Neuroscience 169 (2010) 1268–1278

ENDOCANNABINOID-MEDIATED SYNAPTICALLY EVOKED SUPPRESSION OF GABAERGIC TRANSMISSION IN THE CEREBELLAR CORTEX

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Abstract—Presynaptic CB_1 cannabinoid receptors are frequently targets of endogenous cannabinoids (endocannabinoids) released from postsynaptic neurons. It is known that the glutamatergic afferent input to a neuron can trigger endocannabinoid production and that the released endocannabinoid can suppress the glutamatergic input. We tested the hypothesis that activation of the glutamatergic input to a neuron leads to an endocannabinoid-mediated suppression of the GABAergic afferent input to the same neuron. Spontaneous postsynaptic currents (sPSCs) were recorded with patch-clamp techniques in Purkinje cells in mouse cerebellar brain slices. Activation of the climbing fiber-mediated glutamatergic input to Purkinje cells led to a suppression of the sPSCs by 34±3%. This suppression was mostly due to suppression of GABAergic spontaneous inhibitory postsynaptic current (sIPSCs), because 93% of the sPSCs recorded in Purkinje cells were GABAergic sIPSCs. Blockade of ionotropic, but not metabotropic glutamate receptors, prevented the suppression. The climbing fiber activation led to an increase in calcium concentration in the Purkinje cells, and this increase was necessary for the suppression of sPSCs, because the suppression did not occur when the calcium increase was prevented by BAPTA. No sPSC suppression was observed in the presence of the CB_1 antagonist rimonabant or the diacylglycerol lipase inhibitor orlistat. In a further series of experiments GABAergic sIPSCs were recorded: these sIPSCs were also suppressed after climbing fiber activation, and the suppression was sensitive to the CB_1 antagonist SLV319. Finally, the GABAergic synaptic transmission between molecular layer interneurons and Purkinje cells was directly studied on simultaneously patch-clamped neuron pairs. Climbing fiber activation led to suppression of the interneuron → Purkinje cell synaptic transmission. The results point to a novel form of endocannabinoid-mediated heterosynaptic plasticity. The endocannabinoid production in a neuron is triggered by its glutamatergic synaptic input and is dependent on an increase in intracellular calcium concentration. The produced endocannabinoid, in turn, suppresses the GABAergic synaptic input to the neuron by activating CB_1 cannabinoid receptors. © 2010 IBRO. Published by Elsevier Ltd. All rights reserved.

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Abbreviations: ACSF, artificial cerebrospinal fluid; DMSO, dimethylsulfoxide; DNQX, 6,7-dinitroquinoxaline-2,3-dione; DSE, depolarization-induced suppression of excitation; DSI, depolarization-induced suppression of inhibition; mGluR, metabotropic glutamate receptor; IPSPs, inhibitory postsynaptic potentials; PRE, initial reference value; sIPSC, spontaneous inhibitory postsynaptic current; sPSC, spontaneous postsynaptic current.

Key words: 2-arachidonoylglycerol, CB_1 cannabinoid receptor, endocannabinoid, retrograde signaling, synaptic plasticity, voltage-gated calcium channels.

The $G\alpha_{i/o}$ protein-coupled CB_1 cannabinoid receptor is probably the most abundant G protein-coupled receptor in the CNS. It is the primary neuronal target of the phytocannabinoid Δ^9-tetrahydrocannabinol and the endogenous cannabinoids (endocannabinoids) (Howlett et al., 2002; Pertwee, 2005). The two best characterized endocannabinoids are anandamide and 2-arachidonoylglycerol (Piomelli, 2003; De Petrocellis et al., 2004; Sugiura et al., 2006). Activation of CB_1 receptors leads to presynaptic inhibition of synaptic transmission in many regions of the nervous system (Freund et al., 2003; Szabo and Schlicker, 2005).

Endocannabinoids and CB_1 receptors play an important physiological role in both short- and long-term synaptic plasticity. The basis of these actions is endocannabinoid-mediated retrograde signaling: endocannabinoids produced by postsynaptic neurons diffuse to presynaptic axon terminals and inhibit transmitter release by activating presynaptic CB_1 receptors (for review see Alger, 2002; Chevaleyre et al., 2006; Lovinger, 2008; Kano et al., 2009). Two mechanisms can trigger endocannabinoid production in postsynaptic neurons.

One mechanism triggering endocannabinoid production is activation of $G\alpha_{q/11}$ protein-coupled receptors. For example, activation of metabotropic glutamate receptors (mGluR1 and mGluR5) and muscarinic acetylcholine receptors (M_1 and M_3) by exogenous agonists leads to endocannabinoid release and retrograde signaling (Maejima et al., 2001; Varma et al., 2001; Galante and Diana, 2004; Straiker and Mackie, 2007).

The second mechanism leading to endocannabinoid production is elevation of the intracellular calcium concentration. During depolarization-induced suppression of inhibition (DSI) and depolarization-induced suppression of excitation (DSE), depolarization of postsynaptic neurons via the patch-clamp pipette leads to opening of voltage-gated calcium channels, endocannabinoid production and subsequent endocannabinoid-mediated synaptic suppression (e.g. Wilson and Nicoll, 2001; Wallmichrath and Szabo, 2002; Diana and Marty, 2003; Kim and Alger, 2004). Calcium increase occurring physiologically during action potential series can also trigger endocannabinoid production in postsynaptic neurons (Fortin et al., 2004; Brenowitz et al., 2006).

0306-4522/10 $ - see front matter
doi:10.1016/j.neuroscience.2010.05.036

Activity of the glutamatergic synaptic input to a neuron can also lead to endocannabinoid production; the combination of activation of $mGluR_{1/5}$ receptors and calcium influx (which follows depolarization due to activation of ionotropic glutamate receptors) is an especially strong trigger of endocannabinoid production. The released endocannabinoid, in turn, suppresses glutamate release from the same axons, the activity of which triggered endocannabinoid production (Brown et al., 2003; Melis et al., 2004; Maejima et al., 2005; Marcaggi and Attwell, 2005; Brenowitz and Regehr, 2005; Rancz and Häusser, 2006).

The hypothesis of the present experiments was that the glutamatergic synaptic input to a neuron elicits endocannabinoid production and that the endocannabinoid suppresses the GABAergic synaptic input to the neuron. For testing the hypothesis we recorded the GABAergic input to cerebellar cortical Purkinje cells with patch-clamped techniques. Activation of the climbing fiber-mediated glutamatergic input to the Purkinje cells led to a CB_1 receptor-mediated suppression of the GABAergic synaptic input. Some of the results have been published previously in abstract form (Szabo and Urbanski, 2007).

EXPERIMENTAL PROCEDURES

The experiments conformed to the European Communities Council Directive of November 24, 1986 (86/609/EEC). All efforts were made to minimize the number of animals and their suffering. The methods were similar to those described previously (Freiman et al., 2006; Szabo et al., 2006).

Brain slices

Ten to 18-days-old NMRI mice were anaesthetized with isoflurane (>3%) and decapitated. The brains were rapidly removed and placed in ice-cold artificial cerebrospinal fluid (ACSF) of the following composition (mM): NaCl 126, NaH_2PO_4 1.2, KCl 3, $MgCl_2$ 5, $CaCl_2$ 1, $NaHCO_3$ 26, glucose 20, Na-lactate 4, pH 7.3–7.4 (after the solution was gassed with 95% O_2/5% CO_2). 250 μm thick sagittal slices of the cerebellar vermis were cut. After cutting, the slices were stored in a Gibb chamber containing ACSF of the following composition (mM): NaCl 126, NaH_2PO_4 1.2, KCl 3, $MgCl_2$ 1, $CaCl_2$ 2.5, $NaHCO_3$ 26, glucose 10, Na-lactate 4, pH 7.3–7.4. For patch-clamping, brain slices were superfused with ACSF at 20–24 °C at a flow rate of 1.5 ml min^{-1} with ACSF of the following composition (mM): NaCl 126, NaH_2PO_4 1.2, KCl 3, $MgCl_2$ 1, $CaCl_2$ 2.5, $NaHCO_3$ 26, glucose 10, pH 7.3–7.4.

Patch-clamping

Neurons in slices were visualized with infrared video microscopy. Patch-clamp recordings were obtained with an EPC-9 amplifier under the control of TIDA software (HEKA Elektronik, Lambrecht, Germany). Series resistance compensation of 50% was usually applied. Series resistance was measured before and after recordings and experiments with major changes in series resistance (>20%) were discarded. Pipettes filled with ACSF were positioned in the granule cell layer for stimulation of climbing fibers by trains of 50 pulses (P) at 5 Hz or 10 P/50 Hz (see Fig. 1A). Similar patterns were used to stimulate climbing fibers in previous brain slice experiments (Satake et al., 2000; Rancz and Häusser, 2006; Duguid et al., 2007). Notably, climbing fibers usually fire less intensively *in vivo* (e.g. Maruta et al., 2007; Mathy et al., 2009; for review see De Zeeuw et al., 1998).

Recording of spontaneous postsynaptic currents (sPSCs) and spontaneous inhibitory postsynaptic currents (sIPSCs)

sPSCs were recorded (Figs. 1–4) at a holding potential of −70 mV with pipettes containing (mM): CsCl 147, $MgCl_2$ 1, HEPES 10, EGTA 1, ATP–Na_2 4, GTP–Na 0.4, N-ethyl-lidocaine Cl 2, pH 7.4. Under these experimental conditions, both GABAergic sIPSCs and glutamatergic sEPSCs are inward currents. In some experiments, 40 mM BAPTA was included in the pipette solution, and the concentration of CsCl was lowered appropriately. sIPSCs were recorded (Fig. 5) at a holding potential of −60 mV with pipettes containing (mM): K gluconate 145, $CaCl_2$ 0.1, $MgCl_2$ 2, HEPES 5, EGTA 1.1, ATP–Mg 5, GTP–Tris 0.3, pH 7.4. Under these experimental conditions, GABAergic sIPSCs are outward currents and glutamatergic sEPSCs are inward currents. sPSCs and sIPSCs were detected with the MiniAnalysis software (version 6.0.1; Synaptosoft, Decatur, Ga., USA); the program allowed analysis of complex peaks consisting of several currents. Amplitudes and recording times of sPSCs and sIPSCs were transferred from MiniAnalysis to SigmaPlot (SPSS, Chicago, IL, USA), and a program written by us in Sigmaplot calculated cumulative amplitudes by summating amplitudes of synaptic events for defined periods.

Paired recordings

Paired recording was used to study the GABAergic interneuron → Purkinje cell synaptic connection (see Fig. 6A). Because transmitter release from axon terminals of interneurons runs down in the whole-cell patch-clamp mode (Than and Szabo, 2002; Diana and Marty, 2003; Szabo et al., 2004), the perforated patch-clamp mode was used for patch-clamping the interneurons. The pipettes contained (mM): K gluconate 145, $CaCl_2$ 0.1, $MgCl_2$ 2, HEPES 5, EGTA 1.1, ATP–Mg 5, GTP–Tris 0.3, pH 7.4. Amphotericin B (300 μg ml^{-1}) was included in the pipette for establishing perforated patches. Sodium currents in presynaptic neurons were elicited in voltage-clamp mode by depolarizing steps (from −70 to 0 mV for 4 ms). The postsynaptic Purkinje cells were patched with pipettes containing (mM): KCl 70, K gluconate 75, $MgCl_2$ 3, HEPES 7, EGTA 1, ATP–Na_2 4, GTP–Tris 0.4, N-ethyl-lidocaine Cl 2, pH 7.4. For recording inhibitory postsynaptic potentials (IPSPs), the Purkinje cells were clamped in the current-clamp mode, and the membrane potential was adjusted to −70 mV by appropriate current injection.

Fluorescence measurement of calcium concentrations in Purkinje cells

In addition to the intracellular solution used for recording postsynaptic currents, the patch pipette contained the low affinity calcium indicator Oregon green 488 BAPTA-5N (K_d for calcium, 2×10^{-5} M; final concentration, 2×10^{-4} M). Fluorescence intensity in Purkinje cells was determined with an imaging system consisting of a Polychrome IV monochromatic light source, a cooled IMAGO VGA CCD camera and TILLvision imaging software (TILL Photonics, Gräfelfing, Germany). For measuring Oregon green fluorescence, the excitation wavelength was adjusted to 495 nm, and a 505DRLP dichroic filter and a 535AF45 bandpass emission filter were used (Omega Optical, Brattleboro, VT, USA). Fluorescence changes were evaluated in regions of interest (ROIs). Fluorescence values were corrected for background fluorescence. For further evaluation, ratios between stimulation-evoked fluorescence changes (ΔF) and baseline fluorescence measured immediately before stimulation (F_0) were calculated ($\Delta F/F_0$ ratios).

Protocols and statistics

Recordings started 15 (electrophysiological recordings) or 40 min (calcium imaging) after establishment of the whole-cell configura-