Published in final edited form as:

Alcohol Clin Exp Res. 2014 November ; 38(11): 2763–2769. doi:10.1111/acer.12542.

Ethanol attenuation of long term depression in the nucleus accumbens can be overcome by activation of TRPV1 receptors

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Abstract

Background—Altered expression of synaptic plasticity within the nucleus accumbens (NAc) constitutes a critical neuroadaptive response to ethanol and other drugs of abuse. We have previously reported that NMDA receptor-dependent long-term depression (LTD) is markedly affected by chronic intermittent ethanol exposure in vivo; however, endocannabinoid (eCB)-dependent synaptic depression, despite being very well-documented in the dorsal striatum, is much less well understood in the NAc.

Methods—Whole cell patch clamp electrophysiology was used to investigate interactions between these different plasticity-induction systems. Excitatory postsynaptic currents (EPSCs) were measured in the NAc shell and NMDAR-LTD was induced by a pairing protocol (500 stimuli @ 1 Hz stimulation (LFS) paired with postsynaptic depolarization to −50 mV). AM251, a CB1 receptor antagonist, was used to determine whether this form of LTD is modulated by eCBs. To determine the effect of ethanol on a purely eCB-dependent response in the NAc, depolarization-induced suppression of excitation (DSE) was used in the presence of 40 mM ethanol. Finally, we determined whether the enhancement of eCB signaling with URB597, a fatty acid amide hydrolase inhibitor, and AM404, an anandamide reuptake inhibitor would also modulate LFS LTD in the presence of NMDA-receptor blockade or ethanol.

Results—In the presence of AM251, the LFS pairing protocol resulted in NMDA receptor-dependent long-term potentiation (LTP) that was blocked with either ethanol or DL-APV. We also found that DSE in the NAc shell was blocked by AM251 and suppressed by ethanol. Enhanced eCB signaling rescued NAc LTD expression in the presence of ethanol through a distinct mechanism requiring activation of TRPV1 receptors.

Conclusion—Ethanol modulation of synaptic plasticity in the NAc is dependent upon a complex interplay between NMDA receptors, eCBs and TRPV1 receptors. These findings demonstrate a

novel form of TRPV1-dependent LTD in the NAc shell that may be critical for ethanol dependence.

Keywords

Endocannabinoid; Plasticity; TRPV1; DSE; Electrophysiology

Introduction

Neuroadaptations within the mesocorticolimbic system have been implicated in the formation of drug dependence and the expression of drug related behaviors as several studies have shown alterations in excitatory signaling and plasticity after chronic drug exposure (Luscher and Malenka, 2011). In the nucleus accumbens (NAc), NMDAR-dependent long-term depression (LTD) is the most reliably induced and best described form of plasticity (Thomas et al., 2000). Considerable evidence indicates that the alteration of the expression of NMDAR-dependent LTD in the NAc constitutes an important neuroadaptation in response to chronic drug and alcohol exposure (Thomas et al., 2001; Brebner et al., 2005; Kasanetz et al., 2010; Jeanes et al., 2011; Abrahao et al., 2013). Despite the established importance of NAc LTD, the great majority of research in this area has focused on NMDAR-dependent forms of LTD to the neglect of other well-known, non-NMDAR-dependent forms of LTD. In particular, the role of endocannabinoid (eCB) and metabotropic glutamate receptor (mGluR) dependent synaptic transmission in NAc plasticity remain largely unexplored. There are a limited number of reports documenting endocannabinoid mediated plasticity in the NAc. One report documented a NAc LTD dependent upon the activation of presynaptic CB1 receptors (Robbe et al., 2002); whereas another report demonstrated a form of NAc-LTD that instead required the activation of both CB1 receptors and postsynaptic TRPV1 receptors (Grueter et al., 2010). Thus, due to the postsynaptic expression of this latter form of LTD, TRPV1 receptor-dependent LTD appears more likely to share similarities to the postsynaptic LTD activated by NMDARs. In order to understand how drugs and alcohol alter NAc synaptic plasticity, the mechanisms underlying how these synapses are modulated via different signaling systems must be further elucidated. With the ability of the NAc to express both eCB-mediated LTD as well as NMDAR-dependent LTD, we asked whether any mechanistic crossover exists, allowing eCBs to modulate NMDAR-dependent LTD.

We have previously shown that the expression of NMDAR-dependent LTD induced by low frequency stimulation is blocked by acute ethanol, while in vivo chronic intermittent ethanol (CIE) exposure with the same stimulation protocol results in long term potentiation (LTP) (Jeanes et al., 2011). It is unknown if the effects of ethanol on this form of NMDAR-dependent LTD involve the modulation of eCB signaling. However, it is known that ethanol can affect eCB production and/or release although the direction of modulation remains unclear. Some investigators have observed a decrease in both endogenous eCBs, anandamide (AEA) and 2-arachidonylglycerol (2-AG) after acute ethanol (Ferrer et al., 2007; Rubio et al., 2007) while others have observed an increase in endocannabinoids (Basavarajappa et al., 2008). To test the acute effects of ethanol on eCB signaling we used a

short-term form of eCB-mediated synaptic plasticity, depolarization-induced suppression of excitation (DSE).

Several reports have shown that modulation of the eCB system results in altered alcohol intake and alcohol seeking behaviors (Pava and Woodward, 2012). Thus, because ethanol can alter eCB signaling as well as NMDAR-dependent LTD in the NAc, we investigated whether eCBs could modulate ethanol regulation of NMDAR-dependent LTD. To test the hypothesis that ethanol attenuates eCB signaling, we attempt to rescue the expression of NMDAR-dependent LTD by increasing eCB signaling in the presence of ethanol.

Materials and Methods

Brain slice preparation

Parasagittal slices (220–240 μm thick) containing the NAc were prepared using a Leica vibrating microtome from male C57BL/6 mice (Jackson Labs, Bar Harbor, ME). Mice used in the experiments were 4 to 8 weeks old. Mice were anesthetized by inhalation of isoflurane and the brains were rapidly removed and placed in 4°C oxygenated ACSF containing the following (in mM): 110 choline, 25 NaHCO_3 , 1.25 NaH_2PO_4 , 2.5 KCl, 7 MgCl_2 , 0.5 CaCl_2 , 25 dextrose, 11.6 Na^+ -ascorbate, and 3.1 Na^+ -pyruvate, bubbled with 95% O_2 /5% CO_2 . Slices were transferred to a different ACSF solution for incubation containing the following (in mM): 120 NaCl, 25 NaHCO_3 , 1.23 NaH_2PO_4 , 3.3 KCl, 2.4 MgCl_2 , 1.8 CaCl_2 , 10 dextrose, continuously bubbled with 95% O_2 /5% CO_2 ; pH 7.4, 32°C; and were maintained in this solution for 60 minutes prior to recording.

Patch clamp electrophysiology

Whole cell voltage clamp recordings were made in the nucleus accumbens shell. Cells were identified using the MRK200 Modular Imaging system (Siskiyou Corporation, Grants Pass, OR) mounted on a vibration isolation table. Recordings were made in ACSF containing (in mM): 120 NaCl, 25 NaHCO_3 , 1.23 NaH_2PO_4 , 3.3 KCl, 0.9 MgCl_2 , 2.0 CaCl_2 , 10 dextrose, bubbled with 95% O_2 /5% CO_2 . ACSF was continuously perfused at a rate of 2.0 mL/min and maintained at a temperature of 32°C. GABA_A receptor-mediated synaptic currents were blocked with 50 μM picrotoxin (Sigma-Aldrich). Recording electrodes (thin-wall glass, WPI, Sarasota, FL) were made using a Brown-Flaming model P-88 electrode puller (Sutter Instruments, San Rafael, CA) to yield resistances between 3–5 M Ω . Electrodes were filled with (in mM): 135 KMeSO_4 , 12 NaCl, 0.5 EGTA, 10 HEPES, 2 Mg-ATP, 0.3 Tris-GTP, pH 7.3, 260–270 mOsm. Input resistance and access resistance were monitored throughout the experiments. Cells in which input and access resistance varied more than 20% were not included for analysis. The following drugs were purchased from Tocris (Bristol, UK): capsaizepine, URB597, AM404, DHPG, DL-APV, and AM251.

Data analysis and acquisition

Glutamatergic afferents were stimulated with a stainless steel bipolar stimulating electrode (FHC, Inc., Bowdoin, ME) placed between the recorded MSN and prefrontal cortex, about 150–300 μm from the cell body. EPSCs were acquired using an Axopatch 200B amplifier (Axon Instruments, Foster City, CA), filtered at 1 kHz, and digitized at 10–20 kHz via a

Digidata 1440A interface board using pClamp 10.2 (Axon Instruments, Foster City, CA). In experiments for long term plasticity, EPSCs were evoked by local stimulation for at least 10 minutes at 0.1 Hz, to ensure stable recordings. To induce LTD, a conditioning stimuli of 500 pulses at 1 Hz was paired with continuous postsynaptic depolarization to -50 mV. EPSCs were then monitored for 30 minutes after pairing at a rate of 0.1 Hz. The magnitude of LTD or LTP was calculated by averaging normalized EPSC values from 20 to 30 minutes after the pairing protocol and comparing that value to the average normalized EPSCs during the last 10 minutes of baseline. Plasticity was determined if the average EPSCs between 20 to 30 minutes post-pairing is greater than 2 standard deviations away from the 10 minute baseline recorded. Data from each neuron within a treatment group was combined and represented as percent baseline values. For DSE, EPSCs were evoked at 0.25 Hz for a 20 second baseline, followed by depolarization for 5 seconds. After depolarization, EPSCs were monitored for 80 seconds at a rate of 0.25 Hz. For each neuron data was averaged over 3 trials. Summary data are presented as mean $\pm$ SEM. Statistical significance from baseline for within each treatment group is defined as $p < 0.05$ using a two-tailed Student's *t* test (assuming equal variance). Statistical significance for between treatment group comparisons is defined as $p < 0.05$ using a single-factor ANOVA with Bonferroni post-hoc analyses.

Results

NMDA receptor and CB1 receptor dependent LTD

In untreated brain slices, pairing 1 Hz stimulation with postsynaptic depolarization to -50 mV for 500 seconds results in robust LTD that is NMDA receptor dependent and has previously been shown to be blocked by 40 mM EtOH (Jeanes et al., 2011). Using this pairing protocol, LTD has been replicated in Figure 1 (closed circles, $67.3 \pm 9.7\%$ of baseline, $n=10$). In the presence of a CB1R antagonist, AM251, LFS elicited synaptic potentiation (Figure 1, open circles, $127.1 \pm 12.0\%$ of baseline, $n=14$). LTP did not involve a change in presynaptic neurotransmitter release as there was no change in paired-pulse ratio (PPR) with an interstimulus interval of 50 ms (Figure 1E, baseline PPR 1.21 ± 0.11 , post-pairing PPR 1.25 ± 0.08 , $n=4$). Synaptic potentiation with AM251 is blocked by 40 mM EtOH (Figure 2, closed circles, $107.6 \pm 9.7\%$ of baseline, $n=6$) as well as by the non-selective NMDAR antagonist, 100 μ M DL-APV (Figure 2, open circles, $102.1 \pm 6.6\%$ of baseline, $n=6$).

Ethanol decreases the magnitude of DSE

In light of the complex interactions between eCBs, ethanol, and the pre- and postsynaptic mechanisms of LTD expression, we sought a means to directly measure whether acute ethanol modulates eCB signaling. We chose depolarization-induced suppression of excitation (DSE) as an assay of the effects of ethanol on eCB signaling (Figure 3). To our knowledge, this is the first known report of DSE in the NAc shell. To induce DSE in the NAc shell, 5 μ M DHPG was used to activate group 1 mGlu receptors, in the presence of both DL-APV (50 μ M) and picrotoxin (50 μ M) (Wang et al., 2010). Depolarization of NAc shell neurons from -80 mV to 0 mV for 5 seconds resulted in DSE (Figure 3, closed circles, 24.5 ± 1.9 , $n=10$) that was significantly reduced in the presence of CB1 receptor antagonist AM251 (Figure 3, open circles, 7.1 ± 3.4 , $n=8$). A significant reduction in the magnitude of

DSE was also observed in the presence of 40mM ethanol (Figure 4, open circles, 14.4 ± 4.2 , $n=5$).

Increased eCB signaling results in TRPV1 dependent LTD that is insensitive to ethanol

We have previously reported that 40 mM EtOH blocks the expression of LTD (Jeanes et. al. 2011). This experiment has been replicated and it was similarly found that LTD is blocked by ethanol using a concentration of 40 mM (Figure 5, $97.8 \pm 1.6\%$ of baseline, $n=4$). Ethanol did not have a direct effect on AMPA-mediated EPSCs as a stable baseline was recorded in the presence of 40 mM ethanol. Ethanol-induced inhibition of NAc LTD may have resulted from a reduction in eCB signaling; thus, we enhanced synaptic eCBs in an attempt to rescue LTD expression. To increase eCB signaling, URB597 (1 μ M) and AM404 (1 μ M), a fatty acid amide hydrolase inhibitor and anandamide reuptake inhibitor respectively, were applied in the presence of 40 mM ethanol. Increased eCB signaling rescued the expression of LTD in the presence of 40 mM ethanol (Figure 5, $78.4 \pm 7.5\%$ of baseline, $n=10$). Interestingly, this new form of LTD expression in the presence of enhanced eCB synaptic tone required activation of TRPV1 receptors but not NMDARs. This new form of LTD was blocked by TRPV1 antagonist, capsaizepine (CPZ) (Figure 6 closed circles, $87.9 \pm 7.0\%$ of baseline, $n=5$) but not by NMDAR antagonist, DL-APV or CB1R antagonist, AM251 (Figure 6, open circles, $56.7 \pm 3.8\%$ of baseline, $n=4$).

Discussion

Nucleus accumbens LTD involves both pre- and postsynaptic signaling mechanisms. Our current report demonstrates that in addition to the influence of postsynaptic NMDA receptors, presynaptic CB1 receptor activity plays a significant role in determining the set-point of synaptic plasticity expression in the NAc shell. We observed that a NMDA receptor antagonist inhibits the expression of both LTD and a novel form of LTP resulting from CB1 receptor blockade. The requirement for activation of CB1 receptors for LTD seems to be necessary only during the pairing protocol. The CB1 antagonist SR 141716A, has previously been shown to exert no effect on basal synaptic transmission in the nucleus accumbens (Robbe et al., 2001) and we were also able to record stable EPSCs in the presence of AM251 suggesting that CB1 receptors are not tonically active. The mechanism by which LTP is induced in the presence of AM251 is unclear, although it is unlikely that there is a long-term alteration in presynaptic neurotransmitter release as we found that LTP requires the activation of NMDA receptors and we observed no change in PPR after the pairing protocol. In the absence of inhibitory CB1 receptor activity, the pairing protocol that normally induced LTD may instead cause a substantial increase in postsynaptic calcium influx through NMDA receptors resulting in the insertion of AMPA receptors and synaptic potentiation (Malenka, 2003). This switch in the polarity of synaptic plasticity is reminiscent of the changes in plasticity observed after chronic intermittent ethanol (CIE) exposure in which LFS resulted in LTP, 24 hours after ethanol exposure (Jeanes et al., 2011) and also replicated in our lab (unpublished observations). Synaptic potentiation observed after CIE may involve modulation of eCB signaling since several studies have shown that in vivo ethanol exposure can modulate CB1 receptor expression (Erdozain and Callado, 2011).

The specific interactions between acute ethanol and eCBs are somewhat unclear as some groups demonstrate an increase in eCBs while others show a decrease in 2-AG and AEA. The discrepancy in the effects of ethanol on eCBs may be due to the differences in the experimental preparations or the particular brain regions under study (Pava and Woodward, 2012). We observed a reduction in the magnitude of DSE, an eCB-dependent form of plasticity, in the presence of ethanol; however, whether ethanol specifically modulates the production/release of eCBs or the CB1 receptor itself requires further investigation. Given the substantial evidence that eCBs may be necessary for the reinforcing effects of ethanol and CB1 activation is required for ethanol self-administration (Erdozain and Callado, 2011; Pava and Woodward, 2012), it was surprising to find that ethanol decreased the magnitude of DSE. It would be necessary to test the effects of chronic ethanol exposure on the expression of DSE in the NAc to further understand how eCB signaling in the NAc contributes to the expression of ethanol related behaviors.

Unexpectedly, we observed a form of LTD with increased eCB signaling that was independent of NMDA or CB1 receptors but instead required activation of TRPV1 receptors. Several studies have demonstrated the functional significance of TRPV1 in various brain regions including the hippocampus, amygdala, dorsal striatum, ventral tegmental area and nucleus accumbens core (Marinelli et al., 2005; Gibson et al., 2008; Musella et al., 2009; Chavez et al., 2010; Grueter et al., 2010; Zschenderlein et al., 2011; Brown et al., 2013). To our knowledge, this is the first known report of TRPV1-dependent LTD in the NAc shell. The involvement of TRPV1 receptors in NAc LTD expression was dependent upon an increase in eCB signaling. In our experience, TRPV1 receptors do not appear to contribute to normal LFS-LTD in the NAc shell since that form of LTD is unaffected by TRPV1 antagonist (supplemental Figure 1) and is completely blocked by NMDAR antagonists. Thus, we conclude that unless eCB production is markedly enhanced, TRPV1-dependent LTD must remain quiescent under the stimulation conditions used herein.

Importantly, we show that TRPV1-dependent LTD is insensitive to acute ethanol exposure. Activation of TRPV1 receptors may be important in attenuating ethanol intake as TRPV1 knockout mice were found to have increased ethanol preference and consumption (Blednov and Harris, 2009). Modulation of TRPV1 signaling could prove to be a valuable target in the development of effective treatments for alcoholism. Certainly, the synaptic pathways within the nucleus accumbens represent a complex and adaptable system, capable of expressing varying degrees of plasticity. Future work directed at an understanding of how these different signaling systems interact with each other may ultimately elucidate the molecular neuroadaptations underlying the development of alcohol dependence.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

Support: This work was supported by the Alcohol Training Grant T32 AA07471 (RR) and the National Institutes of Health RO1AA15167 (RAM).

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FIGURE 1. Pairing protocol results in LTP in the presence of CB1 receptor antagonist, AM251
 Pairing low frequency stimulation (1 Hz) with post synaptic depolarization (–50 mV) for 500 seconds results in a long term depression of AMPA receptor mediated EPSCs (A. open circles, $67.3 \pm 9.7\%$ of baseline, $n=10$). In the presence of CB1 receptor antagonist, AM251, the same pairing protocol results in LTP (A. closed circles, $127.1 \pm 12.0\%$ of baseline, $n=14$). B. Sample EPSCs of a single neuron from control conditions (left) and with AM251 (right). Scale bars represent 5 ms (horizontal) and 50 pA (vertical). C. Bar graph representing the percentage change $\pm$ S.E.M. for average EPSC amplitude between baseline (min 0–10) and post-pairing (min 40–50). * $p < 0.05$ versus baseline. D. Sample EPSCs of a single neuron during paired-pulse stimulation (50 ms) in the presence of AM251 during baseline (left) and post-pairing (right). Scale bars represent 5 ms (horizontal) and 50 pA (vertical). E. Bar graph representing the paired-pulse ratio (PPR) during baseline (min 0–10) and post-pairing (min 40–50).

FIGURE 2. Ethanol (40 mM) and NMDA receptor antagonist (100 μM DL-APV) block the expression of LTP in the NAc shell

The pairing protocol (1 Hz stimulation paired with post synaptic depolarization to -50 mV) in the presence of AM251 (2 μM AM251) results in potentiation of AMPA receptor mediated EPSCs (Figure 1.) and is dependent on the activation of NMDA receptors (A. open circles, $104.7 \pm 6.6\%$ of baseline, $n=6$). Similarly, LTP is blocked by 40 mM ethanol (A. closed circles, $107.6 \pm 9.7\%$ of baseline, $n=6$). B. Sample EPSCs from a single neuron in each treatment group. Scale bars represent 5 ms (horizontal) and 50 pA (vertical). C. Bar graph representing the percentage change $\pm$ S.E.M. for average EPSC amplitude between baseline (min 0–10) and post-pairing (min 40–50).

FIGURE 3. DSE in the NAc shell is dependent on activation of CB1 receptors

A. Post synaptic depolarization step from -80 mV to 0 mV for 5 seconds results in a short term depression of EPSCs (closed circles, 24.5 ± 1.9 , $n=10$) that is dependent on the activation of CB1 receptors (open circles, 7.1 ± 3.3 , $n=8$). B. Sample EPSCs from a single neuron in control conditions (left) and with AM251 (right) before and after depolarization. Scale bars represent 5 ms (horizontal) and 50 pA (vertical). C. Bar graph representing the % DSE [$100 - (\text{mean of three consecutive normalized EPSC amplitudes after depolarization}) / (\text{mean of 6 consecutive normalized EPSC amplitudes before depolarization}) \pm \text{S.E.M.}$]. * $p < 0.05$ versus baseline, † $p < 0.05$ versus control.

FIGURE 4. Ethanol (40 mM) reduces the expression of DSE in the NAc shell

A Post synaptic depolarization for 5 seconds results in a short term depression of AMPA receptor mediated EPSCs (closed circles, 24.5 ± 1.9 , $n=10$) that is reduced with 40 mM ethanol (open circles, 14.4 ± 4.2 , $n=5$). B. Sample EPSCs from a single neuron in each treatment group. Scale bars represent 5 ms (horizontal) and 50 pA (vertical). C. Bar graph representing the % DSE [100– (mean of three consecutive normalized EPSC amplitudes after depolarization)/(mean of 6 consecutive normalized EPSC amplitudes before depolarization)] $\pm$ S.E.M. * $p < 0.05$ versus baseline, † $p < 0.05$ versus control.

FIGURE 5. Increasing eCB signaling (1 μ M URB597 and 1 μ M AM404) rescues the expression of LTD in the presence of ethanol (40 mM)

A. 40 mM ethanol blocks the expression of LTD (closed circles, $97.8 \pm 1.6\%$ of baseline, $n=4$). In the presence of URB597 and AM404, the pairing protocol results in LTD that is unaffected by 40 mM ethanol ($78.4 \pm 7.5\%$ of baseline, $n=10$). B. Sample traces from a representative neuron in each treatment group. Scale bars represent 5 ms (horizontal) and 50 pA (vertical). C. Bar graph representing the percentage change $\pm$ S.E.M. for average EPSC amplitude between baseline (min 0–10) and post-pairing (min 40–50). * $p < 0.05$ versus baseline.

FIGURE 6. Increasing eCB signaling with URB597 (1 μ M) and AM404 (1 μ M) results in LTD through activation of TRPV1 receptors

A. The pairing protocol in the presence of URB597 and AM404 results in LTD that is independent of NMDA receptor and CB1 receptor activation (open circles, $56.7 \pm 3.8\%$ of baseline, $n=4$) but requires activation of TRPV1 receptors (closed circles) as it is blocked with 10 μ M capsazepine (CPZ) ($87.9 \pm 7.0\%$ of baseline, $n=5$). B. Sample EPSCs from a single neuron in each treatment group. Scale bars represent 5 ms (horizontal) and 50 pA (vertical). C. Bar graph representing the percentage change $\pm$ S.E.M. for average EPSC amplitude between baseline (min 0–10) and post-pairing (min 40–50). * $p < 0.05$ versus baseline, † $p < 0.05$ versus drug treatment group without CPZ.