Published in final edited form as:

Hippocampus. 2012 February ; 22(2): 209–221. doi:10.1002/hipo.20884.

A novel non-CB1/TRPV1 endocannabinoid-mediated mechanism depresses excitatory synapses on hippocampal CA1 interneurons

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Abstract

Endocannabinoids (eCBs) mediate various forms of synaptic plasticity at excitatory and inhibitory synapses in the brain. The eCB anandamide binds to several receptors including the transient receptor potential vanilloid 1 (TRPV1) and cannabinoid receptor 1 (CB1). We recently identified that TRPV1 is required for long-term depression at excitatory synapses on hippocampal stratum radiatum interneurons. Here we performed whole-cell patch clamp recordings from CA1 stratum radiatum interneurons in rat brain slices to investigate the effect of the eCB anandamide on excitatory synapses as well the involvement of group I metabotropic glutamate receptors (mGluRs), which have been reported to produce eCBs endogenously. Application of the non-hydrolysable anandamide analogue R-methanandamide depressed excitatory transmission to CA1 stratum radiatum interneurons by approximately 50%. The group I mGluR agonist DHPG also depressed excitatory glutamatergic transmission onto interneurons to a similar degree, and this depression was blocked by the mGluR5 antagonist MPEP (10 μM) but not by the mGluR1 antagonist CPCCOEt (50 μM). Interestingly, however, neither DHPG-mediated nor R-methanandamide-mediated depression was blocked by the TRPV1 antagonist capsazepine (10 μM), the CB1 antagonist AM-251 (2 μM) or a combination of both, suggesting the presence of a novel eCB receptor or anandamide target at excitatory hippocampal synapses. DHPG also occluded R-methanandamide depression, suggesting the possibility that the two drugs elicit synaptic depression via a shared signaling mechanism. Collectively, this study illustrates a novel CB1/TRPV1-independent eCB pathway present in the hippocampus that mediates depression at excitatory synapses on CA1 stratum radiatum interneurons.

Keywords

hippocampus; plasticity; anandamide; mGluR5; THC

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Introduction

Changes in synaptic plasticity are important to the development of the CNS and are thought to be an essential cellular mechanism mediating learning and memory formation in response to experience. Long-term changes in synaptic plasticity such as long-term potentiation (Bliss and Lomo, 1973) and long-term depression (Dudek and Bear, 1992; Mulkey and Malenka, 1992) change synaptic efficacy at excitatory synapses. In the hippocampus, a brain region required for spatial and declarative memory, a major mechanism underlying LTD is the activation of group I metabotropic glutamate receptors (mGluRs). At excitatory synapses on hippocampal CA1 pyramidal cells, mGluR activation initiates LTD following application of the group I mGluR agonist, DHPG. The inhibition of excitatory transmission can result either from a postsynaptic alteration mediated by α -amino-3-hydroxyl-5-methyl-4-isoxazole-propionate receptor (AMPA) endocytosis (Huang et al., 2004; Huber et al., 2000; Snyder et al., 2001; Xiao et al., 2001) or from a presynaptic decrease in glutamate release (Bolshakov and Siegelbaum, 1994; Faas et al., 2002; Feinmark et al., 2003; Fitzjohn et al., 2001; Rammes et al., 2003; Rouach and Nicoll, 2003; Volk et al., 2006; Watabe et al., 2002; Zakharenko et al., 2002).

At excitatory synapses on CA1 pyramidal cells, the presynaptic mechanism underlying the acute phase of DHPG-induced inhibition is mediated in part by endocannabinoids (eCBs) and possibly additional retrograde signaling mechanisms (Rouach and Nicoll, 2003). Endocannabinoids are common and widespread retrograde signaling molecules in the CNS, most often produced postsynaptically and traveling retrogradely to activate different presynaptic receptors such as the cannabinoid receptor, CB1. CB1 can be activated by the eCBs anandamide, 2-arachidonylglycerol (Huang et al., 2002) and the exogenous cannabinoid ligand Δ^9 -tetrahydrocannabinol (THC), the main psychoactive component of marijuana. In the hippocampus, CB1 activation inhibits GABA release from cholecystikinin (CCK)-containing interneurons onto CA1 pyramidal cells (Hajos et al., 2001; Katona et al., 1999) and while initially CB1 activation was not thought to inhibit glutamate release at the CA3-CA1 pyramidal cell excitatory synapse, more recent findings have identified CB1-mediated depression at the CA3-CA1 pyramidal cell excitatory synapse as well (Domenici et al., 2006; Katona et al., 2006; Kawamura et al., 2006; Takahashi and Castillo, 2006). Interestingly, in DHPG-induced synaptic depression at the CA3-CA1 pyramidal cell excitatory synapse it was proposed that the acute phase of depression was mediated by eCB binding to a non-CB1 receptor (Rouach and Nicoll, 2003). A candidate non-CB1 cannabinoid receptor is transient receptor potential vanilloid 1 (TRPV1), which can also be activated by lipid signaling molecules including anandamide, *N*-arachidonyldopamine (NADA) and 12-(S)-HPETE, a lipoxygenase metabolite of arachidonic acid. Recent work has indicated that functional TRPV1 channels regulate excitatory synaptic transmission in the hippocampus (Gibson et al., 2008; Lin et al., 2008; Marsch et al., 2007). The involvement of TRPV1 channels in specifically mediating eCB-induced depression at hippocampal excitatory synapses is a possible explanation of some previous findings as well (Al-Hayani et al., 2001; Hajos and Freund, 2002b; Rouach and Nicoll, 2003). Collectively, these studies suggest that while there are types of eCB-mediated inhibition of glutamate release in the hippocampus that are CB1-dependent, others are CB1-independent that could involve TRPV1, although a single study demonstrated CB1 and TRPV1-independent inhibition of K^+ -induced glutamate release (Kofalvi et al., 2003).

An understanding of synaptic plasticity in CA1 interneurons is important because these interneurons play a critical role in the local control of pyramidal cells by feedback and feedforward inhibition (Sik et al., 1995), as well as in synchronizing or entraining pyramidal cells (Cobb et al., 1995), and generating oscillations via interconnected interneuron networks (Whittington and Traub, 2003). Synapses on interneurons exhibit forms of

plasticity that are often different from those seen at synapses on neighboring pyramidal cells (Kullmann and Lamsa, 2007; Lamsa et al., 2005; McBain et al., 1994; McMahon and Kauer, 1997; Pelkey et al., 2005; Perez et al., 2001; Topolnik et al., 2006). For example, group I mGluR activation initiates LTP rather than LTD at excitatory synapses on stratum oriens interneurons, and requires simultaneous activation of both group I mGluR subtypes, mGluR1 and mGluR5 (Le Vasseur et al., 2008; McBain et al., 1994). To date, there has been no demonstration of DHPG initiated LTD at excitatory synapses onto interneurons, as seen in CA1 pyramidal cells.

Here we report that the endocannabinoid analogue, R-methanandamide, depresses excitatory synapses on stratum radiatum interneurons in area CA1. This R-methanandamide-mediated depression is distinct from synaptically-activated LTD previously reported (Gibson et al., 2008) in these interneurons as it appears to be independent of both TRPV1 receptors and CB1 receptors. Furthermore, we find that the group I mGluR agonist, DHPG, elicits synaptic depression with a pharmacological profile similar to anandamide, suggesting that anandamide could be the endogenous ligand produced by mGluRs mediating this DHPG-induced synaptic depression. The DHPG-induced synaptic depression also differed from synaptically-activated LTD in CA1 interneurons in that it was mediated by mGluR5 rather than mGluR1 (Gibson et al., 2008). These findings emphasize that DHPG initiates LTD in CA1 stratum radiatum interneurons as in CA1 pyramidal cells, in contrast to the response of synapses on interneurons of CA1 stratum oriens (Le Vasseur et al., 2008).

In summary, our findings support what others have noted, that of general CB1-dependent or independent endocannabinoid-mediated mechanisms that inhibit excitatory glutamatergic transmission in several brain regions including the neocortex (Auclair et al., 2000), hippocampus (Domenici et al., 2006; Hajos and Freund, 2002a; Hajos and Freund, 2002b; Takahashi and Castillo, 2006) and amygdala (Pistis et al., 2004), suggesting that it is a common neuromodulatory feature of importance in normal CNS function. Our findings are the first to demonstrate a novel mechanism of this type in the CNS, being a CB1/TRPV1-independent, anandamide-dependent pathway mediating inhibition of glutamate release at excitatory synapses in the hippocampus as well as the first to show DHPG-LTD in stratum radiatum interneurons and an additional site of action for the cannabinoid THC in the hippocampus.

Experimental Procedures

Preparation of brain slices

The basic methods have been detailed previously (McMahon and Kauer, 1997; Gibson et al., 2008). Sprague-Dawley rats (15–27 days old) were deeply anaesthetized using halothane or isoflurane and quickly decapitated in accordance with the Brown University Institutional Animal Care and Use Committee guidelines. The brain was rapidly removed and 300–350 μm thick coronal slices were prepared and stored for at least one hour submerged on a net in artificial cerebrospinal fluid (ACSF) containing in mM: 119 NaCl, 26 NaHCO₃, 2.5 KCl, 1.0 NaH₂PO₄, 2.5 CaCl₂, 1.3 MgSO₄ and 11 dextrose, saturated with 95% O₂/5% CO₂ (pH 7.4). A surgical cut was made between the CA3 and CA1 regions.

Electrophysiological recordings from interneurons

After 1 hour, slices were transferred to a submerged recording chamber and bathed in oxygenated ACSF (28–32° C) containing elevated divalent cations to reduce epileptiform activity (4 mM CaCl₂ and 4 mM MgCl₂, replacing MgSO₄). Slices were continuously perfused with ACSF at a flow rate of 1–2 ml/min. Picrotoxin (100 μM) and D-AP5 (50 μM) were added to block GABA_A receptor- and NMDAR-mediated synaptic transmission.

Whole-cell patch clamp recordings were made from interneurons identified visually in the CA1 stratum radiatum of the hippocampus. No specific cell morphology was targeted, although we avoided recording from cells with the “giant cell” morphology as these have been reported to be glutamatergic interneurons (Gulyas et al., 1998). Input resistance and other electrophysiological properties were used to ensure recordings were from the tightly ‘space clamped’ interneurons. Patch pipettes were filled with internal recording solution containing in mM: 117 cesium gluconate, 2.8 NaCl, 5 MgCl₂, 20 HEPES, 2 ATP-Na⁺, 0.3 GTP-Na⁺ and 0.6 EGTA. Excitatory postsynaptic currents (EPSCs) were stimulated at 0.1 Hz (100 μsec) using a bipolar stainless steel stimulating electrode placed in stratum radiatum at least 200 μm from the recorded cell. Interneurons were voltage clamped at -65 mV (not corrected for the liquid junction potential, of ~10 mV), and EPSCs were evoked by paired pulses with an interval of 50 msec (stimulus intensity typically 50–400 μA). Receptor antagonists were added directly to the ACSF at known concentrations for at least 10 minutes prior to agonist application. Control experiments were interleaved with all others. The cell input resistance and series resistance were monitored throughout each experiment; cells were discarded if these values changed by more than 10% during the experiment. EPSCs were amplified using an AxoClamp 2B amplifier (Axon instruments) and Brownlee Precision Model 410 post-amplifier (AutoMate Scientific), low-pass filtered at 3 kHz and digitally sampled to a PC at 30 kHz using an analogue to digital interface (National Instruments). Field recordings were performed similar to whole cell recordings, except that 2 M NaCl was used in the field electrode, and an axopatch 200B amplifier was used in current clamp mode to measure excitatory postsynaptic potentials (EPSPs).

Analysis

The peak amplitude of each EPSC was measured by comparing a 10 msec time period immediately prior to the stimulus with the peak of the EPSC using a LabVIEW-based program (National Instruments) or pClamp10 (Axon Instruments) in whole cell recordings. If field experiments the slope was measured and reported. Occasionally polysynaptic responses were evoked, and in these cases, only the initial monosynaptic event was measured. To calculate the effects of DHPG, R-methanandamide and THC application on basal excitatory glutamatergic transmission, normalized EPSC amplitudes were averaged in the final 5 minutes of drug application after at least 10 minutes exposure to drug, and compared with averaged EPSCs 5 minutes prior to drug application using a t-test (unpaired, two-tailed, with Welch’s correction if the variances between the groups were unequal; GraphPad Prism, Version 4). The *n*-values reported refer to the number of slices and not number of animals, with only one individual experiment carried out per slice. All combined data are expressed as mean ± the standard error of the mean (SEM). All results reported in this study were significant to at least $P < 0.05$. Paired-pulse ratios (PPR; EPSC2/EPSC1) and coefficient of variation ($1/CV^2$) were calculated within 5 minute epochs of 30 EPSCs each, starting 5 minutes immediately before addition of drug. The PPR was calculated by dividing the mean of all 30 EPSC2 amplitudes by the mean of all 30 corresponding EPSC1 amplitudes within each epoch. $1/CV^2$ was determined by dividing the squared mean amplitude of 30 EPSCs within 5 minute epochs by the variance of these EPSC amplitudes. For statistical analysis of significance of the changes in non-normalized values of $1/CV^2$ and PPR, distribution-free, non-parametric inferential statistics were used (Wilcoxon Matched-Pairs Signed-Ranks Test) to assess these values obtained from the same cell before and after drug application with a significance level of $P < 0.05$. Non-parametric statistics were used since the response values did not meet assumptions of normality (D’Agostino-Pearson omnibus test) and homogeneity of variance (Bartlett’s test).

Materials

AM-251, capsazepine, CPCCOEt, D-AP5, DHPG, MPEP and R-methanandamide were obtained from Tocris Bioscience. SR141716A was generously provided by NIDA, and Δ^9 -THC (THC) was obtained from the Brown University Institutional Animal Care and Use Committee. All other chemicals were purchased from Sigma-Aldrich. THC was supplied in EtOH (159 mM) and then diluted to its final concentration in ACSF. AM-251, capsazepine, CPCCOEt, MPEP and SR141716A were dissolved in DMSO and then diluted at least 1:1000 to the final concentration in ACSF. DMSO and ethanol were determined previously to not effect synaptic transmission in our preparation (Gibson et al., 2008).

Results

We recently reported that high-frequency stimulation (HFS) of the Schaffer collateral pathway elicits long-term depression (LTD) of excitatory synapses on CA1 stratum radiatum interneurons that requires postsynaptic activation of mGluR1 (Gibson et al., 2008). Activation of mGluR1 promotes the formation of the arachidonic acid metabolite, 12-(S)-HPETE, which most likely travels retrogradely to activate presynaptic TRPV1 receptors, persistently depressing glutamate release. Another endogenous activator of TRPV1 is the endocannabinoid, anandamide. Recording from the same synapses, we therefore tested the effects of R-methanandamide (50 μ M), an anandamide analogue that is not broken down by fatty acid amide hydrolase. Application of R-methanandamide depressed EPSCs recorded in all but two stratum radiatum interneurons by an average of 50%, (Figure 1A) with no change in the holding current, similar in magnitude to the depression observed during synaptically-elicited LTD or elicited by the TRPV1 channel agonist 12-(S)-HPETE (Gibson et al., 2008). Interestingly, R-methanandamide had no depressing effect on synapses on pyramidal cells (figure 1B) as measured with field excitatory postsynaptic potentials (EPSPs) in the stratum radiatum, suggesting the R-methanandamide mediated depression is specific for the CA3-interneuron, but not CA3-CA1 pyramidal cell synapse. To investigate whether R-methanandamide acts presynaptically to inhibit glutamate release, we quantified paired pulse ratios (PPRs) and the coefficient of variance (CV) of evoked EPSCs, which can both be indicators of altered presynaptic function. We found both a significant ($P < 0.05$) decrease in $1/CV^2$ and a significant ($P < 0.05$) increase in PPR, both of which can be accounted for by a persistent reduction in transmitter release (Figure 1C&D). To confirm our hypothesis that the synaptic depression by R-methanandamide was in fact mediated by TRPV1 activation, we next tested its effects in the presence of the TRPV1 antagonist capsazepine (10 μ M). Unexpectedly, capsazepine was entirely ineffective at blocking the synaptic depression produced by R-methanandamide (Figure 2A), even though this concentration of capsazepine is sufficient to block effects of another endogenous TRPV1 agonist, 12-(S)-HPETE, under the same experimental conditions (Gibson et al., 2008). R-methanandamide also activates CB1 receptors, and our previous work demonstrated that activation of CB1 receptors can also depress the excitatory synapse on CA1 stratum radiatum interneurons (Gibson et al., 2008). In order to explore the involvement of CB1 receptors, we examined whether R-methanandamide-mediated depression was blocked by the CB1 receptor antagonists, SR141716A (1 μ M) or AM-251 (2 μ M), all at concentrations shown previously to be effective in this preparation (Gibson et al., 2008). Again, however, neither of these drugs blocked the synaptic depression elicited by R-methanandamide (Figure 2B & C). Even the combination of AM-251 and capsazepine failed to prevent R-methanandamide-mediated synaptic depression (Figure 2D), indicating that R-methanandamide activates a target that is neither the TRPV1 nor the CB1 receptor.

Anandamide can be produced by activation of mGluRs, and brief exposure to the mGluR1/5 agonist, DHPG, triggers LTD at synapses on CA1 pyramidal cells. We therefore next asked

whether activation of group I mGluRs with DHPG can elicit synaptic depression at excitatory synapses on interneurons. Application of DHPG to hippocampal slices did indeed depress these synapses in all cells but one, again with no significant change in the holding current (Figure 3A). The PPRs were increased and coefficient of variance were significantly ($P < 0.05$) decreased, as expected for a presynaptic decrease in neurotransmitter release (Figure 3B & C).

Again, given that activation of group I mGluRs by DHPG can result in the production of lipid signaling molecules including eCBs (Varma et al., 2001) that bind to CB1 and TRPV1 receptors, we next investigated the involvement of these receptors in DHPG-mediated synaptic depression. Similarly to what we found with R-methanandamide, neither the TRPV1 specific antagonist capsazepine (10 μ M), the CB1/TRPV1 antagonist SR141716A (2 μ M) (Gibson et al., 2008), the CB1-specific antagonist AM-251 (2 μ M), nor a combination of AM-251 and capsazepine significantly blocked DHPG-mediated depression (Figure 3D–G). DHPG can activate both mGluR1 and mGluR5, so we used antagonists of each receptor subtype to determine which triggered the DHPG-mediated depression at excitatory interneuron synapses. Whereas in the presence of the mGluR1 antagonist CPCCOEt, DHPG still elicited robust depression (Figure 4A), the mGluR5 antagonist, MPEP, entirely blocked the DHPG-induced depression (Figure 4B). Together these data suggest that mGluR5 activation triggers synaptic depression that is not mediated by either TRPV1 or CB1, and furthermore shares properties with the synaptic depression elicited by R-methanandamide. If DHPG and R-methanandamide act via a common signaling pathway, pre-exposure to one drug would be expected to prevent or occlude further synaptic depression by the other drug. Consistent with this idea, following synaptic depression elicited by DHPG, no further depression of EPSCs was evoked by R-methanandamide (Figure 4C).

Paired pulse ratio and coefficient of variance data from R-methanandamide and DHPG suggest that depression is mediated presynaptically (at least at its final step). We anticipate that postsynaptic mGluR5 activation stimulates production of an EC, possibly anandamide, which then activates a presynaptic receptor decreasing transmitter release. This is analogous to iLTD that we reported previously (Gibson et. al., 2008), mediated by postsynaptic mGluR1 activation followed by presynaptic TRPV1 activation. To investigate this possibility we performed experiments with R-methanandamide and DHPG in the presence of GDP β S (500 μ M) in our internal solution to block postsynaptic G protein coupled receptors. Previously we reported that a similar concentration of GDP β S (250 μ M) was sufficient to significantly block interneuron LTD (Gibson et. al., 2008), serving as a positive control. Intracellular GDP β S did not significantly alter R- methanandamide-mediated depression. This result suggests that R-methanandamide is not likely acting through a postsynaptic G-protein coupled receptor (figure 5A), and is consistent with R-methanandamide acting directly at a presynaptic receptor. Interestingly, in the presence of intracellular GDP β S, DHPG-induced depression was also reversible, which was not observed when GDP β S was absent (see figure 3A). These results suggest that postsynaptic mGluR5 within the recorded interneurons is essential for maintaining depression (figure 5B).

We have identified a signaling pathway that does not involve either CB1 or TRPV1 receptors, yet is activated by mGluR5 or R-methanandamide. We were curious whether or not this CB1/TRPV1-independent pathway was also activated in response to THC, the active component in marijuana. We found that indeed THC (1 μ M) resulted in a depression of this synapse; however the magnitude of depression was small and more variable than with either R-methanandamide or DHPG (Figure 6A). Furthermore, unlike DHPG or R-methanandamide, depression elicited with THC was entirely blocked by the CB1 receptor

antagonist, AM-251 (Figure 6B). This is the first demonstration that THC depresses excitatory synapses in hippocampal slices.

Discussion

Collectively, our data suggest that in CA1 stratum radiatum interneurons, synaptic depression induced by mGluR5 activation involving a CB1/TRPV1-independent mechanism, is potentially activated endogenously by the eCB anandamide or by a parallel signaling pathway activated by anandamide. This modulatory pathway is distinct from that underlying the synaptically-induced LTD at the same synapses, mediated by presynaptic TRPV1 receptors activated downstream from mGluR1 activation (Gibson et al., 2008).

Is there an analogous CB1 and TRPV1-independent eCB signaling mechanism at excitatory synapses on CA1 pyramidal cells? It has been reported in CA1 pyramidal cells that there is at least a similar CB1-independent, cannabinoid-mediated pathway activated by DHPG or cannabinoid agonists, though it appears it may be TRPV1-dependent. While recording from CA1 pyramidal cells in hippocampal slices, the acute, but not long-term depression induced by DHPG was partially mediated via eCB signaling, since SR141716A (the TRPV1/CB1 antagonist) somewhat attenuated this acute depression. These data suggest either that TRPV1 and/or CB1 receptors may be involved (Rouach and Nicoll, 2003). Several other studies demonstrated a CB1-independent activated eCB pathway in the hippocampus with some discrepancies regarding TRPV1 involvement (Hajos and Freund, 2002a; Kofalvi et al., 2003). It has also been reported that the CB1/TRPV1 antagonist, SR141716A, blocked anandamide-mediated depression in CA1 field potential recordings, thus suggesting TRPV1 and/or CB1 receptor involvement in this anandamide pathway (Ameri et al., 1999). Overall it appears that the CB1/TRPV1-independent mGluR5/anandamide-depression mechanism we report here at the CA3 pyramidal cell-CA1 stratum radiatum interneuron synapse may not be present at the excitatory synapses on CA1 pyramidal cells. Interestingly, a completely CB1/TRPV1-independent pathway, also activated by anandamide, has recently been reported in trigeminal ganglion sensory neurons (Roberts et al., 2008), suggesting that a similar pathway may operate in other CNS/PNS regions.

Anandamide

The synaptic depression caused by DHPG occluded depression by R-methanandamide; that is, once the synapses were depressed by DHPG, R-methanandamide had no further depressant effect. Moreover, both DHPG-induced depression and R-methanandamide-induced depression shared a similar pharmacological profile and both appeared to result from a decrease in presynaptic glutamate release as indicated by PPRs and coefficient of variance data. A presynaptic site of R-methanandamide action and/or a non-postsynaptic G-protein coupled receptor as the anandamide target is also supported by the fact that its depression is not blocked by intracellularly applied GDP β S. It is difficult to say whether R-methanandamide induces true LTD as it is very lipophilic and is likely difficult to washout during the length of time that we could record from the interneurons. The most likely explanation for these findings is that anandamide is the endogenous activator of this CB1/TRPV1-independent pathway, synthesized downstream from mGluR5 activation, which does so by activating a previously unidentified receptor or signaling pathway that depresses glutamate release. While the process of anandamide production is currently under study, mGluR-mediated production of eCBs is often observed (Chevalere and Castillo, 2003; Ohno-Shosaku et al., 2002; Varma et al., 2001). In addition, intracellular calcium and PLC activation are important for anandamide production (Liu et al., 2006; Placzek et al., 2008a; Placzek et al., 2008b), which are increased in response to mGluR5 activation. Therefore, mGluR5 mediated production of anandamide appears to be a good possibility. Because mGluR5 is expressed on CA1 pyramidal cells (Mannaioni et al., 2001; Rae and Irving,

2004) and some CA1 stratum radiatum interneurons (Romano et al., 1995) we cannot be sure which population of mGluR5 receptors is required for the depression of interneuron excitatory transmission reported here and for anandamide production. However, in the presence of GDP β S, the reversal of DHPG-mediated depression suggests an important contribution from mGluR5 receptors on the recorded cell for long-term, but not necessarily short-term depression.

Interestingly, given that anandamide activates both TRPV1 and CB1 receptors, our work suggests that there is also a third target anandamide can activate. It is striking that the anandamide-mediated depression is not even partially blocked by TRPV1 and CB1 antagonists (see figure 2D) despite the fact that it can bind to both receptors and both receptors are present at the CA3-CA1 interneuron synapse. This might be due to the fact that anandamide maximally depresses synaptic transmission by activating this third receptor/target alone, independent of CB1 or TRPV1, stimulating a signaling pathway common among them all. Thus, TRPV1 or CB1 receptor activation produces no further depression. Alternatively, the action of anandamide on voltage-gated ion channels or other targets could be required (Di Marzo et al., 2001; Maccarrone et al., 2008; Meuth et al., 2006).

Metabotropic Glutamate Receptor Plasticity

This is the first report that synapses on CA1 interneurons undergo DHPG-LTD induced by group I mGluRs similar to that reported at synapses on CA1 pyramidal cells. Furthermore, mGluR5 is thought to induce presynaptically-mediated DHPG-LTD in CA1 pyramidal cells (Feinmark et al., 2003), while mGluR1 is thought to be required for postsynaptic DHPG-induced LTD expression and the AMPA-receptor endocytosis associated with it (Volk et al., 2006). Therefore, our finding that CA1 interneuron DHPG-LTD reported here requires mGluR5, but not mGluR1, correlates well with mGluR5 in this report being involved in a form of presynaptic depression. The depression of interneuron excitatory input by mGluR5 activity could potentially be important for hippocampal output because mGluR5 has been reported to strongly regulate hippocampal spatial memory and plasticity of pyramidal cells (Manahan-Vaughan and Braunewell, 2005) and the site of mGluR5 involved, whether in interneurons or pyramidal cells that both express mGluR5, is still not known.

Is there a similar mGluR5-induced pathway modulating excitatory synaptic transmission to other hippocampal interneurons? In CA3 interneurons it was demonstrated previously that at synapses on hilar interneurons in the dentate gyrus, DHPG also decreased the evoked EPSC amplitude, although in these neurons the DHPG-induced synaptic depression appeared reversible, suggesting it was a different mechanism (Doherty and Dingledine, 1998). In CA1 stratum oriens interneurons it is interesting to note that both group I mGluRs (mGluR1 and mGluR5) are required for LTP and do not induce LTD (Le Vasseur et al., 2008) in contrast to our results in stratum radiatum interneurons, suggesting a significant difference in mGluR transduction or downstream effectors at synapses on interneurons in distinct layers of area CA1.

Presynaptic Depression

Many studies in CA1 pyramidal cells have noted that mGluR-LTD initiated by postsynaptic mGluRs or anandamide-mediated depression are maintained by presynaptic depression of glutamate release (Ameri et al., 1999; Feinmark et al., 2003; Fitzjohn et al., 2001; Oliet et al., 1997; Rammes et al., 2003; Watabe et al., 2002; Zakharenko et al., 2002), although postsynaptically maintained LTD is also widely reported (Huang et al., 2004; Huber et al., 2000; Snyder et al., 2001; Xiao et al., 2001). Our data, including PPR and coefficient of variance, suggest that in CA1 stratum radiatum interneurons, mGluR-LTD and anandamide-mediated depression are caused by decreased presynaptic glutamate release. Although

postsynaptic AMPA receptor endocytosis can also decrease the CV, PPR changes are most easily explained by a decrease in presynaptic glutamate release. We also noted no significant change in holding current in any of the included experiments suggesting this pathway if activated by a receptor is not expressed within the interneurons. We did not record holding currents in the presence of anandamide in CA3 pyramidal cells. It is possible there is a current response in the somatodendritic compartment, but such a response would not guarantee a similar response in the nerve terminals, and the converse is also true. It is possible that the relevant receptors are targeted specifically to nerve terminals on interneurons, as has been described for mGluR7 receptors in area CA3 (Pelkey et al., 2005; Pelkey and McBain, 2008), and as we observed with TRPV1 receptors located presynaptically at the CA3-interneuron synapse, but surprisingly not at the CA3-CA1 pyramidal cells synapse (Gibson et al., 2008).

Our data correlates with that of others and suggests that the relevant mGluR5 receptors are located on postsynaptic cells in the region, since mGluR5 is expressed in CA1 pyramidal cells and hippocampal interneurons, and produces a retrograde messenger that diffuses and acts presynaptically to decrease transmitter release. We cannot, however, rule out the possibility that the mGluR5 receptors themselves could be located presynaptically and induce depression directly. However, mGluR5 has never been reported to be expressed presynaptically in the hippocampus by others and our GDP β S data in the presence of DHPG suggest some postsynaptic mGluR5 receptors are at least involved to some degree. Interestingly, the initial depression of DHPG in the presence of GDP β S could be the result of production and diffusion of lipophilic anandamide from other nearby interneurons and pyramidal cells, because they also express mGluR5, which then depresses the excitatory synapses onto the interneurons we are recording from. Hippocampal astrocytes also express mGluR5 (Schools and Kimelberg, 1999; Servitja et al., 1999) and thus could be involved in producing anandamide as well. This reversal, not noted in the absence of GDP β S, suggests that activation of mGluR5 within the recorded interneuron is required for continuous mGluR5 activation and production of anandamide. One hypothesis that could account for our results is that mGluR5 activation leads to anandamide production that must be maintained to continually depress glutamate release. The lack of a washout effect with R-methanandamide in the presence of GDP β S suggests that anandamide does not likely target a postsynaptic G protein receptor, but likely has a presynaptic target.

CB1 Receptors

We recently identified that WIN55,212-2 caused a depression of glutamate release at excitatory synapses on stratum radiatum interneurons (Gibson et al., 2008), suggesting for the first time that CB1 receptors are present at the CA3 pyramidal cell-CA1 interneuron synapse. Previously, CB1 expression in the CA1 region was shown to occur at inhibitory synapses (Hajos and Freund, 2002a; Katona et al., 1999; Wilson and Nicoll, 2001), while more recently others have identified CB1 activity at the CA3-CA1 pyramidal cell synapse as well (Domenici et al., 2006; Katona et al., 2006; Kawamura et al., 2006; Takahashi and Castillo, 2006). The ability of THC to also induce depression of EPSCs at the CA3-CA1 interneuron synapse via CB1 supports our prior result and suggests that drugs of abuse can alter plasticity or activity levels at this synapse as well. While data regarding the THC effect at excitatory synapses in culture has been mixed (Kelley and Thayer, 2004; Straiker and Mackie, 2005), our finding demonstrates an additional site of action for the active component in marijuana. Since interneurons are involved in regulating network activity (Cobb et al., 1995; Traub et al., 2005; Whittington and Traub, 2003) and because cannabinoids alter hippocampal and neocortical network activity (Ameri, 1999; Wilson and Nicoll, 2002), which affect cognitive functions such as short-term memory formation, it is

reasonable to suggest that alterations at excitatory synapses on hippocampal interneurons may contribute to the cognitive impairment in humans caused by marijuana.

A CB1/TRPV1-independent eCB Receptor?

The mGluR5 activated CB1/TRPV1-independent pathway we report here appears to be unique to synapses on hippocampal CA1 interneurons. While a somewhat similar mechanism could exist at synapses on CA1 pyramidal cells, it appears less likely. This is rather surprising, as the major excitatory input to both cell classes in area CA1 is thought to be the Shaffer collaterals originating from CA3 pyramidal cells.

So what site is anandamide acting on? Some have claimed the existence of a yet to be cloned G-protein, non-CB1 receptor sometimes called CB3 (Hajos et al., 2001), and anandamide could be working at this site. Other possible anandamide targets include ion channels such as voltage-gated calcium, sodium and potassium channels known to be activated by anandamide (Chemin et al., 2001; Kim et al., 2005; Poling et al., 1996), or perhaps a non-TRPV1 TRP channel. While we currently do not know the molecular identity of this CB1/TRPV1-independent, anandamide activated pathway, a similar anandamide-activated pathway was described in sensory neurons of the PNS, and while they also could not determine the receptor involved, it appears that it does not require G proteins (Roberts et al., 2008).

Conclusions

A better understanding of the mechanism of interneuron eCB-mediated depression reported here is likely important considering that altering interneuron activity regulates pyramidal cell activity beyond the local area where eCB diffusion can occur. This is because of the extensive interneuron axonal arbors that spread beyond the eCB area of release (Kreitzer et al., 2002) and the potent effects on bursting caused by transient depression of excitatory input to pyramidal cells by interneurons (Traub et al., 2005). Interestingly, endocannabinoid-mediated mechanisms that inhibit excitatory glutamatergic transmission, which are CB1-dependent or independent, appear to be a common neuromodulatory feature in CNS (Breivogel et al., 2001; Di Marzo et al., 2000) including the neocortex (Auclair et al., 2000), hippocampus (Domenici et al., 2006; Hajos and Freund, 2002a; Hajos and Freund, 2002b; Takahashi and Castillo, 2006) and amygdala (Pistis et al., 2004), as well as sensory ganglia (Roberts et al., 2008), suggesting that it is an important component in normal PNS/CNS function. How the pathway we report here is activated *in vivo* is still unknown. Based on our current data it is apparently not activated by our HFS protocol, which was identified to induce depression via TRPV1 at this synapse (Gibson et al., 2008). It is possible that activation occurs in response to changes in mGluR5 expression that occur, for example, following ischemia, or alternatively that presynaptic TRPV1 when activated by HFS could be canceling out this presynaptic anandamide-induced pathway. An understanding of the signaling pathway of TRPV1 and this anandamide-induced depression will likely be required in order to fully understand the *in vivo* activation of this pathway. In conclusion, our findings are the first to demonstrate this novel anandamide-dependent pathway mediating inhibition of glutamate release at excitatory synapses in the hippocampus, though the pathway mediating depression remains elusive to identify at this point. This is also the first report demonstrating DHPG-LTD in stratum radiatum interneurons and an additional site of action for the cannabinoid THC in the hippocampus, demonstrating depression of EPSCs.

Acknowledgments

The authors thank Dr. Kevin Gormley at NIDA for providing SR141716A. This work was supported by National Institutes of Health grants DA11289, NS050570 (JK) and NS049779 (JE), and a BYU MEG grant (JE).

Reference List

- Al-Hayani A, Wease KN, Ross RA, Pertwee RG, Davies SN. The endogenous cannabinoid anandamide activates vanilloid receptors in the rat hippocampal slice. *Neuropharmacology*. 2001; 41(8):1000–1005. [PubMed: 11747904]
- Ameri A. The effects of cannabinoids on the brain. *Progress in Neurobiology*. 1999; 58(4):315–348. [PubMed: 10368032]
- Ameri A, Wilhelm A, Simmet T. Effects of the endogenous cannabinoid, anandamide, on neuronal activity in rat hippocampal slices. *Br J Pharmacol*. 1999; 126(8):1831–1839. [PubMed: 10372827]
- Auclair N, Otani S, Soubrie P, Crepel F. Cannabinoids Modulate Synaptic Strength and Plasticity at Glutamatergic Synapses of Rat Prefrontal Cortex Pyramidal Neurons. *J Neurophysiol*. 2000; 83(6):3287–3293. [PubMed: 10848548]
- Bliss TVP, Lomo T. Long-lasting potentiation of synaptic transmission in the dentate area of the anaesthetized rabbit following stimulation of the perforant path. *J Physiol*. 1973; 232(2):331–356. [PubMed: 4727084]
- Bolshakov V, Siegelbaum S. Postsynaptic induction and presynaptic expression of hippocampal long-term depression. *Science*. 1994; 264(5162):1148–1152. [PubMed: 7909958]
- Breivogel CS, Griffin G, Di Marzo V, Martin BR. Evidence for a New G Protein-Coupled Cannabinoid Receptor in Mouse Brain. *Mol Pharmacol*. 2001; 60(1):155–163. [PubMed: 11408610]
- Chemin J, Monteil A, Perez-Reyes E, Nargeot J, Lory P. Direct inhibition of T-type calcium channels by the endogenous cannabinoid anandamide. *The EMBO Journal*. 2001; 20:7033–7040. [PubMed: 11742980]
- Chevalere V, Castillo PE. Heterosynaptic LTD of Hippocampal GABAergic Synapses: A Novel Role of Endocannabinoids in Regulating Excitability. *Neuron*. 2003; 38(3):461–472. [PubMed: 12741992]
- Cobb SR, Buhl EH, Halasy K, Paulsen O, Somogyi P. Synchronization of neuronal activity in hippocampus by individual GABAergic interneurons. *Nature*. 1995; 378(6552):75–78. [PubMed: 7477292]
- Di Marzo V, Bisogno T, De Petrocellis L. Anandamide: some like it hot. *Trends in Pharmacological Sciences*. 2001; 22(7):346–349. [PubMed: 11431028]
- Di Marzo V, Breivogel CS, Tao Q, Bridgen DT, Razdan RK, Zimmer AM, Zimmer A, Martin BR. Levels, Metabolism, and Pharmacological Activity of Anandamide in CB1 Cannabinoid Receptor Knockout Mice. Evidence for Non-CB1, Non-CB2 Receptor-Mediated Actions of Anandamide in Mouse Brain. *Journal of Neurochemistry*. 2000; 75(6):2434–2444. [PubMed: 11080195]
- Doherty J, Dingledine R. Differential Regulation of Synaptic Inputs to Dentate Hilar Border Interneurons by Metabotropic Glutamate Receptors. *J Neurophysiol*. 1998; 79(6):2903–2910. [PubMed: 9636096]
- Domenici MR, Azad SC, Marsicano G, Schierloh A, Wotjak CT, Dodt H-U, Zieglansberger W, Lutz B, Rammes G. Cannabinoid Receptor Type 1 Located on Presynaptic Terminals of Principal Neurons in the Forebrain Controls Glutamatergic Synaptic Transmission. *J Neurosci*. 2006; 26(21):5794–5799. [PubMed: 16723537]
- Dudek SM, Bear MF. Homosynaptic Long-Term Depression in Area CA1 of Hippocampus and Effects of N-Methyl-D-Aspartate Receptor Blockade. *Proceedings of the National Academy of Sciences*. 1992; 89(10):4363–4367.
- Faas GC, Adwanikar H, Gereau RWIV, Saggau P. Modulation of Presynaptic Calcium Transients by Metabotropic Glutamate Receptor Activation: A Differential Role in Acute Depression of Synaptic Transmission and Long-Term Depression. *J Neurosci*. 2002; 22(16):6885–6890. [PubMed: 12177186]

- Feinmark SJ, Begum R, Tsvetkov E, Goussakov I, Funk CD, Siegelbaum SA, Bolshakov VY. 12-Lipoxygenase Metabolites of Arachidonic Acid Mediate Metabotropic Glutamate Receptor-Dependent Long-Term Depression at Hippocampal CA3-CA1 Synapses. *J Neurosci.* 2003; 23(36): 11427–11435. [PubMed: 14673007]
- Fitzjohn SM, Palmer MJ, May JER, Neeson A, Morris SAC, Collingridge GL. A characterisation of long-term depression induced by metabotropic glutamate receptor activation in the rat hippocampus in vitro. *Journal of Physiology.* 2001; 537:421–430. [PubMed: 11731575]
- Gibson HE, Edwards JG, Page RS, Van Hook MJ, Kauer JA. TRPV1 Channels Mediate Long-Term Depression at Synapses on Hippocampal Interneurons. *Neuron.* 2008; 57(5):746–759. [PubMed: 18341994]
- Hajos N, Freund TF. Distinct cannabinoid sensitive receptors regulate hippocampal excitation and inhibition. *Chemistry and Physics of Lipids.* 2002a; 121(1–2):73–82. [PubMed: 12505692]
- Hajos N, Freund TF. Pharmacological separation of cannabinoid sensitive receptors on hippocampal excitatory and inhibitory fibers. *Neuropharmacology.* 2002b; 43(4):503–510. [PubMed: 12367597]
- Hajos N, Ledent C, Freund TF. Novel cannabinoid-sensitive receptor mediates inhibition of glutamatergic synaptic transmission in the hippocampus. *Neuroscience.* 2001; 106(1):1–4. [PubMed: 11564411]
- Huang C-C, You J-L, Wu M-Y, Hsu K-S. Rap1-induced p38 Mitogen-activated Protein Kinase Activation Facilitates AMPA Receptor Trafficking via the GDI{middle dot}Rab5 Complex: POTENTIAL ROLE IN (S)-3,5-DIHYDROXYPHENYLGLYCINE-INDUCED LONG TERM DEPRESSION. *J Biol Chem.* 2004; 279(13):12286–12292. [PubMed: 14709549]
- Huang SM, Bisogno T, Trevisani M, Al-Hayani A, De Petrocellis L, Fezza F, Tognetto M, Petros TJ, Krey JF, Chu CJ, et al. An endogenous capsaicin-like substance with high potency at recombinant and native vanilloid VR1 receptors. *Proc Natl Acad Sci U S A.* 2002; 99(12):8400–5. [PubMed: 12060783]
- Huber KM, Kayser MS, Bear MF. Role for Rapid Dendritic Protein Synthesis in Hippocampal mGluR-Dependent Long-Term Depression. *Science.* 2000; 288(5469):1254–1256. [PubMed: 10818003]
- Katona I, Sperlagh B, Sik A, Kafalvi A, Vizi ES, Mackie K, Freund TF. Presynaptically Located CB1 Cannabinoid Receptors Regulate GABA Release from Axon Terminals of Specific Hippocampal Interneurons. *J Neurosci.* 1999; 19(11):4544–4558. [PubMed: 10341254]
- Katona I, Urban GM, Wallace M, Ledent C, Jung K-M, Piomelli D, Mackie K, Freund TF. Molecular Composition of the Endocannabinoid System at Glutamatergic Synapses. *J Neurosci.* 2006; 26(21):5628–5637. [PubMed: 16723519]
- Kawamura Y, Fukaya M, Maejima T, Yoshida T, Miura E, Watanabe M, Ohno-Shosaku T, Kano M. The CB1 Cannabinoid Receptor Is the Major Cannabinoid Receptor at Excitatory Presynaptic Sites in the Hippocampus and Cerebellum. *J Neurosci.* 2006; 26(11):2991–3001. [PubMed: 16540577]
- Kelley BG, Thayer SA. [Delta]9-Tetrahydrocannabinol antagonizes endocannabinoid modulation of synaptic transmission between hippocampal neurons in culture. *Neuropharmacology.* 2004; 46(5): 709–715. [PubMed: 14996548]
- Kim HI, Kim TH, Shin YK, Lee CS, Park M, Song J-H. Anandamide suppression of Na⁺ currents in rat dorsal root ganglion neurons. *Brain Research.* 2005; 1062(1–2):39–47. [PubMed: 16256960]
- Kofalvi A, Vizi ES, Ledent C, Sperlagh B. Cannabinoids inhibit the release of [3H]glutamate from rodent hippocampal synaptosomes via a novel CB1 receptor-independent action. *European Journal of Neuroscience.* 2003; 18(7):1973–1978. [PubMed: 14622229]
- Kreitzer AC, Carter AG, Regehr WG. Inhibition of Interneuron Firing Extends the Spread of Endocannabinoid Signaling in the Cerebellum. *Neuron.* 2002; 34(5):787–796. [PubMed: 12062024]
- Kullmann DM, Lamsa KP. Long-term synaptic plasticity in hippocampal interneurons. *Nat Rev Neurosci.* 2007; 8(9):687–699. [PubMed: 17704811]
- Lamsa K, Heeroma JH, Kullmann DM. Hebbian LTP in feed-forward inhibitory interneurons and the temporal fidelity of input discrimination. *Nat Neurosci.* 2005; 8(7):916–924. [PubMed: 15937481]

- Le Vasseur M, Ran I, Lacaille JC. Selective induction of metabotropic glutamate receptor 1- and metabotropic glutamate receptor 5-dependent chemical long-term potentiation at oriens/alveus interneuron synapses of mouse hippocampus. *Neuroscience*. 2008; 151(1):28–42. [PubMed: 18035501]
- Lin MT, Lujan R, Watanabe M, Adelman JP, Maylie J. SK2 channel plasticity contributes to LTP at Schaffer collateral-CA1 synapses. *Nat Neurosci*. 2008; 11(2):170–177. [PubMed: 18204442]
- Liu J, Wang L, Harvey-White J, Osei-Hyiaman D, Razdan R, Gong Q, Chan AC, Zhou Z, Huang BX, Kim H-Y, et al. A biosynthetic pathway for anandamide. *Proceedings of the National Academy of Sciences*. 2006; 103(36):13345–13350.
- Maccarrone M, Rossi S, Bari M, De Chiara V, Fezza F, Musella A, Gasperi V, Prosperetti C, Bernardi G, Finazzi-Agro A, et al. Anandamide inhibits metabolism and physiological actions of 2-arachidonoylglycerol in the striatum. *Nat Neurosci*. 2008; 11(2):152–159. [PubMed: 18204441]
- Manahan-Vaughan D, Braunewell K-H. The Metabotropic Glutamate Receptor, mGluR5, is a Key Determinant of Good and Bad Spatial Learning Performance and Hippocampal Synaptic Plasticity. *Cereb Cortex*. 2005; 15(11):1703–1713. [PubMed: 15703249]
- Mannaioni G, Marino MJ, Valenti O, Traynelis SF, Conn PJ. Metabotropic Glutamate Receptors 1 and 5 Differentially Regulate CA1 Pyramidal Cell Function. *J Neurosci*. 2001; 21(16):5925–5934. [PubMed: 11487615]
- Marsch R, Foeller E, Rammes G, Bunck M, Kossel M, Holsboer F, Zieglgansberger W, Landgraf R, Lutz B, Wotjak CT. Reduced Anxiety, Conditioned Fear, and Hippocampal Long-Term Potentiation in Transient Receptor Potential Vanilloid Type 1 Receptor-Deficient Mice. *J Neurosci*. 2007; 27(4):832–839. [PubMed: 17251423]
- McBain CJ, DiChiara TJ, Kauer JA. Activation of metabotropic glutamate receptors differentially affects two classes of hippocampal interneurons and potentiates excitatory synaptic transmission. *J Neurosci*. 1994; 14(7):4433–4445. [PubMed: 7517996]
- McMahon LL, Kauer JA. Hippocampal Interneurons Express a Novel Form of Synaptic Plasticity. *Neuron*. 1997; 18(2):295–305. [PubMed: 9052799]
- Meuth SG, Aller MI, Munsch T, Schuhmacher T, Seidenbecher T, Meuth P, Kleinschmitz C, Pape H-C, Wiendl H, Wisden W, et al. The Contribution of TWIK-Related Acid-Sensitive K⁺-Containing Channels to the Function of Dorsal Lateral Geniculate Thalamocortical Relay Neurons. *Molecular Pharmacology*. 2006; 69(4):1468–1476. [PubMed: 16424077]
- Mulkey RM, Malenka RC. Mechanisms underlying induction of homosynaptic long-term depression in area CA1 of the hippocampus. *Neuron*. 1992; 9(5):967–975. [PubMed: 1419003]
- Ohno-Shosaku T, Shosaku J, Tsubokawa H, Kano M. Cooperative endocannabinoid production by neuronal depolarization and group I metabotropic glutamate receptor activation. *European Journal of Neuroscience*. 2002; 15(6):953–961. [PubMed: 11918654]
- Oliet SHR, Malenka RC, Nicoll RA. Two Distinct Forms of Long-Term Depression Coexist in CA1 Hippocampal Pyramidal Cells. *Neuron*. 1997; 18(6):969–982. [PubMed: 9208864]
- Pelkey KA, Lavezzari G, Racca C, Roche KW, McBain CJ. mGluR7 Is a Metaplastic Switch Controlling Bidirectional Plasticity of Feedforward Inhibition. *Neuron*. 2005; 46(1):89–102. [PubMed: 15820696]
- Pelkey KA, McBain CJ. Target-cell-dependent plasticity within the mossy fibre—CA3 circuit reveals compartmentalized regulation of presynaptic function at divergent release sites. *The Journal of Physiology*. 2008; 586(6):1495–1502. [PubMed: 18079156]
- Perez Y, Morin F, Lacaille J-C. A hebbian form of long-term potentiation dependent on mGluR1a in hippocampal inhibitory interneurons. *PNAS*. 2001; 98(16):9401–9406. [PubMed: 11447296]
- Pistis M, Perra S, Pillolla G, Melis M, Gessa GL, Muntoni AL. Cannabinoids modulate neuronal firing in the rat basolateral amygdala: evidence for CB1- and non-CB1-mediated actions. *Neuropharmacology*. 2004; 46(1):115–125. [PubMed: 14654103]
- Placzek EA, Okamoto Y, Ueda N, Barker EL. Mechanisms for recycling and biosynthesis of endogenous cannabinoids anandamide and 2-arachidonoylglycerol. *Journal of Neurochemistry*. 2008a; 107(4):987–1000. [PubMed: 18778304]

- Placzek EA, Okamoto Y, Ueda N, Barker EL. Membrane microdomains and metabolic pathways that define anandamide and 2-arachidonyl glycerol biosynthesis and breakdown. *Neuropharmacology*. 2008b; 55(7):1095–1104. [PubMed: 18760289]
- Poling JS, Rogawski MA, Salem N, Vicini S. Anandamide, an Endogenous Cannabinoid, Inhibits Shaker-related Voltage-gated K⁺ Channels. *Neuropharmacology*. 1996; 35(7):983–991. [PubMed: 8938728]
- Rae MG, Irving AJ. Both mGluR1 and mGluR5 mediate Ca²⁺ release and inward currents in hippocampal CA1 pyramidal neurons. *Neuropharmacology*. 2004; 46(8):1057–1069. [PubMed: 15111012]
- Rammes G, Palmer M, Eder M, Dodt H-U, Zieglgänsberger W, Collingridge GL. Activation of mGlu receptors induces LTD without affecting postsynaptic sensitivity of CA1 neurons in rat hippocampal slices. *The Journal of Physiology*. 2003; 546(2):455–460. [PubMed: 12527731]
- Roberts LA, Ross HR, Connor M. Methanandamide activation of a novel current in mouse trigeminal ganglion sensory neurons in vitro. *Neuropharmacology*. 2008; 54(1):172–80. [PubMed: 17631916]
- Romano C, Sesma MA, McDonald CT, O'malley K, Pol ANvd, Olney JW. Distribution of metabotropic glutamate receptor mGluR5 immunoreactivity in rat brain. *The Journal of Comparative Neurology*. 1995; 355(3):455–469. [PubMed: 7636025]
- Rouach N, Nicoll RA. Endocannabinoids contribute to short-term but not long-term mGluR-induced depression in the hippocampus. *European Journal of Neuroscience*. 2003; 18(4):1017–1020. [PubMed: 12925027]
- Schools GP, Kimelberg HK. mGluR3 and mGluR5 are the predominant metabotropic glutamate receptor mRNAs expressed in hippocampal astrocytes acutely isolated from young rats. *Journal of Neuroscience Research*. 1999; 58(4):533–543. [PubMed: 10533045]
- Servitja J-M, Masgrau R, Sarri E, Picatoste F. Group I Metabotropic Glutamate Receptors Mediate Phospholipase D Stimulation in Rat Cultured Astrocytes. *Journal of Neurochemistry*. 1999; 72(4):1441–1447. [PubMed: 10098847]
- Sik A, Penttonen M, Ylinen A, Buzsaki G. Hippocampal CA1 interneurons: an in vivo intracellular labeling study. *J Neurosci*. 1995; 15(10):6651–6665. [PubMed: 7472426]
- Snyder EM, Philpot BD, Huber KM, Dong X, Fallon JR, Bear MF. Internalization of ionotropic glutamate receptors in response to mGluR activation. *Nat Neurosci*. 2001; 4(11):1079–1085. [PubMed: 11687813]
- Straiker A, Mackie K. Depolarization-induced suppression of excitation in murine autaptic hippocampal neurones. *The Journal of Physiology*. 2005; 569(2):501–517. [PubMed: 16179366]
- Takahashi KA, Castillo PE. The CB1 cannabinoid receptor mediates glutamatergic synaptic suppression in the hippocampus. *Neuroscience*. 2006; 139(3):795–802. [PubMed: 16527424]
- Topolnik L, Azzi M, Morin F, Kougioumoutzakis A, Lacaille J-C. mGluR1/5 subtype-specific calcium signalling and induction of long-term potentiation in rat hippocampal oriens/alveus interneurons. *J Physiol*. 2006; 575(1):115–131. [PubMed: 16740609]
- Traub RD, Pais I, Bibbig A, LeBeau FEN, Buhl EH, Garner H, Monyer H, Whittington MA. Transient Depression of Excitatory Synapses on Interneurons Contributes to Epileptiform Bursts During Gamma Oscillations in the Mouse Hippocampal Slice. *J Neurophysiol*. 2005; 94(2):1225–1235. [PubMed: 15728773]
- Varma N, Carlson GC, Ledent C, Alger BE. Metabotropic Glutamate Receptors Drive the Endocannabinoid System in Hippocampus. *J Neurosci*. 2001; 21(24):188RC.
- Volk LJ, Daly CA, Huber KM. Differential Roles for Group 1 mGluR Subtypes in Induction and Expression of Chemically Induced Hippocampal Long-Term Depression. *J Neurophysiol*. 2006; 95(4):2427–2438. [PubMed: 16421200]
- Watabe AM, Carlisle HJ, O'Dell TJ. Postsynaptic Induction and Presynaptic Expression of Group 1 mGluR-Dependent LTD in the Hippocampal CA1 Region. *J Neurophysiol*. 2002; 87(3):1395–1403. [PubMed: 11877514]
- Whittington MA, Traub RD. Interneuron Diversity series: Inhibitory interneurons and network oscillations in vitro. *Trends in Neurosciences*. 2003; 26(12):676–682. [PubMed: 14624852]
- Wilson RI, Nicoll RA. Endogenous cannabinoids mediate retrograde signalling at hippocampal synapses. *Nature*. 2001; 410(6828):588–592. [PubMed: 11279497]

- Wilson RI, Nicoll RA. Endocannabinoid Signaling in the Brain. *Science*. 2002; 296(5568):678–682. [PubMed: 11976437]
- Xiao M-Y, Zhou Q, Nicoll RA. Metabotropic glutamate receptor activation causes a rapid redistribution of AMPA receptors. *Neuropharmacology*. 2001; 41(6):664–671. [PubMed: 11640920]
- Zakharenko SS, Zablow L, Siegelbaum SA. Altered Presynaptic Vesicle Release and Cycling during mGluR-Dependent LTD. *Neuron*. 2002; 35(6):1099–1110. [PubMed: 12354399]

Figure 1.

The endocannabinoid analogue, R-methanandamide, mediates depression of excitatory post synaptic currents (EPSCs) on CA1 stratum radiatum interneurons. A: application of R-methanandamide (50 μ M) induces significant ($P < 0.05$) depression (EPSC amplitudes after 10–15 minutes in R-methanandamide: $55.3 \pm 13.4\%$ of pre-drug control values, $n = 14$) of EPSCs. B: R-methanandamide (50 μ M) did not induce a significant ($P > 0.7$) depression of excitatory postsynaptic potentials (EPSPs) recorded from CA1 stratum radiatum field recordings (EPSP amplitudes after 10–15 minutes in R-methanandamide were $98.6\% \pm 7.8\%$ of baseline, $n=5$;) that were used to measure the effect of R-methanandamide on CA1 pyramidal cells. C: application of R-methanandamide induced a significant ($P < 0.05$; *) increase in paired pulse ratio (PPR; ratio was 0.8 ± 0.1 before R-methanandamide application and 1.0 ± 0.2 after, a 19% increase), suggesting a presynaptic site for its action. D: application of R-methanandamide also induced a significant ($P < 0.01$; **) decrease in coefficient of variance ($1/CV^2$). Non-normalized values of PPR and $1/CV^2$ from each interneuron are shown (open circles). The thick black line and filled circles indicate the mean value for all cells. In individual experiments the PPR significantly increased in 8 of 13 cells. See methods for a detailed description of $1/CV^2$ and PPR determination. Error bars indicate SEM. Scale bars: A, 100 pA, 10 ms; B, 0.1 mV, 10 ms.

Figure 2.

R-methanandamide-induced depression is not dependent on cannabinoid receptor 1 (CB1) or transient receptor potential vanilloid 1 (TRPV1) receptor activation. R-methanandamide-induced depression is not blocked by pre-exposure to either A, the TRPV1 specific antagonist capsazepine (10 μ M; EPSC amplitudes after 10–15 minutes in R-methanandamide and capsazepine: $54.6 \pm 8.6\%$ of pre-R-methanandamide control values, $n = 6$; $P = 0.97$ compared to R-methanandamide depression in the absence of capsazepine), B, the CB1/TRPV1 antagonist SR141716A (1 μ M; EPSC amplitudes after 10–15 minutes in R-methanandamide and SR141716A: $37.3 \pm 12.1\%$ of pre-R-methanandamide control values, $n = 5$; $P = 0.35$ compared to R-methanandamide depression in the absence of SR141716A), C, the CB1 specific antagonist AM-251 (2 μ M; EPSC amplitudes after 10–15 minutes in R-methanandamide and AM251: $51.1 \pm 4.1\%$ of pre-R-methanandamide control values, $n = 5$; $P = 0.76$ compared to R-methanandamide depression in the absence of AM251), or D, a combination of AM-251 and capsazepine (EPSC amplitudes after 10–15 minutes in R-methanandamide and AM251 plus capsazepine: $33.7 \pm 7.7\%$ of pre-R-methanandamide control values, $n = 6$; $P = 0.20$ compared to R-methanandamide depression in the absence of AM251 plus capsazepine). Error bars indicate SEM. Insets: representative paired pulse EPSCs (average of 10 consecutive) before (black) and after drug application (gray). Scale bar: 100 pA, 10 ms.

Figure 3.

DHPG-mediated depression of excitatory synapses on CA1 stratum radiatum interneurons has a similar pharmacological profile to that of R-methanandamide. **A:** DHPG (50–100 μ M) depresses EPSCs recorded from interneurons ($62.5 \pm 8.7\%$ of pre-drug control values; $P < 0.001$, $n = 17$), which lasts beyond the time of drug application ($n = 16$). **B & C:** PPRs were increased (ratio was 1.2 ± 0.1 before R-methanandamide application and 1.3 ± 0.1 after, a 5% increase) and CVs were significantly decreased ($P < 0.05$) in DHPG. Non-normalized values of PPR (**B**) and $1/CV^2$ (**C**) from each interneuron are shown (open circles). The thick black line and filled circles indicate the mean value for all cells. In individual experiments the PPR significantly increased in 9 of 13 cells. DHPG-induced LTD is not blocked by pre-exposure to either **D**, the TRPV1 specific antagonist capsazepine (10 μ M; EPSC amplitudes after 10–15 minutes in DHPG and in the presence of capsazepine: $58.3 \pm 11.6\%$ of pre-DHPG control values, $n = 5$; $P = 0.82$ compared to DHPG depression in the absence of capsazepine), **E**, the CB1/TRPV1 antagonist SR141716A (1 μ M; EPSC amplitudes after 10–15 minutes in DHPG and SR141716A: $62.3 \pm 7.8\%$ of pre-DHPG control values, $n = 8$; $P = 0.99$ compared to DHPG depression in the absence of SR141716A), **F**, the CB1 specific antagonist AM251 (2 μ M; EPSC amplitudes after 10–15 minutes in DHPG and AM251: $32.0 \pm 11.9\%$ of pre-DHPG control values, $n = 5$; $P = 0.11$ compared to DHPG depression in the absence of AM251), or **G**, a combination of AM251 and capsazepine (EPSC amplitudes after 10–15 minutes in DHPG and AM251 plus capsazepine: $47.0 \pm 16.6\%$ of pre-DHPG control values, $n = 5$; $P = 0.42$ compared to DHPG depression in the absence of AM251 plus

capsazepine). Note that cell to cell differences in the degree of DHPG-induced depression can be seen as differences in overall depression in each of these experiments; however DHPG depresses all cells to some degree, which is never blocked by these antagonists. Error bars indicate SEM. Insets: representative paired pulse EPSCs (average of 10 consecutive) before (black) and after drug application (gray). Scale bar: 100 pA, 10 ms.

Figure 4.

The effects of DHPG are mediated by mGluR5 and occlude synaptic depression by R-methanandamide. A: DHPG (50 μ M) continues to induce LTD even during pre-exposure to the mGluR1 antagonist CPCCOEt (50 μ M; EPSC amplitudes after 10–15 minutes in DHPG and CPCCOEt: $52.7 \pm 9.7\%$ of pre-DHPG control values, $n = 8$; $P = 0.51$ compared to DHPG depression in the absence of CPCCOEt). B: in contrast, DHPG does not induce LTD during pre-exposure of the mGluR5 antagonist MPEP (10 μ M; EPSC amplitudes after 10–15 minutes in DHPG and MPEP: $97.8 \pm 13.1\%$ of pre-DHPG control values, $n = 7$; $P < 0.05$ compared to DHPG depression in the absence of MPEP). C: pre-exposure of DHPG for at least 15 minutes occludes R-methanandamide mediated depression (EPSC amplitudes after

10–15 minutes in R-methanandamide: $108.5 \pm 22.0\%$ of pre-drug control values post-DHPG depression; $P < 0.05$ compared to control R-methanandamide depression, $n = 8$). Error bars indicate SEM. Insets: representative paired pulse EPSCs (average of 10 consecutive) before (black) and after drug application (gray). Scale bar: 100 pA, 10 ms.

Figure 5.

Intracellular application of GDP β S inhibits long lasting depression mediated by DHPG. A) Short-term application of R-methanandamide in the presence and absence of internal GDP β S ($n=8$; $n=5$ with GDP β S and $n=3$ without; data were combined because there was no statistical or visual difference between the two groups) resulted in a significant ($P < 0.05$) long lasting depression. The depression of combined (with and without GDP β S) or separated data, at 10–15 post R-methanandamide application were not significantly different ($P > 0.5$) from R-methanandamide application in Fig. 1A where no washout effect was investigated. Error bars indicate SEM. Insets: representative paired pulse EPSCs (average of 15 consecutive) before (black) and during R-methanandamide application (light gray) in the presence of GDP β S. Scale bar: 50 pA, 10 ms. B) Acute depression mediated by DHPG is not significantly different ($P > 0.7$) in the presence of GDP β S as compared to in its absence (see Fig. 3A). However, in the presence of GDP β S, DHPG-mediated long-term depression

as measured using EPSCs 25–30 minutes after washout, were significantly greater ($P < 0.05$) as compared to DHPG in the absence of GDP β S, indicating a significant washout effect. Error bars indicate SEM. Insets: representative paired pulse EPSCs (average of 15 consecutive) before (black), after DHPG application (light gray) and after washout (dark gray) in the presence of GDP β S. Scale bar: 50 pA, 10 ms.

Figure 6.

THC mediates depression of interneuron EPSCs via CB1 receptors. A: application of THC (1 μ M) induces significant depression of EPSCs (EPSC amplitudes after 10–15 minutes in THC: $80.3 \pm 9.0\%$ of pre-drug control values; $P < 0.05$, $n = 13$). B: THC-mediated depression is blocked by pre-exposure to AM-251 (2 μ M; EPSC amplitudes after 10–15 minutes in THC and AM251: $100.3 \pm 9.0\%$ of pre-THC control values, $n = 7$; $P < 0.05$ compared to THC depression in the absence of AM251). Error bars indicate SEM. Insets: representative paired pulse EPSCs (average of 10 consecutive) before (black) and after drug application (gray). Scale bar: 100 pA, 10 ms.