GABAergic TRANSMISSION MODULATES ETHANOL EXCITATION OF VENTRAL TEGMENTAL AREA DOPAMINE NEURONS

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Abstract—Activation of the dopaminergic (DA) neurons of the ventral tegmental area (VTA) by ethanol has been implicated in its rewarding and reinforcing effects. We previously demonstrated that ethanol enhances GABA release onto VTA-DA neurons via activation of 5-HT2C receptors and subsequent release of calcium from intracellular stores. Here we demonstrate that excitation of VTA-DA neurons by ethanol is limited by an ethanol-enhancement in GABA release. In this study, we performed whole-cell voltage clamp recordings of miniature inhibitory postsynaptic currents (mIPSCs) and cell-attached recordings of action potential firing from VTA-DA neurons in midbrain slices from young Long Evans rats. Acute exposure to ethanol (75 mM) transiently enhanced the firing rate of VTA-DA neurons as well as the frequency of mIPSCs. Simultaneous blockade of both GABA A and GABA B receptors (Picrotoxin (75 μM) and SCH50911 (20 μM)) disinhibited VTA-DA firing rate whereas a GABA B agonist (muscimol, 1 μM) strongly inhibited firing rate. In the presence of picrotoxin, ethanol enhanced VTA-DA firing rate more than in the absence of picrotoxin. Additionally, a sub-maximal concentration of muscimol together with ethanol inhibited VTA-DA firing rate more than muscimol alone. DAMGO (3 μM) inhibited mIPSC frequency but did not block the ethanol-enhancement in mIPSC frequency. DAMGO (1 and 3 μM) had no effect on VTA-DA firing rate. Naltrexone (60 μM) had no effect on basal or ethanol-enhancement of mIPSC frequency. Additionally, naltrexone (20 and 60 μM) did not block the ethanol-enhancement in VTA-DA firing rate. Overall, the present results indicate that the ethanol enhancement in GABA release onto VTA-DA neurons limits the stimulatory effect of ethanol on VTA-DA neuron activity and may have implications for the rewarding properties of ethanol. © 2011 IBRO. Published by Elsevier Ltd. All rights reserved.

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Activation of dopaminergic (DA) neurons of the ventral tegmental area (VTA) by ethanol and other drugs of abuse is believed to be a critical component in the development and expression of drug dependence and addiction (Gatto et al., 1994; Robbins and Everitt, 1996; Wise, 1996; Koob et al., 1998; Schultz, 2002; Appel et al., 2004). The VTA is situated in the midbrain and sends projections to the nucleus accumbens (NAc), prefrontal cortex, basolateral amygdala and a variety of other corticolimbic structures (Albanese and Minciacchi, 1983; Oades and Halliday, 1987). The VTA also receives reciprocal excitatory and inhibitory innervations from multiple brain regions. Local VTA GABAergic neurons (interneurons) and GABAergic projections arising from the NAc and ventral pallidum synapse onto VTA-DA neurons and represent a potential target for ethanol modulation of DA activity.

While relatively little controversy surrounds the direct actions of ethanol to enhance DA signaling in the midbrain (Gessa et al., 1985; Brodie et al., 1990; Weiss et al., 1993; Brodie and Appel, 2000), quite disparate findings concerning ethanol modulation of VTA GABAergic transmission have been documented. We and others have reported that acute ethanol exposure enhances GABA release onto midbrain DA neurons (Meils et al., 2002; Criswell et al., 2008; Theile et al., 2008, 2009). Conversely, other groups have presented opposite results. Important observations by Ye and colleagues demonstrated that ethanol (10–40 mM) may have differential effects on VTA action potential-dependent GABA release. Although under control conditions ethanol decreased GABA release, an increase in spontaneous inhibitory postsynaptic current (sIPSC) frequency was observed in the presence of saturating concentrations of the mu-opioid receptor (MOR) agonist DAMGO (Xiao and Ye, 2008). Ye and colleagues also show that ethanol decreased the firing rate of GABA interneurons (Xiao et al., 2007). Steffensen and colleagues demonstrated an ethanol inhibition of GABAergic interneuron excitability believed to be mediated through inhibition of NMDA receptors (Stobbs et al., 2004). However, they also demonstrated that low dose i.v. ethanol (0.01–0.03 g/kg) enhanced VTA-GABA neuron firing rate in rats (Steffensen et al., 2009). GABA A receptors are present on VTA-DA neurons and tonic GABA release inhibits these neurons (Yim and Mogenson, 1980; Johnson and North, 1992a,b; Westerink et al., 1996). Therefore, we hypothesized that if ethanol enhances GABAergic transmission onto VTA DA neurons, then stimulation or blockade of GABA receptors...
may modulate the overall stimulatory effect of ethanol on VTA-DA neuron activity.

Furthermore, it has been demonstrated that activation of MORs on GABAergic interneurons in the VTA disinhibits VTA-DA neuron activity (Johnson and North, 1992a; Margolis et al., 2003; Xiao et al., 2007; Xiao and Ye, 2008). The MOR agonist, DAMGO, reduced the frequency of miniature and spontaneous IPSCs through inhibition of the secretory process at the nerve terminal of GABAergic cells (Bergevin et al., 2002). In the central amygdala (CeA), ethanol increased GABA release and this effect was enhanced in delta-opioid receptor (DOR) knockout mice and in the presence of DOR antagonists (Kang-Park et al., 2007). That study suggests that endogenous opioid release negatively modulates spontaneous and ethanol-induced GABA release in the CeA. It is possible that blocking opioid activation in the VTA may also enhance spontaneous and ethanol-mediated GABA release. As a result, we hypothesize that the ethanol-induced increase in GABA release we observe is limited by basal opioid-activation of presynaptic MORs, thus inhibiting GABA release. Therefore, inhibition of presynaptic MORs with the non-selective opioid antagonist, naltrexone, may remove tonic inhibition of GABA release, thus amplifying the ethanol-induced increase in GABA release. The resultant increase in GABA transmission may then overcome the direct effect of ethanol on DA neuron excitability, thereby providing a possible mechanism to explain the ability of naltrexone to block ethanol-induced increases in dialysate dopamine levels in the NAc of male Wistar rats (Gonzales and Weiss, 1998), and possibly may contribute to the clinical efficacy of naltrexone on preventing relapse in recovering alcoholics.

**EXPERIMENTAL PROCEDURES**

**Slice preparation**

All experiments were carried out in accordance with NIH animal use guidelines and were approved by the University of Texas Institutional Animal Care and Use Committee. Slices used in this study were prepared from male Long Evans rats (postnatal day 21 to 28). Rats were anesthetized with halothane, decapitated, and the brain was rapidly removed and placed in an ice-cold choline-based, oxygenated artificial cerebrospinal fluid (aCSF) containing (in mM): 110 choline Cl, 25 NaHCO3, 1.25 NaH2PO4, 2.5 KCl, 25 dextrose, 7 MgSO4, 0.5 CaCl2, 11.6 Na-ascorbate and 3.1 Na-pyruvate, bubbled with 95% O2/5% CO2 (all chemicals obtained from Sigma-Aldrich, St Louis, MO, USA). Horizontal midbrain slices (210 μm) were prepared using a vibrating slicer (VT1000S; Leica, Nussloch, Germany). The slices were then maintained at 32 °C before electrophysiological recordings for a minimum of 60 min in aCSF containing (in mM): 120 NaCl, 25 NaHCO3, 3.3 KCl, 1.23 NaH2PO4, 10 dextrose, 2.4 MgSO4, and 1.8 CaCl2, bubbled with 95% O2/5% CO2.

**Electrophysiological recordings of VTA-DA neurons**

Individual slices were transferred to a recording chamber and perfused with oxygenated aCSF (30–32 °C) at a flow rate of ~2 ml/min. Recording aCSF was as described above except it contained 0.9 mM MgSO4 and 2 mM CaCl2. Cells were visualized using IR–DIC optics on an Olympus BX-50WI microscope (Leeds Instruments, Irving, TX, USA). The VTA was identified as being medial to the medial terminal nucleus of the accessory optic tract (MT) and rostral to the oculomotor nerve and the medial lemniscus. The majority of recordings were conducted in the lateral VTA, just medial to the MT. For action potential recordings, putative DA neurons were identified by their characteristic slow (1–5 Hz) pacemaking activity. Additionally, whole-cell access was obtained and putative DA neurons were further identified by the presence of a large hyperpolarization-induced Ih current (~200 pA) that was measured immediately following break-in by application of a 1.5-s hyperpolarizing step from ~60 to ~110 mV (Johnson and North, 1992b). For mIPSC recordings, DA neurons were identified via the presence of an Ih current before the experiment was carried out. Recording electrodes were made from thin-walled borosilicate glass (TW 150F-4, WPI, Sarasota, FL, USA, 1.5–2.5 MΩ) and contained (in mM): 135 KCl, 12 NaCl, 0.5 EGTA, 10 HEPES, 2 Mg-ATP, and 0.3 Tris-GTP, pH 7.3 with KOH (all chemicals obtained from Sigma, St Louis, MO, USA). Data were collected by an Axon Instruments Model 2001 amplifier filtered at 1 kHz and digitized at 20 kHz (for mIPSCs) and 50 kHz (for action potentials) with pClamp interfaced using pClamp v10.2 (Molecular Devices, Sunnyvale, CA, USA).

Firing rate recordings were conducted in the absence of any channel/receptor drugs except where mentioned. Action potential firing rate was measured using tight-seal current clamp recordings because rupture of the cell membrane under traditional whole-cell recordings significantly disrupts the pacemaking activity of DA neurons (Morikawa et al., 2003). Following formation of a tight seal and a stable 10-min baseline (control), drugs were bath applied through the aCSF perfusion line and a continuous 15–17-min recording epoch was used to detect changes in firing rate. A 10-min washout followed drug application.

GABAergic mIPSCs were pharmacologically isolated with kynurenic acid (1 mM) to inhibit AMPA- and NMDA receptor-mediated currents. Tetrodotoxin (TTX; 0.5 μM) and eticlopride (250 mM) were included to block Na+ currents and D2 receptor-mediated currents, respectively. Under these conditions, mIPSCs were inward at a holding potential of ~60 mV and in an initial set of experiments their identity as GABAergic events was verified by testing for block with picrotinox or bicuculline (data not shown). Following break-in and a stable 10-min baseline (control) recording, drugs were bath-applied through the aCSF perfusion line and a continuous 10–15-min recording epoch was used to detect changes in mIPSC frequency and amplitude. A 4-min drug wash-on preceded the start of data collection in each treatment condition, and a 12-min washout period followed drug application. The number of neurons used per each treatment condition is represented as n with only one neuron used per slice. SCH50911, muscimol and DAMGO were obtained from Tocris Bioscience (Ellisville, MO, USA). Kynurenic acid, naltrexone hydrochloride and picrotinox were obtained from Sigma-Aldrich (St. Louis, MO, USA). TTX was obtained from Alomone Labs (Jerusalem, Israel).

**Data analysis**

For firing rate recordings, action potentials (30–50 sweeps each condition, 20 s/sweep) were detected using a threshold detection protocol contained with pClampfit (pClamp v10.2, Axon Inst., Molecular Devices). The peak 5 min of the drug effect and the last 5 min of washout were normalized to the last 5 min of the baseline (control) and represented as a percentage of the control. Averaged values for all data sets are expressed as mean ± SEM and were compared statistically using paired Student’s t-test, unpaired Student’s t-test and one-way analysis of variance (ANOVA) test where mentioned.

For mIPSC recordings, quantal events (90–120 sweeps each condition, 5 s/sweep) were detected using the template mIPSC detection protocol contained within pClampfit. Access resistance ranged from 8 to 25 MΩ and was monitored throughout the experiment. Experiments where access resistance changed (>20% at anytime during the experiment) were excluded from this