospritzer) from a pipette containing 0.5 mM glycine or GABA. All drugs were obtained from Sigma (St. Louis, MO).

Fully dark-adapted eyecup and slice preparations were made from animals that had been dark-adapted overnight, with all procedures done in total darkness using infrared illumination. Where indicated, data were obtained from light-adapted preparations that had been previously dark-adapted but exposed to room light before the experiment, so that the degree of light adaptation was variable.

RESULTS

Picrotoxin causes a slow enhancement of glycinergic inputs to ganglion cells in dark-adapted retinal slices

Because light stimuli elicit both GABA- and glycine-mediated responses in ganglion cells, we used picrotoxin to block GABA receptors and isolate glycinergic IPSCs. Figure 1*A* shows whole-cell patch recordings from an on-off ganglion cell in a dark-adapted retinal slice preparation. The cell was voltage clamped at 0 mV to eliminate excitatory currents, and IPSCs were elicited by full-field light stimuli. In the slice preparation the light stimuli produced transient IPSCs at light on and light off, although only the responses at light onset are shown here. Responses to the same light stimulus were recorded in normal Ringer's solution (control), at 1, 2, and 9 min after the onset of continuous superfusion with 150 μM picrotoxin, and finally after addition of 2 μM strychnine while still in picrotoxin. Immediately after the addition of picrotoxin (PTX 1') the IPSC was reduced in amplitude

because the GABA-mediated component was blocked. The small glycinergic IPSC then slowly increased in amplitude and reached a maximum after 9 min in picrotoxin (PTX 9'). The IPSC in picrotoxin was completely abolished by the subsequent addition of strychnine (PTX + STR). The effects of picrotoxin on the IPSCs at light off (data not shown) were similar.

We also tested the effect of picrotoxin on IPSCs elicited by focal electrical stimulation (zaps) in the outer plexiform layer directly over the recorded ganglion cell. Picrotoxin also caused a slow enhancement of the zap-evoked IPSCs (Fig. 1*B*). Because the zap electrodes directly stimulate bipolar cells (Higgs and Lukasiewicz, 1999), the slow enhancement of the glycinergic IPSCs was not caused by an action of picrotoxin in the outer retina.

The time course of the slow enhancement of light- and zap-evoked IPSCs by picrotoxin, and their subsequent block by strychnine, is shown in more detail in the Figure 1 *inset* on the right. In other experiments (data not shown) addition of strychnine at any time after the onset of picrotoxin eliminated the IPSCs, indicating that all of these responses were glycinergic.

The time course of the enhancement of glycinergic IPSCs from 16 dark-adapted preparations is summarized in Figure 2*A* (filled circles). Because the amplitudes of the IPSCs and the amount of enhancement were different in different cells, the responses of each cell were normalized to the first IPSC recorded in the presence of picrotoxin (1 min after onset of picrotoxin), which represents the control value for the glycinergic IPSC.

Similar experiments were done in seven light-adapted preparations (Fig. 2*A*, open circles). In light-adapted preparations the initial reduction of the IPSC was smaller, and there was very little slow enhancement in the continued presence of picrotoxin. This is consistent with the fact that glycinergic inhibition is already enhanced in light-adapted retinas.

Figure 2*B* shows that the results were similar when the IPSCs were elicited by zaps. In dark-adapted preparations, zap-evoked IPSCs (filled circles) were initially reduced and then slowly enhanced during continuous superfusion with picrotoxin, and this effect was minimal in light-adapted retinas (open circles).

Although picrotoxin caused little slow enhancement of glycinergic IPSCs in light-adapted retinas, it did have a separate, rapid

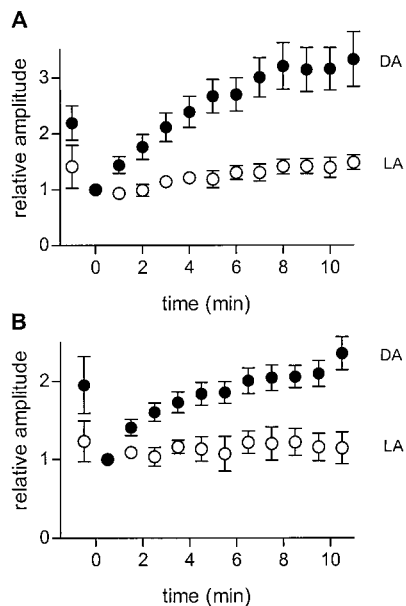


Figure 2. Enhancement of glycinergic IPSCs by picrotoxin is greater in dark- than light-adapted retinas. *A*, IPSCs elicited by full-field light flashes at different times after onset of continuous superfusion with picrotoxin. Data are averaged from 16 dark-adapted retinas (filled circles) and 7 light-adapted retinas (open circles). For each cell, peak IPSC amplitudes were normalized to that of the first response recorded in the presence of picrotoxin, which was recorded within 1 min after switching the superfusate to picrotoxin. Superfusion with picrotoxin begins at $t = 0$. In 11 of the cells tested (8 dark-adapted, 3 light-adapted) $2 \mu\text{M}$ strychnine was added after the enhancement had reached a maximum; in all cases strychnine completely blocked the IPSCs, indicating that they were glycinergic. Error bars indicate 1 SEM. The maximum enhancement was $3.57 (\pm 0.33)$ -fold in dark-adapted preparations ($n = 16$) and $1.49 (\pm 0.15)$ -fold in light-adapted preparations ($n = 7$). The probability that this difference was caused by chance was < 0.003 (Student's unpaired t test). *B*, Same as *A*, except that the IPSCs were elicited by focal electrical stimuli ($+0.5 \mu\text{A}$; 1 msec) as described in Figure 1. The maximum enhancement was $2.38 (\pm 0.20)$ -fold for dark-adapted preparations ($n = 4$) and $1.12 (\pm 0.28)$ -fold for light-adapted preparations ($n = 4$). The probability that this difference was caused by chance was < 0.001 (Student's unpaired t test). *DA*, Dark-adapted; *LA*, light-adapted.

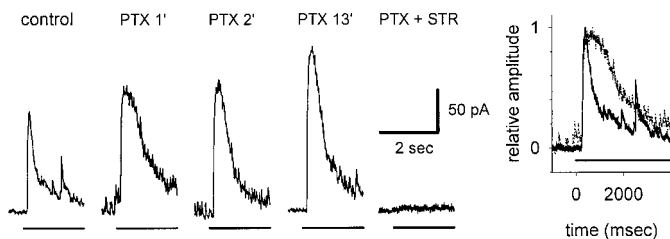


Figure 3. Effect of picrotoxin on ganglion cell IPSCs in a light-adapted retinal slice. *Left*, Details in Figure 1, except that this retina had been light adapted by exposure to background illumination for several minutes. *Inset, Right*, Superimposed traces of the first two responses (control and *PTX 1'*) scaled to the same amplitude to better illustrate the difference in time courses.

effect on glycinergic IPSCs in some light-adapted preparations. In three of the seven light-adapted ganglion cells, picrotoxin caused a rapid increase in the amplitude and duration of the glycinergic IPSC. An example is shown in Figure 3, which shows light-evoked IPSCs in a light-adapted ganglion cell. In this cell the first IPSC recorded after the addition of picrotoxin was larger and more

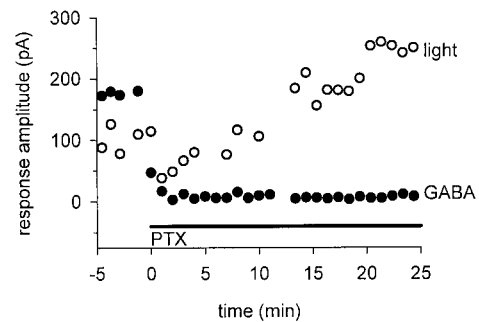


Figure 4. Picrotoxin slowly enhances light-evoked glycinergic responses but rapidly blocks responses to directly applied GABA. Responses are IPSCs evoked by alternating full-field light flashes (open circles) and puffs of GABA (filled circles). Picrotoxin (*PTX*) rapidly blocked the responses to GABA puffs, but the light-evoked IPSCs were initially reduced and then slowly enhanced over the next 20 min. Similar results were seen in all of the four cells tested.

prolonged than the control IPSC. The increase in duration is shown more clearly in the Figure 3 *inset* on the *right*, in which the first two traces (control and 1 min after application of picrotoxin) were scaled to the same amplitude and superimposed. Similar rapid changes in amplitude and kinetics of glycinergic responses have been described in light-adapted retinas by others and were attributed to blocking the GABAergic inhibition of glycinergic amacrine cells (Zhang et al., 1997; Roska et al., 1998). This is, however, a separate effect of picrotoxin that is not related to the slow enhancement of glycinergic inhibition described above (see Discussion). After the initial rapid effect of picrotoxin there was little additional increase in IPSC amplitude during the next 17 min, after which it was blocked by the addition of strychnine.

Picrotoxin rapidly blocks GABA receptors

Although picrotoxin caused a slow enhancement of glycinergic inputs to ganglion cells in dark-adapted retinas, this was not caused by a slow action of picrotoxin in blocking GABA receptors. Figure 4 shows inhibitory currents elicited by full-field light flashes or puffs of GABA onto the ganglion cell dendrites. The light and puff stimuli were given alternately every 30 sec. The IPSCs elicited by light stimuli (open circles) were slowly enhanced in the presence of picrotoxin as described above, but the responses to direct application of GABA (filled circles) were completely blocked almost immediately after picrotoxin was added.

Bicuculline does not cause slow enhancement of glycinergic IPSCs

Because picrotoxin blocks both GABA_A and GABA_C receptors, we also used bicuculline ($150\text{--}200 \mu\text{M}$), which blocks only GABA_A receptors, to test whether the effect of picrotoxin was caused by blocking GABA_A or GABA_C receptors. Figure 5*A* shows the effects of bicuculline on the light-evoked IPSCs in four ganglion cells. Bicuculline (open circles) rapidly reduced the light-evoked IPSCs but did not cause a subsequent enhancement of these responses. For comparison, the filled circles show the IPSCs elicited by the same light stimuli during superfusion with picrotoxin (data from Fig. 2*A*, filled circles). Bicuculline also failed to enhance zap-evoked IPSCs (Fig. 5*B*). These results indicate that the enhancement of glycinergic IPSCs by picrotoxin was caused by blocking GABA_C receptors.

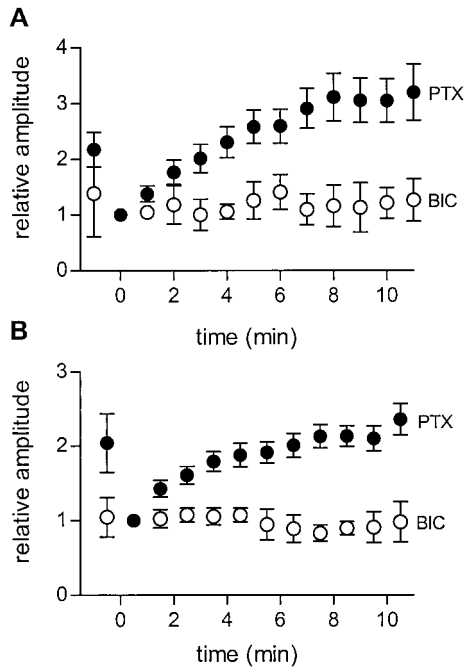


Figure 5. Bicuculline does not enhance glycinergic IPSCs in ganglion cells. *A*, Light-evoked IPSCs. *Open circles* show the amplitude of IPSCs at various times after onset of continuous superfusion with 200 μ M bicuculline (*BIC*). For comparison, *filled circles* show the results (from Fig. 2*A*) obtained during superfusion with 150 μ M picrotoxin (*PTX*). Data were averaged from 16 cells for *PTX* and 5 cells for *BIC*. For each cell, peak IPSC amplitudes were normalized to the first response recorded in the presence of picrotoxin. *B*, Same as *A*, except that the IPSCs were elicited by zaps (+0.5 μ A; 1 msec) as described in Figure 1.

Effect of picrotoxin on excitatory inputs to ganglion cells

Because GABA_C receptors are present on salamander bipolar cell terminals (Lukasiewicz and Werblin, 1994) it is possible that the slow enhancement of glycinergic inputs to ganglion cells in the presence of picrotoxin was caused by removal of tonic inhibition onto bipolar terminals, which would cause increased release of glutamate from bipolar terminals onto glycinergic amacrine cells. To test this possibility we looked for an effect of picrotoxin on glutamate release from bipolar cells by measuring the EPSCs in ganglion cells. To isolate EPSCs the ganglion cells were clamped at the reversal potential for chloride, which eliminated GABA- and glycine-mediated inhibitory currents in the recorded cell. Figure 6*A* shows the effect of picrotoxin on EPSCs elicited by full-field light stimuli. Picrotoxin caused an initial increase in EPSC amplitude ($t = 2$ min), but after that the EPSCs gradually became smaller in amplitude and shorter in duration ($t = 17$ min). However, the decrease in EPSC amplitude and duration was reversed when strychnine was added (*PTX + STR*), suggesting that the slow decrease in EPSC was caused by slowly increasing glycinergic inhibition of bipolar terminals. Similar results were seen in four of the six ganglion cells in which light- and zap-evoked EPSCs were studied (Fig. 6*B*). Thus the slow enhancement of glycinergic IPSCs in picrotoxin was not caused by an increase in glutamate release from bipolar cells.

Picrotoxin does not increase ganglion cell sensitivity to glycine

Figure 7 compares the effect of picrotoxin on ganglion cell IPSCs elicited by full-field light stimuli and by puffs of glycine onto the

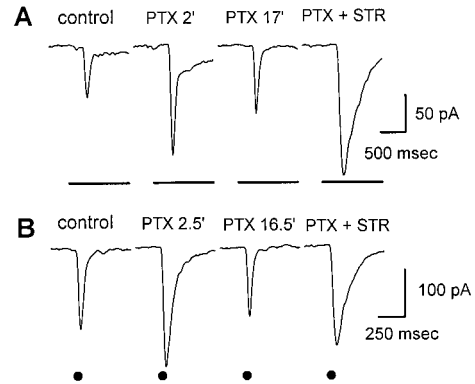


Figure 6. Effect of picrotoxin on excitatory currents in an on-off ganglion cell. The cell was voltage clamped at the chloride reversal potential (-65 mV) to eliminate GABA- and glycine-mediated inhibitory currents. Other details are as described in Figure 1. *A*, EPSCs that were elicited by 4 sec full-field light stimuli. After the onset of picrotoxin the EPSCs were initially enhanced (*PTX 2'*) but then gradually declined (*PTX 17'*). Subsequent addition of 2 μ M strychnine in the continued presence of picrotoxin (*PTX + STR*) partially reversed the slow decline in EPSC amplitude caused by picrotoxin, indicating that the slowly developing suppression of the EPSC was mediated by glycine. The holding current was -52 pA. The effects on the off responses (data not shown) were similar. *B*, Same as *A*, except that the EPSCs were elicited by zaps (+0.5 μ A; 1 msec) as described in Figure 1.

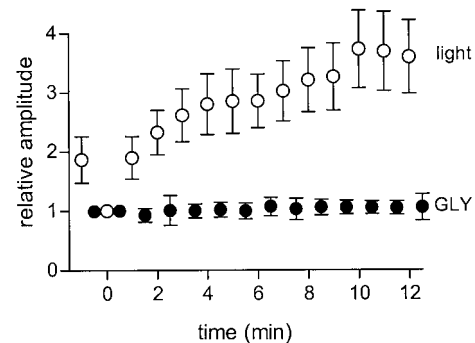


Figure 7. Picrotoxin does not affect the ganglion cell response to direct application of glycine. *Data points* indicate the average peak amplitude of IPSCs elicited by full-field illumination (*open circles*) and puffs of glycine (*filled circles*) in four cells at various times after onset of continuous superfusion with picrotoxin at $t = 0$. For each cell, responses were normalized to the first response after the application of picrotoxin in each cell. *GLY*, Glycine.

ganglion cell dendrites. Responses are averaged from four cells that were alternately stimulated by light and glycine puffs. After application of picrotoxin the IPSCs elicited by light stimuli (*open circles*) were initially reduced and then slowly enhanced to nearly twice that size over the time course of several minutes. However, the responses to puffs of glycine (*filled circles*) were not affected by picrotoxin.

The enhancement of glycinergic inhibition induced by picrotoxin does not involve dopamine

Because dopamine modulates several different retinal changes associated with light adaptation we wanted to determine whether it is involved in the picrotoxin-induced enhancement of glycinergic inhibition, particularly because this enhancement is similar to that observed during light adaptation. We therefore tested the effects of dopamine and dopamine antagonists on the picrotoxin-induced enhancement of glycinergic inhibition of ganglion cells in

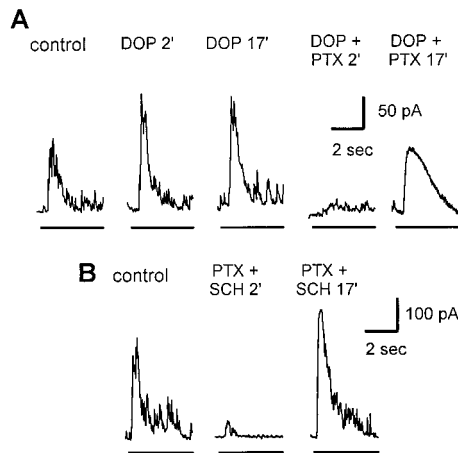


Figure 8. Effect of dopamine and dopamine antagonists on the ability of picrotoxin to cause slow enhancement of glycinergic IPSCs. Light stimuli and recording conditions are as described in Figure 1. *A*, Addition of 20 μ M dopamine caused a rapid increase in IPSC amplitude within 2 min (*DOP 2'*), but there was no further increase after 15 additional minutes in dopamine (*DOP 17'*). The initial enhancement of the IPSC by dopamine was seen in only two of the four cells tested; in all four cells the mean enhancement was 1.13 (\pm 0.32)-fold ($p = 0.65$). After 18 min in dopamine, addition of 150 μ M picrotoxin caused an immediate strong reduction in the IPSC (*DOP + PTX 2'*). In the continued presence of *PTX* the IPSC was slowly enhanced (*DOP + PTX 17'*). The mean enhancement by *PTX* was 4.02 (\pm 1.48)-fold ($p = 0.02$; $n = 4$). *B*, Addition of 15 μ M SCH23390 and 150 μ M *PTX* caused an immediate reduction of the IPSC (*SCH + PTX 2'*), but in the continued presence of *PTX* and *SCH* the response slowly became enhanced (*SCH + PTX 17'*). In the five cells tested, the maximum enhancement by *PTX* in the presence of *SCH* was 4.42 (\pm 1.24)-fold ($p = 0.02$). In both *A* and *B* the enhanced IPSC in *PTX* was completely blocked by 2 μ M strychnine in all cells (data not shown).

the slice preparation. Dopamine (20 μ M) caused an immediate enhancement of light-evoked IPSCs (Fig. 8*A*) in two of the four cells tested, probably because of its upmodulation of glutamate release from bipolar cells (Wellis and Werblin, 1995). However, in all four cells there was no further enhancement during the next 15 min, and the IPSCs were dramatically reduced by the subsequent addition of picrotoxin, indicating that they were mainly GABAergic and that dopamine had not caused any enhancement of glycinergic input. After the addition of picrotoxin the small, glycinergic IPSC was slowly enhanced over the next 15 min, which was similar to the effect of picrotoxin in the absence of dopamine (compare Fig. 1).

The dopamine antagonist SCH23390 did not prevent the enhancement of glycinergic IPSCs by picrotoxin (Fig. 8*B*). In the presence of SCH23390 (15 μ M), picrotoxin caused an initial reduction of the light-evoked IPSC, followed by a gradual enhancement of the IPSC over the next 15 min ($n = 5$). This was similar to the effect of picrotoxin added to control Ringer's solution (compare Fig. 1).

Picrotoxin causes a slow enhancement of glycinergic inhibition and TLI in ganglion cells in dark-adapted eyecup preparations

The slow enhancement of glycinergic IPSCs by picrotoxin in dark-adapted retinal slice preparations suggests that GABA_C receptors may be involved in the slow enhancement of TLI by adapting light in intact retinas. To test this possibility we investigated whether picrotoxin could mimic the ability of an adapting light to enhance TLI in dark-adapted eyecup preparations. The results of one such experiment are shown in Figure 9*A*.

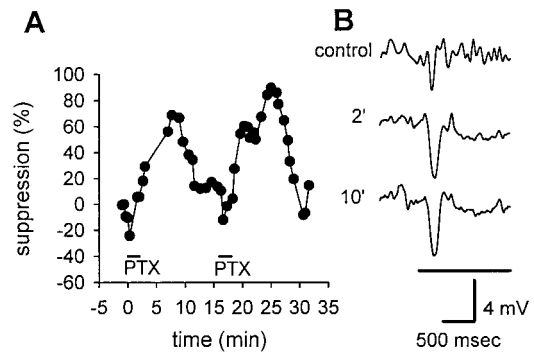


Figure 9. Effect of picrotoxin on transient lateral inhibition and glycinergic IPSPs in on-off ganglion cells in eyecup preparations. *A*, Effect of 150 μ M picrotoxin (*PTX*) on TLI in a dark-adapted eyecup. TLI was measured as the suppression of the response to a small test spot in the receptive field center by rotation of a broken annulus (windmill) pattern. *B*, Effect of picrotoxin on the transient IPSP elicited at the onset of an annulus (i.d., 500 μ m; o.d., 2600 μ m) in a light-adapted eyecup. *Traces* show the IPSP response in control Ringer's solution and at 2 and 10 min after onset of continuous superfusion with 150 μ M picrotoxin. The horizontal line below the response traces indicates the initial portion of the 4 sec light stimulus.

TLI was measured as the percent suppression of a test response (to a small spot flashed in the receptive field center) by the rotation of a concentric windmill (broken annulus) stimulus, as described in detail elsewhere (Cook and McReynolds, 1998). In the dark-adapted retina, rotation of the windmill did not cause suppression of the test response. A 2 min exposure to picrotoxin caused a transient decrease in the suppressive effect of the rotating windmill, followed by a gradual increase in suppression, which reached a maximum after 8 min and then declined again. A second application of picrotoxin produced essentially the same effect. In this experiment we used a brief exposure to picrotoxin to verify that the effect was reversible, because picrotoxin was difficult to wash out after prolonged application. The effect of continuous superfusion with picrotoxin (data not shown) was similar ($n = 9$ cells) except that the suppression remained high rather than declining again. The maximum amount of suppression and the time course of its development (2–12 min in 9 cells) were quite variable, even with continuous exposure to picrotoxin.

In light-adapted eyecup preparations, TLI was already enhanced, and no slow development of TLI was observed when picrotoxin was added. However, in light-adapted retinas picrotoxin did have a rapid effect on ganglion cell IPSPs, similar to the rapid effect of picrotoxin on IPSCs in light-adapted slices. An example is shown in Figure 9*B*, in which picrotoxin caused a rapid increase in both the amplitude and duration of the transient IPSP elicited by an annular light stimulus. These changes occurred within 2 min after addition of picrotoxin, but there was no further increase even after 10 min in the continued presence of picrotoxin. Picrotoxin had similar effects on the IPSPs elicited by annular stimuli in three other ganglion cells. In all cases the IPSPs were blocked by strychnine, indicating that they were glycinergic.

DISCUSSION

In dark-adapted retinas picrotoxin caused a slow enhancement of glycinergic inhibition that is similar in action and time course to the effect of light adaptation. Picrotoxin slowly enhanced glycinergic inhibition and TLI in ganglion cells in the eyecup and also slowly enhanced both light- and electrically evoked glycinergic IPSCs in ganglion cells in the slice. The time courses of these

actions in the eyecup and slice were similar. The slow decline in ganglion cell EPSC amplitudes during superfusion with picrotoxin and their rapid recovery after subsequent addition of strychnine suggest that picrotoxin also enhances glycinergic feedback to bipolar terminals. The fact that bicuculline did not produce these effects indicates that the enhancement of glycinergic activity was caused by blocking GABA_C receptors, although the possibility that the enhancement requires blocking both GABA_C and GABA_A receptors cannot be ruled out.

The enhancement of glycinergic inhibition was very slow compared with the block of GABA receptors by picrotoxin. The immediate block of ganglion cell responses to GABA puffs indicates that GABA_A receptors were rapidly blocked. Because GABAergic feedback to bipolar cell terminals in salamander retina is mediated mainly by GABA_C receptors (Lukasiewicz et al., 1994; Dong and Werblin, 1998) the rapid enhancement of EPSCs indicates that GABA_C receptors were also rapidly blocked. Other studies have also shown that GABA_C receptors are rapidly blocked by picrotoxin (C. R. Shields and P. D. Lukasiewicz, unpublished observations) (see also Feigenspan and Bormann, 1994). The very slow time course of the enhancement of glycinergic inhibition relative to the blocking of GABA receptors suggests that the modulation of glycinergic activity by GABA is indirect.

Where does the modulation of glycinergic inhibition by GABA occur?

The fact that picrotoxin does not enhance the responsiveness of glycine receptors on ganglion cells indicates that the output of the glycinergic amacrine cells is enhanced. This could be caused by an increase in bipolar cell output to glycinergic amacrine cells or by some change in the glycinergic amacrine cells themselves. Two findings suggest that the modulation does not result from increased glutamate release from bipolar terminals. First, prolonged exposure to picrotoxin caused a slow decrease in the amplitude of the EPSCs, which reflects a decrease in glutamate release from bipolar terminals, even though it slowly enhanced glycinergic IPSCs during the same time period. Second, dopamine did not enhance glycinergic IPSCs, even though it increases glutamate release from bipolar cells (Wellis and Werblin, 1985). Therefore, although we cannot rule out the possibility that the excitatory input to glycinergic amacrine cells is from a separate population of bipolar terminals that are not affected by dopamine and do not receive glycinergic feedback, it seems likely that the site of modulation is the glycinergic amacrine cell. The cellular mechanism that is modulated could be the glycinergic amacrine cell's responsiveness to glutamate, its ability to release glycine, or some intervening step. Recording from glycinergic transient amacrine cells in dark- and light-adapted preparations, and in the presence and absence of picrotoxin, may provide additional information about the cellular mechanism involved.

How might blocking GABA_C receptors cause changes in the glycinergic amacrine cells? One possibility is that these cells have an unusual type of picrotoxin-sensitive GABA receptor that has slow metabotropic effects. Although metabotropic actions have been described at some "ionotropic" receptors (Wang et al., 1997; Kawai and Sterling, 1999), no such actions have been reported at picrotoxin-sensitive GABA receptors. A more likely possibility is that the modulation is mediated by another, unknown substance and that GABA may act to suppress the synthesis or release of this substance. In such a scheme, the GABA_C receptors that control the modulation could be located on the modulatory neu-

rons or on neurons that provide input to these cells. Blocking GABA_C receptors on bipolar terminals could also increase release of a modulatory substance by increasing excitatory input to the neurons that release the substance, but again this would require a separate population of bipolar terminals that were not affected by dopamine and did not receive glycinergic feedback.

The slow time course of the enhancement of glycinergic inhibition could be attributable to the time required for synthesis of the modulatory substance or activation of its release mechanism. It is also possible that the action of the substance on its target cells, presumably glycinergic amacrine cells, is slow. The identity of the postulated modulator substance is unknown. Although dopamine modulates several different types of retinal changes associated with light adaptation, it does not seem to be involved in the slow modulation of glycinergic inhibition. No other substances have yet been tested in this regard, and identification of the modulatory substance may be difficult. Possible candidates include nitric oxide, serotonin, or one of the several neuropeptides that have been found in retinal neurons, particularly amacrine cells, but have no clearly defined functional role.

What causes the rapid enhancement of glycinergic IPSCs by picrotoxin in light-adapted retinas?

In three of the seven experiments in light-adapted retinas the addition of picrotoxin caused an immediate increase in the amplitude and duration of glycinergic IPSCs. Similar changes in the amplitude and kinetics of glycinergic responses by GABA antagonists in light-adapted preparations were attributed to blocking GABA_A receptors on glycinergic amacrine cells (Zhang et al., 1997; Roska et al., 1998). Blocking GABA_C receptors on bipolar terminals, which increases excitatory input from bipolar cells to third-order neurons (Lukasiewicz and Werblin, 1994; Dong and Werblin, 1998), could also cause a rapid increase in glycinergic IPSCs. The fact that picrotoxin never caused a rapid enhancement of glycinergic IPSCs in dark-adapted retinas supports the idea that in dark-adapted retinas the glycinergic amacrine cells are suppressed by a separate action of GABA.

Network adaptation in the retina has been studied for over 20 years, but the neural basis for many of the adaptive changes is still not well understood. Here we have demonstrated that adaptive changes in a specific neural circuit in the inner retina are controlled by GABA_C receptors. Other aspects of this adaptation, including the identity of the postulated neuromodulator substance, have yet to be determined.

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