**Ethanol Suppresses Fast Potentiation of Glycine Currents by Glutamate**

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**ABSTRACT**

Excitatory (glutamate) and inhibitory (GABA<sub>A</sub> and glycine) receptor/channels coexist in many neurons. To assess effects of ethanol on the interaction of glutamate and glycine receptors, glycine-induced current (i<sub>Gly</sub>) was recorded by a whole-cell patch-clamp technique from neurons freshly dissociated from the ventral tegmental area of rats. A conditioning prepulse of glutamate (1–3 s, 1 mM) significantly and reversibly potentiated responses to a pulse of glycine. This potentiation was increased when extracellular calcium was raised to 12 mM and reduced by including 10 mM 1,2-bis-(2-aminophenoxy)ethane-N,N,N',N'-tetraacetic acid in the internal recording medium. It was not affected by NMDA, non-NMDA (AMPA), GABA<sub>A</sub>, glycine, 5-HT<sub>3</sub>, and nicotinic acetylcholine receptors (Narahashi et al., 2001). Modulation of these receptors by ethanol may be responsible for its behavioral effects. Because ethanol acts at many sites in the central nervous system, studies of the effects of ethanol on interactions between excitatory and inhibitory synaptic mechanisms are crucial.

The ventral tegmental area (VTA) contains the cells of origin of the mesolimbic system, which is important for the rewarding properties of drugs of abuse like ethanol (Gatto et al., 1994; Wise, 1996). There are two main types of neurons in the VTA: dopamine and nondopamine neurons (Lacey et al., 1989; Johnson and North, 1992). Both receive monosynaptic glutamatergic innervation from prefrontal cortex and have NMDA and non-NMDA receptors (Wang and French, 1993, 1995). According to Floresco et al. (2001), glutamatergic afferents from the hippocampus to the nucleus accumbens strongly excite VTA dopamine neurons.

We have already reported that glycine-activated current (I<sub>Gly</sub>) can be recorded in most VTA neurons, and that ethanol (0.1–40 mM) potentiated I<sub>Gly</sub> in VTA neurons of 5- to 14-day-old rats and thus alters their excitability (Ye et al., 2001a). Bearing in mind that ethanol alters intracellular Ca<sup>2+</sup> (for review, see Little, 1991; Simasko et al., 1999; Mennerick and Zorumski, 2000), interactions between ethanol and glycine receptors may involve mechanisms linked to intracellular Ca<sup>2+</sup>. Three recent studies have reported that glutamate-induced Ca<sup>2+</sup> entry greatly potentiates I<sub>Gly</sub> in spinal neurons or oocytes expressing glycine receptors (Xu et al., 1999, 2000; Fucile et al., 2000). However, the effects of glutamate on VTA glycine receptors have not been examined. In view of the important function of glycine receptors in the VTA and the pivotal role of the VTA in drug addiction, we initiated the current study on freshly dissociated VTA neurons to examine...
ine: 1) the enhancement of $I_{\text{Gly}}$ by glutamate and 2) the effects of ethanol on this potentiating action of glutamate.

Materials and Methods

Isolation of Neurons and Electrophysiological Recording.

The care and use of animals and the experimental protocol of this study were approved by the Institutional Animal Care and Use Committee of the University of Medicine and Dentistry of New Jersey (protocol number 00074). Sprague-Dawley rats (5- to 14-day-old) were decapitated as described earlier (Ye et al., 2001a). The brains were quickly excised, placed into ice-cold saline saturated with 95% $O_2$ and 5% $CO_2$, and then cut into two 1 mm-thick slices. The slices were held at 4°C for 7–10 min before being transferred to the standard external solution containing 1 mg of pronase/6 ml and saturated with $O_2$ and incubated at 31°C for 20 min. After 20 min of additional incubation in 1 mg of thermolysin/6 ml, the VTA was identified medial to the accessory optic tract and lateral to the fasciculus retroflexus under a dissecting microscope. Micro-punches of the VTA were isolated and transferred to a 35-mm culture dish. Mild trituration through heat-polished pipettes of progressively smaller tip diameters dissociated single neurons. Within 20 min of trituration, isolated neurons attached to the bottom of the culture dish and were ready for electrophysiological experiments.

Under the light microscope, the cells acutely isolated from the VTA were of two types: bipolar and multipolar. The majority was bipolar, with one to three dendritic processes emerging from each end of the fusiform soma (20–40 μm in length and 15–25 μm in diameter). The multipolar neurons were larger, with a diameter of 35 to 60 μm, and had four to five major dendrites. Most of the cells were tyrosine hydroxylase-positive and therefore presumed to be dopaminergic. This is in good agreement with the recent report of Brodie et al. (1999). There were no appreciable differences in the response of these two groups of neurons to ethanol.

The saline in which the brain was dissected contained 128 mM NaCl, 5 mM KCl, 1.2 mM NaHPO$_4$, 26 mM NaHCO$_3$, 9 mM MgCl$_2$, 0.3 mM CaCl$_2$, and 2.5 mM glucose. The standard external solution contained 140 mM NaCl, 5 mM KCl, 1 mM MgCl$_2$, 2 mM CaCl$_2$, 10 mM glucose, and 10 mM HEPES. The pH was adjusted to 7.4 with Tris base and the osmolality to 320 mM with sucrose. The patch pipette solution contained 120 mM CsCl, 21 mM tetraethylammonium chloride, 4 mM MgCl$_2$, 11 or 4 mM EGTA, 1 mM CaCl$_2$, 10 mM HEPES, and 2 mM Mg-ATP. The pH was adjusted to 7.2 with Tris base and the osmolarity to 340 mM with sucrose. The bath solutions was nulled just before forming the giga-seal.

To determine whether this was a true potentiation, we examined the reversal potential of $I_{\text{Gly}}$. In agreement with previous observations, most neonatal VTA neurons (82%) were sensitive to glycine. Glycine-induced current ($I_{\text{Gly}}$) was antagonized by 0.1 μM strychnine (Ye et al., 1998). Glutamate (0.1 and 1 mM) elicited inward currents in all VTA neurons tested. At a holding potential of −50 mV, larger peak currents were induced by 30 μM glycine (−520 ± 68 pA, n = 42) than by 1 mM glutamate (−338 ± 44 pA, n = 41). To examine the effect of glutamate on $I_{\text{Gly}}$, a pulse of glycine (10–1000 μM) was preceded by a brief conditioning pulse of glutamate (1–3 s). For this purpose, 1 mM glutamate was routinely used, because of its predictable action and its previous use in comparable experiments (Fucile et al., 2000). However, substantial potentiation of $I_{\text{Gly}}$ could be obtained with 100 μM glutamate (see below).

The traces in Fig. 1A illustrate the effect of a glutamate prepulse (1 mM) on an immediately following $I_{\text{Gly}}$, recorded from a neuron in standard external solution (2 mM Ca$^{2+}$) and with 11 mM EGTA in the pipette. Because the glutamate-induced current returned to baseline immediately upon washout of glutamate, the potentiation of $I_{\text{Gly}}$ cannot be the result of simple addition of the two currents, as illustrated in Fig. 1B (and also Fig. 3A; see below). Because $I_{\text{Gly}}$ rapidly returned to the control level after glutamate washout, the duration of glutamate’s effect could not be measured accurately. Glutamate (1 mM) potentiated $I_{\text{Gly}}$ (induced by 30 μM glycine) in 85 of 131 (65%) of VTA neurons tested. The potentiation varied from 10 to >150% (Fig. 1C). $I_{\text{Gly}}$ was increased from a control mean of 487 ± 50 to 643 ± 62 pA; that is, to 153 ± 8% of control ($P < 0.001, n = 72$, median 122%). To determine whether this was a true potentiation, we examined the reversal potential of $I_{\text{Gly}}$. Plots of the current-voltage relationships of $I_{\text{Gly}}$ were not changed after the glutamate pretreatment (Fig. 1, D–G), the reversal potential remaining close to the [Cl$^-$/Nernst potential of 0 mV calculated for our solutions. This is in agreement with the previous report by Xu et al. (1999).

Involvement of Ca$^{2+}$ in the Glutamate-Induced Potentiation of $I_{\text{Gly}}$. According to recent reports, Ca$^{2+}$ exerts...
a powerful and rapid modulation of glycine receptor/channels (Xu et al., 1999, 2000; Fucile et al., 2000). The following results suggest that Ca²⁺ also plays a role in I_{Gly} potentiation in VTA neurons.

**Potentiation Was Greater when Extracellular Ca²⁺ Was Increased to 12 mM.** As shown in Fig. 2, when [Ca²⁺]₀ was raised locally to 12 mM, I_{Gly} increased by 65 ± 4% (P < 0.01, n = 4) and the magnitude of I_{Gly} potentiation by glutamate by 55 ± 10% of control tests of glutamate in 2 mM [Ca²⁺]₀ (Fig. 2B, P < 0.01, n = 4). This effect of [Ca²⁺]₀ was...
reversible: both \( I_{Gly} \) and the potentiation of \( I_{Gly} \) returned to control values when 2 mM \([Ca^{2+}]_o\) was restored.

**Reducing the Internal Concentration of EGTA Enhanced Both the Magnitude and Duration of Glutamate’s Effect.** In recordings with pipettes containing 4 mM EGTA (instead of the usual 11 mM), the potentiation of peak \( I_{Gly} \) induced by a 3-s glutamate prepulse reached 405 ± 128% \((n = 14; \text{median } 164\%)\) of control. Note the unusually large effects of glutamate in traces A of Fig. 3, obtained with an electrode containing 4 mM EGTA; the potentiation persisted for more than 8 s (Fig. 3B). When \( I_{Gly} \) was recorded with such pipettes, even 100 \( \mu \)M glutamate induced a marked potentiation \((146 ± 9\%, n = 4; \text{Fig. } 3, \text{C and D}).

**There Was Less Potentiation when Recording with Electrodes Containing 10 to 30 mM BAPTA.** In these recordings, the potentiation induced by glutamate decreased with time: 4 min after the start of whole-cell recording, \( I_{Gly} \) was potentiated to 153 ± 8% of control, but by 16 min, to only 126 ± 4% of control \((\text{paired } t \text{ test}, P = 0.029, n = 4; \text{cf. traces in Fig. } 3E \text{ and histograms in Fig. } 3F)\). Presumably, this reflects the time required for BAPTA to equilibrate at the intracellular site of \( Ca^{2+} \) action.

Although the glutamate-induced potentiation of \( I_{Gly} \) thus seems to depend on an increase in intracellular free \( Ca^{2+} \)-2, it was not affected by pretreating cells for 8 min with 5 \( \mu \)M KN-62, a selective calcium/calmodulin-dependent protein kinase II inhibitor: the large potentiations observed in the same five cells before and after applying KN-62 were to 372 ± 18 and 387 ± 21% of control, respectively \((P = 0.67; \text{Fig. } 2, \text{C and D}).

**Glutamate-Induced Potentiation Is Sensitive to Glycine Concentration.** Glutamate could augment \( I_{Gly} \) either by increasing the number or conductance of functional glycine receptor channels or by modifying their sensitivity to glycine. To distinguish between these possibilities, we examined the effect of glutamate on \( I_{Gly} \) induced by 10 to 1000 \( \mu \)M glycine. The traces in Fig. 4 show \( I_{Gly} \) evoked by 30, 100, and 300 \( \mu \)M glycine, in the absence \((\text{Fig. } 4A) \) and presence \((\text{Fig. } 4B)\) of prepulses of glutamate (1 mM). Glutamate strongly potentiated \( I_{Gly} \) induced by submaximal concentrations of glycine \((30 \text{ and } 100 \mu \text{M, \text{Fig. } 4, \text{a and b}); but it had a weaker effect on \( I_{Gly} \) induced by supramaximal concentrations of glycine \((\geq 300 \mu \text{M, \text{Fig. } 4C})\). On average, 1 mM glutamate potentiated peak \( I_{Gly} \) elicited by 30, 100, 300, and 1000 \( \mu \)M glycine to 180 ± 21, 152 ± 22, 118 ± 22, and 121 ± 20%, respectively \((n = 4, \text{Fig. } 4D)).

Concentration-response data obtained in the absence and presence of conditioning pulses of glutamate \((1 \text{ mM})\) are illustrated in Fig. 4C. The \( EC_{50} \) and Hill coefficient were 87 \( \mu \)M and 1.96 in the absence of glutamate, and 47 \( \mu \)M and 1.83 after glutamate conditioning pulses. Thus glutamate reduced the \( EC_{50} \) by nearly 50%. Similar observations on three other cells gave a mean reduction to 64 ± 3% \((n = 4)\). The conditioning pulses of glutamate thus increased the apparent affinity of the glycine receptor for its agonist. In addition, maximal \( I_{Gly} \) was also larger after glutamate \((121 ± 8\%, n = 4); as found by \text{Xu et al. (1999).}

**Glutamate Alters the Kinetics of \( I_{Gly} \).** Changes in either agonist affinity or channel opening efficacy can alter the \( EC_{50} \) values of agonists \((\text{Colquhoun, } 1998)\). Indeed, data from human embryonic kidney-AMPA cells transfected with cH1 demonstrate different kinetics of \( I_{Gly} \) before and after a glutamate prepulse \((\text{Fucile et al., } 2000)\). Therefore, we examined \( I_{Gly} \) channel activation, deactivation, and desensitization, before and after glutamate conditioning pulses. To allow accurate measurement within the limits of the fast perfusion system \((\text{time constant of } \approx \text{10 ms); we applied glycine at a concentration of 30 } \mu \text{M (Fig. } 5A)\). As previously observed \((\text{Ye et al., } 2001a)\), both the onset and the decay of \( I_{Gly} \) could be fitted by a single exponential function \((\text{Fig. } 5, \text{C and D}). The activation time constant \((\tau_{\text{on}}) \) was significantly shortened by a glutamate prepulse, from 340 ± 14 ms to 183 ± 22 ms \((\text{paired } t \text{ test}, P < 0.01, n = 8)\). In contrast, glutamate prolonged the deactivation time constant \((\tau_{\text{off}}) \) from 261 ± 19 ms to 350 ± 31 ms \((\text{Fig. } 5B; \text{paired } t \text{ test}, P < 0.01, n = 8)\). The slower decay indicates that glutamate increases the affinity of glycine for its receptor \((\text{Fucile et al., } 2000)\).

**Glutamate Accelerates Glycine Receptor Desensitization.** The potentiation of \( I_{Gly} \) by glutamate could result from a slower rate of receptor desensitization. To test for this possibility, we compared \( I_{Gly} \) desensitization in the absence and presence of glutamate prepulses. As shown in Fig. 5E, the current activated by a long pulse of glycine \((30 \mu \text{M})\) decayed more rapidly when applied after a brief pulse of glutamate. The ratio of the decay time constants \((\tau_{\text{gly}}/\tau_{\text{control}}) \) in Fig. 5E was 0.56. For six neurons \((\text{Fig. } 5F)\), 1 mM glutamate significantly shortened the time constant of desensitization from 6.9 ± 1.4 to 4.7 ± 0.8 s \((\text{paired } t \text{ test}, P < 0.05)\).

Because glutamate enhanced the peak more than the steady-state \( I_{Gly} \), the ratio of steady-state to peak current amplitude declined from 0.92 ± 0.02 to 0.71 ± 0.04 \((\text{paired } t \text{ test}, P < 0.01, n = 17)\).

**Ethanol Potentiates \( I_{Gly} \) But Inhibits Glutamate Current.** In agreement with our previous findings \((\text{Ye et al., } 2001a)\), 0.1 to 100 mM ethanol enhanced \( I_{Gly} \) in 35% of VTA neurons from 5- to 14-day-old rats. This effect is illustrated in Fig. 6A, where \( I_{Gly} \) evoked by 30 \( \mu \)M glycine was potentiated by 0.1, 1, and 10 mM ethanol \((\text{Fig. } 6, \text{A-b-A-d}). After washout of ethanol, \( I_{Gly} \) recovered to control amplitude \((\text{Fig. } 6, \text{A-e}). For a series of neuron, 0.1, 1, 10, and 100 mM ethanol enhanced peak \( I_{Gly} \) to 116 ± 5% \((n = 3)\), 135 ± 4% \((n = 34)\), 127 ± 3% \((n = 34)\), and 117 ± 6% \((n = 4)\) of control, respectively. When a brief pulse of 10 mM ethanol was coapplied during a longer pulse of glycine, there was an immediate and rapidly reversible increase in \( I_{Gly} \) \((\text{Fig. } 6B). In contrast to this potentiation of \( I_{Gly} \), 10 mM ethanol depressed glutamate-evoked current to 74 ± 3% \((n = 10)\) of control \((\text{Fig. } 6C), in agreement with previous reports \((\text{Narahashi et al., } 2001)\).

**Ethanol Suppresses the Glutamate-Induced Potentiation of \( I_{Gly} \).** Records a and b of Fig. 6D illustrate the usual potentiation of \( I_{Gly} \) by a conditioning prepulse of glutamate. When 10 mM ethanol was coapplied with glutamate and glycine \((\text{Fig. } 6D-d)\), the potentiation of \( I_{Gly} \) by glutamate was significantly reduced: from 188 ± 72% of control in the absence of ethanol to 146 ± 26% in its presence \((\text{Fig. } 6E; P < 0.05, n = 4)\).

In the experiments illustrated in Fig. 6D, ethanol was present when glutamate was applied. Therefore, the suppression of glutamate-induced potentiation of \( I_{Gly} \) could result from ethanol-induced reduction of \( Ca^{2+} \) entry via glutamate receptors. To assess this possibility, ethanol was coapplied with glycine immediately after the end of the glutamate prepulse \((\text{Fig. } 7A). Although either glutamate or ethanol alone increased \( I_{Gly} \) \((\text{Fig. } 7, \text{A-b and A-c}), there was no fur-
ther enhancement of $I_{\text{Gly}}$ by ethanol (Fig. 7A-d). The data from 13 cells studied with this protocol are summarized in Fig. 7B: there was a similar potentiation of $I_{\text{Gly}}$ by condition-
ing pulses of glutamate (as in Fig. 7A-b; 146 ± 12%), ethanol alone (Fig. 7A-c; 145 ± 11%), and ethanol applied after the glutamate conditioning pulse (Fig. 7A-d; 148 ± 16%). These findings suggest different mechanisms of potentiation by eth-
anol and by glutamate. As shown in Fig. 7A-b, glutamate enhanced mainly peak $I_{\text{Gly}}$ and its rate of decay, whereas $I_{\text{Gly}}$ potentiated both peak and steady-state $I_{\text{Gly}}$ (Fig. 7A-c). When ethanol was coapplied with glycine after a con-
ditioning pulse of glutamate, both peak and steady-state $I_{\text{Gly}}$ were enhanced, as during ethanol treatment alone (Fig. 7A-
d).

It is unlikely that maximal activation of glycine receptors explains these observations because the outcome was the

Fig. 3. When recording with pipettes containing 4 instead of 11 mM EGTA, potentiation by glutamate was enhanced, could be elicited with 100 μM glutamate, but was reduced when pipettes also contained 10 mM BAPTA. A, current traces obtained with pipette containing 4 mM EGTA from a VTA neuron of a 10-day-old rat. B, time course of change of peak $I_{\text{Gly}}$ after glutamate prepulse from neuron illustrated in A. Similar data were obtained from another three cells. C, current traces obtained from a VTA neuron of a 15-day-old rat produced by 30 μM glycine alone (a and c) and immediately after a conditioning prepulse of 100 μM glutamate (b). D, histogram shows mean potentiation of $I_{\text{Gly}}$ (evoked by 30 μM glycine) by 100 μM glutamate ($n = 4$). E, current traces obtained from a VTA neuron of an 11-day-old rat with 10 mM BAPTA-containing pipette illustrate progressive reduction of glutamate-induced potentiation after 4 min of whole-cell recording (traces a and b) and after 16 min (traces c and d). F, mean data from four cells show significantly reduced potentiation by 16 min. All data in this figure were obtained with 4 mM EGTA pipette solution.

Fig. 4. Glutamate prepulse reduces EC$_{50}$ and increases maximal $I_{\text{Gly}}$. A and B, current traces (obtained from a neuron of an 11-day-old rat) induced by increasing glycine concentrations without (A) and with (B) preceding pulse of 1 mM glutamate. C, concentration-response curves for glycine alone (open circles) or glycine after glutamate prepulse (filled circles), from the same neuron as in A and B. Continuous lines are fits of the Hill equation: $I = I_{\text{max}}/[1 + (EC_{50}/C)^n]$, where $I$ is $I_{\text{Gly}}$, $I_{\text{max}}$ is maximal $I_{\text{Gly}}$, $C$ is glycine concentration, $EC_{50}$ is glycine concentration for half-
maximal response, and $n$ is the Hill coefficient. D, mean potentiation of $I_{\text{Gly}}$ (four neurons) by 1 mM glutamate, at various concentrations of glycine. Before pooling data, amplitudes of peak $I_{\text{Gly}}$ were normalized to response elicited by 30 μM glycine alone.
same when ethanol was applied at a lower concentration (1 mM) (data not shown). Moreover, ethanol suppressed the potentiation by glutamate even in cells that showed no enhancement of \( I_{\text{Gly}} \) by ethanol alone. This is illustrated in Fig. 7C, where 10 mM ethanol did not alter \( I_{\text{Gly}} \) (Fig. 7C-c) but suppressed its enhancement by glutamate (cf. Fig. 7, A-b and A-d). The results obtained from five cells are summarized in Fig. 7D, in which \( I_{\text{Gly}} \) was not altered by ethanol alone (\( I_{\text{Gly}} \) was 103 \pm 1\% of control), although after glutamate, \( I_{\text{Gly}} \) was enhanced to 124 \pm 4\%. When glycine + ethanol were applied after the glutamate prepulse, \( I_{\text{Gly}} \) was 105 \pm 5\% of control, although peak glutamate currents were the same for the first and second pulse (274 \pm 91 and 271 \pm 97 pA; \( P > 0.1, n = 5 \)). Thus, even when ethanol did not change the response to glycine, it still prevented the interaction between glutamate and glycine.

Fig. 5. Glutamate prepulse reduces deactivation rate and accelerates desensitization of \( I_{\text{Gly}} \). A, whole-cell currents activated by 30 \( \mu \)M glycine before (a) and after (b) glutamate prepulse (from a VTA neuron of an 11-day-old rat). B, histograms show that glutamate prepulse (1 mM, 2 s) decreased the activation time constant (\( t_{\text{on}} \)) and increased the deactivation time constant (\( t_{\text{off}} \)) of \( I_{\text{Gly}} \); data are from eight neurons. C and D are from the same neuron as in A. Both activation (C) and deactivation (D) could be fitted by a single exponential (continuous curves). E, current traces from another neuron of an 11-day-old rat. \( I_{\text{Gly}} \) was elicited by 30 \( \mu \)M glycine before (a) and immediately after a conditioning prepulse of 1 mM glutamate (b). Superimposed traces in c illustrate decay of \( I_{\text{Gly}} \) elicited by long application of 30 \( \mu \)M glycine, with and without glutamate prepulse. F, histograms indicate average values of time constants of decay (\( t_{\text{d}} \)) for currents activated by 30 \( \mu \)M glycine; values obtained with and without 1 mM glutamate prepulse differed significantly (\( P < 0.01, n = 6 \)).

Fig. 6. Ethanol enhances \( I_{\text{Gly}} \), depresses \( I_{\text{Glu}} \), and suppresses glutamate-induced potentiation of \( I_{\text{Gly}} \). A, traces from a VTA neuron of a 7-day-old rat show \( I_{\text{Gly}} \) elicited by 30 \( \mu \)M glycine alone (a and e, open horizontal bars) or together with 0.1 (b), 1 (c), and 10 mM ethanol (d, hatched bars). B, brief pulse of 10 mM ethanol potentiated \( I_{\text{Gly}} \), induced by 30 \( \mu \)M glycine (from a VTA neuron of an 8-day-old rat). C, brief pulse of 10 mM ethanol inhibited \( I_{\text{Gly}} \), induced by 1 mM glutamate (from the same neuron as in B). D, in a VTA neuron of a 12-day-old rat, \( I_{\text{Gly}} \) was elicited by 30 \( \mu \)M glycine alone (a), immediately after 1 mM glutamate prepulse (b), together with 10 mM ethanol (c), and immediately after coapplication of 1 mM glutamate and 10 mM ethanol (d). E, mean values of peak \( I_{\text{Gly}} \), activated by 30 \( \mu \)M glycine obtained in the three recording conditions as in D-b, c, and d. Before plotting, all values were normalized to the value of peak \( I_{\text{Gly}} \) obtained in the control condition (D-a).
Discussion

Our previous research revealed glycine receptors in the majority of freshly dissociated VTA neurons (Ye et al., 1998), all of which also respond to glutamate (Wang and French, 1993; Wu and Johnson, 1996; Ye et al., 2001b). The present results show that in such VTA neurons, glutamate consistently enhances $I_{\text{Gly}}$, as previously observed in spinal neurons by Xu et al. (1999, 2000), and spinal and transfected cells by Fucile et al. (2000). Our principal new finding is that ethanol suppresses the glutamate-induced potentiation of $I_{\text{Gly}}$ in VTA neurons. In keeping with the previous authors, our results point to the involvement of Ca$^{2+}$, perhaps Ca$^{2+}$ influx, in this phenomenon, although probably not calcium/calmodulin-dependent protein kinase II.

Comparison with Previous Reports of Interactions between Glutamate and Glycine. The previous studies of glutamate-induced fast potentiation of $I_{\text{Gly}}$ (Xu et al., 1999, 2000; Fucile et al., 2000) attributed this effect to a rise in intracellular free Ca$^{2+}$. Although outwardly similar, these reports differed in some important respects. The results of the perforated-patch recordings from freshly dissociated spinal neurons (Xu et al., 1999, 2000) led to the conclusion that the increase in $I_{\text{Gly}}$ is not caused by a change in the affinity of glycine for its receptor and that the potentiation is mediated by activation of CAMKII. In contrast, conventional whole-cell recordings from transfected cells (Fucile et al., 2000) suggested potentiation was due to a higher affinity of glycine receptors and not mediated by known protein kinases. In our experiments, an increase in glycine receptor affinity was indicated by the slower deactivation of $I_{\text{Gly}}$ and the consistent reduction in $EC_{50}$. However, the glycine concentration-response plots also showed a clear increase in the maximal $I_{\text{Gly}}$. In VTA neurons, the potentiation thus appeared to be mediated by both mechanisms.

Is the Potentiating Action of Glutamate in VTA Neurons Mediated by Intracellular Ca$^{2+}$? Our findings that glutamate was more effective when [Ca$^{2+}$]$_i$ was raised to 12 mM and less effective when a stronger Ca$^{2+}$ buffer, BAPTA or 11 mM EGTA (as compared with 4 mM EGTA) was present in the electrodes are consistent with some involvement of Ca$^{2+}$. However, the relatively modest effects produced by these manipulations, especially when compared with those seen in the experiments of Xu et al. (1999, 2000) and Fucile et al. (2000), seem more in keeping with a Ca$^{2+}$-sensitive than a Ca$^{2+}$-dependent process. Admittedly, by buffering slow changes in [Ca$^{2+}$]$_i$, the routine presence of EGTA in the electrodes would tend to reduce Ca$^{2+}$-mediated mechanisms. This may, at least in part, account for the relatively small and transient effects of glutamate observed in the current study as compared with the potentiation observed in the previous studies, where weaker or no buffers were used. Since even 30 mM BAPTA failed to abolish the action of glutamate, the potentiation of glycine receptors may occur at a site that is not entirely intracellular, or at least not easily accessible to intracellular chelators. Judging by the lack of effect of KN-62, CAMKII is probably not the agent of Ca$^{2+}$-mediated modulation. Whether phosphorylation or dephosphorylation plays a significant role should be clarified by further tests of selective blockers.

Mechanisms of Ethanol’s Actions. Because ethanol alone inhibits glutamate-induced currents, it could exert its
effect by a direct depression of glutamate receptor/channels and consequently a smaller rise in [Ca\(^{2+}\)]\(_i\) (Gruol et al., 1997). This is unlikely because ethanol suppressed glutamate-induced potentiation when ethanol was applied after the end of the glutamate prepulse. Therefore, ethanol probably exerts its suppressant action at a site closer to the glycine receptors; for example, where Ca\(^{2+}\)-sensitive phosphorylation occurs.

In VTA neurons, both glutamate prepulses and ethanol potentiated glycine responses. These effects showed several similarities. First, the potentiation of glycine receptors by either ethanol (Ye et al., 2001a) or glutamate decreases with higher glycine concentrations. Second, both agents lower the EC\(_{50}\) of glycine. Third, they both accelerate glycine receptor desensitization. Thus, some occlusion between glutamate- and ethanol-induced potentiations is highly probable. However, because ethanol was effective in neurons that showed no potentiation of \(I_{\text{gly}}\) by ethanol alone (Fig. 7 D), another mechanism is also likely involved.

Consequences of Ethanol’s Inhibition of Glutamate-Induced Potentiation of Glycine Responses and Other Cellular Activities. Because excitatory and inhibitory receptors coexist in many neurons, it is essential for us to understand how they interact. Modulation of glycine receptors by agents such as glutamate and ethanol is important because changes in the efficacy of glycinergic transmission have pathophysiological implications in nociception and motor behavior (Breitinger and Becker, 1998; Simpson and Huang, 1998). Moreover, Ca\(^{2+}\)-dependent clustering of glycine receptors during synaptogenesis has been demonstrated (Kirsch and Betz, 1998). Being present in most VTA neurons, glycine receptors are likely to play an important role in modulating the excitability of VTA dopaminergic and nondopaminergic neurons and, consequently, the release of dopamine and other agents in the brain.

In conclusion, glutamate induced a fast potentiation of \(I_{\text{gly}}\) in VTA neurons of 5- to 14-day-old rats. This was enhanced when extracellular Ca\(^{2+}\) was raised from 2 to 12 mM and diminished by intraneuronal chelators, indicating the possible involvement of intracellular Ca\(^{2+}\). Glutamate increased both the apparent affinity of the glycine receptor for its agonist and the maximal response. Ethanol suppressed these effects of glutamate, even when ethanol was applied after a glutamate prepulse. Therefore, ethanol may act on a Ca\(^{2+}\)-sensitive kinase or other pathways that regulate the function of glycine receptor channels.

References


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