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# The effects of GABA and related drugs on horizontal cells in the isolated turtle retina

IDO PERLMAN[1] AND RICHARD A. NORMANN[2]

[1]The Rappaport Family Institute for Research in the Medical Sciences and Department of Physiology, Faculty of Medicine, Technion-Israel Institute of Technology, Haifa, Israel
[2]Departments of Bioengineering, Physiology, and Ophthalmology, The University of Utah, Salt Lake City

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**Abstract**

The role of GABA in the outer plexiform layer of the turtle retina has been examined by intracellular recordings from L- and C-type horizontal cells in the isolated retina preparation.

GABA (1–5 mM) slightly depolarized the L-type horizontal cells, reduced the amplitude of their photoresponses, and slowed down the rate of hyperpolarization during the ON component of the photoresponse. These effects could not be replicated by either muscimol or baclofen. When synaptic transmission from the photoreceptors had been blocked by either kynurenic acid or cobalt ions, GABA depolarized L-type horizontal cells and augmented the remaining photoresponses. Neither muscimol nor baclofen exerted any effect on L-type horizontal cells under these conditions. Nipecotic acid, a competitive inhibitor of the GABA-uptake system, induced effects on turtle L-type horizontal cells which were similar to those exerted by GABA. Thus, the complex GABA effect on turtle L-type horizontal cells seems to represent the summation of at least two actions; an indirect one mediated by the red cones via $GABA_a$-type receptors and a direct one which probably reflects the activation of an electrogenic GABA-uptake system.

GABA (1–5 mM) induced a transient depolarization in C-type horizontal cells but eliminated color opponency in only three cells out of seven studied. This observation is inconsistent with the notion that the only neural mechanism responsible for the chromatic properties of C-type horizontal cells in the turtle retina is a GABAergic negative feedback from the L-type horizontal cells onto the green ones.

**Keywords:** Retina, Horizontal cells, Synapse, Negative feedback, GABA

## Introduction

Information processing in the vertebrate visual system is based upon extensive neural interactions. At the first stage of the visual pathway, the photoreceptors and the horizontal cells form a neural network which has provided a simple explanation for the genesis of color-opponent photoresponses in chromaticity-type horizontal cells in the turtle (Fuortes & Simon, 1974) and in the fish retinas (Burkhardt, 1977; Burkhardt & Hassin, 1983). According to this model, red cones directly excite L-type horizontal cells while green cones directly excite R/G C-type horizontal cells. The L-type horizontal cells provide negative feedback onto both red and green cones (Baylor et al., 1971; O'Bryan, 1973; Fuortes & Simon, 1974; Piccolino et al., 1980). Thus, in the R/G C-type horizontal cell, green stimuli evoke hyperpolarizing responses via direct green-cone excitation while the depolarizing red responses are mediated by the negative-feedback pathway from L-type horizontal cells onto the green cones.

Reprint requests to: Ido Perlman, Department of Physiology and Biophysics, Faculty of Medicine, Technion-Israel Institute of Technology, POB 9649, Haifa, 31096, Israel.

A number of observations argue against the negative-feedback model as being solely responsible for color opponency in C-type horizontal cells. Green cones would be expected to manifest the same color opponency as C-type horizontal cells, especially considering the tight coupling between the cone cell body, where intracellular recordings are probably made, and its pedicle, where the negative feedback action is exerted (Lasater et al., 1989). However, while C-type horizontal cells manifest prominent depolarizing responses to red light stimuli (Fuortes & Simon, 1974; Kato, 1979; Burkhardt & Hassin, 1983; Gottesman & Burkhardt, 1987), green cones usually exhibit hyperpolarizing responses (Baylor & Hodgkin, 1973; Burkhardt, 1977; Burkhardt & Hassin, 1983; Kaneko & Tachibana, 1985) and show depolarizing ones only under restricted spatial and color conditions (Baylor et al., 1971; O'Bryan, 1973). Furthermore, in some species, negative feedback onto cones cannot be demonstrated physiologically yet color opponency is observed in the horizontal cells (Burkhardt & Hassin, 1978). These considerations suggest that while the negative-feedback model may partially account for color opponency of C-type horizontal cells, other neural mechanisms, possibly of a feedforward nature, may also be involved (Burkhardt & Hassin, 1978).

Gamma-aminobutyric acid (GABA) has been suggested as

the synaptic neurotransmitter which mediates horizontal cell negative feedback onto cones (Lam et al., 1978; Marc et al., 1978). Recent experimental evidence has supported this suggestion. The horizontal cells have been shown to accumulate GABA by a high-affinity uptake system, to contain a high concentration of the enzyme glutamic-acid dehydrogenase (GAD) and to release GABA when depolarized (Yazulla, 1986). In the isolated fish retina, GABA hyperpolarized green cones and cone horizontal cells and eliminated color opponency of C-type horizontal cells (Djamgoz & Ruddock, 1979; Negishi & Drujan, 1979; Wu & Dowling, 1980; Murakami et al., 1982*a*, *b*). Recent studies on isolated turtle cones in culture demonstrated $GABA_a$ receptors localized to the cone pedicle (Tachibana & Kaneko, 1984; Kaneko & Tachibana, 1986).

GABA also appears to produce effects on retinal horizontal cells which cannot be attributed to its role as the feedback neurotransmitter. In the retinas of the necturus (Miller et al., 1981), skate (Cohen, 1988), and tiger salamander (Yang & Wu, 1989), GABA has been found to depolarize the horizontal cells, to reduce the amplitude of their photoresponses and to slow down the "ON" phase of the photoresponses. These observations have been attributed to a direct action of GABA on the horizontal cells. A direct effect of GABA has been demonstrated in horizontal cells isolated from the skate retina (Lasater et al., 1984) which reflects the activation of electrogenic GABA-uptake mechanisms (Malchow & Ripps, 1990). The contribution of electrogenic GABA-uptake systems to the horizontal cell's membrane potential has also been demonstrated in the carp retina (Kamermans, 1989). In the turtle eyecup, GABA has been shown to modify cellular coupling between adjacent horizontal cells (Piccolino et al., 1982; Normann et al., 1988). This GABA action has been attributed to inner retinal mechanisms involving dopaminergic pathways (Piccolino et al., 1987). However, despite the presence of negative-feedback pathways from L-type horizontal cells to the cones, which have been demonstrated physiologically (Baylor et al., 1971; O'Bryan, 1973; Fuortes & Simon, 1974), GABA, its agonists, and antagonists appear to have little effect on the chromatic properties of horizontal cells (Normann et al., 1988).

In this study, we examined the role of GABA in the outer plexiform layer of the isolated turtle retina (Perlman et al., 1990). In order to identify specific sites of action, we applied GABA agonists and antagonists under conditions where synaptic transmission was unaltered and after it had been blocked with cobalt ions or kynurenic acid. Under normal synaptic transmission, GABA produced a transient depolarization of C-type horizontal cells but did not abolish the chromatic properties in all of the cells studied. Thus, additional pathways, possibly of a feedforward nature may also be involved in the genesis of color opponency in the turtle retina.

L-type horizontal cells, exposed to GABA exhibited a slight but steady depolarization, profound slowing of the ON component of the photoresponses, and reduction in their amplitude. These GABA effects could not be replicated by either muscimol or baclofen. Under conditions where transmission of chemical synapses was minimized, GABA, but not muscimol nor baclofen, depolarized the L-type horizontal cell and augmented the remaining photoresponses. However, nipecotic acid, a competitor of GABA uptake, produced effects similar to those exerted by GABA.

These observations are consistent with the notion that GABA may modulate the horizontal cell physiology in the turtle retina via at least two mechanisms. One may represent an indirect action mediated by the cones and activated by $GABA_a$-type receptors in the cone plasma membrane. A second pathway involves a direct action of GABA on the horizontal cells which is likely mediated by an electrogenic GABA-uptake mechanism located in the L-type horizontal cells.

## Methods

### *The isolated turtle retina preparation*

The preparation of the inverted, isolated turtle retina has been previously described (Perlman et al., 1990). After decapitation and pithing, the turtle head was wrapped in a moist paper towel and refrigerated at 10°C for a period of between 4–8 h in order to facilitate the separation of the retina from the pigment epithelium with minimal damage to the outer segments. After the refrigeration period, the eye was enucleated, hemisected, and the vitreous humor was carefully removed. The eyecup was first bisected along the vertical meridian (just adjacent to the optic disk), and then along the horizontal meridian (parallel to the visual streak) to produce four triangular pieces. One of these pieces was placed vitreal side down on a 1-cm-diameter piece of millipore filter (0.45 $\mu$m pore size). The retina and filter were enclosed in a 5-ml volume for 3 min to promote adherence of the retina to the filter paper. The remaining pieces of eyecup and the head were placed back in the refrigerator for subsequent preparations. The sclera, choroid, and pigment epithelium were detached by pealing the sclera back from the retina. This left the retina attached to the filter paper with the photoreceptors facing up. All of the procedures were performed under very dim illumination to minimize pigment bleaching.

### *The superfusion system*

The superfusion system consisted of four containers which were connected to a four-valve manifold. The outlet of the manifold led to a small caliber inlet tube which was positioned directly above one corner of the retina to assure adequate superfusion of the preparation. The flow rate of the superfusion system was about 0.6 ml/min. The "normal" solution was composed as follows: 110 mM NaCl; 2.6 mM KCL; 2 mM $MgCl_2$; 2 mM $CaCl_2$; 10 mM D-Glucose; 22 mM $NaHCO_3$. The pH of the solution was maintained at 7.5 by constant bubbling of 95% $O_2$ and 5% $CO_2$ through the solution. Test drugs, GABA, muscimol, bicuculline, baclofen, and kynurenic acid, were added to the "normal" solution. The concentrations of the experimental drugs used here were in the low (0.5–5 mM) range, similar to those used in the studies cited in the Introduction. For nipecotic-acid experiments, glucose was omitted from the solution to prevent changes in osmolality. All of the drugs, except baclofen, were purchased from Sigma (St. Louis, MO). Baclofen was kindly supplied to us by Ciba-Geigy.

### *Photostimulation and recording system*

White-light stimuli of 500-ms duration were applied every 3 s to monitor the effects of the test drugs on response amplitude and kinetics. The intensity of the white-light stimulus was calibrated in terms of effective quanta (633 nm)$s^{-1}$ $\mu m^{-2}$ (Normann & Perlman, 1990). Full-field, diffuse retinal illumination was used in order to cover the entire receptive fields of the hor-

izontal cells. After a successful impalement of a horizontal cell, the intensity–response relationship was measured with light stimuli of different intensities. The superfusion was then switched from the "normal" solution to the experimental one. When a "steady-state" effect was achieved, an intensity–response series was recorded before returning to superfusion with "normal" solution.

Intracellular recordings from horizontal cells were achieved with microelectrodes pulled on a modified Livingston electrode puller. When filled with 3 M potassium acetate solution, the microelectrodes had resistances of about 200 MΩ. The membrane potential was continuously monitored on an oscilloscope screen and on a Gould paper recorder and recorded on an FM tape recorder. The data were digitized after the experiment with a small computer system for analysis and plotting of the data.

## Results

We have studied the effects of GABA, its agonists, and antagonists in the isolated turtle retina. In order to separate indirect from direct actions of these agents, we performed the experiments when synaptic transmission was intact and after it had been blocked pharmacologically.

### *Intact synaptic transmission*

The effects of 5 mM GABA on an L-type and a C-type horizontal cell in the isolated turtle retina are shown in Fig. 1A and B, respectively. GABA produced a slight depolarization of the L-type horizontal cell which was accompanied by a reduction in the photoresponse amplitude (Fig. 1A). The most striking effect of GABA was on the kinetics of the responses; the rate of hyperpolarization during the "ON" phase was substantially reduced while the depolarization during the "OFF" phase was virtually unaffected. After returning to "normal" solution, the cell recovered to its pre-exposure condition at a very slow rate suggesting that 5 mM GABA provided an exposure well above threshold. All 21 L-type horizontal cells studied during superfusion with 5 mM GABA exhibited effects similar to those shown in Fig. 1A but to a variable extent: the degree of depolarization varied from 0–15 mV. However, in every cell, GABA reduced the amplitude of the photoresponse and produced a characteristic slowing of its kinetics.

Figure 1B shows the effects of 5 mM GABA on a C-type horizontal cell. The chromatic properties of the cell were monitored by applying alternatingly a pair of 514-nm stimuli, which elicited hyperpolarizing responses and a pair of 694-nm stimuli which evoked depolarizing responses. About 30 s after the superfusion with GABA solution was initiated (the time required for the test solution to reach the retina), a depolarizing wave of about 20 mV was observed. During this phase of the GABA effect, the depolarizing response to red-light stimulation reversed in polarity and became a hyperpolarizing one. These GABA effects were transient; despite continual exposure to the drug, the cell hyperpolarized to a level which was slightly (about 5 mV) more negative than the resting potential measured under control conditions and color opponency recovered. The responses during this phase were smaller in amplitude compared to those recorded under control conditions and exhibited slower kinetics. GABA always produced a transient depolarization in C-type horizontal cells ($N = 7$), but its effect on color opponency varied. In four of the seven cells studied, color opponency was maintained in the presence of GABA, although the amplitude of the responses to any wavelength was reduced. In the other three cells, hyperpolarizing responses were recorded to light stimuli of all wavelengths.

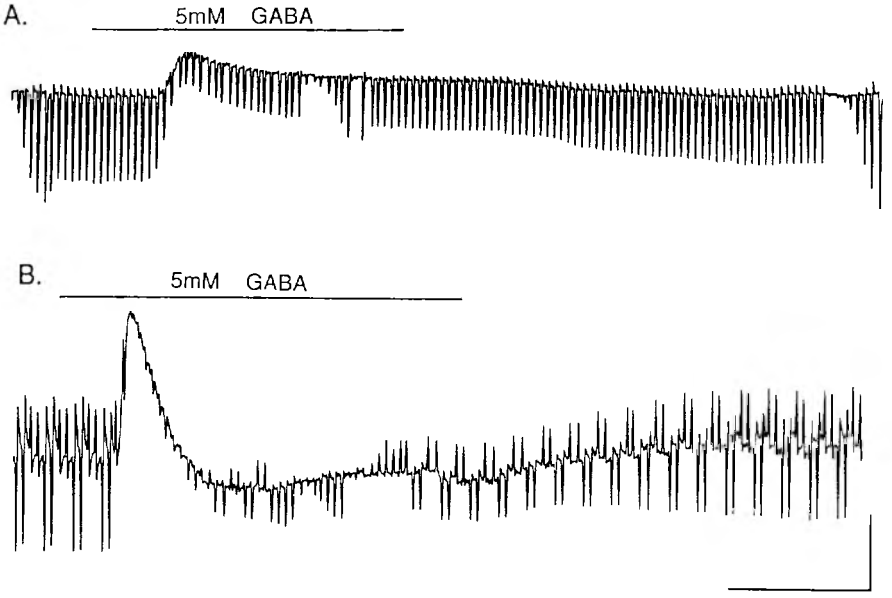


**Fig. 1.** The effects of 5 mM GABA on an L-type horizontal cell (A) and a C-type horizontal cell (B) in the inverted isolated turtle retina. The photoresponses of the L-type horizontal cell were elicited with bright red (633 nm) light stimuli. The C-type cell was stimulated alternatingly with two red (694 nm) stimuli and two green (514 nm) ones which elicit photoresponses of opposite polarity (upward and downward deflections, respectively). To examine the entire dynamic range of the cell, intensity–response series were recorded periodically throughout the experiment using red- or green-light stimuli. Calibration bars: vertical = 20 mV (for A) and 10 mV (for B); horizontal = 1 min.

The effects of GABA on the horizontal cells in the turtle isolated retina (Fig. 1) are difficult to reconcile with its single action in the horizontal cell to cone feedback pathway. It is possible that GABA exerts additional effects, unrelated to synaptic transmission. We, therefore, examined the action of GABA agonists and antagonists on the L-type horizontal cells. These drugs should be less affected by cellular uptake mechanisms and therefore, they should have a more specific action on postsynaptic receptors. Figure 2 shows the effects of 0.1 mM muscimol (upper trace), 1 mM GABA (middle trace), and 0.1 mM bicuculline (lower trace) on an L-type horizontal cell. The data in Fig. 2 represent a continuous recording from a single cell but were separated into three parts for clarity. For each drug, intensity–response series were recorded before, during, and after its application. Figure 3 shows these responses on a faster time scale in order to allow a more accurate examination of the effects of the different drugs on the kinetics of the photoresponses. Muscimol, a specific $GABA_a$ agonist, produced a slight hyperpolarization (about 8 mV) of the horizontal cell and a reduction in the response amplitude (Fig. 2, upper trace). The responses shown in Fig. 3B are of lower amplitude but exhibit normal kinetics as compared to those recorded under "normal" superfusion (Fig. 3A). GABA depolarized the horizontal cell by about 10 mV and produced a significant reduction in the amplitude of the photoresponses (Fig. 2, middle trace). As already discussed, GABA also induced a profound slowing down of the "ON" phase of the photoresponses as shown in Fig. 3C. The $GABA_a$, antagonist, bicuculline, depolarized the horizontal cell by about 10 mV and augmented its photoresponses (Fig. 2, lower trace). The photoresponses recorded during bicuculline application exhibited normal kinetics and sometimes even slight speeding up of the "ON" phase especially in the responses evoked by bright stimuli (Fig. 3D).

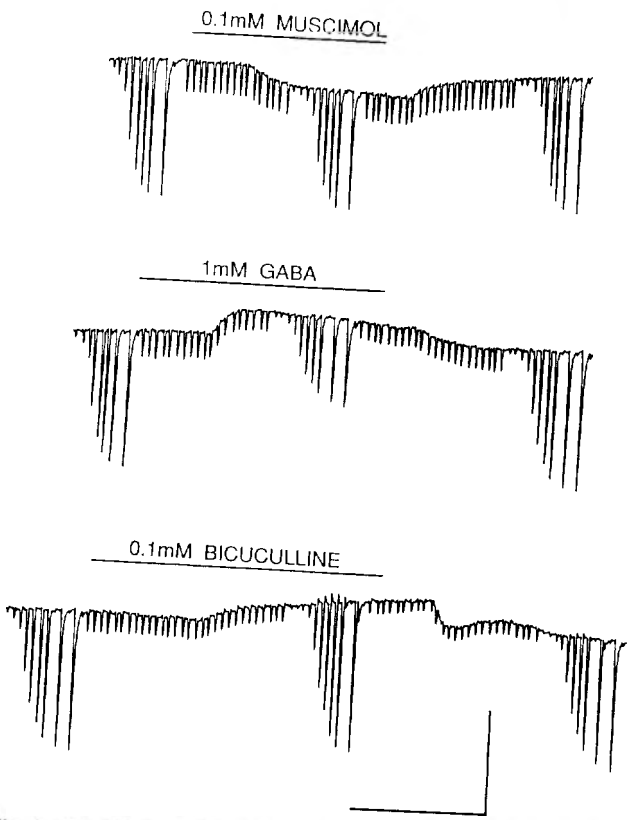


**Fig. 2.** The effects of 0.1 mM muscimol (upper trace), 1 mM GABA (middle trace), and 0.1 mM bicuculline (lower trace) on an L-type horizontal cell in the isolated turtle retina. All three traces describe a continuous record from one cell which were separated for clarity. Before, during, and after the application of each test drug, intensity–response series were measured to examine the entire dynamic range of the cell. Calibration bars: vertical = 30 mV; horizontal = 1 min.

GABA and muscimol induced different effects on L-type horizontal cells (Figs. 2 and 3). The effect of bicuculline was opposite to that of muscimol but not to that of GABA. It is clear from these figures that the action of GABA on L-type horizontal cells is not mediated exclusively by $GABA_a$-type receptors. An additional pathway must exist in the turtle retina to account for the effects observed. This additional pathway may be mediated by $GABA_b$-type receptors. In order to examine this possibility, we superfused the retina with 1 mM baclofen solution. Figure 4 shows the effects of 1 mM GABA and 1 mM baclofen on an L-type horizontal cell. Although GABA exerted its typical effect, baclofen, a specific $GABA_b$ agonist had no measurable effects on the cell. Thus, the difference between GABA action and that of muscimol cannot reflect the existence of both $GABA_a$- and $GABA_b$-type receptors.

Muscimol and baclofen are potent agonists to GABA receptors but do not participate in the GABA-uptake systems. Retinal cells, including horizontal cells, contain extensive mechanisms for GABA uptake (Yazulla, 1986). These mechanisms may interfere with electrophysiological studies of the pharmacological properties of retinal neurons especially if they are electrogenic. In order to examine the role of GABA uptake in horizontal cell physiology, we superfused the isolated retina with a solution containing 10 mM nipecotic acid, a competitive inhibitor of GABA uptake (Johnston et al., 1976; Lam & Ayoub, 1983). A variety of effects were observed. In some cells ($N = 10$), nipecotic acid produced effects similar to those seen with muscimol; hyperpolarization to varying degrees accompanied by reduction in the amplitude of the photoresponses. In other cells ($N = 3$), a slight depolarization and response augmentation were seen during exposure to nipecotic acid. These effects were similar to those seen with bicuculline (Fig. 2, lower trace). The diversity of the nipecotic-acid effects in the isolated retina probably reflects multiple GABA-uptake sites in the outer plexiform layer. Exogenous application of nipecotic acid is expected to activate all these uptake systems and also to modulate the local concentration of endogenously released GABA. The direct action of GABA on horizontal cells and the contribution of electrogenic-uptake mechanisms to horizontal cell's membrane potential can be better assessed by blocking synaptic transmission to the horizontal cells.

### *Blocked synaptic transmission*

To study the direct action of GABA on horizontal cells, we minimized synaptic input to the cells by superfusing the retina with a solution containing either kynurenic acid or cobalt chloride. Kynurenic acid, a glutamate antagonist, blocks the excitatory input from the cones to the horizontal cells by binding to the postsynaptic receptor sites (Coleman et al., 1986). Cobalt ions act on presynaptic terminals to inhibit the release of neurotransmitters. Figure 5 shows the effects of GABA on L-type horizontal cells, when it is delivered during superfusion with a solution containing 1 mM cobalt chloride (A) or during expo-

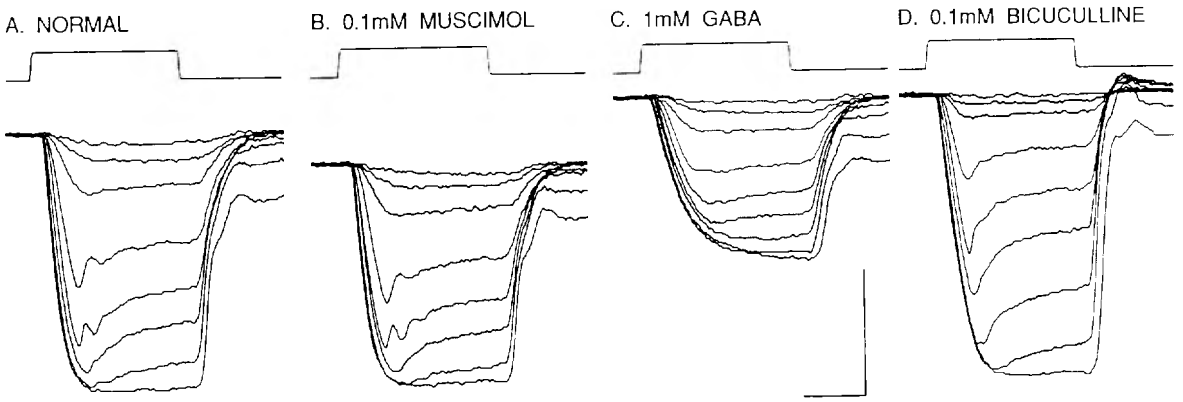


**Fig. 3.** The photoresponses recorded during the intensity–response series of Fig. 2 are shown on a faster time scale to emphasize the effects of the different drugs on response kinetics. The light stimuli used to elicit each set of photoresponses were of 500-ms duration and their intensity was incremented by approximately 0.5 log units steps between successive stimuli. The photoresponses were traced with d.c. fidelity, namely, relative to the dark-resting potential recorded under control (superfusion with "normal" solution) conditions. Calibration bars: vertical = 20 mV; horizontal = 200 ms.

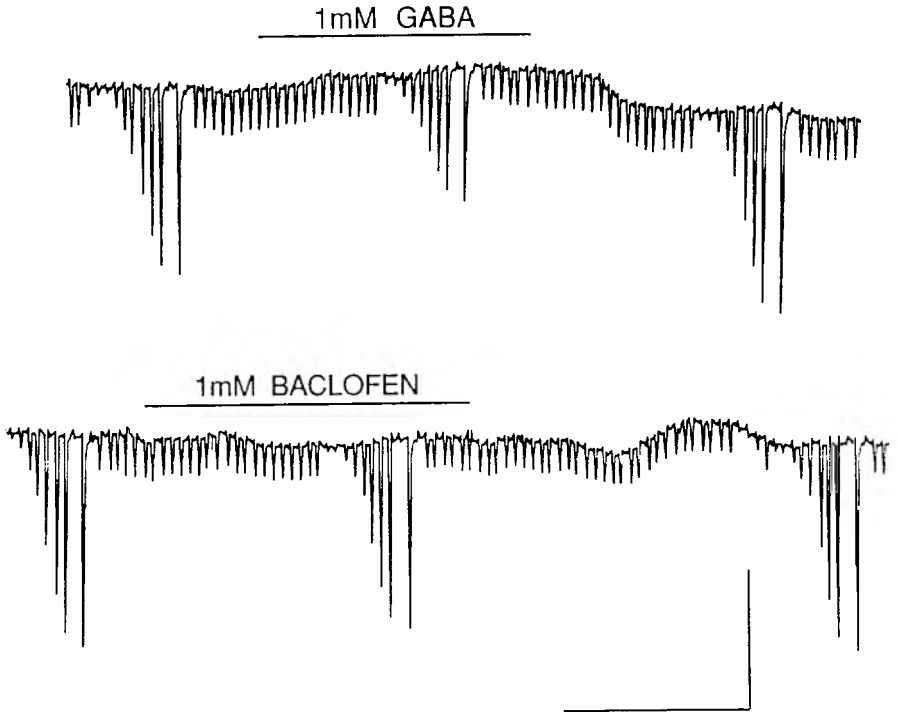


**Fig. 4.** The effects of 1 mM GABA (upper trace) and 1 mM baclofen (lower trace) on an L-type horizontal cell in the isolated turtle retina. While GABA slightly depolarized the cell, reduced the amplitude of the photoresponses, and slowed down the response kinetics, baclofen had virtually no effect on the physiology of the horizontal cell. Calibration bars: vertical = 30 mV; horizontal = 1 min.

sure to 0.5 mM kynurenic-acid solution (B). Cobalt ions and kynurenic acid induced similar effects on L-type horizontal cells; they hyperpolarized the cells and reduced the amplitude of their photoresponses. The cobalt solution used in this study was more potent than the kynurenic-acid solution as reflected in the virtually complete elimination of light-evoked responses in the presence of cobalt. During kynurenic-acid superfusion, measurable photoresponses could be elicited with bright stimuli but these manifested a pronounced overshoot at stimulus offset which was followed by a very slow recovery to the dark-resting potential. Since cobalt ions proved to be toxic to the isolated turtle retina and recovery from their effects was usually incomplete, we mostly used kynurenic acid to reduce the input from cones to horizontal cells. When GABA was added to the superfusion solution after "steady-state" effects with either kynurenic acid or cobalt ions had been achieved, a depolarization was observed accompanied by an increase in the amplitude of the photoresponses (Fig. 5A and 5B). These effects of GABA

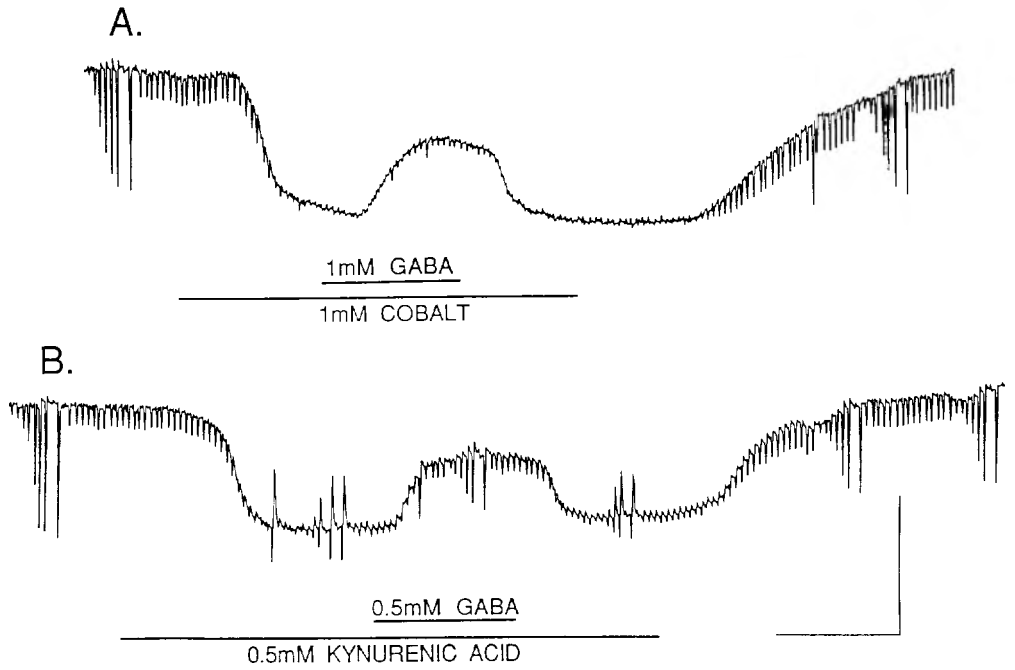


**Fig. 5.** The effects of GABA on L-type horizontal cells in the isolated turtle retina after blocking synaptic input to the cell by either 1 mM cobalt chloride (A) or 0.5 mM kynurenic acid (B). Both drugs produced hyperpolarization of the horizontal cell and substantial reduction of photoresponse amplitude indicating a significant reduction in the cones input to the cell. GABA, applied after blocking the synaptic input to the horizontal cell, depolarized the cell and augmented the remaining photoresponses. Calibration bars: vertical = 30 mV; horizontal = 1 min.

could reflect a simple binding between GABA and the kynurenic acid or cobalt ions which would reduce the effective concentration of the blocking agent. However, two lines of evidence argue against this notion. The rate of depolarization seen when GABA was added was usually faster than that seen after switching the superfusion back to "normal" solution. Furthermore, the growth in the response amplitude did not balance the degree of depolarization; namely, the absolute potential level achieved in response to a very bright light stimulus was reduced. During the return to "normal" superfusion, the growth in response amplitude roughly paralleled the degree of depolarization. The findings shown in Fig. 5 indicate a possible direct action of GABA on L-type horizontal cells in the turtle retina.

In order to identify whether this direct effect of GABA on horizontal cells was mediated by $GABA_a$ or $GABA_b$ receptors, we conducted experiments similar to that shown in Fig. 5 except that muscimol or baclofen were added to the kynurenic-acid solution. The results of such an experiment are shown in Fig. 6. Superfusion with 0.5 mM kynurenic acid solution produced a fast hyperpolarization and almost complete elimination of the photoresponse. Adding either 1 mM baclofen (A) or 0.1 mM muscimol (B) to the superfusate did not produce a significant change in horizontal cell membrane potential or light-evoked responses. It should be noted that we also used 0.5 mM muscimol but no change in the kynurenic-acid effects was observed. These observations suggest than the direct effect of GABA on the horizontal cells was not mediated by either $GABA_a$ or $GABA_b$ receptors.

Figure 7 shows the effects of 1 mM GABA and of 5 mM nipecotic acid on an L-type horizontal cell which has been almost completely isolated from its photoreceptors' input by 1 mM kynurenic-acid solution. Both drugs produced qualitatively similar effects; a slight depolarization (about 8 mV) and augmentation of the photoresponses. It should be noted that the effects of GABA and nipecotic acid under these conditions vary in the time course of development of the effect, while GABA induced a gradual depolarization which reached a steady state. The effect of nipecotic acid developed faster but was more transient in nature. Results, similar to those shown in Fig. 7, have been obtained in five L-type horizontal cells. Thus, nipecotic acid, which is a substrate of GABA-uptake system, may mimic the GABA effects on turtle L-type horizontal cells.

## Discussion

GABA is considered to play a dual role in the outer plexiform layer of the vertebrate retina. It indirectly affects the degree of cellular coupling in the horizontal cell network (Piccolino et al., 1982; Normann et al., 1988) by modifying the activity of dopaminergic neurons in the inner retinal layers (Piccolino et al., 1987), and it has been proposed to mediate the negative-feedback pathway from the L-type horizontal cells back onto the red and green cones (Lam et al., 1978; Marc et al., 1978; Tachibana & Kaneko, 1984; Kaneko & Tachibana, 1986). The goal of this study was to test the role of GABA as the inhibitory horizontal cell to cone neurotransmitter in the turtle retina. Therefore, full-field light stimuli which produced diffuse retinal illumination were used to monitor the action of GABA and its analogues on the horizontal cells. With this mode of photostimulation, changes in cellular coupling, induced by the test drugs, have negligible effects on horizontal cell physiology

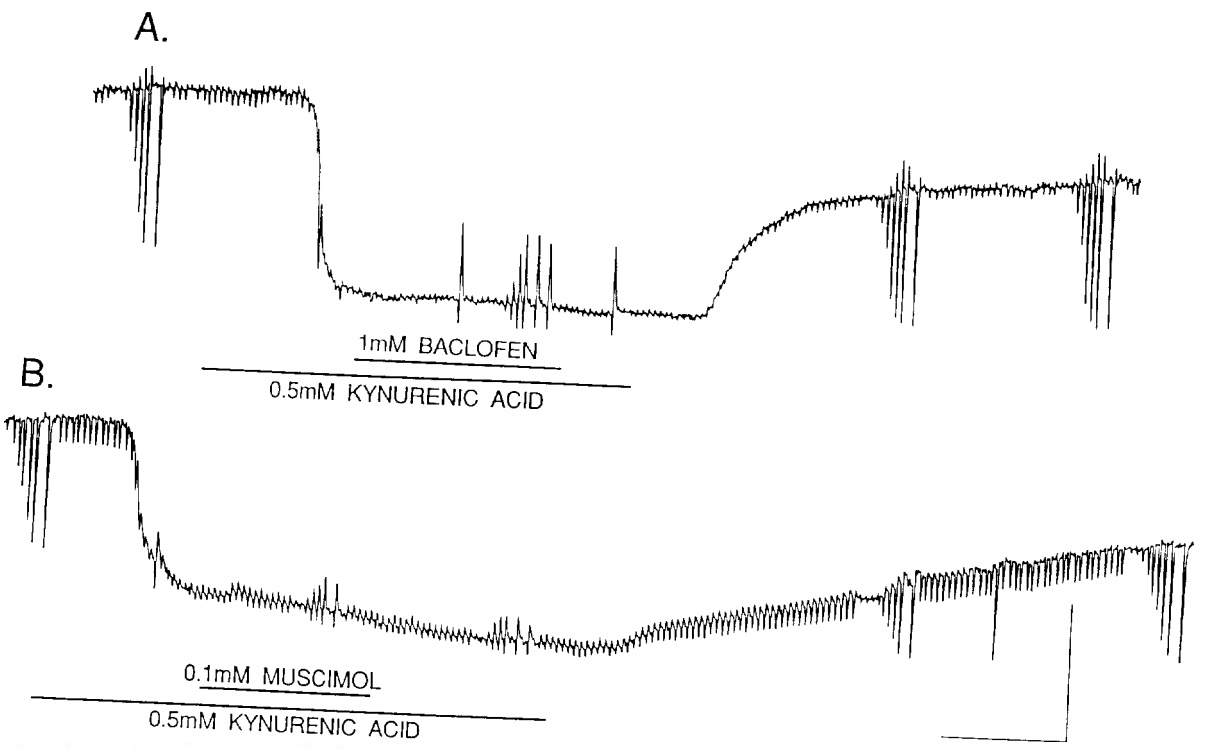


**Fig. 6.** The effects of 1 mM baclofen (upper trace) and 0.1 mM muscimol (lower trace) on L-type horizontal cells in the isolated turtle retina after synaptic input from the cones had been minimized by 0.5 mM kynurenic acid. Neither of these GABA agonists induced any effect on the horizontal cells under these conditions. Calibration bars: vertical = 30 mV; horizontal = 1 min.

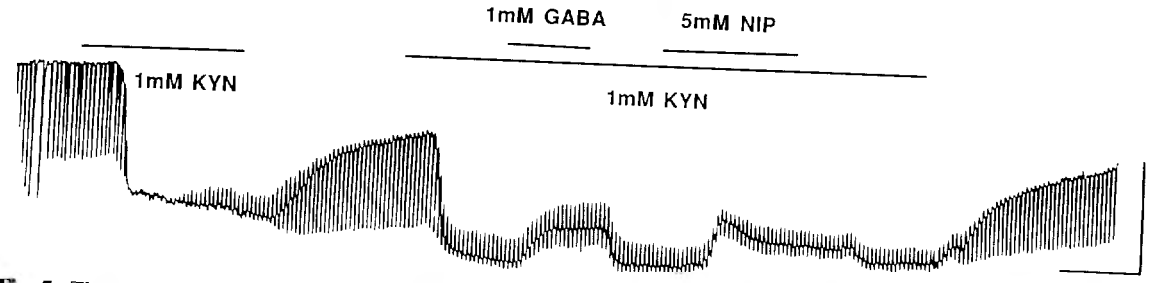


**Fig. 7.** The effects of 5 mM nipecotic acid and 1 mM GABA on an L-type horizontal cell in the isolated turtle retina. The experimental drugs have been applied to the retina after synaptic input from the photoreceptors to the horizontal cells had been reduced by superfusion with 1 mM kynurenic acid. In the experiment illustrated in this figure, the first application of 1 mM kynurenic acid was followed by almost complete recovery under superfusion with "normal" Ringer solution. The experimental drugs, GABA and nipecotic acid, were applied only during the second exposure to kynurenic acid. Calibration bars: vertical = 30 mV; horizontal = 1 min.

and the role of the drugs in synaptic transmission can be explored.

Voltage-clamp studies on turtle cones in culture have demonstrated that GABA opens chloride channels in the cone plasma membrane (Tachibana & Kaneko, 1984; Kaneko & Tachibana, 1986). Since the chloride equilibrium potential has been reported to be more hyperpolarized than the cone resting potential (Kaneko & Tachibana, 1986), GABA is expected to induce cone hyperpolarization and decrease the photoresponse amplitude. According to the cone-horizontal cell cascade network (Fuortes & Simon, 1974), similar effects are expected to be seen in the L-type horizontal cells. The effects of GABA on C-type horizontal cells should reflect the blockage of the long wavelength input to the green cones via the negative-feedback pathway. Accordingly, the C-type horizontal cells are expected to hyperpolarize and to loose their color opponency. The data presented here are in accord with some of these predictions but are at variance with others.

### *Intact synaptic transmission*

GABA produced a slight depolarization in L-type horizontal cells, reduced the amplitude of the photoresponses, and slowed down the "ON" phase of the photoresponses (Figs. 1–3). These GABA effects are similar to those reported for the mudpuppy (Miller et al., 1981), skate (Cohen, 1988), and salamander ret-

inas (Yang & Wu, 1989) but differ from those described in fish retina (Djamgoz & Ruddock, 1979; Negishi & Drujan, 1979; Wu & Dowling, 1980; Murakami et al., 1982*a*,*b*).

We have tested the possibility that the complex GABA effect on turtle L-type horizontal cells reflects the combined action of multiple pathways involving different types of GABA receptors. This goal was achieved by exposing the retina to specific GABA agonists and antagonists. Muscimol, a $GABA_a$ agonist, produced effects in L-type horizontal cells which differed from those exerted by GABA but satisfied the predictions for the inhibitory feedback transmitter. Bicuculline, a $GABA_a$ antagonist, exerted effects opposite to those seen with muscimol (Figs. 2 and 3). Baclofen, a $GABA_b$ agonist, produced no apparent action on turtle L-type horizontal cells (Fig. 4). These observations are compatible with the hypothesis that the observed effect of GABA on turtle L-type horizontal cells reflects the summed action of at least two pathways. An indirect effect is transmitted from the red cones and probably involves the activation of $GABA_a$-type receptors. Muscimol activates only this pathway and bicuculline inhibits it. The second GABA action does not involve either $GABA_a$- or $GABA_b$-type receptors.

The recordings from C-type horizontal cells are also difficult to reconcile with the view that the main role of GABA in the outer plexiform layer of the turtle retina is to mediate the negative-feedback pathway. The transient depolarization and the failure to eliminate color opponency in four out of the seven cells studied raises questions regarding the genesis of color opponency (Fig. 1B). These considerations, in concert with the differences in the action spectra of C-type horizontal cells (Fuortes & Simon, 1974; Kato, 1979; Burkhardt & Hassin, 1983; Gottesman & Burkhardt, 1987) and green cones (Baylor & Hodgkin, 1973; Burkhardt, 1977; Burkhardt & Hassin, 1983; Kaneko & Tachibana, 1985), raise the possibility that color opponency in C-type horizontal cells in the turtle retina may have contributions from two mechanisms; a negative-feedback pathway from the L-type horizontal cells onto the green cones and an inhibitory feedforward input from red cones to the C-type cells.

### *Blocked synaptic transmission*

In order to separate a direct action of GABA on horizontal cells from an indirect one, mediated by the red cones, we added kynurenic acid or cobalt ions to the superfusion media. The modes of action of these drugs differ. Although kynurenic acid interacts with the glutamate receptors on the postsynaptic membrane (Coleman et al., 1986), cobalt ions reduce transmitter release from the presynaptic terminals. The net effect of either of these drugs is to isolate the horizontal cells from the cones. This effect is expressed in horizontal cell hyperpolarization and elimination of its photoresponses (Figs. 5 and 6). GABA, applied during exposure to kynurenic acid or to cobalt ions, depolarized the L-type horizontal cells and slightly augmented their photoresponses (Fig. 5). Similar effects of GABA in the presence of cobalt ions have been observed in retinal horizontal cells of the fish (Hankins & Ruddock, 1984) and Xenopus (Witkovsky & Stone, 1987) but not of the mudpuppy (Miller et al., 1981). The effects of GABA on L-type horizontal cells that had been isolated pharmacologically from their synaptic input support the view that this drug exerts a direct action on these cells. This action of GABA could not be replicated by either muscimol or baclofen (Fig. 6). Thus, it does not involve either of the classical GABA receptors ($GABA_a$ and $GABA_b$).

Nipecotic acid, a competitive inhibitor of GABA-uptake systems (Johnston et al, 1976; Lam & Ayoub, 1983), induced effects on L-type horizontal cells, similar to those exerted by GABA (Fig. 7). These data suggest that electrogenic GABA-uptake mechanisms in the horizontal cells' membrane may contribute to the effect of exogenously applied GABA on turtle L-type horizontal cells. The differences in the time course of development of the GABA's and nipecotic acid's effects may reflect differences in the concentrations used, in the affinity between the drugs and the uptake system and the possible contributions of GABA as a neurotransmitter in the retina. Participation of electrogenic GABA-uptake system in determining the horizontal cells membrane potential has been demonstrated in the carp retina (Kamermans, 1989) and in horizontal cells isolated from the skate retina (Malchow & Ripps, 1990).

The electrogenity of the GABA-uptake mechanisms should be considered when the cone-horizontal cell network is studied. In darkness, when GABA is continuously released from the relatively depolarized horizontal cells, the GABA-uptake system is fully activated and will, therefore, further depolarize the horizontal cells. When light is applied, the horizontal cells hyperpolarize, GABA release is reduced, and therefore the GABA-uptake system will be activated to a lesser extent and will further hyperpolarize the cells This analysis indicates that the GABA-uptake system in the turtle L-type horizontal cells forms a positive feedback mechanism which contributes to the membrane potential and may speed up potential changes evoked by changes in retinal illumination.

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