GABA Transporters and GABA_C-like Receptors on Catfish Cone- but Not Rod-driven Horizontal Cells

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The effects of GABA and related agents were studied in solitary rod- and cone-driven horizontal cells, acutely isolated from the catfish retina using enzymatic and mechanical treatment. Both types of horizontal cells, which normally receive glutamatergic input from photoreceptors, responded to pressure ejection of the glutamate analog kainate (50 μ M) with an inward current of 300–800 pA when voltage-clamped at -70 mV using the whole-cell patch-clamp technique. But pressure ejection of GABA (500 μ M) elicited an inward current only in cone-driven horizontal cells. This current, ranging between 100 and 400 pA, consisted of two components: (1) a GABA receptor-gated chloride current that reversed near the chloride equilibrium potential and was blocked by bath application of picrotoxin (100–500 μ M), and (2) a GABA transporter-mediated current that was picrotoxin resistant but was blocked by NO-711 (1 μ M) and *cis*-4-hydroxynipetotic acid (250 μ M), two potent GABA transporter blockers. The GABA transporter current could also be eliminated when sodium was replaced by either choline or lithium in the bathing medium. The picrotoxin-sensitive receptor-gated current could not be elicited by the GABA_B receptor agonist baclofen, nor could it be blocked by the potent GABA_B receptor antagonist 2-hydroxysaclofen. The picrotoxin-sensitive current could be divided into two components based on their sensitivity to the specific GABA_A receptor antagonist bicuculline methiodide. The bicuculline-sensitive component was found only in some cells, whereas the bicuculline-resistant, picrotoxin-sensitive component was found in all cells tested. The bicuculline-resistant current was insensitive to pentobarbital, an allosteric modulator of GABA_A receptor. To confirm the effectiveness of the specific batch of bicuculline methiodide and pentobarbital, we tested both drugs in ganglion cells in the salamander retinal slice preparation, where the GABA-elicited current is almost exclusively mediated by GABA_A receptors. Bicuculline methiodide almost completely blocked, while pentobarbital significantly enhanced, the GABA current recorded in ganglion cells. Thus, in catfish cone horizontal cells the bicuculline-resistant GABA

receptor current is most likely mediated by the GABA_C receptor based on the above pharmacological profile. The relative effectiveness of GABA, muscimol, *trans*- and *cis*-4-aminocrotonic acid (TACA and CACA) was determined at this GABA_C receptor site after cells were bathed in choline Ringer to eliminate the transporter current and in the presence of 100 μ M bicuculline methiodide to block GABA_A receptor current. The order of effectiveness was GABA > TACA > muscimol >> CACA. Our results demonstrate the presence of GABA transporters as well as GABA_A and GABA_C receptors in catfish cone- but not rod-driven horizontal cells. This suggests that the GABA-mediated autodeedback and chemical coupling described recently in an amphibian retina may also exist in the catfish cone horizontal cell network.

[Key words: GABA, GABA_C receptor, GABA transporter, horizontal cell, retina, catfish, neurotransmission]

GABA is a major inhibitory neurotransmitter in the vertebrate CNS. Its action is believed to be mediated by a bicuculline-sensitive GABA_A receptor, which gates Cl[−] channels and a bicuculline-resistant GABA_B receptor, which modulates potassium and calcium channels (Bormann, 1988; Sieghart, 1992). There is now increasing evidence that there exists a third type of GABA receptor, namely GABA_C receptor, which gates a Cl[−] conductance but is not sensitive to conventional GABA_A receptor antagonists or modulators, such as bicuculline and pentobarbital (Johnston, 1986; Feigenspan et al., 1993; Lukasiewicz et al., 1994; Qian and Dowling, 1993).

GABA is also used as a neurotransmitter in the luminosity-type (L-type) cone horizontal cells that mediate lateral interactions in the outer retina. L-type horizontal cells play an important role in the formation of the antagonistic receptive field surround in cone photoreceptors and bipolar cells through the feedback and feedforward synapses, respectively (Baylor et al., 1971; O'Bryan, 1973; Yang and Wu, 1991). There is strong evidence suggesting that those synapses are GABAergic. For example, GABA is synthesized in L-type horizontal cells (Lam, 1975; Lam et al., 1979) and can be released when these cells are depolarized (Schwartz, 1982; Ayoub and Lam, 1984; Schwartz, 1987). Furthermore, the strength of the antagonistic surround in the cone photoreceptor and bipolar cell is greatly altered in the presence of GABA or GABA receptor antagonists (Murakami et al., 1982; Wu, 1986, 1991). Autoradiographic and electrophysiological studies have demonstrated that GABA is released from and taken up into horizontal cells mainly via a calcium-independent, electrogenic transporter (Schwartz, 1982, 1987; Yazulla and Kleinschmidt, 1983; Ayoub and Lam, 1984).

To understand further the mechanisms of GABAergic trans-

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Figure 1. Morphology of acutely dissociated catfish rod and cone horizontal cells. *Top*, A rod horizontal cell. *Bottom*, A cone horizontal cell. Scale bars, 50 μ m.

mission in the outer retina, we studied and characterized the effects of GABA and related compounds in solitary rod- and cone-driven horizontal cells, acutely isolated from the catfish retina.

Materials and Methods

Cell dissociation. Solitary rod and cone horizontal cells acutely dissociated from the catfish (*Ictalurus punctatus*) retina were used in the present study to avoid possible changes in receptor subtypes and distribution that could occur during the culture period. Cells were obtained through enzymatic treatment of the retinas modified from the procedures described elsewhere (Tachibana, 1981; Linn and Christensen, 1992). Briefly, a catfish was decapitated and pithed. Both eyes were enucleated and excised. The excessive vitreous fluid was carefully removed using filter paper. The eyecups were then cut into four pieces and incubated for 20 min in papain solution containing (in mM) 126

NaCl, 4 KCl, 1 MgCl₂, 15 glucose, 10 HEPES, 5 EGTA, and 5 L-cysteine to which was added 10 U/ml papain (Worthington Biochemical Corp., Freehold, NJ). The pH of the solution was adjusted to 7.4 with NaOH. Afterward, the retina pieces were isolated from the epithelium layer and placed into the fresh papain solution for another 20 min. Following this procedure, the retina pieces were rinsed five times with the normal catfish bathing medium (see below) and kept for up to 6 hr at 4°C until used in the normal bathing medium containing 1 mg/ml bovine serum albumin (Sigma, St. Louis, MO). Cells used for electrical recordings were freshly dissociated from the stored retina pieces by mechanical trituration with a fire-polished glass pipette immediately before recording (Linn and Christensen, 1992).

Morphological and electrophysiological identification. Both cone and rod horizontal cells retained their characteristic morphology (Naka and Carraway, 1975) after the dissociation procedures and could be identified easily under light microscopy. Rod horizontal cells were larger in size with broad, radiating branches and lacked an axon (Fig. 1, top), whereas cone horizontal cells were stellate and had an axon (Fig. 1,

Figure 2. Differences in electrophysiological properties between rod (left) and cone (right) horizontal cells. The upper portions show current-voltage relations of the sustained whole-cell current. The lower portions show the sample current traces recorded when the membrane voltages were stepped from a holding potential of -70 mV to 0 mV and after the leak currents were subtracted. Signals were low-pass filtered at 3000 Hz. Most of the capacitive current was erased for clarity.

bottom). Rod and cone horizontal cells also have strikingly different electrical properties, as illustrated in Figure 2. The upper part of the figure shows the current-voltage (I - V) relations of the sustained whole-cell current obtained from a rod and a cone horizontal cell. The lower part shows two sample current traces recorded when the membrane voltages were stepped from a holding potential of -70 mV to 0 mV and after the leak currents were subtracted. The cone horizontal cell had three prominent voltage-dependent currents: a sustained inward-rectifying current, an inward current activated near -40 mV (see the I - V curve), and a large transient current when the membrane voltage was stepped from -70 mV to 0 mV (see the sample current recording). Previous work has shown that these currents are voltage-dependent K^+ , Ca^{2+} , and Na^+ currents, respectively (Shingai and Christensen, 1986; Schwartz, 1987). On the other hand, rod horizontal cell shows only a small voltage-dependent Ca^{2+} current, which became visible only after leak current subtraction.

Solutions and drug application. Normal bathing solution contained (in mM) 126 NaCl, 3 KCl, 3 $CaCl_2$, 1 $MgCl_2$, 10 HEPES, 10 glucose, with pH adjusted to 7.4 with NaOH. Pharmacological agents (concentrations specified in the text and figure captions) were applied to horizontal cells either by bath application or by pressure ejection (puffing) through a glass pipette. When bath applied, the drugs were added to the normal bathing solution without substitution for NaCl since their concentrations were less than 1 mM. The puffing pipette had a tip diameter of about 1 μ m and was positioned within 50 μ m of the patched cell. The bathing media containing different drugs were driven by gravity and delivered to the recording chamber through a six-way valve at a rate of about 4 ml/min. In addition to the whole-chamber bath perfusion, drugs were also applied to the cells with a local perfusion system in some experiments. Fine polyethylene tubing from seven reservoirs converged to a plastic gel-filling pipette tip (400 μ m i.d.), which was placed about 1 – 2 mm away from the patched cell. The timing of the drug application through the local perfusion system was controlled by an IBM-compatible personal computer via solenoid valves. The flow

rate of local perfusion was 1 – 2 ml/min. The pipette solution used in most whole-cell recordings contained (in mM) 130 KCl, 0.5 $CaCl_2$, 5 EGTA, 10 HEPES, 4 ATP- $2Na^+$, and 0.5 GTP- Mg^{2+} adjusted to pH 7.2 with KOH. Exceptions are noted in the figure captions. *Trans*- and *cis*-4-aminocrotonic acid (TACA and CACA) were purchased from Tocris Neuramin (Bristol, UK); 2-hydroxysaclofen, from Research Biochemicals Inc. (Natick, MA); and GABA, muscimol, bicuculline methiodide, picrotoxin, and baclofen, from Sigma (St. Louis, MO).

Electrophysiological recording and data analysis. Whole-cell patch recordings (Hamill et al., 1981) were performed using a List EPC-7 (Medical Systems Corp., Greenvale, NY) patch-clamp amplifier. Patch electrodes were fabricated from borosilicate filament glass (TW-150F-4, World Precision Instruments Inc., New Haven, CT) with a Flaming-Brown horizontal puller (model P-87, Sutter Instruments, Novato, CA). The resistance of the patch electrodes was between 5 and 10 M Ω in the normal bathing medium when filled with the pipette solution whose composition is given above. Patch electrodes were used without fire polish or coating. The electrode series resistance ranged from 12 to 20 M Ω and was not compensated. Membrane potentials were corrected for liquid junction potential as described by Fenwick et al. (1982).

The software used to generate voltage commands, acquire data, trigger the solenoid for the drug application puffer and the local perfusion system, and analyze data was developed in this lab. The responses and stimuli were digitized by a Data Translation DT2828 analog-to-digital interface and recorded on an IBM-compatible PC. The GABA-elicited whole-cell currents were low-pass filtered at 60 Hz unless otherwise indicated to improve the signal:noise ratio. Statistical data are expressed in the text as mean $\pm$ SEM unless otherwise noted.

Results

GABA affects cone but not rod horizontal cells

In the intact retina, horizontal cells receive glutamatergic inputs from photoreceptors (Cervetto and MacNichol, 1972; Ayoub

Figure 3. Effect of GABA and kainate (KA) on rod (left) and cone (right) horizontal cells. The upward deflections of the horizontal lines above the response traces indicate the timing of a 500 msec puff of normal bathing medium containing 500 μ M GABA or 50 μ M kainate (KA) applied through a glass pipette, which had an inner tip diameter of approximately 1 μ m and was placed within 50 μ m of the patched cell. All current traces represent the average of three recordings. In this and all following figures, GABA-elicited currents were measured with the cells voltage clamped at -70 mV except where indicated in the figure captions.

and Copenhagen, 1991). In order to test the viability of horizontal cells after the dissociation process, the glutamate receptor agonist kainate (KA; 50 μ M) was pressure ejected to rod and cone horizontal cells through a glass pipette (~ 1 μ m tip diameter) placed within 50 μ m of the patched cell. Figure 3 shows the effect of KA and GABA on a rod and a cone horizontal cell. Both cells were voltage clamped at -70 mV. KA elicited an inward current in both cell types, as shown in the upper traces of Figure 3. This KA-induced current reversed between 0 to 10 mV and could be blocked by 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX), non-NMDA glutamate receptor antagonist (data not shown), suggesting that it was mediated by the conventional KA type of glutamate receptors on both rod and cone horizontal cell membranes.

Pressure ejection of 500 μ M GABA through a glass pipette elicited an inward current in cone but not rod horizontal cells, as shown in the lower traces of Figure 3. GABA had no effect in all eight rod horizontal cells tested, whereas it always produced an inward current ranging from 100 to 400 pA in cone horizontal cells ($n > 50$) when the recording electrodes were filled with a nearly symmetrical Cl^- intracellular solution whose composition is given in Materials and Methods. The experiments illustrated in Figure 3 demonstrate that only cone, not rod, horizontal cells respond to GABA in the catfish retina. Rod horizontal cells are probably insensitive to GABA because they do not have GABA receptors (see Discussion).

GABA activates both receptors and transporters in cone horizontal cells

Figure 4 shows that the GABA-elicited current in cone horizontal cells can be divided into receptor- and transporter-mediated components. In the cell shown in Figure 4A, bath application of 500 μ M picrotoxin (PTX), a GABA-gated chloride channel blocker, reduced the GABA-elicited current to 41% of the control value. In 16 cells tested the average reduction by

picrotoxin was $48 \pm 2\%$. The remaining picrotoxin-resistant current was almost completely blocked by the addition to the bathing medium of 1 μ M NO-711 ($n = 5$), or 250 μ M *cis*-4-hydroxyneipicotic acid ($n = 3$; data not shown), two potent GABA-transporter blockers (Larsson et al., 1981; Suzdak et al., 1992), suggesting that the picrotoxin-resistant current was mediated by a GABA transporter.

However, it has been reported that in rat retinal bipolar cells the GABA-gated chloride current is not very sensitive to the picrotoxin blockade (Feigenspan et al., 1993). To demonstrate unequivocally that the picrotoxin-resistant current was truly mediated by GABA transporters, two additional lines of experiment were carried out. First, since it is known that the activity of the GABA transporter is sodium dependent (Yazulla and Kleinschmidt, 1983; Malchow and Ripps, 1990), the picrotoxin-resistant current was measured in bathing media in which sodium was replaced by either choline or lithium. Figure 4B shows the representative recordings from a cell bathed first in the normal bathing medium and then in the choline medium. The picrotoxin-resistant current was completely and reversibly eliminated in the sodium-free medium. Similar results were obtained in four additional cells.

Second, the current-voltage ($I-V$) relations of the picrotoxin-resistant current were examined in normal bathing solution. The transporter-mediated current has a characteristic $I-V$ relation such that the current diminishes as the cell membrane is depolarized and it approaches to zero when the membrane is depolarized to potentials more positive to $+50$ mV (Malchow and Ripps, 1990). A similar $I-V$ relation was observed for the picrotoxin-resistant current in five cone horizontal cells. Current responses recorded at different holding potentials from a typical cell are illustrated in Figure 4C. Figure 4D depicts the $I-V$ relations of the responses. The findings shown in Figure 4 demonstrate that the GABA-elicited response consists of a picrotoxin-sensitive current probably mediated by GABA receptor-

Figure 4. GABA response consists of a receptor- and a transporter-mediated component. *A*, GABA response consists of a picrotoxin (PTX)-sensitive and a picrotoxin-resistant component. The picrotoxin-resistant component was blocked by the GABA transporter blocker NO-711. Three recordings from a cone horizontal cell are aligned for comparison. They were recorded first in the normal bathing medium (*control*), then in the presence of 500 μ M picrotoxin, and finally in 500 μ M picrotoxin plus 1 μ M NO-711 (*PTX + NO-711*). The recovery was 85% of control and is not shown for clarity. The upward deflection of the horizontal line above the response traces indicates the timing of a 500 msec puff of normal bathing medium containing 500 μ M GABA applied through a glass pipette, which had an inner tip diameter approximately 1 μ m and was placed within 50 μ m of the patched cell. *B*, Picrotoxin-resistant current is sodium sensitive. Three recordings from another cone horizontal cell are aligned for comparison. They were recorded first in the normal bathing medium (*control*), then in the presence of 200 μ M picrotoxin (*PTX*), and finally in a choline bathing medium in which all sodium was replaced by choline and 200 μ M picrotoxin was added to it (*PTX + ChR*). The recovery was nearly superimposed with control and is not shown for clarity. The upward deflection of the horizontal line above the response traces indicates the timing of the application of a 50 msec puff of choline bathing medium containing 500 μ M GABA. *C*, Current traces of picrotoxin-resistant current recorded in the presence of 200 μ M picrotoxin and at different holding potentials, which are indicated by the numbers (in mV) to the left of the traces. *D*, Plot of current–voltage (*I*–*V*) relations of the picrotoxin-resistant current traces illustrated in *C*.

gated channels (see below) and a picrotoxin-resistant current mediated by electrogenic GABA transporters.

Measurement of the reversal potentials of the picrotoxin-sensitive current at different intracellular chloride concentrations indicated that it was carried mainly by chloride ions. An example of this type of experiment is shown in Figure 5. The current–voltage relations were obtained by subtracting the ramp recordings in the presence of both GABA and picrotoxin from those in the presence of GABA alone. In Figure 5*A*, the recording electrode was filled with a nearly symmetrical chloride intracellular solution (see Materials and Methods) and the picrotoxin-sensitive current reversed near 0 mV, very close to the calculated E_{Cl} , which was -1 mV. The average chloride reversal potential measured in three cells under conditions similar to those in Figure 5*A* was -2.7 ± 2.0 mV. In another cell, upon partial substitution of the intracellular chloride by equimolar

gluconate the reversal potential of the picrotoxin-sensitive current was shifted to a more negative value at -35 mV near the calculated E_{Cl} (-38 mV), as shown in Figure 5*B*. The average chloride reversal potential measured in three cells under conditions similar to those in Figure 5*B* was -30 ± 2.1 mV. These observations indicate that the picrotoxin-sensitive current is mainly a chloride current, as found in other studies (Olsen, 1981; Lukasiewicz et al., 1994; Qian and Dowling, 1993).

GABA gates both bicuculline-sensitive and bicuculline-insensitive receptors in some cone horizontal cells

In some cells the picrotoxin-sensitive current could be further divided into two components based on their sensitivity to the specific GABA_A receptor antagonist bicuculline methiodide, as illustrated in Figure 6. Figure 6*A* shows that bath application of 200 μ M bicuculline methiodide (BIC) reduced the GABA-

Figure 5. Current-voltage (I - V) relations of the picrotoxin-sensitive current. Membrane potentials of horizontal cells were ramped from -70 mV to 60 mV at 0.18 V/sec in the presence and absence of $150 \mu\text{M}$ picrotoxin during a long GABA ($500 \mu\text{M}$) puff. I - V relations of picrotoxin-sensitive current were obtained by subtracting the I - V curve recorded in the presence of picrotoxin from that in the absence of picrotoxin. I - V curves in both *A* and *B* were an average of five ramps. *A*, I - V relation recorded from a cone horizontal cell with a patch pipette filled with the normal internal solution (see Materials and Methods). The calculated E_{Cl} was -1 mV and indicated by an arrowhead on the abscissa. *B*, I - V relation recorded from another cell with a patch pipette filled with a low-chloride internal solution in which 100 mM Cl^- was replaced by equimolar gluconate. The calculated E_{Cl} was -38 mV, as shown by the arrowhead on the abscissa.

elicited current to 73% of control, whereas bath application of $200 \mu\text{M}$ picrotoxin (PTX) reduced the current to 49% of control in a cone horizontal cell. However, not all cone horizontal cells showed a clear bicuculline-sensitive component. Figure 6*B* summarizes the results from 11 cells where the effects of both bicuculline methiodide and picrotoxin on the GABA-elicited currents were tested. Each pair of gray and black columns represents the responses in one cell recorded in the presence of $200 \mu\text{M}$ bicuculline methiodide or picrotoxin, normalized to their respective control values. The cells were arranged in the order of increasing blocking effect of bicuculline methiodide. In most of the cells, bicuculline methiodide was not very effective in block-

Figure 6. GABA_A receptor-mediated component was present only in some cone horizontal cells. *A*, Superimposed recordings from a cone horizontal cell obtained first in the normal bathing medium (control), then in the presence of $200 \mu\text{M}$ bicuculline methiodide (BIC), and finally in $200 \mu\text{M}$ picrotoxin (PTX). The upward deflection of the horizontal line above the response traces indicates the timing of the application of a 500 msec puff of the normal bathing medium containing $500 \mu\text{M}$ GABA. *B*, Summary of the effects of bicuculline methiodide and picrotoxin in 11 cone horizontal cells. Each pair of gray and black bars represents the responses elicited by a $500 \mu\text{M}$ GABA puff recorded in the presence of $200 \mu\text{M}$ bicuculline methiodide or picrotoxin, normalized to those recorded in the normal bathing medium. Cells are aligned according to increasing effectiveness of bicuculline methiodide.

ing GABA responses. The average reduction was $13 \pm 4\%$. On the other hand, picrotoxin always caused a significant reduction of the GABA responses, even in those cells where bicuculline methiodide was not effective. The average reduction caused by picrotoxin was $56 \pm 3\%$. These data suggest that in some cells part of the chloride current was mediated by GABA_A receptor-gated channels.

Bicuculline-resistant GABA receptors are not of GABA_B subtype

In order to characterize the pharmacological properties of the bicuculline-resistant GABA receptors, we examined the effects of baclofen, a GABA_B receptor agonist, and 2-hydroxysaclofen, a GABA_B receptor antagonist. Puff application of 1 mM baclofen elicited no response in a cone horizontal cell, as shown in Figure 7*A*. Similar results were obtained in four additional cells. Fur-

Figure 7. The bicuculline-resistant GABA receptor is not of the $GABA_B$ subtype. *A*, The $GABA_B$ receptor agonist had no effect on the cone horizontal cell. Puff application of 1 mM baclofen (BAC) elicited no response in a cone horizontal cell. *B*, The $GABA_B$ receptor antagonist did not block GABA-elicited current. Bath application of 500 μ M 2-hydroxysaclofen (SAC) did not alter GABA response elicited by a 500 msec GABA (500 μ M) puff in another cone horizontal cell. The upward deflections of the horizontal lines above the current traces in *A* and *B* indicate the timing of puffs.

thermore, Figure 7*B* shows that bath application of 200–500 μ M 2-hydroxysaclofen had no effect in blocking GABA-elicited currents ($n = 4$). These results suggest that the bicuculline-resistant GABA receptors on the cone horizontal cells are not of the $GABA_B$ receptor subtype.

Bicuculline-resistant GABA receptors are of $GABA_C$ subtype

To characterize further the bicuculline-resistant receptor, we isolated the receptor current by superfusing cells with choline medium to eliminate the transporter current (Fig. 4*B*). Choline medium also significantly improved the quality of recordings and signal:noise ratio. This made possible a more accurate comparison of the effects of different GABAergic compounds. Figure 8 compares the effects of 100 μ M pentobarbital, bicuculline methiodide, and picrotoxin on responses elicited by 30 μ M GABA. These drugs were coapplied with GABA through a computer-controlled local perfusion system (see Materials and Methods). The drugs were given in a random order and the current traces for the amplitude measurement were the averages of two to four recordings to ensure the accuracy of the com-

Figure 8. The bicuculline (BIC)-resistant GABA receptor is of the $GABA_C$ subtype. *A*, The pure GABA receptor current isolated by bathing the cell in a choline medium to eliminate the transporter current was mostly insensitive to the blockade by 100 μ M bicuculline methiodide and to the enhancement by 100 μ M pentobarbital (PTB), but was almost completely blocked by 100 μ M picrotoxin (PTX). GABA at 30 μ M was applied alone or coapplied with 100 μ M bicuculline methiodide, 100 μ M picrotoxin, or 100 μ M pentobarbital through a computer-controlled local perfusion system (see Materials and Methods). The upward deflections of the horizontal lines above each current trace indicate the timing of drug applications. The drugs were applied in a random order and each current trace was an average of two recordings. *B*, Statistical data obtained from five cells recorded under conditions similar to those described in *A*. All averaged responses are normalized to those elicited by the application of 30 μ M GABA. Error bars show 1 SD.

parison. In the cell shown in Figure 8*A*, neither pentobarbital (100 μ M) nor bicuculline methiodide (100 μ M) had significant effects on the GABA-elicited current. Figure 8*B* shows the statistical data from five cells. In the presence of pentobarbital and bicuculline methiodide the GABA receptor currents were $94 \pm 6\%$ (mean $\pm$ SD) and $91 \pm 3\%$ (mean $\pm$ SD) of the control values, whereas 100 μ M picrotoxin almost completely blocked the GABA-elicited current.

To confirm the effectiveness of the specific batch of bicuculline methiodide and pentobarbital, we tested both drugs in ganglion cells in the salamander retinal slice preparation, where the GABA-elicited current is almost exclusively mediated by $GABA_A$ receptors (Lukasiewicz et al., 1994). GABA-elicited currents were

Figure 9. Verification of the effectiveness of bicuculline methiodide (BIC) and pentobarbital (PTB) at GABA_A receptors on ganglion cells in the tiger salamander retinal slice preparation. Synaptic transmission was blocked by substituting 20 mM magnesium for sodium in the bathing medium. GABA (500 μ M dissolved in the bathing medium) was applied through a puff pipette placed within 50 μ m of the cell. The upward deflections of the horizontal lines above each current trace indicate the timing of a 50 msec puff. The bathing medium contained (in mM) 92 NaCl, 2 KCl, 20 MgCl₂, 5 glucose, and 5 HEPES, buffered to pH 7.8. *A*, Bicuculline methiodide at 100 μ M completely and reversibly blocked GABA-elicited current. *B*, Pentobarbital at 100 μ M significantly enhanced the GABA-elicited current.

Figure 10. Relative effectiveness of GABA, muscimol (MUS), trans- and cis-aminocrotonic acid (TACA and CACA) at the GABA_C receptor. The cell was bathed in the choline medium to eliminate the transporter current. Bicuculline methiodide at 100 μ M was added in all drug solutions to block any possible GABA_A current. *A*, Current responses to bath application of GABA, muscimol, TACA, and CACA. Responses to three concentrations of CACA are shown. Drugs were applied through a computer-controlled local perfusion system (see Materials and Methods) and in a random order. Each current trace was an average of two recordings. The upward reflections of the horizontal lines above each trace indicate the timing of drug application. *B*, Statistical data obtained from five cells recorded under conditions similar to those described in *A*. All averaged responses are normalized to those elicited by the application of 30 μ M GABA. Error bars show 1 SD.

recorded from the ganglion cells after synaptic transmission was blocked by 20 mM MgCl₂ (replacing 30 mM NaCl in the bath solution). GABA was applied by pressure ejection through a glass pipette placed within 50 μ m of the patched cell. Bicuculline methiodide virtually completely blocked the effect of GABA, as shown in Figure 9*A*. Pentobarbital significantly enhanced the GABA current recorded in ganglion cells, as shown in Figure 9*B*. Similar results were obtained in a total of seven cells for both bicuculline methiodide and pentobarbital. Based on its unique pharmacological properties the bicuculline-resistant and 2-hydroxysaclofen-resistant GABA receptor in cone horizontal

cells can be classified as the GABA_C receptor subtype (Johnston, 1986).

We also studied the relative effectiveness of GABA, muscimol, TACA, and CACA at the GABA_C receptor. Figure 10*A* illustrates the current recordings (averages of two responses) from a representative cell. Figure 10*B* shows the statistical data from five cells. GABA was the most potent ligand of the four. At 30 μ M it generated an average inward current of 105 pA when cells were clamped at -70 mV. TACA was the second most potent. The averaged current elicited by 30 μ M was 79 pA, 75% of that of GABA. Muscimol was the third (30 pA and 29%) and CACA the least potent of the four and had no effect at 30 μ M (0 pA). Even at 300 μ M the effect of CACA was only about 33% (35 pA) of that of GABA.

Discussion

GABA affects cone-driven, not rod-driven, horizontal cells

The results of this study demonstrate that only cone, not rod, horizontal cells respond to GABA in the catfish retina. Three possible explanations could account for the ineffectiveness of GABA in rod horizontal cells. First, all GABA receptors and/or transporters may be destroyed by papain treatment. Second, GABA receptors and/or transporters may be located only on the fine dendritic processes and could be lost after the mechanical trituration during the dissociation process. Third, rod horizontal cells may simply not have GABA receptors and/or transporters. Because all cone horizontal cells consistently responded to GABA, it seems very unlikely that the enzymatic treatment selectively destroyed the GABA receptors and/or transporters in rod horizontal cells. In fact, the GABA receptor subtypes were well preserved in the acutely dissociated retinal neurons after the papain treatment, similar to that used in our study (Gilbertson et al., 1991; Heidelberger and Matthews, 1992). It is unlikely that the fine processes carrying GABA receptors and/or transporters are all lost, for the following reasons: (1) an autoradiographic study showed that in the intact catfish retina only cone, not rod, horizontal cells take up GABA (Lam et al., 1978), suggesting that rod horizontal cells do not have the GABA transporter; (2) rod horizontal cells receive glutamatergic inputs from rod photoreceptors in the intact retina. Glutamate receptors are probably all located in the rod horizontal cell dendrites since the rod terminals do not make synapses on the soma. The consistent response to kainate in all rod horizontal cells tested indicates that at least some of the dendrites were retained in the isolated cells. Therefore, the lack of GABA response in rod horizontal cells is most likely due to the fact that GABA receptors and transporters are not expressed in those cells.

Our results are opposite from those in a recent report (Qian and Dowling, 1993) in which cultured rod, not cone, horizontal cells dissociated from the white perch retina have GABA receptors pharmacologically similar to those found in catfish cone horizontal cells. We do not know whether this is due to the species difference or a difference between acutely dissociated cells and cultured cells.

Physiological implications of the coexistence of GABA receptors and transporters in cone horizontal cells

Our results provide direct evidence that both GABA receptors and transporters exist in the same cone horizontal cell. GABA released from one horizontal cell could act on the receptor on the same cell to form autotfeedback and on the neighboring cells to form chemical coupling in the horizontal cell network. Al-

though our findings do not directly demonstrate autotfeedback or bidirectional chemical coupling, they are crucial to the existence of these functions in the horizontal cell network (Stockton and Slaughter, 1991; Kamermans and Werblin, 1992). However, although the GABA-mediated autotfeedback has been shown to operate in the isolated salamander retina and retinal slice preparations (Yang and Wu, 1989; Kamermans and Werblin, 1992), the GABA-mediated chemical coupling remains to be directly demonstrated in a physiologically functioning retina preparation. In rod horizontal cells the lack of GABA receptors and transporters precludes GABA-mediated autotfeedback and chemical coupling.

All cone horizontal cells have GABA_C; some also have GABA_A receptor subtypes

Two different GABA receptor subtypes seem to be present in the catfish cone horizontal cells: a bicuculline- and baclofen-insensitive, picrotoxin-sensitive subtype and a bicuculline-sensitive subtype. Bicuculline-sensitive receptors present in only some cells are likely to be of the GABA_A subtype. On the other hand, the bicuculline-insensitive GABA receptors seem to be present in all cells and their pharmacology resembles that of GABA_C receptor subtype described in recent studies (Feigenspan et al., 1993; Lukasiewicz et al., 1993; Qian and Dowling, 1993).

In many species of fish there are several morphologically distinct subtypes of cone horizontal cells (Stell, 1967; Dowling, 1987). Surprisingly, it is believed that in the catfish retina there is only one type of cone horizontal cell (Naka and Nye, 1970). We did not observe any obvious morphological and electrophysiological differences between the cone horizontal cells where only GABA_C receptors were present and those where both GABA_A and GABA_C receptor subtypes coexist. However, it is possible that those cells represent two different subtypes of cone horizontal cells.

Relative effectiveness of GABA, muscimol, TACA, and CACA and effectiveness of picrotoxin at GABA_C receptor

Our results show that GABA is more potent than muscimol, TACA, and CACA at GABA_C receptors in catfish cone horizontal cells. The relative effectiveness of GABA, muscimol, and CACA observed in our study was similar to that in the white perch rod horizontal cells (Qian and Dowling, 1993). At GABA_C receptors on retinal horizontal cells (present study), retinal bipolar cells (Feigenspan et al., 1993; Lukasiewicz et al., 1994), or frog tectal neurons (Sivillotti and Nistri, 1989), TACA, an extended analog of GABA, is always more potent than CACA, a folded analog. This suggests that extended conformation is preferred at the GABA_C receptor as at the GABA_A receptor (Ayoub and Matthews, 1991; Lukasiewicz et al., 1994).

Muscimol, a conventional GABA_A receptor agonist, also seemed to act at the GABA_C site. However, the relative effectiveness of GABA and muscimol appears to be different at GABA_C receptors found in different neuronal tissues. At the GABA_C receptor on the white perch (Qian and Dowling, 1993), catfish retinal horizontal cells, and salamander retinal bipolar cells (Lukasiewicz et al., 1994) muscimol is less potent than GABA, whereas at the bicuculline-resistant but picrotoxin-sensitive GABA receptor sites in frog optic tectum muscimol is much more potent (Nistri and Sivillotti, 1985; Sivillotti and Nistri, 1989). In view of the existence of more than one type of ρ GABA receptor subunit (Cutting et al., 1993), from which the GABA_C

receptor is believed to be assembled (Shimada et al., 1991), it is possible that this discrepancy is due to different subunit compositions of the GABA_C receptors on different neurons.

Recently, Feigenspan et al. (1993) described a GABA_C-like receptor on rat bipolar cells. In contrast to our findings, and those of Qian and Dowling (1993) and Lukasiewicz et al. (1994), the bicuculline-resistant GABA-elicited Cl⁻ current was not very sensitive to the blockade by the noncompetitive GABA receptor antagonist picrotoxinin, the active ingredient of picrotoxin. The difference in the GABA antagonist pharmacology could also be due to different molecular assembly of GABA_C receptors.

Possible physiological role of GABA_C receptor in cone horizontal cells

Catfish cone horizontal cells contain GABA (Lam et al., 1978), and release GABA when depolarized (Schwartz, 1987). Since GABA_C receptor gates a chloride conductance (Fig. 5; see also Feigenspan et al., 1993; Qian and Dowling, 1993) the physiological consequence of the activation of GABA receptors is largely dependent on E_{Cl} . In intact horizontal cells E_{Cl} is 10–20 mV more positive to the dark membrane potential (Miller and Dacheux, 1983; Djamgoz and Laming, 1987). Thus, GABA released from one horizontal cell upon depolarization could generate a depolarizing response leading to positive feedback via the GABA_C receptor, similar to that described in the salamander retina (Kamermans and Werblin, 1992). The positive feedback acts to slow down the onset of the light response, thus shaping its kinetics. Indeed, it has been shown that slowdown of the onset of the horizontal cell light response (Yang and Wu, 1989; Kamermans and Werblin, 1992) was absent whenever the positive feedback loop was opened experimentally (Kamermans and Werblin, 1992).

The GABA_C receptor could also be involved in a chemical coupling between cone horizontal cells. Since E_{Cl} is relatively positive in horizontal cells, GABA release from one horizontal cell upon depolarization could also depolarize the neighboring horizontal cells and vice versa, thus forming a chemical coupling between horizontal cells. This chemical coupling would increase the receptive field dimension of the horizontal cell network, complementing the electrical coupling between those cells.

The GABA response mediated by the GABA_C receptor shows little desensitization (Feigenspan et al., 1993; Qian and Dowling, 1993; present study), whereas the response mediated by the GABA_A receptor desensitizes rapidly to a prolonged exposure to GABA (Gilbertson et al., 1991; Qian and Dowling, 1993). Therefore, the GABA_C receptor may be more suitable for mediating the sustained actions of GABA such as positive auto-feedback and chemical coupling in horizontal cells and in the feedback synapse from the sustained GABAergic amacrine cell to the bipolar cell terminal.

It is interesting to note that in the retina GABA_C receptors are found only in horizontal cells (Qian and Dowling, 1993; present study) and bipolar cells (Feigenspan et al., 1993; Lukasiewicz et al., 1994), the second-order, nonspiking neurons. The GABA receptors found on amacrine cells (Feigenspan et al., 1993) and ganglion cells (Cohen et al., 1989; Lukasiewicz et al., 1994), the third-order and spiking cells, are almost exclusively the GABA_A subtype. Further study is needed to elucidate whether this is an interesting coincidence or has some functional correlation, and whether the cells that have the GABA_C receptors in the other regions of the brain are nonspiking neurons.

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