Endocannabinoids Mediate Rapid Retrograde Signaling At Interneuron → Pyramidal Neuron Synapses of the Neocortex

Joseph Trettel and Eric S. Levine
Department of Pharmacology and Program in Neuroscience, University of Connecticut Health Center, Farmington, Connecticut 06030

Submitted 15 November 2002; accepted in final form 22 December 2002

Trettel, Joseph and Eric S. Levine. Endocannabinoids mediate rapid retrograde signaling at interneuron → pyramidal neuron synapses of the neocortex. J Neurophysiol 89: 2334–2338, 2003; 10.1152/jn.01037.2002. In the neocortex, inhibitory interneurons tightly regulate the firing patterns and integrative properties of pyramidal neurons (PNs). The endocannabinoid system of the neocortex may play an important role in the activity-dependent regulation of inhibitory (i.e., GABAergic) inputs received by PNs. In the present study, using whole cell recordings from layer 2/3 PNs in slices of mouse sensory cortex, we have identified a role for PN-derived endocannabinoids in the control of afferent inhibitory strength. Pairing evoked inhibitory currents with repeated epochs of postsynaptic depolarization led to a transient suppression of inhibition that was induced by a rise in postsynaptic Ca\(^{2+}\) and was expressed as a reduction in presynaptic GABA release. An antagonist (AM251) of the type-1 cannabinoid receptor blocked the depolarization-induced suppression of evoked inhibitory postsynaptic currents (eIPSCs), and the cannabinoid WIN55,212-2 reduced eIPSC amplitude and occluded suppression. The degree of WIN55,212-2-mediated inhibition of eIPSCs was strongly correlated with the magnitude of depolarization-induced suppression of the eIPSCs, suggesting that the WIN-sensitive afferents are suppressed by PN depolarization. Moreover, blocking endocannabinoid uptake with AM404 strongly modulated the kinetics and magnitude of eIPSC suppression. We conclude that the release of endocannabinoids from PNs allows for the postsynaptic control of presynaptic inhibition and could have profound consequences for the integrative properties of neocortical PNs.

The activity of neocortical pyramidal neurons (PNs) is regulated by diverse classes of GABAergic interneurons. These inhibitory neurons innervate functionally segregated domains of PNs to control action potential timing, the efficacy of excitatory inputs, and the synchronous activity of PNs (Larkum et al. 1999; Somogyi et al. 1998; Szabadics et al. 2001). Many interneurons form axosomatic contacts with PNs (Somogyi et al. 1998), and interneuron discharge rates in vivo can be very high (Mountcastle et al. 1969), suggesting that physiological regulation of this inhibition is likely essential for neocortical function. Several lines of evidence indicate that the endocannabinoid system may modulate the strength of inhibition in the neocortex. First, the type 1 cannabinoid receptor (CB1R) is abundantly expressed throughout the cortical mantle (Mailleux and Vanderhaeghen 1992) where it is mostly limited to interneurons of layers 2/3 and 6 (Egertova and Elphick 2000; Marsicano and Lutz 1999). Second, activation of CB1R reduces neocortical GABA levels (Ferraro et al. 2001) by inhibiting Ca\(^{2+}\)-dependent GABA release (Trettel and Levine 2002), consistent with the effects of CB1R agonists in other brain regions (reviewed by Schlicker and Kathmann 2001). Third, neocortical neurons synthesize the endocannabinoid arachidonylethanolamide (anandamide) (Devane et al. 1992) and display carrier-mediated uptake (Beltramo et al. 1997) and enzymatic degradation of this lipid-derived compound (Beltramo and Piomelli 2000). Last, exogenous cannabinoids have pronounced effects on neocortical function (Feldman et al. 1997). Despite this evidence, the role of endocannabinoid signaling in the neocortex is largely unexplored.

In the hippocampus and cerebellum, endocannabinoids function as retrograde messengers that mediate depolarization-induced suppression of inhibition (DSI) (reviewed by Kreitzer and Regehr 2002; Wilson and Nicoll 2002). DSI is a transient reduction in presynaptic GABA release following postsynaptic depolarization (Llano et al. 1991; Pitler and Alger 1992) that enhances neuronal excitability (Wagner and Alger 1996) by allowing target neurons to regulate the strength of afferent inhibition. Stimuli that result in voltage-dependent Ca\(^{2+}\) entry (Alger et al. 1996; Llano et al. 1991) or activation of metabotropic glutamate (mGluR) or muscarinic acetylcholine receptors (Kim et al. 2002; Maejima et al. 2001; Varma et al. 2001) are sufficient to initiate the release of endocannabinoids from hippocampal and cerebellar neurons. These molecules then diffuse retrogradely to presynaptic terminals to activate CB1R, resulting in the depression of GABA release. Such signaling mechanisms may also be essential for the physiological regulation of cortical inhibition. Therefore in the present study, we sought to determine whether depolarization of neocortical PNs triggers endocannabinoid-mediated retrograde signaling at inhibitory synapses.

To determine whether endocannabinoids act as retrograde messengers in the neocortex, we examined synaptic inhibition of layer 2/3 PNs in 250-μm-thick slices of mouse auditory and visual cortex (P12–20; Swiss-Webster, Charles River). Briefly, whole cell voltage-clamp recordings were made from PNs at 32°C, and GABA\(_{\alpha_4}\)-mediated inhibitory postsynaptic currents (IPSCs) were evoked using bipolar stimulation (25–100 μs, 100–900 μA). To assay the effects of PN depolarization, evoked IPSCs (eIPSCs) were paired with repeated, brief epochs of membrane depolarization (pairing protocol; see Fig. 1A). Brain slices were perfused at 1.5–2 ml/min in a submer-
sion-type chamber with normal artificial cerebrospinal fluid (ACSF) containing (in mM) 125 NaCl, 2.5 KCl, 1.25 NaH₂PO₄, 25 NaHCO₃, 2 CaCl₂, 2 MgCl₂, 17.5 glucose, 0.01 6,7-dinitroquinoxaline-2,3-dione (DNQX), and 0.002 3-((+/-)-2-carboxypiperazin-4-yl)propyl-1-phosphonate (CPP), equilibrated with 95% O₂ -5% CO₂ (pH 7.3, 305 mmol/kg).

Borosilicate glass recording pipettes had resistances of 3–5 MΩ when filled with internal solution. For BAPTA experiments, the internal solution contained (in mM) 105 CsCl, 10 HEPES, 1.5 CaCl₂, 1.5 MgCl₂, 3.5 Na₂-ATP, and 0.3 Na-GTP (pH 7.3, 285 mmol/kg). For all other experiments, the internal solution was composed of (in mM) 120 CsCl, 10 HEPES, 2 EGTA, 0.2 CaCl₂, 1.5 MgCl₂, 3.5 Na₂-ATP, and 0.3 Na-GTP (pH 7.3, 282 mmol/kg). Lidocaine N-ethyl bromide (QX-314, 5 mM) was included in the pipette solutions to block gNa. All recordings were made at –70 mV, and synaptic currents were filtered at 2.9 kHz and digitized at ≥6 kHz using a Heka EPC9/2. AM404 and AM251 were generously provided by Dr. Alex Makriyannis (University of Connecticut, Storrs, CT). Neurons were rejected from analyses if the series resistance was >30 MΩ at the time of break-in or >12 MΩ after compensation at 100 µs lag, if input resistance (Ri) changed by ≥15% during the course of an experiment, or if Ri fell <150 MΩ. Off-line analysis was carried out using PulseFit (Heka Elektronik), MiniAnalysis (Synaptosoft), and Origin (OriginLab, Northampton MA) software and significance was tested using the Student’s t-test and one-way ANOVAs. All data are presented as means ± SE.

Somatic whole cell recordings were obtained from 51 layer 2/3 PNs. At Vhold = –70 mV, stimulation near the soma produced large eIPSCs (1.73 ± 0.21 nA) that reversed polarity near EcL and were abolished by bicuculline methiodide (30 µM).
shown in Fig. 1, two pairing trials were sufficient to depress eIPSC amplitude (Fig. 1B; 90.8 ± 1.7% of baseline; \( P < 0.05; n = 10 \)), and at the end of 10 pairings, the suppression reached 63.2 ± 4.8% of baseline (Figs. 1, A and B, and 4; \( P < 0.001; n = 10 \)). There was no relationship between the magnitude of IPSC suppression and the initial size of the eIPSCs (\( P > 0.8 \)). The recovery from suppression was rapid: at 60 s following pairing, eIPSC amplitude was 90.4 ± 5.7% of baseline (\( P > 0.25 \) compared with baseline).

To determine the synaptic locus of the suppression, we first measured the paired-pulse ratio (PPR = eIPSC2/eIPSC1), PN depolarization suppressed eIPSC1, and this was paralleled by a sharp increase in the PPR from 1.11 ± 0.1 to 1.81 ± 0.1 (Fig. 2, A and B; \( P < 0.01; n = 6 \)). These data suggest that the suppression of eIPSC amplitude was associated with a reduction in the probability of presynaptic transmitter release (e.g., Zucker 1989). We also measured whole cell currents evoked by pressure ejection of GABA onto PN soma to determine if PN depolarization resulted in changes in postsynaptic GABA \(_A\) receptors. Immediately following the pairing voltage protocol, the amplitude of the GABA current was 103.5 ± 5.3% of baseline (Fig. 2C; \( P > 0.1; n = 7 \)). Therefore the most likely explanation for the observed suppression of eIPSC amplitude was that PN depolarization affected presynaptic function via retrograde signaling. To investigate the role of postsynaptic Ca\(^{2+}\) in triggering the release of a retrograde messenger, we loaded PNs with 15 mM BAPTA. Under these conditions the suppression of eIPSC amplitude was significantly attenuated (Figs. 1B and 4; 92.3 ± 6.7% of baseline; \( P < 0.001 \) compared with control; \( n = 5 \)). These data suggest that the synthesis and/or release of the retrograde signal required a rise in postsynaptic intracellular Ca\(^{2+}\), similar to the induction of retrograde signaling in other brain systems (Llano et al. 1991; Pittler and Alger 1992).

We next asked whether the retrograde signal was an endocannabinoid. Pretreatment of slices with the selective CB1R antagonist AM251 (2 \( \mu M \); \( n = 8 \)) completely abolished the pairing-induced suppression of eIPSCs (Figs. 3A and 4; 97.4 ± 1.6% of baseline; \( n = 8 \)), while having no effect on baseline eIPSC amplitude (data not shown). In addition, CB1R activation by the synthetic cannabinoid WIN55,212-2 (5 \( \mu M \)) reduced eIPSC amplitude to 54.8 ± 5.0% of baseline (Fig. 3B; \( P < 0.01; n = 6 \)) and occluded the effects of PN depolarization (Figs. 3B and 4; 95.5 ± 2.8% of baseline; \( P > 0.8 \)). The strong correlation between the magnitudes of WIN-mediated inhibition and depolarization-induced suppression of eIPSC amplitude (Fig. 3C; \( r^2 = 0.92; n = 6 \)) suggests that most if not all of the WIN-sensitive inhibitory afferents to PNs are suppressed.
by depolarization. The rapid recovery from eIPSC suppression (i.e., Fig. 1B) prompted us to explore the role of endocannabinoid uptake using a selective inhibitor of the anandamide transporter, AM404 (Beltramo et al. 1997). As shown in Fig. 3D, AM404 (25 μM) reduced the latency to peak suppression, increased the magnitude of the suppression (Fig. 4; 36.9 ± 12.0% of baseline compared with 62.7 ± 5.9% of baseline, P < 0.05; n = 5), and significantly retarded eIPSC recovery (P < 0.05; n = 5). Taken together, these data suggest that CB1R activation by endocannabinoids mediates the transient suppression of eIPScs following repeated epochs of PN depolarization.

To our knowledge, this is the first report of the cellular effects of endocannabinoid signaling in the neocortex. Two other forms of retrograde signaling at neocortical layer 2/3 synapses have been reported previously. In one type, back-propagating action potentials triggered the release of GABA from the dendrites of bipolar interneurons and suppressed glutamate release from afferent PNs via GABA<sub>A</sub> receptor activation (Zilberter et al. 1999). In the other form, glutamate released from depolarized PN dendrites acted on fast-spiking interneurons via mGlurRs to depress GABA release (Zilberter 2000). The present results show that endocannabinoids are also involved in the suppression of GABA release from neocortical interneurons. It is possible that both retrograde glutamate and endocannabinoid signaling occur at an overlapping population of layer 2/3 synapses received by PNs. Alternatively, different retrograde messengers may mediate suppression of distinct classes of interneurons. Because these interneurons innervate segregated functional domains of PNs, e.g., apical dendrites, soma/basal dendrites, and the axon initial segment (Somogyi et al. 1998), selective modulation of this compartmentalized inhibition could play a critical role in shaping the responsiveness and firing patterns of PNs.

Several observations support our conclusion that endocannabinoids mediate the suppression of inhibition following the depolarization of neocortical PNs. First, the suppression of eIPScs was completely blocked by the diarylpyrazole-type CB1R antagonist AM251. Second, the suppression was pre-synaptically expressed, similar to the effects of the cannabino-mimetic WIN55,212-2 in the neocortex (Trettel and Levine 2002). Furthermore, WIN55,212-2 occluded the suppression, suggesting a common target for both WIN55,212-2 and the endogenous retrograde signal. Third, PNs synthesize and release endocannabinoids in a Ca<sup>2+</sup>-dependent manner (Di Marzo et al. 1994; Stella et al. 1997), and the suppression of inhibition in the present study was dependent on a rise in intracellular Ca<sup>2+</sup>. Last, inhibiting endocannabinoid uptake with AM404 had a pronounced effect on eIPSC suppression, suggesting an important role for reuptake during retrograde signaling. Together, these results indicate that in the neocortex, similar to the hippocampus and cerebellum, endocannabinoids released from PNs act retrogradely to suppress GABA release from interneurons that express CB1R.

The physiological significance of endocannabinoid signaling in the neocortex may ultimately be reflected in the activity of PNs. The ability of PNs to fire bursts of action potentials is thought to play a key role in cortical information processing (Kepes et al. 2002; Lisman 1997). As associative neurons, PNs receive excitatory input over widespread, functionally segregated dendritic domains. The integration or coupling of temporally coincident distal (i.e., associative, nonspecific intracortical) and basal (i.e., specific thalamo-cortical) inputs leads to burst firing (Larkum et al. 2001) and is highly sensitive to GABAAergic inhibition (Larkum et al. 1999). Regulation of this inhibition likely plays an essential role in shaping PN dendritic integration and discharge pattern. Thus activity-dependent release of endocannabinoids from PNs could transiently suppress afferent inhibition to a degree that promotes the coupling of excitatory inputs, thereby representing a functional role for endocannabinoid-mediated DSI in the neocortex.

REFERENCES


