25. Hamill, O. P., Marty, A. Neher, E., Sakmann, B. & Sigworth, F. J. *Pflügers Arch.* **391,** 85–100 (1981).
26. Colquhoun, D. & Sigworth, F. J. in *Single-Channel Recording* (eds Sakmann, B. & Neher, E.) 191–263 (Plenum, New York, 1983).

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Novel GABA responses from rod-driven retinal horizontal cells

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γ-AMINOBUTYRIC acid (GABA) is the main inhibitory neurotransmitter in the central nervous system. Two classes of GABA receptors ($GABA_A$ and $GABA_B$) have been identified. $GABA_A$ receptors are ligand-gated chloride channels that are competitively antagonized by bicuculline, noncompetitively blocked by picrotoxin, and often allosterically modulated by barbiturates and benzodiazepines[1–3]. $GABA_B$ receptors regulate potassium and calcium channels through G-protein and intracellular second-messenger pathways[2,3], are selectively activated by baclofen, and are antagonized by phaclofen and 2-hydroxysaclofen[4,5]. For some years, evidence has accumulated that there are GABA receptors, especially prominent along visual pathways, which are neither antagonized by bicuculline nor activated by baclofen, but are activated by certain conformationally restricted analogues of GABA, including *cis*-4-aminocrotonic acid (CACA)[6–9]. These receptors have been designated $GABA_C$ receptors[9]. As yet, membrane current responses from isolated neurons that reflect this novel pharmacology have not been reported, although such responses have been recorded from oocytes injected with retinal messenger RNA[10–13]. Here we describe a chloride-mediated current response from isolated rod-driven horizontal cells (H4) of the white perch retina that has this novel pharmacology.

Figure 1 shows a typical inward current response recorded from an isolated H4 horizontal cell upon application of 100 μM GABA to the cell for 30 seconds. The cell was voltage-clamped with a patch pipette and held at −50 mV. Similar responses were observed in all 96 H4 horizontal cells tested, but not in 10 cone-driven horizontal cells. Most cone-driven horizontal cells in white perch are unresponsive to GABA; a small proportion (<25%) show $GABA_A$ receptor-mediated responses (Z. J. Zhou, G. L. Fain and J.E.D. manuscript submitted. To compare the GABA response of the rod-driven horizontal cell with typical $GABA_A$ mediated current responses, a recording from a white perch bipolar cell is also shown in Fig. 1. The responses differ in two respects; first, the response from the bipolar cell showed a dramatic desensitization during the 30-s GABA exposure (in 21 cells tested, a reduction to 37.1 ± 17.8% of its peak value was observed after 8 s of continuous application of GABA), whereas the response from the horizontal cell showed no desensitization (the response remained at 98.9 ± 6.7% of its initial value after 8 s of continuous application of GABA; $n = 29$). Second, the H4 horizontal cell response recovered quite slowly after GABA was withdrawn (time constant 15.9 ± 6.0 s; $n = 25$), whereas the response from the bipolar cell quickly returned to the baseline (time constant 1.45 ± 1.12 s, $n = 21$).

Figure 2*a* shows a typical current–voltage relation for the GABA response recorded from H4 horizontal cells. The positive slope of the line indicates that a membrane conductance increase occurred during the GABA response. The reversal potential for the horizontal cell response was close to 0 mV when chloride was the main extracellular anion (Fig. 2*a*). Reversal potentials (mean ± s.d.; $n = 4$) determined with various extracellular chloride concentrations are shown in Fig. 2*b*. The straight line plots the values for the chloride equilibrium potentials calculated from the Nernst equation. The excellent correspondence of the data points to the line indicates the response is mediated by chloride.

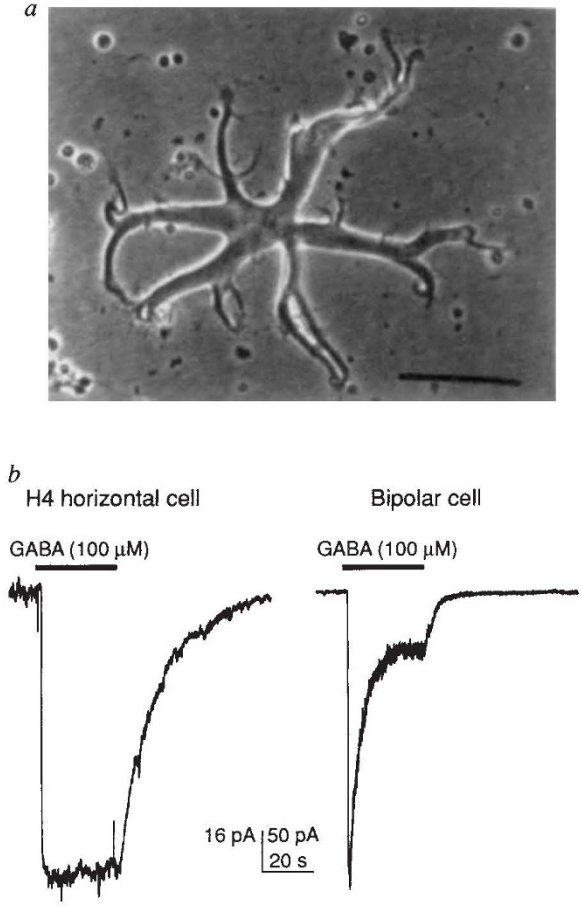

FIG. 1 *a*, A solitary rod-driven horizontal cell (H4) isolated from the white perch retina. The cell has a flat cell body and several primary processes from which many fine processes extend. Scale bar, 50 μm. *b*, GABA responses from an H4 horizontal cell (left) and a bipolar cell (right) isolated from the white perch retina. Both cells were held at −50 mV. GABA was applied onto the cell by bath superfusion, indicated by a bar above each trace. The response from the bipolar cell showed a dramatic desensitization during the application of GABA. The response from the H4 horizontal cell was sustained and did not desensitize during the GABA application.
METHODS. Retinal cells were isolated from the white perch (*Roccus americana*) retina by enzymatic and mechanical treatment as described[26]. Cells were cultured in L-15 medium at room temperature for up to one week. Four subtypes (H1 to H4) of horizontal cells can be identified in culture by their morphology. H1–H3 horizontal cells are cone-driven horizontal cells, whereas H4 horizontal cells are driven exclusively by the rod photoreceptors. The H4 horizontal cell shown in *a* was in culture for 24 h. Before each experiment the culture medium was replaced by Ringer's solution, which contained (in mM) NaCl (110), KCl (2.5), $CaCl_2$ (2.4), $MgCl_2$ (1.5), glucose (10), HEPES (10), pH 7.7. Whole-cell patch clamp recording techniques were used to monitor membrane currents[27]. The intracellular solution in the patch pipette contained (in mM) KCl (124), $CaCl_2$ (1), EGTA (11), $MgCl_2$ (2), HEPES (10), Mg-ATP (1). The resistance of a typical patch pipette was ~2 MΩ. Series resistance during whole-cell recording was ~4 MΩ for horizontal cells and 6 MΩ for bipolar cells. Consequently, a 100 pA current would produce an error of about 0.4 mV in horizontal cell membrane voltage. Recordings were not compensated. Typical input resistance and cell capacitance were about 0.5 GΩ and 250 pF, respectively, for horizontal cells, and 0.5 GΩ and 40 pF for bipolar cells. In some cases, potassium was substituted by caesium to block potassium currents. No difference was found in the GABA-induced currents when the pipettes contained caesium. In most experiments, cells were held at −50 mV and the signals filtered at 30 Hz unless otherwise specified. All values given in the text are expressed as means ± s.d.

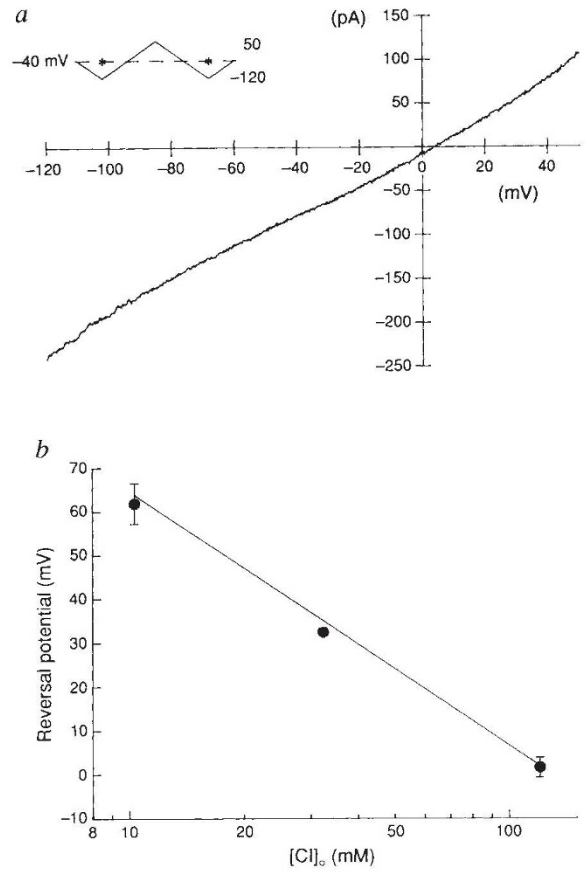

FIG. 2 GABA responses from H4 horizontal cells are mediated by chloride. *a*, Current–voltage relations of the response. Membrane voltage was ramped at 0.5 V s^{-1} according to the protocol shown in the inset. Currents were measured during the time marked by the asterisks; signals were filtered at 1 kHz and averaged 5 times. Current–voltage relations were first recorded in Ringer's and then in Ringer's containing 10 μM GABA. The difference between the two averaged records gave the GABA response. *b*, Reversal potentials of GABA responses (means ± s.d.; $n = 4$) determined with various extracellular chloride concentrations. Extracellular chloride was substituted by isothionate. The straight line is the calculated value of the chloride equilibrium potential according to the Nernst equation.

The GABA responses recorded from H4 horizontal cells were bicuculline-insensitive. Figure 3*a*, *b* and Table 1 show that the GABA responses from H4 horizontal cells were unchanged in the presence of 500 μM bicuculline methochloride, whereas the GABA responses from perch bipolar cells were significantly reduced by the same concentration of bicuculline methochloride. (The GABA responsiveness that persists after bicuculline application to bipolar cells may indicate that bipolar cells in white perch possess some bicuculline-resistant GABA receptors.) Picrotoxin (500 μM), on the other hand, totally blocked the GABA responses from both the horizontal ($n = 12$) and bipolar cells ($n = 4$), indicating that both responses are mediated by chloride channels (Fig. 3*c*, *d*). In addition, neither 10 μM diazepam nor 100 μM pentobarbital had a significant effect on the GABA responses from horizontal cells (Fig. 3*e*, *g*; Table 1), whereas both chemicals dramatically enhanced GABA responses from bipolar cells (Fig. 3*f*, *h*; Table 1). The response was also insensitive to $GABA_B$ receptor agonists and antagonists. Baclofen, a $GABA_B$ agonist, at 100 μM did not induce any response from H4 horizontal cells, nor did it alter the GABA current responses ($n = 8$). Furthermore, neither 500 μM phaclofen ($n = 3$) nor 500 μM hydroxysaclofen ($n = 3$), antagonists of the $GABA_B$ receptors, had any effect on GABA-induced currents in horizontal cells (data not shown). Electrogenic GABA transporters were also excluded as sources of the responses because the GABA-induced current persisted when choline was substituted for extracellular sodium ($n = 6$) (Fig. 3*i*).

A conformationally restricted analogue of GABA, *cis*-4-aminocrotonic acid (CACA), elicted a sustained inward current from H4 horizontal cells similar to the GABA response (Fig. 4*a*). The CACA responses, like those of GABA, were blocked by picrotoxin but insensitive to bicuculline ($n = 2$). But CACA was less potent than GABA. Dose-response curves for GABA, CACA and muscimol are shown in Fig. 4*b*. The EC_{50} (concentration producing 50% of maximum response) for GABA in eliciting the response was 1.87 μM, which is about 10-fold lower than the EC_{50} for GABA at typical $GABA_A$ receptor sites[14–17]. Muscimol, a powerful $GABA_A$ agonist, induced only a small sustained current in H4 horizontal cells. The EC_{50} for muscimol (4.81 μM) was higher than that for GABA itself, and even at 100 μM elicited a response which was only ~42% of that elicited by GABA (Fig. 4*b*). At $GABA_A$ receptors, muscimol is typically more potent than GABA by 2–10 fold[18–20].

The GABA responses rcorded from H4 horizontal cells of the white perch retina fit well with the properties of bicuculline-resistant GABA responses recorded from the frog optic tectum and guinea-pig superior colliculus[8,21]; with GABA responses recorded from *Xenopus* oocytes injected with bovine retinal mRNA[10,11]; and with GABA responses obtained from *Xenopus* oocytes expressing the ρ_1 GABA receptor subunit whose gene has prominent retinal expression[12,13]. Johnston[9] has proposed that the receptors mediating such responses be called $GABA_C$ receptors, and Shimada *et al.*[13] have recently provided arguments supporting this proposal.

TABLE 1 Effect of different drugs on GABA responses in H4 horizontal cells and bipolar cells

Drug	H4 horizontal cells	Bipolar cells
Bicuculline methochloride	95.8 ± 7.6, $n = 10$	17.8 ± 2.9**, $n = 4$
Diazepam	97.5 ± 10.8, $n = 11$	177.4 ± 37.2*, $n = 4$
Pentobarbital	108.4 ± 11.7, $n = 7$	586.4 ± 284.7*, $n = 4$

Data are presented as per cent of the GABA response obtained in Ringer's (means ± s.d.). Drug concentrations were the same as in the experiments shown in Fig. 3.

** $P < 0.001$ compared with 100% control; * $P < 0.05$ compared with 100% control.

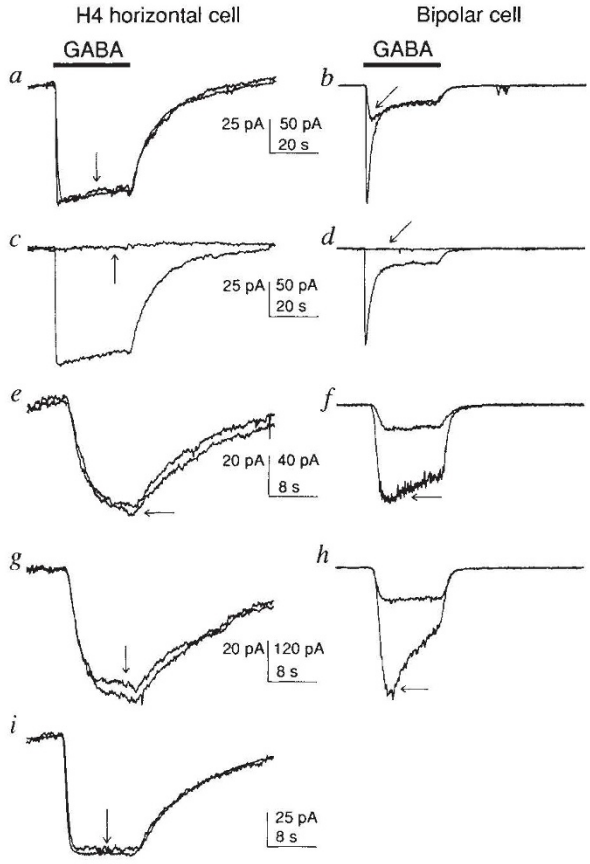

FIG. 3 GABA responses from H4 horizontal cells are pharmacologically distinct from GABA responses of bipolar cells. Arrows point to GABA responses obtained after application of various agents to the cells by bath superfusion. GABA was applied either through a puff pipette filled with 1 mM GABA (*a*–*d*) or co-applied with the test agent by bath superfusion (*e*–*i*). *a*, 500 μM bicuculline methochloride had no effect on the GABA response from the horizontal cell; *b*, 83% of the GABA response from the bipolar cell was blocked by bicuculline methochloride. In five experiments, 10 μM GABA was co-applied by bath supefusion with 500 μM bicuculline methochloride. In these experiments as well, no effects on the GABA responses from H4 horizontal cells were noted, whereas in two bipolar cells tested, there was a reduction of ~81% in the GABA response. Responses from both the horizontal (*c*) and bipolar cell (*d*) were blocked completely by 500 μM picrotoxin. GABA responses elicited with 2 μM GABA were not significantly altered by coapplication of either 10 μM diazepam (*e*) or 100 μM pentobarbital (*g*) onto H4 horizontal cells, whereas these drugs at the same concentrations significantly increased the responses of bipolar cells to 10 μM GABA (*f*, diazepam; *h*, pentobarbital). *i*, 10 μM GABA responses from an H4 horizontal cell in normal Ringer's and in Ringer's in which choline was substituted for sodium. Traces have been adjusted for a slight baseline shift.

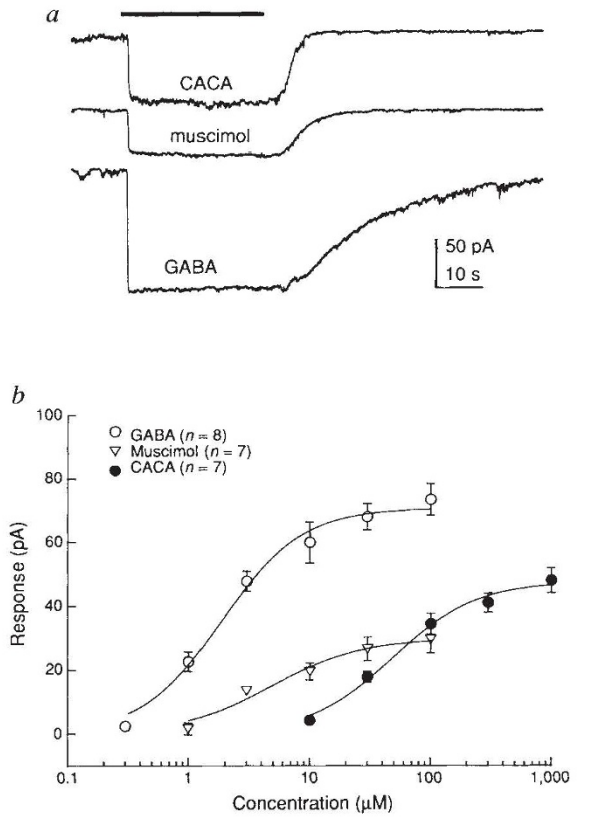

FIG. 4 *a*, Sustained currents elicited in an H4 horizontal cell by bath superfusion of 1 mM *cis*-4-aminocrotonic acid (CACA), 100 μM muscimol and 100 μM GABA. *b*, Dose-response curve for GABA, CACA and muscimol. Data are shown as means ± s.e. The continuous curves are Michaelis functions, with EC_{50} values of 1.87, 48.5 and 4.81 μM, and maximum responses of 70.8, 47.5 and 29.8 pA for GABA, CACA and muscimol respectively.

That the GABA responses recorded from H4 horizontal cells of the white perch retina resemble so closely the GABA responses of oocytes expressing only the ρ_1 GABA receptor subunit suggests that the functional GABA receptor of the rod horizontal cells in perch may be homo-oligomeric. By analogy with nicotinic acetylcholine receptors, GABA receptors are thought to be pentamers, consisting of several different subunits. At least 15 different GABA receptor subunits have so far been identified, and the various subunits impart different properties to the receptor[22–24]. It will be of interest to show unequivocally that GABA receptors made up of only a single subunit are formed in neurons.

The functional significance of these novel GABA receptors is not known. Distal retinal neurons typically respond tonically to prolonged stimuli[25], thus GABA receptors that do not desensitize may be of advantage in the outer retina. It may be for similar reasons that such receptors are found in higher visual structures and elsewhere in the brain. □

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1. Olsen, R. W. *A. Rev. Pharmac. Toxicol.* **22,** 245–277 (1982).
2. Bormann, J. *Trends Neurosci.* **11,** 112–116 (1988).
3. Sivilotti, L. & Nistri, A. *Prog. Neurobiol.* **36,** 35–92 (1991).
4. Hill, D. R. & Bowery, N. G. *Nature* **290,** 149–152 (1981).
5. Kerr, D. I. B., Ong, J., Johnston, G. A. R., Abbenante, J. & Prager, R. H. *Neurosci. Lett.* **92,** 92–96 (1988).
6. Johnston, G. A. R. *et al. J. Neurochem.* **24,** 157–160 (1975).
7. Drew, C. A., Johnston, G. A. R. & Weatherby, R. P. *Neurosci. Lett.* **52,** 317–321 (1984).
8. Sivilotti, I. & Nistri, A. *Eur. J. Pharmac.* **164,** 205–212 (1989).
9. Johnston, G. A. R. in *Benzodiazepine/GABA Receptors and Chloride Channels. Receptor Biochemistry and Methodology* (eds Olsen, R. W. & Venter, J. C.) Vol. 5, 57–71 (Liss, New York, 1986).
10. Polenzani, L., Woodward, R. M. & Miledi, R. *Proc. natn. Acad. Sci. U.S.A.* **88,** 4318–4322 (1991).
11. Woodward, R. M., Polenzani, L. & Miledi, R. *Molec. Pharmac.* **42,** 165–173 (1992).
12. Cutting, G. R. *et al. Proc. natn. Acad. Sci. U.S.A.* **88,** 2673–2677 (1991).
13. Shimada, S., Cutting, G. & Uhl, G. R. *Molec. Pharmac.* **41,** 683–687 (1992).
14. Gallagher, J. P., Higashi, H. & Nishi, S. *J. Physiol., Lond.* **275,** 263–282 (1978).
15. Akaike, N., Hattori, K., Inomata, N. & Oomura, Y. *J. Physiol., Lond.* **360,** 367–386 (1985).
16. Houamed, K. M. *et al. Nature* **310,** 318–321 (1984).
17. Itabashi, S., Aibara, K., Sasaki, H. & Akaike, N. *J. Neurophysiol.* **67,** 1367–1374 (1992).
18. Mathison, R. D. & Dreifuss, J. J. *Brain Res.* **187,** 476–480 (1980).
19. Curtis, D. R., Bornstein, J. C. & Lodge, D. *Brain Res.* **194,** 255–258 (1980).
20. Nakagawa, T., Wakamori, M., Shirasaki, T., Nakaye, T. & Akaike, N. *Am. J. Physiol.* **260,** c745–c749 (1991).
21. Arakawa, T. & Okada, Y. *Eur. J. Pharmac.* **158,** 217–224 (1988).
22. Olsen, R. W. & Tobin, A. J. *FASEB J.* **4,** 1469–1480 (1990).
23. Seeburg, P. H. *et al. Cold Spring Harbor Symp. quant. Biol.* **55,** 29–40 (1990).
24. Burt, D. R. & Kamatchi, G. L. *FASEB J.* **5,** 2916–2923 (1991).
25. Dowling, J. E. *The Retina an Approachable Part of the Brain* (Harvard University Press, Cambridge, Massachusetts, 1987).
26. Dowling, J. E., Pak, M. W. & Lasater, E. M. *Brain Res,* **360,** 331–338 (1985).
27. Hamill, O. P., Marty, A., Neher, E., Sakmann, B. & Sigworth, F. J. *Pflügers Archiv.* **391,** 85–100 (1981).

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