Selective modulation of GABAergic tonic current by dopamine in the nucleus accumbens of alcohol-dependent rats

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Liang J, Marty VN, Mulpuri Y, Olsen RW, Spigelman I. Selective modulation of GABAergic tonic current by dopamine in the nucleus accumbens of alcohol-dependent rats. J Neurophysiol 112: 51–60, 2014. First published April 9, 2014; doi:10.1152/jn.00564.2013.—The nucleus accumbens (NAcc) is a key structure of the mesolimbic dopaminergic reward system and plays an important role in mediating alcohol-seeking behaviors. Alterations in glutamatergic and GABAergic signaling were recently demonstrated in the NAcc of rats after chronic intermittent ethanol (CIE) treatment, a model of alcohol dependence. Here we studied dopamine (DA) modulation of GABAergic signaling and how this modulation might be altered by CIE treatment. We show that the tonic current (I_{tonic}) mediated by extrasynaptic γ-aminobutyric acid type A receptors (GABA_ARs) of medium spiny neurons (MSNs) in the NAcc core is differentially modulated by DA at concentrations in the range of those measured in vivo (0.01–1 μM), without affecting the postsynaptic kinetics of miniature inhibitory postsynaptic currents (mIPSCs). Use of selective D1 receptor (D1R) and D2 receptor (D2R) ligands revealed that I_{tonic} potentiation by DA (10 nM) is mediated by D1Rs while I_{tonic} depression by DA (0.03–1 μM) is mediated by D2Rs in the same MSNs. Addition of guanosine 5′-O-(2-thiodiphosphate) (GDP_\text{S}) to the recording pipettes eliminated I_{tonic} decrease by the selective D2R agonist quinpirole (5 nM), leaving intact the quinpirole effect on mIPSC frequency. Recordings from CIE and vehicle control (CIV) MSNs during application of D1R agonist (SKF 38393, 100 nM) or D2R agonist (quinpirole, 2 nM) revealed that SKF 38393 potentiated I_{tonic} to the same extent, while quinpirole reduced I_{tonic} to a similar extent, in both groups of rats. Our data suggest that the selective modulatory effects of DA on I_{tonic} are unaltered by CIE treatment and withdrawal.

ventral striatum; ethanol; withdrawal; neuroadaptation; tonic current

ALCOHOL (ethanol, EtOH), consumed in moderation, has numerous beneficial effects on health (French and Zavala 2007; Gunzerath et al. 2004), yet excessive alcohol drinking can lead to alcohol dependence and loss of control over alcohol consumption, with serious detrimental health consequences (Room et al. 2005). Chronic alcohol exposure causes marked changes in reinforcement mechanisms and motivational state that are thought to contribute to the development of cravings and relapse during protracted withdrawal (Koob and Le Moal 2008). Relapse to alcohol is a critical problem in treating alcoholism, and effective treatments are yet to be found.

The nucleus accumbens (NAcc) is a key neural substrate for the rewarding actions of many drugs of abuse, including alcohol (Koob et al. 1998; Wise 2004). Enhancement of dopamine (DA) release in several brain regions, most prominently in the NAcc, is a common property of alcohol and other drugs of abuse (Di Chiara and Imperato 1985, 1988). Action potential discharge in the ventral tegmental area (VTA) DA neurons mediates DA release in the NAcc (Sombers et al. 2009); this release is highly regulated by presynaptic auto- and heteroreceptors (Zhang and Sulzer 2012). DA concentrations ([DA]) reported in the NAcc of awake rats range from low nanomolar basal levels to high submicromolar concentrations during phasic DA release (Budygin et al. 2001; Owesson-White et al. 2012; Robinson et al. 2009; Segovia and Mora 2001; Yim and Gonzales 2000). Transient increases in extracellular [DA] measured with fast scanning voltammetry techniques are more frequent after administration of drugs of abuse such as alcohol and cocaine (Robinson et al. 2009; Stuber et al. 2005) and become time-locked to cues predicting reward (Day et al. 2007; Phillips et al. 2003; Stuber et al. 2005). Indeed, the mere expectation of EtOH evokes DA release in the NAcc (Kattner et al. 1996; Melendez et al. 2002; Weiss et al. 1993). Basal DA release is decreased in alcohol-dependent animals, but an EtOH challenge evokes DA release comparable to if not greater than that in nondependent control animals (Budygin et al. 2007; Diana et al. 1992, 1993; Weiss et al. 1996; Yoshimoto et al. 1996). Also, EtOH-induced DA release is greater in rats bred for alcohol preference (Bustamante et al. 2008), and alcohol cue-evoked DA release in the ventral striatum is greatest in men with a greater genetic risk for alcoholism (Oberlin et al. 2013).

Upon release, DA affects signal transmission in the NAcc via activation of various pre- and postsynaptically localized DA receptor (DAR) subtypes. The D1 class of DA receptors (D1Rs and D5Rs) is richly expressed on GABAergic and cholinergic terminals projecting to the ventral pallidum (Beaulieu and Gainetdinov 2011; Sesack and Grace 2010). In addition, there is a population of NAcc MSNs that express both D1Rs and D2Rs (Lu et al. 1998; Perreault et al. 2010, 2011). DARs are also variably expressed on GABAergic and cholinergic interneurons, as well as on glutamatergic terminals projecting to the NAcc. There is also evidence for both D1Rs and D2Rs on striatal astrocytes (Bal et al. 1994; Hosli and Hosli 1987; Miyazaki et al. 2004; Zanassi et al. 1999).

On the basis of recordings in behaving animals it has been suggested that the action potential discharge of MSNs depolarized by excitatory glutamatergic inputs is the time when NAcc neurons execute their behaviorally relevant functions.
(O’Donnell et al. 1999). The patterns of spike discharge are thus sculpted mainly by a combination of glutamatergic excitation, GABAergic inhibition, and modulatory influences of DA. Recently, we demonstrated alterations in the intrinsic electrical membrane properties and enhanced glutamatergic synaptic transmission of MSNs in the NAcc core of rats during protracted withdrawal from chronic intermittent ethanol (CIE) treatment, a model of alcohol dependence (Marty and Spigelman 2012). In the companion article (Liang et al. 2014), we demonstrated CIE-induced alterations in the function and expression of GABA_A receptors (GABA_A Rs). Here we studied the modulatory influence of DA and selective D1R and D2R ligands on GABA_A-R-mediated currents in NAcc core MSNs in CIE rats and vehicle control (CIV) rats after protracted withdrawal with the goal of obtaining a better understanding of alterations in neurotransmission within the mesolimbic reward system in alcohol dependence.

Our studies reveal that at physiologically relevant concentrations DA selectively affects tonic GABA_A-R currents, without effects on synaptic GABA_A-Rs. Furthermore, DA (10 nM) potentiates the tonic GABA_A-R current (I_tonic) in MSNs, whereas higher [DA] (0.03–1 μM) depress this current. These bidirectional effects of DA on I_tonic are mediated by D1Rs (potentiation) and D2Rs (depression), respectively. We also show that modulation of I_tonic by selective D1R and D2R agonists is unaffected by CIE treatment and long-term withdrawal. However, because of the loss of EtOH potentiation of I_tonic in CIE rats, an EtOH challenge in these rats should result in unopposed D2R-mediated depression of I_tonic. Coupled with the demonstrated increases in glutamatergic excitatory neurotransmission after CIE treatment (Jeanes et al. 2011; Marty and Spigelman 2012; Szumilinski et al. 2007), we predict that EtOH-evoked DA release should produce larger increases in the “up-state” excitability of MSNs in alcohol-dependent compared with nondependent animals.

METHODS

The Institutional Animal Care and Use Committee approved all animal experiments. Male Sprague-Dawley rats (Harlan, weighing 190–220 g upon arrival) were housed in the vivarium under a 12:12-h light-dark cycle and had free access to food and water. Rats were administered a chronic intermittent EtOH (CIE) regimen: for the first 5 doses rats received 5 g/kg EtOH as a 25% (wt/vol) solution once every other day and for the following 55 doses 6 g/kg of EtOH 30% (wt/vol) once every day. The CIE control group received water (20 ml/kg). Naive rats age-matched to the CIV/CIE groups (weighing 0.01–1 μM) application (data not shown). The effect of DA on picrotoxin (GABA_AR Cl⁻ channel blocker)-sensitive tonic current (I_tonic). In all MSNs tested, DA (10 nM) produced a significant increase of I_tonic, while higher [DA] (0.03–1 μM) had a significant depressive effect on I_tonic (Fig. 1, A and B). In contrast, DA (0.01–1 μM) had no significant effect on the average amplitude, charge transfer (area), or frequency of mIPSCs (Fig. 1, D–F). The rise time and decay kinetic parameters of mIPSCs were similarly unaffected by DA (0.01–1 μM) application (data not shown). The effect of DA on I_tonic was fully reversible upon washout, irrespective of the [DA] applied (e.g., Fig. 1B).

Potentiation and depression of I_tonic by DA are mediated by D1Rs and D2Rs, respectively. Since the effects of DA at its different receptor subtypes are determined in part by differences in the affinity for DA (Rankin et al. 2010; Seeman and

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The recordings were low-pass filtered (Clampfit software, Molecular Devices) at 2 kHz and analyzed with the aid of the Mini Analysis Program (Synaptosoft, Fort Lee, NJ). Miniature inhibitory postsynaptic currents (mIPSCs) were detected with threshold criteria of 5-pA amplitude and 20-μs charge transfer. Frequency of mIPSCs was determined from all automatically detected events in a given recording period. Tonic current magnitudes were obtained from the mean baseline current of a given recording period. For kinetic analysis of mIPSCs, single events with a stable baseline, sharp rising phase, and exponential decay were chosen. Double and multiple-peak mIPSCs were excluded. The mIPSC kinetics were obtained from analysis of the averaged chosen single events (>120 events/100-s recording period) aligned with half rise time in each cell. The investigators performing the recordings and analysis were blind to the treatment (naive, CIE, or CIV) that the rats received. All summary values are presented as means ± SE. Group differences were evaluated by unpaired Student’s t-test or one-way analysis of variance (ANOVA), where appropriate. P < 0.05 was considered statistically significant.

RESULTS

Selective concentration-dependent effects of DA on tonic GABA_AR current. We first examined the effects of DA application on isolated GABA_AR currents in NAcc MSNs from naive rats (Fig. 1). We chose to use [DA] in the range reported in the NAcc of awake rats at basal conditions and during phasic DA release (Budygin et al. 2001; Owesson-White et al. 2012; Robinson et al. 2009; Segovia and Mora 2001; Yin and Gonzales 2000). Application of DA at increasing concentrations (0.01–1 μM) had a biphasic effect on the picrotoxin (GABA_AR Cl⁻ channel blocker)-sensitive tonic current (I_tonic).

In all MSNs tested, DA (10 nM) produced a significant increase of I_tonic, while higher [DA] (0.03–1 μM) had a significant depressive effect on I_tonic (Fig. 1, A and B). In contrast, DA (0.01–1 μM) had no significant effect on the average amplitude, charge transfer (area), or frequency of mIPSCs (Fig. 1, D–F). The rise time and decay kinetic parameters of mIPSCs were similarly unaffected by DA (0.01–1 μM) application (data not shown). The effect of DA on I_tonic was fully reversible upon washout, irrespective of the [DA] applied (e.g., Fig. 1B).
Van Tol 1994; Undieh 2010), we tested for DA effects on $I_{\text{tonic}}$ in the presence of D1R or D2R antagonists. To that end, we recorded GABA$_{\text{A}}$R current responses to DA first at 10 nM and then at 1 μM during continuous application of the selective D1R antagonist SKF 83566 at 10 nM (Ohlstein and Berkowitz 1985). SKF 83566 application alone had no discernible effects on $I_{\text{tonic}}$ (Fig. 2A). In the continued presence of SKF 83566, application of DA (10 nM) did not increase $I_{\text{tonic}}$, whereas subsequent application of DA (1 μM) had a significant (>30%) depressant effect on $I_{\text{tonic}}$ (Fig. 2, A and C). These pharmacological manipulations had no effect on mIPSC amplitude or charge transfer, with only a small (<3%) effect on mIPSC frequency (Fig. 2D). These data suggested that D1Rs mediate potentiation of $I_{\text{tonic}}$ while D2Rs depress $I_{\text{tonic}}$. To confirm this, we measured GABA$_{\text{A}}$R current responses to DA first at 10 nM and then at 1 μM during continuous application

Fig. 1. Concentration-dependent effects of dopamine (DA) on the tonic GABA type A receptor (GABA$_{\text{A}}$R) current ($I_{\text{tonic}}$). A, bottom: continuous voltage-clamp recording of pharmacologically isolated GABA$_{\text{A}}$R currents in a nucleus accumbens (NAcc) core medium spiny neuron (MSN) from a naive rat. The picrotoxin (PTX, 50 μM)-sensitive $I_{\text{tonic}}$ is indicated by the dashed lines. Note the increase in $I_{\text{tonic}}$ during DA (10 nM) application and the subsequent decrease during DA (30 nM) application. Top: the miniature inhibitory postsynaptic currents (mIPSCs) averaged over the indicated 100-s recording intervals are unaffected by DA applications. B: in another MSN recording from a naive rat, application of DA (100 nM) reversibly decreases $I_{\text{tonic}}$ (bottom) without affecting mIPSCs (top). C: summary graph of concentration-dependent DA effects on $I_{\text{tonic}}$. *P < 0.05 (n = 2–6 neurons from 2 rats, 1-way ANOVA). D–F: summary graphs of DA effects on mIPSC amplitude (D), area (E), and frequency (F).
of the selective D2R antagonist sulpiride at 30 nM (Seeman and Van Tol 1994). Sulpiride application alone had no discernible effects on $I_{\text{tonic}}$ (Fig. 2, B and E). However, in the continued presence of sulpiride, application of DA (10 nM) significantly increased $I_{\text{tonic}}$ by >20% (Fig. 2E). During the subsequent application of DA (1 μM), the $I_{\text{tonic}}$ remained significantly potentiated above baseline (Fig. 2, B and E). During these pharmacological manipulations, only the amplitude of mIPSCs during the combined sulpiride + 10 nM DA application was significantly increased (<8%), while the frequency of mIPSCs was unaffected by application of sulpiride or sulpiride-DA combinations (Fig. 2F). The charge transfer, rise time, and decay kinetic parameters of mIPSCs were similarly unaffected by sulpiride-DA application (not shown). Together, these data suggested that potentiation and depression of $I_{\text{tonic}}$ by DA are mediated by D1Rs and D2Rs, respectively.

D2R-mediated effects on $I_{\text{tonic}}$ are blocked in recordings with intrapipette guanosine 5'-O-(2-thiodiphosphate). Modulation of $I_{\text{tonic}}$ magnitude by DA could be due to modulation of postsynaptic GABA$_A$R function by postsynaptic DA receptors or to DA-induced changes in ambient GABA concentrations ([GABA]). To help differentiate between these two possibil-
ties we measured responses to the application of a selective D2R agonist, quinpirole at 5 nM (Seeman and Van Tol 1994), in recordings from naive rat NAcc MSNs with patch pipettes containing a control internal solution and compared them to recordings with pipettes containing guanosine 5'-O-(2-thiodiphosphate) (GDPβS), which antagonizes G protein signaling within the recorded neurons (Schiffmann et al. 1995; Yan et al. 1997). For these recordings, we used CsCl-based intrapipette solutions and voltage-clamped the MSNs at −70 mV to demonstrate that the effects of DAR activation on \( I_{\text{tonic}} \) are independent of the GABAAR Cl⁻ current reversal potential. In control recordings, quinpirole (5 nM) application produced large reversible decreases in the basal holding current (Fig. 3A). Quinpirole had no significant effect on any of the postsynaptic mIPSC kinetic parameters (rise time: 90.8 ± 3.6, amplitude: 98.1 ± 10.2, decay \( \tau_1 \): 102.4 ± 3.1, decay \( \tau_2 \): 108.5 ± 13.1, charge transfer: 103.2 ± 20.3; all expressed as % of baseline). There was a trend toward decreased mIPSC frequency: 88.5 ± 7.3 of baseline, which did not reach statistical significance (paired t-test). In contrast, the quinpirole effect on the tonic holding current was almost completely abolished in recordings with GDPβS-containing patch pipettes (Fig. 3A, B and D). In these recordings, quinpirole also had no significant effect on any of the postsynaptic mIPSC parameters (rise time: 112.7 ± 14.3, amplitude: 99.9 ± 11.3, decay \( \tau_1 \): 108.1 ± 22.3, decay \( \tau_2 \): 79.0 ± 39.5, charge transfer: 105.0 ± 27.1; expressed as % of baseline), but we did observe a significant (\( P < 0.01 \), paired t-test) decrease in mIPSC frequency (59.1 ± 7.4 of baseline), consistent with activation of presynaptic D2Rs (Delle Donne et al. 1997; Zhang and Sulzer 2012). We also showed that during recordings with control CsCl-based pipettes application of quinpirole (5 nM) during continuous bath application of picrotoxin had no effect on the remaining holding current (Fig. 3, C and D). Together, these data suggested that DA-mediated modulation of \( I_{\text{tonic}} \) requires the activation of postsynaptic G protein-coupled DARs, which is separate from the D2R-mediated presynaptic effects on mIPSC frequency.

DA-mediated depression of \( I_{\text{tonic}} \) is unaffected by CIE treatment. We next compared the D2R-mediated depressant effect of DA (30 nM) in NAcc MSN recordings from CIV- and CIE-treated rats. In the same recordings we also measured the effect of acute EtOH (10 and 60 mM) application during coapplication with DA (Fig. 4A). Similar to recordings from naive rats, DA (30 nM) application produced a significant depression of \( I_{\text{tonic}} \) in the MSNs from CIV rats without significant changes in postsynaptic mIPSC kinetic parameters (rise time: 103.9 ± 3.0, amplitude: 98.8 ± 0.7, decay \( \tau_1 \): 111.6 ± 3.9, decay \( \tau_2 \): 136.5 ± 36.4, charge transfer: 103.2 ± 20.3; all expressed as % of baseline).
expressed as % of baseline). However, there was a small (92.3 ± 1.9% of baseline) but significant (*P < 0.02, paired t-test) decrease in mIPSC frequency after DA application, which was not observed in the initial set of DA application recordings (cf. Fig. 1).

During continuous application of DA, application of EtOH, first at 10 mM and then at 60 mM, resulted in significant concentration-dependent increases in I\textsubscript{tonic} during recordings from MSNs of CIV rats (Fig. 4). When comparing recordings from CIE rats, DA (30 nM) still produced a significant depression of I\textsubscript{tonic} (Fig. 4B), while EtOH (10–60 mM) no longer potentiated I\textsubscript{tonic} (Fig. 4C). In these recordings from CIE rat MSNs, DA application again had no significant effect on postsynaptic mIPSC kinetic parameters (rise time: 97.4 ± 6.0, amplitude: 99.0 ± 0.4, decay τ\textsubscript{1}: 100.6 ± 3.6, decay τ\textsubscript{2}: 112.9 ± 23.5, charge transfer: 103.2 ± 20.3; expressed as % of baseline), although we again observed a significant (*P < 0.01, paired t-test) decrease (84.4 ± 2.2% of baseline) in mIPSC frequency after DA application.

CIE treatment does not alter I\textsubscript{tonic} response to selective D1R and D2R agonists. To further assess whether CIE treatment alters dopaminergic modulation of I\textsubscript{tonic}, we compared GABA\textsubscript{A}R current responses to application of the selective D1R agonist SKF 38393 (0.1 μM) or separately to application of the selective D2R agonist quinpirole at 2 nM (Seeman and Van Tol 1994). Application of SKF 38393 produced an enhancement of the tonic current in the MSNs of CIE and CIV rats (Fig. 5). Subsequent coapplication of the selective D1R antagonist SKF 83566 (0.1 μM) reversed this effect (Fig. 5A), confirming selectivity of D1R activation for I\textsubscript{tonic} potentiation. In recordings from CIV rats, SKF 38393 (0.1 μM) application had no significant effect on any mIPSC parameters (rise time: 105.2 ± 8.7, amplitude: 106.7 ± 3.5, decay τ\textsubscript{1}: 110.9 ± 12.5, decay τ\textsubscript{2}: 133.7 ± 33.9, charge transfer: 105.8 ± 7.8, frequency: 101.8 ± 1.0; expressed as % of baseline). A similar lack of effects was observed in recordings from CIE rats (rise time: 106.8 ± 12.1, amplitude: 100.5 ± 2.1, decay τ\textsubscript{1}: 135.8 ± 17.7, charge transfer: 111.8 ± 5.5, frequency: 99.2 ± 1.2; expressed as % of baseline), with the exception of mIPSC decay τ\textsubscript{2}, which was significantly (*P < 0.05, paired t-test) increased (174.6 ± 23.1% of baseline).

Application of quinpirole suppressed I\textsubscript{tonic} in MSNs from CIV and CIE rats, and this suppression was reversed by coapplication of the D2R antagonist sulpiride (30 nM) (Fig. 4B). Quinpirole application in recordings from CIV rat MSNs had no significant effect on postsynaptic mIPSC kinetic parameters (rise time: 116.9 ± 7.0, amplitude: 101.5 ± 0.7, decay τ\textsubscript{1}: 100.0 ± 8.0, decay τ\textsubscript{2}: 114.4 ± 28.3, charge transfer: 107.9 ± 8.7; expressed as % of baseline), although there was a very small, but significant (*P < 0.05, paired t-test) decrease in mIPSC frequency (98.6 ± 0.6 of baseline), consistent with previous observations. A mostly similar lack of effects was observed in recordings from CIE rat MSNs (rise time: 114.4 ± 7.1, amplitude: 101.3 ± 1.1, decay τ\textsubscript{1}: 111.9 ± 1.8, decay τ\textsubscript{2}: 147.3 ± 24.8, frequency: 99.2 ± 1.2; expressed as % of baseline). Surprisingly, mIPSC charge transfer was signifi-

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**Fig. 5.** CIE treatment does not alter D1R- and D2R-mediated modulation of I\textsubscript{tonic}. A: representative recording (CsCl-based patch electrode) of selective D1R agonist (SKF 38393, 0.1 μM) potentiation of the PTX-sensitive I\textsubscript{tonic} (dashed lines) in a NAcc MSN from a CIE-treated rat. Note that coapplication of the selective D1R antagonist SKF 83566 (0.1 μM) reversed this potentiation. Horizontal brackets indicate the 100-s recording periods used for I\textsubscript{tonic} and mIPSC analysis. C: summary graph illustrates comparable potentiation of I\textsubscript{tonic} by SKF 38393 (1 μM) in CIV and CIE rats. *P < 0.05 (n = 4–6 neurons from 2–4 rats/group, t-test vs. control). D: summary graph illustrates comparable depression of I\textsubscript{tonic} by quinpirole (2 nM) in CIV and CIE rats. *P < 0.05 (n = 9–14 neurons from 4–6 rats/group, t-test vs. control).

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cantly (P < 0.001, paired t-test) increased (111.9 ± 1.8% of baseline), which was in contrast to the large decreases in I_{tonic} observed in these recordings.

Importantly, comparison of D1R-mediated potentiation of I_{tonic} between CIE and CIV rats did not reveal significant group differences (Fig. 4C). Similarly, D2R-mediated suppression of I_{tonic} was not different between CIE and CIV rats (Fig. 4D). These data suggested that CIE treatment does not modify the modulatory effect of DA on GABA_A-mediated I_{tonic}.

**DISCUSSION**

Studies utilizing microdialysis and fast scanning voltammetry techniques demonstrated that [DA] measured in the NAcc of awake rats range from low nanomolar basal levels to high submicromolar concentrations during phasic DA release (Budygin et al. 2001; Owesson-White et al. 2012; Robinson et al. 2009; Segovia and Mora 2001; Yim and Gonzales 2000). Here we demonstrate that application of DA in NAcc slices at such physiologically relevant concentrations (0.01–1 μM) preferentially modulates extrasynaptic GABA_ARs that mediate the tonic current, without significant effects on synaptic GABA_ARs. Studies in murine MSNs in the dorsal striatum have also suggested preferential modulation of I_{tonic} by DA receptor ligands compared with synaptic currents (Janssen et al. 2009). Analogous to the hippocampus (Glykys et al. 2008; Liang et al. 2007; Scimemi et al. 2005), I_{tonic} in NAcc MSNs is mediated by several different combinations of extrasynaptic GABA_A subunits that demonstrate considerable alterations after CIE treatment (companion article, Liang et al. 2014). These low-conductance, high-affinity extrasynaptic GABA_ARs are activated by ambient GABA whose sources may include spillover from the synaptic cleft during synaptic transmission (Glykys and Mody 2007; however, see Bright et al. 2011) and channel-mediated release from astrocytes (Lee et al. 2010).

The selectivity of DA actions on I_{tonic} could result from DA modulation of extracellular [GABA]. Changes in extracellular [GABA] may result from changes in the amount of released GABA or its uptake. Presynaptic effects of DA on neuronal GABA release are well-established (Feuerstein 2008; Zhang and Sulzer 2012), and we did observe decreases in frequency of mIPSCs after DA (30 nM) or quinpirole application, without concomitant changes in postsynaptic mIPSC kinetic parameters. These data suggested that the observed DA actions on I_{tonic} might be mediated by changes in synaptic GABA release. GABA may also be released from astrocytes via Bestrophin 1 channels (Lee et al. 2010). Striatal astrocytes were demonstrated to express both D1Rs and D2Rs (Bal et al. 1994; Hosli and Hosli 1987; Miyazaki et al. 2004; Zanassi et al. 1999). Conceivably, activation of these DARs could modulate GABA release from astrocytes. Indeed, D1R activation was shown to increase extracellular [GABA] via nonvesicular transporter-mediated GABA release in striatal slices and primary cultures (Schoffelmeer et al. 2000). However, we demonstrated that blockade of postsynaptic G protein signaling in the recorded MSNs with GDPβS abolished D2R-receptor-mediated decreases in I_{tonic} (Fig. 3), without affecting postsynaptic mIPSC kinetic parameters. Notably, during this blockade of postsynaptic G protein signaling in the recorded MSNs, mIPSC frequency could still be decreased by quinpirole application. These experiments provided concrete evidence for a postsynaptic mechanism by which extrasynaptic GABA_ARs are modulated by DAR activation in MSNs. A postsynaptic mechanism of action was previously demonstrated for the D3R-mediated suppression of GABA_A R currents in the NAcc (Chen et al. 2006). Also, functional postsynaptic G protein-dependent cross talk between GABA_A R and GABA_A Rs that mediate I_{tonic} has been recently demonstrated in thalamic, dentate gyrus, and cerebellar neurons (Connelly et al. 2013).

The preferential DA responsiveness of extrasynaptic GABA_A Rs in NAcc MSNs could result from preferential removal of extracellular synaptic DA by DA transporters localized around GABAergic synapses. However, extensive cellular and subcellular studies have failed to localize DA transporter to any synaptic active zones in the striatum, suggesting that striatal DA reuptake likely occurs outside of synaptic specializations once DA diffuses from the synaptic cleft (Hersch et al. 1997). Furthermore, the fact that I_{tonic} is also preferentially modulated by selective D1R and D2R agonists that are not subject to DA transporter-mediated removal makes this an unlikely possibility. More likely is the differential coupling of second messenger mechanisms to the unique subunit composition of extrasynaptic GABA_A Rs versus synaptic GABA_A Rs. In the NAcc as in other forebrain regions, extrasynaptic GABA_A Rs include, together with the ubiquitous β subunits, various combinations of α4, α5, and δ subunits. Isoform-specific phosphorylation of individual GABA_A R subunits by protein kinase A (PKA) and protein kinase C (PKC) has been shown to differentially affect activation, pharmacological responsiveness, and membrane recycling of extrasynaptic versus synaptic GABA_A Rs (Brandon et al. 1999; Kia et al. 2011; Werner et al. 2011). More studies are needed to precisely identify the extrasynaptic GABA_A R subunit combinations affected by DAR activation.

The observed selective potentiation of postsynaptic GABA_A Rs by D1Rs and their selective depression by D2Rs is supported by previous studies in mice with genetic ablation of D1Rs or D2Rs (Centonze et al. 2003), which showed a loss of DA-dependent depression of IPSCs evoked in striatal interneurons of D2R-null mice. Differential D1R versus D2R regulation of IPSCs by DA was also demonstrated in recordings from rat prefrontal cortex neurons (Trantham-Davidson et al. 2004). It is noteworthy that while activation of both synaptic and extrasynaptic GABA_A Rs contributes to the action potential-dependent evoked IPSCs measured in the above studies, GABA_A Rs giving rise to action potential-independent synaptic mIPSCs were separable from the extrasynaptic GABA_A Rs giving rise to I_{tonic} in our recordings. Recent studies in the NAcc of mice have confirmed both D1R-mediated potentiation and D2R-mediated depression of extrasynaptic α4β3 GABA_A R activity and also showed that such differential effects on MSN neuron excitability can modulate the behavioral response to psycho-stimulants (Maguire et al. 2014). The differential responsiveness of GABA_A Rs to D1R or D2R activation is likely due to the activation of different intracellular signaling pathways by these DAR subtypes. The D1-class DARs (D1R and D5R) are generally known to activate the G_{lof} family of G proteins to stimulate adenylyl cyclase (AC) and PKA activity, whereas the D2-class DARs (D2R, D3R, and D4R) couple to G_{lof} proteins, resulting in inhibition of AC and a decrease in PKA activity (Beaulieu and Gainetdinov 2011). Amplification of PKA signaling is mediated by the DA- and cAMP-regulated phosphoprotein (DARPP-32), which is highly expressed in MSNs,
where it acts as an integrator on modulatory responses to multiple neurotransmitters, including DA (Bateup et al. 2008; Nishi et al. 1997; Svenningsson et al. 2004). Coexpression of D1Rs and D2Rs may lead to formation of D1R:D2R heterodimers that, acting through G_{o,q}, can regulate downstream calcium signaling (Rashid et al. 2007).

Numerous studies have shown that striatal MSNs, including those in the NAcc core, form two principal subgroups that are defined by their selective expression of DAR subtypes and their projection targets (reviewed in Beaulieu and Gainetdinov 2011; Valjent et al. 2009). For example, murine NAcc MSNs projecting to the VTA selectively express D1Rs, thus resembling the striatonigral (direct pathway) MSNs, while MSNs projecting to the ventral pallidum express D2Rs and thus resemble the striatopallidal (indirect pathway) MSNs (Gangarossa et al. 2013). However, in addition to the two main subgroups there is a population of MSNs that express both D1Rs and D2Rs, with the highest incidence in NAcc (Lu et al. 1998; Perreault et al. 2010, 2011). Partial coexpression of D1R with D3R, particularly in the NAcc, has also been described (Le Moine and Bloch 1996). In our experiments, all recorded NAcc core MSNs tested with an application of a selective D1R agonist or 10 nM DA responded with I_{tonic} enhancement (n = 14), and conversely all MSNs tested with a selective D2R agonist or 0.03–1 μM DA responded with a decrease in I_{tonic} (n = 40). The probability that our “blind” patch recordings selectively targeted a particular subgroup of MSNs is very small, suggesting instead that the ability of DA and selective DAR agonists to bidirectionally modulate the activity of extrasynaptic GABA_{A}Rs is common to all NAcc core MSNs. This is in agreement with the immunohistochemically and functionally demonstrated coexistence of D1Rs and D2Rs in virtually all embryonic rat striatal neurons after 3 wk in culture (Aizman et al. 2000) and with the functional and single-cell RNA amplification demonstrations of D1R, D2R, and D3R coexpression in retrogradely labeled single striatongral rat MSNs (Surmeier et al. 1992). Taken together, these data suggest that there may be important species differences in the colocalization of DAR subtypes in direct and indirect pathway NAcc MSNs between rats and mice. Furthermore, the extensive colocalization of DARPP-32 with both D1R- and D2R-positive MSNs in the rat striatum (Rajput et al. 2009) provides a plausible intracellular substrate by which bidirectional modulation of I_{tonic} might be achieved. This conjecture would have to be thoroughly tested in future studies. It would also be of interest to determine whether there are differences in DA modulation of I_{tonic} between rat MSNs projecting to the VTA and those projecting to the ventral pallidum.

In MSNs at their hyperpolarized resting (“down”) state, similar to hippocampal interneurons (Song et al. 2011), the GABA_{A}R current reversal potential is depolarizing, making baseline I_{tonic} excitatory. Indeed, excitatory synaptic activation of single action potentials in MSNs is reduced during application of L655,703, which preferentially antagonizes I_{tonic} in MSNs (Ade et al. 2008). In contrast, when MSNs are in their depolarized “up” state of action potential discharge, the GABA_{A}R current reversal potential is hyperpolarizing, making baseline I_{tonic} inhibitory. Therefore, low basal levels of extracellular DA (≤10 nM) would be expected to exert an excitatory influence on MSNs at their “down” state by D1R-mediated potentiation of I_{tonic}, but when MSNs are in their depolarized “up” state D1R-mediated potentiation of I_{tonic} should have a hyperpolarizing inhibitory effect. Furthermore, phasic increases in DA release resulting in higher [DA] (≥30 nM) would be expected to block I_{tonic} via D2Rs. If phasic DA release were time-locked with activation of excitatory glutamatergic inputs in the NAcc, the D2R-mediated block of I_{tonic} would then effectively accentuate the “up” state of affected MSNs. Evidence for the synchronized release of glutamate and DA includes the demonstration that dopaminergic terminals in the NAcc (but not the dorsal striatum) corelease glutamate, such that their selective optogenetic activation results in robust glutamate-mediated excitatory postsynaptic currents (EPSCs) in NAcc (but not dorsal striatum) MSNs (Stuber et al. 2010). Other studies have shown that cholinergic interneurons also corelease glutamate and that their selective optogenetic activation not only elicits EPSCs in MSNs (Higley et al. 2011) but also elicits DA release directly from DA terminals in the NAcc, this release being dependent on glutamatergic receptor activity (Cachope et al. 2012). Also, in vivo recordings of NAcc neuron activity coupled with fast scanning voltammetry measurements showed that phasic DA selectively modulates excitatory responses of NAcc neurons during cue-evoked sucrose-seeking behavior (Cacciapaglia et al. 2011). On the basis of these studies and our present findings, we propose that during basal DA release D1R-mediated potentiation of I_{tonic} will either increase or decrease the excitability of MSNs depending on their membrane potential. In contrast, D2R activation, which should be greatest during peak phasic DA release, will increase MSN excitability by blocking I_{tonic} thereby accentuating the throughput of strong glutamatergic inputs.

Neither the magnitude of I_{tonic} nor the relative effectiveness of D1R- and D2R-mediated modulation of I_{tonic} is altered by CIE treatment (Fig. 5 and Liang et al. 2014). The observed lack of CIE-induced changes in modulation of I_{tonic} by selective DAR agonists is consistent with the reported absence of changes in D2Rs after a chronic EtOH diet (Tajuddin and Druse 1996). Also, in alcohol-dependent animals basal DA release is decreased, yet an EtOH challenge evokes comparable if not greater DA release than in nondependent control animals (Budygin et al. 2007; Diana et al. 1992, 1993; Weiss et al. 1996; Yoshimoto et al. 1996). However, EtOH potentiation of I_{tonic} is lost in CIE rats (Fig. 4 and Liang et al. 2014), and therefore an EtOH challenge in alcohol-dependent rats should result in unopposed D2R-mediated block of I_{tonic}. Coupled with the demonstrated increases in glutamatergic excitatory neurotransmission after CIE treatment (Jeanes et al. 2011; Marty and Spigelman 2012; Szumlinski et al. 2007), we predict that EtOH-evoked DA release should produce larger increases in the “up-state” excitability of MSNs in CIE rats compared with control rats. These neuroadaptive changes could contribute to the high levels of voluntary EtOH intake previously demonstrated in CIE rats (Rimondini et al. 2003). Analogous changes in the human NAcc could contribute to the development of cravings and relapse during protracted withdrawal in alcohol dependence.

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CONFLICTS OF INTEREST

No conflicts of interest, financial or otherwise, are declared by the author(s).

AUTHOR CONTRIBUTIONS


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