Retrograde Signalling by Endocannabinoids

C.W. Vaughan · M.J. Christie (✉)

Pain Management Research Institute, Northern Clinical School,
University of Sydney at Royal North Shore Hospital, 2006 NSW,Sydney, Australia
chrisv@med.usyd.edu.au

Abstract The cannabinoid neurotransmitter system comprises cannabinoid G protein-coupled membrane receptors (CB_1 and CB_2), endogenous cannabinoids (endocannabinoids), as well as mechanisms for their synthesis, membrane transport and metabolism. Within the brain the marijuana constituent Δ^9-tetrahydrocannabinol (THC) produces its pharmacological actions by acting on cannabinoid CB_1 receptors. THC modulates neuronal excitability by inhibiting synaptic trans-

mission via presynaptic CB_1-mediated mechanisms. More recently, it has been established that physiological stimulation of neurons can induce the synthesis of endocannabinoids, which also modulate synaptic transmission via cannabinoid CB_1 and other receptor systems. These endogenously synthesised endocannabinoids appear to act as retrograde signalling agents, reducing synaptic inputs onto the stimulated neuron in a highly selective and restricted manner. In this review we describe the cellular mechanisms underlying retrograde endocannabinoid signalling.

Keywords Endocannabinoid · Synaptic transmission · Retrograde signalling · TRP · mGluR

1 Endocannabinoids

The main psychoactive ingredient of cannabis, Δ^9-tetrahydrocannabinol (THC), is known to produce its actions in the central nervous system by acting on the body's own cannabinoid neurotransmitter system, predominantly via interaction with cannabinoid G protein-coupled CB_1 receptors. Like other neurotransmitter systems, the components of the cannabinoid signalling system also include endogenous cannabinoids (endocannabinoids), as well as mechanisms for their synthesis, membrane transport and metabolism (for recent reviews see Freund et al. 2003; Piomelli 2003; Petrocellis et al. 2004). Some of the endocannabinoids identified to date include anandamide, 2-arachidonoyl glycerol (2-AG), noladin ether and virodhamine. Within the central nervous system, endocannabinoids produce their biological effects by acting at least in part on cannabinoid CB_1 receptors. In this section we briefly describe some of the factors involved in the production and degradation of endocannabinoids and their locus of action, which are relevant to retrograde endocannabinoid signalling.

1.1 Endocannabinoid Synthesis, Release and Degradation

The production and degradation of endocannabinoids proceeds via a number of discrete steps that remain to be fully elucidated (Schmid 2000; Sugiura et al. 2002; Cravatt and Lichtman 2003; Glaser et al. 2003; Hillard and Jarrahian 2003; Piomelli 2003; Petrocellis et al. 2004). Endocannabinoids are thought to be produced on demand from membrane-bound phospholipids as a result of specific stimuli, and to be released in a non-vesicular manner. Briefly, anandamide is thought to be formed in a Ca^{2+}-dependent manner by a specific isoform of the enzyme phospholipase D (PLD) (Okamoto et al. 2004). 2-AG, on the other hand is thought to be formed via the phospholipase C (PLC)/DAG (*sn*-1-acyl-2-arachidonoylglycerol) lipase cascade in a Ca^{2+}-dependent manner (Bisogno et al. 1997; Stella et al. 1997). However, there are other potential synthetic pathways for these endocannabinoids. The biological

activity of anandamide and 2-AG is terminated by uptake and subsequent degradation. It has been proposed that these endocannabinoids are removed from the extracellular space by an anandamide membrane transporter (AMT). However, the AMT remains to be identified, and it has been suggested that uptake occurs by passive diffusion maintained through intracellular degradation (Glaser et al. 2003; Hillard and Jarrahian 2003). Once within the cell, the degradation of anandamide and 2-AG appears to be catalysed by at least two distinct enzymes, fatty acid amide hydrolase (FAAH) and monoacylglycerol lipase (MAGL). The biosynthetic and degradation pathways of the other identified endocannabinoids have not been examined.

1.2 Endocannabinoids Act via Presynaptic Cannabinoid CB_1 Receptors to Inhibit Transmitter Release

THC and a number of synthetic non-selective cannabinoid receptor agonists (such as WIN 55,212-2 and HU-210) modulate neuronal excitability by presynaptic CB_1-mediated short-term inhibition of glutamatergic and γ-aminobutyric acid (GABA)ergic synaptic transmission (for more detailed reviews see Schlicker and Kathmann 2001; Alger 2002). Release studies have demonstrated that cannabinoids also modulate other neurotransmitter systems. In accordance with the electrophysiological evidence, anatomical studies have demonstrated that cannabinoid CB_1 receptors are located presynaptically on nerve terminals in numerous brain structures. Exogenous application of the endocannabinoids anandamide and 2-AG also modulates synaptic transmission within a number of regions throughout the central nervous system, including the hippocampus, midbrain periaqueductal grey, spinal and medullary dorsal horn, and the substantia nigra (Shen et al. 1996; Vaughan et al. 2000; Morisset et al. 2001; Jennings et al. 2003; Marinelli et al. 2003). While the inhibitory effects of endocannabinoids are mediated by presynaptic cannabinoid CB_1 receptors, endocannabinoids have complex effects on synaptic transmission that are also mediated by presynaptic vanilloid TRPV1 (transient receptor potential vanilloid type 1) receptors and potentially other mechanisms (Di Marzo et al. 2001, see also Sect. 5).

In addition to their short-term effects, cannabinoids also modulate the induction of long-term synaptic plasticity. Administration of THC and the endocannabinoids anandamide and 2-AG inhibits the induction of long-term potentiation (LTP) in the hippocampus (Nowicky et al. 1987; Terranova et al. 1995; Stella et al. 1997) and long-term depression (LTD) within the cerebellum and nucleus accumbens (Levenes et al. 1998; Hoffman et al. 2003a).

2 Endocannabinoid as Retrograde Transmitters

The importance of endocannabinoid signalling elements remained uncertain for some time because of a lack of direct evidence for physiologically relevant synthesis,

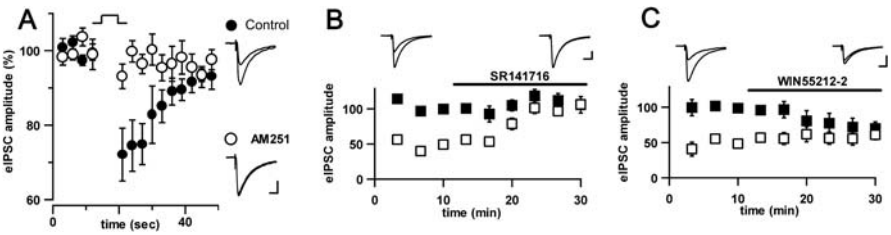

Fig. 1A–C DSI is mediated by endogenous cannabinoids. **A** A 5-s depolarising step produces a transient reduction in the amplitude of evoked inhibitory postsynaptic currents (*eIPSC*, control, *filled circles*) in hippocampal pyramidal neurons that is abolished by the CB_1 antagonist AM251 (2 μM, *open circles*). **B** and **C** Time plots of the eIPSC amplitude just before (*filled squares*) and just after (*open squares*) a 5-s depolarisation over 30 min. **B** DSI is blocked by the CB_1 antagonist SR141716 (2 μM). **C** The CB agonist WIN55212-2 (800 nM) inhibits eIPSCs and occludes DSI. The *insets* show average eIPSCs for the 10 s before and 10 s after the depolarising step (**A**), and in the same at 0 min and 30 min (**B** and **C**). *Scale bars* in inserts are 200 pA, 20 ms. (Modified from Wilson and Nicoll 2001, by permission)

release and activity of endocannabinoids on CB_1 receptors at the level of the synapse. Such evidence has recently arisen from the prior in vitro observation that strong depolarisation of hippocampal pyramidal and cerebellar Purkinje neurons produces a subsequent transient (from <10 s to 120 s), short-term inhibition of GABAergic synaptic inputs impinging upon these cells, observed as a decrease in the amplitude of evoked synaptic currents in the depolarised neuron (Llano et al. 1991; Pitler and Alger 1992) (Fig. 1A). This depolarisation-induced suppression of inhibition (DSI) was likely to be mediated by a retrograde messenger because it had a *postsynaptic* origin, but a *presynaptic* locus of action. However, the specific retrograde transmitter involved in DSI remained elusive (for review see Alger 2002).

2.1 Depolarisation-Induced Transient, Short-Term Retrograde Endocannabinoid Signalling

Wilson and Nicoll (2001) and Ohno-Shosahu et al. (2001) independently established that physiologically relevant stimulation of single hippocampal pyramidal neurons produces an endocannabinoid or endocannabinoids that diffuse onto the terminals of presynaptic GABAergic interneurons, where they act upon cannabinoid CB_1 receptors to produce transient, short-term inhibition of neurotransmitter release. Endocannabinoids also mediate depolarisation-induced retrograde signalling of interneuronal inhibitory GABAergic synapses onto cerebellar Purkinje cells (Kreitzer and Regehr 2001a; Diana et al. 2002; Yoshida et al. 2002), onto neocortical pyramidal cells (Trettel and Levine 2003) and onto substantia nigra neurons (Yoshida et al. 2002). In addition, retrograde endocannabinoid signalling-mediated depolarisation-induced suppression of excitation (DSE) has been demonstrated for excitatory glutamatergic synaptic inputs onto cerebellar Purkinje cells from climbing fibre and parallel fibre inputs (Kreitzer and Regehr 2001b; Maejima et al. 2001)

and onto dopaminergic neurons within the ventral tegmental area (Melis et al. 2004). Endocannabinoids thus belong to a small but growing club of retrograde neurotransmitters that act back to modulate presynaptic inputs impinging upon a neuron (Alger 2002).

A role for endocannabinoids in DSI and DSE has been established indirectly by pharmacological antagonism, and mimicry/occlusion with agonists (e.g. Kreitzer and Regehr 2001a,b; Ohno-Shosaku et al. 2001; Varma et al. 2001; Wilson and Nicoll 2001; Diana et al. 2002; Yoshida et al. 2002; Hampson et al. 2003; Yanovsky et al. 2003; Melis et al. 2004). In these studies, the transient inhibition of evoked glutamatergic excitatory and GABAergic inhibitory postsynaptic currents (IPSCs and EPSCs) produced by postsynaptic depolarisation is abolished by the cannabinoid receptor antagonists SR141716, AM251 and AM281 (Fig. 1A and B). The non-selective cannabinoid receptor agonist WIN 55,212-2 inhibits synaptic transmission and occludes DSI and DSE (Fig. 1C). In addition, DSI is absent in mice with a CB_1 receptor deletion (Varma et al. 2001; Wilson et al. 2001; Kim et al. 2002; Yoshida et al. 2002). Thus, DSI and DSE are mediated by a yet-to-be-identified endocannabinoid(s) that acts via cannabinoid CB_1 receptors.

It has long been known that DSI satisfies the criteria for retrograde signalling. First, both DSI and DSE are induced in the postsynaptic cell because they are caused by depolarisation-induced increases in postsynaptic cytoplasmic Ca^{2+} (see Sect. 3.1). Second, DSI and DSE are expressed presynaptically (Kreitzer and Regehr 2001a,b; Ohno-Shosaku et al. 2001; Wilson and Nicoll 2001; Diana et al. 2002; Kreitzer et al. 2002; Yoshida et al. 2002; Diana and Marty 2003; Trettel and Levine 2003). In these studies presynaptic inhibition has been demonstrated using standard electrophysiological techniques, including an increase in the paired-pulse ratio of electrically evoked synaptic currents, an increase in failure rate and variance of evoked synaptic currents using paired recordings and a reduction in the rate, but not the amplitude of tetrodotoxin (TTX)-resistant miniature synaptic currents and of Sr^{2+}-induced evoked asynchronous synaptic currents. Thus, postsynaptic elevations in cytoplasmic Ca^{2+} produce a reduction in the probability of transmitter release from presynaptic terminals impinging upon the depolarised neuron. Retrogradely released endocannabinoids might act directly on the cell bodies of interneurons (Kreitzer et al. 2002; Diana and Marty 2003).

Thus, DSI and DSE satisfy the three criteria of retrograde endocannabinoid signalling: they are (1) mediated by endocannabinoid(s), (2) induced postsynaptically and (3) expressed presynaptically. It might be noted that other transmitters have been implicated in retrograde signalling (e.g. Yanovsky et al. 2003).

2.2 Activation of Postsynaptic Metabotropic Receptors Induces Short-Term Retrograde Endocannabinoid Signalling

Some studies have also shown that retrograde endocannabinoid signalling can also be induced by postsynaptic activation of metabotropic glutamatergic receptors (mGluRs) and muscarinic acetylcholine receptors (mAChRs). Like the

cannabinoid receptors, metabotropic glutamate receptors (mGluR) belong to the G protein-coupled membrane receptor superfamily and have been classified into three main groups on the basis of their sequence homology and their biochemical and pharmacological profiles (Conn and Pin 1997). Group II and III mGluRs are located on presynaptic nerve terminals and act as autoreceptors to inhibit glutamate release in numerous brain regions. While group I mGluRs are located mainly on the cell bodies of neurons, activation of group I mGluRs (either with agonists or indirectly by high frequency stimulation of glutamatergic inputs) produces short- and long-term presynaptic inhibition of glutamatergic and GABAergic synaptic transmission within a number of brain regions (for review see Doherty and Dingledine 2003).

The divergence between the anatomical localisation (postsynaptic) and electrophysiological locus of action (presynaptic) of group I mGluRs can be reconciled by retrograde signalling. A role for endocannabinoids in postsynaptic mGluR-induced retrograde signalling has been demonstrated using similar pharmacological and knockout techniques to those described for DSI and DSE. In these studies, the inhibition of evoked synaptic currents produced by the selective group I mGluR agonist 3,5-dihydroxyphenylglycine (DHPG) (and by stimulation-evoked release from glutamatergic inputs) is abolished by cannabinoid CB_1 antagonism and deletion, and is mimicked/occluded by cannabinoid receptor agonists (Maejima et al. 2001; Varma et al. 2001; Ohno-Shosaku et al. 2002; Robbe et al. 2002; but see Chevaleyre and Castillo 2003; Rouach and Nicoll 2003). Similar techniques have demonstrated that endocannabinoids mediate retrograde signalling produced by postsynaptic mAChRs activation (Kim et al. 2002).

Like DSI and DSE, mGluR/stimulation-induced short-term depression is mediated by a retrograde signalling process. The group I mGluR-mediated inhibition is induced at a postsynaptic site because the presynaptic inhibition produced by the group I mGluR agonist DHPG (but not group II agonists) and by high frequency stimulation is abolished by disrupting postsynaptic G protein coupling with guanosine triphosphate (GTP)-γS, or guanosine diphosphate (GDP)-βS (Maejima et al. 2001; Ohno-Shosaku et al. 2002). However, group I mGluR-evoked presynaptic inhibition is not dependent upon postsynaptic elevations in intracellular Ca^{2+} (Maejima et al. 2001; Ohno-Shosaku et al. 2002, and see Sect. 3.2). Also, evidence similar to that described in Sect. 2.2 has demonstrated that mGluR-induced endocannabinoid-mediated inhibition is expressed presynaptically (Maejima et al. 2001; Ohno-Shosaku et al. 2002; Chevaleyre and Castillo 2003).

2.3 Activation of Postsynaptic Metabotropic Receptors Potentiates Depolarisation-Induced Retrograde Endocannabinoid Signalling

Do mGluR-induced and depolarisation-induced retrograde endocannabinoid signalling work independently? While depolarisation-induced DSI does not require activation of mGluRs (e.g. Wilson and Nicoll 2001), there is an interaction between the postsynaptic depolarisation and group I mGluR-induced presynaptic inhibi-

tion. Low concentrations of mGluR and mAChR agonists, which alone produce little presynaptic inhibition, potentiate DSI (Varma et al. 2001; Kim et al. 2002; Hoffman et al. 2003b). Given that the two forms of retrograde endocannabinoid signalling are mediated by distinct postsynaptic mechanisms (see Sects. 3.1 and 3.2), there is the potential for synergistic presynaptic inhibition.

2.4 A Role for Retrograde Endocannabinoids in Long-Term Synaptic Plasticity

It is becoming apparent that, in addition to short-term modulation of synaptic transmission, retrograde endocannabinoid signalling is involved in some forms of long-term synaptic plasticity because both short- and long-term modulation are abolished by cannabinoid CB_1 antagonists and are absent in mice with a CB_1 receptor deletion (Fig. 2A). Endocannabinoids mediate high-frequency stimulation-induced LTD of glutamatergic synaptic transmission in the striatum (Gerdeman et al. 2002) and nucleus accumbens (Robbe et al. 2002), stimulation-induced LTD of GABAergic synaptic transmission in the amygdala and hippocampus (Marsicano et al. 2002; Chevaleyre and Castillo 2003), timing-dependent LTD within the neocortex (Sjostrom et al. 2003) and amphetamine-induced LTD of glutamatergic synaptic transmission in the amygdala (Huang et al. 2003). However, endocannabinoids are not involved in all forms of long-term synaptic plasticity (e.g. Carlson et al. 2002; Marsicano et al. 2002; Rouach and Nicoll 2003) and it is therefore likely to be region- and synapse-specific.

Stimulation-induced LTD is induced at a postsynaptic site and is expressed presynaptically. LTD is induced postsynaptically because it is abolished by disrupting postsynaptic G protein coupling (Chevaleyre and Castillo 2003) (Figure 2C). Some forms of stimulation-induced LTD are caused by endogenously released glutamate acting on postsynaptic group I mGluR receptors because they are abolished by group I antagonists (Fig. 2B) and can be mimicked by the application of

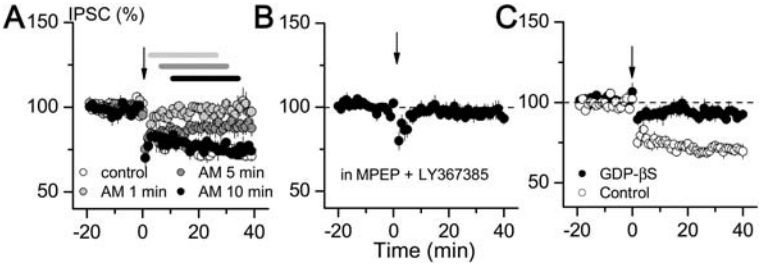

Fig. 2A–C LTD is mediated by postsynaptic mGluR-induced release of endocannabinoids that act on presynaptic CB_1 receptors. **A** High-frequency stimulation (HFS)-induced LTD of evoked IPSCs (e*IPSC*s) in hippocampal pyramidal neurons is abolished by the CB_1 antagonist AM251 (*AM*, 4 μM) when applied prior to, or within 10 min of LTD induction. The HFS-induced LTP is abolished by pre-application of (**B**) group I mGluR antagonists *MPEP* (4 μM) plus LY367385 (100 μM), and by (**C**) disrupting postsynaptic G protein coupling with intracellular *GDP-βS*. (Modified from Chevaleyre and Castillo 2003, by permission)

group I mGluR agonists (Robbe et al. 2002; Chevaleyre and Castillo 2003). Evidence in favour of presynaptic expression of LTD is similar to that described above for short-term depression (Choi and Lovinger 1997; Gerdeman et al. 2002; Marsicano et al. 2002; Robbe et al. 2002; Chevaleyre and Castillo 2003).

Endocannabinoid activation of CB_1 receptors is likely to be involved in the induction, but not in the maintenance of long-term synaptic plasticity because CB_1 antagonists impair long-term depression only when applied either prior to, or within 5 to 10 min of LTD induction (Chevaleyre and Castillo 2003; Ronesi et al. 2004) (Fig. 2A). The difference between short- and long-term synaptic effects of endocannabinoids may be due to the duration (or type) of endocannabinoid release, and sustained (for several minutes) activation of presynaptic CB_1 receptors (note DSI has a shorter time course). The mechanisms by which CB_1 activation induces long-term changes remain to be resolved, but the induction is unlikely to be solely due to CB_1 activation (Ronesi et al. 2004).

3
Production and Release of Endocannabinoids in Retrograde Endocannabinoid Signalling

While the identity of the endocannabinoid(s) involved in retrograde signalling remain to be determined directly, some clues have come from our understanding of the biosynthetic pathways involved in their production and degradation. Below we discuss the mechanisms underlying the postsynaptic production and release of endocannabinoids in relation to retrograde signalling. Depolarisation and mGluR-induced retrograde signalling are mediated by distinct Ca^{2+}-dependent and -independent intracellular cascades that provide some clues to the endocannabinoids involved in these processes. However, many issues remain to be resolved.

3.1
Ca^{2+}-Dependent and Ca^{2+}-Independent Endocannabinoid Production

Depolarisation-induced retrograde endocannabinoid signalling is dependent upon an increase in postsynaptic Ca^{2+}. First, uncaging postsynaptic Ca^{2+} from a photolabile chelator (with flash photolysis) in single neurons produces endocannabinoid-mediated DSI in the absence of depolarisation (Wilson and Nicoll 2001). Second, DSI and DSE are abolished by chelating postsynaptic Ca^{2+} with ethyleneglycoltetraacetic acid (EGTA), or ethylenedioxybis(o-phenylenenitrilo)tetraacetic acid (BAPTA) (Pitler and Alger 1992; Kreitzer and Regehr 2001b; Ohno-Shosaku et al. 2001; Kim et al. 2002; Trettel and Levine 2003).

The postsynaptic intracellular processes involved in mGluR-induced endocannabinoid-mediated retrograde signalling are diverse. Short-term mGluR-induced presynaptic inhibition does not require postsynaptic Ca^{2+} increases because postsynaptic BAPTA does not abolish group I mGluR-induced short-term in-

hibition of GABAergic and glutamatergic synaptic transmission in the hippocampus and nucleus accumbens, respectively (Maejima et al. 2001; Varma et al. 2001; Kim et al. 2002; Ohno-Shosaku et al. 2002). In contrast, both Ca^{2+}-dependent and Ca^{2+}-independent endocannabinoid-mediated long-term plasticity has been observed. Postsynaptic BAPTA abolishes stimulation-induced LTD of glutamatergic synaptic transmission in the striatum and nucleus accumbens (Gerdeman et al. 2002; Robbe et al. 2002; see also Huang et al. 2003), but not of GABAergic synaptic transmission in the hippocampus (Chevaleyre and Castillo 2003).

3.2 Depolarisation and Stimulation/mGluR-Induced Depression: Distinct Intracellular Cascades and Endocannabinoids?

A major question to be addressed is which endocannabinoids mediate retrograde endocannabinoid signalling? Both anandamide and 2-AG are potential candidates as retrograde signallers because their exogenous application produces presynaptic inhibition, albeit with reduced potency and efficacy compared to synthetic agonists (see Sect. 1.2). The Ca^{2+}-dependent depolarisation-induced short-term retrograde endocannabinoid signalling is not mediated by postsynaptic G protein-coupled processes and the PLC/DAG lipase cascade (Kim et al. 2002; Chevaleyre and Castillo 2003). It might be speculated that anandamide mediates depolarisation-induced short-term depression because anandamide formation/release can be induced by neuronal activation and Ca^{2+} influx. However, a role for 2-AG cannot be entirely ruled out because cellular Ca^{2+} influx also promotes its formation.

Stimulation-induced LTD within the hippocampus and nucleus accumbens is likely to be mediated by activation of group I mGluRs (see Sect. 2.4). Group I mGluRs are coupled via G_q-proteins/PLC to produce DAG and to increase intracellular Ca^{2+} via IP_3 and ryanodine sensitive stores (Conn and Pin 1997). The Ca^{2+}-independent endocannabinoid-mediated LTD within the hippocampus is mediated by the PLC/DAG cascade because it is abolished by PLC and DAG lipase inhibitors (Chevaleyre and Castillo 2003). On the other hand, the Ca^{2+}-dependent endocannabinoid-mediated LTD in the nucleus accumbens is mediated by the PLC mobilisation of intracellular Ca^{2+} because it is abolished thapsigargin and ryanodine (Robbe et al. 2002). It might be speculated that 2-AG mediates stimulation/mGluR-induced depression because 2-AG formation is activated via the PLC cascade (see Sect. 1.1) and high-frequency stimulation-induced hippocampal LTP is associated with an increase in 2-AG, but not anandamide production (Stella et al. 1997). However, a role for anandamide cannot be entirely ruled out because neuronal activation, Ca^{2+} influx and G protein-coupled receptor activation can also trigger anandamide (Piomelli 2003). In addition, striatal LTD is mimicked by loading the postsynaptic cell with either anandamide, or 2-AG (Gerdeman et al. 2002; Ronesi et al. 2004).

In summary, the endocannabinoids involved in the types of retrograde endocannabinoid signalling described to date are not fully understood. This will only be resolved by more fully comparing the intracellular cascades involved in the

processes of endocannabinoid production and retrograde signalling. Ultimately, the issue of which endocannabinoid(s) mediates retrograde signalling will only be resolved by direct identification.

4
Spread of Retrograde Endocannabinoid Signalling

Under normal conditions, retrograde endocannabinoid signalling is spatially restricted in a manner likely to be determined by diffusion, uptake and enzymatic degradation.

4.1
Endocannabinoid Signalling Is Spatially Restricted

Inhibition of synaptic inputs onto a cell is dominated by endocannabinoids generated by that cell. There is little heterosynaptic 'spill-over' of endocannabinoids from adjacent cells because retrograde signalling is abolished by disrupting postsynaptic endocannabinoid production (see Sects. 2 and 3). In contrast, other studies have demonstrated that there is some heterosynaptic endocannabinoid 'spill-over'. Using paired patch-clamp recordings, Wilson and Nicoll (2001) and Kreitzer et al. (2002) have exploited the parallel alignment of principal hippocampal and cerebellar neurons to estimate the range of influence of the endocannabinoid(s) released by a single neuron. In these studies, depolarisation of one neuron produces heterosynaptic inhibition of GABAergic synaptic inputs onto an adjacent neuron. Heterosynaptic effects are only observed for inter-electrode distances less than 20–75 μm from the stimulated neuron (the separation between cells is likely to be much less because this distance includes the radius of both cells). The heterosynaptic presynaptic depression is likely to be solely due to diffusion because it is independent of whether the two neurons receive common presynaptic inputs.

4.2
Factors Influencing Endocannabinoid Spread

The spatial restriction of endocannabinoid signalling is likely to be influenced by a number of factors including temperature and the mode of retrograde endocannabinoid induction. Differences in temperature between studies are likely to affect uptake (via the AMT). Diffusion is spatially restricted by uptake/degradation at physiological temperatures (in comparison to room temperature), although this is likely to be influenced by synaptic geometry and diffusion barriers (Kreitzer et al. 2002). Another factor likely to influence the spatial extent of retrograde endocannabinoid signalling is the mode and strength of induction, both of which are likely to affect endocannabinoid production.

4.3 Inhibitors of Uptake and Metabolism

External application of the AMT transport inhibitor AM404 alone causes CB_1-mediated presynaptic inhibition (Wilson and Nicoll 2001; Robbe et al. 2002; Huang et al. 2003) and potentiates amphetamine-induced LTD in the amygdala, but has no effect on stimulation-induced LTD in the striatum (Gerdeman et al. 2002; Huang et al. 2003; Ronesi et al. 2004). Blockade of uptake might alter the spatial influence of endogenously released endocannabinoids. Indeed this seems to be the case for some forms of retrograde endocannabinoid signalling. External AM404 and VDM-11 restore Ca^{2+}-sensitive LTD in EGTA, or BAPTA loaded cells in both the striatum and amygdala. Thus, while endocannabinoid signalling is normally spatially restricted, blockade of uptake allows significant endocannabinoid 'spillover'. It might be noted that while the molecular components involved in endocannabinoid metabolism have been more fully characterised (see Sect. 1.1), their role in retrograde endocannabinoid signalling has not been examined. There is a strong possibility that transport or metabolism inhibitors will increase the range or intensity of retrograde endocannabinoid signalling at cannabinoid sensitive synapses throughout the nervous system. Experimental manipulation of potential enzymes involved in breakdown of endocannabinoids, e.g. FAAH and MAGL inhibitors, may also help to narrow the range of candidate endocannabinoids in future studies.

5 Other Endocannabinoid Targets: TRP Channels

In all of the above studies, endocannabinoid signalling is mediated largely via presynaptic activation of cannabinoid CB_1 receptors. However, endocannabinoids such as anandamide have some affinity for other receptors, such as the TRPV1 'noxious heat/capsaicin' receptor (Di Marzo et al. 2001). Besides their predicted primary afferent localisation, TRPV1 receptors are present within discrete brain regions (Sasamura et al. 1998; Mezey et al. 2000). Thus, in addition to CB_1-mediated presynaptic effects, exogenously applied anandamide acts via TRPV1 receptors to increase spontaneous glutamatergic synaptic transmission not only within the spinal and medullary dorsal horn (Morisset et al. 2001; Jennings et al. 2003) but also within brain regions such as the hippocampus and substantia nigra (Al-Hayani et al. 2001; Marinelli et al. 2003) (Fig. 3A).

Recently, it has been demonstrated that the TRPV1 antagonists capsazepine and iodoresiniferatoxin increase glutamatergic synaptic transmission within the substantia nigra (Marinelli et al. 2003) (Fig. 3B). This suggests that an endogenously released endocannabinoid, possibly anandamide, can produce both inhibitory CB_1 and excitatory TRPV1-mediated presynaptic effects. However, activation of presynaptic ligand-gated ion channels has complex effects on synaptic transmission (Engelman and MacDermott 2004). It also remains to be determined whether this presynaptic TRPV1 action is produced via a retrograde signalling process.

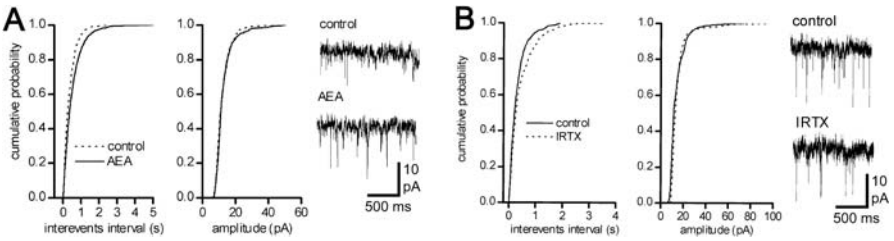

Fig. 3A, B Tonic release of anandamide presynaptically facilitates glutamatergic synaptic transmission in substantia nigra by activating TRPV1 receptors. **A** Anandamide (*AEA*, 30 μM) increases the rate (leftward shift in the cumulative distribution of the inter-event interval), but has no effect on the amplitude distribution of spontaneous EPSCs (sEPSCs). **B** The TRPV1 antagonist iodoresiniferatoxin (*IRTX*, 300 nM) decreases the rate (rightward shift in the cumulative distribution of the inter-event interval), but has no effect on the amplitude distribution of sEPSCs. *Insets* show raw traces of sEPSCs before (control) and during AEA and IRTX. (Modified from Marinelli et al. 2003, by permission)

6
What Is the Functional Significance of Retrograde Endocannabinoid Signalling?

It might be asked whether retrograde endocannabinoid signalling is physiologically relevant. It has been suggested that physiologically relevant action potential firing patterns do not induce DSI in the hippocampus (Hampson et al. 2003). While the depolarisation protocols used in DSI/DSE studies are unphysiological, it has been demonstrated that physiological action potential firing and electrical stimulation in the hippocampus is sufficient to produce DSI, which in turn has a significant disinhibitory effect on LTP (Ohno-Shosaku et al. 2001; Chevaleyre and Castillo 2003). In addition, synaptic activation of cerebellar parallel fibres at physiological rates induces retrograde endocannabinoid signalling of glutamatergic synaptic inputs onto Purkinje cells (Maejima et al. 2001; Brown et al. 2003). However, physiological relevance will only ultimately be determined by establishing retrograde endocannabinoid signalling in vivo using 'natural' stimuli.

7
Summary and Implications

There is compelling evidence to indicate that endocannabinoids are involved in retrograde signalling within a number of brain regions, including those involved in memory, motor control and reward/addiction. It is possible that retrograde endocannabinoid signalling occurs in other 'cannabinoid-sensitive' regions of the central nervous system and might prove to be a general phenomenon. Briefly, the events underlying retrograde endocannabinoid signalling can be described as follows (Fig. 4). Distinct stimuli, including postsynaptic depolarisation and activation of metabotropic receptors (group I mGluRs, mAChRs) appear to activate distinct intracellular postsynaptic cascades which induce de novo production and

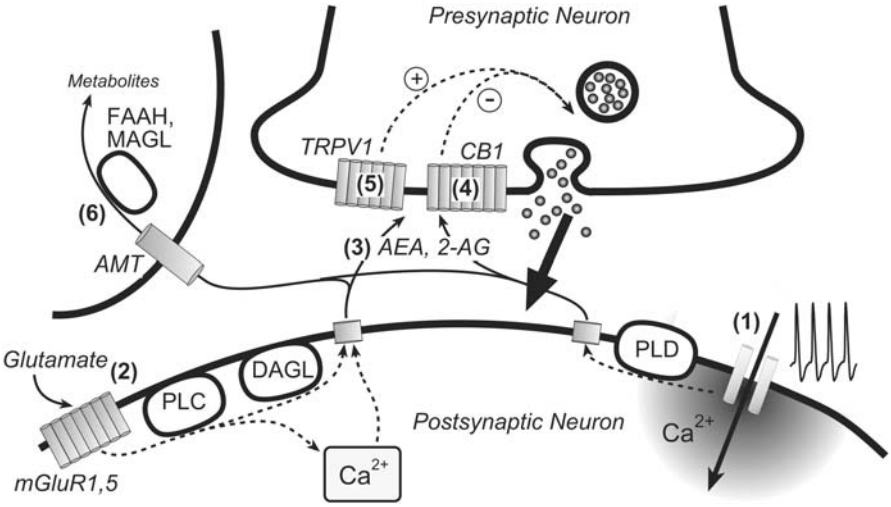

Fig. 4. Model depicting how endogenously released cannabinoids act as retrograde messengers within the brain. *(1)* Neuronal depolarisation induces an influx of Ca^{2+} via voltage dependent Ca^{2+} channels that stimulates phospholipase D (*PLD*). *(2)* Endogenously released glutamate, or synthetic agonist-induced activation of group I mGluRs, stimulates phospholipase C (*PLC*) to activate Ca^{2+} release from internal stores and to activate DAG-lipase in a Ca^{2+}-independent manner. *(3)* These postsynaptic events cause cleavage of membrane lipid precursors to induce de novo synthesis and release of endocannabinoids such as anandamide (*AEA*) and 2-arachidonoyl glycerol (*2-AG*) into the synaptic cleft. *(4)* These endocannabinoids activate cannabinoid CB_1 receptors located on presynaptic terminals of neurons which reduces release of neurotransmitters (such as GABA and glutamate) onto the postsynaptic neuron. *(5)* Endogenously released cannabinoids might also act via TRP ligand gated ion channels (e.g. *TPRV1*) to increase transmitter release. *(6)* Endocannabinoids are taken back up into neuronal and glial cells, possibly by a selective carrier-mediated transporter (*AMT*), and then degraded by enzymes such as fatty acid amide hydrolase (*FAAH*) and MAG-lipase (*MAGL*)

release of endocannabinoid(s). These endocannabinoids diffuse from the post-synaptic cell and act upon presynaptic cannabinoid CB_1 receptors to suppresses specific synaptic inputs impinging upon that cell, producing either short- and/or long-term changes. The action of endocannabinoid(s) is terminated by uptake and degradation. However, a number of issues remain to be resolved, such as the endocannabinoid(s) involved in these processes, the postsynaptic mechanisms of endocannabinoid production/release, other presynaptic endocannabinoid targets (e.g. TRP-like ion channels), and the precise mechanisms involved in uptake and degradation.

Increased understanding of the organisation of endocannabinoid signalling has raised hopes that therapeutic agents without the unwanted side-effects of cannabis could be developed. Natural and synthetic cannabinoids produce a range of pharmacological effects with potential therapeutic applications in the treatment of pain, migraine, muscle spasticity associated with multiple sclerosis, glaucoma, nausea and vomiting, and stimulation of appetite. Unfortunately, the CB_1 receptor is widely distributed throughout the brain and accounts for almost all of the effects of cannabis, including non-therapeutic effects on memory, cognition and motor

coordination. The possibility that drugs selective for one cannabinoid receptor type could overcome the unwanted psychotropic actions of cannabis is therefore limited. Another therapeutic possibility relates to the finding that retrograde endocannabinoid signalling is restricted by uptake and degradation. There is a strong possibility that transport or metabolism inhibitors will increase the range/intensity of retrograde endocannabinoid signalling. Transport and degradation inhibitors may provide novel therapeutic agents in a manner analogous to the clinically useful inhibitors associated with other neurotransmitter systems (e.g. Kathuria et al. 2003). In doing so, endocannabinoid transport or metabolism inhibitors might intensify retrograde signalling at specific synapses, in contrast to the disruptive effects of globally acting cannabinoid CB_1 receptor agonists.

References

Al-Hayani A, Wease KN, Ross RA, Pertwee RG, Davies SN (2001) The endogenous cannabinoid anandamide activates vanilloid receptors in the rat hippocampal slice. Neuropharmacology 41:1000–1005

Alger BE (2002) Retrograde signaling in the regulation of synaptic transmission: focus on endocannabinoids. Prog Neurobiol 68:247–286

Bisogno T, Sepe N, Melck D, Maurelli S, De Petrocellis L, Di Marzo V (1997) Biosynthesis, release and degradation of the novel endogenous cannabimimetic metabolite 2-arachidonoylglycerol in mouse neuroblastoma cells. Biochem J 322:671–677

Brown SP, Brenowitz SD, Regehr WG (2003) Brief presynaptic bursts evoke synapse-specific retrograde inhibition mediated by endogenous cannabinoids. Nat Neurosci 6:1048–1057

Carlson G, Wang Y, Alger BE (2002) Endocannabinoids facilitate the induction of LTP in the hippocampus. Nat Neurosci 5:723–724

Chevaleyre V, Castillo PE (2003) Heterosynaptic LTD of hippocampal GABAergic synapses: a novel role of endocannabinoids in regulating excitability. Neuron 38:461–472

Choi S, Lovinger DM (1997) Decreased frequency but not amplitude of quantal synaptic responses associated with expression of corticostriatal long-term depression. J Neurosci 17:8613–8620

Conn PJ, Pin JP (1997) Pharmacology and functions of metabotropic glutamate receptors. Annu Rev Pharmacol Toxicol 37:205–237

Cravatt BF, Lichtman AH (2003) Fatty acid amide hydrolase: an emerging therapeutic target in the endocannabinoid system. Curr Opin Chem Biol 7:469–475

Di Marzo V, Bisogno T, De Petrocellis L (2001) Anandamide: some like it hot. Trends Pharmacol Sci 22:346–349

Diana MA, Marty A (2003) Characterization of depolarization-induced suppression of inhibition using paired interneuron-Purkinje cell recordings. J Neurosci 23:5906–5918

Diana MA, Levenes C, Mackie K, Marty A (2002) Short-term retrograde inhibition of GABAergic synaptic currents in rat Purkinje cells is mediated by endogenous cannabinoids. J Neurosci 22:200–208

Doherty J, Dingledine R (2003) Functional interactions between cannabinoid and metabotropic glutamate receptors in the central nervous system. Curr Opin Pharmacol 3:46–53

Engelman HS, MacDermott AB (2004) Presynaptic ionotropic receptors and control of transmitter release. Nat Rev Neurosci 5:135–145

Freund TF, Katona I, Piomelli D (2003) Role of endogenous cannabinoids in synaptic signaling. Physiol Rev 83:1017–1066

Gerdeman GL, Ronesi J, Lovinger DM (2002) Postsynaptic endocannabinoid release is critical to long-term depression in the striatum. Nat Neurosci 5:446–451

Glaser ST, Abumrad NA, Fatade F, Kaczocha M, Studholme KM, Deutsch DG (2003) Evidence against the presence of an anandamide transporter. Proc Natl Acad Sci USA 100:4269–4274

Hampson RE, Zhuang SY, Weiner JL, Deadwyler SA (2003) Functional significance of cannabinoid-mediated, depolarization-induced suppression of inhibition (DSI) in the hippocampus. J Neurophysiol 90:55–64

Hillard CJ, Jarrahian A (2003) Cellular accumulation of anandamide: consensus and controversy. Br J Pharmacol 140:802–808

Hoffman AF, Oz M, Caulder T, Lupica CR (2003a) Functional tolerance and blockade of long-term depression at synapses in the nucleus accumbens after chronic cannabinoid exposure. J Neurosci 23:4815–4820

Hoffman AF, Riegel AC, Lupica CR (2003b) Functional localization of cannabinoid receptors and endogenous cannabinoid production in distinct neuron populations of the hippocampus. Eur J Neurosci 18:524–534

Huang YC, Wang SJ, Chiou LC, Gean PW (2003) Mediation of amphetamine-induced long-term depression of synaptic transmission by CB1 cannabinoid receptors in the rat amygdala. J Neurosci 23:10311–10320

Jennings EA, Vaughan CW, Roberts LA, Christie MJ (2003) The actions of anandamide on rat superficial medullary dorsal horn neurons in vitro. J Physiol (Lond) 548:121–129

Kathuria S, Gaetani S, Fegley D, Valino F, Duranti A, Tontini A, Mor M, Tarzia G, La Rana G, Calignano A, Giustino A, Tattoli M, Palmery M, Cuomo V, Piomelli D (2003) Modulation of anxiety through blockade of anandamide hydrolysis. Nat Med 9:76–81

Kim J, Isokawa M, Ledent C, Alger BE (2002) Activation of muscarinic acetylcholine receptors enhances the release of endogenous cannabinoids in the hippocampus. J Neurosci 22:10182–10191

Kreitzer AC, Regehr WG (2001a) Cerebellar depolarization-induced suppression of inhibition is mediated by endogenous cannabinoids. J Neurosci 21:RC174

Kreitzer AC, Regehr WG (2001b) Retrograde inhibition of presynaptic calcium influx by endogenous cannabinoids at excitatory synapses onto Purkinje cells. Neuron 29:717–727

Kreitzer AC, Carter AG, Regehr WG (2002) Inhibition of interneuron firing extends the spread of endocannabinoid signaling in the cerebellum. Neuron 34:787–796

Levenes C, Daniel H, Soubrie P, Crepel F (1998) Cannabinoids decrease excitatory synaptic transmission and impair long-term depression in rat cerebellar Purkinje cells. J Physiol (Lond) 510:867–879

Llano I, Leresche N, Marty A (1991) Calcium entry increases the sensitivity of cerebellar Purkinje cells to applied GABA and decreases inhibitory synaptic currents. Neuron 6:565–574

Maejima T, Hashimoto K, Yoshida T, Aiba A, Kano M (2001) Presynaptic inhibition caused by retrograde signal from metabotropic glutamate to cannabinoid receptors. Neuron 31:463–475

Marinelli S, Di Marzo V, Berretta N, Matias I, Maccarrone M, Bernardi G, Mercuri NB (2003) Presynaptic facilitation of glutamatergic synapses to dopaminergic neurons of the rat substantia nigra by endogenous stimulation of vanilloid receptors. J Neurosci 23:3136–3144

Marsicano G, Wotjak CT, Azad SC, Bisogno T, Rammes G, Cascio MG, Hermann H, Tang JR, Hofmann C, Zieglgansberger W, Di Marzo V, Lutz B (2002) The endogenous cannabinoid system controls extinction of aversive memories. Nature 418:530–534

Melis M, Pistis M, Perra S, Muntoni AL, Pillolla G, Gessa GL (2004) Endocannabinoids mediate presynaptic inhibition of glutamatergic transmission in rat ventral tegmental area dopamine neurons through activation of CB1 receptors. J Neurosci 24:53–62

Mezey E, Toth ZE, Cortright DN, Arzubi MK, Krause JE, Elde R, Guo A, Blumberg PM, Szallasi A (2000) Distribution of mRNA for vanilloid receptor subtype 1 (VR1), and VR1-like immunoreactivity, in the central nervous system of the rat and human. Proc Natl Acad Sci USA 97:3655–3660

Morisset V, Ahluwalia J, Nagy I, Urban L (2001) Possible mechanisms of cannabinoid-induced antinociception in the spinal cord. Eur J Pharmacol 429:93–100
Nowicky AV, Teyler TJ, Vardaris RM (1987) The modulation of long-term potentiation by delta-9-tetrahydrocannabinol in the rat hippocampus, in vitro. Brain Res Bull 19:663–672
Ohno-Shosaku T, Maejima T, Kano M (2001) Endogenous cannabinoids mediate retrograde signals from depolarized postsynaptic neurons to presynaptic terminals. Neuron 29:729–738
Ohno-Shosaku T, Shosaku J, Tsubokawa H, Kano M (2002) Cooperative endocannabinoid production by neuronal depolarization and group I metabotropic glutamate receptor activation. Eur J Neurosci 15:953–961
Okamoto Y, Morishita J, Tsuboi K, Tonai T, Ueda N (2004) Molecular characterization of a phospholipase D generating anandamide and its congeners. J Biol Chem 279:5298–5305
Petrocellis LD, Cascio MG, Marzo VD (2004) The endocannabinoid system: a general view and latest additions. Br J Pharmacol 141:765–774
Piomelli D (2003) The molecular logic of endocannabinoid signalling. Nat Rev Neurosci 4:873–884
Pitler TA, Alger BE (1992) Postsynaptic spike firing reduces synaptic GABAA responses in hippocampal pyramidal cells. J Neurosci 12:4122–4132
Robbe D, Kopf M, Remaury A, Bockaert J, Manzoni OJ (2002) Endogenous cannabinoids mediate long-term synaptic depression in the nucleus accumbens. Proc Natl Acad Sci USA 99:8384–8388
Ronesi J, Gerdeman GL, Lovinger DM (2004) Disruption of endocannabinoid release and striatal long-term depression by postsynaptic blockade of endocannabinoid membrane transport. J Neurosci 24:1673–1679
Rouach N, Nicoll RA (2003) Endocannabinoids contribute to short-term but not long-term mGluR-induced depression in the hippocampus. Eur J Neurosci 18:1017–1020
Sasamura T, Sasaki M, Tohda C, Kuraishi Y (1998) Existence of capsaicin-sensitive glutamatergic terminals in rat hypothalamus. Neuroreport 9:2045–2048
Schlicker E, Kathmann M (2001) Modulation of transmitter release via presynaptic cannabinoid receptors. Trends Pharmacol Sci 22:565–572
Schmid HHO (2000) Pathways and mechanisms of N-acylethanolamine biosynthesis: can anandamide be generated selectively? Chem Phys Lipids 108:71–87
Shen M, Piser TM, Seybold VS, Thayer SA (1996) Cannabinoid receptor agonists inhibit glutamatergic synaptic transmission in rat hippocampal cultures. J Neurosci 16:4322–4334
Sjostrom PJ, Turrigiano GG, Nelson SB (2003) Neocortical LTD via coincident activation of presynaptic NMDA and cannabinoid receptors. Neuron 39:641–654
Stella N, Schweitzer P, Piomelli D (1997) A second endogenous cannabinoid that modulates long-term potentiation. Nature 388:773–778
Sugiura T, Kobayashi Y, Oka S, Waku K (2002) Biosynthesis and degradation of anandamide and 2-arachidonoylglycerol and their possible physiological significance. Prostaglandins Leukot Essent Fatty Acids 66:173–192
Terranova JP, Michaud JC, Le Fur G, Soubrie P (1995) Inhibition of long-term potentiation in rat hippocampal slices by anandamide and WIN55212–2: reversal by SR141716 A, a selective antagonist of CB1 cannabinoid receptors. Naunyn Schmiedebergs Arch Pharmacol 352:576–579
Trettel J, Levine ES (2003) Endocannabinoids mediate rapid retrograde signaling at interneuron→pyramidal neuron synapses of the neocortex. J Neurophysiol 89:2334–2338
Varma N, Carlson GC, Ledent C, Alger BE (2001) Metabotropic glutamate receptors drive the endocannabinoid system in hippocampus. J Neurosci 21:RC188
Vaughan CW, Connor M, Bagley EE, Christie MJ (2000) Actions of cannabinoids on membrane properties and synaptic transmission in rat periaqueductal gray neurons in vitro. Mol Pharmacol 57:288–295

Wilson RI, Nicoll RA (2001) Endogenous cannabinoids mediate retrograde signalling at hippocampal synapses. Nature 410:588–592
Wilson RI, Kunos G, Nicoll RA (2001) Presynaptic specificity of endocannabinoid signaling in the hippocampus. Neuron 31:453–462
Yanovsky Y, Mades S, Misgeld U (2003) Retrograde signaling changes the venue of postsynaptic inhibition in rat substantia nigra. Neuroscience 122:317–328
Yoshida T, Hashimoto K, Zimmer A, Maejima T, Araishi K, Kano M (2002) The cannabinoid CB1 receptor mediates retrograde signals for depolarization-induced suppression of inhibition in cerebellar Purkinje cells. J Neurosci 22:1690–1697