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GLYCINE BLOCKS LONG-TERM POTENTIATION OF GABAergic SYNAPSES IN THE VENTRAL TEGMENTAL AREA

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Abstract—The mesocorticolimbic dopamine system, originating in the ventral tegmental area (VTA) is normally constrained by GABA-mediated synaptic inhibition. Accumulating evidence indicates that long-term potentiation of GABAergic synapses (LTP_{GABA}) in VTA dopamine neurons plays an important role in the actions of drugs of abuse, including ethanol. We previously showed that a single infusion of glycine into the VTA of rats strongly reduces ethanol intake for 24 h. In the current study, we examined the effect of glycine on the electrophysiological activities of putative dopamine VTA neurons in midbrain slices from ethanol-naïve rats. We report here that a 15-min exposure to 10 μM glycine prevented trains of high-frequency stimulation (HFS) from producing LTP_{GABA}, which was rescued by the glycine receptor (GlyR) antagonist strychnine. Glycine also concentration-dependently decreased the frequency of spontaneous excitatory postsynaptic currents (sEPSCs). By contrast, glycine pretreatment did not prevent potentiation of inhibitory postsynaptic currents (IPSCs) during a continuous exposure to the nitric oxide (NO) donor, SNAP (S-nitroso-N-acetylpenicillamine), or a brief exposure to 10 μM glycine and 10 μM NMDA (N-methyl-D-aspartate), an agonist of NMDA-type glutamate receptors. Thus, the blockade of LTP_{GABA} by glycine is probably resulted from suppressing glutamate release by activating the GlyRs on the glutamatergic terminals. This effect of glycine may contribute to the reduction in ethanol intake induced by intra-VTA glycine observed *in vivo*.

Key words: synaptic plasticity, mesolimbic system, EPSCs, long-term potentiation, GABA.

INTRODUCTION

The mesocorticolimbic dopaminergic system, originating in the ventral tegmental area (VTA), plays an important role in alcohol use disorders (Gilpin and Koob, 2008). VTA dopamine neurons are normally constrained by powerful gamma-aminobutyric acid (GABA)-mediated synaptic inhibition (Johnson and North, 1992; Tepper et al., 1998). Both $GABA_A$ and $GABA_B$ receptor subtypes have been identified within the VTA. Activation of $GABA_A$ receptor ($GABA_AR$) on GABAergic interneurons increases VTA dopamine neuron activity through disinhibition. Conversely, GABAergic neurons decrease spike output of dopamine neurons via $GABA_B$ receptors on dopamine neurons.

GABAergic signaling in the VTA has been linked to ethanol drinking behaviors. Intra-VTA administration of $GABA_AR$ antagonists decreases ethanol intake in rodents (Nowak et al., 1998; Melon and Boehm, 2011). Importantly, VTA GABA neurons become hyperexcitable during ethanol withdrawal (Gallegos et al., 1999). Interestingly, rodents readily self-administer $GABA_AR$ antagonists into the VTA (Ikemoto et al., 1997; Gavello-Baudy et al., 2008) and this blockade of $GABA_ARs$ in the VTA increases dopamine levels in the nucleus accumbens (Ikemoto et al., 1997), producing robust rewarding effects (Laviolette and van der Kooy, 2001). Changes in synaptic strength are important for persistent pathologies such as drug addiction. In particular, accumulating evidence indicates that long-term potentiation of GABAergic synapses (LTP_{GABA}) in VTA dopamine neurons increases or decreases in response to the actions of drugs of abuse, including ethanol (Nugent et al., 2007, 2009; Guan and Ye, 2010).

Like GABA, glycine is also a major inhibitory neurotransmitter in the CNS. Functional glycine receptors (GlyRs) are found throughout the mammalian CNS, including the mesocorticolimbic dopamine system (Zheng and Johnson, 2001; Ye et al., 2002; Lewis and O'Donnell, 2003; Wang et al., 2005). Our previous *in vitro* electrophysiological data indicate that GlyRs exist on the GABAergic terminals, which make synapses on VTA dopamine neurons, and activation of these GlyRs reduces GABAergic transmission and increases VTA dopamine neuron activity (Ye et al., 2004). *In vivo* studies have shown that GlyRs in the mesolimbic dopamine system are involved in ethanol drinking behaviors.

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Abbreviations: aCSF, artificial cerebrospinal fluid; DNQX, 6,7-dinitroquinoxaline-2,3-dione; eIPSC, evoked inhibitory postsynaptic current; GABA, gamma-aminobutyric acid; $GABA_AR$, $GABA_A$ receptor; GlyR, glycine receptor; GLYTs, glycine transporters; HFS, high-frequency stimulation; IPSC, inhibitory postsynaptic current; LTP_{GABA}, long-term potentiation of GABA-mediated synapses; NMDA, N-methyl-D-aspartate; NO, nitric oxide; PPR, paired pulse ratio; sEPSC, spontaneous excitatory postsynaptic current; SNAP, S-nitroso-N-acetylpenicillamine; VTA, ventral tegmental area.

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Activation of GlyRs in the nucleus accumbens substantially decreases ethanol intake (Molander et al., 2005; Molander and Soderpalm, 2005a,b). Remarkably, we have shown that a single intra-VTA infusion of glycine substantially reduces ethanol intake for 24 h in rats, in three different drinking models: intermittent access, continuous access, and operant self-administration (Li et al., 2012). However, the underlying neuronal basis has not been well explored. Glycine could alter the activity of dopamine neurons by activating the GlyRs on the GABAergic terminals, which make synapses on VTA dopamine neurons (Ye et al., 2004). However, given that extracellular glycine is probably removed rapidly by reuptake, additional mechanisms must be involved.

In an attempt to understand the mechanisms underlying the long-lasting effect of intra-VTA perfusion of glycine on ethanol drinking, we examined glycine's effects on the electrophysiological activities of putative dopamine neurons in the VTA in midbrain slices from ethanol-naïve rats.

EXPERIMENTAL PROCEDURES

Animals

The experiments were carried out on Sprague–Dawley rats, in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals, and were approved by the Institutional Animal Care and Use Committee of the Rutgers, the State University of New Jersey, New Jersey Medical School.

Chemicals and applications

DNQX (6,7-dinitroquinoxaline-2,3-dione), gabazine (SR-95531), glycine, NMDA (N-methyl-D-aspartic acid), SNAP (S-nitroso-N-acetylpenicillamine), strychnine, and all common salts were obtained from Sigma–Aldrich Chemical Company (St Louis, MO, USA). Drugs were added to the superfusate at final concentrations. Chemicals were applied to the recorded neurons at the stated concentrations through bath perfusion.

Brain slice preparation

The midbrain slices were prepared as described previously (Guan and Ye, 2010). Briefly, Sprague–Dawley rats (21–35 d old) were anesthetized using ketamine/xylazine and then sacrificed. Coronal midbrain slices (200–250-mm-thick) were cut using a VF-200 slicer (Precisionary Instruments, Greenville, NC, USA) and prepared in ice-cold glycerol-based artificial cerebrospinal fluid (aCSF) containing (in mM): 250 glycerol, 1.6 KCl, 1.2 NaH_2PO_4, 1.2 $MgCl_2$, 2.4 $CaCl_2$, 25 $NaHCO_3$, and 11 glucose, and saturated with 95% O_2/5% CO_2 (carbogen) (Ye et al., 2006). Slices were allowed to recover for at least 1 h in a holding chamber at 32 °C in carbogen-saturated regular aCSF, which had the same composition as glycerol-based aCSF, except that glycerol was replaced by 125 mM NaCl.

Electrophysiological recording

Electrical signals were obtained with a MultiClamp 700 A amplifier (Molecular Devices Co., Union City, CA, USA), and pCLAMP software (Molecular Devices Co.). Data were filtered at 1 kHz and sampled at 5 kHz, stored on a computer, and analyzed offline. The amplitudes of inhibitory postsynaptic currents (IPSCs) were calculated by taking the mean of a 2–4-ms window around the peak and comparing it with the mean of a 2–8-ms window immediately before the stimulation artifact, using Clampfit (Molecular Devices).

Patch pipettes (4–6 MΩ) for recording IPSCs contained (in mM): 125 KCl, 2.8 NaCl, 2 $MgCl_2$, 0.6 EGTA, 10 HEPES, 2 Mg ATP-Na and 0.2 GTP-Na; and for EPSCs contained (in mM): 140 Cs-methanesulfonate, 5 KCl, 2 $MgCl_2$, 10 HEPES, 2 MgATP, 0.2 NaGTP, pH 7.2. The pH was adjusted to 7.2 with Tris base, and osmolality to 300 m Osmol/l with sucrose. A single slice was transferred to a 0.4-ml recording chamber, where a platinum ring held it down. Throughout the experiments, the bath was continually perfused with warm (32 °C) carbogenated aCSF (1.5–2.0 ml/min).

Under infrared video microscopy (E600FN; Nikon Corporation, Tokyo, Japan), the VTA was identified medial to the accessory optic tract and lateral to the fasciculus retroflexus. Currents were recorded using whole-cell mode. Experiments were carried out only after series resistance had stabilized. Series resistance and input resistance were monitored continuously online with a −4 mV hyperpolarizing step (50 ms), which was given after every afferent stimulus, and experiments were discarded if these values changed by 20% during the experiment. Putative dopamine neurons in the current study were identified by the presence of a large Ih current (Johnson and North, 1992) that was assayed immediately after break-in, using a series of incremental 10 mV hyperpolarizing steps from a holding potential of −50 mV. Specifically, if the steady-state h-current was greater than 60 pA during a step from −50 to −100 mV, the neuron was considered a dopamine neuron. A recent study showed that expression of Ih alone is not sufficient to identify dopamine cells unequivocally (Margolis et al., 2006). Therefore, in each set of our experiments, a subset of the neurons recorded from and reported here are possibly non-dopaminergic neurons (Nugent et al., 2009).

$GABA_A$R-mediated IPSCs were stimulated at 0.1 Hz using a bipolar stainless steel-stimulating electrode placed 200–400 μm away from the recording site in the VTA. As previously described (Guan and Ye, 2010), LTP_{GABA} was induced by stimulating afferents at 100 Hz for 1 s, and the train was repeated twice, 20 s apart (high-frequency stimulation; HFS). After recording the baseline currents, during the drug application and washout, synaptic stimulation was stopped and the recorded neuron was taken from voltage-clamp into bridge mode. The HFS trains were also delivered under bridge mode.