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Modulation of endocannabinoid-mediated long-lasting disinhibition of striatal output by cholinergic interneurons

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ABSTRACT

The frequency and duration of glutamatergic inputs to the striatum are strong determinants of the net effect of retrograde endocannabinoid (eCB) signaling, and key factors in determining if long-term depression (LTD) has a net disinhibitory or inhibitory action in striatum. Low to moderate frequency stimulation in the dorsolateral striatum elevates eCB levels to an extent that primarily depresses transmitter release at inhibitory synapses, leading to a long-lasting disinhibition (DLL) of synaptic output. The aim of this study was to further characterize the basic features of endocannabinoid-mediated DLL of striatal output induced by moderate frequency stimulation (5 Hz, 60 s). DLL was inhibited in slices treated with the group 1 metabotropic glutamate receptor (mGluR) antagonists MPEP (40 μM) and CPCCOEt (40 μM), the dopamine D2 receptor antagonist sulpiride (5 μM), the L-type calcium channel blocker nifedipine (20 μM), the nicotinic receptor antagonist mecamylamine (10 μM), the muscarinic agonist oxotremorine sesquifumarate (10 μM), and strychnine (0.1 μM). Strychnine did not block DLL induced by WIN55,212-2 (250 nM), showing that glycine receptor-mediated modulation of eCB signaling occurs upstream from CB_1R activation. Scopolamine (10 μM) restored DLL in strychnine-treated slices, suggesting that inhibition of glycine receptors on cholinergic interneurons could modulate eCB signaling by enhancing muscarinic receptor activation and reducing the opening of L-type calcium channels in response to depolarization. These data suggests that similar activation points are required for stimulation-induced DLL as for LTD at excitatory striatal synapses, and that cholinergic interneurons are key modulators of stimulation-induced eCB signaling in the striatum.

1. Introduction

A growing body of psychiatric research has emerged focusing on the role of the endocannabinoid (eCB)-system in major psychiatric disorders like schizophrenia, bipolar disorder, major depression, and anxiety disorders (Leweke and Koethe, 2008; Hill and Gorzalka, 2009; Koethe et al., 2009). The eCB system is also necessary for behavioral adaptations of motor behavior to changing reward-position contingencies (Balleine et al., 2007; Hilario et al., 2007), and could be a possible candidate linking neuronal adaptations elicited by drugs of abuse with behavioral consequences of that adaptation (Gerdeman et al., 2003; Vinod et al., 2008).

eCBs are synthesized on demand, are postsynaptically released, and act on presynaptic cannabinoid 1 receptors (CB_1R), which leads to a reduction in release probability of excitatory and inhibitory neurotransmitters (Hillard and Campbell, 1997; Gerdeman and Lovinger, 2001; Adermark and Lovinger, 2007b). CB_1Rs are found throughout the brain but are especially abundant in the striatum (Herkenham et al., 1990), a key structure for controlling habit formation and reward based learning (Balleine et al., 2007; Yin et al., 2008). The crucial molecular switch for eCB production at excitatory synapses in the striatum is activation of L-type calcium channels and the subsequent increase in postsynaptic $[Ca^{2+}]_i$ (Adermark and Lovinger, 2007a), but the formation of long-term depression (LTD) also requires eCB transporters, protein translation, and concomitant pre- and postsynaptic activity at both inhibitory and excitatory synapses (Ronesi et al., 2004; Yin et al., 2006; Adermark and Lovinger, 2007b; Singla et al., 2007; Adermark et al., 2009). eCB signaling in the striatum is also regulated by acetylcholine (Narushima et al., 2007; Wang et al., 2006; Partridge et al., 2002), and it has been suggested that activation of dopamine D2 receptors is required in order to pause cholinergic interneuron activity to enhance the opening of postsynaptic L-type calcium channels in response to synaptic depolarization (Wang et al., 2006).

GABAergic synapses on medium spiny neurons (MSNs) are more sensitive than glutamatergic synapses on the same cells to eCB

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signaling, and protocols that induce short-lasting CB_1R-dependent depression at glutamatergic synapses have been shown to be sufficient to induce LTD at GABAergic synapses (Adermark and Lovinger, 2009). For instance, low to moderate frequency stimulation (1–5 Hz) of afferent fibers during shorter time frames (<2 min) induces an eCB-mediated decrease in transmitter release that primarily occurs at inhibitory synapses, thus causing a long-lasting disinhibition (DLL) of striatal output (Adermark and Lovinger, 2009). In other words, the frequency and duration of glutamatergic inputs are strong determinants of the net effect of eCB signaling, and key factors in determining if LTD has a net disinhibitory or inhibitory action in the striatum (Adermark and Lovinger, 2009; Adermark et al., 2009). The balance between excitation and inhibition in striatal medium spiny neurons might thus be shifted by eCBs, making repetitive activity self-reinforcing (Surmeier, 2009).

The goal of this study was to further characterize the basic features of endocannabinoid-mediated DLL of striatal output, and to address the role of cholinergic interneurons in modulating eCB signaling in the striatum. The data presented here show that the basic properties of DLL and HFS-LTD are similar, and that cholinergic interneurons are important regulators of striatal plasticity.

2. Materials and methods

2.1. Brain slice preparation

Experiments were carried out in accordance with the guidelines laid down by the Swedish Research Council regarding the care and use of animals for experimental procedures and were approved by the Local Ethics Committee of University of Gothenburg (302/09, 2009-11-18). Striatal slices (400 μm) were prepared from 20 to 23-day-old Wistar rats (Breeding performed at Gothenburg University, rats originating from Charles River, Germany), as previously described (Clarke and Adermark, 2010). In brief, animals were anesthetized with isoflurane and decapitated. The brains were placed in ice-cold modified artificial cerebrospinal fluid (aCSF) containing (in mM); 194 sucrose, 30 NaCl, 4.5 KCl, 1 $MgCl_2$, 26 $NaHCO_3$, 1.2 NaH_2PO_4 and 10 D-glucose, saturated with oxygen, before sectioned coronally. Before initiating any recordings, brain slices were allowed to equilibrate for at least 1 h at room temperature in normal aCSF containing (in mM); 124 NaCl, 4.5 KCl, 2 $CaCl_2$, 1 $MgCl_2$, 26 $NaHCO_3$, 1.2 NaH_2PO_4 and 10 D-glucose, continuously bubbled with a mixture of 95% O_2/5% CO_2 gas.

2.2. Striatal field potential recordings

Field potential recordings were performed as previously described (Clarke and Adermark, 2010). In brief, one hemisphere of a coronal brain slice was transferred to a recording chamber (2 ml) and perfused at a constant rate of 2.6 ml/min with pre-warmed aCSF kept at 30 °C. In striatal field potential recordings the relative synaptic efficacy is estimated by measuring the amplitude of the population spike (PS, also known as N2), rather than field EPSP amplitude or slope. PSs were induced at a frequency of 0.05 Hz by a stimulation electrode placed at the border of the dorsolateral striatum and the overlying white matter (World Precision Instruments, FL, USA, type TM33B). The intensity was set to yield a PS amplitude of approximately half the size of the maximal evoked response. DLL was induced by a train of pulses delivered at 5 Hz for 60 s (60 s-5 Hz). In the majority of recordings, the NMDA receptor inhibitor D,L-2-amino-5-phosphonopentanoic acid (APV; 50 μM) was added to the buffer. The activation points required for DLL were evaluated in slices treated with the $GABA_A$ receptor inhibitor (+)-bicuculline (2 μM), the CB_1R antagonist AM251 (2 μM), the metabotropic glutamate (mGlu) group 1 receptor antagonists 7-(hydroxyimino)cyclopropa[b]chromen-1a-carboxylate ethyl ester (CPCCOEt, 40 μM, $mGlu_1R$) and 2-methyl-6-(phenylethynyl)pyridine hydrochloride (MPEP, 40 μM, $mGlu_5R$), the dopamine D1 and D2 receptor antagonists SCH 23390 (500 nM) and S-(−)-sulpiride (5 μM), the nicotinic acetylcholine receptor inhibitors mecamylamine (10 μM) and the α7 neuronal nicotinic acetylcholine receptor antagonist methyllycaconitine citrate (MLA, 40 nM). Slices were also treated with the muscarinic acetylcholine receptor antagonist scopolamine (10 μM), the muscarinic agonist oxotremorine sesquifumarate (10 μM), the L-type calcium channel blocker nifedipine (20 μM), and the glycine receptor antagonist strychnine (0.1 μM). CB_1R-mediated disinhibition of striatal output was also induced by a low concentration of WIN55,212-2 (250 nM). All drugs were bought from Sigma Aldrich (St. Louis, MO, USA) or Tocris (Ellisville, MO, USA), applied for at least 20 min before the DLL-induction protocol was instigated, and remained in the bath until the end of the experiment. Signals were amplified by a custom-made amplifier, filtered at 3 kHz, digitized and transferred to a PC for analysis.

2.3. Data analysis

Data was analyzed with Clampex 10.1 (Molecular Devices, Foster City, CA), and graphs were assembled in GraphPad Prism (GraphPad Software, Inc., San Diego, CA), and Photoshop. Data is presented in text as mean values at $t = 25–30$ min compared to baseline ($t = 1–6$ min) with 95% confidence interval (CI), while time course figures are plotted as mean PS amplitude per minute compared to baseline, with standard error of the mean (SEM). Two-tailed paired t-test was used for statistical analysis unless otherwise is stated.

3. Results

3.1. Modulation of DLL by NMDA receptors

Moderate activation of glutamatergic afferents with a train of pulses delivered at 5 Hz for 60 s (60 s-5 Hz) enhanced PS amplitude (PS amplitude at $t = 25–30$ min = 116 ± 8.6% of baseline level, $t = 3.66$, $n = 10$, $p < 0.01$). This potentiation was prevented in slices treated with the $GABA_A$ receptor antagonist bicuculline (2 μM; PS amplitude at $t = 25–30$ min = 99 ± 3.74% of bicuculline-treated baseline, $t = 0.71$, $n = 7$, $p > 0.05$; un-paired t-test: control vs. bicuculline-treated at $t = 25–30$ min, $t = 3.14$, df = 16, $p < 0.01$) (Fig. 1A).

The increase in PS amplitude induced by 60 s-5 Hz was significantly enhanced in slices treated with the NMDA receptor antagonist APV (50 μM) (PS amplitude at $t = 25–30$ min = 132 ± 7.4% of APV-treated baseline, $t = 8.34$, $n = 8$, $p < 0.001$; un-paired t-test: control vs. APV-treated, $t = 2.74$, df = 15, $p < 0.05$) (Fig. 1B), suggesting that DLL is counterbalanced by mechanisms involving NMDA receptor activation. In the following experiments, 50 μM APV was added to the aCSF unless anything else is clearly stated. The amplitude of the presynaptic volley, in the striatum known as the N1 component of the population spike, did not change significantly throughout these recordings, indicating that the number of axons activated remained stable.

3.2. eCB signaling at GABAergic synapses underlies DLL

In the next set of experiments the mechanisms underlying DLL were evaluated in APV-treated slices. In all experiments, drugs were applied for at least 20 min before DLL-induction, and remained in the bath throughout the experiment. In line with previous findings, the CB_1R antagonist AM251 (2 μM) prevented DLL (PS amplitude at $t = 25–30$ min = 103 ± 3.7% of AM251-treated baseline, $t = 1.33$, $n = 8$, $p > 0.05$; un-paired t-test: control slices vs. AM251-treated, $t = 4.28$, df = 14, $p < 0.001$) (Fig. 2A) (Adermark and Lovinger, 2009). DLL was also blocked by bicuculline (2 μM) (PS amplitude at $t = 25–30$ min = 101 ± 3.0% of bicuculline-treated baseline, $t = 0.59$, $n = 6$, $p > 0.05$; un-paired t-test: control vs. bicuculline-treated, $t = 3.88$, df = 11, $p < 0.01$), suggesting that eCB signaling at inhibitory synapses underlies DLL (Fig. 2B). Baseline striatal output was not significantly modulated by this low concentration of bicuculline (PS amplitude at $t = 25–30 = 96 ± 5.0$% of baseline, $t = 1.70$, $n = 10$, $p > 0.05$; data not shown).

3.3. DLL-requires mGluRs and D2 receptor activation

Activation of mGlu receptor group 1 has previously been shown to be vital for stimulation-induced eCB signaling at both excitatory and inhibitory striatal synapses (Kreitzer and Malenka, 2005; Adermark and Lovinger, 2009). Supporting these data we found that treatment with a cocktail of mGlu group 1 receptor inhibitors containing CPCCOEt ($mGlu_1$; 40 μM) and MPEP ($mGlu_5$; 40 μM) blocked DLL-formation (PS amplitude at $t = 25–30$ min = 101 ± 3.1% of baseline level, $t = 0.50$, $n = 8$, $p > 0.05$) (Fig. 3A). DLL remained in slices treated with the D1 receptor antagonist SCH 23390 (500 nM; PS amplitude at $t = 25–30$ min = 124 ± 8.3% of SCH 23390-treated