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Neuropharmacology 49 (2005) 1170–1177
NEURO PHARMACOLOGY
www.elsevier.com/locate/neuropharm

Δ^9-Tetrahydrocannabinol-induced desensitization of cannabinoid-mediated inhibition of synaptic transmission between hippocampal neurons in culture

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Received 14 June 2005; accepted 12 July 2005

Abstract

Prolonged exposure to cannabinoids results in desensitization of cannabinoid receptors. Here, we compared the desensitization produced by the partial agonist, Δ^9-tetrahydrocannabinol (THC) to that produced by the full agonist Win55,212-2 on cannabinoid-mediated inhibition of glutamatergic synaptic transmission. Synaptic activity between rat hippocampal neurons was determined from network-driven increases in the intracellular Ca^{2+} concentration ($[Ca^{2+}]_i$ spikes). To assess the effects of prolonged treatment, cultures were incubated with cannabinoids, washed in 0.5% fatty-acid-free bovine serum albumin to ensure the removal of the lipophilic drug and then tested for inhibition of $[Ca^{2+}]_i$ spiking by Win55,212-2. In control experiments, 0.1 μM Win55,212-2 inhibited $[Ca^{2+}]_i$ spiking by 93 ± 5%. Win55,212-2 produced significantly less inhibition of $[Ca^{2+}]_i$ spiking following 18–24 h treatment with 1 μM THC (48 ± 5%) or treatment with 1 μM Win55,212-2 (29 ± 6%). Thus, THC produced significantly less functional desensitization than Win55,212-2. The desensitization produced by THC was maximal at 0.3 μM, remained stable between 1 and 7 days of preincubation and shifted the EC_{50} of acute inhibition by Win55,212-2 from 27 to 251 nM. Differences in the long-term effects of cannabinoid receptor agonists on synaptic transmission may prove important for evaluating their therapeutic and abuse potential.

Keywords: Cannabinoid; CB1; Desensitization; Hippocampus; Tetrahydrocannabinol; Win55,212-2

1. Introduction

Cannabinoids act on presynaptic CB1 receptors to inhibit glutamatergic (Shen et al., 1996) and GABAergic (Chan et al., 1998) neurotransmission. Prolonged exposure to cannabinoid agonists results in desensitization of effects mediated by CB1 receptors including activation of GTPase activity (Sim-Selley, 2003), activation of K^+ channels (Jin et al., 1999) and inhibition of synaptic transmission (Kouznetsova et al., 2002). These cellular changes may underlie the development of tolerance to cannabinoid effects on behavior such as antinociception, hypomotility, hypothermia, and catalepsy following repeated administration (Bass and Martin, 2000; Fan et al., 1994). Desensitization of G-protein-coupled receptors is generally thought to be influenced by the intrinsic activity of the agonist (Clark et al., 1999), yet the rate of CB1 desensitization is independent of agonist efficacy (Luk et al., 2004).

Here, we studied the effects of two cannabinoid receptor agonists of different efficacies on glutamatergic

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0028-3908/$ - see front matter © 2005 Elsevier Ltd. All rights reserved.
doi:10.1016/j.neuropharm.2005.07.012

activity in the synaptic network formed by rat hippocampal neurons grown in primary culture. The objective of this study was to determine whether THC, a partial agonist at CB1 receptors (Shen and Thayer, 1999), would produce less desensitization at steady state than the full agonist Win55,212-2. Differences in the long-term effects of cannabinoids on synaptic transmission may prove important for evaluating the therapeutic and abuse potential of these drugs.

2. Methods

2.1. Cell culture

Rat hippocampal neurons were grown in primary culture as described previously (Wang et al., 1994) with minor modifications. Fetuses were removed on embryonic day 17 from maternal rats, anesthetized with CO_2, and killed by decapitation under a protocol approved by the University of Minnesota Institutional Animal Care and Use Committee in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals. Hippocampi were dissected and placed in Ca^{2+}- and Mg^{2+}-free HEPES buffered Hanks' salt solution (HHSS), pH 7.45. HHSS was composed of the following (in mM): HEPES 20, NaCl 137, $CaCl_2$ 1.3, $MgSO_4$ 0.4, $MgCl_2$ 0.5, KCl 5.0, KH_2PO_4 0.4, Na_2HPO_4 0.6, $NaHCO_3$ 3.0, and glucose 5.6. Cells were dissociated by trituration through a 5-ml pipette and a flame-narrowed Pasteur pipette. Cells were pelleted and resuspended in Dulbecco's modified Eagle Medium (DMEM; GIBCO Labs, Grand Island, NY) without glutamine, supplemented with 10% fetal bovine serum (FBS; Sigma, St. Louis, MO) and penicillin/streptomycin (100 U/ml and 100 μg/ml, respectively). Dissociated cells then were plated at a density of 10,000–15,000 cells/well onto 25-mm-round cover glasses that had been coated with poly-D-lysine (0.1 mg/ml; Sigma), and washed with H_2O. Neurons were grown in a humidified atmosphere of 10% CO_2 and 90% air (pH 7.4) at 37 °C, and fed at days 1 and 6 by exchange of 75% of the media with DMEM supplemented with 10% horse serum (GIBCO) and penicillin/streptomycin. Cells used in these experiments were cultured without mitotic inhibitors for a minimum of 10 days and a maximum of 15 days.

2.2. $[Ca^{2+}]_i$ measurement

$[Ca^{2+}]_i$ was determined using a previously described dual-emission microfluorimeter (Werth and Thayer, 1994) to monitor indo-1 (Grynkiewicz et al., 1985). Cells were loaded with 2 μM indo-1 acetoxymethyl ester for 45 min at 37 °C in HHSS containing 0.5% bovine serum albumin. Loaded cells were placed in a flow-through chamber (Thayer et al., 1988), and experiments were performed at room temperature (22 °C). The chamber was mounted on an inverted microscope and cells were superfused with HHSS containing 10 μM glycine at a rate of 1–2 ml/min for 15 min before starting an experiment. Either bovine serum albumin at a concentration of 0.1% or fatty acid-free bovine serum albumin at a concentration of 0.5% was present in all the solutions to prevent adherence of lipophilic compounds to the solution delivery system. Superfusion solutions were selected with a multiport valve coupled to several reservoirs.

For excitation of indo-1, light from a 75-W Xe arc lamp was passed through a 350/10-nm band-pass filter (Omega Optical, Brattleboro, VT), reflected from a dichroic mirror (380 nm), and focused with a 70× phase-contrast oil immersion objective (Leitz, numerical aperture 1.15). Emitted light was reflected sequentially from dichroic mirrors (440 and 516 nm), through band-pass filters (405/20 and 495/20 nm, respectively), to photomultiplier tubes operating in photon-counting mode (Thorn EMI, Fairfield, NJ). Cells were illuminated with transmitted light (580 nm long pass) and visualized with a video camera placed after the second dichroic mirror. Recordings were defined spatially with a rectangular diaphragm. The 5-V photomultiplier output was integrated by passing the signal through an eight-pole Bessel filter at 2.5 Hz. This signal then was input into two channels of an analog-to-digital converter (Indec Systems, Sunnyvale, CA) sampling at 1 Hz.

After completion of each experiment, cells were wiped from the microscope field using a cotton-tipped applicator, and background light levels were determined (typically less than 5% of cell intensity). Autofluorescence from cells that had not been loaded with dye was not detectable. Records were later corrected for background and the ratios recalculated. Values of R_{min}, R_{max}, and β were 1.16, 10.4, and 4.33, respectively.

2.3. Data analysis

The frequency of $[Ca^{2+}]_i$ spiking was calculated from data collected during the last 10 min in 0.1 mM $[Mg^{2+}]_o$ for control ($F_{control}$) and from a 5-min window starting 5 min after drug application for drug treated (F). Percent inhibition (I) was calculated by the formula $I = ([F_{control} - (F \times 2)]/F_{control}) \times 100$. Concentration–response curves were fit by a logistic equation of the form $I = [(I_{max} - I_{min})/(1 + (X/EC_{50})^b)] + I_{min}$, where X is the agonist concentration, I_{min} and I_{max} are the percent inhibition calculated for $X = 0$, and for an 'infinite' concentration, respectively, and b is a slope factor that determines the steepness of the curve. EC_{50} values were calculated by a nonlinear, least squares curve-fitting algorithm using Origin 6.0 software (Microcal). Data are presented as mean ± S.E.M. and significance was determined by Student's t-test or