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Biosynthesis of Endocannabinoids and Their Modes of Action in Neurodegenerative Diseases

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Endocannabinoids are thought to function as retrograde messengers, which modulate neurotransmitter release by activating presynaptic cannabinoid receptors. Anandamide and 2-arachidonoylglycerol (2-AG) are the two best studied endogenous lipids which can act as endocannabinoids. Together with the proteins responsible for their biosynthesis, inactivation and the cannabinoid receptors, these lipids constitute the endocannabinoid system. This system is proposed to be involved in various neurodegenerative diseases such as Parkinson's and Huntington's diseases as well as Multiple Sclerosis. It has been demonstrated that the endocannabinoid system can protect neurons against glutamate excitotoxicity and acute neuronal damage in both *in vitro* and *in vivo* models. In this paper we review the data concerning the involvement of the endocannabinoid system in neurodegenerative diseases in which neuronal cell death may be elicited by excitotoxicity. We focus on the biosynthesis of endocannabinoids and on their modes of action in animal models of these neurodegenerative diseases.

Keywords: Anandamide; 2-Arachidonylglycerol; Endocannabinoids; Tetrahydrocannabinol; Parkinson's disease' Huntington's disease; Multiple sclerosis

INTRODUCTION

The Endocannabinoid System

Since the first isolation of an endogenous substance exhibiting the pharmacological characteristics of plant-derived psychoactive cannabinoids, several candidates are now proposed to be endogenous ligands for the cannabinoid receptors. To date, all endocannabinoids identified are of lipid origin and structurally represented in lipid families that previously have not received much interest. Two lipid families are broadly recognized as having molecular constituents which mimic the distinct pharmacological effects of the plant-derived psychoactive cannabinoids, although they bear only very little structural resemblance to the principal psychoactive cannabinoid of natural origin, Δ^9-tetrahydrocannabinol (Δ^9-THC, FIG. 1). Among the constituents of these lipid families, *N*-acylethanolamines (NAEs) and 2-monoacylglycerols (2-MAGs), two substances have gained substantial interest, *i.e. N*-arachidonoylethanolamine (anandamide) and 2-arachidonoylglycerol (2-AG, FIG. 1). These arachidonate-derived molecules bind with nanomolar affinities to both cannabinoid CB_1 and CB_2 receptors (Devane *et al.*, 1992; Mechoulam *et al.*, 1995)

Other arachidonic acid derivatives are reported to be potential endogenous cannabimimetic substances, *i.e.* 2-arachidonoylglyceryl ether (noladin ether) (Hanus *et al.*, 2001) and *O*-arachidonoylethanolamine (virodhamine) (Porter *et al.*, 2002). In addition, the polyunsaturated NAEs *N*-docosatetraenoylethanolamine and *N*-dihomo-γ-linolenoylethanolamine have been demonstrated to exhibit a cannabimimetic profile (Hanus *et al.*, 1993). It is presently not known whether these four substances may be classified as endocannabinoids as their pharmacology has been explored to a much lesser extent than anandamide and 2-AG. In addition, other NAEs have gained renewed interest in endocannabinoid research. Thus, *N*-palmitoylethanolamine was suggested to exclusively bind to the peripheral cannabinoid CB_2 receptor (Facci *et al.*, 1995; Calignano *et al.*, 1998), however, due to conflicting reports on the receptor affinity and phar-

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macology, this theory is now challenged with the suggestion that it may instead be a ligand of a yet unknown cannabinoid receptor subtype (Lambert and Di Marzo, 1999).

See Table I for an overview of selected substances with effects on the (endo)cannabinoid system.

The Endocannabinoid Biosynthetic Pathways in the Brain

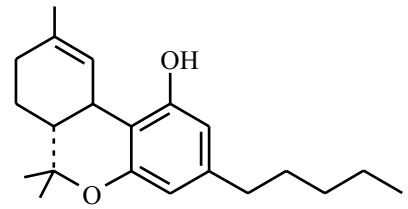
Endocannabinoids are formed in cell membranes, presumably also the plasma membrane, stored in the form of their respective lipid precursors and released following activation of the enzymes catalyzing the hydrolysis of their precursors (Natarajan *et al.*, 1983; Schmid *et al.*, 1983; Cadas *et al.*, 1996a; Sugiura *et al.*, 1996). As a consequence of the highly lipophilic nature of endocannabinoids they are therefore unlikely to be stored in synaptic vesicles. Obviously, these characteristics of endocannabinoid biochemistry deviate from the classical definition of neurotransmitter biochemistry, which has led to the notion that endocannabinoids should be rather classified as neuromodulators (Di Marzo *et al.*, 1999).

There is now considerable evidence for phospholipid-derived, however diverging, pathways for the cerebral biosynthesis of anandamide and 2-AG. The biosynthetic concept of anandamide (FIG. 2) implicates that it is released from the phosphate moiety of a corresponding phospholipid precursor molecule, *N*-arachidonoyl-phosphatidylethanolamine, by hydrolytic action of a specific phosphodiesterase subtype of the phospholipase D (PLD) family (Schmid *et al.*, 1983; Sugiura *et al.*, 1996). This NAPE-PLD enzyme is likely a novel PLD subtype because it exhibits substrate specificity for the *N*-acylethanolamine phospholipid (NAPE) family (Schmid *et al.*, 1983), and does not share key catalytic specifics with other known PLDs (Petersen and Hansen, 1999). NAPEs are derived from phospholipid metabolism by which ethanolamine phospholipids are amino-acylated via transacylase action of a NAPE-specific *N*-acyltransferase (NAT) catalyzing *sn*-1 stereospecific transfer of arachidonate from a donor glycerophospholipid, preferentially phosphatidylcholine (PC), to the amino position of the ethanolamine phospholipid (Natarajan *et al.*, 1983; Sugiura *et al.*, 1996). Since the mammalian NAT does not discriminate between different *sn*-1 acyl donor groups and shows no preferences between the diverse ethanolamine phospholipid acceptor molecules, the variation of the *N*-acyl moiety of NAPE is determined solely by the acyl variation of the *sn*-1 position of the donor phospholipid (Sugiura *et al.*, 1996). These characteristics of NAT explain why the *N*-arachidonoyl moiety of NAPE and NAE is found in only trace amounts in the rodent and human brain, as arachidonate only makes up about 1% of all esterified acyl groups of PC (Sugiura *et al.*, 1996). As predicted by the *sn*-1 acyl composition of PC, the palmitoyl, stearoyl, and oleoyl moeities make up the bulk volume of *N*-acyl groups of both NAPEs and NAEs in the mammalian brain (Schmid *et al.*, 1995; Sugiura *et al.*, 1996).

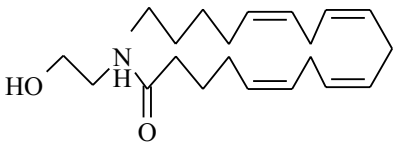
As opposed to anandamide biosynthesis, 2-AG can be formed from several sources of arachidonic acid-enriched membrane phospholipids. Predominantly, the lipid species of the 2-MAG family, including 2-AG, are likely formed via stereoselective hydrolysis of diacylglycerol (DAG). DAG is a degradation product from phospholipase C catalyzed hydrolysis of inositol phospholipids and PC (Stella *et al.*, 1997; Kondo *et al.*, 1998), but other sources of DAG may come from the hydrolysis of phosphatidic acid (Bisogno *et al.*, 1999b). Via action of a *sn*-1-selective DAG lipase, the removal of the *sn*-1 positioned fatty acyl group of DAG results in the formation of 2-MAGs (Bisogno *et al.*, 1997; Stella *et al.*, 1997). Cerebral *sn*-2 arachidonate is very abundant in the phospholipid precursors of DAG, in particular inositol phospholipids where it makes up 40-50% of all *sn*-2 acyl groups (Kerwin *et al.*, 1994). However, it cannot be ruled out that 2-MAG may also be generated via involvement of other lipases, such as phospholipase A_1 (which implicates the removal of the *sn*-1 acyl group of phospholipids prior to removal of the proximal phosphate bond) or triacylglycerol lipase (Stella *et al.*, 1997). In combination with the likelihood that several pathways may contribute to the production of DAGs with *sn*-2-arachidonate this explains why 2-AG is the major constituent of the 2-MAG family, accounting for 20-30% of all 2-MAGs in rat whole-brain preparations (Kondo *et al.*, 1998). Thus, 2-AG is usually determined at basal levels of 3-4 nmol per gram rat brain tissue, *i.e.* approximately 200-fold higher than that of anandamide (for quantitative data, see reviews by Hansen *et al.* (2000) and Sugiura *et al.* (2002)).

Regulation of Endocannabinoid Enzyme Activity

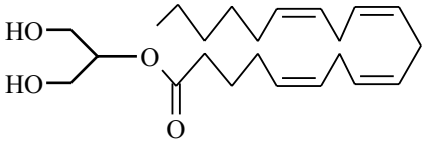
It is becoming evident that the endocannabinoid biosynthetic pathways operate in a variety of tissues and cells under various conditions of exogenous stimuli. However, very little is known about the endogenous regulation of the enzymes involved in cerebral endocannabinoid homeostasis. The catalytic activity of NAT and NAPE-PLD undergoes ontogenetic changes, as the neonatal rat brain shows much higher capability of synthesizing NAPE than NAE while this interrelationship reverses as the rat brain



Δ^9-tetrahydrocannabinol



N-arachidonoylethanolamine (anandamide)



2-arachidonoylglycerol (2-AG)

FIGURE 1 Molecular structure of the principal psychoactive cannabinoid of plant origin, Δ^9-tetrahydrocannabinol (Δ^9-THC), and the two best known endocannabinoids, N-arachidonoylethanolamine (anandamide) and 2-arachidonoylglycerol (2-AG).

matures (Moesgaard *et al.*, 2000). Also, anandamide-generating enzymes of the heart display rather large species-related activity differences (Moesgaard *et al.*, 2002), but this variation may not be applicaple to the brain. The basis for the ontogenetic and species influence on NAPE/NAE formation is unknown.

NAT activity is highest in the brain (Sugiura *et al.*, 1996; Cadas *et al.*, 1997), which correlates with the relatively high abundance of anandamide and other NAEs found in a variety of mammalian brain regions (Felder *et al.*, 1996; Bisogno *et al.*, 1999a). Presently, it is recognized that the calcium- (Ca^{2+}-)stimulated NAT reaction is the rate-limiting step of anandamide formation, because it is uncertain whether Ca^{2+} activates the NAPE-PLD *in situ* or if the enzyme is constitutively acting at a much lower catalytic rate than NAT (Schmid *et al.*, 1983; Sugiura *et al.*, 1996). Additionally, 2-AG synthesis is also stimulated by the presence of Ca^{2+} (Bisogno *et al.*, 1997; Kondo *et al.*, 1998), with the phosphoinositide-specific phospholipase C likely being the predominant Ca^{2+}-stimulated enzyme of the 2-AG biosynthetic pathway, as Ca^{2+} stimulation leads to a parallel accumulation of DAG (Stella *et al.*, 1997).

Although it is unclear how newly synthesized endocannabinoids are induced to leave the plasma membrane, Ca^{2+} influx may also constitute a key stimulus for endocannabinoid release. The critical determinant for extracellular accumulation of endocannabinoids is likely the preceding Ca^{2+}-stimulated synthesis of their precursors, which provides a link to the involvement of membrane depolarization in endocannabinoid homeostasis, signifying that endocannabinoids are synthesized and released in an activity-dependent manner. Anandamide concentrations are elevated in rat brain microdialysates following electrical stimulation and potassium-induced depolarization (Giuffrida *et al.*, 1999; Walker *et al.*, 1999), although 2-AG has yet not been found co-released under similar conditions (Giuffrida *et al.*, 1999). As expected from the variety of NAEs produced in the brain, non-endocannabinoid NAEs are also released with anandamide (Di Marzo *et al.*, 1994; Giuffrida *et al.*, 1999), which makes the basis for the notion that simultaneous efflux of non-endocannabinoid NAEs and 2-MAGs may prolong endocannabinoid actions by delaying endocannabinoid reuptake and degradation (Mechoulam *et al.*, 2002).

Effects of Endocannabinoids on Neurotransmission

CB_2 receptors are not expressed in the normal brain which makes CB_1 receptors the principal cannabinoid receptor subtype in the CNS. CB_1 receptors are far more abundant at presynaptic nerve terminals than on cell somata, in particular on GABAergic basal ganglia projections and hippocampal interneurons (Tsou *et al.*, 1999; Irving *et al.*, 2000). CB_1 receptors are also, however to a lesser extent, localized on glutamatergic neurons, as suggested by CB_1 receptor immunoreactivity on rat corticopyramidal glutamatergic efferents (Tsou *et al.*, 1998; Rodríguez *et al.*, 2001). Correspondingly, higher basal anandamide and 2-AG levels are found in the basal ganglia and hippocampus than in most other brain areas examined (Bisogno *et al.*, 1999a).

The major (endo)cannabinoid receptor-specific actions in the CNS are considered mediated by presynaptic CB_1 receptors resulting in modulation of cation channel conductivity (Mackie *et al.*, 1993; Shen and Thayer, 1998b) (FIG. 3). Cannabinoid- and endocannabinoid-induced CB_1 receptor-$G_{i/o}$ protein-α-subunit association evokes diminished Ca^{2+} entry through (1) voltage-dependent N- and P/Q-type Ca^{2+} channels; (2) increased potassium efflux by stimulation of inwardly

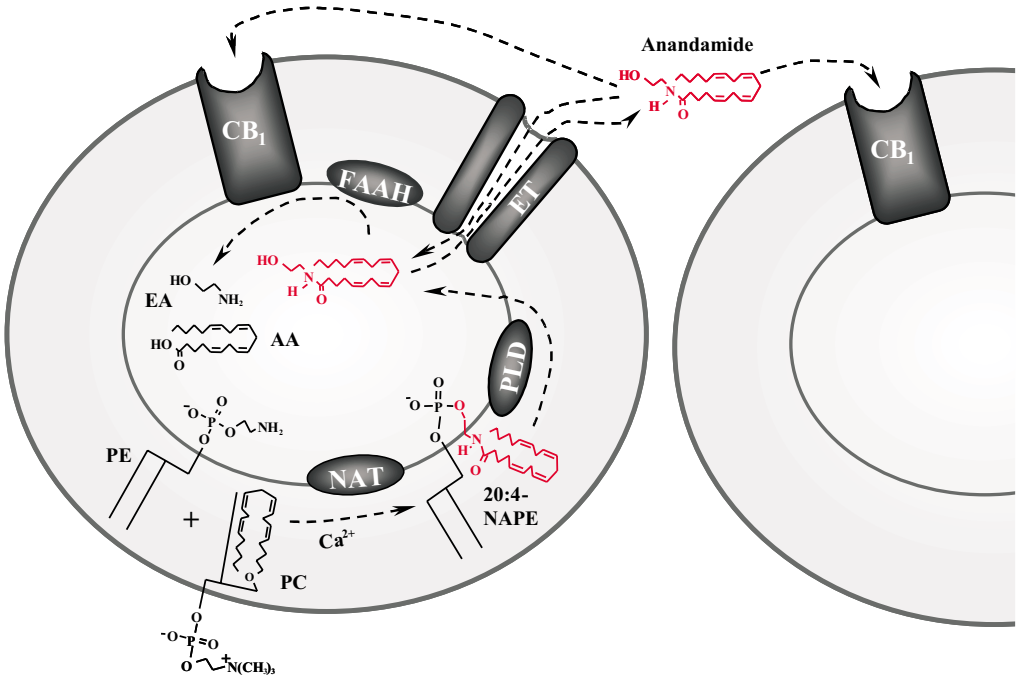


FIGURE 2 Phospholipid biosynthetic route of anandamide (and other *N*-acylethanolamines) and the cellular mechanisms likely to be involved in the release and termination of endocannabinoid activity in the brain.
PE, ethanolamine phospholipid; 20:4-NAPE, *N*-arachidonoyl-phosphatidylethanolamine; PC, 1-arachidonoyl-2-*O*-acyl-phosphatidylcholine; NAT, *N*-acyltransferase; PLD, NAPE-specific phospholipase D; CB_1, cannabinoid CB_1 receptor; AT, anandamide transporter; FAAH, fatty acid amidohydrolase; AA, arachidonic acid; EA, ethanolamine. For a schematic outline of 2-AG biosynthetic pathways, see review by Sugiura *et al.* (2002)

rectifying K^+ channels (K^+_{ir}), and (3) reversal of voltage-dependent A-type K^+ channels (K^+_A). The effect on K^+_A channels is not directly linked to G-protein coupling. It includes downstream inhibition of adenylate cyclase ATP hydrolysis, thereby reducing cAMP levels, which then in turn reduces the protein kinase A (PKA)-mediated phosphorylation of the K^+ channel, leading to a negative shift in voltage-dependence. Thus, the overall consequence of the interference with presynaptic ion channel activity is understood to slow down Ca^{2+}-influx-mediated neurotransmitter release from nerve terminals thereby decreasing neuronal excitability. The arrangement of CB_1 receptors at the nerve terminal therefore provides a mechanism by which the receptors inhibit the release of several neurotransmitters, including glutamate, γ-amino-butyric acid (GABA), and dopamine (Rodriguez de Fonseca *et al.*, 2001; Schlicker and Kathmann, 2001).

The modulatory effect of endocannabinoids on presynaptic neurotransmitter release is controlled by their retrograde transport from postsynaptic loci, presumably from depolarized somata and dendrites (Egertova *et al.*, 1998). Endocannabinoids are likely acting as short-range inhibitory retrograde messengers by activating presynaptic CB_1 receptors, which results in a substantial, transient or long-term, inhibitory effect on presynaptic GABAergic and excitatory transmission. Retrograde endocannabinoid function has so far been demonstrated in the striatum, hippocampus and cerebellum (Kreitzer and Regehr, 2001; Varma *et al.*, 2001; Gerdeman *et al.*, 2002). Presumably, the retrograde messenger effect is mediated by CB_1 receptor-induced reduction of presynaptic Ca^{2+} influx (Diana *et al.*, 2002).

It is unclear whether depolarizing stimuli trigger directly endocannabinoid release, but recent studies have demonstrated that metabotropic glutamate receptor stimulation may constitute a signal for postsynaptic endocannabinoid efflux (Maejima *et al.*, 2001; Varma *et al.*, 2001). This tentatively suggests that glutamate release drives postsynaptic endocannabinoid release. The direction of endocannabinoid movement appears also to be driven by the concentration gradient across the plasma membrane, as endocannabinoid efflux and reuptake via a high-affinity transmembrane endocannabinoid transporter (FIG. 2) exhibit similar kinetics (Rakhshan *et al.*, 2000). It is therefore also thought that the equilibrium of release and reuptake is coupled to the activity of the intracellular endocannabinoid-degrading enzymes, fatty acid amide hydrolase (FAAH, FIG. 2) and MAG lipase (Deutsch *et al.*, 2000; Dinh *et al.*, 2002), indicating that the degree of endocannabinoid-induced modulation of synaptic neurotransmission depends on their synthesis and degradation rate.

Table I Selected substances with effects on the (endo)cannabinoid system. Substances in italics are mentioned in this review. 1) endogenous substances which are generally accepted as behaving as endogenous cannabimimetic ligands; 2) putative endogenous substances with cannabimimetic effects not yet fully characterized; a) affinity for CB_2 and VR_1 receptor not reported; b) the substance has weak or negligible affinity for the CB_1, CB_2 and VR_1 receptors; c) affinity for cannabinoid/vanilloid receptors not reported. Abbreviations: CB_1, cannabinoid CB_1 receptor; CB_2, cannabinoid CB_2 receptor; VR_1, vanilloid VR1 receptor; FAAH, fatty acid amidohydrolase; FAAH inh., fatty acid amidohydrolase inhibitor; transport inh., endocannabinoid transmembrane transport inhibitor. See comprehensive reviews by Pertwee (1997), Davies *et al.* (2002), and Fowler *et al.* (2001; 2002) for the pharmacology of substances mentioned in the table.

Classification	Targets & selectivity
Endocannabinoids[1]	
anandamide	$CB_1 \approx> CB_2 > VR_1$
2-AG	$CB_1 = CB_2$
Putative endocannabinoids[2]	
noladin ether	$CB_1 > CB_2$
virodhamine	$CB_2 > CB_1$
N-docosatetraenoylethanolamine	$CB_1 >> VR_1$
N-dihomo-γ-linolenoylethanolamine	CB_1[a]
N-palmitoylethanolamine	unknown cannabinoid-like receptor ?
Plant-derived cannabinoids	
Δ^9-THC	$CB_1 = CB_2$
cannabinol	$CB_1 = CB_2$
cannabidiol	CB/VR neglectible[b]
Synthetic cannabinoid receptor agonists	
Methanandamide	$CB_1 > CB_2 > VR_1$
arachidonoyl-2'-chloroethylamide (ACEA)	$CB_1 >> CB_2$
arachidonoylcyclopropylamide (ACPA)	$CB_1 >> CB_2$
nabilone	$CB_1 = CB_2$
O-1812	$CB_1 >> CB_2 > VR_1$
O-1057	$CB_1 = CB_2$
HU-210	$CB_1 = CB_2$
WIN55,212-2	$CB_1 \approx < CB_2$
CP55,940	$CB_1 = CB_2$
BAY38-7271	$CB_1 = CB_2$
JWH015	$CB_2 >> CB_1$
JWH133	$CB_2 >> CB_1$
L-759633	$CB_2 >> CB_1$
L-759656	$CB_2 >> CB_1$
HU-308	$CB_2 >> CB_1$
N-arachidonoyldopamine	$VR_1 >> CB_1 >> CB_2 >$ transport inh. = FAAH inh.
arvanil	$VR_1 > CB_1 >>$ transport inh.
Synthetic cannabinoid receptor antagonists/inverse agonists	
SR141716A	$CB_1 >> CB_2$
AM251	$CB_1 >> CB_2$
AM281	$CB_1 >> CB_2$
LY320135	$CB_1 > CB_2$
SR144528	$CB_2 >> CB_1$
AM630	$CB_2 >> CB_1$
Synthetic endocannabinoid transport inhibitors	
AM404[a]	transport inh. = $CB_1 > VR_1$
VDM11	transport inh. = $CB_1 >> VR_1$
palmitylisopropylamide	transport inh. > FAAH inh.
FAAH inhibitors	
N-arachidonoylglycine	FAAH inh. >> CB_1, CB_2, transport inh.
phenylmethylsulfonyl fluoride (PMSF)	FAAH inh.
arachidonoyl trifluoromethyl ketone (ATMK)	FAAH inh. = CB_1
methylarachidonoyl fluorophosphonate (MAFP)	FAAH inh. >> CB_1
palmitylsulfonyl fluoride (AM374)	FAAH inh. >> CB_1
α-keto heterocycles	FAAH inh.[c]

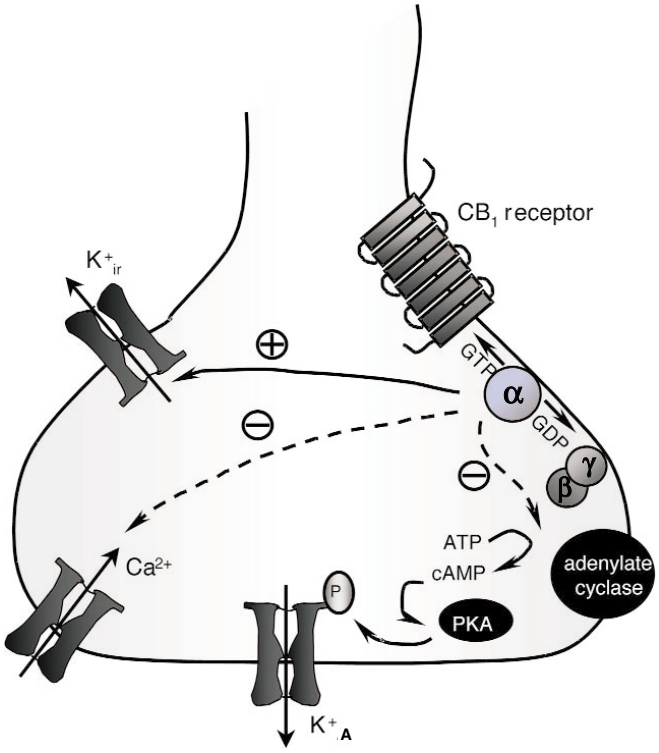


FIGURE 3 Down-stream actions of CB_1 receptors on presynaptic cation channels. Enhanced receptor-effector coupling is marked by unbroken lines and those which are diminished are marked by dotted lines.

It is becoming increasingly clear that (endo)cannabinoids may also act on a number of cellular targets distinct from cannabinoid receptors, which may well be relevant for the effects of endocannabinoid on neuronal excitability: (1) based on cannabinoid receptor agonist WIN-55.212-2 binding profiles and behavioral assays in CB_1 receptor knock-out mice at least one non-cannabinoid G-protein-coupled receptor is suggested to be an additional receptor target for anandamide in the rodent brain (Breivogel *et al.*, 2001). Interestingly, WIN55,212-2, inhibits hippocampal glutamatergic postsynaptic currents in CB_1 receptor knock-out mice, suggesting that a so far unknown non-CB_1 receptor may participate in the (endo)cannabinoid-mediated downregulation of excitatory neurotransmission (Hajos *et al.*, 2001); (2) by contrast, anandamide also activates glutamatergic NMDA receptor Ca^{2+} currents in rat cortical brain slices by a CB_1 receptor-independent mechanism (Hampson *et al.*, 1998a) and induce neuronal depolarization via direct inhibition of K^+ channels (Maingret *et al.*, 2001); (3) anandamide, but not 2-AG, is considered being an endogenous activator of the ionotropic vanilloid VR_1 receptor (Zygmunt *et al.*, 1999), possibly activating the receptor from an intracellular binding domain following cellular uptake of anandamide (De Petrocellis *et al.*, 2001; Ross *et al.*, 2001). The VR_1 receptors are expressed in several brain areas, including the hippocampus and basal ganglia (Mezey *et al.*, 2000), and recent findings indicate that anandamide causes a VR_1 receptor antagonist-sensitive increase in inhibitory GABAergic synaptic transmission in rat hippocampal slices (Al-Hayani *et al.*, 2001); (4) anandamide directly inhibits astrocyte gap junctions, thereby interfering with astrocyte intercellular Ca^{2+} communication (Venance *et al.*, 1995); (5) anandamide lipoxygenase metabolites inhibit brain L-type Ca^{2+} channels and stimulate bronchial ionotropic vanilloid VR_1 receptor activity (Shimasue *et al.*, 1996; Craib *et al.*, 2001), suggesting that oxidative endocannabinoid metabolism can contribute to endocannabinoid-like modulatory effects on neurotransmission.

THE ENDOCANNABINOID SYSTEM IN ACUTE NEURODEGENERATION

The central nervous system is highly vulnerable to ischemia induced by a stroke or traumatic brain injury. Neuronal death caused by ischemia is executed via a complex array of processes in which excitotoxicity plays a major role. In excitotoxicity, cell death is triggered by the overstimulation of excitatory amino acid receptors by glutamate. This leads to cytotoxic levels of Ca^{2+} and subsequent activation of destructive pathways, involving among others caspases, calpains and generation of reac-

tive oxygen species (Dirnagl *et al.*, 1999; Doble, 1999). Several lines of evidence reveal a connection between (endo)cannabinoids and excitotoxicity (Grundy *et al.*, 2001; Mechoulam *et al.*, 2002; van der Stelt *et al.*, 2002).

Biosynthesis of Endocannabinoids in Acute Neurodegeneration

The physiological cytosolic Ca^{2+} level is very low (~100 nM) (Doble, 1999), which is far below the level required to facilitate detectable basal biosynthesis of anandamide and 2-AG. This may therefore explain why unstimulated endocannabinoid levels are present in only trace amounts. Since unphysiologically high Ca^{2+} concentrations are required to trigger significant accumulation of the endocannabinoid precursors NAPE and DAG *in vitro* (Kondo *et al.*, 1998; Moesgaard *et al.*, 2000; 2002), it is believed that only strong depolarizing stimuli lead to a significant raise of basal extracellular endocannabinoid concentrations. Hence, neuronal exposure to Ca^{2+} ionophores and high K^+ concentrations induce intra- and extracellular accumulation of endocannabinoids *in vitro* (Cadas *et al.*, 1996b; Hansen *et al.*, 1997) and *in vivo* (Giuffrida *et al.*, 1999). Correspondingly, endocannabinoid biosynthesis occurs following activation of excitatory amino acid receptors, of which the *N*-methyl-D-aspartate (NMDA) receptor induces a very strong signal for endocannabinoid accumulation (Di Marzo *et al.*, 1994; Hansen *et al.*, 1997, 1999). Coactivation of cholinergic and NMDA receptors results in more elevated endocannabinoid synthesis in mouse cortical neurons, than following stimulation of NMDA receptors alone (Stella and Piomelli, 2001; Dinh *et al.*, 2002), indicating that several neurotransmitter systems can act synergistically on endocannabinoid synthesis.

Overstimulation of excitatory amino acid receptors has a substantial effect on endocannabinoid synthesis. Anandamide, but not 2-AG, accumulates in the neonatal rat brain following exposure to excitotoxic concentrations NMDA *in vivo* (Hansen *et al.*, 2001b). Similarly, neonatal traumatic brain injury induces an upregulation of anandamide concentrations, while 2-AG levels are unaffected (Hansen *et al.*, 2001b). Since a range of NAEs and NAPEs are co-produced in a parallel fashion in different rat models of pediatric brain damage (Hansen *et al.*, 2001a,b), this provides a strong indication that the NAPE/NAE pathway is exceedingly responsive to cerebral events that instigate damage to the brain. This notion is supported by different lines of research. Data from rat whole brain preparations suggest that the NAT/NAPE-PLD pathway is exceedingly active during post-decaptive ischemia (Natarajan *et al.*, 1986; Moesgaard *et al.*, 1999); neocortical cells continues to produce NAEs and NAPEs (including anandamide and its precursor) in conditions of severe neuronal damage (Hansen *et al.*, 1997; 1999); and apoptotic cell damage also triggers NAE and NAPE accumulation (Berdyshev *et al.*, 2000). The sustained accumulation of the anandamide phospholipid precursor contrasts the common observation that glycerophospholipids are subjected to rapid degradation following a variety of cellular insults, which result in liberation of free fatty acids, including arachidonic acid (Farooqui *et al.*, 2000). It is known that enzymatic condensation of free arachidonic acid and ethanolamine occurs by reversed action of FAAH (Arreaza *et al.*, 1997), but this process requires unphysiologically high concentrations of cytosolic free arachidonic acid and ethanolamine (Sugiura *et al.*, 1996), implying that this 'anandamide synthase' pathway may potentially only be active under pathophysiological conditions involving severe membrane damage. However, it remains to be demonstrated that this alternative pathway is relevant, as synthesis of the anandamide phospholipid precursor and anandamide typically accelerates in a parallel fashion during neurodegeneration (Hansen *et al.*, 2000).

In contrast to neonatal rat models of neurodegeneration, 2-AG levels are reported to rise significantly as a consequence of traumatic brain injury in the adult mouse (Panikashvili *et al.*, 2001), and these contradictory results have caused some confusion (Mechoulam *et al.*, 2002). Although it is likely that the brain maturational level could be a critical parameter for the responsiveness of the 2-AG biosynthetic pathways, the discovery of a novel enzyme, that directly acylates glycerol utilising acyl-CoA as substrate (Lee *et al.*, 2001), may offer an additional explanation for the discrepancies. This novel enzyme prefers arachidonoyl-CoA and the acylation process occurs at the *sn*-2 position, thereby generating 2-AG. The enzyme has a rather high K_m-value for glycerol, but it is well-known that glycerol accumulates during brain ischemia (Frykholm *et al.*, 2001), as will arachidonoyl-CoA (Deutsch *et al.*, 1997) and the activity of the enzyme may thus be dependent on the endogenous levels of arachidonoyl-CoA and glycerol in the different models of brain injury used. It is well known that high intracellular Ca^{2+} concentrations activate several phospholipases, thereby possibly also generating glycerol (Farooqui *et al.*, 2000). Thus, phospholipase A_2-potentiated formation of 2-AG in Ca^{2+} ionophore-stimulated neuroblastoma cells (Bisogno *et al.*, 1997) could possibly be explained by excessive formation of glycerol and arachidonoyl-CoA, because phospholipase A_2 may liberate arachidonic acid to facilitate increased formation of arachidonoyl-CoA.

In summary, strong depolarizing stimuli, such as occurring during excitotoxin-induced neurodegeneration

and after post-decapative ischemia, may lead to a rise in endocannabinoid formation.

Neuroprotective Properties of (Endo)cannabinoids in Acute Neurodegeneration

Several *in vitro* studies have reported neuroprotection with classical and synthetic (endo)cannabinoids. Depending on the model used, cannabinoids were shown to protect neurons via CB_1-mediated inhibition of glutamate exocytosis (Shen and Thayer, 1998a), CB_1 receptor-mediated closing of voltage sensitive Ca^{2+} channels (Abood *et al.*, 2001; Hampson *et al.*, 1998a), anti-oxidant activity (Hampson *et al.*, 1998b; Marsicano *et al.*, 2002), and suppression of the formation of tumour necrosis factor alpha (TNF-α) (Gallily *et al.*, 2000; Venters *et al.* 2000). Anandamide and 2-AG have been shown to rescue cerebral neurons from *in vitro* hypoxia and glucose deprivation via a CB_1 and CB_2 receptor-independent pathway (Sinor *et al.*, 2000). By contrast, some *in vitro* studies do not support a neuroprotective action of cannabinoids. Anandamide was shown to be ineffective to protect neurons against prolonged exposure to toxic levels of glutamate (Andersson *et al.*, 2000; Skaper *et al.*, 1996). Hampson and Grimaldi (2001) have suggested that the varying and uncontrolled levels of cAMP in *in vitro* models of neurotoxicity may explain the lack of neuroprotection by cannabinoids.

Neuroprotective effects of (endo)cannabinoids in *in vivo* studies have also been observed. Panikashvili *et al.* (2001) demonstrated that 2-AG prevented neuronal damage in a mouse model of closed head injury by acting at least in part via the CB_1 receptor. 2-AG administered in a dose range of 0.1-10 mg/kg could reduce brain edema and at a dose of 5 mg/kg it significantly improved clinical recovery, reduced infarct volume and reduced hippocampal cell death compared to controls after 7 days. The reduction in brain edema by 2-AG was dose-dependently attenuated by SR141716A. Various mechanisms may contribute to the neuroprotective actions of 2-AG, such as reduction in excitotoxicity, inhibition of production of pro-inflammatory TNF-α and reactive oxygen species and lowering of cerebral vasoconstriction. Application of the CB_1 receptor antagonist SR141716A (20 mg/kg) alone did not increase the volume of edematous tissue, which might indicate that endogenously released 2-AG exerts its protective actions through non-CB_1-mediated mechanisms (Panikashvili, *et al.*, 2001).

Similar results were obtained in another *in vivo* model of secondary excitotoxicity. It was demonstrated that Δ^9-THC (1 mg/kg, *i.p.*) and anandamide (10 mg/kg, *i.p.*) could significantly reduce the volume of cytotoxic edema in neonatal rats in the acute phase after the intrastriatal injection with the Na^+/K^+-ATPase inhibitor ouabain (van der Stelt *et al.*, 2001a,b). The effect of Δ^9-THC was antagonized by blocking the CB_1 receptor, whereas the reduction in cellular swelling by anandamide was insensitive to SR141716A. After seven days the infarct volume in THC- and anandamide-treated rats was reduced by ~40% and ~64%, respectively, as compared to control animals. When assessed at this time point, the neuroprotective actions of both anandamide and Δ^9-THC were abolished by SR141716A. A CB_1 receptor-mediated reduction in Ca^{2+} influx and glutamate release were thought to be responsible for the neuroprotection in the hippocampus, striatum and cortex in the late phase (van der Stelt *et al.*, 2001a,b). There seems to be no endogenous endocannabinoid tone controlling neuronal damage, because application of anandamide-uptake inhibitor, VDM11, or SR141716A alone neither affected lesion volumes at day 0 nor at day 7 compared to control animals (van der Stelt, *et al.*, 2001a,b).

The protective effects of modulation of the endocannabinoid system after stroke have also been investigated. CB_1 receptor expression was enhanced in the arterial boundary zone of the cortical mantle after a 20 min occlusion of the middle cerebral artery in rats (Jin *et al.*, 2000). Chronic Δ^9-THC administration reduces the impact of an ischemic insult evoked by lowering blood pressure and 12 min bilateral carotid artery occlusion (Louw *et al.*, 2000). The mixed CB_1/CB_2 cannabinoid receptor agonist WIN55,212-2 afforded protection to hippocampal and cortical neurons in CB_1 receptor-dependent manner in rats with a permanent middle cerebral artery occlusion or global ischemia (Nagayama *et al.*, 1999). No increase in infarct volume upon application of SR141716 was found in these global and focal ischemia models, which suggests that there is no endogenous protection of endocannabinoids (Nagayama, *et al.*, 1999). In gerbils subjected to transient global ischemia pretreatment with the CB_1 receptor agonist CP55,940 reduced ischemia-induced hyperlocomotion and improved electroencephalographic (EEG) spectral power after 24h, which lasted for at least 7 days (Braida *et al.*, 2000). Recently, it was demonstrated that a novel CB_1/CB_2 receptor agonist, BAY38-7271, also exhibited neuroprotective properties. It could reduce infarct volume in rats when applied with a 3-h delay after the induction of a subdural hematoma and in rats with a permanent middle cerebral artery-occlusion (Mauler *et al.*, 2002).

Although exogenously applied (endo)cannabinoids are able to prevent neuronal death, endogenously released endocannabinoids do not seem to be able to reduce neuronal damage via activation of the CB_1 receptor (see above). Endogenously released endocannabinoids may

even be toxic to neurons (Hansen *et al.*, 2002). It was demonstrated that SR141716A-pretreatment of neonatal rats prior to an unilateral intrastriatal microinjection of NMDA evoked a robust neuroprotective response by reducing the ipsilateral infarct area and the number of degenerating cortical neurons (Hansen, *et al.*, 2002). This effect was abolished by co-injection of WIN55,212-2, indicating that blockade of cannabinoid receptor activity inhibited caudal propagation of the excitotoxic response. In another study, CB_1 receptor antagonists were also protective, but not WIN55,212-2, when the middle cerebral artery in rats was occluded for 2 h (Muthian and Hillard, 2000).

Several reasons may explain the apparent paradox that exogenous application of (endo)cannabinoids may protect the brain in a CB_1-dependent manner, while endogenously released cannabinoid may not. (1) The neurodegenerative insult may not always lead to an up-regulation of endocannabinoid biosynthesis, the occurrence of which seems to be dependent on the species and on the type of injury (see above) (Hansen *et al.*, 2001a,b; Panikashvili *et al.*, 2001; van der Stelt *et al.*, 2001b). (2) Neuroprotective action of endocannabinoids may result from actions via molecular targets distinct from CB_1 receptors (see above). For example, novel yet-unidentified cannabinoid receptors may also reduce glutamatergic transmission (Breivogel *et al.*, 2001; Hajos *et al.*, 2001) (3) Presynaptic CB_1 receptors are ineffective against an exogenously induced glutamate receptor stimulation (Shen and Thayer; 1998a; van der Stelt, *et al.*, 2001b; Hansen *et al.*, 2002). (4) The ability of endocannabinoids to influence downstream effects of increased Ca^{2+} concentrations is also dependent on the cell type, strength, duration, and stimulus type (Hampson *et al.*, 1998a; Netzeband *et al.*, 1999). (5) The inflicted damage in the *in vivo* models of acute neuronal injury might be too severe, which leads to a loss of CB_1 receptor mRNA expression and ligand binding capacity (Hansen *et al.*, 2001b). (6) CB_1 receptor-induced altered GABAergic transmission may be involved in the degenerative process. (7) By acting on non CB_1/CB_2 receptors, endocannabinoids may exert a neurotoxic effect that partially masks the tonic neuroprotective effect mediated by cannabinoid receptors. For example, anandamide has been shown to induce cell death by activation of vanilloid receptors (Maccarrone *et al.*, 2000; Grant *et al.*, 2002). (8) Activation of cannabinoid receptors may have a different impact on the biochemical pathways underlying apoptosis na necrosis. In fact, cannabinoid receptor activation has been shown to induce apoptosis (Chan *et al.*, 1998; Downer *et al.*, 2001; Guzman *et al.*, 2001). Anti-apoptotic strategies have been identified as potentially beneficial in limiting ischemic neuronal injury (Mattson *et al.*, 2001), which might imply that CB_1 receptor antagonism might also be beneficial in neurodegenerative insults in which apoptosis is the major cause of death. However, it is unclear whether endocannabinoids induce apoptosis during acute neuronal injury.

THE ENDOCANNABINOID SYSTEM IN SLOWLY PROGRESSING NEURODEGENERATION

The excitotoxicity hypothesis is also used to explain the common biochemical basis of neuronal death caused by many chronic neurodegenerative disorders such as amyotrophic lateral sclerosis, Parkinson's, Huntington's and Alzheimer's diseases (Dirnagl *et al.*, 1999 Doble, 1999; Nicotera *et al.*, 1999). Recently, a number of studies have appeared in which the relationship between the endocannabinoid system and these diseases has been investigated (See also reviews by Fernandez-Ruiz *et al.* (2002) and Glass (2001)). These studies were aimed at finding evidence at a molecular level to verify the anecdotal reports and small-scale clinical trials in which marijuana and cannabinoids were tested to alleviate symptoms of multiple sclerosis, Parkinson's and Huntington's diseases. These studies characterized the endocannabinoid system in patient *post-mortem* material and in animal models of the diseases. Most of the animal models used were capable of displaying one or more aspects of the symptoms of the diseases (face validity), whereas a few provided also a biochemical substrate for studying the etiology of the disease (construct validity), *e.g.* genetic models such as huntingin overexpressing mice. Therefore, relatively little is known about the involvement of the endocannabinoid system in the pathogenesis of these neurodegenerative diseases.

Huntington's Disease

Huntington's disease (HD) is an autosomal-dominant disorder in which a mutation in a gene located on chromosome 4 (4p16.3), its product is named huntingin (IT-15), is responsible for an unstable expansion in a poly-CAG repeat, and the development of progressive involuntary choreiform movements and cognitive deficits. The functions of the normal and mutated gene are still unknown, but expression of the mutated form in early and pre-symptomatic cases of HD leads to selective loss of GABA/enkephalin-containing medium spiny neurons projecting from the caudate putamen to the globus pallidus externa, and GABA/Substance P-containing striatal neurons projecting to the substantia nigra, while there is a relative sparing of GABA/Substance P-neurons project-

ing to the globus pallidus interna. However, in severe grades of HD all striatal projections show extensive loss. The progression of the disease and the age of onset are dependent on the number of the CAG-copy numbers (Feigin and Zgaljardic, 2002)

In *post mortem* brains of patients with HD a relative loss of CB_1 and dopamine receptors was observed through several grades of pathology in the globus pallidus and substantia nigra (Glass *et al.*, 2000). Within the globus pallidus CB_1 receptor binding was highly decreased in the globus pallidus externa in the early HD cases, which exceeded the loss of binding in the globus pallidus interna. In the latter region, the loss of CB_1 receptor preceded the terminal atrophy, because it occurred before the loss of co-localized dopamine receptors was observed. Interestingly, a cell-specific and time-dependent regulation of the CB_1 receptor mRNA as a result of the expression of the mutated huntingtin has recently been observed in two different strains of transgenic mouse models of HD (Denovan-Wright and Robertson, 2000; Lastres-Becker *et al.*, 2002a). It was shown that prior to the development of either HD phenotype or neuronal degeneration CB_1 receptor mRNA was down-regulated in the lateral striatum, cortical regions and in a subset of hippocampal neurons in the brain of R6/2 mice. (Denovan-Wright and Robertson, 2000). In HD94 mice, which overexpress a smaller poly-CAG repeat (94 copies) than the R6/2-mice (>115 copies), CB_1 receptor mRNA was also decreased in the caudate putamen, but not in the cerebral cortex and hippocampus (Lastres-Becker *et al.*, 2002a). This transcriptional effect was accompanied by a reduction in receptor levels, as measured with [^{3}H]CP55.940 in the striatum and in its projections areas such as the globus pallidus, entopeduncular nucleus and substantia nigra pars reticulata. The decrease in CB_1 receptor levels was paralleled by a decrease in proenkephalin mRNA, but not in substance P mRNA levels (Lastres-Becker *et al.*, 2002a). The efficacy of receptor activation was only significantly reduced in the globus pallidus, suggesting that there is a difference in vulnerability in the two striatal efferent pathways.

By contrast, in rat models of HD where 3-nitropropionic acid (3-NP), a mitochondrial neurotoxin, is used to produce striatal lesions, the loss of CB_1 receptors was associated with neuronal death (Page *et al.*, 2000; Lastres-Becker *et al.*, 2001b). CB_1 receptor binding sites were lost and the CB_1 receptor-mediated activation of GTP-binding proteins were reduced in the selectively damaged striatal GABAergic efferent neurons (Lastres-Becker *et al.*, 2001b). Enkephalin- and substance P-containing neurons were equally affected. These changes were accompanied by a decrease of anandamide and 2-AG levels in the striatum, whereas normal endocannabinoid levels were found in the non-lesioned cerebral cortex (Lastres-Becker *et al.*, 2001a). At the moment it is unclear to which extent these observations contribute to the symptomology of HD, or whether they are side effects of toxin-induced destruction of striatal GABAergic neurons (Lastres-Becker *et al.*, 2001b; 2002b).

It is speculated that the early down regulation of cannabinoid receptors is a compensatory mechanism in HD, because it might increase GABA release, which could counteract the initial loss of GABAergic neurons (Glass, 2001). This hypothesis has not been verified yet. If the hypothesis is valid, blockade of CB_1 receptor function should be able to slow down the progression of HD. On the other hand, it may also be speculated that the early loss of CB_1 receptors in the striatum results in an imbalanced glutamatergic transmission, thereby eliciting excitotoxicity and subsequent neurodegeneration. As yet, no CB_1 receptor agonists have been tested to prevent neurodegeneration in animal models of HD. It was shown that the anandamide uptake inhibitor AM404 could attenuate motor disturbances in the early phase of hyperactivity in 3-NP treated rats by restoring GABA and dopamine transmission (Lastres-Becker *et al.*, 2002c). At the moment the mechanism of action of AM404 is not clear, because apart from being an anandamide uptake inhibitor, AM404 has also been shown to act as a full agonist of the VR_1 receptor, thereby modulating neurotransmission and motor behaviour (Zygmunt P.M. *et al.*, 2000). To date, small clinical trials using cannabidiol or nabilone have failed to ameliorate the symptoms of HD patients (Glass, 2001; Fernandez-Ruiz *et al.*, 2002).

Parkinson's Disease

Parkinson's disease (PD) is a chronic, progressive disorder of late life, which is characterized by rigidity, unintentional tremor, and bradykinesia. There is a selective degeneration of dopaminergic neurons in the nigrostriatal pathway, which is thought to be related to their particular vulnerability to oxidative stress. The resulting dopamine deficiency in the caudate- putamen leads to imbalances in the basal ganglia physiology, which include an overactivation of the indirect pathway, *i.e.* increased GABAergic transmission in the globus pallidus. Increased activation of the GABAergic neurons in the globus pallidus is thought to contribute to the symptoms of Parkinson's disease (Blandini *et al.*, 2000)

In *post-mortem* brains of PD patients, treated chronically with L-DOPA, there was an increased binding of [^{3}H]CP55,940, a selective CB_1 receptor agonist, in the caudate-putamen, whereas an increased G-protein coupling was also observed in the lateral globus pallidus and

in the substantia nigra (Lastres-Becker *et al.*, 2001a). CB_1 receptor mRNA expression has also been studied in different animal models of PD, but yielded varying results. CB_1 receptor mRNA levels in cell bodies of striatal efferent neurons were markedly increased in rats unilaterally injected with 6-hydroxydopamine (6-OHDA) to deplete dopamine, but this did not result in changes in CB_1 receptor binding capacity and activation of intracellular signal transduction mechanisms (Mailleux and Vanderhaeghen, 1993; Romero *et al.*, 2000). Zeng *et al.* (1999) also observed increased striatal CB_1 mRNA in 6-OHDA-lesioned rats, but only after chronic L-DOPA treatment. By contrast, CB_1 receptor transcription was increased in an 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) marmoset model of PD, and the mRNA levels returned to control values upon chronic L-DOPA treatment (Lastres-Becker *et al.*, 2001a). Decreased striatal CB_1 receptor mRNA levels were found in reserpine-treated rats, an acute neurotransmitter-depletion model of PD (Silverdale *et al.*, 2001). In summary, there seems to be a dopamine transmission-controlled mRNA expression of the CB_1 receptor, but its functional consequences remain to be clarified.

Presently, it is thought that an increased endocannabinoid tonus in the globus pallidus contributes to the symptoms of PD (Di Marzo *et al.*, 2000). It was shown that intrapallidal administration of cannabinoids reduced the uptake of GABA from striatopallidal terminals and voluntary movement, thereby reproducing PD-like symptoms (Maneuf *et al.*, 1996a,b). Agonist stimulation of CB_1 receptors increased catalepsy produced by administration of dopamine receptor antagonists (Anderson *et al.*, 1996) and reduced the anti-parkinson-like actions of D_2 dopamine receptor agonists (Maneuf *et al.*, 1997; Rodriguez de Fonseca *et al.*, 2001). This was in line with the finding of increased 2-AG levels in the globus pallidus of reserpine-treated rats. Furthermore, SR141716A increased the efficacy of anti-parkinson-like effects of dopamine receptor agonists in these rats. In MPTP-treated primates SR141716A was also therapeutically favorable by reducing L-DOPA-induced dyskinesia (Brotchie, 1998). However, it should be noted that according to recent studies with non-human primates, SR141716A failed to alleviate the parkinson-like symptoms (Meschler *et al.*, 2000; 2001). Furthermore, it has recently been shown that the enhanced corticostriatal glutamatergic transmission in 6-OHDA-lesioned animals could be attenuated by stimulation of the endocannabinoid system (i.e. by application of the CB_1 receptor agonist HU-210, uptake inhibitors AM404 and VDM11, and FAAH-inhibitor methylarachidonoylfluorophosphonate) (Gubellini *et al.*, 2002). An increased striatal level of anandamide, but not of 2-AG, was found in the lesioned animals, which was paralleled by an abnormal downregulation of anandamide membrane transport and FAAH-activity, without any significant changes in anandamide binding to CB_1 receptors. It was suggested that the elevated anandamide levels were a compensatory mechanism to reduce the increased cortical glutamatergic transmission and that inhibition of anandamide hydrolysis might represent a possible target to decrease the glutamatergic drive in PD (Gubellini *et al.*, 2002). However, in an *in vivo* study unilaterally pallidal or striatal infusions of CP55,940 did not alter motor behavioral effects in control rats compared to rats receiving 6-OHDA (Sanudo-Pena *et al.*, 1998). Differences in dose-regimen, methods to deplete dopamine, and species differences in endogenous cannabinoids levels might explain the differences between various rodent and primate models.

Presently, only two small-scale clinical trials have shown that marijuana was ineffective in reducing Parkinsonian tremor, but recently a randomized, double blind, placebo-controlled, cross-over trial with the cannabinoid receptor agonist nabilone showed an improvement in L-DOPA-induced dyskinesia in patients with PD (Sieradzan *et al.*, 2001). This obviously contrasts the animal studies that suggest that CB_1 receptor antagonists may be used to reduce the adverse actions of L-DOPA. Further research is therefore warranted to understand the involvement of the endocannabinoid system in human basal ganglia physiology. In addition, relatively little is known about the involvement of the endocannabinoid system in the pathogenesis of PD. Cannabinoids might be useful to slow down neurodegeneration in PD, because different cannabinoids have anti-oxidative properties (Hampson *et al.*, 1998b) and are able to reduce excitotoxicity (see above). However, CB_1 receptor agonists have yet not been tested in animal models of PD to establish whether they are able to reduce PD-associated neurodegeneration.

Multiple Sclerosis

Multiple Sclerosis (MS) is a chronic neurological disorder caused by demyelination of neurons in the CNS. Any part of the white matter in the CNS may be affected, thereby producing highly variable and disseminating neurological symptoms, such as visual loss, cognitive impairment, motor weakness, sensory loss, tremor and spasticity. The disease has an age-onset between 20 and 40 and is characterized by relapses and remission, which may proceed into a progressive form. The etiology of MS remains unknown, but it is thought that the immune system is primed to attack the myelin of the CNS (Rieckmann and Smith, 2001). Noteworthy, it is recent-

ly proposed that glutamate excitotoxicity is an important mechanism in autoimmune demyelination (Pitt *et al.*, 2000).

It has been demonstrated in a Lewis rat model of experimental allergic encephalomyelitis (EAE), which is often used to mimic the symptoms of MS in animals, that CB_1 receptor mRNA and [^{3}H]CP55,940 binding was downregulated in the caudate-putamen and in both deep and superficial layers of the cerebral cortex, but not in the hippocampus, limbic structures or cerebellum (Berrendero *et al.*, 2001). The decrease in striatal CB_1 receptor mRNA was not accompanied by reduced [^{3}H]CP55,940 binding in the nuclei of striatal output neurons, whereas it was paralleled by a decrease in the caudate-putamen itself. This suggested that changes in CB_1 receptor transcription and binding capacity were located on cell bodies of striatal neurons. Also, the remaining striatal and cortical CB_1 receptors were more efficiently G-protein coupled. In combination, the changes were not directly correlated to brain areas that suffered from demyelination. Thus, it is unknown whether these alterations are associated to the pathogenesis of the disease. It was suggested that the changes in CB_1 receptors may act as a compensatory mechanism and may be related to the alleviation of some motor signs observed after the treatment with cannabinoid receptor agonists (Berrendero *et al.*, 2001).

It has been shown that THC was able to inhibit the progression of EAE in rodents. The presumed mode of protection was its immunosuppressive action rather than a direct effect on paralysis itself (Lyman *et al.*, 1989; Wirguin *et al.*, 1994). However, recent studies indicate that Δ^9-THC as well as synthetic cannabinoids can ameliorate both tremor and spasticity in mice with chronic relapsing experimental allergic encephalomyelitis (CREAE) via CB_1 and in part via CB_2 receptors (Baker *et al.*, 2000). The transient enhancement of tremor and spasticity by cannabinoid receptor antagonists suggested that there was an endogenous endocannabinoid tonus controlling these symptoms in the CREAE model. Indeed, in the brain and spinal cord of spastic mice, increased levels of anandamide, 2-AG and *N*-palmitoylethanolamine were found, whereas comparable levels of these compounds were found in normal and non-spastic mice (Baker *et al.*, 2001). In addition, it was demonstrated that exogenous anandamide, 2-AG, *N*-palmitoylethanolamine, and arvanil (a mixed CB_1/VR_1-receptor agonist) ameliorated spasticity (Baker *et al.*, 2001; Brooks *et al.*, 2002). In line with these observations, inhibitors of endocannabinoid hydrolysis and uptake could ameliorate spasticity to an extent comparable with that observed with potent synthetic cannabinoid receptor agonists.

Currently, the precise mechanisms of action of (endo)cannabinoids, *N*-palmitoylethanolamine, and arvanil have not been resolved. It is suggested that arvanil may act through novel sites of action different from cannabinoid and vanilloid receptors (Brooks *et al.*, 2002). Furthermore, the relative role of the CB_1 and CB_2 receptors in the reduction of tremor and spasticity needs clarification. The putative involvement of the CB_2 receptor in the amelioration of spasticity, as demonstrated by the selective CB_2 receptor agonist JHW133 and antagonist SR144528, is surprising, because the CB_2 receptor is mainly found in cells of the immune system. The presence of these receptors on microglia and brain resident mast cells (Carlisle *et al.*, 2002) might be an interesting starting point to study the involvement of the CB_2 receptor in the reduction of spasticity without induction of psychotropic side-effects. Since it has been shown that glutamate excitotoxicity contributes to clinical symptoms and cell death of oligodendrocytes in a mice EAE-model of MS (Pitt *et al.*, 2000), it is tempting to suggest that the amelioration of the symptoms in AEA-models by (endo)cannabinoids is due to their anti-excitotoxic properties (see above). Altogether, data from experimental animals do provide a scientific basis for the anecdotal reports in which MS patients alleviate their symptoms by using marijuana, but results from small- and short-term clinical trials have been equivocal (Killestein *et al.*, 2002).

CONCLUSIONS AND PERSPECTIVES

Recent developments, such as the generation of CB_1 and CB_2 receptor knock-out mice and the synthesis of selective cannabinoid receptor antagonists/reverse agonists have provided insight into some (patho)physiological roles of the endocannabinoid system. However, some important fundamental aspects of the endogenous cannabinoid system remain to be elucidated. For example, the proteins responsible for the biosynthesis of endocannabinoids and their transport into cells remain to be isolated and cloned. The regulation of the biosynthetic and inactivation pathways of anandamide and 2-AG is also largely unknown. It is likely that novel cannabinoid receptor subtypes, as well as novel endogenous ligands, will be found. The understanding of the complex interplay of the endocannabinoid system with other neurotransmitters in the CNS and their function as retrograde messengers will greatly enhance our knowledge about the physiological roles of the endocannabinoid system. This may provide useful information to exploit the cannabinoid system for therapeutic intervention in various diseases.

The studies reported in this review indicate that (par-

tial) CB_1 receptor agonists might prove to be useful to improve the therapeutic outcome after acute brain damage and slow down the gradual progression of slowly neurodegenerative diseases such as HD, PD and MS. However, the involvement of the endocannabinoid system in the pathogenesis of neurodegenerative diseases awaits further clarification, ligands of the cannabinoid receptors and compounds, which interfere with endocannabinoid biosynthesis and inactivation may be useful to alleviate symptoms of the neurodegenerative diseases. To date, there is a lack of data from human (*post-mortem*) studies and small clinical trials directed at amelioration of the symptoms of various neurodegenerative diseases have not proven to be successful yet. The route of administration of the cannabinoids, their psychotropic activity and possible pro-degenerative effects (*e.g.* induction of apoptosis) are other factors that deserve further attention, before compounds which modulate the activity of the endocannabinoid system are therapeutically useful in the treatment of acute and slowly progressive neurodegenerative diseases.

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References

Abood ME, G Rizvi, N Sallapudi and SD McAllister (2001) Activation of the CB(1) Cannabinoid receptor protects cultured mouse spinal neurons against excitotoxicity. *Neurosci. Lett.* **309,** 197-201.

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

Anderson JJ, AM Kask and TN Chase (1996) Effects of cannabinoid receptor stimulation and blockade on catalepsy produced by dopamine receptor antagonists. *Eur. J. Pharmacol.* **295,** 163-168.

Andersson M, SO Jacobsson, KO Jonsson, G Tiger and CJ Fowler (2000) Neurotoxicity of glutamate in chick telencephalon neurons: reduction of toxicity by preincubation with carbachol, but not by the endogenous fatty acid amides anandamide and palmitoylethanolamide. *Arch. Toxicol.* **74,** 161-164.

Arreaza G, WA Devane, RL Omeir, G Sajnani, J Kunz, BF Cravatt and DG Deutsch (1997) The cloned rat hydrolytic enzyme responsible for the breakdown of anandamide also catalyzes its formation via the condensation of arachidonic acid and ethanolamine. *Neurosci. Lett.* **234,** 59-62.

Baker D, G Pryce, JL Croxford, P Brown, RG Pertwee, JW Huffman and L Layward (2000) Cannabinoids control spasticity and tremor in a multiple sclerosis model. *Nature* **404,** 84-87.

Baker D, G Pryce, JL Croxford, P Brown, RG Pertwee, A Makriyannis, A Khanolkar, L Layward, F Fezza, T Bisogno and V Di Marzo (2001) Endocannabinoids control spasticity in a multiple sclerosis model. *FASEB J.* **15,** 300-302.

Berdyshev EV, PC Schmid, Z Dong and HHO Schmid (2000) Stress-induced generation of *N*-acylethanolamines in mouse epidermal JB6 P^+ cells. *Biochem. J.* **346,** 369-374.

Berrendero F, A Sanchez, A Cabranes, C Puerta, JA Ramos A Garcia-Merino and J Fernandez-Ruiz (2001) Changes in cannabinoid CB(1) receptors in striatal and cortical regions of rats with experimental allergic encephalomyelitis, an animal model of multiple sclerosis. *Synapse* **41,** 195-202.

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

Bisogno T, F Berrendero, G Ambrosino, M Cebeira, JA Ramos, JJ Fernández-Ruiz and V Di Marzo (1999a) Brain regional distribution of endocannabinoids: implications for their biosynthesis and biological function. *Biochem. Biophys. Res. Commun.* **256,** 377-380.

Bisogno T, D Melck, L De Petrocellis and V Di Marzo (1999b) Phosphatidic acid as the biosynthetic precursor of the endocannabinoid 2-arachidonoylglycerol in intact mouse neuroblastoma cells stimulated with ionomycin. *J. Neurochem.* **72,** 2113-2119.

Blandini F, G Nappi, C Tassorelli and E Martignoni (2000) Functional changes of the basal ganglia circuitry in Parkinson's disease. *Prog. Neurobiol.* **62**, 63-88

Braida D, M Pozzi and M Sala (2000) CP 55,940 protects against ischemia-induced electroencephalographic flattening and hyperlocomotion in Mongolian gerbils. *Neurosci. Lett.* **296,** 69-72.

Breivogel CS, G Griffin, V Di Marzo and BR Martin (2001) Evidence for a new G protein-coupled cannabinoid receptor in mouse brain. *Mol. Pharmacol.* **60,** 155-163.

Brooks JW, G Pryce, T Bisogno, SI Jaggar, DJ Hankey, P Brown, D Bridges, C Ledent, M Bifulco, AS Rice, V Di Marzo and D Baker (2002) Arvanil-induced inhibition of spasticity and persistent pain: evidence for therapeutic sites of action different from the vanilloid VR1 receptor and cannabinoid CB(1)/CB(2) receptors. *Eur. J. Pharmacol.* **439,** 83-92.

Brotchie JM (1998) Adjuncts to dopamine replacement: a pragmatic approach to reducing the problem of dyskinesia in Parkinson's disease. *Mov. Disord.* **13,** 871-876.

Cadas H, S Schinelli and D Piomelli (1996a) Membrane localization of *N*-acylphosphatidylethanolamine in central neurons: studies

with exogenous phospholipases. *J. Lipid Mediat. Cell Signal.* **14**, 63-70.

Cadas H, S Gaillet, M Beltramo, L Venance and D Piomelli (1996b) Biosynthesis of an endogenous cannabinoid precursor in neurons and its control by calcium and cAMP. *J. Neurosci.* **16,** 3934-3942.

Cadas H, E di Tomaso and D Piomelli (1997) Occurrence and biosynthesis of endogenous cannabinoid precursor, *N*-arachidonoyl phosphatidylethanolamine, in rat brain. *J. Neurosci.* **17,** 1226-1242.

Calignano A, G La Rana, A Giuffrida and D Piomelli (1998) Control of pain initiation by endogenous cannabinoids. *Nature* **394,** 277-281.

Carlisle SJ, F Marciano-Cabral, A Staab, C Ludwick and GA Cabral (2002) Differential expression of the CB2 cannabinoid receptor by rodent macrophages and macrophage-like cells in relation to cell activation. *Int. Immunopharmacol.* **2**, 69-82.

Chan GC, TR Hinds, S Impey and DR Storm (1998) Hippocampal neurotoxicity of Delta9-tetrahydrocannabinol. *J. Neurosci.* **18,** 5322-5332.

Craib SJ, HC Ellington, RG Pertwee and RA Ross (2001) A possible role of lipoxygenase in the activation of vanilloid receptors by anandamide in the guinea-pig bronchus. *Br. J. Pharmacol.* **134,** 30-37.

Davies SN, RG Pertwee and G Riedel (2002) Functions of cannabinoid receptors in the hippocampus. *Neuropharmacol.* **42,** 993-1007.

De Petrocellis L, T Bisogno, M Maccarrone, JB Davis, A Finazzi-Agro and V Di Marzo (2001) The activity of anandamide at vanilloid VR_1 receptors requires facilitated transport across the cell membrane and is limited by intracellular metabolism. *J. Biol. Chem.* **276,** 12856-12863.

Denovan-Wright EM and HA Robertson (2000) Cannabinoid receptor messenger RNA levels decrease in a subset of neurons of the lateral striatum, cortex and hippocampus of transgenic Huntington's disease mice. *Neuroscience* **98,** 705-713.

Deutsch DG, ST Glaser, JM Howell, JS Kunz, RA Puffenbarger, CJ Hillard and N Abumrad (2000) The cellular uptake of anandamide is coupled to its breakdown by fatty acid amide hydrolase (FAAH). *J. Biol. Chem.* **276,** 6967-6773.

Deutsch J, SI Rapoport and AD Purdon (1997) Relation between free fatty acid and acyl-CoA concentrations in rat brain following decapitation. *Neurochem. Res.* **22,** 759-765.

Devane WA, L Hanus, A Breuer, RG Pertwee, LA Stevenson, G Griffin, D Gibson, A Mandelbaum, A Etinger and R Mechoulam (1992) Isolation and structure of a brain constituent that binds to the cannabinoid receptor. *Science* **258,** 1946-1949.

Di Marzo V, A Fontana, H Cadas, S Schinelli, G Cimino, J-C Schwartz and D Piomelli (1994) Formation and inactivation of endogenous cannabinoid anandamide in central neurons. *Nature* **372,** 686-691.

Di Marzo V, D Melck, T Bisogno and L De Petrocellis (1999) Endocannabinoids: endogenous cannabinoid receptor ligands with neuromodulatory action. *Trends Neurosci.* **22,** 80.

Di Marzo V, MP Hill, T Bisogno, AR Crossman and JM Brotchie (2000) Enhanced levels of endogenous cannabinoids in the globus pallidus are associated with a reduction in movement in an animal model of Parkinson's disease. *FASEB J.* **14,** 1432-1438.

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

Dinh TP, D Carpenter, FM Leslie, TF Freund, I Katona, SL Sensi, S Kathuria and D Piomelli (2002) Brain monoacylglyceride lipase participating in endocannabinoid inactivation. *Proc. Natl. Acad. Sci. USA* **99**, 10819-10824**.**

Dirnagl U, C Iadecola and MA Moskowitz (1999) Pathobiology of ischaemic stroke: an integrated view. *Trends Neurosci.* **22,** 391-397.

Doble A (1999) The role of excitotoxicity in neurodegenerative disease: implications for therapy. *Pharmacol. Ther.* **81,** 163-221.

Downer E, B Boland, M Fogarty and V Campbell (2001) Δ^9-tetrahydrocannabinol induces the apoptotic pathway in cultured cortical neurones via activation of the CB_1 receptor. *NeuroReport* **12,** 3973-3978.

Egertova M, DK Giang, BF Cravatt and MR Elphick (1998) A new perspective on cannabinoid signalling: complementary localization of fatty acid amide hydrolase and the CB1 receptor in rat brain. *Proc. R Soc. Lond. [Biol]* **265,** 2081-2085.

Facci L, R Dal Toso, S Romanello, A Buriani, SD Skaper and A Leon (1995) Mast cells express a peripheral cannabinoid receptor with differential sensitivity to anandamide and palmitoylethanolamide. *Proc. Natl. Acad. Sci. USA* **92,** 3376-3380.

Farooqui AA, LA Horrocks and T Farooqui (2000) Glycerophospholipids in brain: their metabolism, incorporation into membranes, functions, and involvement in neurological disorders. *Chem. Phys. Lipids* **106,** 1-29.

Feigin A and D Zgaljardic (2002) Recent advances in Huntington's disease: implications for experimental therapeutics. *Curr. Opin. Neurol.* **15**, 483-489.

Felder C, A Nielsen, EM Briley, M Palkovits, J Priller, J Axelrod, DN Nguyen, JM Richardson, RM Riggin, GA Koppel, SM Paul and GW Becker (1996) Isolation and measurement of the endogenous cannabinoid receptor agonist, anandamide, in brain and peripheral tissues of human and rat. *FEBS Lett.* **393,** 231-235.

Fernandez-Ruiz J, I Lastres-Becker, A Cabranes, S Gonzalez and JA Ramos (2002) Endocannabinoids and basal ganglia functionality. *Prostagl.Leukotr. Essent. Fatty Acids* **66,** 257-267.

Fowler CJ, K-O Jonsson and G Tiger (2001) Fatty acid amide hydrolase: biochemistry, pharmacology, and therapeutic possibilities for an enzyme hydrolyzing anandamide, 2-arachidonoylglycerol, palmitoylethanolamide, and oleamide. *Biochem. Pharmacol.* **62**, 517-526.

Fowler CJ and SOP Jacobsson (2002) Cellular transport of anandamide, 2-arachidonoylglycerol, and palmitoylethanolamide – targets for drug development? *Prostagl. Leukotr. Essent. Fatty Acids* **66**, 193-200.

Frykholm P, L Hillered, B Långström, L Persson, J Valtysson, Y Watanabe and P Enblad (2001) Increase of interstitial glycerol reflects the degree of ischaemic brain damage: a PET and microdialysis study in a middle cerebral artery occlusion-reperfusion primate model. *J. Neurol. Neurosurg. Psychiatr.* **71,** 455-461.

Gallily R, A Breuer and R Mechoulam (2000) 2-Arachidonylglycerol, an endogenous cannabinoid, inhibits tumor necrosis factor-alpha production in murine macrophages, and in mice. *Eur. J. Pharmacol.* **406,** R5-R7.

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

Giuffrida A, LH Parsons, TM Kerr, F Rodriguez de Fonseca, M Navarro and D Piomelli (1999) Dopamine activation of endogenous cannabinoid signaling in dorsal striatum. *Nat. Neurosci.* **2,** 358-363.

Glass M (2001) The role of cannabinoids in neurodegenerative diseases. *Prog. Neuropsychopharmacol. Biol. Psychiatr.* **25,** 743-765.

Glass M, M Dragunow and RL Faull (2000) The pattern of neurodegeneration in Huntington's disease: a comparative study of cannabinoid, dopamine, adenosine and GABA(A) receptor alterations in the human basal ganglia in Huntington's disease. *Neuroscience* **97**, 505-519.

Grant ER, AE Dubin, SP Zhang, RA Zivin and Z Zhong (2002) Simultaneous intracellular calcium and sodium flux imaging in human vanilloid receptor 1 (VR1)-transfected human embryonic

kidney cells: a method to resolve ionic dependence of VR1-mediated cell death. *J. Pharmacol. Exp. Ther.* **300,** 9-17.

Grundy RI, M Rabuffetti and M Beltramo (2001) Cannabinoids and neuroprotection. *Mol. Neurobiol.* **24,** 29-51.

Gubellini P, B Picconi, M Bari, N Battista, P Calabresi, D Centonze, G Bernardi, A Finazzi-Agrò and M Maccarrone (2002) Experimental Parkinsonism alters endocannabinoid degradation: implications for striatal glutamatergic transmission. *J. Neurosci.* **22**, 6900-6907.

Guzman M, C Sanchez and I Galve-Roperh (2001) Control of the cell survival/death decision by cannabinoids. *J. Mol. Med.* **78**, 613-625.

Hajos N, C Ledent and TF Freund (2001) Novel cannabinoid-sensitive receptor mediates inhibition of glutamatergic synaptic transmission in the hippocampus. *Neuroscience* **106,** 1-4.

Hampson AJ and M Grimaldi (2001) Cannabinoid receptor activation and elevated cyclic AMP reduce glutamate neurotoxicity. *Eur. J. Neurosci.* **13,** 1529-1536.

Hampson RE, TC Foster and SA Deadwyler (1989) Effects of delta-9-tetrahydrocannabinol on sensory evoked hippocampal activity in the rat: principal components analysis and sequential dependency. *J. Pharmacol. Exp. Ther.* **251,** 870-877.

Hampson AJ, LM Bornheim, M Scanziani, CS Yost, AT Gray, BM Hansen, DJ Leonoudakis and PE Bickler (1998a) Dual effects of anandamide on NMDA receptor-mediated responses and neurotransmission. *J. Neurochem.* **70,** 671-676.

Hampson AJ, M Grimaldi, J Axelrod and D Wink (1998b) Cannabidiol and (-)Delta9-tetrahydrocannabinol are neuroprotective antioxidants. *Proc. Natl. Acad. Sci. USA* **95,** 8268-8273.

Hansen HS, L Lauritzen, AM Strand, AM Vinggaard, A Frandsen and A Schousboe (1997) Characterization of glutamate-induced formation of *N*-acylphosphatidylethanolamine and *N*-acylethanolamine in cultured neocortical neurons. *J. Neurochem.* **69,** 753-761.

Hansen HS, B Moesgaard, HH Hansen, A Schousboe and G Petersen (1999) Formation of *N*-acyl-phosphatidylethanolamine and *N*-acylethanolamine (including anandamide) during glutamate-induced neurotoxicity. *Lipids* **34,** S327-S330.

Hansen HS, B Moesgaard, HH Hansen and G Petersen (2000) *N*-acylethanolamines and precursors – relation to cell injury. *Chem. Phys. Lipids* **108,** 135-150.

Hansen HH, C Ikonomidou, P Bittigau, SH Hansen and HS Hansen (2001a) Accumulation of the anandamide precursor and other *N*-acylethanolamine phospholipids in infant rat models of *in vivo* necrotic and apoptotic neuronal cell death. *J. Neurochem.* **76,** 39-46.

Hansen HH, PC Schmid, P Bittigau, I Lastres-Becker, F Berrendero, J Manzanares, C Ikonomidou, HHO Schmid, JJ Fernández-Ruiz and HS Hansen (2001b) Anandamide, but not 2-arachidonoylglycerol, accumulates during *in vivo* neurodegeneration. *J. Neurochem.* **78,** 1415-1427.

Hansen HH, I Azcoitia, S Pons, J Romero, LM Garcia-Segura, JA Ramos, HS Hansen and J Fernandez-Ruiz (2002) Blockade of cannabinoid CB(1) receptor function protects against in vivo disseminating brain damage following NMDA-induced excitotoxicity. *J. Neurochem.* **82,** 154-158.

Hanus L, A Gopher, S Almog and R Mechoulam (1993) Two new unsaturated fatty acid ethanolamides in brain that bind to the cannabinoid receptor. *J. Med. Chem.* **36,** 3032-3034.

Hanus L, S Abu-Lafi, E Fride, A Breuer, Z Vogel, DE Shalev, I Kustanovich and R Mechoulam (2001) 2-arachidonoyl glyceryl ether, an endogenous agonist of the cannabinoid CB_1 receptor. *Proc. Natl. Acad. Sci. USA* **98,** 3662-3665.

Irving AJ, AA Coutts, J Harvey, MG Rae, K Mackie, GS Bewick and RG Pertwee (2000) Functional expression of cell surface cannabinoid CB_1 receptors on presynaptic inhibitory terminals in cultures rat hippocampal neurons. *Neuroscience* **98,** 253-262.

Jin KL, XO Mao, PC Goldsmith and DA Greenberg (2000) CB_1 cannabinoid receptor induction in experimental stroke. *Ann. Neurol.* **48,** 257-261.

Kerwin JL, AR Tuininga and LH Ericsson (1994) Identification of molecular species of glycerophospholipids and sphingomyelin using electrospray mass spectrometry. *J. Lipid Res.* **35,** 1102-1114.

Killestein J, ELJ Hoogervorst, M Reif, NF Kalkers, AC van Loenen, PGM Staats, RW Gorter, BMJ Uitdehaag and CH Polman (2002) Safety, tolerability, and efficacy of orally administered cannabinoids in MS. *Neurology* **58**, 1404-1407.

Kondo S, H Kondo, S Nakane, T Kodaka, A Tokumura, K Waku and T Sugiura (1998) 2-arachidonoylglycerol, an endogenous cannabinoid receptor agonist: identification as one of the major species of monoacylglycerols in various rat tissues, and evidence for its generation through Ca^{2+}-dependent and -independent mechanisms. *FEBS Lett.* **429,** 152-156.

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

Lambert DG and V Di Marzo (1999) The palmitoylethanolamide and oleamide enigmas: Are these two fatty acid amides cannabimimetic? *Curr. Med. Chem.* **6,** 757-773.

Lastres-Becker I, M Cebeira, ML de Ceballos, BY Zeng, P Jenner, JA Ramos and JJ Fernandez-Ruiz (2001a) Increased cannabinoid CB_1 receptor binding and activation of GTP- binding proteins in the basal ganglia of patients with Parkinson's syndrome and of MPTP-treated marmosets. *Eur. J. Neurosci.* **14,** 1827-1832.

Lastres-Becker I, F Fezza, M Cebeira, T Bisogno, JA Ramos, A Milone, J Fernandez-Ruiz and VD Marzo (2001b) Changes in endocannabinoid transmission in the basal ganglia in a rat model of Huntington's disease. *NeuroReport* **12,** 2125-2129.

Lastres-Becker I, F Berrendero, JJ Lucas, E Martin-Aparicio, A Yamamoto, JA Ramos and JJ Fernandez-Ruiz (2002a) Loss of mRNA levels, binding and activation of GTP-binding proteins for cannabinoid CB (1) receptors in the basal ganglia of a transgenic model of Huntington's disease. *Brain Res.* **929,** 236-242.

Lastres-Becker I, M Gomez, R De Miguel, JA Ramos and J Fernandez-Ruiz (2002b) Loss of cannabinoid CB_1 receptors in the basal ganglia in the late akinetic phase of rats with experimental Huntington's disease. *Neurotoxicity Res.* **4**, 601-608.

Lastres-Becker I, HH Hansen, F Berrendero, R De Miguel, A Perez-Rosado, J Manzanares, JA Ramos and J Fernandez-Ruiz (2002c) Alleviation of motor hyperactivity and neurochemical deficits by endocannabinoid uptake inhibition in a rat model of Huntington's disease. *Synapse* **44,** 23-35.

Lee DP, AS Deonarine.,M Kienetz, QS Zhu, M Skrzypczak, M Chan and PC Choy (2001) A novel pathway for lipid biosynthesis: the direct acylation of glycerol. *J. Lipid Res.* **42,** 1979-1986.

Louw DF, FW Yang and GR Sutherland (2000) The effect of delta-9-tetrahydrocannabinol on forebrain ischemia in rat. *Brain Res.* **857,** 183-187.

Lyman WD, JR Sonett, CF Brosnan, R Elkin and MB Bornstein (1989) Delta 9-tetrahydrocannabinol: a novel treatment for experimental autoimmune encephalomyelitis. *J. Neuroimmunol.* **23,** 73-81.

Maccarrone M, T Lorenzon, M Bari, G Melino and A Finazzi-Agro (2000) Anandamide induces apoptosis in human cells via vanilloid receptors. Evidence for a protective role of cannabinoid receptors. *J. Biol. Chem.* **275,** 31938-31945.

Mackie K, WA Devane and B Hille (1993) Anandamide, an endogenous cannabinoid, inhibits calcium currents as a partial agonist in N18 neuroblastoma cells. *Mol. Pharmacol.* **44,** 498-500.

Maejima T, T Ohno-Shosaku and M Kano (2001) Endogenous cannabinoid as a retrograde messenger from depolarized postsynaptic neurons to presynaptic terminals. *Neurosci. Res.* **40,** 205-

210.

Mailleux P and JJ Vanderhaeghen (1993) Dopaminergic regulation of cannabinoid receptor mRNA levels in the rat caudate-putamen: an *in situ* hybridization study. *J. Neurochem.* **61,** 1705-1712.

Maingret F, AJ Pate, M Lazdunski and E Honore (2001) The endocannabinoid anandamide is a direct and selective blocker of the background K(+) channel TASK-1. *EMBO J.* **20,** 47-54.

Maneuf YP, AR Crossman and JM Brotchie (1996a) Modulation of GABAergic transmission in the globus pallidus by the synthetic cannabinoid WIN 55,212-2. *Synapse* **22,** 382-385.

Maneuf YP, JE Nash, AR Crossman and JM Brotchie (1996b) Activation of the cannabinoid receptor by delta 9-tetrahydrocannabinol reduces gamma-aminobutyric acid uptake in the globus pallidus. *Eur. J. Pharmacol.* **308,** 161-164.

Maneuf YP, AR Crossman and JM Brotchie (1997) The cannabinoid receptor agonist WIN 55,212-2 reduces D_2, but not D_1, dopamine receptor-mediated alleviation of akinesia in the reserpine- treated rat model of Parkinson's disease. *Exp. Neurol.* **148,** 265-270.

Marsicano G, B Moosmann, H Hermann, B Lutz and C Behl (2002) Neuroprotective properties of cannabinoids against oxidative stress: role of the cannabinoid receptor CB1. *J. Neurochem.* **80,** 448-456.

Mattson MP, W Duan, WA Pedersen anc C Culmsee (2001) Neurodegenerative disorders and ischemic brain diseases. *Apoptosis* **6**, 69-81.

Mauler F, J Mittendorf, E Horvath and J De Vry (2002) Characterization of the diarylether sulfonylester (-)-(R)-3-(2-hydroxymethylindanyl-4-oxy)phenyl-4,4,4-trifluoro-1-sulfonate (BAY 38-7271) as a potent cannabinoid receptor agonist with neuroprotective properties. *J. Pharmacol. Exp. Ther.* **302,** 359-368.

Mechoulam R, S Ben-Shabat, L Hanus, M Ligumsky, NE Kaminski, AR Schatz, A Gopher, S Almog, BR Martin, DR Compton, RG Pertwee, G Griffin, M Bayewitch, J Barg J and Z Vogel (1995) Identification of an endogenous 2-monoglyceride, present in canine gut, that binds to cannabinoid receptors. *Biochem. Pharmacol.* **50,** 83-90.

Mechoulam R, D Panikashvili and E Shohami (2002) Cannabinoids and brain injury: therapeutic implications. *Trends Mol. Med.* **8,** 58-61.

Meschler JP, FA Clarkson, PJ Mathews, AC Howlett and BK Madras (2000) D(2), but not D(1) dopamine receptor agonists potentiate cannabinoid- induced sedation in nonhuman primates. *J. Pharmacol. Exp. Ther.* **292,** 952-959.

Meschler JP, AC Howlett and BK Madras (2001) Cannabinoid receptor agonist and antagonist effects on motor function in normal and 1-methyl-4-phenyl-1,2,5,6-tetrahydropyridine (MPTP)-treated non-human primates. *Psychopharmacology (Berl.)* **156,** 79-85.

Mezey É., ZE Tóth, DN Cortright, MK Arzybi, JE Krause, R Elde, A Guo, PM Blumberg and A Szallasi (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.

Moesgaard B, JW Jaroszewski and HS Hansen (1999) Accumulation of *N*-acyl-ethanolamine phospholipids in rat brains during post-decapitative ischemia: a ^{31}P NMR study. *J. Lipid Res.* **40,** 515-521.

Moesgaard B, G Petersen, JW Jaroszewski and HS Hansen (2000) Age-dependent accumulation of *N*-acyl-ethanolamine phospholipids in ischemic rat brain: a ^{31}P NMR and enzyme activity study. *J. Lipid Res.* **41,** 985-990.

Moesgaard B, G Petersen, SA Mortensen and HS Hansen (2002) Substantial species differences in relation to formation and degradation of *N*-acyl-ethanolamine phospholipids in heart tissue: an enzyme activity study. *Comp. Biochem. Physiol. B* **131,** 475-482.

Muthian S and CJ Hillard (2000) CB1 receptor antagonists are neuroprotective in focal cerebral ischemia-reperfusion injury. Symposium on the Cannabinoids (ICRS, Burlington, VT), p 107.

Nagayama T, AD Sinor, RP Simon, J Chen, SH Graham, K Jin and DA Greenberg (1999) Cannabinoids and neuroprotection in global and focal cerebral ischemia and in neuronal cultures. *J. Neurosci.* **19,** 2987-2995.

Natarajan V, PC Schmid, PV Reddy, ML Zuarte-Augustin and HHO Schmid (1983) Biosynthesis of *N*-acylethanolamine phospholipids by dog brain preparations. *J. Neurochem.* **41,** 1303-1312.

Natarajan V, PC Schmid and HHO Schmid (1986) *N*-Acylethanolamine phospholipid metabolism in normal and ischemic rat brain. *Biochim. Biophys. Acta* **878,** 32-41.

Netzeband JG, SM Conroy, KL Parsons and DL Gruol (1999) Cannabinoids enhance NMDA-elicited Ca^{2+} signals in cerebellar granule neurons in culture. *J. Neurosci.* **19,** 8765-8777.

Nicotera P, M Leist and L Manzo (1999) Neuronal cell death: a demise with different shapes. *Trends Pharmacol. Sci.* **20,** 46-51.

Page KJ, L Besret, M Jain, EM Monaghan, SB Dunnett and BJ Everitt (2000) Effects of systemic 3-nitropropionic acid-induced lesions of the dorsal striatum on cannabinoid and mu-opioid receptor binding in the basal ganglia. *Exp. Brain Res.* **130,** 142-150.

Panikashvili D, C Simeonidou, S Ben-Shabat, L Hanus, A Breuer, R Mechoulam and E Shohami (2001) An endogenous cannabinoid (2-AG) is neuroprotective after brain injury. *Nature* **413,** 527-531.

Pertwee RG (1997) Pharmacology of cannabinoid CB1 and CB2 receptors. *Pharmacol. Ther.* **74**, 129-180.

Petersen G and HS Hansen (1999) *N*-acylphosphatidylethanolamine-hydrolysing phospholipase D lacks the ability to transphosphatidylate. *FEBS Lett.* **455,** 41-44.

Pitt D, P Werner and C Raine (2000) Glutamate excitotoxicity in a model of multiple sclerosis. *Nature Med.* **6**, 67-70.

Porter AM, J-M Sauer, MD Knierman, GW Becker, MJ Berna, J Bao, GG Nomikos, P Carter, FP Bymaster, AB Leese and CC Felder (2002) Characterization of a novel endocannabinoid, virodhamine, with antagonist activity at the CB1 receptor. *J. Pharmacol. Exp. Ther.* **301,** 1020-1024.

Rakhshan F, TA Day, RD Blakely and EL Barker (2000) Carrier-mediated uptake of the endogenous cannabinoid anandamide in RBL-2H3 cells. *J. Pharmacol. Exp. Ther.* **292,** 3960-3967.

Rieckmann P and KJ Smith (2001) Multiple sclerosis: more than inflammation and demyelination. *Trends Neurosci.* **24,** 435-437.

Rodriguez de Fonseca F, MA Gorriti, A Bilbao, L Escuredo, LM Garcia-Segura, D Piomelli and M Navarro (2001) Role of the endogenous cannabinoid system as a modulator of dopamine transmission: implications for Parkinson's disease and schizophrenia. *Neurotoxicity Res.* **3**, 23-35.

Romero J, F Berrendero, A Perez-Rosado, J Manzanares, A Rojo, JJ Fernandez-Ruiz, JG de Yebenes and JA Ramos (2000) Unilateral 6-hydroxydopamine lesions of nigrostriatal dopaminergic neurons increased CB1 receptor mRNA levels in the caudate-putamen. *Life Sci.* **66,** 485-494.

Ross RA, TM Gibson, HC Brockie, M Leslie, G Pashmi, SJ Craib, V Di Marzo and RG Pertwee (2001) Structure-activity relationship for the endogenous cannabinoid, anandamide, and certain of its analogues at vanilloid receptors in transfected cells and vas deferens. *Br. J. Pharmacol.* **132,** 631-640.

Sanudo-Pena MC, SL Patrick, S Khen, RL Patrick, K Tsou and JM Walker (1998) Cannabinoid effects in basal ganglia in a rat model of Parkinson's disease. *Neurosci. Lett.* **248,** 171-174.

Schlicker E and M Kathmann (2001) Modulation of transmitter release via presynaptic cannabinoid receptors. *Trends Pharmacol. Sci.* **22,** 565-572.

Schmid PC, PV Reddy, V Natarajan and HHO Schmid (1983) Metabolism of *N*-acylethanolamine phospholipids by mammalian

phosphodiesterase of the phospholipase D type. *J. Biol. Chem.* **258,** 9302-9306.

Schmid PC, RJ Krebsbach, SR Perry, TM Dettmer, JL Maasson and HHO Schmid (1995) Occurrence and postmortem generation of anandamide and other long-chain *N*-acylethanolamines in mammalian brain. *FEBS Lett.* **375,** 117-120. Erratum appears in *FEBS Lett.* **385**: 125, 1996.

Shen M and SA Thayer (1998a) Cannabinoid receptor agonists protect cultured rat hippocampal neurons from excitotoxicity. *Mol. Pharmacol.* **54,** 459-462.

Shen M and SA Thayer (1998b) The cannabinoid agonist Win55,212-2 inhibits calcium channels by receptor-mediated and direct pathways in cultured rat hippocampal neurons. *Brain Res.* **783,** 77-84.

Shimasue K, T Urushidani, M Hagiwara and T Nagao (1996) Effects of anandamide and arachidonic acid on specific binding of (+)-PN200-110, diltiazem and (-)-desmethoxyverapamil to L-type Ca^{2+} channel. *Eur. J. Pharmacol.* **296,** 347-350.

Sieradzan KA, SH Fox, M Hill, JP Dick, AR Crossman and JM Brotchie (2001) Cannabinoids reduce levodopa-induced dyskinesia in Parkinson's disease: a pilot study. *Neurology* **57,** 2108-2111.

Silverdale MA, S McGuire, A McInnes, AR Crossman and JM Brotchie (2001) Striatal cannabinoid CB_1 receptor mRNA expression is decreased in the reserpine-treated rat model of Parkinson's disease. *Exp. Neurol.* **169,** 400-406.

Sinor AD, SM Irvin and DA Greenberg (2000) Endocannabinoids protect cerebral cortical neurons from *in vitro* ischemia in rats. *Neurosci. Lett.* **278,** 157-160.

Skaper SD, A Buriani, R Dal Toso, L Petrelli, S Romanello, L Facci and A Leon (1996) The aliamide palmitoylethanolamide and cannabinoids, but not anandamide, are protective in a delayed postglutamate paradigm of excitotoxic death in cerebellar granule neurons. *Proc. Natl. Acad. Sci. USA* **93,** 3984-3989.

Stella N and D Piomelli (2001) Receptor-dependent formation of endogenous cannabinoids in cortical neurons. *Eur. J. Pharmacol.* **425,** 189-196.

Stella N, P Schweitzer and D Piomelli (1997) A second endogenous cannabinoid that modulates long-term potentiation. *Nature* **388,** 773-778.

Sugiura T, S Kondo, A Sukagawa, T Tonegawa, S Nakane, A Yamashita, Y Ishima and K Waku (1996) Transacylase-mediated and phosphodiesterase-mediated synthesis of *N*-arachidonoylethanolamine, an endogenous cannabinoid- receptor ligand, in rat brain microsomes - Comparison with synthesis from free arachidonic acid and ethanolamine. *Eur. J. Biochem.* **240,** 53-62.

Sugiura T, Y Kobayashi, S Oka and K Waku (2002) Biosynthesis and degradation of anandamide and 2-arachidonoylglycerol and their possible physiological relevance. *Prostagl. Leukotr. Essent. Fatty Acids* **66,** 173-192.

Tsou K, S Brown, MC Sañudo-Peña, K Mackie and JM Walker (1998) Immunohistochemical distribution of cannabinoid CB1 receptors in the rat central nervous system. *Neuroscience* **83,** 393-411.

Tsou K, K Mackie, MC Sañudo-Peña and JM Walker (1999) Cannabinoid CB1 receptors are localized primarily on cholecystokinin-containing GABAergic interneurons in the rat hippocampal formation. *Neuroscience* **93,** 969-975.

van der Stelt M, WB Veldhuis, PR Bar, GA Veldink, JF Vliegenthart and K Nicolay (2001a) Neuroprotection by Delta9-tetrahydrocannabinol, the main active compound in marijuana, against ouabain-induced *in vivo* excitotoxicity. *J. Neurosci.* **21,** 6475-6479.

van der Stelt M, WB Veldhuis, GW van Haaften, F Fezza, T Bisogno, PR Bar, GA Veldink, JF Vliegenthart, V Di Marzo and K Nicolay (2001b) Exogenous anandamide protects rat brain against acute neuronal injury *in vivo. J. Neurosci.* **21,** 8765-8771.

van der Stelt M, WB Veldhuis, M Maccarrone, PR Bär, K Nicolay, GA Veldink, V Di Marzo and JFG Vliegenthart (2002) Acute neuronal injury, excitotoxicity and the endocannabinoid system. *Mol. Neurobiol.* **26**, 317-346.

Varma N, GC Carlson, C Ledent and BE Alger (2001) Metabotropic glutamate receptors drive the endocannabinoid system in hippocampus. *J. Neurochem.* **21,** 1-5.

Venance L, D Piomelli, J Glowinski and C Giaume (1995) Inhibition by anandamide of gap junctions and intercellular calcium signalling in striatal astrocytes. *Nature* **376,** 590-594.

Venters HD, R Dantzer and KW Kelley (2000) A new concept in neurodegeneration: TNFalpha is a silencer of survival signals. *Trends Neurosci.* **23**, 175-180.

Walker JM, SM Huang, NM Strangman, K Tsou and MC Sañudo-Peña (1999) Pain modulation by release of the endogenous cannabinoid anandamide. *Proc. Natl. Acad. Sci. USA* **96,** 12198-12203.

Wirguin I, R Mechoulam, A Breuer, E Schezen, J Weidenfeld and T Brenner (1994) Suppression of experimental autoimmune encephalomyelitis by cannabinoids. *Immunopharmacol.* **28,** 209-214.

Zeng BY, B Dass, A Owen, S Rose, C Cannizzaro, BC Tel and P Jenner (1999) Chronic L-DOPA treatment increases striatal cannabinoid CB1 receptor mRNA expression in 6-hydroxydopamine-lesioned rats. *Neurosci. Lett.* **276,** 71-74.

Zygmunt PM, J Petersson, DA Andersson, H Chuang, M Sorgard, V Di Marzo, D Julius and ED Hogestatt (1999) Vanilloid receptors on sensory nerves mediate the vasodilator action of anandamide. *Nature* **400,** 452-457.

Zygmunt PM, H Chuang H, P Movahed D Julius and ED Hogestatt (2000) The anandamide transport inhibitor AM404 activates vanilloid receptors. *Eur. J. Pharmacol.* **396**, 39-42.