Available online at www.sciencedirect.com
ScienceDirect
Neuropharmacology 54 (2008) 51–57
NEURO PHARMACOLOGY
www.elsevier.com/locate/neuropharm

CB_1 receptor-dependent and -independent inhibition of excitatory postsynaptic currents in the hippocampus by WIN 55,212-2

Beáta Németh [a], Catherine Ledent [b], Tamás F. Freund [a], Norbert Hájos [a,*]

[a] *Institute of Experimental Medicine, Hungarian Academy of Sciences, Szigony u. 43, Budapest H-1450, Hungary*
[b] *Institut de Recherche Interdisciplinaire en Biologie Humaine et Moleculaire, Universite Libre de Bruxelles, 1070 Brussels, Belgium*

Received 26 March 2007; received in revised form 27 June 2007; accepted 4 July 2007

Abstract

We investigated the effect of a synthetic cannabinoid, WIN 55,212-2 on excitatory postsynaptic currents (EPSCs) evoked by stimulation of Schaffer collaterals in CA1 pyramidal cells. Bath application of WIN 55,212-2 reduced the amplitude of EPSCs in dose-dependent manner tested between 0.01 nM and 30 μM. In rats and mice, this cannabinoid ligand inhibited excitatory synapses in two steps at the nM and μM concentrations. When the function of CB_1 cannabinoid receptors (CB_1R) was impaired, either by the application of a CB_1R antagonist AM251, or by using CB_1R knockout mice, WIN 55,212-2 in μM concentrations could still significantly reduced the amplitude of EPSCs. WIN 55,212-2 likely affected the efficacy of excitatory transmission only at presynaptic sites, since both at low and high doses the paired pulse ratio of EPSC amplitude was significantly increased. The inactive enantiomer, WIN 55,212-3, mimicked the effect of WIN 55,212-2 applied in high doses. In further experiments we found that the CB_1R-independent effect of 10 μM WIN 55,212-2 at glutamatergic synapses was fully abolished, when slices were pre-treated with ω-conotoxin GVIA, but not with ω-agatoxin IVA.

These data suggest that, in the hippocampus, WIN 55,212-2 reduces glutamate release from Schaffer collaterals solely via CB_1Rs in the nM concentration range, whereas in μM concentrations, WIN 55,212-2 suppresses excitatory transmission, in addition to activation of CB_1Rs, by directly blocking N-type voltage-gated Ca^{2+} channels independent of CB_1Rs.

Keywords: Brain slices; Glutamate; Transmitter release; Hippocampus; Pyramidal cell; Cannabinoids

1. Introduction

The type 1 cannabinoid receptors (CB_1Rs) have been shown to control the release of different neurotransmitters, but the mechanisms underlying the regulation of synaptic communication could substantially vary between brain regions (Freund et al., 2003). Pharmacological results, suggesting a presynaptic locus of action of cannabinoid receptor ligands, have been fully supported by immunohistochemical data. Several studies demonstrated at the electron microscopic level that CB_1Rs decorated both inhibitory and excitatory axon terminals (Katona et al., 1999, 2006; Kawamura et al., 2006). In addition, recent high-resolution quantitative studies established that CB_1Rs were found all around the axon membrane, but were enriched in the perisynaptic annulus and on preterminal segments, whereas immunolabelling was weaker in the synaptic active zone (Nyiri et al., 2005; Kawamura et al., 2006). This subcellular distribution of CB_1Rs might imply an action on several regulatory mechanisms of transmitter release, including the control of Ca^{2+} entry via voltage-dependent Ca^{2+} channels (primarily by receptors located in the perisynaptic annulus), the reduction of axonal conduction (by receptors present on the preterminal segments), or a direct action on exocytosis (Wilson et al., 2001; Diana and Marty, 2003).

In spite of the direct anatomical evidence, several pharmacological observations suggest that some synthetic

* Corresponding author. Tel.: +36 1 2109 400/387; fax: +36 1 210 9412.
E-mail address: hajos@koki.hu (N. Hájos).

0028-3908/$ - see front matter © 2007 Elsevier Ltd. All rights reserved.
doi:10.1016/j.neuropharm.2007.07.003

cannabinoid agonists (mainly WIN 55,212-2) could also have a CB_1R-independent action on synaptic glutamate release. This possibility has been fuelled primarily by experiments using CB_1R knockout mice. Our laboratory was the first to show that, in the absence of CB_1Rs, WIN 55,212-2 was still able to reduce excitatory, but not inhibitory postsynaptic currents in CA1 pyramidal neurons (Hájos et al., 2001). Moreover, WIN 55,212-2 was more potent in suppressing GABAergic than glutamatergic transmission (Hoffman and Lupica, 2000; Ohno-Shosaku et al., 2002; Hájos and Freund, 2002), providing further support for the possible presence of CB_1R-independent binding site at excitatory synapses. Importantly, AM251, a CB_1R antagonist prevented the reduction of synaptic inhibition after application of WIN 55,212-2, whereas glutamatergic transmission could still be suppressed by about 50% in the presence of AM251 (Hájos and Freund, 2002). In contrast to the above findings showing that hippocampal glutamatergic synapses were effectively regulated independent of CB_1Rs, electrophysiological data from other groups suggested that CB_1Rs were solely responsible for the cannabinoid modulation of excitatory synaptic transmission in the hippocampus (Ohno-Shosaku et al., 2002; Domenici et al., 2006; Takahashi and Castillo, 2006).

To shed light on the reasons behind the contradictory findings regarding the involvement of CB_1R-dependent vs. -independent mechanisms in the regulation of hippocampal excitatory synapses, we re-examined the effect of WIN 55,212-2 on monosynaptically evoked excitatory postsynaptic currents (EPSCs) in CA1 pyramidal cells. All these experiments were performed in a modified submerged recording conditions (Hájos et al., 2005).

2. Methods

Experiments were carried out according to the guidelines of the institutional ethical code and the Hungarian Act of Animal Care and Experimentation (1998. XXVIII. section 243/1998.). Male Wistar rats (14–18 days old), as well as wild type and CB_1R knockout mice (15–25 days old, CD1 strain) were used. The animals were deeply anaesthetized with isoflurane followed by decapitation. After opening the skull, the brain was quickly removed and immersed into ice-cold cutting solution containing (in mM: sucrose 252; KCl 2.5; $NaHCO_3$ 26; $CaCl_2$ 0.5; $MgCl_2$ 5; NaH_2PO_4 1.25; glucose 10). The solution had been bubbled with 95% O_2/5% CO_2 (carbogen gas) for at least 30 min before use. Thick horizontal slices (350 μm from mice and 400 μm from rats) were prepared using a Leica VT1000S Vibratome. The CA3 region was removed to prevent epileptic burst firings. The slices were stored in an interface type chamber containing ACSF (in mM: 126 NaCl, 2.5 KCl, 26 $NaHCO_3$, 2 $CaCl_2$, 2 $MgCl_2$, 1.25 NaH_2PO_4, and 10 glucose) at room temperature for at least 1 h before recording. After the initial incubation period, slices were transferred individually into a submerged type recording chamber.

Whole-cell patch-clamp recordings were obtained at 30–32 °C from CA1 pyramidal cells visualized by infrared DIC videomicroscopy (Zeiss Axioscope, Germany). Patch electrodes were pulled from borosilicate glass capillaries with an inner filament (1.5 mm O.D.; 1.12 mm I.D., Hilgenberg, Germany) using a Sutter P-87 puller. Electrodes (~3–6 MΩ) were filled with a solution containing (in mM) 80 CsCl, 60 Cs-gluconate, 3 NaCl, 1 $MgCl_2$, 10 HEPES, 2 Mg-ATP, and 5 QX-314 (pH 7.2–7.3 adjusted with CsOH; osmolarity 275–290 mOsm). Excitatory postsynaptic currents (EPSCs) were recorded at a holding potential of −65 mV. Slices were perfused with ACSF containing 70–100 μM picrotoxin to block inhibitory neurotransmission. The solution was bubbled with carbogen gas at room temperature and perfused at a flow rate of 3–4.5 ml/min in a slice chamber optimized for laminar flow to ensure the stability of the amplitude of evoked currents and a better oxygenation of submerged slices (Hájos et al., 2005). To evoke EPSCs, the stimulating electrode was placed in the stratum radiatum of CA1. Pairs of electrical stimuli separated by 50 ms were delivered via a theta glass pipette (Sutter Instrument Company, Novato, CA) filled with ACSF at 0.1 Hz using a Supertech timer and isolator (Supertech LTD, Pécs, Hungary, http://www.superte.ch). Access resistances (between 4 and 18 MΩ, compensated 65–70%) were frequently monitored and remained constant (±20%) during the period of analysis. Signals were recorded with a Multiclamp 700A (Molecular Devices, Sunnyvale, CA), filtered at 2 kHz, digitized at 6 kHz (National Instruments PCI-6024E A/D board, Austin, TX), and analyzed off-line with the EVAN program (courtesy of Prof. I. Mody, UCLA, CA).

The drug was perfused in a given concentration until the maximal effect was reached. The time needed for maximal inhibition was usually 6–8 min. To avoid the possible effect of a changing pH, we added the same amount of HCl to the control solution. The concentration response relationship for WIN 55,212-2 was obtained as follows: control EPSC amplitudes in a 2–3 min time window were compared to those measured after 10 min drug application for the same period of time. Only those experiments were included that had stable amplitudes at least for 10 min before drug application. After each experiment, the tubing made of Teflon was washed with ethanol for 10 min and with ACSF for 15 min. Each data point represents the mean ± SEM of the maximal inhibition of the evoked EPSCs ($n = 3–7$). EC_{50} values were estimated by fitting a curve to the points of the dose response plots obtained in rats or wild type mice using the equation of $f(x) = a/(1 + \exp(-(x - c)/b)) + (100 - a)/(1 + \exp(-(x - e)/d))$, where '$c$' and '$e$' give the values for high and low affinity binding sites, respectively. The data points obtained in the presence of AM251 or in CB1 knockout mice were fitted by the equation of $f(x) = a/(1 + \exp(-(x - c)/b))$, where '$c$' gives the value of EC_{50}. The curve fitting was done using Origin 7.5 (OriginLab Corporation, MA). The paired pulse ratio was calculated from the mean amplitude of the second EPSCs divided by the mean amplitude of the first EPSCs. The paired pulse ratio after drug treatment was compared with the control using Wilcoxon matched pairs test in Statistica 6.1 (Statsoft, Inc., Tulsa, OK). Data are presented as mean ± SEM.

Picrotoxin, WIN 55,212-2 and WIN 55,212-3 were purchased from Sigma-Aldrich, AM251 was obtained from Tocris, while ω-conotoxin GVIA and ω-agatonix IVA from Alomone Labs. For all experiments, WIN-55,212-2 was dissolved in 0.1N HCl giving a 20 mM stock solution stored at 4 °C. AM251 was dissolved in DMSO (100 mM) and stored at −20 °C. WIN 55,212-3 dissolved in DMSO (100 mM) was stored at 4 °C. From these stock solutions, the final dilution of drugs was done in ACSF containing picrotoxin under constant stirring and the prepared solution was bath applied. In control solutions, the vehicle was diluted in the same concentration as in the solutions containing drugs. Bovine serum albumin (BSA) was added in a concentration of 0.1 mg/ml to the solutions used for experiments with WIN 55,212-3.

3. Results

The effects of the cannabinoid agonist WIN 55,212-2 on EPSCs evoked by focal stimulation of Schaffer collaterals were measured in hippocampal CA1 pyramidal cells. First we performed concentration response analyses for the inhibitory effects of WIN 55,212-2 on evoked EPSC in rat slices (Fig. 1a). WIN 55,212-2 bath applied between the concentrations of 0.1 nM and 30 μM suppressed the amplitude of EPSCs in two steps. The apparent EC_{50} values from the fitted curve were 2.91 nM and 3.77 μM (Fig. 1c). Then we investigated the WIN 55,212-2-sensitivity of EPSCs, when AM251, a CB_1R specific antagonist was added to the bath solution in the concentration of 2 μM. In spite of the presence of AM251, the cannabinoid agonist could still reduce the amplitude of evoked currents, but only in the μM range (Fig. 1a). The estimated EC_{50} value for this effect was 1.69 μM (Fig. 1c).