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Asymmetric Cross-inhibition between $GABA_A$ and Glycine Receptors in Rat Spinal Dorsal Horn Neurons*

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Presynaptic nerve terminals of inhibitory synapses in the dorsal horn of the spinal cord and brain stem can release both GABA and glycine, leading to coactivation of postsynaptic $GABA_A$ and glycine receptors. In the present study we have analyzed functional interactions between $GABA_A$ and glycine receptors in acutely dissociated neurons from rat sacral dorsal commissural nucleus. Although the application of GABA and glycine activates pharmacologically distinct receptors, the current induced by a simultaneous application of these two transmitters was less than the sum of currents induced by applying two transmitters separately. Sequential application of glycine and GABA revealed that the GABA-evoked current is more affected by glycine than glycine-evoked responses by GABA. Activation of glycine receptors decreased the amplitude and accelerated the rate of desensitization of GABA-induced currents. This asymmetric cross-inhibition is reversible, dependent on the agonist concentration applied, but independent of both membrane potential and intracellular calcium concentration or changes in the chloride equilibrium potential. During sequential applications, the asymmetric cross-inhibition was prevented by selective $GABA_A$ or glycine receptor antagonists, suggesting that occupation of binding sites did not suffice to induce glycine and $GABA_A$ receptors functional interaction, and receptor channel activation is required. Furthermore, inhibition of phosphatase 2B, but not phosphatase 1 or 2A, prevented $GABA_A$ receptor inhibition by glycine receptor activation, whereas inhibition of phosphorylation pathways rendered cross-talk irreversible. Taken together, our results demonstrated that there is an asymmetric cross-inhibition between glycine and $GABA_A$ receptors and that a selective modulation of the state of phosphorylation of $GABA_A$ receptor and/or mediator proteins underlies the asymmetry in the cross-inhibition.

Although neurotransmission involves specific activation of receptor channels by distinct neurotransmitters, different classes of receptor can be colocalized at the same postsynaptic site and may be activated by the corelease of more than one type of neurotransmitter from the same presynaptic nerve terminal. Recently it has been demonstrated that simultaneous activation of different postsynaptic receptors by the coapplication of their specific neurotransmitter induces cross-modulation of their activation properties. This cross-talk phenomenon has been proposed to represent a fast adaptive process in controlling signal transmission (1). Negative cross-talk was demonstrated between ATP P2X and nicotinic acetylcholine receptors (2–5), between dopamine and adenosine receptors (6), between γ-aminobutyric acid type A ($GABA_A$)[1] and dopamine (1) or P2X receptors (7), as well as between dopamine and *N*-methyl-D-aspartic acid receptors (8). In general, the cross-talk between these receptors is characterized by a partial occlusion of transmitter-evoked currents, *i.e.* the sum of the amplitudes of responses evoked by each agonist is larger than the amplitude of responses evoked by coapplication of the two neurotransmitters. The molecular mechanism involved in the interaction between P2X and nicotinic acetylcholine receptors is unknown. Negative cross-talk between dopamine and adenosine receptors, between $GABA_A$ and dopamine receptors, and between dopamine and *N*-methyl-D-aspartic acid receptors involves direct intramembranous protein-protein interaction (1, 6, 8), whereas cross-talk between P2X receptor and $GABA_A$ receptors ($GABA_AR$) is modulated by chloride efflux and intracellular Ca^{2+} (7).

In the spinal cord, brain stem (9–12), and cerebellum (13), GABA and glycine can be coreleased by the same synaptic terminal, whereas $GABA_AR$ and glycine receptors (GlyR) can be coaggregated at the same postsynaptic site (13, 14). The ionotropic $GABA_A$ and GlyR both gate chloride-permeable channels but have distinct structures, resulting from oligomerization of specific subunits (15, 16). There is evidence suggesting that $GABA_AR$ and GlyR can interact negatively in mammalian (17, 18) or lamprey (19) spinal cord neurons, in olfactory bulb cells (20), and in the hippocampus (21), *i.e.* the effects of GABA and glycine are less than additive when they are coapplied. The mechanisms responsible for this cross-inhibition between GABA and glycine remain poorly understood. One possibility is that it reflects the presence, at least in the olfac-

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[1] The abbreviations used are: $GABA_A$, γ-aminobutyric acid type A; ATPγS, adenosine 5′-*O*-(3-thiotriphosphate); BAPTA, 1,2-bis(2-aminophenoxy)ethane-*N*,*N*,*N*′,*N*′-tetraacetic acid; CSPN, cyclosporin A; E_{Cl}, theoretical Cl^- equilibrium potential; E_{GABA} and E_{Gly}, GABA- and glycine-induced currents, respectively; $GABA_AR$, $GABA_A$ receptor(s); GlyR, glycine receptor(s); I_{GABA} and I_{Gly}, GABA- and glycine-induced Cl^- currents, respectively; OA, okadaic acid; V_h, holding potential.

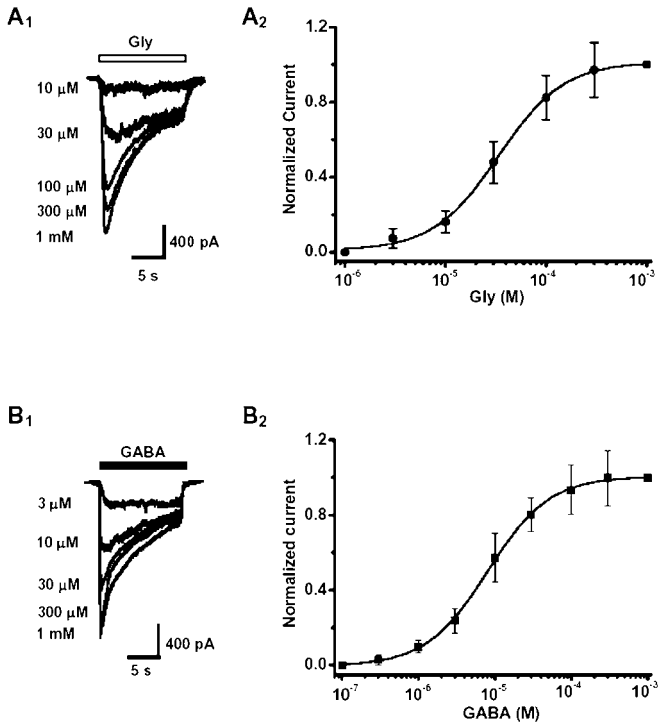

FIG. 1. **Glycine- and GABA-evoked currents in dissociated sacral dorsal commissural nucleus neurons.** A_1 and B_1, sample recordings demonstrating inward currents induced by glycine and GABA at various concentrations. The *bars* above each *trace* indicate the drug application period. A_2 and B_2, concentration-response relationships for I_{Gly} and I_{GABA}. I_{Gly} and I_{GABA} were normalized to the peak current amplitude induced by 1 mM Gly (A_2) and 1 mM GABA (B_2), respectively. Each point represents the mean ± S.E. of six to eight neurons. In this and subsequent figures, unless otherwise noted, the V_h was −50 mV.

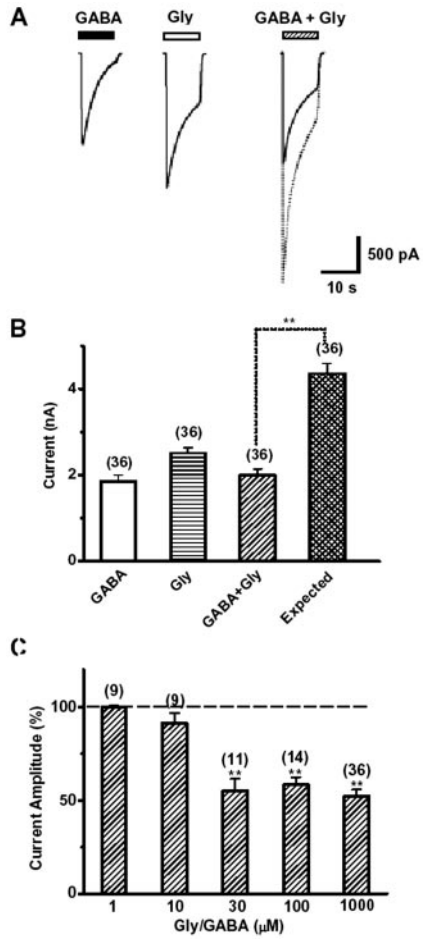

FIG. 2. **Cross-inhibition between I_{GABA} and I_{Gly}.** *A*, whole cell currents were induced by application of either 1 mM GABA or 1 mM Gly or by the simultaneous application of 1 mM GABA and 1 mM Gly. All three current traces were obtained from one neuron. The *dashed trace* represents the expected summation of the I_{GABA} and I_{Gly}. *B*, mean values of 36 experiments shown in *A*. *C*, summary of current inhibition data of different concentrations of GABA plus Gly. *Ordinates* represent $I_{GABA\ +\ Gly}/(I_{GABA} + I_{Gly}) \times 100\%$. In each *bar*, the concentrations of GABA and Gly were the same. The *dashed line* indicates $(I_{GABA} + I_{Gly})/I_{EX}$. In this and subsequent figures, the number of experiments is shown in *parentheses*. **, $p < 0.01$.

tory bulb, of a receptor subpopulation that can bind either GABA or glycine (20). However the identity of these postulated receptors remains unclear.

In the present study, we explored the mechanisms of the functional interaction between GlyR and GABA$_A$R by analyzing cross-inhibition between glycine- and GABA-induced Cl^- currents (I_{Gly} and I_{GABA}) in acutely dissociated rat sacral dorsal commissural nucleus neurons, using whole cell, patch clamp recording. Our results showed that cross-inhibition is asymmetric between GABA$_A$R and GlyR and that glycine-induced inhibition of GABA responses depends largely on protein dephosphorylation processes.

EXPERIMENTAL PROCEDURES

Cell Preparation—Rat sacral dorsal commissural nucleus neurons were acutely dissociated according to the method of Wu *et al.* (11). In brief, pentobarbital-sodium-anesthetized (45–50 mg kg^{-1}, intraperitoneally) Wistar rats (2 weeks old) were decapitated. A segment about 10–15 mm long of lumbosacral (L_5-S_3) spinal cord was quickly dissected out and immersed in the standard external solution at freezing temperatures. After removing attached dorsal rootlets and the pia matter on the lateral aspects of the cord, the spinal segment was fixed with cyanoacrylic glue to a 15 × 15-mm^2 agar block to support the spinal cord tissue. The tissue block was then placed in the cutting chamber of a vibratome tissue slicer (LEICA VT1000S, Leica Instruments Ltd., Wetzlar, Germany). A cold standard external solution (about 4 °C) bubbled with O_2 was subsequently placed in the chamber to immerse the tissue block. The spinal segment was sectioned to yield several transverse slices of thickness 400 μm. Slices were preincubated in oxygenated incubation solution for 30 min at room temperature (22–25 °C) and then treated enzymatically in oxygenated incubation solution containing Pronase (1 mg/5 ml) for 20 min at 31 °C. This treatment was followed by exposure to thermolysin (1 mg/5 ml) for 15 min. After the enzyme treatment, slices were kept in enzyme-free incubation solution for 1 h. Then a portion of dorsal horn region was micropunched out and transferred into a culture dish filled with the standard external solution. Neurons were mechanically dissociated with fire-polished Pasteur pipettes under visual guidance under a phase contrast microscope (IX70, Olympus Optical Co., Ltd., Tokyo, Japan). Within 20 min, isolated neurons had attached to the bottom of the culture dish and were ready for electrical recording. The care and use of animals in these experiments followed guidelines and protocols approved by our institutional Animal Care and Use Committee.

Electrophysiology—Whole cell, voltage clamp recordings were made at room temperature (22–25 °C). Culture dishes (Corning 430165, Corning, Inc., Corning, NY) were used as recording chambers and were perfused at 0.5–2.0 ml/min with the standard external solution. Patch pipettes were pulled from glass capillaries with an outer diameter of 1.5 mm (Narishige, Tokyo, Japan) on a two-stage puller (PP-830, Narishige), and had a resistance of 4–6 megohms. Membrane potentials were corrected for the liquid junction potential, which had a measured value of 3–4 mV. The series resistance (R_s), estimated from visual cancellation of the capacity transient, was 5–15 megohms. In most experiments, 80–90% series resistance compensation was applied. Artifacts caused by inadequate voltage clamp and space clamp were minimized by selecting for experiment neurons bearing no or short processes. Transmitter-evoked currents recorded from the cell soma normally did not exceed 5 nA. Unless otherwise noted, CsCl solution was used in the recording pipette. After taking these precautions, the largest recorded currents did not differ in time course from the smaller ones. To ensure cell dialysis, data for measurements were obtained at

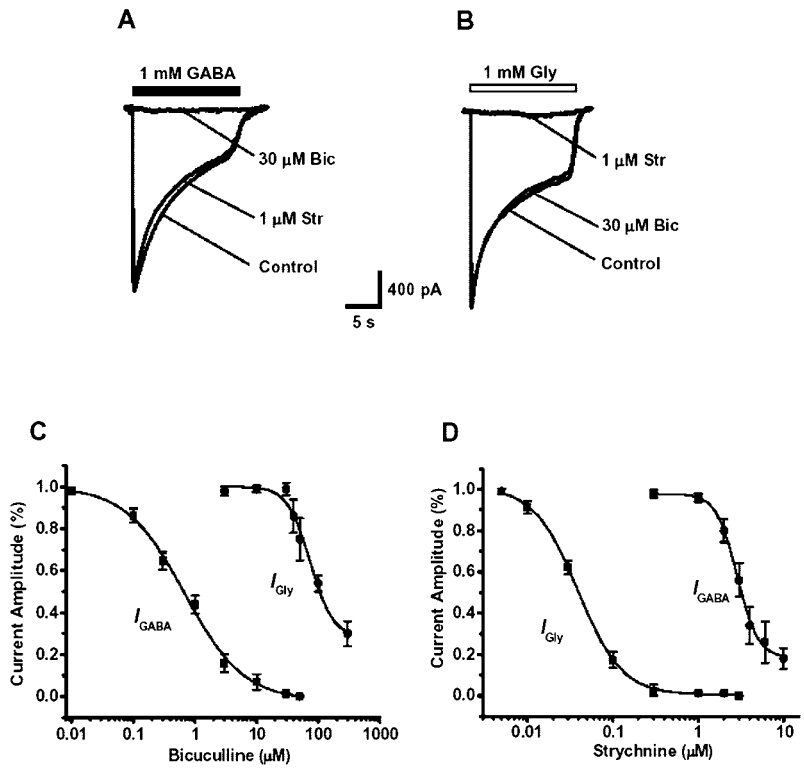

FIG. 3. **Effect of bicuculline and strychnine on I_{GABA} and I_{Gly}.** *A* and *B*, 30 μM bicuculline (*Bic*) was an effective antagonist of 1 mM I_{GABA} but had little effect on 1 mM I_{Gly}. To the contrary, 1 μM strychnine (*Str*) had little effect on 1 mM I_{GABA} but inhibited 1 mM I_{Gly}. *C* and *D*, concentration-response relationships for inhibition of 1 mM I_{GABA} and 1 mM I_{Gly} by bicuculline (*C*) and strychnine (*D*), respectively. The antagonists were perfused 30 s before simultaneous application of the agonists and the antagonists. The amplitudes of I_{GABA} and I_{Gly} were measured at the peak and expressed as values relative to the control response induced by 1 mM GABA or 1 mM Gly alone, respectively. Each *point* represents the mean of 6–10 neurons.

least 3–5 min after the whole cell configuration was established.

Solutions—The composition of incubation solution was (in mM) 124 NaCl, 24 $NaHCO_3$, 5 KCl, 1.2 KH_2PO_4, 2.4 $CaCl_2$, 1.3 $MgSO_4$, and 10 glucose, and it was aerated with 95% O_2 and 5% CO_2 to a final pH of 7.4. The standard external solution contained (in mM) 150 NaCl, 5 KCl, 1 $MgCl_2$, 2 $CaCl_2$, 10 HEPES, and 10 glucose. The pH was adjusted to 7.4 with Tris. The osmolarity of all bath solutions was adjusted to 310–320 mosm/liter with sucrose. The ionic composition of the internal solution medium was (in mM) 120 CsCl, 30 NaCl, 0.5 $CaCl_2 2H_2O$, 1 $MgCl_2 6H_2O$, 5 EGTA, 2 MgATP, and 10 HEPES with the pH adjusted to 7.2. Stocks of MgATP stored at −20 °C were dissolved in the intracellular solution shortly before use to a final concentration of 2 mM. Unless otherwise noted, the membrane potential was held at −50 mV in the voltage clamp studies.

Drugs and Application System—Cyclosporin A (CSPN), staurosporine, and $Li_4ATP\gamma S$ were obtained from Biomol. Okadaic acid (OA) and GF 109203X were from Tocris (Bristol, UK), and H89, KN93, and genistein were from Sigma. All other drugs were from Sigma. Phosphatase inhibitors and protein kinase inhibitors were added to the pipette solution. Agonists or antagonists of GlyR and GABA$_A$R were diluted with extracellular solution to a final concentration and applied via the "Y-tube" method as described previously (22). The tip of the drug tube was positioned between 50 and 100 μm away from the patched neurons. This system allows a complete exchange of external solution surrounding a neuron within 20 ms. Throughout the experiment the bath was perfused continuously with the standard external solution.

Data Acquisition and Analysis—Signals were filtered at 1 kHz, data were collected with an Axopatch 200B patch clamp amplifier (Axon Instruments, Foster City, CA), which was connected to a Pentium III computer equipped with Digidata 1320A, Clampex, and Clampfit software (Axon Instruments). Data were analyzed with the pCLAMP software (Axon Instruments) and ORIGIN for Windows (Microcal Software, Northampton, MA). Results are presented as the mean ± S.E. (n = number of cells), with statistical significance assessed by Student's t test for two groups' comparison or one-way analysis of variance test for multiple comparisons. A p value of <0.05 or 0.01 was considered statistically significant. To evaluate the strychnine and bicuculline concentrations at half-maximal inhibition of I_{Gly} and I_{GABA} (IC_{50}), the mirror image of the Michaelis-Menten equation

$$I/I_{max} = 1/(1 + (C/IC_{50})^n) \quad \text{(Eq. 1)}$$

was fitted to the data by the least squares method. Current desensitization was fitted by exponential functions, beginning shortly after the peak of response, using Clampfit 8.0 software. Curve fitting was performed using a simplex algorithm least squares exponential fitting routine with single or double exponential equations of the form

$$I(t) = I_f\exp(-t/\tau_f) + I_s\exp(-t/\tau_s) + I_p \quad \text{(Eq. 2)}$$

where I_f and I_s are the amplitudes of the fast and slow components, respectively; I_p was the amplitude of the nondesensitized current; and τ_f and τ_s are respective time constants.

RESULTS

GABA- and Glycine-induced Responses—Cross-talk between GABA$_A$R and GlyR was analyzed on acutely dissociated sacral dorsal commissural nucleus neurons from P14 rats. At this age, neurons express the mature form of GABA$_A$R and GlyR (15, 23). Whole cell recording at the holding potential (V_h) of −50 mV showed that application of GABA or glycine evoked inward chloride currents in all cells tested. The reversal potentials of GABA- and glycine-induced currents (E_{GABA} and E_{Gly}) were −2.5 ± 1.1 (S.E., n = 6) and −1.3 ± 0.8 mV (n = 5), respectively, and were not significantly different ($p > 0.1$; unpaired t test). They were close to the theoretical Cl^- equilibrium potential (E_{Cl}) calculated from the Nernst equation (−1.3 mV), based on the external and internal Cl^- concentration used in the recording (see "Experimental Procedures").

The saturating concentration and EC_{50} for GABA or glycine were determined by analyzing concentration-response curves for responses to a 10-s application of agonists (Fig. 1). I_{Gly} and I_{GABA} were normalized to the peak amplitude of the currents evoked by the application of glycine and GABA at 1 mM, respectively. The normalized data were fitted using a single isotherm function of the form,

$$I/I_{max} = 1/(1 + (EC_{50}/[\text{agonist}])^H) \quad \text{(Eq. 3)}$$

where I_{max} is the maximum current amplitude, I/I_{max} is the normalized current amplitude, EC_{50} is the agonist concentration producing 50% of the I_{max}, and H is the Hill coefficient. This fit produced an EC_{50} of 8.1 and 34 μM for GABA and glycine, respectively. The Hill coefficients for GABA and glycine were 1.18 and 1.27, respectively.

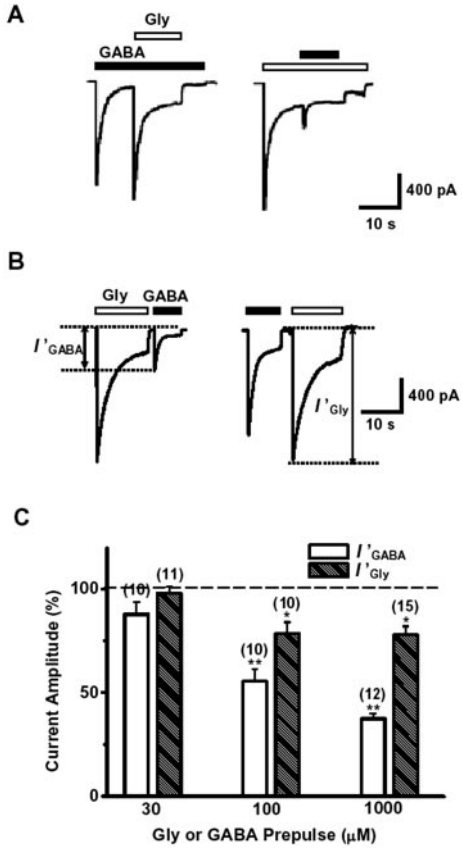

FIG. 4. **Asymmetric cross-interaction between I_{GABA} and I_{Gly}.** *A* and *B*, sample recordings demonstrating application of 1 mM GABA and 1 mM Gly with two different drug application modes. *C*, pooled percentage inhibition of I_{GABA} or I_{Gly} after Gly (I'_{GABA} in *B*) or GABA (I'_{Gly} in *B*) prepulse. Ordinates represent I'_{GABA}/I_{GABA} or $I'_{Gly}/I_{Gly} \times 100\%$. The *dashed line* indicates the control I_{GABA} or I_{Gly} in the absence of Gly or GABA prepulse. Note the much stronger reduction of I_{GABA} by Gly than contrariwise. In this and subsequent figures, unless otherwise noted, sequential application means that one agonist was applied immediately after (200–400 ms) another agonist application. *, $p < 0.05$; **, $p < 0.01$.

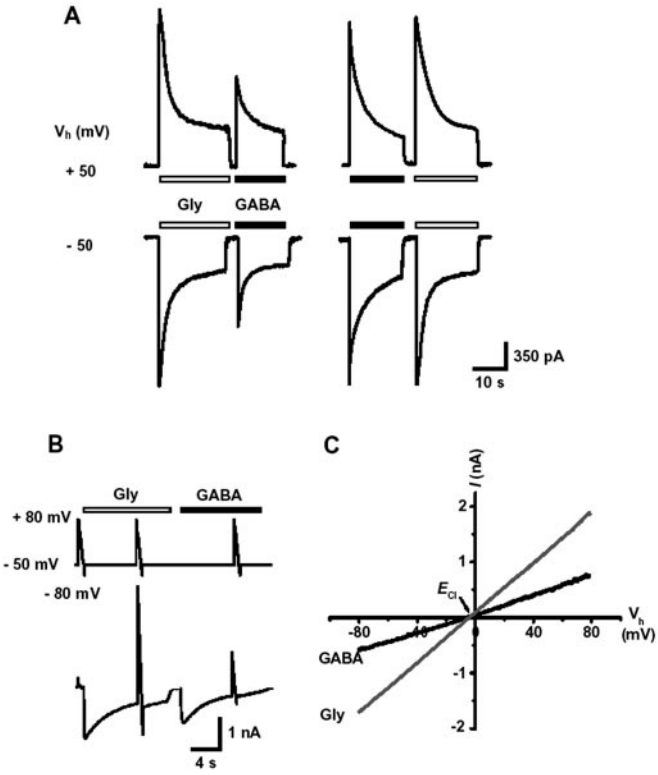

FIG. 5. **Direction of current flow did not influence cross-inhibition.** *A*, *left*, inhibition of I_{GABA} by Gly prepulse was similar at −50 mV and at +50 mV (E_{Cl} = 0 mV). *A*, *right*, the inhibition of I_{Gly} by GABA prepulse was similar at −50 mV and at +50 mV. The traces in *A* were from the same cells. *B*, representative current trace obtained from the ramp voltage command. The experimental protocol is shown above the current trace. Three voltage ramps ranging from −80 to +80 mV were applied. Gly and GABA were applied to the cell and covered the last two ramps. Traces obtained from the first ramp measured background or leakage currents. *C*, current-voltage (I-V) curves for I_{GABA} and I_{Gly} obtained from traces shown in *B*.

Cross-inhibition between I_{GABA} and I_{Gly}—The above experiments suggest that the saturating concentration for GABA or glycine for inducing neuronal responses was ≥1 mM. This suggests that responses evoked by the application of 1 mM GABA or 1 mM glycine should be additive if they are induced by the activation of independent receptors. We therefore compared peak amplitudes of currents induced by the application of either GABA or glycine (at 1 mM) and by the simultaneous application of both agonists. As shown in Fig. 2*A*, coapplication of GABA and glycine evoked currents with an amplitude that was significantly lower than the expected sum (I_{EX}) of currents evoked separately by GABA and glycine (Fig. 2*B*), suggesting a cross-inhibition between I_{GABA} and I_{Gly} (Fig. 2*B*; $n = 36$; $p < 0.01$; *t* test). A quantitative analysis of this cross-inhibition and its dependence on concentrations of both agonists is shown in Fig. 2*C*. At 10 μM GABA and glycine, no significant cross-inhibition occurred (91.5 ± 5.3% of the I_{EX}; $n = 9$; $p > 0.05$). Significant cross-inhibition was observed at 30 μM GABA and glycine (55.1 ± 6.4% of the I_{EX}; $n = 11$; $p < 0.01$), and the effect appears to saturate beyond 30 μM concentrations of the two agonists.

Cross-inhibition Was Not Caused by Nonspecific Receptor Activation by GABA and Glycine—It has been suggested that at high concentrations GABA may activate GlyR (24, 25), resulting in an apparent cross-talk between GABA$_A$R and GlyR (20). We therefore used selective antagonists to determine the specificity of GABA and glycine in their receptor activation (Fig. 3). At 30 μM bicuculline, a selective GABA$_A$R antagonist, the I_{GABA} induced by 1 mM GABA was totally abolished, whereas the I_{Gly} induced by 1 mM glycine was unaffected (Fig. 3, *A* and *B*). Conversely, 1 μM strychnine, a potent GlyR antagonist, completely abolished I_{Gly} without affecting I_{GABA}. Dose-response curves for bicuculline and strychnine inhibition were obtained from currents evoked by glycine or GABA at 1 mM. The bicuculline inhibited I_{GABA} in a concentration-dependent manner with an IC_{50} of 0.68 μM (Fig. 3*C*). Strychnine also inhibited I_{GABA}, but only at concentrations higher than 1 μM (Fig. 3*D*), with IC_{50} of 2.83 μM (Fig. 3*D*). The inhibitory effect of bicuculline on I_{Gly} was also evident only at concentrations higher than 30 μM (Fig. 3*C*), a concentration that completely suppressed I_{GABA}. Strychnine was effective in suppressing I_{Gly} at concentrations ≥ 0.01 μM (Fig. 3*D*). In contrast, the IC_{50} values for the inhibition of I_{Gly} were 0.04 and 74.2 μM for strychnine and bicuculline, respectively. Taken together, these results suggest that at a concentration of 1 mM, GABA and glycine are unlikely to cross-activate GlyR or GABA$_A$R, respectively, significantly.

Cross-inhibition between GABA$_A$R and GlyR Was Asymmetric—A different mode of drug application was used to explore further the interactions between I_{Gly} and I_{GABA} and to examine relative contributions of GABA$_A$R and GlyR to the cross-inhibition. As shown in Fig. 4*A*, GABA or glycine responses obtained in the presence of the other agonist were reduced in amplitude, with GABA-evoked responses more strongly affected by glycine than the reverse condition. The cross-inhibi-

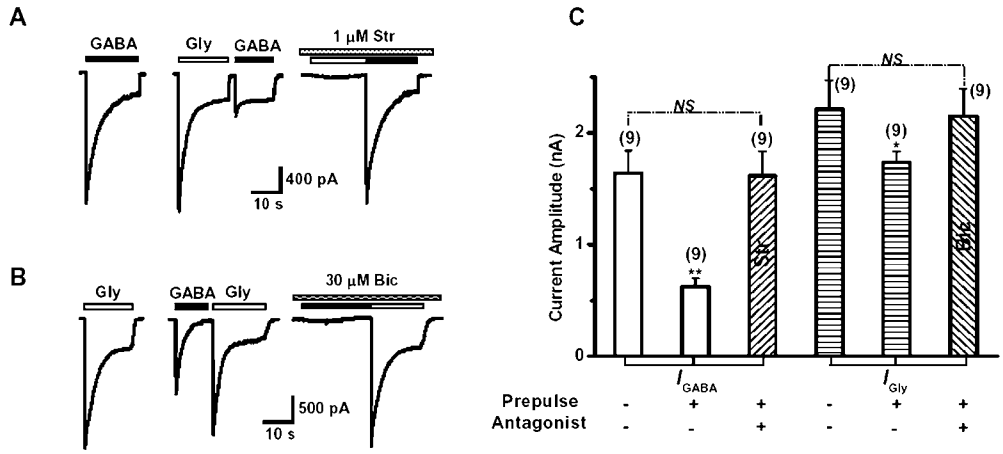

FIG. 6. **Functional receptors are required for the current inhibition.** *A*, whole cell currents induced by 1 mM GABA and sequential application of Gly and GABA at 1 mM in the absence or presence of strychnine (*Str*). *B*, whole cell currents induced by 1 mM Gly and sequential application of GABA and Gly at 1 mM in the absence or presence of bicuculline (*Bic*). In the presence of 1 μM strychnine or 30 μM bicuculline, the sequential application of GABA and Gly induced a current with amplitude and kinetics similar to the current induced by GABA or Gly alone. The recordings in *A* and *B* were from different neurons. *C*, statistical data showing the pooled results obtained on nine cells. Strychnine or bicuculline was pretreated for 30 s before simultaneous application with GABA or Gly. *NS* indicates no statistical significance. *, $p < 0.05$; **, $p < 0.01$.

tion was also observed during sequential application of 1 mM GABA and 1 mM glycine or vice versa. As shown in Fig. 4*B*, when glycine was applied before GABA, the amplitude of I_{GABA} became 37.4 ± 2.5% ($n = 12$; $p < 0.01$) of the control values observed in the absence of glycine. Similarly, I_{Gly} was reduced to 77.9 ± 4.0% ($n = 15$; $p < 0.05$) of the control value after a prepulse of 1 mM GABA. Thus the suppression of I_{GABA} by GlyR activation was significantly larger than inhibition of I_{Gly} by GABA$_A$R activation ($p < 0.01$). Fig. 4*C* summarizes the percent reduction in the mean amplitude of I_{GABA} and I_{Gly} when different prepulse concentrations of glycine and GABA were used, respectively. Glycine became effective in cross-inhibition at concentrations ≥ 30 μM, whereas GABA was effective at 100 μM. This asymmetric cross-inhibition between GABA$_A$R and GlyR did not depend on chloride fluxes or changes in E_{Cl} during receptor activation. For E_{Cl} close to 0 mV, cross-inhibition was observed at both $V_h = -50$ and +50 mV (Fig. 5*A*). Preactivation of GlyR decreased I_{GABA} to 37.4 ± 2.5% of the control at $V_h = -50$ mV ($n = 12$) and to 38.5 ± 4.1% of control at $V_h = +50$ mV ($n = 12$). Similarly, preactivation of GABA$_A$R decreased I_{Gly} to 77.9 ± 4.0% of the control at $V_h = -50$ mV ($n = 10$) and to 79.5 ± 4.5% of the control at $V_h = +50$ mV ($n = 10$). The differences between percent changes at two V_h values were all not significant ($p = 0.1$, paired *t* test). Reversal potentials of response evoked by successive applications of glycine and GABA at 1 mM were estimated by voltage ramp application during the steady-state phase of the evoked currents (Fig. 5*B*). As shown in Fig. 5*C*, successive glycine- and GABA-evoked responses had similar reversal potentials. During the glycine prepulse E_{Cl} was −4.15 ± 0.4 mV, whereas it was −3.98 ± 0.58 mV during successive GABA application ($n = 8$).

Receptor Channel Activation Is Required for Cross-inhibition—The data shown in Fig. 4 suggest that cross-inhibition depends on the prepulse agonist concentration. However, it remained unclear whether specific receptor channel opening and thus changes in receptor conformation triggered by the prepulse application are required for asymmetric cross-inhibition. To address this issue, we have used specific competitive antagonists for GABA$_A$R and GlyR, bicuculline and strychnine, respectively. Sequential applications of glycine-GABA at 1 mM (Fig. 6*A*), or GABA-glycine at 1 mM (Fig. 6*B*) were performed in the presence of 1 μM strychnine and 30 μM bicuculline, respectively, at a concentration that selectively inhibits GlyR or GABA$_A$R completely (Fig. 3, *C* and *D*). As shown in Fig. 6, *A* and *B*, during sequential applications of transmitters, application of the antagonist known to compete with transmitter binding of the preactivated receptor prevented cross-inhibition. Fig. 6*C* summarizes the results from all experiments ($n = 9$) by comparing the amplitudes of control currents (in the absence of prepulses and antagonists) and currents observed after prepulse application, in the absence or presence of either strychnine (for I_{GABA}) or bicuculline (I_{Gly}). We found that in the presence of bicuculline, I_{Gly} observed after the GABA prepulse had the same amplitude as the control I_{Gly} (2.151 ± 0.243 *versus* 2.213 ± 0.259 with or without bicuculline, $p > 0.1$). Similarly, in the presence of strychnine, I_{GABA} observed after the glycine prepulse had the same amplitude as the control I_{GABA} (1.615 ± 0.226 *versus* 1.638 ± 0.204 with or without strychnine, $p > 0.1$). These data indicate that cross-inhibition between GlyR and GABA$_A$R cannot be explained solely by ligand binding and suggest that activation of receptor channel is required.

Kinetics of Cross-inhibition—Inhibition of I_{GABA} by preactivation of GlyR was accompanied by significant changes in the time course of the desensitizing I_{GABA} component. This was not the case for I_{Gly} when GABA$_A$R was preactivated. To examine potential changes in the desensitization of I_{Gly} or I_{GABA} during cross-inhibition, time constants of the desensitizing phase of the corresponding current were measured. The desensitization time course of I_{Gly} evoked by 10-s applications of 1 mM glycine with or without GABA prepulses could be fitted by the sum of two exponential curves with time constants τ_f and τ_s. As shown in Fig. 7, preactivation of GlyR accelerated the desensitization of GABA$_A$R (Fig. 7, *A* and *B*). In contrast, preactivation of GABA$_A$R did not change GlyR desensitization kinetics significantly (Fig. 7*C*).

We have also examined whether cross-inhibition between GlyR and GABA$_A$R was reversible and whether it requires simultaneous activation of the two amino acid receptors. As shown in Fig. 8A, we found that increasing the time interval between sequential applications of glycine and GABA at 1 mM (or vice versa) resulted in a progressively reduced cross-inhibition. The effect of time interval between successive applications of agonist on cross-inhibition was analyzed on 12 and 14 cells for I_{GABA} and I_{Gly} inhibition, respectively. Half-inhibition of I_{GABA} was obtained at interpulse intervals close to 41 s (Fig. 8B_1). Cross-inhibition cannot be evoked for I_{GABA} for interpulse intervals ≥55 s. The time interval for I_{Gly} inhibition evoked by

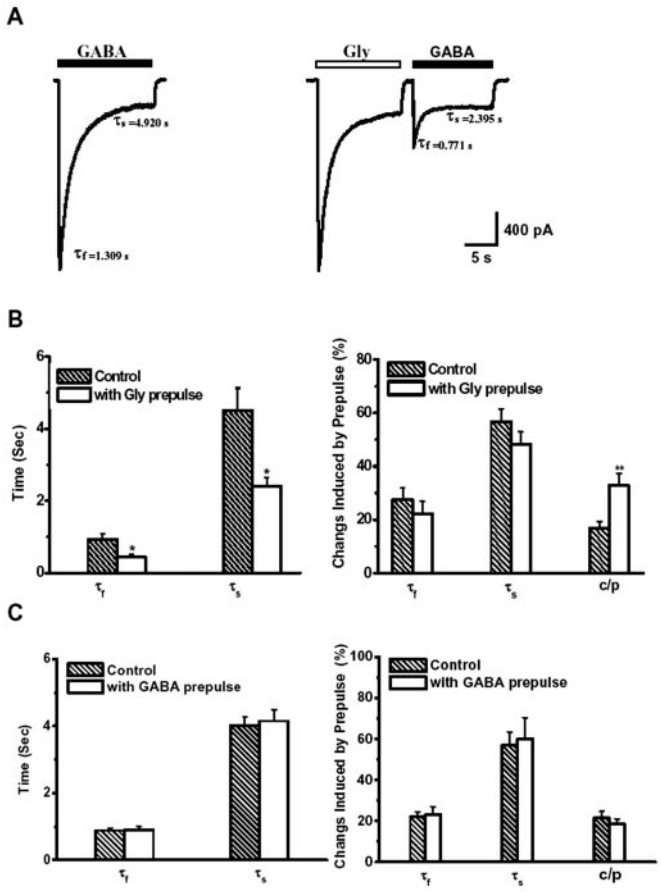

FIG. 7. **Effect of preapplication of Gly or GABA on I_{GABA} or I_{Gly} desensitization.** *A*, 1 mM I_{GABA}-desensitized time course was fitted with the sum of two exponential curves alone. I_{GABA} recorded after 1 mM Gly prepulse displayed a marked decreased in desensitization time constants. *B*, pooled data obtained on 10 different cells. τ_f and τ_s are the fast and slow desensitization time constants of I_{GABA} before (*dashed bar*) and after Gly prepulse (*empty bar*). The relative amplitude of τ_f and τ_s is shown without (*dashed bar*) and after the Gly prepulse (*empty bar*). *c/p* represents the percentage of nondesensitized I_{GABA} component without (*dashed bar*) and after Gly prepulse (*empty bar*) ($n = 10$). *C*, pooled data illustrating the desensitization time constants and relative amplitude of I_{Gly} without (*dashed bar*) and after GABA prepulse (*empty bar*) ($n = 26$).

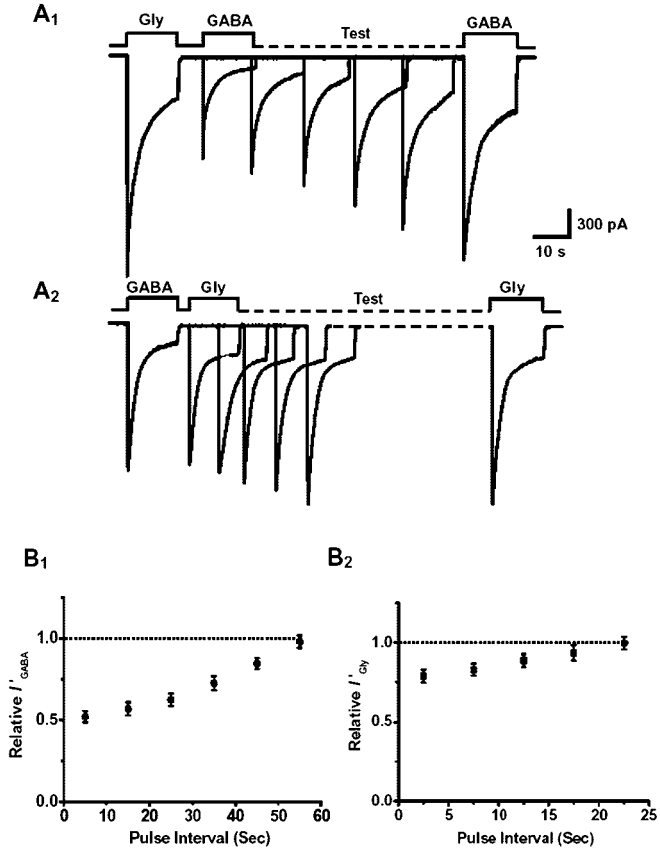

FIG. 8. **Time-dependent recovery of I_{GABA} or I_{Gly} with various intervals between Gly and GABA applications.** A_1 and A_2, illustration of the experimental protocols. Conditioning responses were obtained by applying a saturating concentration of GABA or glycine (1 mM for 10 s). Conditioning pulses were followed at various time intervals from 2 to 60 s by application of Gly or GABA. B_1 and B_2, normalized amplitude of I_{GABA}, I_{Gly} measured at various time intervals after application of Gly (B_1, I'_{GABA}, $n = 12$) or GABA (B_2, I'_{Gly}, $n = 14$).

GABA prepulses at which no cross-talk occurred was shortened (≥22.5). Half-inhibition of I_{Gly} occurred at time intervals close to 14 s (Fig. 8B_2).

Inhibition of I_{GABA} by GlyR Activation Depends on Phosphatase 2B—It is known that phosphatase 2B (calcineurin) activity can inhibit GABA$_A$R activation (26–28) and modulate GABA$_A$R desensitization kinetics (29, 30). We therefore asked whether phosphatase activity might be involved in the cross-inhibition between GABA$_A$R and GlyR. We first examined the effect of the phosphatase 2B inhibitor CSPN on the asymmetric cross-inhibition elicited by sequential application of 1 mM glycine and GABA at 1-s intervals. When loaded into neurons via the patch electrode, 500 nM CSPN prevented I_{GABA} inhibition by preactivation of GlyR. In contrast, CSPN had no significant effect on the inhibition of I_{Gly} evoked by GABA$_A$R activation (Fig. 9, *A1* and *B*). When glycine and GABA were coapplied, 500 nM CSPN blocked cross-talk by 84.6 ± 5.2% ($n = 9$).

Loading the neuron with 1 μM okadaic acid at a concentration that specifically blocks phosphatase 1 and 2A (31) did not prevent I_{GABA} inhibition by preapplication of glycine or I_{Gly} inhibition by preapplication of GABA. In contrast, loading the neuron with a concentration of OA (5 μM) known to inhibit phosphatase 2B (30) prevented I_{GABA} inhibition by GlyR preactivation (Fig. 9, *A2*, *A3*, and *B*). OA became nonspecific at concentration higher than 1 μM and mimics the effect of CSPN. Similar to that found for CSPN, 5 μM OA had no significant effect on I_{Gly} inhibition evoked by GABA prepulses. Because phosphatase 2B activation may depend on intracellular Ca^{2+} concentration, we have examined the effect of buffering intracellular Ca^{2+} with BAPTA. When the neurons were loaded with 15 mM BAPTA via the recording pipette, I_{GABA} was decreased to 39.7 ± 5.3% of its control value, whereas I_{Gly} was reduced to 81.2 ± 4.5% of the control following the glycine or GABA prepulse, respectively (Fig. 9, *A4* and *B*). These values did not differ significantly from the cross-inhibition obtained in control conditions ($p > 0.1$; unpaired t test). Thus the results indicate that changes in intracellular Ca^{2+} were not directly involved in the cross-inhibition. Inhibition of I_{GABA}, but not of I_{Gly}, depends on phosphatase 2B activity, whereas phosphatase 1 and 2A did not appear to be involved.

The level of substrate phosphorylation depends on the action of kinases and phosphatases in the neuronal cytoplasm. Prevention of cross-inhibition by phosphatase 2B inhibition may result from a shift in balance in favor of phosphorylation of the substrate by protein kinases. We first tested this hypothesis by loading the neuron with ATPγS, which facilitates protein phosphorylation by donating a thiophosphate group in a kinase-mediated reaction that resists hydrolysis by phosphatases (32). As shown in Fig. 10, loading the cells with 150 μM ATPγS (with Li^+) prevented I_{GABA} inhibition induced by prepulse activation of GlyR. The effect of ATPγS on I_{GABA} inhibition increased with

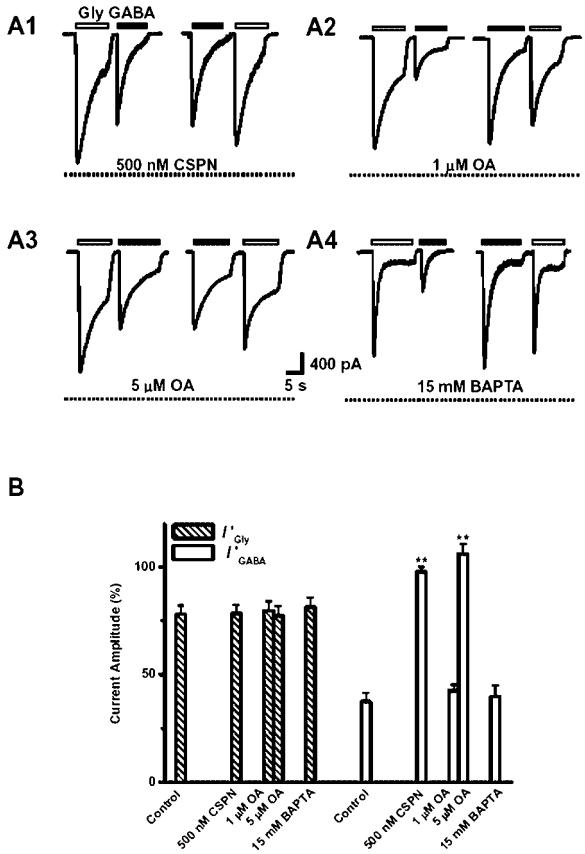

FIG. 9. **Inhibition of phosphatase 2B prevented cross-inhibition.** *A1*, example of currents evoked on the same cell by sequential applications of glycine then GABA (*left*) or GABA then glycine (*right*) in the presence of 500 nM CSPN. The same protocol was applied to study the effect of 1 μM OA (*A2*), 5 μM OA (*A3*), or 15 mM BAPTA (*A4*). *B*, pooled data illustrating percent inhibition of I_{GABA} or I_{Gly} after Gly or GABA prepulse in the presence of CSPN, 1 μM or 5 μM OA, or BAPTA. Each *bar* represents the mean of 10–16 neurons. Note that CSPN and 5 μM OA prevented the inhibition of I_{GABA} by Gly preapplication, whereas 1 μM OA and BAPTA had no effect on the cross-inhibition. **, $p < 0.01$.

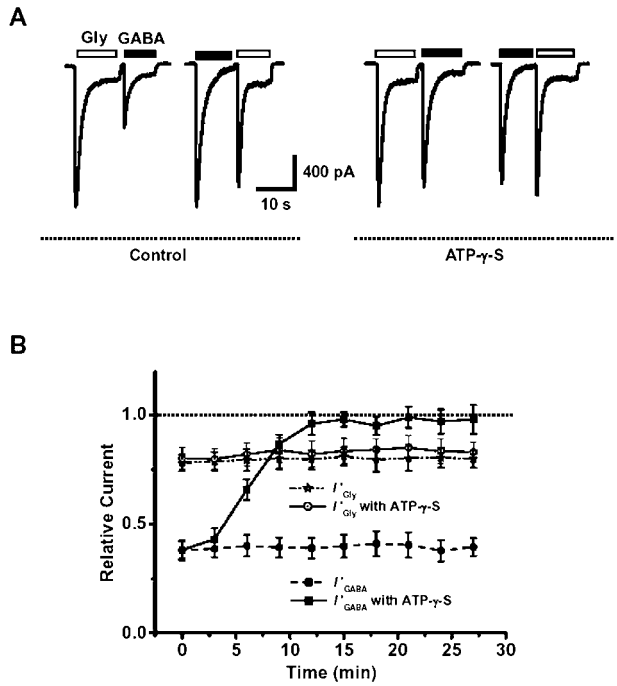

FIG. 10. **Inhibition of cross-talk by nonhydrolyzable ATP.** *A*, sample recordings demonstrating cross-talk between GABA$_A$ and GlyR in the absence (*left*) and in the presence of intracellular $Li_4ATP\gamma S$ (*right*). Note that adding ATPγS in the intracellular recording solution can prevent the inhibition of I_{GABA} by glycine preapplication only. *B*, pooled data illustrating the percentage of inhibition of I_{GABA} or I_{Gly} after glycine or GABA prepulse in the presence of ATPγS changes with time ($n = 6$–10). The full effect of ATPγS on the inhibition of I_{GABA} by glycine prepulse occurred 10 min after the beginning of the intracellular dialysis. Note that ATPγS had no effect on the inhibition of I_{Gly} by GABA preapplication.

time after the onset of whole cell configuration and completely abolished the inhibition within 15 min. The effect is unlikely to result from the loading of Li^+ because loading of LiCl (up to 20 mM) did not affect the cross-inhibition (data not shown; $n = 4$). In contrast to the effect on I_{GABA} cross-inhibition, I_{Gly} inhibition evoked by GABA prepulse application was not affected by loading of ATPγS (Fig. 10).

To confirm further the role of protein phosphorylation, we analyzed the effect of 5 μM staurosporine, a nonselective protein kinase inhibitor, on the cross-inhibition between GABA$_A$R and GlyR (Fig. 11). If changes in GABA$_A$R activation properties resulted from changes in the balance between phosphorylation and dephosphorylation processes, protein kinase inhibition should render cross-inhibition irreversible. The protocol used to determine time interval dependence of cross-inhibition in the presence of staurosporine was identical to that described in Fig. 8. In control conditions, recovery from cross-inhibition occurred in less than 50 s (Fig. 8). However, staurosporine evoked a rundown of I_{GABA} when applied alone. This was not the case for I_{Gly}. A 50% rundown of I_{GABA} was found after 40–50 min. To overcome this problem, the effects of staurosporine on cross-inhibition were tested when I_{GABA} amplitude had declined to 50%. Change in GABA response amplitude evoked by glycine preapplication in the presence of staurosporine was compared with change in GABA response amplitude with time in the presence of staurosporine but without preapplication of glycine. As shown in Fig. 11*B*, I_{GABA} was only slightly decreased 100 s after attaining 50% rundown. Staurosporine cannot block the inhibition of I_{GABA} or I_{Gly} evoked by preactivation of GlyR or GABA$_A$R, respectively. However, in these conditions we observed no recovery from I_{GABA} inhibition up to 2 min after evoking cross-inhibition. In contrast, staurosporine had no effect on the recovery of I_{Gly} (Fig. 11*B*). These results indicate that kinase activity is needed for GABA$_A$R but not for GlyR to recover from cross-inhibition. Interestingly, specific antagonists of protein kinase A (H89, 10 μM, $n = 6$ cells), protein kinase C (GF 109203X, 3 μM, $n = 6$ cells), tyrosine kinase (genistein, 5 μM, $n = 10$ cells) or calmodulin-dependent protein kinase II (KN93, 10 μM, $n = 10$ cells) had no effect on cross-inhibition and its recovery. However, applying a mixture of all four kinase inhibitors mimicked the staurosporine effect on the recovery from cross-inhibition.

DISCUSSION

In this study, we have demonstrated that activation of GABA$_A$R and GlyR in acutely dissociated rat spinal dorsal horn neurons can induce specific asymmetric cross-inhibition of currents induced by GABA and glycine. Our results reveal a novel form of interaction between signal events mediated by two distinct anionic channels and show that specific involvement of intracellular phosphorylation pathways triggered by GlyR activation underlies the asymmetry in the cross-inhibition between the GABA$_A$R and GlyR.

Cross-inhibition Depends on Intracellular GABA$_A$R and GlyR Signaling—Although negative cross-talk between GABA$_A$R and GlyR has been suspected previously (20, 21, 33), the underlying mechanisms remain controversial. Trombley *et*

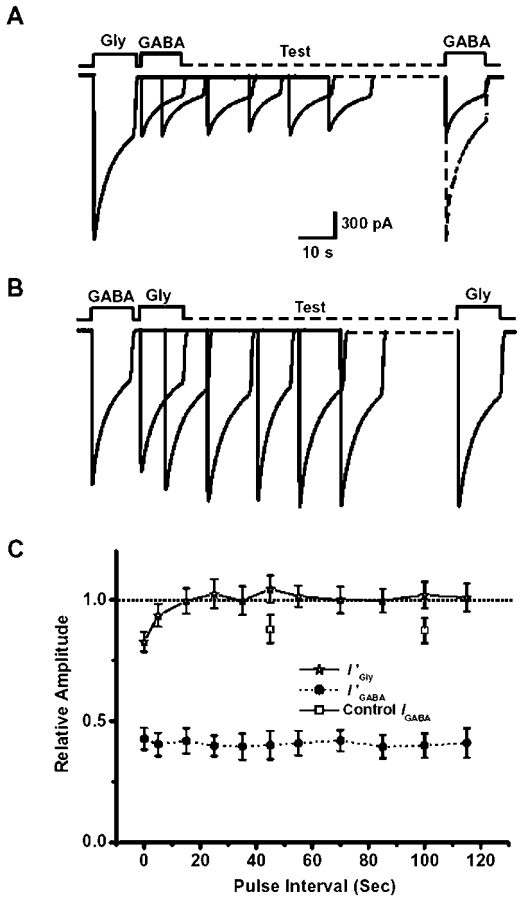

FIG. 11. **Inhibition of I_{GABA} by GlyR activation became irreversible after the inhibition of protein kinases.** *A* and *B*, illustrations of the experimental protocols. *A*, in the presence of staurosporine (a nonspecific protein kinase), the I_{GABA} decreased with time. To overcome this problem paired application of glycine and GABA was performed when I_{GABA} reached 50% of the control response amplitude (measured at the beginning of the recording). The response amplitudes were then compared with currents recorded on different cells without paired application at the same latency after the beginning on the recordings (*empty squares* in *C*). In the presence of staurosporine preapplication of glycine can still inhibit I_{GABA}, but in this case inhibitory cross-talk became irreversible for interpulse intervals up to 2 min. *B*, in the presence of staurosporine the preapplication of GABA can still inhibit I_{GABA}, but in this case inhibitory cross-talk was still reversible. *C*, normalized amplitude of I_{GABA} and I_{Gly} after glycine or GABA prepulse recorded at various time intervals (n = 6–12). *Empty squares* are normalized control amplitude values of I_{GABA} without glycine prepulse.

al. (20) proposed that the interactions between I_{GABA} and I_{Gly} in the olfactory bulb reflect direct actions of GABA on GlyR or glycine on GABA$_A$R. This is not the case in rat spinal dorsal horn neurons. In this spinal cord preparation, we found a small minority of neurons lacking either glycine or GABA responses. The currents evoked by GABA and glycine were not affected by prepulse of glycine and GABA, respectively (data not shown). Similar findings were reported for acutely isolated hippocampal neurons (21). In the present study, we have shown that the specific competitive antagonist bicuculline, when applied at a concentration that blocked 100% of I_{GABA}, did not alter I_{Gly}. Similarly, I_{GABA} was not modified by strychnine when applied at a concentration that totally suppressed I_{Gly}. Furthermore, the inhibition of I_{GABA} by the application of bicuculline prevented the inhibition of I_{Gly} by preapplication of GABA. At a prepulse concentration of 1 mM, there is no direct effect of GABA and glycine on GlyR and GABA$_A$R, respectively. Likewise, I_{GABA} was not depressed by prepulse application of glycine in the presence of strychnine. These results indicate that activation of GABA$_A$ and glycine receptor channels is required for the cross-inhibition.

What downstream signaling events associated with the receptor channel activation are involved in the cross-inhibition? It is unlikely that alterations in the Cl^- gradient resulting from channel activation mediate the cross-inhibition, as proposed previously by Grassi (33). We found that the inhibition remained when I_{Gly} and I_{GABA} were changed from inward to outward, indicating that the movement of Cl^- is not critical for the inhibition. Furthermore, measurements of agonist-induced Cl^- currents with the application of a voltage ramp also showed no change in the reversal potential for the Cl^- current (34). These data indicate that cross-inhibition is a receptor-mediated event unrelated to the Cl^- flux across the membrane.

Asymmetry in Cross-inhibition—When GABA and glycine were coapplied, the Cl^- current was smaller than the sum of the two individual currents evoked by the application of each agonist. The occlusion level depended on the concentration of the two agonists, and it was nearly maximal when the concentration was ≥30 μM. Thus, the intensity of the cross-inhibition between GABA$_A$R and GlyR might be determined by the number of activated receptors, as proposed previously for the interactions between P2X and GABA$_A$R (7).

To determine the respective contribution of the two agonists to the cross-inhibition, we examined the responses evoked by sequential application of these two compounds. Preapplication of glycine more strongly inhibited I_{GABA} than preapplication of GABA did to I_{Gly}. The level of current inhibition depended on the concentration of the prepulse agonist, in a manner consistent with that found when GABA and glycine were coapplied. Furthermore, the effect of prepulse glycine on I_{GABA} is accompanied by a decrease in the desensitization time constants of the current response. This was not the case for the effect of GABA prepulse on I_{Gly}. Because the effect of desensitization was observed during successive applications of the two agonists, the changes in GABA$_A$R desensitization kinetics by GlyR activation remain even when GlyR is fully deactivated. This effect on the nonactivated GABA$_A$R appears to persist for more than 45 s after the end of the application of glycine. This effect cannot be accounted for simply by cross-desensitization between these two receptors (35) because only GABA$_A$R desensitization properties were modified. These results are also in sharp contrast to the previous reports on the inhibitory cross-talk between ionotropic receptors. No changes in receptor desensitization properties were observed during cross-inhibition between GABA$_A$R and P2X (7) or between P2X and nicotinic acetylcholine receptors (2).

Cross-inhibition of GABA$_A$R by Glycine Depends on Dephosphorylation—Our results indicate that inhibition of I_{GABA} by preapplied glycine involves phosphatase 2B activity, whereas its recovery requires protein kinase activity. Our findings suggest the following model of cross-inhibition: Glycine preapplication results in dephosphorylation by phosphatase 2B and inhibition of I_{GABA}, whereas recovery from inhibition depends on rephosphorylation of GABA$_A$R or of associated proteins. Activation of phosphatase 2B requires the activation of GlyR, but rephosphorylation of GABA$_A$R or of an associated protein does not require the activation of either GlyR or GABA$_A$R. Our observations also indicate that in the absence of glycine prepulse, the balance between basal phosphorylation/dephosphorylation results in stable kinetic properties of the GABA$_A$R during repetitive applications of GABA.

There are three potential mechanisms by which GlyR activation may evoke dephosphorylation by phosphatase 2B. First, activated GlyR may directly activate phosphatase 2B. To our knowledge, there is no existing evidence in support of this

possibility. Alternatively, phosphatase 2B is activated indirectly by elevation of the intracellular Ca^{2+} concentration (27). Although activation of immature GlyR could elevate the intracellular Ca^{2+} concentration of neurons (36), it is unlikely that glycine application evoked a Ca^{2+} increase in the present neuronal preparation obtained from mature animal in the voltage clamp mode. Moreover, loading the neuron with BAPTA, a high affinity calcium chelator, did not modify the cross-inhibition between the two receptors. Thus the activation of phosphatase 2B by glycine prepulse does not seem to be mediated by a calcium-dependent process. Finally, GlyR activation may directly change $GABA_AR$ conformation through receptor-receptor interaction as proposed previously for some ionotropic receptors as for P2X2 and 5-HT3 receptors (37). This could result in the exposure of phosphorylation sites of $GABA_AR$ favorable to phosphatase 2B and/or unfavorable to protein kinase binding.

A large fraction of phosphatase 2B is known to be associated with the plasma membrane (38), a condition favorable for its rapid modulation of membrane receptors. Effectively, phosphatase 2B can modulate $GABA_AR$ desensitization kinetics (29) even during outside-out recordings (30). The slow recovery from cross-inhibition may reflect a slower process of rephosphorylation by cytoplasmic protein kinases. However, such a mechanism would require that GlyR is closely associated with $GABA_AR$. Indeed, $GABA_AR$ and GlyR are colocalized at inhibitory synapses in the spinal cord and brain stem (10, 39), and it has been demonstrated recently that GABA and glycine can be coreleased from inhibitory synapses in rat sacral dorsal commissural nucleus (11).

Cross-inhibition of GlyR Function by GABA—GABA preapplication did not change GlyR desensitization kinetics, its effect on current amplitude was less pronounced compared with the effect of glycine on I_{GABA}, and the recovery from inhibition was faster for I_{Gly} than I_{GABA}. The effects of GlyR subunit phosphorylation on receptor function remain controversial, and they largely depend on subunit combination (15). Our results indicate that inhibition of GlyR activity by GABA is unlikely to result from receptor subunit dephosphorylation or phosphorylation processes because inhibition of phosphatases or protein kinases did not alter the GABA effect on I_{Gly}. The most likely mechanism for the inhibition of I_{Gly} by $GABA_AR$ activation is through direct coupling between these two receptors. However, we cannot exclude the possibility that other unknown cytoplasmic signaling molecules may mediate the functional interaction between these two receptors.

Physiological Significance of the Cross-inhibition—GABA and glycine can be colocalized in the same presynaptic terminals (40) and can be coreleased in several brain regions (9–13), and their associated receptors are colocalized in the postsynaptic density (39), thus providing the physiological conditions for the cross-talk between $GABA_AR$ and GlyR. A recent study demonstrating that GABA and glycine interact at the synaptic level is in favor of such a speculation (11). The cross-inhibition of these two major inhibitory neurotransmitter systems could have important functional implications because it may represent an alternative means of limiting excessive inhibition at the synapses where GABA and glycine act as cotransmitters. In particular, GlyR activation and the subsequent phosphatase 2B-dependent $GABA_AR$ dephosphorylation would influence the shape and amplitude of GABAergic postsynaptic currents by speeding up $GABA_AR$ desensitization kinetics. Indeed, $GABA_AR$ desensitization is fast enough to control the time course of GABA-evoked synaptic events (41). Interestingly, a recent work has demonstrated that activity-dependent physical and functional interaction between phosphatase 2B and $GABA_AR$ is necessary and sufficient for inducing long term depression at hippocampal CA1 inhibitory synapses (42). Although corelease of GABA and glycine has not yet been detected in the hippocampus, a functional cross-inhibition has been shown for hippocampal $GABA_AR$ and GlyR (21). Whether or not the phosphatase 2B-dependent cross-inhibition between $GABA_AR$ and GlyR observed in the present study could regulate the synaptic transmission and plasticity remains to be established. Finally, because both $GABA_AR$ and GlyR are targets for many clinical therapeutics, it would be of interest to know whether and how drugs acting at one receptor may shift the synaptic influence toward the other transmitter.

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REFERENCES

1. Liu, F., Wan, Q., Pristupa, Z. B., Yu, X. M., Wang, Y. T., and Niznik, H. B. (2000) *Nature* **403,** 274–280
2. Nakazawa, K. (1994) *J. Neurosci.* **14,** 740–750
3. Barajas-Lopez, C., Espinosa-Luna, R., and Zhu, Y. (1998) *J. Physiol.* (*Lond.*) **513,** 671–683
4. Zhou, X., and Galligan, J. J. (1998) *J. Physiol.* (*Lond.*) **513,** 685–697
5. Khakh, B. S., Zhou, X., Sydes, J., Galligan, J. J., and Lester, H. A. (2000) *Nature* **406,** 405–410
6. Ginés, S., Hillion, J., Torvinen, M., Le Crom. S., Casadó, V., Canela, E. I., Rondin, S., Lew, J. Y., Watson, S., Zoli, M., Agnati, L. F., Vernierá, P., Lluis, C., Ferré, S., Fuxe, K., and Franco, R. (2000) *Proc. Natl. Acad. Sci. U. S. A.* **97**, 8606–8611
7. Sokolova, E., Nistri, A., and Giniatullin, R. (2001) *J. Neurosci.* **21,** 4958–4968
8. Lee, F. J., Xue, S., Pei, L., Vukusic, B., Chery, N., Wang, Y., Wang, Y. T., Niznik, H. B., Yu, X. M., and Liu, F. (2002) *Cell* **111,** 219–230
9. Jonas, P., Bischofberger, J., and Sandkuhler, J. (1998) *Science* **281,** 419–424
10. O'Brien, J. A., and Berger, A. J. (1999) *J. Neurophysiol.* **82,** 1638–1641
11. Wu, L. J., Li, Y., and Xu, T. L. (2002) *Neuroreport* **13**, 977–981
12. Russier, M., Kopysova, I. L., Ankri, N., Ferrand, N., and Debanne, D. (2002) *J. Physiol.* (*Lond.*) **541,** 123–137
13. Dumoulin, A., Triller, A., and Dieudonne, S. (2001) *J. Neurosci.* **21**, 6045–6057
14. Dumoulin, A., Lévi, S., Riveau, B., Gasnier, B., and Triller, A. (2000) *Eur. J. Neurosci.* **12,** 3883–3892
15. Legendre, P. (2001) *Cell. Mol. Life Sci.* **58,** 760–793
16. Korpi, E. R., Grunder, G., and Luddens, H. (2002) *Prog. Neurobiol.* **67,** 113–159
17. Barker, J. L., and McBurney, R. N. (1979) *Nature* **277,** 234–236
18. Lewis, C. A., and Faber, D. S. (1993) *Neuroscience* **52,** 83–96
19. Baev, K. V., Rusin, K. I., and Safronov, B. V. (1992) *Neuroscience* **46,** 931–941
20. Trombley, P. Q., Hill, B. J., and Horning, M. S. (1999) *J. Neurophysiol.* **82,** 3417–3422
21. Li, Y., and Xu, T. L. (2002) *Neuroreport* **13,** 223–226
22. Xu, T. L., Dong, X. P., and Wang, D. S. (2000) *Eur. J. Neurosci.* **12**, 1647–1653
23. Ma, W., Saunders, P. A., Somogyi, R., Poulter, M. O., and Barker, J. L. (1993) *J. Comp. Neurol.* **338,** 337–359
24. Fucile, S., de Saint Jan, D., David-Watine, B., Korn, H., and Bregestovski, P. (1999) *J. Physiol.* (*Lond.*) **517,** 369–383
25. de Saint Jan, D., David-Watine, B., Korn, H., and Bregestovski, P. (2001) *J. Physiol.* (*Lond.*) **535**, 741–755
26. Stelzer, A., and Shi, H. (1994) *Neuroscience* **62,** 813–828
27. Chen, Q. X., and Wong, R. K. (1995) *J. Physiol.* (*Lond.*) **482,** 353–362
28. Lu, Y. M., Mansuy, I. M., Kandel, E. R., and Roder, J. (2000) *Neuron* **26,** 197–205
29. Martina, M., Mozrzymas, J. W., Boddeke, H. W., and Cherubini, E. (1996) *Neurosci. Lett.* **215,** 95–98
30. Jones, M. V., and Westbrook, G. L. (1997) *J. Neurosci.* **17**, 7626–7633
31. Bialojan, C., and Takai, A. (1988) *Biochem. J.* **256,** 283–290
32. Eckstein, F. (1985) *Annu. Rev. Biochem.* **54,** 367–402
33. Grassi, F. (1992) *Brain Res.* **594,** 115–123
34. Akaike, N., Inomata, N., and Tokutomi, N. (1987) *J. Physiol.* (*Lond.*) **391,** 219–234
35. Ariens, E. J., and Simonis, A. M. (1964) *J. Pharm. Pharmacol.* **16**, 289–312
36. Kirsch, J., and Betz, H. (1998) *Nature* **392**, 717–720
37. Boué-Grabot, E., Barajas-López, C., Chakfe, Y., Blais, D., Bélanger, D., Émerit, M. B., and Séguéla, P. (2003) *J. Neurosci.* **23**, 1246–1253
38. Yakel, J. L. (1997) *Trends Pharmacol. Sci.* **18**, 124–134
39. Todd, A. J., Watt, C., Spike, R. C., and Sieghart, W. (1996) *J. Neurosci.* **16**, 974–982
40. Burger, P. M., Hell, J., Mehl, E., Krasel, C., Lottspeich, F., and Jahn, R. (1991) *Neuron* **7**, 287–293
41. Jones, M. V., and Westbrook, G. L. (1995) *Neuron* **15**, 181–191
42. Wang, J., Liu, S. H., Haditsch, U., Tu, W. H., Cochrane, K., Ahmadian, G., Tran, L., Paw, J., Wang, Y. T., Mansuy, I., Salter, M. M., and Lu, Y. M., (2003) *J. Neurosci.* **23,** 826–836