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Pharmacological activation of CB1 receptor modulates long term potentiation by interfering with protein synthesis

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ABSTRACT

Cognitive impairment is one of the most important side effects associated with cannabis drug abuse, as well as the serious issue concerning the therapeutic use of cannabinoids. Cognitive impairments and neuropsychiatric symptoms are caused by early synaptic dysfunctions, such as loss of synaptic connections in different brain structures including the hippocampus, a region that is believed to play an important role in certain forms of learning and memory. We report here that metaplastic priming of synapses with a cannabinoid type 1 receptor (CB1 receptor) agonist, WIN55,212-2 (WIN55), significantly impaired long-term potentiation in the apical dendrites of CA1 pyramidal neurons. Interestingly, the CB1 receptor exerts its effect by altering the balance of protein synthesis machinery towards higher protein production. Therefore the activation of CB1 receptor, prior to strong tetanization, increased the propensity to produce new proteins. In addition, WIN55 priming resulted in the expression of late-LTP in a synaptic input that would have normally expressed early-LTP, thus confirming that WIN55 priming of LTP induces new synthesis of plasticity-related proteins. Furthermore, in addition to the effects on protein translation, WIN55 also induced synaptic deficits due to the ability of CB1 receptors to inhibit the release of acetylcholine, mediated by both muscarinic and nicotinic acetylcholine receptors. Taken together this supports the notion that the modulation of cholinergic activity by CB1 receptor activation is one mechanism that regulates the synthesis of plasticity-related proteins.

1. Introduction

Endogenous cannabinoids are lipid molecules that target G-protein coupled cannabinoid-1 (CB1) receptors in the 'central nervous system (CNS)' CB1 receptors are distributed in all regions of the CNS including the neocortex, cerebellum and hippocampus (Howlett, 1998; Howlett et al., 2002). These lipid molecules are synthesized on demand from the membrane precursors in the postsynaptic cell and then diffuse retrogradely to the presynaptic cell (Moreira and Lutz, 2008; Piomelli, 2003), where their activation leads to the suppression of neurotransmitter release including glutamate and GABA (Schlicker and Kathmann, 2001). CB1 receptors are localized in both glutamatergic and GABAergic nerve terminals and exert different physiological and behavioral consequences (Domenici et al., 2006; Katona et al., 1999; Monory et al., 2006). Schlicker and Kathmann showed that exogenous cannabinoids inhibits neurotransmitter release via presynaptic CB1 receptors (Schlicker et al., 1994) while endogenous cannabinoids inhibits presynaptic calcium channels thereby inhibiting the neurotransmitter release through direct G protein inhibition (Wilson et al., 2001). CB1 receptor activation can lead to the inhibition of N-type and P/Q-type calcium channel activity (Mackie et al., 1995), activation of A-type and inwardly rectifying potassium channels (Deadwyler et al., 1993; Mackie et al., 1993), and can inhibit the synthesis of cAMP by negatively regulating adenylate cyclases, thus downregulating PKA signaling (Childers and Deadwyler, 1996; van Beugen et al., 2006).

Excessive CB1 receptor activation in the hippocampus is known to impair memory formation and consolidation by transiently modulating the mammalian target of rapamycin (mTOR)/p70S6K (Puighermanal et al., 2009). In the mouse hippocampus, CB1 receptor activation triggers the activation of the mTOR pathway and thus it might lead to an imbalance in the protein synthesis machinery through a glutamatergic mechanism, which in the end, induces amnesia. Cannabinoid activation also increases the phosphorylation of S6, and the translation initiation factors eIF4E (S209), eIF4G (S1108) and eIF4B (S422) in the hippocampus, all of which are related to increased local protein translation in dendrites (Puighermanal et al., 2009). Inhibition of protein synthesis reversed these amnesic effects induced by the excessive activation of CB1 receptors (Puighermanal et al., 2009).

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Protein synthesis dependent forms of long-term potentiation/long-term depression (LTP/LTD) are considered to be the cellular mechanisms underlying learning and memory formation (Malenka and Bear, 2004). Exogenous cannabinoids have been implicated in disturbing behavioral learning by blocking the induction of LTP in the hippocampus in vivo (Abush and Akirav, 2010; Robinson et al., 2007), while endogenous cannabinoids are known to facilitate the induction of LTP in single neurons of the hippocampus (Carlson et al., 2002). Exogenous cannabinoids are applied globally and they affect all cells with cannabinoid receptors, including those with excitatory and inhibitory synapses and might disrupt the temporal and spatial selectivity of coding processes (Carlson et al., 2002). Taken together, endocannabinoids by suppressing inhibition in a restricted dendritic area can locally and persistently facilitate the induction of LTP at excitatory synapses (Chevaleyre et al., 2007). Endogenous cannabinoids not only enhances excitability (Chevaleyre and Castillo, 2003), but also mediates metaplasticity, whereby a synapse's previous history determines its current plasticity (Abraham and Bear, 1996; Chevaleyre and Castillo, 2003; Sajikumar and Korte, 2011).

Part of the CB1 receptor effect on LTP might be linked to an interaction with the neuromodulatory system of cholinergic neurons (Goonawardena et al., 2010). This is supported by the fact that presynaptic CB1 receptors are expressed on cholinergic nerve terminals, suggesting a direct inhibition of acetylcholine release (Gifford et al., 2000). Cholinergic system plays a major role in the expression of LTP in the adult brain (Motooka et al., 2001). Both the endogenous release of acetylcholine in vivo (Leung et al., 2003; Ovsepian et al., 2004) and the exogenous application of mAChR (muscarinic acetylcholine receptor) agonists in vitro facilitate the induction of LTP (Boddeke et al., 1992; Shimoshige et al., 1997). Moreover activation of muscarinic acetylcholine receptors by using carbachol enhances the release of endogenous cannabinoids in the CA1 region of hippocampus (Kim et al., 2002). A recent study by Robinson et al. (2010) have shown that WIN55,212-2, a CB1 receptor agonist induces deficits in spatial learning, due to a reduction in cholinergic function.

We assert that the activation of CB1 receptors impaired late-LTP by increasing the protein synthesis. We used synaptic tagging/capture model system, a mechanism which explains how short-term memories are consolidated (Redondo and Morris, 2011; Frey and Morris, 1997), to show that priming of synapses with a CB1 receptor agonist, WIN55, before a strong tetanization protocol that should lead to late-LTP, expressed only early-LTP. The impairment of LTP is due to the enhanced synthesis of plasticity related proteins (PRPs), and it can be rescued by inhibiting the new synthesis of PRPs during LTP induction. We also report that the hypofunction of the cholinergic system through both muscarinic and nicotinic receptors was found to be responsible for the impairment of LTP.

2. Materials and methods

All experiments were carried out according to the guidelines from Animal Committee on Ethics in the Care and Use of Laboratory animals of TU-Braunschweig. Hippocampal slices were prepared from male Wistar rats (6–7 week old). Eighty nine transverse hippocampal slices prepared from the same number of rats were used for the experiments. Rats were anaesthetized in a chamber containing CO_2 for a few seconds and then decapitated immediately. Hippocampal slices of 400 μm were prepared from the right hippocampus by using a manual tissue chopper (Stoelting) and the slices were incubated in an interface chamber maintained at 32 °C (Scientific System Design), which was modified for long-term experiments (Sajikumar et al., 2009). All preparations were carried out in carbogenated artificial cerebrospinal fluid (ACSF) maintained at 4 °C. Flow rate of the chamber was maintained at 0.69 ml/min to keep the slices wet. The ACSF contained the following (in millimolars): 124 NaCl, 4.9 KCl, 1.2 KH_2PO_4, 2.0 $MgSO_4$, 2.0 $CaCl_2$, 24.6 $NaHCO_3$, 10 D-glucose, and was equilibrated with 95% O_2 and 5% CO_2 (17 L/h). Two monopolar stainless steel electrodes (AM Systems, USA) were positioned at an adequate distance in the stratum radiatum of the CA1 region for stimulating two separate and independent synaptic inputs S1 and S2 to a single neuronal population. For recording the field EPSP, one electrode (AM Systems, USA) was placed in the CA1 stratum radiatum layer (see Fig. 1A). The signals were amplified by using a differential amplifier (Model 1700, AM System) and were digitized using a CED 1401 analog-to-digital converter (Cambridge Electronic Design).

Slices were preincubated for 3 h (for details see (Sajikumar et al., 2005)). For setting the test stimulus intensity, a fEPSP of 40% of the maximal amplitude was determined for both synaptic inputs, S1 and S2. Biphasic constant current pulses were used for stimulation. Early-LTP was induced in the stratum radiatum by using a single weak tetanus (WTET) protocol consisting of 100 Hz duration, and 21 biphasic constant current pulses, of 0.2 ms/polarity. Late-LTP was induced using a strong tetanus (STET) protocol consisting of three trains of 100 Hz, 100 biphasic constant current pulses, of 0.2 ms/polarity at an interval of 10 min. The slope of the fEPSP was monitored online. A stable baseline was recorded for a minimum of 60 min. Four 0.2-Hz biphasic constant current pulses (0.1 ms/polarity) were used for baseline recording and were delivered at 1, 3, 5, 11, 15, 21, 25, 30 and every 5 min thereafter and continued until 4–6 h.

2.1. Pharmacology

WIN55,212-2 mesylate salt ((*R*)-(+)-(2,3-Dihydro-5-methyl-3-(4-morpholinylmethyl)pyrrolo (1,2,3-*de*)-1,4-benzoxazin-6-yl)-1-naphthalenyl methanone mesylate) was used at a concentration of 2 μM (Sigma Aldrich) (dissolved in ACSF and 0.1% DMSO). It had been reported earlier that 1 μM WIN55 blocked late-LTP and late-LTD from slices that were kept in submerged chamber (Misner and Sullivan, 1999). Interestingly, in our set-up (interface chamber), we used 2 μM WIN55, as 1 μM concentration did not show any effect when it was bath applied. We had proved earlier that 0.1% DMSO did not have any nonspecific effects on baseline potentials (Navakkode et al., 2005). AM251, 1-(2,4-Dichlorophenyl)-5-(4-iodophenyl)-4-methyl-N-1-piperidinyl-1H-pyrazole-3-carboxamide, a cannabinoid receptor antagonist was used at a concentration of 1 μM (Hoffman et al., 2007) (dissolved in ACSF and 0.1% DMSO) (Tocris Cookson, Bristol, UK). D-2-Amino-5-phosphonopentanoic acid (AP-5) (Tocris Cookson, Bristol, UK) was used at a concentration of 50 μM (dissolved in ACSF) to block the NMDA receptor. Anisomycin (Sigma), a reversible protein synthesis inhibitor, was used at a concentration of 25 μM (dissolved in ACSF and 0.1% DMSO). Emetine (Tocris Cookson) was used at a concentration of 20 μM (dissolved in ACSF and 0.1% DMSO) (Navakkode and Korte, 2012). Carbachol (Tocris Cookson, Bristol, UK), a cholinergic agonist, was used at a concentration of 200 nM [dissolved in ACSF and 0.1% DMSO]. Scopolamine hydrobromide, a muscarinic receptor antagonist was used at a concentration of 10 μM (dissolved in ACSF). Methyllycaconitine citrate (both scopolamine and MLA purchased from Tocris Cookson, Bristol, UK) a nicotinic receptor antagonist was used at a concentration of 10 nM (dissolved in ACSF) (Navakkode and Korte, 2012).

2.2. Statistics

The average values of the slope function of the field EPSP (mV/ms) per time point were analyzed either using the Wilcoxon signed rank test when compared within one group, or the Mann–Whitney *U*-test when data were compared between groups whereas $P < 0.05$ was considered as statistically different or statistically significant.

3. Results

3.1. Endocannabinoids modulate long-term potentiation

It was shown earlier that a priming stimulus by low frequency stimulation (LFS) can inhibit the subsequent induction of late-LTP (Young and Nguyen, 2005) and these metaplastic effects can regulate the integration of synaptic events for a longer period of time. We confirmed these earlier findings of Young and Nguyen under our experimental conditions by applying an LFS of 5 Hz for 15 min before strong tetanization (STET) in S1. This resulted in the expression of an early-LTP, not of late-LTP (Fig. 1B, filled circles). S1 showed statistically significant potentiation from 1 min (Wilcoxon test, $p = 0.02$) after STET and remained significant until 165 min (Wilcoxon test, $p = 0.02$). This result is in line with the fact that protein phosphatases are preferentially activated during LFS (Morishita et al., 2001; Mulkey et al., 1993) and long-lasting activation of these phosphatases by prior LFS can suppress the subsequent expression of late-LTP without affecting early-LTP (Woo and Nguyen, 2002). Apart from the role of phosphatases, the mechanisms behind the LFS mediated impairments of late-LTP are not known. Interestingly, endocannabinoids are known to be involved in LFS induced LTD at the Schaffer collateral synapses of CA1