Ethanol Suppression of Ventral Tegmental Area GABA Neuron Electrical Transmission Involves N-Methyl-D-aspartate Receptors


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ABSTRACT

Ventral tegmental area (VTA) GABA neurons are critical substrates modulating the mesocorticolimbic dopamine system implicated in natural and drug reward. The aim of this study was to evaluate the effects of ethanol on glutamatergic and GABAergic modulation of VTA GABA neuron electrical synaptic transmission. We evaluated the effects of systemic ethanol (0.05–2.0 g/kg i.p.), the N-methyl-D-aspartate (NMDA) receptor antagonist dizocilpine (MK-801; 0.05–0.2 mg/kg i.v.), the connexin-36 gap junction blocker quinidine (5–20 mg/kg i.v.), the fast-acting barbiturate methohexital (Brevital; 5–10 mg/kg i.v.), and the benzodiazepine chlordiazepoxide (Librium; 5–10 mg/kg i.v.), as well as in situ VTA administration of NMDA and the GABA_A receptor agonist muscimol, on VTA GABA neuron spontaneous activity and internal capsule stimulus-induced poststimulus spike discharges (ICPSDs). Systemic ethanol, quinidine, and dizocilpine reduced, whereas local NMDA enhanced, and the systemic and local GABA_A receptor modulators did not significantly alter VTA GABA neuron ICPSDs. Ethanol potentiated dizocilpine inhibition of VTA GABA neuron ICPSDs, but not quinidine inhibition. In situ microelectrophoretic application of dopamine markedly enhanced VTA GABA neuron firing rate (131%), spike duration (124%), and spike coupling, which were blocked by systemic quinidine. These findings indicate that VTA GABA neurons are coupled electrically via gap junctions and that the inhibitory effect of ethanol on electrical transmission is primarily via inhibition of NMDA receptor-mediated excitation, not via enhancement of GABA receptor-mediated inhibition. Thus, the rewarding properties of ethanol may result from inhibitory effects on excitatory glutamatergic neurotransmission between electrically coupled networks of midbrain GABA neurons.

The two major types of membrane-bound proteins that are directly affected by physiologically relevant levels of ethanol (i.e., concentrations up to 100 mM or 460 mg/dl, at which point ethanol can be lethal in humans) are ligand-gated ion channels and voltage-dependent calcium channels (Harris, 1999). Ligand-gated ion channels, including γ-GABA, N-methyl-D-aspartate (NMDA), glycine, nicotinic cholinergic, and 5-hydroxytryptamine type 3 receptors play a major role in synaptic transmission and have been shown to be directly modulated by ethanol. In particular, the intoxicating and rewarding properties of ethanol seem to result from either attenuation of NMDA receptor-mediated and/or enhancement of GABA receptor-mediated neurotransmission (for reviews, see Chester and Cunningham, 2002; Davies, 2003).

The ventral tegmental area (VTA) is the neuronal origin of the mesocorticolimbic dopamine projection and has been implicated in locomotor activity, cognition, and in the reinforcing/rewarding properties of drugs of abuse (Wise, 1996), including ethanol. With drugs such as cocaine and amphetamine, the link to dopamine systems is readily apparent; however, with other drugs such as opiates and ethanol, the link to dopamine systems is not as clear. Nonetheless, ethanol increases the firing rate of midbrain dopamine neurons both in vivo and in vitro (Brodie et al., 1990; Brodie and Appel, 1998) and enhances dopamine release in the nucleus accumbens (Wozniak et al., 1991; Diana et al., 1993; McBride et al., 1993; Weiss et al., 1993), similar to what has been observed for other drugs of abuse. Although mesencephalic dopamine neurons in the substantia nigra compacta are excited by ethanol, it has been suggested that their excitation may be attributed to disinhibition produced by a primary inhibitory effect on GABA-containing neurons of the...
substantia nigra pars reticulata (Mereu and Gessa, 1985). It has been previously shown that VTA GABA neurons, presumed to be analogous to reticulata neurons, are sensitive to low-dose ethanol (Mereu and Gessa, 1985). In addition, tolerance develops to ethanol inhibition of their firing rate, and their firing rate increases during ethanol withdrawal (Diana et al., 1993, 1996; Gallegos et al., 1999), suggesting that adaptation of VTA GABA neurons may lead to long-term changes in mesocorticolimbic dopamine neurotransmission.

Midbrain dopamine neurons fire bursts of activity in response to sensory stimuli, including those associated with primary reward (Overton and Clark, 1997; Kitai et al., 1999; Di Chiara, 2002). The bursting pattern of dopamine neurons is felt to be regulated by glutamatergic NMDA receptor-mediated input from the prefrontal cortex; however, cholinergic and glutamatergic neurons in the segmental pedunculopontine nucleus, and/or rhythmic inhibitory GABAergic projections, are also involved in modulating dopamine neuron firing behavior (Kitai et al., 1999). Activation of glutamate receptors transforms a temporally dispersed synaptic GABA input into midbrain dopamine neurons into a rhythmic pattern, probably through a mechanism involving coupling through electrical synapses via gap junctions (Berretta et al., 2001). We have previously demonstrated that a subpopulation of VTA GABA neurons occurs in clusters of synchronized spikes (Steffensen et al., 1998). Their synchronization is likely governed by corticotegmental glutamatergic synaptic transmission, because their firing rate and afferent driven activity are suppressed by NMDA receptor antagonists (Steffensen et al., 1998). In preliminary studies, we have demonstrated that synaptic activation of VTA GABA neurons is blocked by gap junction antagonists and low-dose ethanol, as well as NMDA antagonists (Steffensen et al., 2003), suggesting that VTA GABA neurons are part of a network of GABA neurons, connected by electrical synapses, but governed by NMDA receptor-mediated glutamatergic neurotransmission. The aim of this study was to further characterize the pharmacology of acute ethanol on electrical, glutamate, and GABA synaptic transmission in the VTA.

Materials and Methods

Animal Care and Surgical Procedure. Male Sprague-Dawley rats (Charles River Laboratory, Hollister, CA), weighing 250 to 400 g, were housed three to a cage with ad libitum access to food and water. The room was temperature controlled (22–25°C) and maintained on a reverse 12-h light/dark cycle (off 8:00 AM, on 8:00 PM). All care and procedures were in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals. Rats were anesthetized with halothane (3.0%–4.0%), a jugular catheter was inserted, and the rats were placed into a stereotaxic apparatus. Body temperature was monitored and maintained at 37.4°C by a feedback regulated heating pad. The skull was exposed. A micropipette with its tip (4–6 μm outside diameter) protruding 10 μm distal from the tip of the five-barrel pipette. Potentials were amplified with a Multiclamp 700A amplifier (Axon Instruments, Union City, CA). Microelectrodes were oriented, via stereotaxic coordinates, into the VTA (from brain atlas, P, 1.0 to 1.3; L, 0.5–1.0; V, 6.5–7.5) with a piezoelectric microdrive (EXFO Burleigh 8200 controller and Inchworm, Victor, NY). Single-unit activity was filtered at 0.3 to 10 kHz (–3dB) for “filtered” recordings and 0.1 Hz to 10 kHz for “unfiltered” recordings with the Multiclamp 700A amplifier and displayed on Tektronix (Beaverton, OR) digital oscilloscopes. Potentials were sampled at 20 kHz (12-bit resolution) with National Instruments data acquisition boards (Austin, TX) in Macintosh computers (Apple Computer, Cupertino, CA). Extracellularly recorded action potentials were discriminated with a WP-121 spike discriminator (WPI, Sarasota, FL) and converted to computer-level pulses. Single-unit potentials, discriminated spikes, and stimulation events were captured by National Instruments NB-MIO-16 digital I/O and counter/timer data acquisition boards in Macintosh computers.

VTA GABA neurons were identified by previously established stereotaxic coordinates (Steffensen et al., 1998), and by response to in situ iontophoretic dopamine (Steffensen et al., 2003). They included relatively fast firing rate (>10 Hz), ON-OFF phasic nonbursting activity, spike duration less than 25 μs; and multiple poststimulus spike discharges (PSDs) produced by stimulation of the internal capsule (P, –1.0 to 1.3; L, 2.3–3.0; and V, 5.0–6.0). In addition, VTA GABA neuron firing rate was markedly enhanced by microiontophoretic application of dopamine (5–20 nA; 100 mM in pipette) through the recording electrode (Steffensen et al., 2003) via fast-switching current ejection through the Axon Instruments Multiclamp 700A amplifier. We evaluated only those spikes that had greater than 4:1 signal-to-noise ratio. Corticostriatal activation of VTA GABA neurons was accomplished by stimulation of the internal capsule with insulated, bipolar stainless steel electrodes. All VTA GABA neuron spikes recorded in this study produced internal capsule stimulus-induced PSDs (ICPSDs). Square-wave constant current stimulus pulses (50–2000 μA; 0.15-ms duration; average frequency, 0.1 Hz) were generated by an AMPI IsoFlex isolation unit controlled by an AMPI MASTER-8 pulse generator (Jerusalem, Israel) or by computer. Stimulation was performed at an intensity that produced 50% maximum VTA GABA neuron ICPSDs (with 10 pulses at 200 Hz; Steffensen et al., 1998; 2003).

Drug Delivery. For systemic administration of drugs, ethanol (16%) was administered intraperitoneally, whereas chloralozepoxide (HCl) (Librium), methohexital sodium (Brevital), quinidine sulfate (5–20 mg/kg), and dizocilpine (MK-801; 0.05–0.2 mg/kg) were delivered intravenously through an indwelling jugular catheter. For in situ microiontophoretic application of drugs in the VTA, muscimol hydrobromide (0.5 mM in pipette) and NMDA (40 mM in pipette) were iontophoresed by current injection (25–100 nA) through individual barrels of five-barrel micropipettes (20–40 MΩ) with Kation Scientific (Minneapolis, MN) Union-36 iontophoretic pumps. All drugs were solubilized in 0.9% saline and were obtained from Sigma-Aldrich (St. Louis, MO).

Analysis of Responses. Waveforms, discriminated spikes, and stimulation events were processed with National Instruments LabVIEW and IGOR Pro software (Wavemetrics, Lake Oswego, OR). Spike durations were measured by orienting cursors on the waveforms at half-maximum peak amplitude of the negative-going spike. Ratemeter records were analyzed by orienting cursors on the rate-meter records to integrate the average firing rate of VTA GABA neurons over 5-min epochs before drug, during drug, and after recovery. Peristimulus spike histograms (PSHs) were constructed for determinations of the number of VTA GABA neuron ICPSDs. The histograms were normalized to number of internal capsule stimulation before and after drug treatment (12 stimulation trains at 10-s intervals, 1-s epoch, 2-ms bin width). The number of driven spikes after internal capsule stimulation was determined by rectangular integration using IGOR Pro software. As the number of discharges...
varied across neurons within each animal and across animals, we integrated spikes on PSHs falling in bins immediately after the stimulation epoch and extending to a point on the PSH where the discharges seemed to be just above the floor of spontaneous activity (range 250–600 ms beyond the stimulus artifact). To further reduce variability across treatment groups, we standardized ICPSDs to percentage of control. The results for control and drug treatment groups were derived from calculations performed on spontaneous firing rate and PSHs and expressed as means ± S.E.M. The results were compared before and after drug treatment using the paired two-sample for means t test, and single factor a analysis of variance, for comparisons between groups of unequal sample size. The criterion of significance was set at \( P < 0.05 \).

**Results**

**Acute Systemic Ethanol Blocks VTA GABA Neuron ICPSDs.** We have previously found that VTA GABA neuron firing rate is suppressed by systemic and local ethanol in a dose-dependent manner (Gallegos et al., 1999). We sought to evaluate the dose-dependent effects of ethanol (0.05–2 g/kg) on VTA GABA neuron ICPSDs. The mean number of VTA GABA neuron ICPSDs at 50% maximum stimulus level (200 Hz and 10 pulses) for all experiments in this study was 68 ± 3; \( n = 112 \). Intrapерitoneal administration of acute intoxicating doses of ethanol (e.g., 1 g/kg ethanol dose level resulted in blood alcohol levels of approximately 100 mg/100 ml) significantly decreased VTA GABA neuron ICPSDs in a dose-dependent manner [Fig. 1; 0.05 g/kg ethanol dose: \( P = 0.343, t_{(2,5)} = 1.05 \); mean saline ICPSDs, 58.0 ± 10.5; mean ethanol ICPSDs, 55.2 ± 10.5; 0.25 g/kg ethanol dose: \( P = 0.00341, t_{(2,6)} = 4.68 \); mean saline ICPSDs, 55.3 ± 9.6; mean ethanol ICPSDs, 38.5 ± 7.3; 1.0 g/kg ethanol dose: \( P = 5.94E-4, t_{(2,10)} = 4.93 \); mean saline ICPSDs, 66.9 ± 11.3; mean ethanol ICPSDs, 35.3 ± 6.1; 2.0 g/kg ethanol dose: \( P = 0.00188, t_{(2,5)} = 5.97 \); mean saline ICPSDs, 82.4 ± 13.2; mean ethanol ICPSDs, 12.8 ± 3.1]. The IC\(_{50}\) for ethanol inhibition of VTA GABA neuron ICPSDs, as determined by extrapolation from this curve in Fig. 1, was approximately 1.1 g/kg. The inhibitory effect of ethanol peaked in 5 to 10 min. Concomitant with its inhibitory effect on ICPSDs, ethanol also significantly decreased VTA GABA neuron firing rate, as demonstrated previously (Gallegos et al., 1999).

**ICPSDs Are Suppressed by Connexin-36 Gap Junction Blockers.** We evaluated the effects of the connexin-36 junction blocker quinidine (Srinivas et al., 2001; 5–20 mg/kg) on VTA GABA neuron ICPSDs. Intravenous administration of quinidine significantly decreased VTA GABA neuron ICPSDs in a dose-dependent manner [Fig. 2; 5 mg/kg quinidine dose: \( P = 0.055, t_{(2,6)} = 2.40 \); mean saline ICPSDs, 62.6 ± 12.4; mean quinidine ICPSDs, 48.6 ± 9.5; 10 mg/kg quinidine dose: \( P = 4.41E-6, t_{(2,11)} = 8.34 \); mean saline ICPSDs, 60.8 ± 8.1; mean quinidine ICPSDs, 36.1 ± 8.1; 20 mg/kg quinidine dose: \( P = 0.0069, t_{(2,5)} = 4.02 \); mean saline ICPSDs, 41.5 ± 10.4; mean quinidine ICPSDs, 10.2 ± 2.6]. The IC\(_{50}\) for quinidine inhibition of VTA GABA neuron ICPSDs, as determined by extrapolation from this curve in Fig. 2, was approximately 13 mg/kg. The inhibitory effect of quinidine was rapid in onset (1–2 min), fully reversible, and the duration of effect was dependent on dose (e.g., at 10 mg/kg the inhibition recovered in less than 20 min).

**Dopamine Couples VTA GABA Neurons via Connexin-36 Gap Junctions.** VTA GABA neuron spikes recorded in halothane-anesthetized rats were characterized according to stringent electrophysiological and pharmacological (activation of firing rate by dopamine) criteria (Steffensen et al., 1998; see Materials and Methods). We evaluated the effects of in situ microelectrophoretic application of dopamine on VTA GABA neuron firing rate, spike duration, and spike coupling. Microelectrophoretic application of dopamine markedly increased VTA GABA neuron firing rate 131% at +20 nA iontophoretic current \( [P = 0.0001, t_{(2,51)} = 6.2] \); mean baseline firing rate, 26 ± 3.1 Hz; mean dopamine firing rate, 60 ± 4.5 Hz). Concomitant with the enhancement of firing rate, in situ microelectrophoretic application of dopamine markedly enhanced VTA GABA neuron spike duration 124% (Fig. 3). The widening of the spike by dopamine would often
result in distinct spikes on the wave form. These trailing spikes were often smaller spikes that comprised one, or sometimes two, of the background spikes in a cluster of phasic, mildly synchronized neurons. Systemic application of quinidine (10 mg/kg i.v.) reversibly blocked dopamine enhancement of VTA GABA neuron spike duration and recruitment of trailing spikes. Figure 3C summarizes the effects of systemic quinidine on VTA GABA neuron ICPSDs compared with saline control. Asterisk (*) indicates significance level $P < 0.01$.

Other GABA Modulating Sedative/Hypnotics Do Not Alter VTA GABA Neuron ICPSDs. We sought to compare the effects of the benzodiazepine chlordiazepoxide and the barbiturate methohexital to those of ethanol on VTA GABA neuron ICPSDs. Intravenous administration of chlordiazepoxide, at a dose level previously determined to produce similar behavioral sedation as 1 g/kg ethanol (i.e., 5 mg/kg; Steffensen et al., 2002), and that also significantly reduced VTA GABA neuron firing rate approximately 50%, similar to 1 g/kg ethanol, did not significantly decrease VTA GABA neuron ICPSDs.
neuron ICPSDs [Fig. 4; \( P = 0.066, t_{(2.5)} = 2.3 \); mean saline ICPSDs, 33.4 ± 5.7; mean chlordiazepoxide ICPSDs, 27.4 ± 3.9]. The inhibitory effect of chlordiazepoxide on VTA GABA neuron firing rate, but not ICPSDs, was rapid in onset (10–30 s), and the duration of effect was greater than 1 h.

Continuous intravenous administration of methohexital, at a dose level previously determined to produce similar behavioral sedation as 1 g/kg ethanol (i.e., 5 mg/ml/min; Steffensen et al., 2002) and that also significantly reduced VTA GABA neuron firing rate approximately 50%, similar to 1 g/kg ethanol, did not significantly decrease VTA GABA neuron ICPSDs [Fig. 5; \( P = 0.593, t_{(2.7)} = 0.56 \); mean saline ICPSDs, 96.2 ± 17.4; mean methohexital ICPSDs, 99.2 ± 20.1]. The inhibitory effect of methohexital on VTA GABA neuron firing rate, but not ICPSDs, was rapid in onset (10–30 s), fully reversible, and the duration of effect persisted for as long as it was continuously infused.

**Effects of Local NMDA and GABA Receptor Modulators on VTA GABA Neuron ICPSDs.** We have previously shown that microelectrophoretic application of the GABA\(_A\) agonist muscimol or ethanol suppress the firing rate of VTA GABA neurons (Steffensen et al., 2000). We sought to evaluate the effects of local application of NMDA and muscimol on VTA GABA neuron ICPSDs. At an iontophoretic NMDA current level that doubled VTA GABA neuron firing rate (Steffensen et al., 2000), NMDA (50 nA) mildly, but significantly, increased VTA GABA neuron ICPSDs [\( P = 0.026, t_{(2.7)} = 2.81 \); mean saline ICPSDs, 59.6 ± 12.2; mean NMDA ICPSDs, 70.7 ± 15.5]. At a muscimol current level that decreased VTA GABA neuron firing rate 50%, muscimol slightly, but not significantly [\( P = 0.053, t_{(2.9)} = 2.22 \); mean saline ICPSDs, 63.1 ± 9.8; mean muscimol ICPSDs, 49.3 ± 9.6] reduced VTA GABA neuron ICPSDs.

**Ethanol Suppression of VTA GABA Neuron ICPSDs Involves NMDA Receptors.** Because the GABA\(_A\) receptor modulators (i.e., systemic chlordiazepoxide, systemic methohexital, and local muscimol) did not seem to be affecting VTA GABA neuron ICPSDs, whereas NMDA antagonists and quinidine seemed to have effects similar to ethanol, we studied the effects of combined treatment of ethanol and the NMDA antagonist dizocilpine or quinidine to determine interactions that might suggest possible sites of ethanol action (Fig. 6). At a dose level of dizocilpine (i.e., 0.1 mg/kg) that

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**Fig. 4.** Benzodiazepine chlordiazepoxide has little effect on internal capsule-induced multiple discharging of VTA GABA neurons (A). The representative trace and PSH show the effects of intravenous saline control on individual and cumulated VTA GABA neuron ICPSDs. Representative trace and PSH show the effects of intravenous administration of the benzodiazepine chlordiazepoxide on individual and cumulated VTA GABA neuron ICPSDs (B). Summary the effects of systemic chlordiazepoxide on VTA GABA neuron ICPSDs (C). Chlordiazepoxide did not significantly reduce VTA GABA neuron ICPSDs compared with saline control, despite significantly reducing firing rate (data not shown).

**Fig. 5.** Fast-acting barbiturate methohexital has little effect on internal capsule-induced multiple discharging of VTA GABA neurons (A). The representative trace and PSH show the effects of intravenous saline control on individual and cumulated VTA GABA neuron ICPSDs. Representative trace and PSH show the effects of continuous intravenous infusion of the fast-acting barbiturate methohexital (0.5 mg/ml/1.5 min) on individual and cumulated VTA GABA neuron ICPSDs (B). Summary of the effects of systemic methohexital on VTA GABA neuron PSDs and firing rate (C). Methohexital did not significantly reduce VTA GABA ICPSDs compared with saline control, despite significantly reducing firing rate (data not shown).
nonbursting, short-duration action potential (represent a homogeneous population of phasic, rapid-firing, GABA neurons, recorded in halothane-anesthetized rats, described previously (Steffensen et al., 1998). In brief, VTA tural characterization of VTA GABA neurons has been de-

inhibition. The combination of dizocilpine an equipotent dose of ethanol only produced a fraction more VTA GABA neuron ICPSDs approximately 40%, addition of an equipotent dose of ethanol nearly doubled the inhibition. However, at a dose level of quinidine that reduced VTA GABA neuron ICPSDs approximately 40%, addition of an equipotent dose of ethanol only produced a fraction more inhibition. In other words, dizocilpine + ethanol produced a similar response as repeated dosing of dizocilpine. However, quinidine + ethanol produced inhibition that was less than that produced by the other combinations. The inhibition of VTA GABA neuron ICPSDs by the combination of dizocilpine + ethanol was significantly greater than that produced by quinidine + ethanol. Asterisk (*) indicates significance level P < 0.001.

reduced VTA GABA neuron ICPSDs approximately 40%, doubling the dose nearly doubled the inhibition. Similarly, at a dose level of dizocilpine that reduced VTA GABA neuron ICPSDs approximately 40%, addition of an equipotent dose of ethanol (i.e., 1.0 g/kg) nearly doubled the inhibition. However, at a dose level of quinidine (i.e., 10 mg/kg) that reduced VTA GABA neuron ICPSDs approximately 40%, addition of an equipotent dose of ethanol only produced a fraction more inhibition. The combination of dizocilpine + ethanol produced significantly more inhibition of VTA GABA neuron ICPSDs than the combination of quinidine + ethanol (P = 0.0008, $F_{(1,12)} = 21.2$).

Discussion

The electrophysiological, neurochemical, and ultrastructural characterization of VTA GABA neurons has been de-

cribed previously (Steffensen et al., 1998). In brief, VTA GABA neurons, recorded in halothane-anesthetized rats, represent a homogeneous population of phasic, rapid-firing, nonbursting, short-duration action potential (<250 μs), GABAergic projection neurons that project to and receive input from the cortex, nucleus accumbens, and hippocampus. Upon brief, high frequency stimulation of the internal capsular a unique phenomenon emerges. In addition to the elicitation of orthodromic or antidromic VTA GABA neuron spikes (at 1–3 ms latencies) with each stimulus pulse, multiple spike discharges follow the internal capsule stimulus train at select stimulus frequencies, often for hundreds of milliseconds, and potentially evoking hundreds of VTA GABA neuron ICPSDs (Steffensen et al., 1998). VTA GABA neuron ICPSDs are blocked by application of NMDA receptor antagonists (Steffensen et al., 1998) and can be elicited by stimulation of the parietal cortex or the mediodorsolateral nucleus of the thalamus (Steffensen et al., 2003), as well as the internal capsule, suggesting that they involve fibers pro-jecting from the cortex to the midbrain tegmentum. The number of VTA GABA neuron ICPSDs is monotonically related to the product of pulse number times stimulus intensity, and four to five internal capsule stimulus pulses are needed for threshold activation of ICPSDs (Steffensen et al., 1998, 2003).

We have previously reported that acute systemic ethanol inhibits the firing rate of VTA GABA neurons in anesthetized rats with an IC$_{50}$ of 1.2 g/kg (Gallegos et al., 1999). Here, we report that acute systemic ethanol also significantly reduces VTA GABA neuron ICPSDs at doses as low as 0.25 g/kg, and with a similar IC$_{50}$ value of 1.1 g/kg. Given the prevailing dogma that ethanol acts either to increase GABA$_{A}$ receptor neurotransmission, or to decrease NMDA receptor neuro-

transmission, we hypothesized that ethanol was acting to suppress VTA GABA neuron ICPSDs by acting through these mechanisms and/or through blocking gap junctions. Accord-

ingly, we evaluated the effects of select GABA agonists/mod-

ulators on VTA GABA neurons ICPSDs. At doses that induce mild ataxia, and produce 50% inhibition of VTA GABA neu-

ron firing rate, similar to 1.2 g/kg ethanol, the benzodiaz-

epine clordiazepoxide and the barbiturate methohexital did not significantly affect VTA GABA neuron ICPSDs, suggest-

ing that ethanol was not reducing discharges by acting on GABA$_{A}$ receptors. This obtained despite similar inhibitions in firing rate. To further convince ourselves that ethanol was not acting through GABA$_{A}$ receptors, we tested the effects of in situ microelectrophoretic application of the GABA$_{A}$ agonist muscimol. As reported previously, muscimol markedly inhibited VTA GABA neuron firing rate (Steffensen et al., 2000). We adjusted the iontophoretic current to levels that produced a 50% reduction in VTA GABA neuron firing rate before evaluating its effects on VTA GABA neuron ICPSDs. Muscimol did not significantly alter VTA GABA neuron ICPSDs, providing further evidence that ethanol is not acting like a GABA$_{A}$ agonist to decrease VTA GABA neuron ICPSDs.

We have hypothesized that VTA GABA neuron ICPSDs represent a manifestation of recurrent excitatory electrical synaptic activity (Steffensen et al., 2003). This is supported by our previous findings showing pronounced VTA GABA neuron spike synchronization behavior (Steffensen et al., 1998, 2003), and the findings presented here demonstrating dopamine-induced widening/coupling of spike waveforms. Furthermore, quinidine’s suppression of ICPSDs provides additional pharmacological evidence for electrical coupling between VTA GABA neurons. Microelectrophoretic dopamine markedly increases VTA GABA neuron firing rate and spike durations, often resulting in the evolution, or recruit-

ment, of distinct trailing spikes. This is not due to an en-

hancement in bursting activity, typically characterized by spike amplitude decrement and interspike interval incre-

ment, as the trailing spikes are often greater in amplitude than the leading spikes (Fig. 3B). Moreover, we have never seen VTA GABA neurons undergo spontaneous bursting ac-
tivity. The leading spike waveforms are unchanged by dopamine suggesting that it is coupling spikes. Modulation of gap junction neurotransmission by dopamine is well established in the retina (for review, see Weiler et al., 2000) and nucleus accumbens (for review, see O'Donnell et al., 1999). The pharmacological evidence supports the dopamine coupling hypothesis, as systemic administration of the neuronal-specific connexin-36 gap junction blocker quinidine suppressed dopamine enhancement of spike durations. Quinidine's well-known block of delayed rectifier $K^+$ channels, as well as block of ethanol excitation of VTA dopamine neurons (Appel et al., 2003), could not account for its block of dopamine-induced VTA GABA neuron spike widening, because its effects on spike waveforms would be opposite in direction.

Electrical synaptic transmission between neurons via gap junctions is an important mode of intercellular communication in immature and mature nervous systems (for review, see Roerig and Feller, 2000; Rozental et al., 2000; Bennett, 2002). In particular, networks of GABA neurons may regulate oscillatory patterns in the brain through mechanisms often dependent on gap junctions (Buzsaki and Chrobak, 1995; Tamas et al., 2000; Galarreta and Hestrin, 2001). Studies on network oscillatory behavior have mostly involved the hippocampus, thalamus, limbic system, and neocortex; however, neurons of the ventral midbrain may also discharge in a rhythmic oscillatory mode (Overton and Clark, 1997; Kitai et al., 1999). Quinidine, or its optical isomer quinine, has been shown to block connexin-36 and connexin-50 junctional currents in a reversible and concentration-dependent manner (Srinivas et al., 2001). Connexin-36 is the first connexin to be localized only in neurons (Rash et al., 2001). Quinidine, or quinine, block epileptiform transients in the hippocampus (Uusisaari et al., 2002), similar to other gap junction blockers (Perez-Velazquez et al., 1994), apparently by reducing electrical synaptic transmission between GABAergic inhibitory interneurons connected by connexin-36 gap junctions (Tamas et al., 2000; Galarreta and Hestrin, 2001; Yang and Michelson, 2001).

We have previously reported that VTA GABA neuron ICPSDs are sensitive to NMDA receptor antagonists (Steffensen et al., 1998). Thus, to determine whether ethanol was acting as an NMDA receptor antagonist, we tested the effects of in situ microelectrophoretic NMDA on VTA GABA neuron ICPSDs, as well as interactions with dizocilpine. At doses that doubled the firing rate of VTA GABA neurons, microelectrophoretic NMDA mildly, but significantly, increased VTA GABA neuron ICPSDs, providing further evidence that VTA GABA neuron ICPSDs are governed by NMDA receptors. We hypothesized that if ethanol was acting on NMDA receptors it would potentiate the inhibitory effects of dizocilpine on VTA GABA neuron ICPSDs. Compared with an IC$_{50}$ dose of ethanol, an IC$_{50}$ dose of dizocilpine + ethanol added to the inhibition, whereas an IC$_{50}$ dose of quinidine + ethanol had little effect on VTA GABA neuron ICPSDs, suggesting that ethanol is acting upstream from gap junctions, likely on the NMDA receptors that govern their excitability. However, we cannot discount the possibility that ethanol is acting at gap junctions as well. Accordingly, it is well known that long-chain alcohols and halocarbon anesthetics block gap junctions in neuronal and non-neuronal cells (Mantz et al., 1993; Spray and Dermietzel, 1996). Of more relevance to neuronal systems, gap junction inhibitors block hippocampal seizures after ethanol withdrawal, suggesting that ethanol alters gap junctions, or the neurotransmitter receptor systems that govern them (Carlen et al., 2000). Sensitivity to ethanol may result from inhibitory effects on NMDA neurotransmission to electrically coupled VTA GABA neurons.

In conclusion, VTA GABA neuron ICPSDs were suppressed by ethanol as well as quinidine and NMDA receptor blockers, suggesting that VTA GABA neurons are in a synctium, or reticulum, of electrical synapses whose network properties (i.e., synchronization) may be governed by NMDA receptor-mediated excitation from corticollimbic structures, but sustained by connexin-36 gap junctions. Additional evidence for electrical synapses between VTA GABA neurons was demonstrated by quinidine block of dopamine-induced spike coupling. The pharmacological evidence on ICPSDs suggests that ethanol may be acting in the VTA to directly affect NMDA receptor-mediated activation of GABA networks connected by gap junctions. We presume that glutamatergic modulation of GABA electrical networks is upstream from the gap junctions and is driving the network. Therefore, ethanol effects at NMDA receptors would mask potential effects at gap junctions. Although the in vivo pharmacological evidence presented here suggests a possible mechanism for ethanol effects on VTA GABA neuron ICPSDs, and electrical transmission between VTA GABA neurons, direct evidence for or against ethanol actions at gap junctions needs to be corroborated and extended in reduced in vitro systems wherein coupling between neurons can be isolated. The elicitation of VTA GABA neuron ICPSDs is an artificial response with questionable physiological relevance. However, we have observed spontaneous paroxysms of VTA GABA neuron PSDS, as well as spontaneous coupling of spikes (Steffensen et al., 2003). These phenomena, as well as other previously described characteristics of VTA GABA neurons, including spike synchronization, and corticogemal NMDA receptor-mediated glutamate drive, are physiologically relevant and could profoundly influence dopamine neural activity and subsequent release, given the prevailing dogma that VTA GABA neurons inhibit dopamine neurons. By virtue of their wide dynamic range, widespread distribution, and potential connectivity to midbrain dopamine neurons, VTA GABA neurons may be critical neuronal substrates along the continuum of alcohol intoxication, reinforcement, and dependence.

References


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