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TRANSIENT RECEPTOR POTENTIAL VANILLOID 1 CHANNELS CONTROL ACETYLCHOLINE/2-ARACHIDONOYLGLICEROL COUPLING IN THE STRIATUM

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Abstract—The neurotransmitter acetylcholine (Ach) controls both excitatory and inhibitory synaptic transmission in the striatum. Here, we investigated the involvement of the endocannabinoid system in Ach-mediated inhibition of striatal GABA transmission, and the potential role of transient receptor potential vanilloid 1 (TRPV1) channels in the control of Ach-endocannabinoid coupling. We found that inhibition of Ach degradation and direct pharmacological stimulation of muscarinic M1 receptors reduced striatal inhibitory postsynaptic currents (IPSCs) through the stimulation of 2-arachidonoylglicerol (2AG) synthesis and the activation of cannabinoid CB1 receptors. The effects of M1 receptor activation on IPSCs were occlusive with those of metabotropic glutamate receptor 5 stimulation, and were prevented in the presence of capsaicin, agonist of TRPV1 channels. Elevation of anandamide (AEA) tone with URB597, a blocker of fatty acid amide hydrolase, mimicked the effects of capsaicin, indicating that endogenous AEA acts as an endovanilloid substance in the control of M1-dependent 2AG-mediated synaptic effects in the striatum. Accordingly, both capsaicin and URB597 effects were absent in mice lacking TRPV1 channels. Pharmacological interventions targeting AEA metabolism and TRPV1 channels might be considered alternative therapeutic routes in disorders of striatal cholinergic or endocannabinoid neurotransmission.

Key words: anandamide, CB1 receptors, endocannabinoids, IPSC, muscarinic receptors, vanilloid receptors.

Striatal neuron activity is finely regulated by the neurotransmitter acetylcholine (Ach), which acts through pre- and postsynaptic actions and both short- and long-term effects. In fact, stimulation of muscarinic receptors depolarizes striatal neurons by inhibiting K^+ conductances (Dodt and Misgeld, 1986; Lin et al., 2004), reduces voltage-activated Ca^{2+} channels (Howe and Surmeier, 1995), and enhances NMDA receptor-mediated postsynaptic responses (Calabresi et al., 1998). Furthermore, Ach modulates both long-term depression (LTD) and long-term potentiation (LTP) of corticostriatal transmission acting on both muscarinic and nicotinic receptors (Calabresi et al., 2000; Partridge et al., 2002; Wang et al., 2006; Bonsi et al., 2008), and reduces presynaptically glutamate- (Pakhotin and Bracci, 2007) and GABA-mediated synaptic events (Koós and Tepper, 2002).

Recent evidence convincingly showed that Ach-mediated inhibition of GABA synapses in the striatum is largely mediated by the muscarinic M1 receptor-dependent stimulation of endocannabinoid release (likely 2-arachidonoylglicerol, 2AG), and the resulting activation of cannabinoid CB1 receptors on presynaptic nerve terminals (Narushima et al., 2007; Uchigashima et al., 2007).

In the striatum, 2AG production and endocannabinoid-mediated inhibition of GABA transmission also follow the activation of metabotropic glutamate receptor 5 (mGluR5) (Jung et al., 2005, 2007; Centonze et al., 2007b; Uchigashima et al., 2007; Maccarrone et al., 2008), whose distribution in the somatodendritic region of striatal spiny neurons does not overlap with that of M1. Accordingly, mGluR5 is highly enriched in close proximity of the postsynaptic region of dendritic spines, whereas M1 density is lower in spines than in dendritic shafts and somata (Narushima et al., 2007; Uchigashima et al., 2007). Thus, although activation of both mGluR5 and M1 leads to diacylglycerol lipase (DAGL)-mediated 2AG production, the physiological roles of mGluR5-2AG and M1-2AG coupling in the control of striatal synaptic transmission are significantly different (Narushima et al., 2007; Uchigashima et al., 2007). These observations raise the question of whether the regulatory mechanisms of mGluR5-2AG and M1-2AG interactions are similar or distinct.

We have recently reported that the other main endocannabinoid anandamide (AEA) interferes with mGluR5-stimulated 2AG synthesis and synaptic activity, by activating transient receptor potential vanilloid 1 (TRPV1) channels (Maccarrone et al., 2008). Thus, aim of the present study was to scrutinize whether TRPV1 channels also control M1-2AG positive interaction. We found that stimulation of TRPV1 channels by capsaicin or pharmacological inhibition of AEA degradation was able to prevent Ach- and M1-dependent 2AG synthesis and CB1-mediated inhibition of GABAergic inhibitory postsynaptic currents (IPSCs).

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Abbreviations: Ach, acetylcholine; AEA, anandamide; DAGL, diacylglycerol lipase; FAAH, fatty acid amide hydrolase; IPSC, inhibitory postsynaptic current; LTD, long-term depression; LTP, long-term potentiation; mGluR5, metabotropic glutamate receptor 5; mIPSCs, miniature IPSCs; sEPSCs, spontaneous excitatory postsynaptic currents; sIPSCs, spontaneous IPSCs; TRPV1, transient receptor potential vanilloid 1; 2AG, 2-arachidonoylglicerol.

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Our data indicate a widespread role of TRPV1 channels in the regulation of 2AG activity in the striatum, and suggest that AEA might control synthesis and activity of the other endocannabinoid 2AG in multiple physiological and pathological contexts.

EXPERIMENTAL PROCEDURES

Corticostriatal coronal slices (200 μm) were prepared from brain blocks of C57/bl6 mice (6–7 weeks old) with the use of a vibratome. Mice lacking TRPV1 channels (TRPV1−/−; from the Jackson Laboratory, Bar Harbor, ME, USA) were also used along with their matched wild-type (WT) counterparts (Maccarrone et al., 2008; Musella et al., 2009). All efforts were made to minimize animal suffering and to reduce the number of mice used, in accordance with the European Communities Council Directive of November 24, 1986 (86/609/EEC).

Slices were transferred to a recording chamber and submerged in a continuously flowing artificial cerebrospinal fluid (ACSF) (33 °C, 2–3 ml/min) gassed with 95% O_2–5% CO_2. The composition of the bathing solution was (in mM): (126) NaCl, (2.5) KCl, (1.2) $MgCl_2$, (1.2) NaH_2PO_4, (2.4) $CaCl_2$, (11) Glucose, (25) $NaHCO_3$.

The striatum could be readily identified under low power magnification, whereas individual neurons were visualized in situ using a differential interference contrast (Nomarski) optical system. This employed Olympus BX50WI (Tokyo, Japan) or Leica DM LFS (Solms, Germany) upright microscopes with ×40 water immersion objective combined with an infra-red filter, a monochrome CCD camera (COHU 4912), and a PC compatible system for analysis of images and contrast enhancement (WinVision, 2000, Delta Sistem, Italy). Recording pipettes were advanced towards individual striatal cells in the slice under positive pressure and, on contact, tight GΩ seals were made by applying negative pressure. The membrane patch was then ruptured by suction and membrane current and potential monitored using an Axopatch 1D patch clamp amplifier (Molecular Devices, Foster City, CA, USA). Whole-cell access resistances measured in voltage clamp were in the range of 5–20 MΩ.

Whole-cell patch clamp recordings were made with borosilicate glass pipettes (1.8 mm o.d.; 2–5 MΩ). To study spontaneous and miniature $GABA_A$-mediated IPSCs (sIPSCs, mIPSCs), the recording pipettes were advanced toward medium-sized (20–30 μm) striatal neurons and filled with internal solution of the following composition (mM): CsCl (110), K^+-gluconate (30), ethylene glycol-bis (β-aminoethyl ether)-N,N,N',N'-tetra-acetic acid (EGTA; 1.1), HEPES (10), $CaCl_2$ (0.1), Mg-ATP (4), Na-GTP (0.3). MK-801 and CNQX were added to the external solution to block, respectively, NMDA and non-NMDA glutamate receptors. Shortly after the beginning of the recordings, cesium-based patch pipettes significantly altered the action potential properties of the neurons thereby preventing the physiological identification of the different striatal neuron subtypes. In particular, we were unable to distinguish GABA interneurons from principal neurons, while putative cholinergic interneurons were easily recognized in striatal slices because of their large somata (35–55 μm diameter). The identification of striatal principal neurons included in the present study was therefore achieved immediately after rupture of the GΩ seal by evaluating the firing response to the injection of depolarizing current.

To study spontaneous glutamate-mediated excitatory postsynaptic currents (sEPSCs), the recording pipettes were filled with internal solution of the following composition (mM): K^+-gluconate (125), NaCl (10), $CaCl_2$, (1.0), $MgCl_2$ (2.0), 1,2-bis (2-aminophenoxy) ethane-N,N,N,N-tetra-acetic acid (BAPTA; 0.5), HEPES (19), guanosine triphosphate (GTP; 0.3), Mg-adenosine triphosphate (Mg-ATP; 1.0), adjusted to pH 7.3 with KOH. Bicuculline was added to the perfusing solution to block $GABA_A$-mediated transmission. sIPSCs, mIPSCs, and sEPSCs were recorded at the holding potential of −80 mV, stored by using P-CLAMP 10 (Molecular Devices), and analyzed off line on a personal computer with Mini Analysis 5.1 (Synaptosoft, Leonia, NJ, USA) software. The detection threshold of these events was set at twice the baseline noise. The fact that no false events would be identified was confirmed by visual inspection for each experiment. Offline analysis was performed on spontaneous and miniature synaptic events recorded during a fixed time epoch (1 to 2 min), sampled every 2 or 3 min before (two to four samplings) and after (10 to 15 samplings) the application of the drugs. Only cells that exhibited stable frequencies in control (less than 20% changes during the control samplings) were taken into account. Events with complex peaks were eliminated. Figures were prepared by using the digitally acquired neurophysiological traces without further manipulations.

For data presented as the mean±SEM, statistical analysis was performed using a paired or unpaired Student's *t*-test or Wilcoxon's test. The significance level was established at $P<0.05$. Unless otherwise specified, *P*-values refer to pre-drug values. To determine whether two cumulative distributions of spontaneous synaptic activity were significantly different, the Kolmogorov–Smirnov (K–S test) was used. Throughout the text, *n* refers to the number of neurons. One to six neurons were recorded from a single mouse. At least four distinct mice were used for each type of experiment.

Drugs were applied by dissolving them to the desired final concentration in the bathing ACSF. The concentrations of the various drugs were chosen according to previous *in vitro* studies on corticostriatal brain slices (Calabresi et al., 1998; Centonze et al., 2007a,b; Maccarrone et al., 2008; Musella et al., 2009), and were as follows: capsazepine (10 μM), CNQX (10 μM), 3,5-DHPG (DHPG, 50 μM), AM251 (10 μM), HU210 (1 μM), MK-801 (30 μM), tetrodotoxin (TTX, 1 μM), pirenzepine dihydrochloride (pirenzepine, 100 nM), McN-A-343 (3 μM) (from Tocris Cookson, Bristol, UK); bicuculline (10 μM), orlistat (5 μM), neostigmine bromide (neostigmine, 3 μM) (from Sigma-RBI, St. Louis, USA); URB597 (1 μM) (from Alexis Biochemicals, San Diego, CA, USA).

RESULTS

Inhibition of Ach degradation depresses striatal GABA transmission via CB1 receptors

Ach innervation of striatal projection neurons is essentially intrinsic, mainly arising from tonically active cholinergic interneurons (Kawaguchi, 1992; Kawaguchi et al., 1995; Tepper and Bolam, 2004; Bernácer et al., 2007; Pisani et al., 2007). Thus, we tested whether inhibition of ambient Ach degradation was able to affect GABA transmission in the striatum. Neostigmine, a selective Ach-esterase inhibitor, reduced the frequency of sIPSCs ($n=9$, 56±4.0% of pre-drug value at 10 min drug application, $P<0.01$; Fig. 1A) and of mIPSC ($n=10$, 70±4.2% of pre-drug value, $P<0.05$; not shown) recorded from putative striatal projection neurons. sIPSC amplitude-frequency histogram indicated that the decrease in frequency was attributable to a significant reduction in currents in the 0–50 pA range (Fig. 1C). According to previous reports showing that the effects of endogenous Ach on striatal GABA transmission were mediated by endocannabinoids acting on presynaptic GABA terminals (Narushima et al., 2007; Uchigashima et al., 2007), neostigmine did not change sIPSC ($n=9$, 97±3.1% of pre-drug value, $P>0.05$; not shown) and mIPSC ($n=10$, 101±2.3% of pre-drug value, $P>0.05$; not shown)