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Cannabinoid-induced depression of synaptic transmission is switched to stimulation when dopaminergic tone is increased in the globus pallidus of the rodent

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ABSTRACT

Because activation of D2 receptors reverses the neurochemical effects of cannabinoids, we examined whether increasing dopaminergic tone in the globus pallidus (GPe) switches cannabinoid induced depression of synaptic transmission. GABAergic synaptic currents evoked in pallidal neurons by stimulation of striatal projections (IPSCs) were depressed by perfusion with the CB1R agonist ACEA. Coactivation of D2Rs with quinpirole converted the depression into stimulation. Pretreatment with pertussis toxin (PTX) to limit $G_{i/o}$ protein coupling also switched the CB1R-induced depression of IPSCs. The stimulation of IPSCs was blocked by the selective PKA blocker H89. Changes in the paired pulse ratio during both inhibitory and stimulatory responses indicate that the effects are due to changes in transmitter release. Postsynaptic depolarization induces endocannabinoid release that inhibits transmitter release (DSI). When D2Rs were activated with quinpirole, depolarization increased transmission instead of depressing it. This increase was blocked by AM251. We also examined the effects of CB1R/D2R coactivation on cAMP accumulation in the GPe to further verify that the AC/PKA cascade is involved. CB1R/D2R coactivation converted the inhibition of cAMP seen when each receptor is stimulated alone into a stimulation. We also determined the effects on turning behavior of unilateral injection of ACEA into the GPe of awake animals and its modification by dopamine antagonists. Blockade of D2 family receptors with sulpiride antagonized the motor effects of ACEA. We show, for the first time, that cannabinoid-inhibition of synaptic transmission in the GPe becomes a stimulation after D2Rs or PTX treatment and that the switch is probably relevant for the control of motor behavior.

1. Introduction

Cannabinoid receptors are the most numerous of any receptor in the brain (Herkenham et al., 1991; Mailleux and Vanderhaeghen, 1992; Tsou et al., 1998). The most prevalent effect of their activation is depression of transmitter release by presynaptic endings (Freund et al., 2003; Szabo and Schlicker, 2005; Lovinger, 2008). The depression is a retrograde process: endocannabinoids released by depolarization of the postsynaptic membrane diffuse back to inhibit presynaptic function.

Stimulation of CB1Rs activates pertussis toxin-sensitive $G_{i/o}$ proteins (Howlett et al., 1986; Prather et al., 2000; Mukhopadhyay and Howlett, 2005) that in turn trigger several inhibitory mechanisms, including inhibition of voltage-gated calcium channels, activation of potassium channels, inhibition of the vesicle fusion

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process, depression of adenylyl cyclase activity and reduced production of cAMP (Howlett and Fleming, 1984; Howlett et al., 1986; Wade et al., 2004; Lovinger, 2008).

The signaling pathway of CB1Rs in the basal ganglia is switched when the availability of $G_{i/o}$ protein changes (Glass and Felder, 1997; Gonzalez et al., 2009). During restriction of $G_{i/o}$ protein availability, stimulation of CB1Rs activates G_s instead of $G_{i/o}$ proteins leading to enhanced adenylyl cyclase activity and increased cAMP production (Glass and Felder, 1997; Sulcova et al., 1998; Hampson et al., 2000). Since increases in cAMP levels lead to increased GABA release by nerve terminals (Shindou et al., 2002; Neve et al., 2004; Nava-Asbell et al., 2007; Recillas-Morales et al., 2014), we previously examined whether the inhibitory effect of CB1Rs activation on K^+-depolarization-induced [^{3}H] GABA release in the GPe is switched to stimulation by conditions that alter cAMP/PKA signaling. We found that restricting $G_{i/o}$ protein availability in striatopallidal terminals by either pertussis toxin (PTX) treatment or stimulation of dopamine D2Rs, switches the effect of CB1R stimulation on [^{3}H] GABA release (Gonzalez et al., 2009).

Electrophysiological determinations of the effects of CB1R stimulation show that the more general effect is depression of synaptic transmission and not stimulation (Howlett and Fleming, 1984; Howlett et al., 1986; Wade et al., 2004; Lovinger, 2008). Indeed, activation of CB1Rs in the GPe, with either exogenous cannabinoid agonists or endogenous cannabinoids, released by postsynaptic membrane depolarization (DSI), depresses IPSCs (Engler et al., 2006).

Although the switch in signaling cascades is well established, it is not known whether conditions that switch the cascade also transform the effect of CB1R activation on synaptic transmission and motor behavior. We therefore examined the effects of conditions that induce the signaling switch on these parameters. Such studies could reveal whether cannabinoids can have different behavioral effects depending on the state of the animal. In particular, we determined whether the motor effects induced by unilateral intrapallidal injections of ACEA are modified by changes in dopaminergic tone.

As part of the study we also examined whether the activity of the cAMP/PKA cascade was changed in the GPe during conditions that switch the response. A preliminary publication of these results has been made (Caballero et al., 2015).

2. Methods

2.1. Animals

All procedures were carried out in accordance with the National Institutes of Health Guide for Care and Use of Laboratory Animals and approved by the Institutional Animal Care Committee of the CINVESTAV-IPN. Because of the limited availability of rats the electrophysiological experiments were performed in mice that were readily available. However, the assays of cAMP accumulation and the behavioral tests were performed in rats rather than mice because: a) the larger brains provide greater amount of tissue to isolate synaptosomes and greater reliability in the placement of cannulas. b) Comparison of electrophysiological responses in the current study with our previous measurements of [^{3}H] GABA release in the rat GPe (Gonzalez et al., 2009) show that the effects of activating CB1Rs and D2Rs in the GPe are similar in rats and mice.

2.2. Slice preparation

Brain slices were obtained from mice (12–14 days postnatal). In brief, the mice were anesthetized and decapitated and oblique-sagittal slices (300 μM thick) including both the globus pallidus and the caudate-putamen were cut with a vibroslicer at an angle of 20° to the midline. The slices were next incubated in oxygenated normal ACSF solution (in mM/l: 125 NaCl, 3 KCl, 1.25 KH2PO4, 26 NaHCO3, 1 MgCl2, 2.5 CaCl2 and 10 glucose equilibrated with 95% O2 + 5% CO_2, pH 7.4) where they remained for at least 1 h at room temperature (about 25 °C).

2.3. Electrophysiological recordings

The procedures used in the laboratory have been described previously (Gasca-Martinez et al., 2010). In brief, single slices were transferred to a recording chamber placed on the stage of an upright microscope (Zeiss Axioscope) where GPe neurons were visually identified and sampled. GABA-receptor-mediated currents were isolated by incubating with NMDA receptor (6-cyano-7-nitroquinoxaline-2,3-dione, CNQX, 10 μM) and AMPA receptor antagonists, (DL-2-amino5phosphonopentanoic acid, DL-AP-5, 100 μM). Evoked IPSCs (IPSCs) were recorded in whole-cell configuration at a holding potential of −50 mV with borosilicate pipettes (3–6 MΩ resistance) containing (in mM) 135 K-Gluconate, 4 NaCl, 0.4 GTP, 2 Mg-ATP, 5 EGTA, HEPES 10, pH, 7.25. IPSCs were elicited by delivering a pair of pulses every 15 s with a 50 msec interval, using a bipolar platinum/iridium electrode positioned in the caudate-putamen. Single rectangular electrical pulses (10–100 mV intensity, 0.5 msec pulse duration) were delivered through an isolated stimulation unit.

2.4. Basic properties of globus pallidus neurons

Previous studies show that at least three types of neuron can be identified in the GPe of the rat on the basis of their electrophysiological properties (for references see Cooper and Stanford, 2000). In the GPe of mice two of these neuronal populations have been identified (Shin et al., 2003). In agreement with these investigators we detected no differences in the effects of applied agonists or antagonists among the various cell types and therefore, all data from the different GPe neuronal phenotypes were pooled.

2.5. Depolarization-induced suppression of inhibition (DSI)

DSI was elicited by raising the membrane potential of globus pallidus neurons from −60 mV to 0 mV for periods lasting 10 s. Our aim was to elicit a more robust DSI response than the one observed by Engler et al. (2006) in the GPe. Comparison of the observations of Engler et al. (2006) with the findings of Yanovsky et al. (2003) suggested that longer depolarizing pulses and low [EGTA] within the clamp pipette might lead to robust responses. For this reason in all experiments in which DSI was examined, except those labeled high EGTA in Fig. 5 (carried out with 5 mM EGTA in the pipette), were carried out with pipette solutions containing only 0.2 mM of EGTA.

2.6. Pertussis toxin treatment

For Pertussis toxin (PTX) experiments the slices where pre-incubated between 12 and 18 h in a solution containing pertussis toxin at a concentration of 5 μg/ml continuously bubbled with O_2/CO_2 95% (Azad et al., 2003; Misner and Sullivan, 1999).

2.7. cAMP accumulation assay

cAMP accumulation assays were performed as previously described (Alexander, 1995; Rangel-Barajas et al., 2011). Synaptosomal fractions were isolated from GPe slices obtained from the rat brain. The slices were homogenized in buffer (sucrose, 0.32 M;