Proc. Natl. Acad. Sci. USA
Vol. 88, pp. 7135–7139, August 1991
Neurobiology

Inhibition of calcium influx and calcium current by γ-aminobutyric acid in single synaptic terminals

RUTH HEIDELBERGER AND GARY MATTHEWS

Department of Neurobiology and Behavior, The State University of New York, Stony Brook, NY 11794-5230

Communicated by Torsten N. Wiesel, May 28, 1991 (received for review January 31, 1991)

ABSTRACT **Inhibition of Ca influx and Ca current by γ-aminobutyric acid (GABA) was studied in single synaptic terminals of isolated retinal bipolar neurons. Measurements of intracellular Ca concentration ($[Ca]_i$) using the fluorescent Ca indicator fura-2 showed that GABA potently inhibited Ca influx into the terminal elicited by high extracellular K concentration ($[K]_o$). This inhibition was attributed to GABA type A ($GABA_A$) receptor-activated chloride ion conductance that prevented bipolar neurons from depolarizing sufficiently to activate the Ca current, even in response to increased $[K]_o$. Patch-clamp recordings of the Ca current revealed a second effect of GABA: GTP-dependent inhibition of the Ca current. This inhibition was not mediated by $GABA_A$ receptors, but baclofen, which binds to the GABA type B ($GABA_B$) receptor and is known to inhibit the Ca current in other systems, was not able to mimic the action of GABA. This suggests the involvement of a different type of $GABA_B$-like receptor in the inhibition of Ca current by GABA. GABA did not cause an overall suppression of the Ca current; rather, the voltage-dependence of Ca-channel activation was shifted to more depolarized potentials. Thus, maximal inhibition of the Ca current by GABA occurred in the physiological range of potential.**

In the central nervous system (CNS), neurotransmitter actions on presynaptic Ca influx are thought to be an important mechanism by which the presynaptic release of transmitter can be modulated. Because most CNS synaptic terminals are small and inaccessible, information about transmitter actions on Ca influx in neurons has come for the most part from studies of neuronal somata (1–4), and it is not clear to what extent the results extend to presynaptic terminals. In this paper, we report direct studies of the effects of a CNS inhibitory transmitter, γ-aminobutyric acid (GABA), on Ca influx and Ca currents in single synaptic terminals of a CNS interneuron, the type-Mb1 bipolar neuron (5, 6) from goldfish retina. The synaptic terminal of this type of bipolar cell is large and round (8- to 12-μm diameter) and thus is well suited to physiological study of Ca influx in single terminals. Previously, we have used both fura-2 measurements of intracellular Ca concentration ($[Ca]_i$) and patch-clamp measurements of Ca currents to show that Ca influx in these terminals occurs via a single type of slowly inactivating, dihydropyridine-sensitive Ca channel (7, 8). In addition, the terminals of Mb1 bipolar neurons are known to receive direct GABAergic feedback synapses from amacrine cells (9, 10), making the terminal a likely candidate for study of presynaptic actions of GABA. We find that GABA has a dual effect on presynaptic Ca influx in these cells. First, GABA activates a large chloride ion conductance that clamps the membrane potential at a more negative potential than the activation range of the Ca channels in the terminal. Second, GABA reduces the presynaptic Ca current via a mechanism that requires internal GTP and that does not involve GABA type A ($GABA_A$) receptors. Although this second effect of GABA is reminiscent of GABA type B ($GABA_B$) receptor actions in other systems, it cannot be mimicked by the $GABA_B$ agonist baclofen or blocked by the $GABA_B$ antagonist 2-hydroxysaclofen, suggesting that it is not mediated by a classical $GABA_B$ receptor. The inhibition of Ca current by GABA was not an overall suppression of the current; rather, the degree of suppression was largest at more negative potentials, with little effect at more depolarized levels. The result was a shift in the current–voltage relation toward more positive potentials.

MATERIALS AND METHODS

Bipolar neurons were acutely isolated from goldfish retina by mechanical trituration after papain digestion (11). Cells were loaded with fura-2 acetoxymethyl ester (AM) by incubation for 10–30 min in 1 μM fura-2 AM in Ringer's solution containing 120 mM NaCl, 2.6 mM KCl, 1.0 mM $MgCl_2$, 0.2 mM $CaCl_2$, 3 mM Hepes, and 10 mM glucose (pH 7.3). Cells were washed thoroughly and stored in the same solution without fura-2 for 20–30 min before experiments were started. For experiments, the Ringer's solution typically contained 90 mM NaCl, 30 mM choline chloride, 2.6 mM KCl, 1.0 mM $MgCl_2$, 2.5 mM $CaCl_2$, 3 mM Hepes, and 10 mM glucose (pH 7.3). In some experiments, 30 mM NaCl replaced the choline chloride. To depolarize the cells and activate Ca influx, we increased the extracellular K concentration ($[K]_o$) by 30 mM to 32.6 mM, with a corresponding reduction in choline chloride or NaCl. The fluorescence microscopy/photomultiplier system for measurement of fura-2 fluorescence was similar to that described by Neher (12); excitation wavelengths were about 360 and 380 nm. Emitted light was collected from a 30 μm-diameter region of the field of view; with the terminal in the center of this region, no light emitted from the soma and dendrites was measured. The formula of Grynkiewicz *et al.* (13) was used for calculation of $[Ca]_i$, with calibration constants (including the effective fura-2–Ca dissociation constant) determined by filling cells via a whole-cell patch pipette with solutions containing fura-2 and known, highly buffered amounts of Ca; minimum and maximum 360/380 nm fluorescence emission ratios at 0 and high Ca concentrations determined in this way were 2.0 and 9.2. Quenching of fluorescence with Mn (loaded into cells with 4Br-A23187) showed that ≈94% of the fura-2 was Ca sensitive.

Patch-clamp recordings of whole-cell membrane currents were made by standard techniques (14). For experiments in which Ca current was isolated, patch pipettes (10–30 MΩ) were filled with a solution containing 120 mM CsCl, 10 mM tetraethylammonium chloride, 3 mM Hepes, 5 mM Cs_2EGTA, 2 mM $MgCl_2$, 2 mM Na_2ATP, and 0.3 mM GTP (pH 7.2). In some experiments, cesium gluconate replaced

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

Abbreviations: GABA, γ-aminobutyric acid; $[Ca]_i$, intracellular calcium concentration; $[K]_o$, extracellular K concentration; $GABA_A$ and $GABA_B$ receptors; GABA receptors types A and B.

the CsCl. For experiments in which it was not desired to block K currents, the pipette solution contained 130 mM potassium gluconate, 3 mM Hepes, 5 mM K_2EGTA, 2 mM $MgCl_2$, 2 mM Na_2ATP, and 0.3 mM GTP (pH 7.2). Only cells with a series access resistance <50 MΩ were accepted; automatic g-series compensation of the EPC-9 patch-clamp amplifier was used to compensate ≈50% of series resistance, resulting in an estimated voltage error of <25 μV/pA. Cells were normally bathed in Ringer's solution containing 120 mM NaCl, 2.6 mM KCl, 1.0 mM $MgCl_2$, 2.5 mM $CaCl_2$, 3 mM Hepes, and 10 mM glucose (pH 7.3). Drugs were applied by local superfusion, typically only to the synaptic terminal, by using a pressure-application pipette.

RESULTS

To study the effect of GABA on Ca influx into synaptic terminals of intact bipolar neurons, acutely isolated cells were loaded with fura-2, AM (13). When cells were depolarized with increased $[K]_o$ (Fig. 1), $[Ca]_i$ in the terminal increased because of an influx of Ca via dihydropyridine-sensitive Ca channels (7, 8). As shown in Fig. 1*a*, the K-induced increase in $[Ca]_i$ was strongly suppressed by GABA. In 21 experiments, the increase in intraterminal Ca elicited by 32.6 mM $[K]_o$ was reduced by 3 μM GABA to 13 ± 4% (mean ± SEM) of the control increase observed in the same cells without GABA. Because goldfish bipolar neurons have been reported to have a large $GABA_A$ receptor-activated Cl conductance (15), we examined whether the observed suppression of Ca influx might be due to activation of this Cl conductance. As shown in Fig. 1*a*, the $GABA_A$ agonist muscimol was as effective as GABA in suppressing Ca influx. In eight such experiments, 1 μM muscimol reduced K-induced Ca influx to 9 ± 6% of control, compared with 10 ± 4% for 3 μM GABA in the same cells. When applied together with 3 μM GABA, the $GABA_A$ antagonist picrotoxin (30 μM) was able to block the suppression of Ca influx by GABA (Fig. 1*b*). In 11 experiments, the K-induced increase in $[Ca]_i$ observed in the presence of GABA and picrotoxin was 81 ± 11% (mean ± SEM) of the control level, compared with 15 ± 7% for GABA alone in the same cells. These results suggest that $GABA_A$ receptors are important in the suppression by GABA of Ca influx induced by increased $[K]_o$.

Because the $GABA_B$ agonist baclofen has been reported to inhibit the Ca current in amphibian bipolar cells (16), we examined the effects of $GABA_B$ drugs on Ca influx. As shown in Fig. 1*c*, baclofen had little effect on the K-induced increase in $[Ca]_i$, whereas GABA suppressed Ca influx as usual in the same cell. In 11 such experiments, the increase in $[Ca]_i$ with high $[K]_o$ and 50–200 μM baclofen was 97 ± 9% of the control response with high $[K]_o$ alone. The $GABA_B$ antagonist 2-hydroxysaclofen had no effect on the suppression of Ca influx by GABA (Fig. 1*d*). Thus, there was no evidence of an additional effect of GABA beyond the $GABA_A$ receptor effect described above.

To establish the mechanism by which GABA antagonized Ca influx into terminals, we made patch-clamp recordings from acutely isolated bipolar cells and examined the effects of high $[K]_o$ and GABA. To mimic the ionic conditions of the fura-2 experiments on intact cells, a high-potassium, low-chloride (potassium gluconate) pipette solution was used for these experiments. Under voltage-clamp, the zero-current potential (corresponding to the resting potential under current clamp) averaged −60 ± 3 mV (mean ± SEM; n = 7) with a normal $[K]_o$ of 2.6 mM. When $[K]_o$ was increased to 32.6 mM, the average zero-current potential shifted to −16 ± 1 mV (see the trace in Fig. 2*a* labeled "high K"). Such a depolarization strongly activates the Ca current in bipolar cells (refs. 7 and 8; note the inward component of current in Fig. 2*a*, even in the absence of K-current blockers); this accounts for the increase in $[Ca]_i$ elicited by 32.6 mM $[K]_o$ in intact cells in the fura-2 experiments (Fig. 1). In the same seven cells, however, addition of 5 μM GABA to the 32.6 mM $[K]_o$ shifted the zero-current potential back to near the resting level (−57 ± 2 mV; see the trace in Fig. 2*a* labeled "high K + GABA"). This is more negative than the activation range of the Ca current, accounting for the ability of GABA to suppress Ca influx in the fura-2 experiments (Fig. 1). The same pattern was also observed with even higher $[K]_o$: the zero-current potential shifted from −12 ± 2 mV in 62.6 mM $[K]_o$ to −56 ± 3 mV in 62.6 mM $[K]_o$/5 μM GABA (n = 7).

At 5 μM, GABA increased the input conductance an average of 7.4-fold, from 304 ± 27 pS to 2249 ± 337 pS (n = 15); thus, the GABA-activated conductance was sufficiently large to be able to clamp the cell at a negative potential, even in the face of increased $[K]_o$. In agreement with previous results (15), the GABA-activated conductance was a Cl conductance, with a reversal potential near −60 mV with a potassium gluconate pipette solution and near 0 mV with $[Cl]_i$ = $[Cl]_o$. The

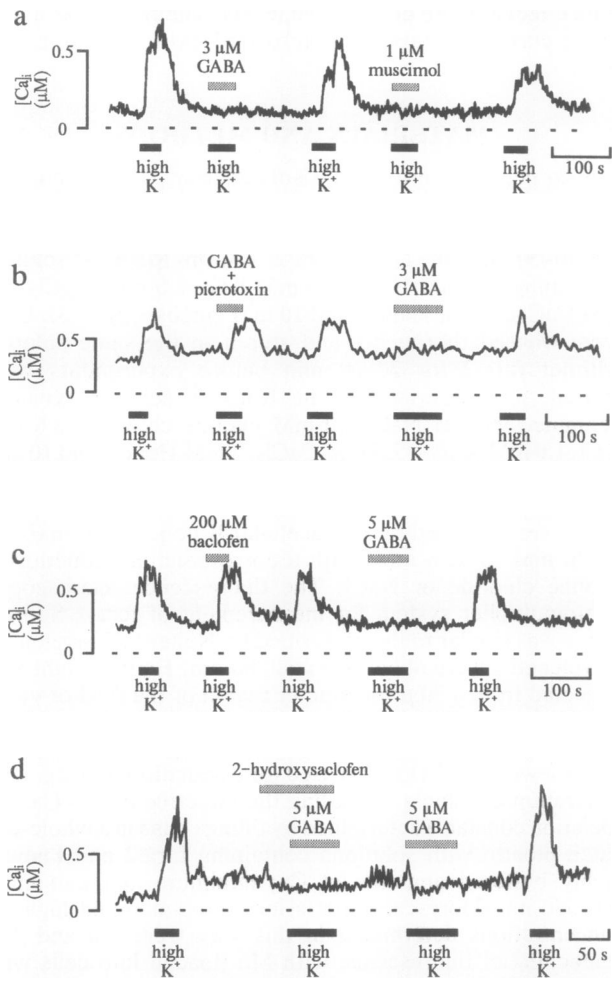

FIG. 1. $[Ca]_i$ measured in single synaptic terminals of acutely isolated Mb1 bipolar neurons with fura-2 fluorescence. All solutions were applied by bath exchange at the indicated times; applications of high $[K]_o$ (32.6 mM) are shown by dark bars below the traces, while other drugs are marked by gray bars above the traces. Dashed lines indicate the zero level for each trace. (*a*) GABA (3 μM) was able to prevent the K-induced increase in $[Ca]_i$ as was 1 μM muscimol, a $GABA_A$ receptor agonist. (*b*) Picrotoxin (30 μM) blocked the inhibitory effect of 3 μM GABA on K-induced Ca influx; subsequently, 3 μM GABA alone had its usual effect. (*c*) The $GABA_B$ receptor agonist baclofen had no effect on K-induced Ca influx into the terminal. (*d*) The $GABA_B$ receptor antagonist 2-hydroxysaclofen (50 μM) had no effect on the inhibitory action of 5 μM GABA.

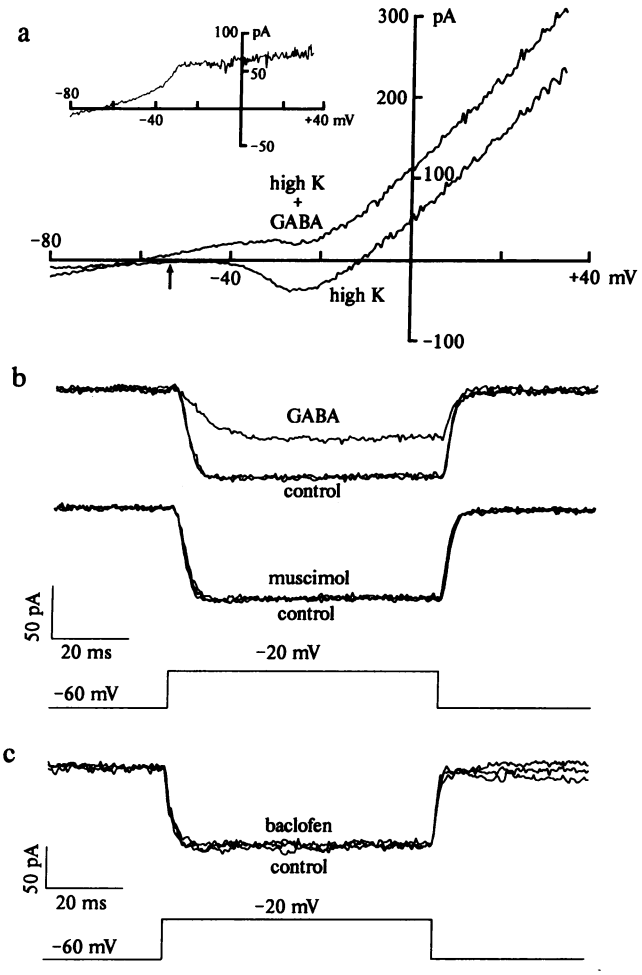

FIG. 2. Patch-clamp measures of whole-cell currents in acutely isolated bipolar neurons. (*a*) Current–voltage relations, obtained with a linear voltage ramp at 100 mV/sec. Currents are total membrane currents without subtraction of leak or capacitative current. High K, 32.6 mM $[K]_o$; high K + GABA, 32.6 mM $[K]_o$ + 5 μM GABA. The arrow indicates the zero-current potential with normal $[K]_o$ (2.6 mM). The pipette solution had no K-current blockers (potassium gluconate solution), and the external solution had no picrotoxin. (*Inset*) Difference current giving the GABA-dependent current, obtained by subtracting the trace in high K from the trace in high K + GABA. (*b*) Effect of GABA and muscimol on the Ca current. Traces: top, current in 5 μM GABA + 100 μM picrotoxin and control traces before and after GABA application; middle, current before, during, and after 5 μM muscimol + 100 μM picrotoxin in the same cell as in *a*; bottom, voltage protocol. A CsCl pipette solution was used. Capacitative and leak currents in *b* and *c* were subtracted by using a P/6 protocol. GABA and muscimol were applied locally to the synaptic terminal by means of a pressure-application pipette. (*c*) Effect of 0.5 mM baclofen, applied to the synaptic terminal, on whole-cell Ca current. Control traces were recorded before and after application of baclofen. Not shown is the effect of GABA, which reduced the Ca current by 33% at −20 mV in the same cell. This is a different cell from that in *b*.

conductance was blocked by picrotoxin and activated by muscimol, indicating that it is due to a $GABA_A$ receptor.

In Fig. 2*a*, an additional effect of GABA beyond the $GABA_A$ receptor-mediated conductance increase can be seen: the inward component of current that activated at potentials positive to −50 mV was reduced by GABA. This can be seen more clearly in Fig. 2*a Inset*, which shows the GABA-dependent membrane current. The inward component in the current–voltage relation, which was reduced by GABA, is a Ca current (7, 8). Because the experiment of Fig. 2*a* was done without K-current blockers and without picrotoxin, the Ca current was largely masked by a prominent outward current and the $GABA_A$-activated Cl conductance. To examine the effect of GABA on Ca current in more detail, whole-cell recordings were made with K-current blockers to allow isolation of the Ca current and with picrotoxin to eliminate the GABA-activated Cl conductance; picrotoxin alone had no effect on Ca current. As shown in Fig. 2*b*, 5 μM GABA reversibly reduced the Ca current, but the $GABA_A$ agonist muscimol had no effect on Ca current in the same cell. In 21 cells, the Ca current measured at −20 mV (which is near the potential at which peak Ca current is observed) was reduced by GABA to 71 ± 3% of control (mean ± SEM). With muscimol, the Ca current was not significantly affected (106 ± 3% of control; n = 13). Thus, the effect of GABA on Ca current was not mediated via $GABA_A$ receptors. The inhibition of Ca current by GABA was slow in both onset and recovery, typically requiring tens of seconds to reach completion, in contrast with the rapid action of GABA on Cl conductance. In addition, the effect of GABA on Ca current depended on the presence of GTP in the pipette solution. In the 21 experiments just mentioned, 0.3 mM GTP was included in the pipette solution; without GTP, 5 μM GABA had little effect on Ca current, reducing the current to 90 ± 6% of control (n = 11). The ineffectiveness of muscimol, the slow speed of the response, and the requirement for GTP all point to the involvement of a GABA-initiated second messenger cascade in GABA's inhibition of Ca current; therefore, we examined the effect of the $GABA_B$ agonist baclofen on Ca current. Baclofen (0.2–1.0 mM) had little effect on Ca current (Fig. 2*c*); in 12 cells, the Ca current in baclofen averaged 94 ± 3% of control. This is in agreement with the lack of effect of baclofen on K-induced Ca influx in the fura-2 experiments (Fig. 1*c*). The $GABA_B$ antagonist, 2-hydroxysaclofen, also had no effect on the suppression of Ca current by GABA (not shown). A simple interpretation is that in goldfish bipolar cell synaptic terminals, GABA reduces Ca current via a $GABA_B$-like receptor for which baclofen is not an effective agonist (see *Discussion*). In the fura-2 experiments (Fig. 1*b*), we found that picrotoxin antagonized GABA's inhibition of K-induced Ca influx, but not completely; picrotoxin restored the influx in the presence of GABA to about 80% of control level. Perhaps the remaining inhibitory effect of GABA in the presence of picrotoxin is due to the suppression of the Ca current shown in Fig. 2*b*; alternatively, the incomplete restoration of Ca influx could reflect incomplete block of Cl conductance by 30 μM picrotoxin.

To characterize the inhibitory effect of GABA on Ca current more fully, the effect of GABA on the current–voltage relation for Ca current was examined. An example is shown in Fig. 3*a*, which shows that GABA did not produce a simple reduction in the amplitude of the Ca current throughout its range of activation. Rather, the degree of inhibition was greatest in the voltage range from about −50 to −20 mV; indeed, at potentials more positive than about −10 mV, there was little effect of GABA on the current. In 17 experiments with 1 μM GABA, the average membrane potential at which the peak suppression of current occurred was −33 ± 1 mV, and the current at that peak potential was reduced to 51 ± 4% of control (mean ± SEM). With 0.25 μM GABA, the peak suppression of the Ca current was smaller, averaging 69 ± 4% of control, but the potential at which peak suppression occurred was no different (−34.6 ± 1 mV; mean ± SEM; n = 17). To establish whether the shift in the current–voltage relation produced by GABA was due to suppression of Ca current, GABA was applied after the Ca current was eliminated by external Cd. GABA had no effect on the current–voltage relation in the presence of 200 μM Cd (Fig. 3*b*).

The inhibition of Ca current by GABA was observed in a range of GABA concentration below that necessary to produce appreciable activation of the $GABA_A$ receptor-

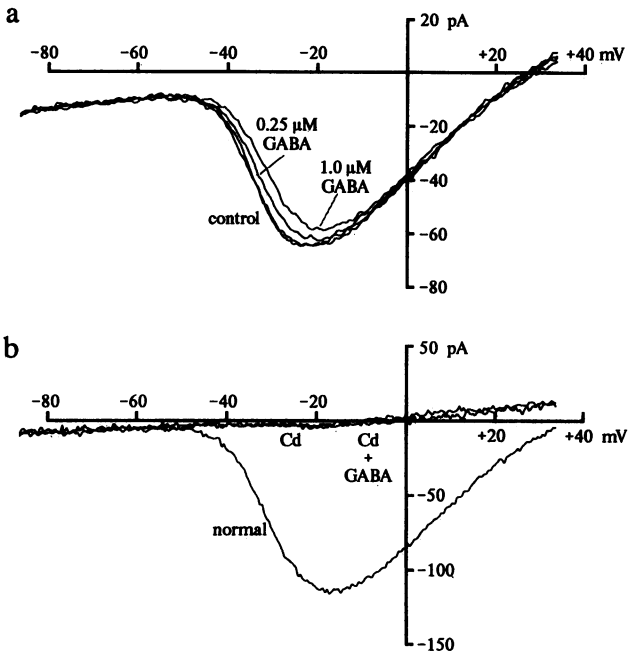

FIG. 3. Effect of GABA on whole-cell Ca current in Mb1 bipolar neurons. Current–voltage relations were obtained with a linear voltage ramp at 100 mV/sec. Currents are total whole-cell currents without subtraction of leak or capacitative current. Pipette solution was cesium gluconate solution. (*a*) Inhibition of Ca current by 0.25 and 1.0 μM GABA. GABA was applied locally to the synaptic terminal, and the superimposed control traces were recorded before and after GABA applications. Unlike most experiments on the effect of GABA on Ca current, in this experiment picrotoxin was omitted from the external solutions so that any GABA-activated change in conductance would be apparent. (*b*) Lack of effect of 3 μM GABA on the current–voltage relation when the Ca current was blocked by 200 μM external Cd. The cell was different from that in *a*; 100 μM picrotoxin was present in all solutions in *b*.

activated Cl conductance. Typically, the dose for measurable activation of Cl conductance was 1–3 μM, while significant inhibition of the Ca current could be observed at 0.25 μM (e.g., see Fig. 3*a*). In fact, the experiment of Fig. 3*a* was done without the Cl-channel blocker picrotoxin, so that any change in conductance due to $GABA_A$-receptor activation would have been apparent; as shown by the superposition of the curves negative to −60 mV, no increase in conductance was produced by GABA. This demonstrates that the receptor responsible for GABA's action on Ca current is more sensitive to GABA than is the $GABA_A$ receptor-activated Cl conductance.

DISCUSSION

Type-Mb1 bipolar neurons of goldfish retina are nonspiking interneurons that respond to light with a sustained and graded depolarization and provide excitatory input to ganglion and amacrine cells (17, 18). Therefore, in terms of retinal signal processing, the GABAergic feedback inhibition of Ca influx in bipolar synaptic terminals will tend to convert sustained responses to steady illumination into transient responses in amacrine and ganglion cells, thereby enhancing response to change. We found two mechanisms that underlie this inhibition: a $GABA_A$ receptor-activated Cl conductance (15) and a GTP-dependent inhibition of Ca current. This latter effect of GABA on Ca current was strongest in the voltage range from approximately −50 to −20 mV, which is the range of physiological interest in the bipolar neuron. Thus, GABA shifted the range of voltage activation of Ca channels to a more depolarized level, in a manner similar to the effects of neurotransmitters on Ca currents in dorsal root ganglion cells (19) and in sympathetic neurons (20). The $GABA_B$ receptor agonist baclofen was unable to mimic this effect of GABA on the Ca current. However, in preliminary experiments, we recently found that *cis*-4-aminocrotonic acid, a reported agonist for GABA receptors that are insensitive to both bicuculline and baclofen ($GABA_C$ receptors; ref. 21), is as potent as GABA in inhibiting Ca current, without activating the $GABA_A$ receptor Cl conductance. This suggests that a $GABA_C$ receptor underlies GABA-mediated inhibition of Ca current.

Why should there be two different GABA-dependent feedback mechanisms that tend to reduce Ca influx into the bipolar cell synaptic terminal? Several possibilities can be suggested. Muscimol, which has no effect on Ca current (Fig. 2*b*), can quite effectively inhibit Ca influx into the terminal (Fig. 1*a*); thus, it is clear that the $GABA_A$ receptor mechanism is by itself capable of mediating feedback inhibition. But at low light levels, when activity of amacrine cells and thus the feedback release of GABA is low, the increase in Cl conductance may be insufficient to overcome depolarizing influences; under these conditions, the contribution of the second mechanism (inhibition of Ca current) might be of more importance. In this regard, it is significant that inhibition of Ca current occurs at a lower concentration of GABA than does activation of Cl conductance. Also, at higher levels of GABA, the $GABA_A$-activated Cl conductance shows pronounced desensitization typical of agonist-gated channels, so that the effectiveness of the $GABA_A$ receptor mechanism might decline with time during steady illumination; it is interesting that the onset of the GABA-inhibition of Ca current occurs on a time scale similar to that of this desensitization. Another possibility is that the two actions of GABA serve qualitatively different functions; because the effect on Ca current is to shift the range of voltage activation, this mechanism might keep the range of steep activation near the steady-state membrane potential (more depolarized in the presence of background illumination, more hyperpolarized in the dark). In this view, the $GABA_A$ receptor-activated Cl conductance would be responsible for actual feedback inhibition of Ca influx, while the effect of GABA on Ca current serves to alter the set-point for activation of Ca entry; such alteration in activation range might be important, for example, in dark and light adaptation. Finally, an electrical mechanism—such as the $GABA_A$ receptor-activated Cl conductance—is inherently global in its action, affecting all of the many individual synaptic output sites made by the bipolar terminal onto many different postsynaptic cells. However, the effect of GABA on Ca current would likely be more local, allowing activity of an individual amacrine cell to affect its own input more selectively. In this view, it is interesting that feedback synapses made by an amacrine cell onto the Mb1 terminal are typically found in close proximity to output synapses from the bipolar terminal to the same amacrine cell process (9).

We thank Dr. S. Yazulla for introducing us to the Mb1 bipolar neuron and for advice about goldfish retina. This work was supported by Grant EY03821 from the National Eye Institute.

1. Dunlap, K. & Fischbach, G. (1981) *J. Physiol. (London)* **317**, 519–535.
2. Scott, R. H. & Dolphin, A. C. (1986) *Neurosci. Lett.* **69**, 59–64.
3. Tsien, R. W., Lipscombe, D., Madison, D. V., Bley, K. R. & Fox, A. P. (1988) *Trends Neurosci.* **11**, 431–437.
4. Dolphin, A. C. (1990) *Annu. Rev. Physiol.* **52**, 243–255.
5. Stell, W. K. (1976) *Invest. Ophthal.* **15**, 895–908.
6. Ishida, A. T., Stell, W. K. & Lightfoot, D. O. (1980) *J. Comp. Neurol.* **191**, 315–335.
7. Heidelberger, R. & Matthews, G. (1990) *Invest. Ophthal. Vis. Sci.* **31**, 389 (abstr.).

8. Matthews, G. & Heidelberger, R. (1990) *Soc. Neurosci. Abstr.* **16,** 1273.
9. Marc, R. E., Stell, W. K., Bok, D. & Lam, D. M. K. (1978) *J. Comp. Neurol.* **182,** 221–246.
10. Yazulla, S., Studholme, K. M. & Wu, J.-Y. (1987) *Brain Res.* **411,** 400–405.
11. Tachibana, M. (1983) *J. Physiol. (London)* **345,** 329–351.
12. Neher, E. (1989) in *Neuromuscular Junction*, eds. Sellin, L. C., Libelius, R. & Thesleff, S. (Elsevier, Amsterdam), pp. 65–76.
13. Grynkiewicz, G., Poenie, M. & Tsien, R. Y. (1985) *J. Biol. Chem.* **260,** 3440–3450.
14. Hamill, O. P., Marty, A., Neher, E., Sakmann, B. & Sigworth, F. J. (1981) *Pflügers Arch.* **391,** 85–100.
15. Tachibana, M. & Kaneko, A. (1987) *Proc. Natl. Acad. Sci. USA* **84,** 3501–3503.
16. Maguire, G., Maple, B., Lukasiewicz, P. & Werblin, F. (1989) *Proc. Natl. Acad. Sci. USA* **86,** 10144–10147.
17. Saito, T., Kondo, H. & Toyoda, J. (1979) *J. Gen. Physiol.* **73,** 73–90.
18. Saito, T., Kujiraoka, T. & Yonaha, T. (1983) *Vision Res.* **23,** 353–362.
19. Bean, B. P. (1989) *Nature (London)* **340,** 153–156.
20. Elmslie, K. S., Zhou, W. & Jones, S. W. (1990) *Neuron* **5,** 75–80.
21. Drew, C. A., Johnston, G. A. R. & Weatherby, R. P. (1984) *Neurosci. Lett.* **52,** 317–321.