The Journal of Neuroscience, February 1995, 15(2): 1384–1391

Differential Modulation by Sulfhydryl Redox Agents and Glutathione of GABA- and Glycine-Evoked Currents in Rat Retinal Ganglion Cells

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Some areas of the mammalian CNS, such as the retina, contain not one but two fast inhibitory neurotransmitter systems whose actions are mediated by GABA and glycine. Each inhibitory receptor system is encoded by a separate gene family and has a unique set of agonists and antagonists. Therefore, in rat retinal ganglion cells we were surprised to find that a single agent, extracellular glutathione, was capable of modulating currents activated by either $GABA_A$ or glycine receptor stimulation. Both oxidized and reduced glutathione influence inhibitory neurotransmission in a manner similar to that of the sulfhydryl redox agents dithiothreitol (DTT) and 5,5′-dithio-bis-(2-nitrobenzoic acid) (DTNB). Remarkably, the actions of glutathione are diametrically opposed on the $GABA_A$ and glycine systems. In whole-cell recordings of single retinal ganglion cells with patch pipettes, reduced glutathione enhances GABA-evoked currents but decreases glycine-evoked currents. These findings suggest that endogenous redox agents, such as glutathione, may constitute a novel modulatory system for the differential regulation of inhibitory neurotransmission in the mammalian retina.

[Key words: $GABA_A$ receptor, glycine receptor, redox modulation, glutathione, retinal ganglion cells]

GABA and glycine are the two major inhibitory neurotransmitters in the CNS (Aprison and Daly, 1978; Olsen and Venter, 1986). In the mammalian retina, stimulation of $GABA_A$ or glycine receptors on ganglion cells modulates their receptive field properties (Daw et al., 1982; Massey and Redburn, 1987; Wässle and Boycott, 1991). Molecular studies have shown that the subunits comprising the $GABA_A$ and glycine receptor proteins share significant structural homology between themselves as well as between the nicotinic acetylcholine (nACh) and glutamate receptors, but are in distinct gene families (Noda et al., 1983; Grenningloh et al., 1987; Schofield et al., 1987; Meguro et al., 1992; Monyer et al., 1992). Particularly striking is the existence of the two extracellular cysteine residues which are thought to form a disulfide bond in a conserved domain of all subunits of the $GABA_A$, glycine, and nACh receptor proteins (Claudio et al., 1983; Noda et al., 1983; Barnard et al., 1987; Olsen and Tobin, 1990; Betz, 1991).

Disulfide bonds and/or sulfhydryl groups, which exist in all ligand-gated channel proteins, as well as in many voltage-gated channel proteins, have been shown to play an important functional role (Karlin and Bartels, 1966; Aronstam et al., 1978; Moxham and Malbon, 1985; Kiskin et al., 1986; Sidhu et al., 1986; Braestrup and Andersen, 1987; Aizenman et al., 1989; Ruppersberg et al., 1991; Laube et al., 1993). The role of thiol groups has been extensively studied in the nACh receptor. Early receptor binding studies found that disulfide-reducing agents (to break disulfide bonds into their component sulfhydryl groups) decrease acetylcholine receptor binding affinity (Karlin, 1969; Ben-Haim et al., 1975; Barrantes, 1980; Walker et al., 1981). More recent electrophysiological studies have shown that these agents inhibit acetylcholine-evoked responses (Steinacker, 1979; Steinacker and Zuazaga, 1981; Aizenman et al., 1989; Bouzat et al., 1991; Rojas et al., 1991). Recently, the *N*-methyl-D-aspartate (NMDA) subtype of glutamate receptor was also shown to be selectively modulated by chemical reducing and oxidizing agents, including oxidized glutathione, and such modulation plays an important role in neuroprotection and neurotoxicity mechanisms *in vitro* and *in vivo* (Aizenman et al., 1989; Gilbert et al., 1991; Sucher and Lipton, 1991; Lei et al., 1992; Lipton et al., 1993; Sathi et al., 1993). Very recently, site-directed mutagenesis of the NMDAR1 subunit has demonstrated at least two cysteine residues that affect redox modulation of NMDA-evoked currents (Köhr et al., 1994; Sullivan et al., 1994).

However, very few studies concerning redox modulation of $GABA_A$ and glycine receptors have been performed. Although receptor binding studies have indicated that sulfhydryl and disulfide groups may play some role in the responses to GABA (Marangos and Martino, 1981; Nishimura et al., 1982; Allan and Baier, 1992) and glycine (Ruiz-Gómez et al., 1991), only preliminary reports of electrophysiological effects have appeared (Porter et al., 1991; Pan and Lipton, 1992, 1993), and their functional implications are not clear. Recently, glutathione, a physiological reducing agent, was found to be released from neuronal tissue in a Ca^{2+}-dependent manner at concentrations approaching those of neurotransmitters (Zängrle et al., 1992). Extracellular levels of neurotransmitters in the low micromolar range are known to reflect millimolar concentrations in the synaptic cleft

Received May 27, 1994; revised Aug. 4, 1994; accepted Aug. 12, 1994.

We thank Drs. N. J. Sucher and H-S. V. Chen for helpful discussions. This study was supported in part by NIH Grant R01 EY05477 (to S.A.L.).

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(Clements et al., 1992), suggesting that glutathione may also approach these levels in various regions of the CNS such as the retina (Organisciak et al., 1984). Therefore, in this study we investigated the effects of sulfhydryl reagents, including glutathione, on GABA- and glycine-evoked responses in rat retinal ganglion cells.

Materials and Methods

Retinal cell cultures. Retinal ganglion cell neurons from 1–2-week-old postnatal Long–Evans rats were identified with specific fluorescent labels, dissociated from the retina, and cultured as previously described (Leifer et al., 1984; Lipton and Tauck, 1987). Acutely dissociated cells (used within 4 hr) or cells in culture for 48 hr yielded similar results. Prior to electrophysiological recording, the culture medium was replaced with a saline based upon Hanks' balanced salts (composition, in mM: NaCl, 137; $NaHCO_3$, 1; Na_2HPO_4, 0.34; KCl, 5.36; KH_2PO_4, 0.44; $CaCl_2$, 1.25; $MgSO_4$, 0.5; $MgCl_2$, 0.5; HEPES-NaOH, 5; glucose, 22.2; with or without phenol red, 0.001% v/v; adjusted to pH 7.2 with 0.3 N NaOH).

Retinal whole-mount or "stripe" preparation. The methods for preparing the isolated rodent retina for electrical recordings have been described previously (Rörig and Grantyn, 1993; Bähring et al., 1994). In brief, C57B1/6H mice were used at the age of 1–6 postnatal days. Animals were decapitated prior to enucleation. Retinas were dissected free and maintained intact (P1–P3) or cut into stripes (P4–P6). Retinal tissue was then transferred to a storage chamber and maintained at room temperature in oxygenated bicarbonate-buffered saline solution. The standard saline contained (in mM) NaCl, 125; KCl, 5; $CaCl_2$, 1.5; $MgSO_4$, 1; $NaHCO_3$, 25; NaH_2PO_4, 1.25; glucose, 10. When saturated with 95% O_2/5% CO_2, the pH was stable at 7.3–7.4. After incubating the tissue for at least 1 hr in oxygenated standard saline, retinal pieces were placed into the recording chamber and continuously superfused on the stage of an upright microscope. The margins of the tissue pieces were mechanically fixed with a rigid nylon grid glued to a platinum frame.

Electrophysiological recordings. Recordings with patch electrodes in the whole-cell configuration were performed using standard procedures (Hamill et al., 1981), as adapted previously by our laboratories (Lipton and Tauck, 1987; Aizenman et al., 1988; Tauck et al., 1988; Lipton, 1989; Bähring et al., 1994). The intracellular solution for recording from dissociated retinal ganglion cells contained (in mM) CsCl, 120; TEA-Cl, 20; $MgCl_2$, 2; $CaCl_2$, 1; EGTA, 2.25; HEPES, 10; pH 7.2. The recording solution for the whole-mount retina contained (in mM) CsCl, 120; TEA-Cl, 10; $MgCl_2$, 1; $CaCl_2$, 0.5; EGTA, 5; HEPES, 10; glucose, 10; ATP, 2; cAMP, 0.25; phosphocreatine, 20; creatine phosphokinase (50 U/ml); pH 7.4. Whole-cell recordings were performed at room temperature (20–25°C). When experiments were performed on dissociated retinal ganglion cells, GABA and glycine were applied by pneumatic ("puffer") pipettes (Aizenman et al., 1988). When the experiments were performed on retinal whole-mounts, these agents were applied locally to the investigated neurons by using gravity-driven superfusion pipettes (Bähring et al., 1994). The redox reagents were mixed in the saline solution, adjusted to pH 7.2 in dissociated cell recordings and to 7.4 in whole-mount cell recordings, and applied via bath or local superfusion. In order to void possible direct interference of redox reagents with GABA or glycine, GABA or glycine was generally applied after a brief washout of the redox reagent, a procedure we term rinsing.

Results

Effects of reducing and oxidizing agents on GABA- and glycine-evoked currents

As reported previously by our laboratories, rat retinal ganglion cells voltage clamped to −60 mV produce large inward currents in response to GABA or glycine; these currents reverse near 0 mV in symmetrical Cl^- solutions, as expected for Cl-selective $GABA_A$ and glycine channels (Tauck et al., 1988; Lipton, 1989; Bähring et al., 1994). Figure 1 shows typical effects of the disulfide-reducing agent dithiothreitol (DTT) and the strong oxidizing agent 5,5′-dithio-bis-(2-nitrobenzoic acid) (DTNB) on glycine-evoked currents of freshly dissociated retinal ganglion cells. Bath application of 2 mM DTT for 2 min, followed by brief rinsing, significantly attenuated 50 μM glycine-evoked currents (Fig. 1*A*). In contrast, in the same retinal ganglion cell, application of 500 μM DTNB potentiated the ligand-induced current.

Reduced glutathione (GSH) and oxidized glutathione (GSSG) are known to be endogenous redox agents and are present in the CNS in millimolar concentrations (Kosower and Kosower, 1978; Slivka et al., 1987). During whole-cell recordings, GSH (5 mM) mimicked the effects of DTT in suppressing glycine-evoked currents (Fig. 1*B*). Application of 5 mM GSSG, on the other hand, potentiated glycine-induced currents (Fig. 1*C*). The overall effects of GSH and GSSG were qualitatively consistent with those of the DTT and DTNB, respectively. However, the effects of GSH/GSSG appeared weaker than those of DTT/DTNB in agreement with the smaller redox potential of the glutathione congeners (Scott et al., 1963).

In contrast to their effects on glycine-activated currents, the opposite effects of these redox reagents were found on GABA-evoked currents (Fig. 2). For example, application of 500 μM DTNB significantly attenuated GABA-induced currents, while DTT potentiated 5 μM GABA-evoked peak currents (Fig. 2*A*). GSH (5 mM) slightly enhanced GABA-evoked responses (Fig. 2*B*); however, GSSG at 5 mM concentrations had very little if any effect on GABA-evoked currents (data not shown). The modulation polarities of DTT and DTNB on GABA- and glycine-evoked currents were not dependent on the concentration of GABA and glycine (see below), although relatively low concentrations of GABA (less than 15 μM) had to be used in order to prevent significant rundown of the currents.

The opposing effects of DTT or DTNB on GABA- and glycine-evoked currents were also demonstrated on the same retinal ganglion cell (data not illustrated). A composite of the effects of these redox reagents on glycine- and GABA-evoked currents in rat retinal ganglion cells is illustrated in Figure 3.

Interestingly, the effects produced by these redox agents on GABA- and glycine-evoked responses were spontaneously reversible. The typical recovery of the responses to glycine or GABA began within 2–4 min and was complete after 5–10 min of continuous washing (as shown in Figs. 1, 2).

Effect of redox reagents on glycine and GABA dose–response curves

Dose–response curves for glycine and GABA were generated in the continuous presence of DTT or DTNB. DTT (2 mM) shifted the glycine dose–response curve to the right and changed the EC_{50} from 50 μM to 80 μM (Fig. 4*A*). In contrast, DTNB (500 μM) slightly shifted the glycine dose–response curve to the left and diminished the EC_{50} to 40 μM. For GABA responses, DTT (2 mM) shifted the dose–response curve to the left and changed the EC_{50} from 47 μM to 27 μM, while DTNB (500 μM) did not display a significant effect under these conditions (Fig. 4*B*). Neither DTT nor DTNB changed the Hill coefficients of the GABA and glycine dose–response curves.

Effects of redox reagents on the retinal whole-mount preparation

Do these effects of redox reagents on glycine- and GABA-evoked responses represent an artifact of tissue culture preparations? In order to begin to answer this question, the effects of DTT and DTNB on GABA and glycine responses were further examined in the mouse retinal whole-mount preparation. Similar to the results on cultured neurons, DTT and DTNB differentially modulated GABA- and glycine-evoked currents. DTT enhanced

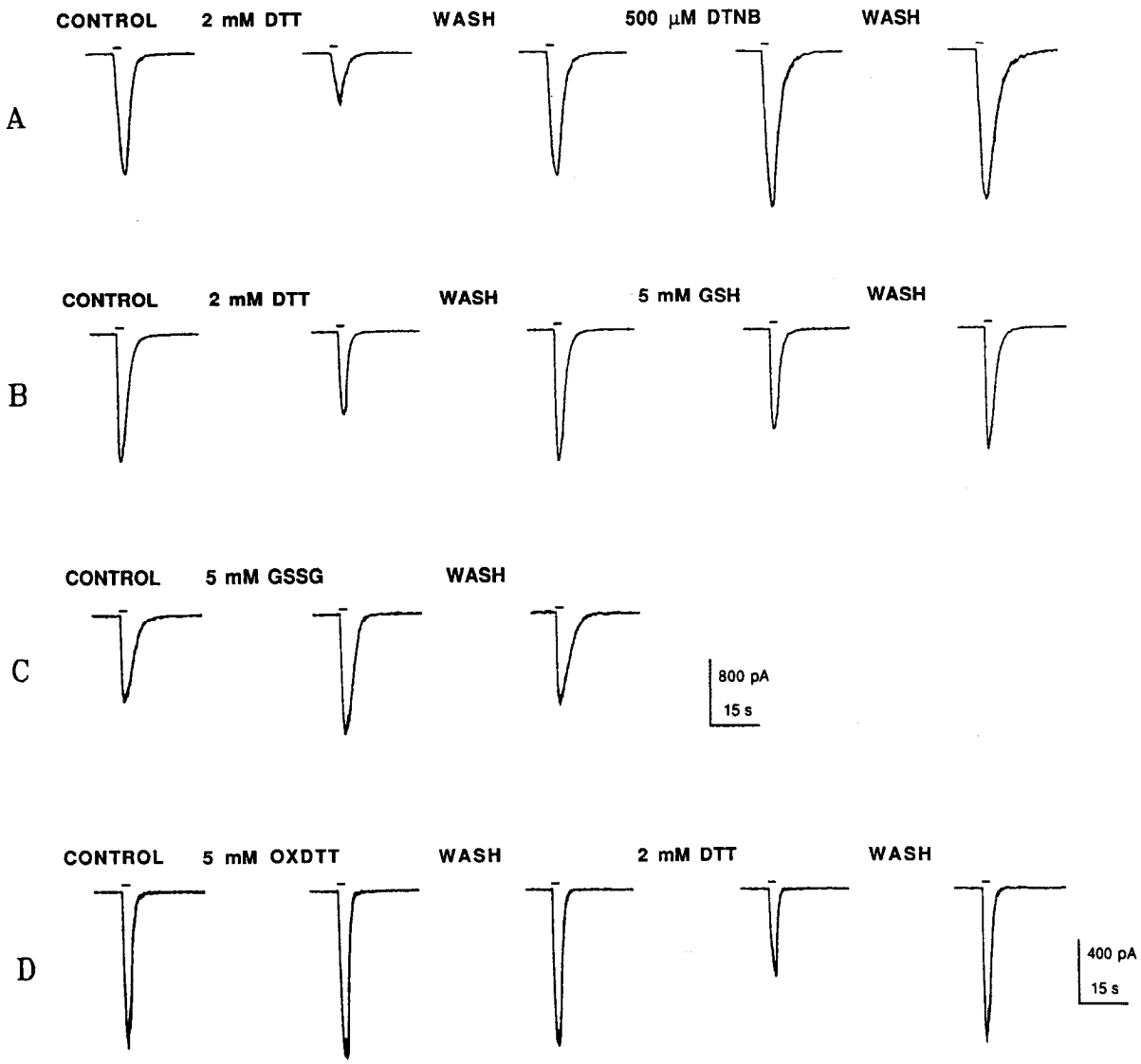

Figure 1. Reducing and oxidizing agents alter glycine-evoked whole-cell currents in rat retinal ganglion cells. *A,* The disulfhydryl-reducing agent DTT attenuated, while the oxidizing agent DTNB potentiated, glycine-activated currents. *B,* Reduced glutathione (*GSH*) mimicked the effects of DTT by attenuating the glycine-evoked current. *C,* Oxidized glutathione (*GSSG*) enhanced the glycine-evoked current. *D,* The oxidized form of DTT (*OXDTT*) slightly enhanced the glycine-evoked current. These experiments are representative of results on 41 retinal ganglion cells. Glycine (50 μM) was applied at intervals of at least 2 min via pneumatic pipettes for the duration indicated by the *horizontal lines.* Reducing and oxidizing agents were administered by bath superfusion for approximately 2 min, followed by rinsing, prior to the application of test pulses of glycine. The effects of the redox reagents began to reverse within 2–4 min of washout.

GABA-activated currents while suppressing glycine-evoked currents. In contrast, DTNB decreased GABA-elicited currents but potentiated glycine-activated currents (Fig. 5). Since exogenous glutathione is only poorly taken up into CNS slices (Rice et al., 1994), the effects of glutathione were not studied in detail in this preparation.

Action of redox reagents on thiol groups

The action of these redox reagents is thought to be quite specific for sulfhydryl groups or disulfide bonds. Several lines of evidence suggest that this is true in these experiments as well. First, application of DTT and DTNB by themselves did not produce any direct effect. We also found that the action of DTT and DTNB on glycine- and GABA-evoked responses was not voltage dependent. Similar effects were observed at both positive (+30 mV) and negative (−60 mV) holding potentials (data not shown). This finding indicates that the effects of these redox reagents are not due to the altering of charge distribution in the receptor–channel complex.

Next, we asked if redox reagents could interfere with the agonist or antagonist binding sites of inhibitory neurotransmitter receptors. We specially addressed this issue in retinal cell cultures on the inhibitory action of DTT on glycine-evoked responses. To test this possibility, we employed an application protocol similar to that described by Wu et al. (1990) using a combination of DTT and strychnine. In our system, 100 nM strychnine readily and reversibly blocked on average 38% (±5% SD, $n = 5$) of the 50 μM glycine-evoked current. In the presence or absence of 2 mM DTT, application of 100 nM strychnine produced a comparable level of inhibition (41 ± 7% SD, $n = 5$). In other words, the strychnine-induced inhibition was unaffected by previous suppression of the glycine response with

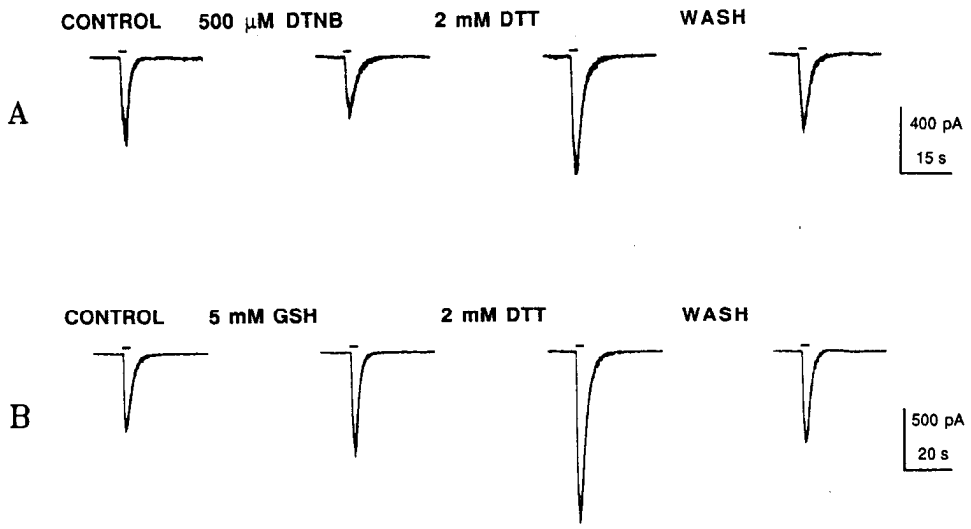

Figure 2. Reducing and oxidizing agents alter GABA-evoked whole-cell currents in rat retinal ganglion cells. *A,* DTNB attenuated while DTT potentiated currents activated by 5 μM GABA. *B,* Reduced glutathione (*GSH*) mimicked the effect of DTT by enhancing GABA-evoked currents. All agents were administered as described in the legend to Figure 1. Results are representative of those encountered in 24 retinal ganglion cells.

DTT. The opposite was also true—strychnine did not interfere with the inhibitory effect of DTT on glycine-evoked responses. The additivity of the effects of DTT and strychnine suggests that DTT does not interfere with the inhibitory effect of strychnine, at least at the concentrations examined.

As further supporting evidence for the specificity of the chemical reducing effect of DTT, oxidized DTT (*trans*-4,5-dihydroxy-1,2-dithiane) did not show any inhibitory effect on glycine-evoked responses of dissociated retinal ganglion cells. Instead, as predicted from its oxidizing nature, oxidized DTT resulted in a slight potentiation of the glycine-evoked current (15 ± 4% SEM, $n = 3$) (Figs. 1*D,* 3*A*). This result strongly suggests that the action of DTT is mediated through modulation of a redox-sensitive site and not by some other non-redox-related mechanism.

Finally, to confirm the notion that the effect of DTT was mediated by reaction with thiol groups (that possibly form a disulfide bond) on glycine and $GABA_A$ receptors, we used the irreversible thiol alkylating agent *N*-ethylmaleimide (NEM). NEM forms covalent bonds with cysteine residues and therefore should prevent further chemical reduction or oxidation of these sites. After chemical reduction with DTT (2 mM), followed by a short washout, NEM (1–5 mM) was superfused onto dissociated retinal ganglion cells. In this preparation, NEM itself was found to produce enhancement of glycine-evoked currents as well as desensitization of this current. This effect of NEM was similar to that of DTNB and was not unexpected since both reagents add bulky groups to cysteine residues, resulting in steric hindrance. The effect of NEM was irreversible, and subsequent application of DTT no longer attenuated glycine-induced currents (Fig. 6*A*). This finding strongly suggests that the inhibitory effect of DTT observed prior to the administration of NEM was indeed mediated by reactions involving thiol groups.

In a parallel series of experiments, NEM was also found to prevent the potentiating effect of DTT on GABA-evoked currents (Fig. 6*B*). The effect of NEM itself on GABA-evoked currents was variable. In some cases, NEM somewhat enhanced GABA-activated currents, similar to its effect on glycine responses. Taken together, the blockade of the effect of DTT on glycine and GABA responses by the alkylating agent NEM provides further evidence that the modulatory effect of redox reagents in this preparation is mediated by reactions with thiol groups. In the absence of NEM, the nature of the slow spontaneous reversibility of the effects of the redox reagents is not clear. One possible explanation for this reversibility could be the presence of endogenous redox agents, such as GSH and GSSG, which would be expected to slowly reverse the effects of the exogenously applied reagents. Moreover, the fact that DTT and DTNB have opposite effects when applied to native cells suggests that under basal conditions the redox state is set at an intermediate level for both $GABA_A$ and glycine receptors on retinal ganglion cells.

Discussion

We report here that GABA- and glycine-evoked responses in rat retinal ganglion cells are modulated by redox reagents. Chemical reducing agents enhance GABA-activated currents while suppressing glycine-activated currents, an observation that we have termed "differential redox modulation." The exact opposite effects on GABA and glycine responses are obtained when oxidizing agents are used instead of reducing agents. Most importantly, endogenous redox compounds, such as reduced and oxidized glutathione (GSH and GSSG), can modulate these ligand-gated currents, which are responsible for fast inhibitory neurotransmission in the CNS. Interestingly, similar differential modulation of GABA- and glycine-induced currents by progesterone have been reported (Wu et al., 1990), but the mechanisms of the modulation in the case of progesterone are not clear. We demonstrate here that the actions of sulfhydryl agents are specifically mediated through modulation of redox-sensitive sites comprised of one or more thiol groups. Sulfhydryl groups appear to be involved because DTT and DTNB modulate the ligand-gated currents, and NEM, a thiol alkylating agent, irreversibly blocks the effects of these redox agents. However, the exact site or sites of redox modulation on the $GABA_A$ and glycine receptors remain unknown. Molecular studies have shown that subunits of the nACh, $GABA_A$, and glycine receptors have two conserved cysteine residues, which form disulfide bonds. These thiol groups are located in an extracellular domain of the membrane receptor proteins (Grenningloh et al., 1987; Schofield et al., 1987; Olsen and Tobin, 1990; Betz, 1991). In addition, there is an additional disulfide bond on the glycine receptor and on the α-subunit of the nACh receptor. Although single cysteine residues could also participate in sulfhydryl modulation, it has

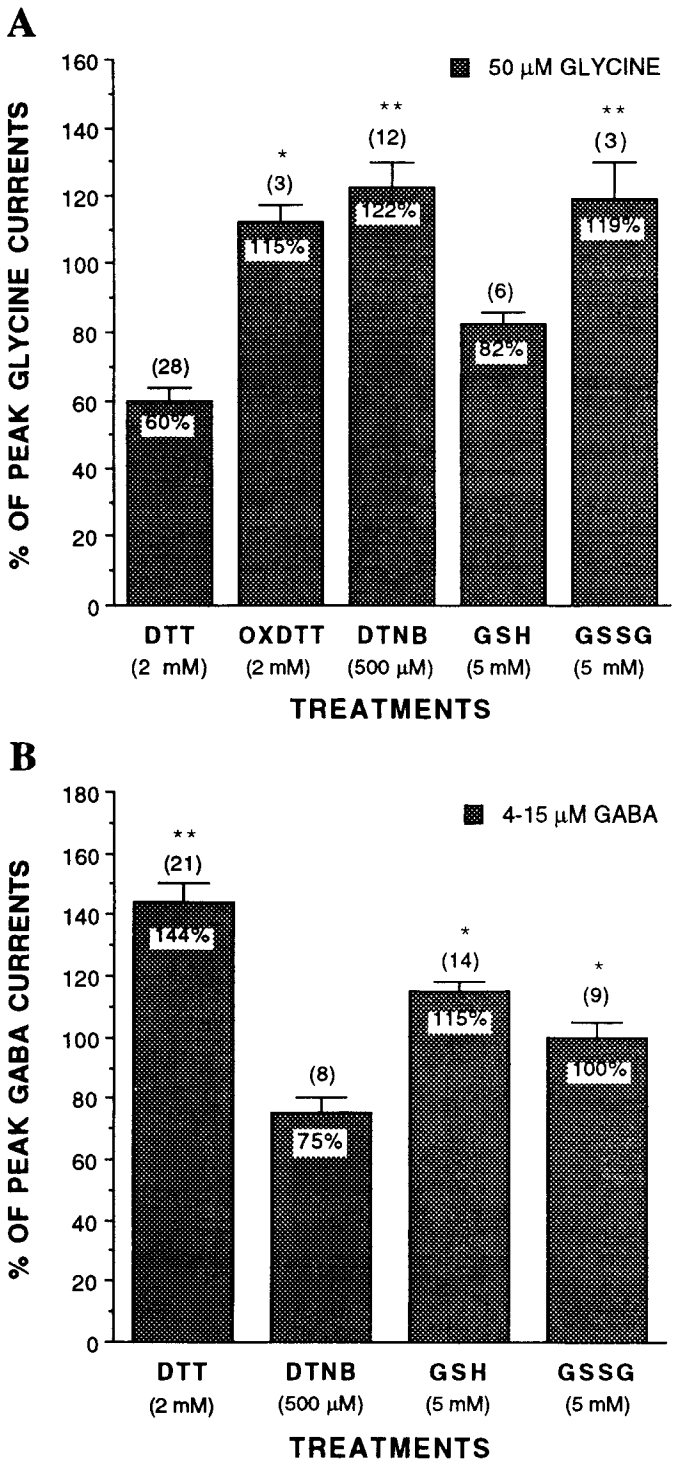

Figure 3. Summary of effects of redox agents on glycine- and GABA-evoked currents. Values are mean ± SEM for the indicated number of retinal ganglion cells. *Asterisks* indicate statistical significance compared to DTT (*A*) or DTNB (*B*) treatment (*$P < 0.005$; **$P < 0.001$ by ANOVA followed with a Scheffé multiple comparison of means). Drug additions were as in the legend to Figure 1. *A*, Oxidizing agents produced larger glycine-evoked responses while reducing agents resulted in smaller currents. *B*, Reducing agents produced larger GABA-evoked responses while oxidizing agents resulted in smaller currents.

been widely speculated that these conserved disulfide bonds are the targets of redox modulation (Laube et al., 1993). The modulation of glycine receptors by redox agents resembles that of the nACh receptor in that their responses are suppressed by chemical reducing agents. In contrast, redox modulation of the $GABA_A$ receptor resembles that of the NMDA receptor, as their currents are potentiated by reducing agents.

The mechanism of redox modulation of GABA- and glycine-evoked currents is not yet entirely clear. We do know that the suppression and potentiation of GABA and glycine responses by reducing and oxidizing agents is accompanied by a shift in the GABA and glycine dose–response curves and therefore a change in the EC_{50} value. Our results are therefore consistent with a mechanism of redox modulation mediated by altering the ligand binding affinities of these receptors, as reported previously for the nACh receptor (Karlin and Bartels, 1966; Karlin, 1969; Ben-Haim et al., 1975; Aronstam et al., 1978). Unfortunately, receptor binding studies of $GABA_A$ receptors have manifested conflicting results. Chemical reducing agents have been reported to decrease agonist binding in one case (Marangos and Martino, 1981) but to have no effect or increase agonist binding in others (Nishimura et al., 1982). In addition, recent studies of the glycine receptor reported that DTT did not significantly change the affinity of glycine binding (Ruiz-Gómez et al., 1991). However, receptor binding studies and our electrophysiological assays are performed under very different conditions, and therefore would not necessarily yield concordant results. Some mechanism other than agonist affinity for the receptor could also explain our results. For example, a recent study indicated that the thiol reagent *p*-chloromercuriphenylsulfonic acid (pCMBS) increases the level of endogenous GABA by increasing GABA release and decreasing its reuptake (Allan and Baier, 1992). However, this mechanism seems unlikely to explain the present data, since an increase in the level of endogenous GABA would desensitize GABA receptors and therefore suppress exogenous responses to GABA. Moreover, our ability to monitor the postsynaptic response to GABA, during patch-clamp recordings of isolated retinal ganglion cells, indicates that the effects that we observe occur at the receptor level. However, alternative mechanisms may exist. For instance, the mechanism of redox modulation of GABA- and glycine-evoked responses could be mediated by changes in coupling efficiency between receptor binding and opening of ion channels, or by a more direct modulation of the channel conductance or gating properties. Future studies at the single-channel level may shed further light on this subject.

In the rodent retina, GABA and glycine have been identified in amacrine cells that make synaptic connections on ganglion cells and modulate their receptive field properties (Pasantes-Morales et al., 1972; Vaughn et al., 1981; Tauck et al., 1988). Both of these transmitters mediate fast synaptic inhibition via the activation of receptor-coupled chloride channels. The reason for the existence of two seemingly redundant inhibitory neurotransmitter systems in the retina is not clear, although several possible explanations have been suggested (Slaughter and Pan, 1991). The differential modulation of $GABA_A$ and glycine receptors by redox agents may offer at least a partial answer to this question. Endogenous redox agents, such as glutathione, may be able to influence these two inhibitory neurotransmitter systems in a diametrically opposing manner. Of course, the physiological relevance of this regulation in the retina is dependent on the existence of endogenous redox agents at appropriate concentrations. High concentrations of glutathione (in the low millimolar

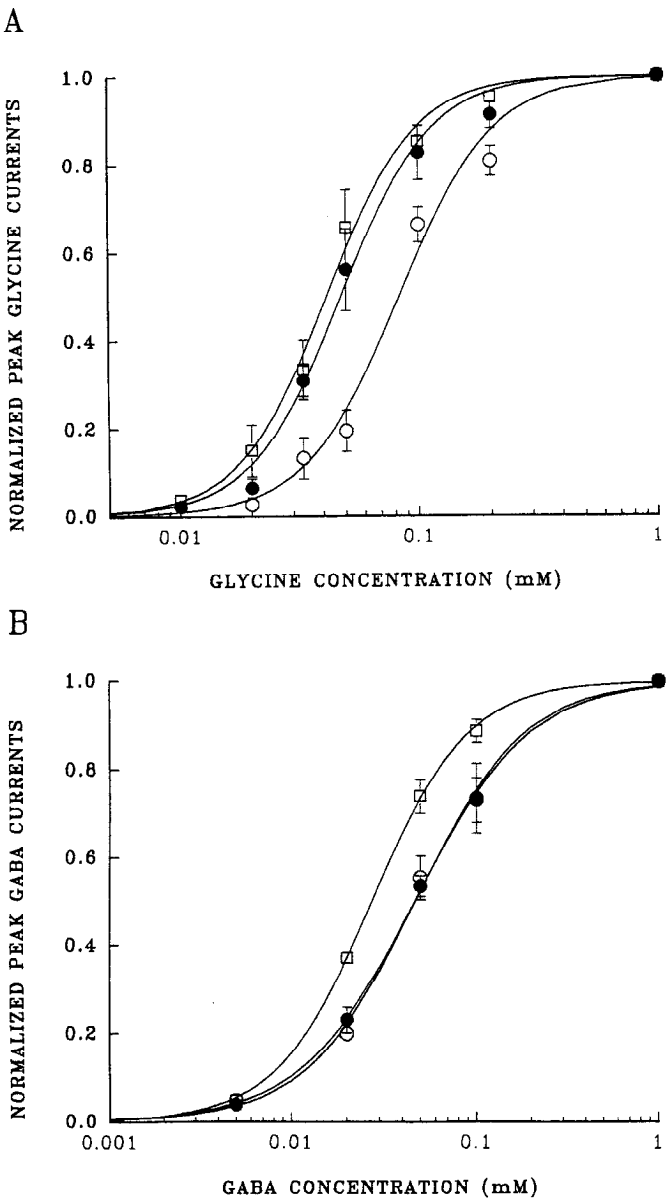

Figure 4. Effects of a reducing agent (DTT) and an oxidizing agent (DTNB) on the glycine and GABA dose–response curve of retinal ganglion cells. *A*, Whole-cell current responses elicited by glycine were normalized to the response to 1 mM glycine for 34 retinal ganglion cells. Each data point was averaged from at least three cells, and error bars are SEM. DTT (2 mM) shifted the glycine dose–response curve to the right (○) and changed the EC_{50} to 80 μM from 50 μM in the control (●). In contrast, DTNB (500 μM) slightly shifted the glycine dose–response curve to the left (□) and changed the EC_{50} to 40 μM. However, neither DTT nor DTNB significantly affected the Hill coefficient (n = 2.3 for control; n = 2.2 for DTT; n = 2.3 for DTNB). *B*, Whole-cell current responses evoked by GABA were normalized to the response to 1 mM GABA for 22 retinal ganglion cells. DTT (2 mM) shifted the dose–response curve to the left (○) and changed the EC_{50} to 27 μM from 47 μM in the control (●). DTNB (500 μM) did not display a significant effect under these conditions (□). Neither DTT nor DTNB significantly changed the Hill coefficient (n = 1.4 for control; n = 1.7 for DTT; n = 1.5 for DTNB). The dose–response curves were fitted by the least sum of the squares of the residuals (SIGMAPLOT computer program).

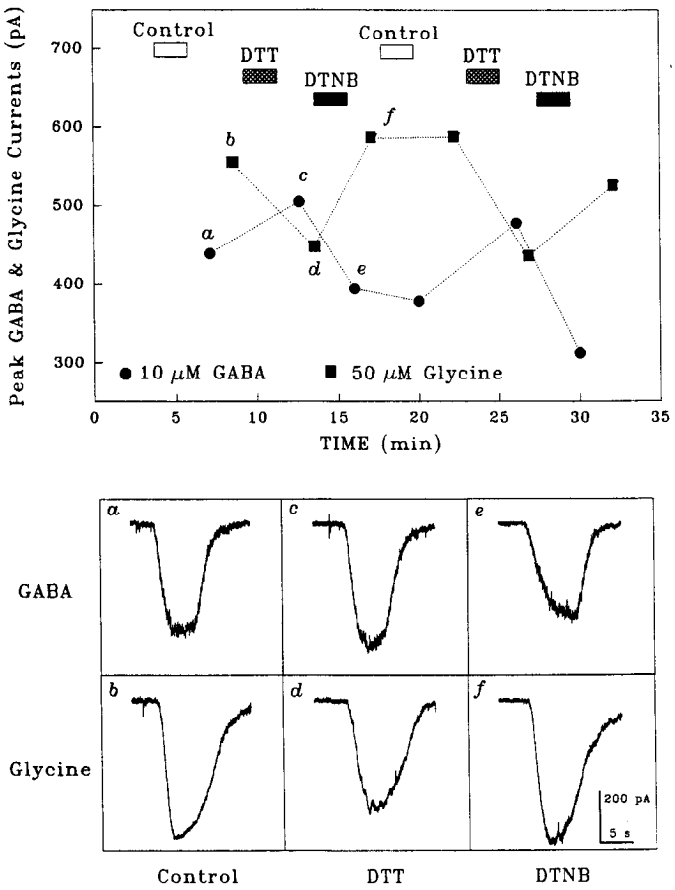

Figure 5. Demonstration of the opposite effects of DTT and DTNB on GABA- and glycine-evoked currents of a single retinal ganglion cell recorded from the mouse retinal whole-mount preparation on postnatal day 5. The responses marked *a–f* in the *bottom panel* represent the raw traces from the marked locations in the *top panel*. The results are representative of nine similar recordings.

range) are known to exist in the CNS in general, and in the retina in particular (Kosower and Kosower, 1978; Organisciak et al., 1984; Slivka et al., 1987). It is also known that GSH and the enzymatic activity associated with GSH metabolism are present in the retina (Herrmann and Moses, 1945; Winkler et al., 1986). Zängerle et al. (1992) recently reported the Ca^{2+}-dependent release of glutathione from depolarized neural tissue. The micromolar concentration of released glutathione measured in the extracellular space approached that of neurotransmitters. Micromolar concentrations of neurotransmitters in the extracellular space are thought to reflect low millimolar concentrations in the synaptic cleft (Clements et al., 1992). Hence, these findings suggest possible physiological relevance of millimolar levels of glutathione to synaptic events.

Moreover, the differential redox modulation of GABA and glycine responses could imply an important physiological role for endogenous redox reagents. GABA and glycine are thought to play distinct physiological roles in the retina (Daw et al., 1982; Massey and Redburn, 1987; Wässle and Boycott, 1991). For example, in the cat retina, GABA has been reported to act mainly as an inhibitory transmitter in the ON pathway while glycine acts mainly in the OFF pathway (Ikeda and Sheardown, 1983). In the mudpuppy retina, GABAergic inhibition is tonic whereas glycinergic inhibition appears to be more phasic (Belgum et al., 1982). GABA and glycine antagonists have also been

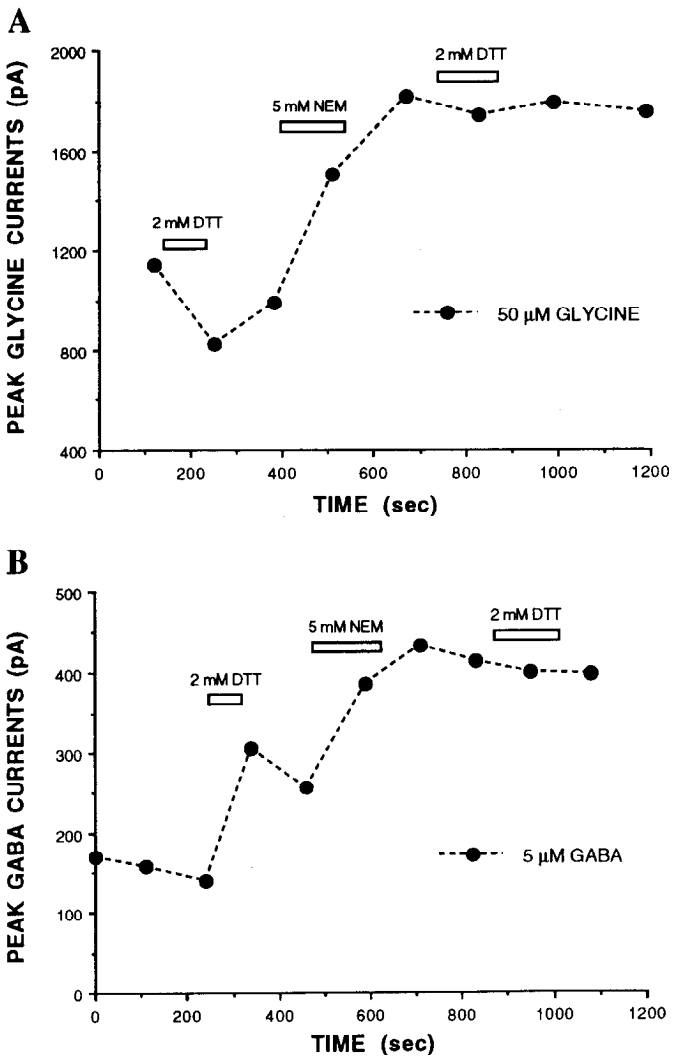

Figure 6. Alkylation of thiol groups with *N*-ethylmaleimide (NEM) prevents the further effect of reducing agents on glycine- or GABA-evoked responses of retinal ganglion cells. *A*, Application of the disulfide-reducing agent DTT (followed by washout) resulted in smaller glycine-evoked currents. Subsequent application of 5 mM NEM (followed by washout) resulted in somewhat enhanced responses to glycine. However, subsequent application of DTT did not significantly attenuate glycine-evoked currents. *B*, The same protocol as in *A* was tested on GABA-evoked currents. Following the application of NEM (and subsequent washout), DTT no longer enhanced GABA-evoked currents.

reported to block differentially the firing properties of retinal ganglion cells (Wyatt and Daw, 1976; Caldwell et al., 1978; Ariel and Adolph, 1985). In the presence of endogenous redox reagents *in vivo*, therefore, the differential modulation of these two inhibitory neurotransmitters could offer a mechanism for the selective regulation of retinal information processing.

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