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The Therapeutic Potential of Drugs That Target Cannabinoid Receptors or Modulate the Tissue Levels or Actions of Endocannabinoids

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ABSTRACT

There are at least 2 types of cannabinoid receptor, CB_1 and CB_2, both G protein coupled. CB_1 receptors are expressed predominantly at nerve terminals and mediate inhibition of transmitter release, whereas CB_2 receptors are found mainly on immune cells, their roles including the modulation of cytokine release and of immune cell migration. Endogenous agonists for cannabinoid receptors also exist. These "endocannabinoids" are synthesized on demand and removed from their sites of action by cellular uptake and intracellular enzymic hydrolysis. Endocannabinoids and their receptors together constitute the endocannabinoid system. This review summarizes evidence that there are certain central and peripheral disorders in which increases take place in the release of endocannabinoids onto their receptors and/or in the density or coupling efficiency of these receptors and that this upregulation is protective in some disorders but can have undesirable consequences in others. It also considers therapeutic strategies by which this upregulation might be modulated to clinical advantage. These strategies include the administration of (1) a CB_1 and/or CB_2 receptor agonist or antagonist that does or does not readily cross the blood brain barrier; (2) a CB_1 and/or CB_2 receptor agonist intrathecally or directly to some other site outside the brain; (3) a partial CB_1 and/or CB_2 receptor agonist rather than a full agonist; (4) a CB_1 and/or CB_2 receptor agonist together with a noncannabinoid, for example, morphine or codeine; (5) an inhibitor or activator of endocannabinoid biosynthesis, cellular uptake, or metabolism; (6) an allosteric modulator of the CB_1 receptor; and (7) a CB_2 receptor inverse agonist.

KEYWORDS: cannabinoid receptors, endocannabinoids, fatty acid amide hydrolase inhibitors, autoprotection, therapeutic strategies

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INTRODUCTION

Mammalian tissues are now known to express at least 2 types of cannabinoid receptor, both of which are G-protein coupled.[1,2] These are CB_1 receptors, cloned in Tom Bonner's laboratory in 1990 (Matsuda et al[3]), and CB_2 receptors, cloned by Sean Munro in 1993.[4] Although CB_1 receptors are expressed by certain nonneuronal cells and tissues, for example, the pituitary gland, immune cells, and reproductive tissues, they are found predominantly at central and peripheral nerve terminals where they mediate inhibition of transmitter release. CB_2 receptors occur mainly on immune cells, their functions including the modulation, both within and outside the central nervous system, of cytokine release and immune cell migration. Thus, one common role of CB_1 and CB_2 receptors appears to be the regulation of ongoing release of chemical messengers, CB_1 receptors mainly from neurones and CB_2 receptors from immune cells. The finding that mammalian tissues express cannabinoid receptors was followed by the discovery of endogenous ligands for these receptors (*Endocannabinoids* section). These endogenous cannabinoids or endocannabinoids are all eicosanoids, 2 notable examples being *N*-arachidonoylethanolamine (anandamide) and 2-arachidonoyl glycerol. Endocannabinoids together with cannabinoid CB_1 and CB_2 receptors constitute the endocannabinoid system.

The discovery of the endocannabinoid system has prompted research directed at establishing its physiological and pathophysiological roles. This research has provided evidence first, that there are certain disorders in which endocannabinoid levels, cannabinoid receptor density, and/or cannabinoid receptor coupling efficiency increase in particular tissues, and second, that this upregulation of the endocannabinoid system often leads to a suppression of unwanted signs and symptoms, and so is autoprotective. The main objectives of this review are to summarize the evidence that the endocannabinoid system can be protective, and then to go on to consider possible therapeutic strategies by which such protection might best be exploited in the clinic. These are strategies in which endocannabinoid-induced protection is mimicked with directly acting CB_1 and/or CB_2 receptor agonists or in which it is augmented with drugs expected to delay the disappearance of endocannabinoids following

their endogenous release or to induce an allosteric enhancement of cannabinoid receptor activation by released endocannabinoids. Evidence that the endocannabinoid system may sometimes be responsible for the production of undesirable effects is also briefly discussed. The review begins with short overviews of first, the pharmacological actions of important cannabinoid receptor ligands; second, the processes by which endocannabinoids are produced and then removed from their sites of action; and third, the progress that has been made to date in the development of drugs that can selectively inhibit one or other of these removal processes.

CANNABINOID RECEPTOR LIGANDS

Several ligands that bind selectively to cannabinoid receptors are now available.[1,2] These fall essentially into 2 categories: the exogenous cannabinoids that are found in cannabis or have been designed and synthesized by chemists and the endogenous cannabinoids that occur naturally in mammalian tissues.

Exogenous Ligands

As detailed elsewhere,[1,2] a large number of CB_1 and CB_2 receptor agonists and antagonists have now been developed. Among the agonists are several compounds that bind more or less equally well to CB_1 and CB_2 receptors, examples being the "classical" cannabinoids, Δ^9-THC and (–)-11-hydroxy-Δ^8-THC-dimethylheptyl (HU-210), the "nonclassical" cannabinoid, CP55940, and the aminoalkylindole cannabinoid, *R*-(+)-WIN55212, which has marginally greater CB_2 than CB_1 affinity. Of these nonselective agonists, HU-210, CP55940, and *R*-(+)-WIN55212 have the highest CB_1 and CB_2 relative intrinsic activities, and HU-210 has the highest affinity for both CB_1 and CB_2 receptors. Δ^9-THC, which is the main psychotropic constituent of cannabis, has lower CB_1 and CB_2 affinities and relative intrinsic activities than these other cannabinoids. Indeed, at both receptor types, Δ^9-THC exhibits the mixed agonist-antagonist properties that are typical of a partial agonist. Another cannabis constituent, the classical cannabinoid cannabinol, also behaves as a partial agonist, at least at CB_1 receptors.[5] Agonists with significant selectivity for CB_1 or CB_2 receptors have also been developed. Notable CB_1-selective agonists include the eicosanoids *R*-(+)-methanandamide, arachidonyl-2'-chloroethylamide (ACEA), arachidonylcyclopropylamide (ACPA), and O-1812, which are all analogs of the endocannabinoid, anandamide (*Endocannabinoids* section). CB_2-selective agonists include L-759633, L-759656, and JWH-133, all structural analogs of Δ^9-THC; other notable examples are the nonclassical cannabinoid, HU-308, and the aminoalkylindoles, JWH-015 and AM1241.

Turning now to cannabinoid receptor antagonists, the compounds most commonly used in research are the CB_1-selective SR141716A, AM251, and AM281 and the CB_2-selective SR144528 and AM630. These are usually all classified as inverse agonists since they can, in at least some cannabinoid receptor-containing systems, produce effects by themselves that are opposite in direction from those produced by agonists for these receptors.[6] It is thought that they may produce these inverse cannabimimetic effects by shifting cannabinoid receptors from a constitutively active "on" state to one or more constitutively inactive "off" states. This putative mechanism relies on the assumption that cannabinoid receptors can exist in a constitutively active state in which they undergo some degree of spontaneous coupling to their effector mechanisms even in the absence of an endogenously released or exogenously added agonist. "Neutral" antagonists have also been developed.[6] These share the ability of the inverse agonists to block responses to cannabinoid receptor agonists but lack the apparent ability of inverse agonists to produce inverse cannabimimetic effects in cannabinoid receptor-containing systems in the absence of any endogenously released or exogenously added cannabinoid receptor agonist. Neutral antagonists are, however, expected to share the ability of CB_1 and CB_2 receptor inverse agonists to produce inverse cannabimimetic effects in tonically active biological systems when this tonic activity arises from ongoing endocannabinoid release onto cannabinoid receptors.

There is evidence that some established exogenous cannabinoid receptor agonists and antagonists have non-CB_1, non-CB_2 pharmacological targets.[2,6,7] These include TRPV1 (vanilloid VR1) receptors for some eicosanoid agonists (see also *Endocannabinoids* section), adenosine A_1 receptors for SR141716A and AM251, and possibly also the following:

- central putative TRPV1-like receptors for CP55940, *R*-(+)-WIN55212 and SR141716A but not AM251;
- central putative non-CB_1, non-CB_2, non-TRPV1 G protein-coupled receptors for *R*-(+)-WIN55212 but not Δ^9-THC, HU-210, or CP55940;
- putative non-CB_1, non-CB_2, non-TRPV1 receptors on perivascular sensory neurons for Δ^9-THC and cannabinol but not HU-210 or CP55940;
- putative non-I_1, non-I_2 imidazoline receptors for CP55940, *R*-(+)-WIN55212, and SR141716A;
- putative abnormal-cannabidiol receptors on mesenteric arteries for *R*-(+)-methanandamide and SR141716A but not for Δ^9-THC or *R*-(+)-WIN55212;
- gap junctions and L-type Ca^{2+} channels for SR141716A;
- Ca^{2+}-activated (BK_{Ca}) and ATP-sensitive potassium channels for SR141716A; and

- putative allosteric sites for certain exogenous cannabinoid receptor ligands on muscarinic M_1 and M_4, 5-HT_2 and 5-HT_3 receptors.

Evidence has also recently emerged for the presence of an allosteric site on the CB_1 receptor. Thus, we have found a series of novel compounds to behave as allosteric CB_1 receptor modulators. These compounds do not displace [^{3}H]CP55940 from CB_1 binding sites but do modulate the rate at which [^{3}H]CP55940 dissociates from these sites.[8] Whether there are also allosteric sites on CB_2 receptors remains to be established.

Endocannabinoids

The most investigated of the endocannabinoids have been the polyunsaturated fatty acid amide, *N*-arachidonoylethanolamine (anandamide), and the polyunsaturated monoacylglycerol, 2-arachidonoyl glycerol.[1,2,9] Of these, anandamide has marginally greater CB_1 than CB_2 affinity and, like the exogenous cannabinoid Δ^9-THC, exhibits relatively low efficacy at CB_1 and CB_2 receptors, behaving as a partial agonist at both receptor types. As to 2-arachidonoyl glycerol, this has been found in several investigations to have affinities for CB_1 and CB_2 receptors similar to those of anandamide but to exhibit higher CB_1 and CB_2 efficacy than anandamide. There has, however, been one recent investigation with human CB_1 receptor-containing tissue in which 2-arachidonoyl glycerol was found both to exhibit lower CB_1 receptor affinity than anandamide and to lack detectable CB_1 receptor efficacy at concentrations of up to 10 μM.[10]

It is likely that anandamide and/or 2-arachidonoyl glycerol have neuromodulatory roles (eg, by serving as retrograde synaptic messengers) and immunomodulatory roles (eg, through the regulation of cytokine release and immune cell migration).[1,2,11-13] It is also likely that both these endocannabinoids play a part in certain pathological processes both within and without the central nervous system.

Cannabinoid CB_1 and CB_2 receptors are not the only pharmacological targets for anandamide.[2,7,14-16] Thus, it is generally accepted that the TRPV1 receptor is activated by anandamide and by synthetic analogs of this endocannabinoid such as methanandamide, arachidonyl-2'-chloroethylamide, and *N*-(4-hydroxybenzyl)arachidonoylamine (AM404), although not by 2-arachidonoyl glycerol or by noneicosanoid cannabinoid receptor agonists such as *R*-(+)-WIN55212, CP55940, or HU-210. As detailed elsewhere,[2,7] additional targets that have been proposed for anandamide include

- central putative non-CB_1, non-CB_2, non-TRPV1 G protein-coupled receptors that are also activated by *R*-(+)-WIN55212;
- putative non-CB_1, non-TRPV1 receptors on neurons of the small intestine;
- putative non-I_1, non-I_2 imidazoline receptors that are also activated by CP55940 and *R*-(+)-WIN55212;
- putative abnormal-cannabidiol receptors on mesenteric arteries that are also activated by *R*-(+)-methanandamide but not by 2-arachidonoyl glycerol;
- putative non-CB_1, non-CB_2, non-TRPV1 receptors on coronary arteries;
- allosteric sites on muscarinic M_1 and M_4 receptors and on 5-HT_3 receptors that are also activated by certain exogenous cannabinoids; and
- allosteric sites on glutamate GLU_{A1} and GLU_{A3} receptors.

Other endocannabinoids are the anandamide analogs, dihomo-γ-linolenoylethanolamide, docosatetraenoylethanolamide, and possibly the CB_1-selective agonist 2-arachidonylglyceryl ether (noladin ether).[9] *N*-arachidonoyl dopamine, *N*-oleoyl dopamine, and oleamide may also be endocannabinoids as there are reports that these endogenous compounds can bind to CB_1 receptors with K_i values that are in the midnanomolar (*N*-arachidonoyl dopamine) or low micromolar range.[9,17,18] *N*-arachidonoyl dopamine can induce both antinociception in the mouse hot-plate test and thermal hyperalgesia, is distributed differently from anandamide in the brain, and shares the ability of anandamide to activate TRPV1 receptors.[9,17] *N*-oleoyl dopamine is also a TRPV1 receptor agonist. Indeed, it activates these receptors with particularly high potency and has also been found to induce both thermal hyperalgesia and allodynia.[9] The monounsaturated fatty acid amide, oleamide, has been shown to activate CB_1 receptors at concentrations above 10 or 100 nM, to block gap junction-mediated cell-cell communication, and to increase food intake, and has been postulated to be involved in sleep regulation.[9,18] One other endogenous fatty acid amide that interacts with cannabinoid receptors at concentrations in the low micromolar range is *O*-arachidonoylethanolamine (virodhamine).[9] In one investigation this ligand was found to activate CB_2 receptors but to exhibit either partial agonist or antagonist activity at CB_1 receptors,[19] whereas in another it behaved as a CB_1 receptor antagonist/inverse agonist.[10]

The Fate of Endocannabinoids and Its Modulation by Drugs

The Biosynthesis and Fate of Endocannabinoids

The biosynthesis of anandamide takes place on demand in response to elevations of intracellular calcium. The immediate precursor to anandamide is *N*-arachidonoyl phosphatidylethanolamine, which is formed from phosphatidylcholine and phosphatidylethanolamine and is

converted to anandamide by the action of *N*-arachidonoyl phosphatidylethanolamine-phospholipase D.[16,20] Anandamide is removed from its sites of action by cellular uptake processes that may involve a transmembrane carrier protein, membrane-associated binding proteins, and/or simple diffusion.[21] It is then metabolized intracellularly. In most tissues, this metabolism is catalyzed by fatty acid amide hydrolase (FAAH),[16,22,23] an enzyme that in central neurons is located mainly on the cytosolic surfaces of smooth endoplasmic reticulum cisternae and mitochondria.[24] Anandamide is also sometimes metabolized by another intracellular enzyme, palmitoylethanolamide-preferring acid amidase (PAA),[22,25,26] and indeed, there is evidence that it is PAA rather than FAAH that is primarily responsible for deactivating anandamide in the mouse duodenum.[27] Other enzymes that can metabolize anandamide are cyclooxygenase-2, lipoxygenases, and cytochrome P450.[28]

Like anandamide, 2-arachidonoyl glycerol is not stored but rather synthesized on demand in a manner that can be triggered by elevations of intracellular calcium.[16,20] Its synthesis depends on the conversion of 2-arachidonate-containing phosphoinositides to diacylglycerols (DAGs), which are then converted to 2-arachidonoyl glycerol by the action of DAG lipase. Also, like anandamide, 2-arachidonoyl glycerol is thought to be removed from its sites of action by cellular uptake and then to be metabolized intracellularly.[16,20,28] Although 2-arachidonoyl glycerol can readily be metabolized by FAAH as well as by cyclooxygenase-2 and lipoxygenases, there is evidence that the enzyme mainly responsible for its metabolism in vivo may be monoacyl glycerol (MAG) lipase.[20,22,29,30] FAAH, MAG lipase, and cannabinoid CB_1 receptors have broadly similar distribution patterns, at least within some brain areas.[24] It is noteworthy, however, that while MAG lipase and cannabinoid CB_1 receptors are located mainly presynaptically in these brain areas, FAAH is found mainly postsynaptically in somata and dendrites of principal neurons. Unlike polyunsaturated fatty acid amides, mono-unsaturated and saturated fatty acid amides are thought to be poor substrates for the putative anandamide transporter.[27] In contrast, cellular uptake may be the main means by which noladin ether is removed from its site of action as this putative endocannabinoid is considered not to be a likely substrate for enzymic hydrolysis.[31] *N*-oleoyl dopamine is also thought to be recognized by the putative anandamide transporter and to be only a poor FAAH substrate.[9] However, 3 other putative endocannabinoids, oleamide, *N*-arachidonoyl dopamine, and virodhamine, all appear to be reasonable FAAH substrates.[23]

Several endogenous fatty acid amides, in addition to anandamide, are metabolized by FAAH.[22,23,26,27] These include not only polyunsaturated fatty acid amides such as *N*-arachidonoyl dopamine but also a range of mono-unsaturated and saturated compounds. One endogenous saturated fatty acid amide that serves as a substrate for FAAH, and also for PAA, is palmitoylethanolamide.[22,25] This has antiinflammatory and antinociceptive properties, can potentiate anandamide-induced antinociception and TRPV1 receptor activation, lacks significant affinity for CB_1 or CB_2 receptors, and is thought to be an agonist for the PPAR-α receptor and possibly also for a putative "CB_2-like" receptor.[7,26,32] A second pharmacologically active endogenous substrate of FAAH and PAA is the mono-unsaturated fatty acid amide, oleoylethanolamide, which can inhibit both anandamide cellular uptake and metabolism. Like palmitoylethanolamide, oleoylethanolamide can activate PPAR-α receptors and has negligible affinity for CB_1 or CB_2 receptors.[9] There is evidence that oleoylethanolamide serves as a satiety hormone, that it is released in the upper small intestine in response to food intake, and that it induces hypophagia by acting through intestinal PPAR-α receptors to activate vagal sensory afferent neurons that innervate higher brain structures involved in the control of energy balance.[26,27] Oleoylethanolamide is also a TRPV1 receptor agonist.[9]

Other pharmacologically active endogenous fatty acid amides include linoleamide, which is a FAAH substrate, may be involved in sleep regulation, and does not interact appreciably with cannabinoid receptors; *N*-arachidonoyl serine, which activates the putative abnormal-cannabidiol receptor; and *N*-palmitoyl dopamine and *N*-stearoyl dopamine, which potentiate anandamide at TRPV1 receptors but do not inhibit its cellular uptake or its metabolism by FAAH.[9] Two other endogenous ligands of interest are *N*-arachidonoyl glycine and the unsaturated fatty acid amide, linoleoylethanolamide, as both have been reported to inhibit FAAH. It has also been found that *N*-arachidonoyl glycine has antinociceptive and anti-edema activity, that it lacks significant affinity for cannabinoid CB_1 receptors, and that it is a FAAH substrate.[9,23,33]

Finally, it is noteworthy that some effects of endogenously released anandamide and 2-arachidonoyl glycerol may be enhanced through an "entourage effect" that relies on the corelease of other endogenous fatty acid derivatives. These derivatives include palmitoylethanolamide and oleamide, which can potentiate anandamide, and 2-linoleyl glycerol and 2-palmitoyl glycerol, which can potentiate 2-arachidonoyl glycerol.[2]

Inhibitors of Endocannabinoid Cellular Uptake and Intracellular Metabolism

The finding that the actions of anandamide and 2-arachidonoyl glycerol are terminated by cellular uptake and intracellular enzymic hydrolysis has been followed by the discovery of several drugs that will inhibit one or other of these processes.[16,23] Several of these inhibitors have been used as pharmacological tools in animal experiments

directed at elucidating the physiological and pathological roles of anandamide or 2-arachidonoyl glycerol when these are released endogenously. These compounds are

- palmitylsulphonyl fluoride (AM374), an irreversible FAAH inhibitor (IC_{50} = 13 or 50 nM) that inhibits this enzyme at concentrations below those at which it binds to CB_1 receptors (K_i = 520 nM)[23];
- methyl arachidonoyl fluorophosphonate (MAFP), an irreversible FAAH inhibitor (IC_{50} = 1 to 3 nM) that has been reported also to inhibit MAG lipase (IC_{50} = 2 to 800 nM) in brain tissue, to bind irreversibly to CB_1 receptors at concentrations in the low nanomolar range,[23] and to behave as an irreversible CB_1 receptor antagonist in one investigation[34] but not in another[35];
- diazomethylarachidonoylketone (DAK), an irreversible FAAH inhibitor (IC_{50} = 500 nM), that has been reported also to inhibit MAG lipase in vitro at 1 μM and to bind to CB_1 receptors (IC_{50} = 1.3 μM)[23,36];
- the alkylcarbamic acid aryl esters, URB532 and URB597, that are irreversible FAAH inhibitors (IC_{50} = 63 and 4.6 nM, respectively) that do not inhibit MAG lipase, acetylcholinesterase, butyrylcholinesterase, or the putative anandamide membrane transporter at concentrations of up to 30 or 300 μM and lack significant affinity both for CB_1 and CB_2 receptors and for several other established receptors[37];
- the α-ketoheterocycle, 1-oxo-1[5-(2-pyridyl)-2-yl]-7-phenylheptane (OL-135), that is a potent reversible FAAH inhibitor (IC_{50} = 2.1 nM), has less potency as an inhibitor of triacylglycerol hydrolase (IC_{50} = 620 nM), and does not inhibit MAG lipase or a selection of other serine hydrolases at concentrations of up to 10 μM or exhibit significant affinity for CB_1 or CB_2 receptors (K_i > 10 μM)[38];
- *N*-arachidonoyl glycine, a FAAH substrate that is found endogenously, inhibits anandamide metabolism (IC_{50} = 4.1 or 7 μM), and lacks significant affinity for CB_1 receptors (K_i > 10 μM)[33,39];
- *N*-arachidonoyl serotonin that inhibits anandamide metabolism (IC_{50} = 5.6, 9, or 12 μM), does not significantly inhibit anandamide cellular uptake at 25 μM, and lacks significant affinity for CB_1 receptors (K_i > 50 μM)[40,41];
- palmitoylisopropylamide that inhibits FAAH (IC_{50} = 12.9 μM) and probably also anandamide cellular uptake and does not readily displace [^{3}H]CP55940 or [^{3}H]*R*-(+)-WIN55212 from CB_1 or CB_2 receptors (IC_{50} > 100 μM)[42];
- *N*-(4-hydroxybenzyl)arachidonoylamine (AM404), an inhibitor of anandamide cellular uptake (IC_{50} = 1 to 11 μM) that, however, also inhibits FAAH (IC_{50} = 0.5-5.9 μM or 22 μM or >30 μM), binds to CB_1 receptors (K_i = 1.76 μM), and potently activates TRPV1 receptors (EC_{50} = 26 to 50 nM)[43-48];
- Two structural analogs of AM404, VDM11 and VDM13, that inhibit anandamide cellular uptake (IC_{50} = 6.1 to 11 μM, and 12 μM, respectively), have less potency as TRPV1 receptor agonists (EC_{50} >> 10 μM) and also as FAAH inhibitors in some experiments (IC_{50} > 50 μM and = 27 μM, respectively) but not others (VDM11 IC_{50} = 1.2 to 3.7 μM, and bind to CB_1 and CB_2 receptors with K_i values that exceed 5 or 10 μM[47,48];
- the (*S*)- and (*R*)-1'-(4-hydroxybenzyl) derivatives of *N*-oleoylethanolamine (OMDM-1 and OMDM-2 respectively) that inhibit anandamide cellular uptake (K_i = 2.4 and 3 μM, respectively; IC_{50} = 2.6 or >20 μM, and 3.2 or 17 μM, respectively), have less potency as FAAH inhibitors (K_i or IC_{50} >50 or >100 μM) or as TRPV1 receptor agonists ($EC_{50} \geq 10$ μM) and bind to CB_1 receptors (K_i = 12 μM and 5 μM, respectively)[48,49];
- *N*-(3-furylmethyl)arachidonoylamine (UCM707) that inhibits anandamide cellular uptake (K_i = 0.8, 25, 41, or >100 μM), is a FAAH substrate and inhibitor (IC_{50} = 30 μM or >100 μM), binds to CB_1 (K_i = 4.7 μM) and CB_2 receptors (K_i = 67 nM), and lacks appreciable affinity for TRPV1 receptors (K_i > 5 μM).[48,50,51]

AM374 is an analog of the irreversible serine protease inhibitor, phenylmethylsulphonyl fluoride (PMSF), which itself inhibits both FAAH (IC_{50} = 290 nM to 15 μM) and MAG lipase (IC_{50} = 155 μM or >500 μM).[23] MAG lipase inhibitors with sufficient potency and selectivity for use as pharmacological tools have yet to be developed.[23]

Results obtained with the FAAH and cellular uptake inhibitors listed above should be interpreted with particular caution as the pharmacological characterization of these compounds is far from complete. An added complication for some of these inhibitors is that they can activate TRPV1 receptors, an action that is known to stimulate anandamide biosynthesis, presumably by increasing the intracellular concentration of calcium,[16] and that will most likely also stimulate the biosynthesis of other endocannabinoids including 2-arachidonoyl glycerol. Based on the available data, compounds that currently show greatest promise as pharmacological tools are AM374, URB532, URB597, and OL-135 for FAAH inhibition, and OMDM-1 for inhibition of the cellular uptake of anandamide.

Evidence for an Upregulation of the Endocannabinoid System in Some Disorders

Since the discovery of anandamide in 1992, there have been several reports that tissue concentrations of this endogenous fatty acid amide increase in some human disorders and in animal models of certain diseases or disorders. More specifically, such increases have been detected in

- brain, blood, or circulating monocytes of patients with disorders that include schizophrenia, stroke, endotoxic (septic) shock, embryo implantation failure, and cancer (Table 1);
- blood in bone cement implantation syndrome (Table 1);
- brain and spinal cord in a mouse model of multiple sclerosis (Table 2);
- lumbar spinal cord in a mouse model of amyotrophic lateral sclerosis (Table 2);
- basal ganglia in a rat model of Parkinson's disease (Table 2);
- ventral mesencephalon in a rat model of Huntington's disease (Table 2);
- periaqueductal gray in a rat model of inflammatory pain (Table 2);
- urinary bladder in a rat model of painful hemorrhagic cystitis (Table 2);
- hypothalamus and uterus in mouse models of obesity (Table 2);
- cardiovascular tissue in experimental models of septic shock, cardiogenic shock, and biliary cirrhosis (Table 3);
- small intestine in rodent models of paralytic ileus, ileitis, and secretory diarrhea (Table 3); and
- lesioned brain areas in rat models of cerebral ischemia and excitotoxicity (Table 4).

In some of these investigations, tissue levels of 2-arachidonoyl glycerol were also monitored. These were found to increase in human colorectal and pituitary adenomas or carcinomas (Table 1); in bone cement implantation syndrome (Table 1); in mouse models of multiple sclerosis, amyotrophic lateral sclerosis, aversive memory extinction, and obesity (Tables 2 and 3); in rat models of hepatic ischemia-reperfusion injury, myocardial infarction, and ileitis (Table 4); and in a model of cardiogenic shock (Table 3). Usually these increases were detected in the same tissues as those in which increases in anandamide levels occurred. However, there have been at least 2 instances in which increases in anandamide and 2-arachidonoyl glycerol were found to have taken place in different cell-types: in a mouse model of septic shock[113] and in experiments with human cancer cells[73] (Tables 1 and 3). There have also been a few instances in which increases in anandamide levels were found not to have been paralleled by any detectable increases in 2-arachidonoyl glycerol (Tables 1, 2, 3, and 4) or in which an increase in 2-arachidonoyl glycerol was not accompanied by any increase in anandamide (Tables 2, 3, and 4). Increased levels of 2-arachidonoyl glycerol in brain tissue have sometimes been observed in experiments in which anandamide levels were not also monitored, for example, in experiments with a mouse model of closed head injury and with a rat model of excitotoxicity (Table 4).

Increases in tissue levels of anandamide and 2-arachidonoyl glycerol are most likely usually triggered by increases in intracellular calcium. However, there is evidence that these increases are sometimes further modulated by changes in the ability of tissues to synthesize these compounds and/or to dispose of them by cellular uptake or subsequent enzymic degradation. Thus, for example, in a mouse model of obesity in which increases in uterine levels of both anandamide and 2-arachidonoyl glycerol were detected, these increases were found to be accompanied by a decrease in uterine tissue both of FAAH and MAG lipase activity and of anandamide transport across membranes[104] (Table 2). An increase in uterine DAG lipase activity was noted as well, suggesting that the increased uterine levels of 2-arachidonoyl glycerol observed in these experiments were partly attributable to an increase in the capacity of the uterus to synthesize this endocannabinoid. A decrease in FAAH activity has also been observed to accompany increases in anandamide levels in the peripheral lymphocytes of women with implantation failure (Table 1) and in rat striatal tissue in a model of Parkinson's disease (Table 2). Also observed in these rat experiments was a decrease in anandamide cellular uptake. However, in experiments using a lipopolysaccharide (LPS) model of endotoxic shock, in one study LPS-induced increases in anandamide levels were reported to be paralleled by a decrease in the gene expression and activity of FAAH (in human peripheral lymphocytes),[115] while in another study, in which a much lower concentration of LPS was used, increases in anandamide levels were found to be paralleled by an *increase* in the expression and activity of this enzyme (in mouse macrophages)[114] (Table 3). In the second of these investigations, increases were also detected in the activities of 2 enzymes that can catalyze anandamide biosynthesis (*N*-acyltranferase and *N*-arachidonoyl phosphatidylethanolamine-phospholipase D). An increase in FAAH activity has also been detected in the small intestine of mice in which intestinal inflammation and increased motility had been induced by orally administered croton oil[117] (Table 3). In these experiments, high levels of anandamide (and

Table 1. Effects of Certain Diseases and Syndromes on the Human Endocannabinoid System*

	Effect of Disease or Syndrome					
Disease or Syndrome	**EC Concentration**		**Protein Expression or Binding Site Density**		**Activity or Protein Expression**	**Reference**
	AEA	2-AG	CB_1	CB_2	FAAH	
Alzheimer's disease (postmortem entorhinal and parahippocampal cortices)	ND	ND	0† (expression)	+†(expression)	+† (activity and expression)	52
Alzheimer's disease (postmortem frontal cortex)	ND	ND	-‡ (expression)	+‡ (expression)	ND	53
Paranoid-type schizophrenia (csf)	+	ND	ND	ND	ND	54
Schizophrenia with psychotic symptoms (csf)	+	0	ND	ND	ND	55
Schizophrenia (postmortem dorsolateral prefrontal cortex)	ND	ND	+ (binding)	§	ND	56
Schizophrenia (postmortem anterior cingulate cortex)	ND	ND	+ (binding)	ND	ND	57
Acute schizophrenia (human blood)	+	ND	ND‖	ND‖	ND‖	58
Depressed suicide (postmortem dorsolateral prefrontal cortex)	ND	ND	+¶ § (expression and binding)	§	ND	59
Alcoholism + suicide (postmortem dorsolateral prefrontal cortex)	+	+	+¶ § (expression and binding)	§	ND	60
Parkinson's disease (postmortem caudate nucleus and putamen)	ND	ND	+¶ § (binding)	§	ND	61
Huntington's disease (postmortem substantia nigra, globus pallidus, putamen, and caudate nucleus)	ND	ND	- § (binding)	§	ND	62,63
Hemispheric stroke (stroke penumbra; microdialysis)	+#	ND	ND	ND	ND	64
Miscarriage (peripheral lymphocytes)	ND	ND	ND	ND	-	65
Implantation failure after in vitro fertilization and embryo transfer (AEA and 2-AG in blood; cannabinoid receptor binding and FAAH in peripheral lymphocytes)	+	0	0§ (binding)	0§ (binding)	- (activity and expression)	66
Endotoxic shock (serum)	+	+	ND	ND	ND	67
Advanced liver cirrhosis: (a) monocytes and (b) hepatic arterial endothelial cells	(a) +	ND	(b) + (mRNA and binding)	ND	ND	68
Liver cirrhosis (plasma)	+	ND	ND	ND	ND	69
Liver cirrhosis (fibrogenic cells in human liver biopsy specimens)	ND	ND	ND	+ (expression)	ND	70
Atherosclerosis (human and mouse atherosclerotic plaques)	ND	ND	0 (expression)	+ (expression)	ND	71
Cancer (human adenomas and/or carcinomas; colorectal biopsies)	+	+	0 (mRNA and expression)	0 (mRNA and expression)	0 (mRNA)	41
Cancer (meningiomas; resected human brain tissue)	-	0	ND	ND	ND	72
Cancer: (a) glioblastomas and (b) meningiomas; resected human brain tissue	(a) + (b) 0	(a) 0 (b) +	ND	ND	(a) - (activity) (b) - (activity)	73

Continued

Table 1. *Continued*

Disease or Syndrome	Effect of Disease or Syndrome: EC Concentration		Protein Expression or Binding Site Density		Activity or Protein Expression	Reference
Cancer (human pituitary adenomas; postmortem)	+	+	**(mRNA and expression)	ND	ND	74
Prostate cancer (human cell lines)	ND	ND	+ (expression)	+ (expression)	ND	75
Bone cement implantation syndrome (blood)	+	+	ND	ND	ND	76

*2-AG indicates 2-arachidonoyl glycerol; AEA, anandamide; EC, endocannabinoid; FAAH, fatty acid amide hydrolase; csf, cerebral spinal fluid; ND, not determined.

†FAAH protein and enzyme activity were overexpressed in neuritic plaque-associated astrocytes; CB_2 receptor protein was overexpressed in neuritic plaque-associated microglia; no change in CB_1 receptor protein density or location in the vicinity of neuritic plaques.

‡Signs of microglial activation; CB_2 receptor protein expressed in tangle-like neurons and dystrophic neurites and CB_1 and CB_2 receptor protein in senile plaques; CB_1-positive neuron density was greatly reduced in areas of microglial activation; cannabinoid receptor coupling efficiency was decreased.

§Binding experiments were perfomed with [^{3}H]CP55940, which binds equally well to CB_1 and CB_2 receptors.

||Pharmacologically-induced clinical improvement was accompanied by decreases in blood levels of anandamide and in FAAH mRNA and CB_2 receptor mRNA but not CB_1 receptor mRNA in mononuclear cells.

¶Increased CB_1 receptor immunoreactivity (not reference 61) and cannabinoid receptor binding site density and coupling efficiency.

#Increased levels of palmitoylethanolamide and oleoylethanolamide were also detected.

**CB_1 receptors were detected in most but not all types of adenoma.

2-arachidonoyl glycerol) were detected in the small intestine of both croton oil-treated and control mice. Some increases in anandamide tissue levels take place in the absence of changes in FAAH expression or activity. Thus, for example, in experiments with human colorectal cancer biopsies (Table 1) and with mouse models of diarrhea and paralytic ileus (Table 3), elevations in anandamide levels observed in the cancerous tissue and in mouse small intestine were not associated with any detectable changes in FAAH expression or activity.

In some disorders or animal models in which endocannabinoid tissue concentrations have been reported to be high, elevated expression levels and/or densities of cannabinoid CB_1 receptors have also been detected. Such upregulation of the CB_1 receptor has been observed in the following:

- postmortem cerebral cortical tissue of suicide victims (Table 1),
- tissue from humans with advanced liver cirrhosis (Table 1),
- brain tissue in a rat model of traumatic head injury (Table 4), and
- small intestine in mouse models of intestinal inflammation, secretory diarrhea, and paralytic ileus (Table 3).

Increases in CB_1 receptor density have also sometimes been detected in experiments in which no increases in endocannabinoid levels were observed or no measurements of endocannabinoid tissue concentrations made. These increases in receptor density were detected in the following:

- brain tissue taken postmortem from patients with schizophrenia or Parkinson's disease (Table 1),
- the striatum in marmoset and rat models of Parkinson's disease (Table 2),
- the spinal cord in a rat model of neuropathic pain (Table 2),
- cortical neurons in a rat model of focal cerebral ischemia (Table 4),
- the hippocampus in a rat febrile seizure model (Table 4),
- the colon in a mouse model of colitis (Table 3),
- mesenteric arteries in a rat model of cirrhosis (Table 3), and
- human prostate cancer cells (Table 1).

It is noteworthy that spontaneously hypertensive rats have been reported to exhibit increased CB_1 receptor density in myocardium and aorta but a decrease in myocardial anandamide levels[111] (Table 3). Conversely, excitotoxicity induced by *N*-methyl-D-aspartate (NMDA) in rats has been found to provoke not only a rise in anandamide concentrations but a fall in CB_1 receptor density and expression level in some brain areas (Table 4). A fall in CB_1 receptor density has also been detected in cerebral cortex and caudate putamen in a rat model of multiple sclerosis (Table 2). However, in this investigation, the reduced population of cannabinoid receptors was found to signal with increased efficiency.

Table 2. Signs of Upregulation or Downregulation of the Endocannabinoid System in Animal In Vivo Models of Multiple Sclerosis, Amyotrophic Lateral Sclerosis, Encephalitis, Alzheimer's Disease, Parkinson's Disease, Huntington's Disease, Pain, Obesity, Feeding, and Fasting*

	Effect Observed in Experimental Model					
Experimental Model	**EC Concentration**		**Protein Expression, mRNA or Binding Site Density**	**Activity or Protein Expression**		**Reference**
	AEA	2-AG	CB_1	FAAH	AMT	
CREAE model of multiple sclerosis (mouse brain and spinal cord)	+†	+†	ND	ND	ND	77
EAE model of multiple sclerosis (rat caudate putamen and/or cerebral cortex)	ND	ND	-§ || (mRNA and binding)	ND	ND	78
EAU model of uveoretinitis (mouse retina)	ND	ND	‡ND	ND	ND	79
Model of amyotrophic lateral sclerosis (lumbar spinal cords of transgenic mice)	+	+	ND	ND	ND	80
Human immunodeficiency virus-induced encephalitis (macaque cerebral cortex)	ND	ND	0¶ (expression)	+¶ (expression)	ND	81
β-Amyloid peptide model of Alzheimer's disease (rat cerebral cortex)	ND	ND	- (expression)	ND	ND	53
Attention-deficit hyperactivity disorder ("Impulsive" spontaneously hypertensive adolescent rats; prefrontal cortex)	ND	ND	- (expression)	ND	ND	82
Reserpine model of Parkinson's disease (rat globus pallidus)	0	+	ND	ND	ND	83
6-Hydroxydopamine model of Parkinson's disease (rat striatum)	+	0	0|| (binding)	- (activity)	- (activity)	84,85
6-Hydroxydopamine model of Parkinson's disease (rat striatum)	ND	ND	ND	- (activity)	ND	86
6-Hydroxydopamine model of Parkinson's disease (rat striatum)	-	0	ND	0 (activity)	ND	87
6-Hydroxydopamine model of Parkinson's disease (rat striatum or substantia nigra)	ND	ND	0|| # (binding)	ND	ND	88,89
6-Hydroxydopamine model of Parkinson's disease (rat striatum)	ND	ND	+ or 0# (mRNA)	ND	ND	89-91
MPTP model of Parkinson's disease (marmoset striatum)	ND	ND	+|| # (binding)	ND	ND	61
Huntington's disease (lateral striata of transgenic mice)	ND	ND	- (mRNA)	ND	ND	92
3-NP model of Huntington's disease (rat basal ganglia)	ND	ND	-|| (binding)	ND	ND	93
3-NP model of Huntington's disease (rat striatum)	-	-	0|| ** (binding)	ND	ND	94
3-NP model of Huntington's disease (rat ventral mesencephalon)	+	0	0|| ** (binding)	ND	ND	94
3-NP model of Huntington's disease (rat striatum)	ND	ND	-|| ** (mRNA and binding)	ND	ND	95
3-NP model of Huntington's disease (rat striatum and/or globus pallidus)	ND	ND	-|| (mRNA and binding)	ND	ND	96
Transgenic model of Huntington's disease (mouse basal ganglia)	ND	ND	-|| **(mRNA and binding)	ND	ND	97

Continued

Table 2. *Continued*

Experimental Model	Effect Observed in Experimental Model: EC Concentration		Protein Expression, mRNA or Binding Site Density	Activity or Protein Expression		Reference
Formalin paw model of inflammatory pain (rat periaqueductal gray)	+	ND	ND	ND	ND	98
Neuropathic pain model (rat spinal cord dorsal horn)	ND	ND	+ (expression)	ND	ND	99
Neuropathic pain model (rat lumbar spinal cord)	ND	ND	††ND	ND	ND	100
Cyclophosphamide model of painful hemorrhagic cystitis (rat bladder)	+	ND	ND	ND	ND	101
Obesity model (Zucker *fa/fa* rat hypothalamus)	0	+†	ND	ND	ND	102
Obesity model (*db/db* mouse hypothalamus)	+	+†	ND	ND	ND	
Obesity model (*ob/ob* mouse hypothalamus)	0	+	ND	ND	ND	
Obesity model (obese *fa/fa* rat adipose tissue and mouse cultured differentiated 3T3 F442A adipocyte cells)	ND	ND	+ (mRNA or expression)	ND	ND	103
Obesity model (*ob/ob* mouse uterus)	+	+†	0‖	- (activity; also MAG lipase)	-	104
Feeding (a) and fasting (b) (rat limbic forebrain and/or hypothalamus)	(a) 0 (b) +	(a) - (b) +	ND	ND	ND	105

*AMT indicates anandamide membrane tranport; CREAE, chronic relapsing experimental allergic encephalomyelitis; EAE, experimental allergic encephalomyelitis; EAU, experimental autoimmune uveoretinitis; MAG lipase, monoacyl glycerol lipase; MPTP, 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine; 3-NP,3-nitropropionic acid; TMEV-IDD, Theiler murine encephalomyelitis virus-induced demyelinating disease. (See first footnote to Table 1 for additional definitions.)

†Increased levels of palmitoylethanolamide were also detected in CREAE mouse spinal cord but not in CREAE mouse brain or in *fa/fa* rat or *db/db* mouse hypothalamus or *ob/ob* mouse uterus.

‡Retinal infiltrated cells, predominantly T cells and macrophages, were positive for the CB_2 receptor.

§Increased cannabinoid receptor coupling efficiency in caudate putamen and cerebral cortex.

‖These binding experiments were perfomed with [^{3}H]CP55940, [^{3}H]*R*-(+)-WIN55212 or [^{3}H]anandamide which have significant affinities for both CB_1 and CB_2 receptors.

¶ FAAH overexpressed in perivascular astrocytes and astrocytic processes and CB_2 receptor immunoreactivity detected in cerebrocortical perivascular macrophages, microglial nodules and T-lymphocytes.

#Increase[61] or no change[89] in cannabinoid receptor coupling efficiency.

**Decrease in cannabinoid receptor coupling efficiency in the globus pallidus[97] or in the area(s) indicated in the left hand column.

†† CB_2 expression detected in the lumbar spinal cord undergoing neuronal damage (CB_2 mRNA and immunoreactivity, probably on activated microglia); CB_2 mRNA was not detected in the spinal cords of rats with inflamed paws.

Increases in the efficiency of CB_1 receptor signaling have also been observed in postmortem brain tissue of patients who had suffered from Parkinson's disease or were suicide victims (Table 1) and in the striatum in a marmoset model of Parkinson's disease (Table 2). Additional examples of human disorders or of animal models of disorders in which signs of *downregulation* rather than upregulation of the endocannabinoid system have been observed are to be found in Tables 1 to 4.

It is likely that expression levels of CB_2 receptors also change in certain disorders. Indeed, Zhang et al[100] have found that when signs of neuropathic pain were produced in rats by sciatic nerve ligation, nonneuronal CB_2-expressing cells became detectable in the lumbar spinal cord. These cells were probably activated microglia that had migrated into this region of the cord. CB_2 expression was detectable only in certain regions of the dorsal and ventral horns, the precise distribution pattern of these receptors within the spinal cord varying

Table 3. Signs of Upregulation or Downregulation of the Endocannabinoid System in Animal Models of Stress, Memory, Aging, Hypertension, Cirrhosis, Septic Shock, Cardiogenic Shock, and Various Intestinal Disorders*

	Effect Observed in Experimental Model				
Experimental Model	**EC Concentration**		**Protein Expression, mRNA, cDNA, Enzymic Activity or Binding Site Density**		**Reference**
	AEA	2-AG	CB_1	FAAH	
Chronic unpredictable stress (rat hippocampus)	0	-	-† (expression and binding)	ND	106
Mouse forebrain after (a) acute or (b) repeated restraint stress or mouse amygdala after (c) acute or (d) repeated restraint stress	(a) 0 (b) 0 (c) - (d) -	(a) 0 (b) + (c) 0 (d) +	ND	ND	107
Extinction of aversive memories (mouse amygdala)	+	+	ND	ND	108
Age: (a) rat entorhinal and temporal cortices and (b) rat postrhinal cortex	ND	ND	(a) + (b) - (expression)	ND	109
Exercise (human plasma)	+	0	ND	ND	110
Spontaneously hypertensive rats (myocardial or aortic tissue)	-	0	+ (expression)	+ (expression)	111
Biliary cirrhosis and hypotension (rat monocytes)	+	ND	ND	ND	68
Cirrhosis (rat mesenteric arteries)	ND	ND	+ (expression)	ND	112
LPS model of septic shock/ hypotension:(a) mouse platelets or (b) mouse macrophages	(a) 0 (b) +	(a) +	ND	ND	113
LPS model of septic shock/ hypotension (mouse macrophages)	+	0	ND	+ (mRNA and activity)	114
LPS model of septic shock/ hypotension (human lymphocytes)	+	ND	0† ‡ (cDNA and binding)	- (expression, cDNA and activity)	115
Cardiogenic shock/hypotension (human monocytes and platelets)	+	+	ND	ND	116
Intestinal inflammation and hypermotility (mouse small intestine)	0§	0§	+ (expression)	+ (activity)	117
Colitis (mouse colon)	ND	ND	+ (mRNA)	ND	118
Cholera toxin-induced secretory diarrhea (mouse small intestine)	+	0§‖	+ (mRNA and expression)	0 (activity)	119
Clostridium difficile toxin A model of ileitis (rat ileum)	+	+	ND	ND	120
Paralytic ileus (mouse small intestine)	+	0§‖	+ (expression)	0 (activity)	121

* LPS indicates lipopolysaccharide. (See first footnote to Table 1 for additional definitions.)
† These binding experiments were perfomed with [^{3}H]CP55940 which has significant affinity for both CB_1 and CB_2 receptors.
‡ Similar CB_2 receptor data were obtained.
§ No higher than in controls in which significant levels were detected.
‖ No increased levels of palmitoylethanolamide.

with the model of experimental neuropathic pain used. No change in CB_2 expression was observed in the spinal cords of rats with inflamed paws. There have also been reports that

- in Alzheimer's disease brain tissue, CB_2 receptors are selectively overexpressed in neuritic plaque-associated microglia and are also expressed in tangle-like neurons and dystrophic neurites but not in normal brain tissue[52,53] (see also Table 1),
- CB_2-expressing immune cells are present in brain tissue samples from macaques with simian

Table 4. Signs of Upregulation or Downregulation of the Endocannabinoid System in Animal Models of Cerebral Ischemia, Myocardial Infarction, Neurotoxicity, and Febrile Seizures*

	Effect Observed in Experimental Model				
	EC Concentration		Protein Expression, mRNA, Enzymic Activity or Binding Site Density		
Experimental Model	AEA	2-AG	CB_1	FAAH	**Reference**
Focal cerebral ischemia induced by transient left middle cerebral artery occlusion (rat cortical neurons)	ND	ND	+† (expression)	ND	122
Focal cerebral ischemia induced by transient left middle cerebral artery occlusion (rat brain)	+	0	ND	ND	123
Hepatic ischemia-reperfusion injury (rat plasma): (a) short ischemia (b) long ischemia	(a) + (b) 0	(a) + (b) +	ND	ND	124
Acute myocardial infarction (rat monocytes and platelets)	+	+	ND	ND	116
(a) Excitotoxicity (intracerebral NMDA) or (b) traumatic head injury (rat pup brain areas)	(a) +‡ (b) +	(a) 0 (b) 0	(a) -§ (b) +§ (mRNA or binding)	ND	125
Traumatic brain injury (closed head injury; mouse brain)	ND	+	ND	ND	126
Picrotoxinin-induced excitotoxicity (rat brain)	ND	+‖	ND	ND	127
Kainic acid-induced excitotoxicity (rat hippocampus)	+	0	ND	ND	128
Febrile seizures (rat hippocampus)	0	0	+ (expression)	0¶ (activity)	129
Human immunodeficiency virus type-1 coat glycoprotein gp120-induced neurotoxicity (rat cerebral cortex)	-	ND	0§ (binding)	+# (expression and activity)	130

* NMDA indicates *N*-methyl-D-aspartate. (See footnote to Table 1 for additional definitions.)
† In the ischemic boundary zone of the cortical mantle but not in the cortical or striatal sectors of the ischemic core.
‡ Increased levels of 16:0, 18:0, 18:1, and 18:2 acyl ethanolamides were also detected.
§ These binding experiments were perfomed with [^{3}H]CP55940 or [^{3}H]anandamide which have significant affinity for both CB_1 and CB_2 receptors.
‖ Increased levels of 1(3)-arachidonoyl glycerol but not of other acyl glycerols such as 2-palmitoyl or 2-oleoyl glycerol were also detected.
¶ The activity of MAG lipase was also not affected.
# There was also an increase in anandamide membrane transport.

immunodeficiency virus-induced encephalitis but not in samples from control animals[81] (see also Table 2),

- CB_2 receptors are expressed in atherosclerotic plaques of human and mouse diseased arteries but not in nondiseased arteries (Table 1),
- CB_2 receptors are highly upregulated in human cirrhotic liver (Table 1),
- CB_2 (and CB_1) receptor expression is higher in human prostate cancer cells than in normal cells[75] (see also Table 1), and
- CB_2 but not CB_1 receptor expression is higher in biopsies from human astrocytomas exhibiting high-grade malignancy than in biopsy tissue exhibiting lower grade malignancy.[131]

Finally, it is important to bear in mind that changes in endocannabinoid tissue levels or in the expression level or density of cannabinoid receptors seems to take place in response not only to certain pathological insults but also to some nonpathological processes or stimuli such as fasting, feeding, stress, aging, and exercise (Tables 2 and 3).

Evidence That the Endocannabinoid System Is Autoprotective

There is evidence not only that tissue concentrations of endocannabinoids, cannabinoid receptor density and/or cannabinoid receptor coupling efficiency increase in a range

of different disorders but also that these increases serve to reduce the severity of signs and symptoms of some of these disorders or even to oppose disease progression. Support for the hypothesis that the endocannabinoid system has such an "autoprotective" role has so far come mainly from experiments concerned with pain, multiple sclerosis, cancer, intestinal, mental and cardiovascular disorders, excitotoxicity, traumatic head injury, and Parkinson's disease.

Pain

Reports are emerging that FAAH inhibitors such as OL-135, URB532, URB597, and *N*-arachidonoylglycine are antinociceptive in various rodent models of pain. These models are the formalin paw test of persistent inflammatory pain[38,39] in which it is already known that central nervous system (CNS) levels of anandamide rise above control values in response to the formalin (Table 2), and the tail-immersion and hot-plate tests of acute thermal pain.[37,38,132] There are reports too that the endocannabinoid cellular uptake inhibitors, OMDM-2 and VDM11, produce antinociception in the rat hot-plate test.[133]

That the antinociception induced by inhibitors of FAAH or endocannabinoid cellular uptake is mediated by endogenous fatty acid ethanolamides is supported by several findings. These include the observations that OL-135, URB597, and *N*-arachidonoylglycine elevate endogenous levels of these amides in rodents[37,38,134] and that exogenously administered anandamide can be potentiated by OL-135 in the mouse tail-immersion test[38] and by AM404 in the mouse hot-plate test.[44] Also consistent with this hypothesis are the findings first, that mice from which FAAH has been genetically deleted ($FAAH^{-/-}$ mice) exhibit reduced sensitivity to noxious stimuli when these are applied in the tail-immersion, hot-plate, or formalin paw test or in the carrageenan model of inflammatory pain[135,136]; and second, that $FAAH^{-/-}$ mice have elevated tissue levels of anandamide and other fatty acid ethanolamides and exhibit greater sensitivity than wild-type mice to the antinociceptive effect of exogenously administered anandamide.[135]

Some experiments have been performed with transgenic (FAAH-NS) mice that express FAAH only within the nervous system. These differ from $FAAH^{-/-}$ mice in not exhibiting enhanced levels of anandamide and other fatty acid ethanolamides in the CNS or reduced nociception in the tail-immersion or hot-plate plate test.[137] Consequently, it is likely that signs of hypoalgesia in these tests that are observed in $FAAH^{-/-}$ mice or are induced by FAAH or endocannabinoid cellular uptake inhibitors in wild-type mice depend on the activation of targets located in the nervous system. It is also likely that these targets are mainly or wholly cannabinoid CB_1 receptors. Thus, Kathuria et al[37] found that the antinociceptive effects of URB532 and URB597 in the mouse hot-plate test were attenuated by SR141716A at a dose that by itself had no effect on nociception. Similarly, it was found by Lichtman et al[38] that SR141716A attenuated OL-135 induced-antinociception in mice in the tail-immersion, hot-plate, and formalin paw tests. None of the FAAH inhibitors used in these 2 investigations exhibited significant affinity for CB_1 (or CB_2) receptors, suggesting that these inhibitors did not produce antinociception by activating cannabinoid receptors directly. It has also been found that after they have been injected with SR141716A, $FAAH^{-/-}$ mice no longer exhibit hypoalgesic responses to noxious stimuli in the tail-immersion test, the hot-plate test, or the formalin paw test (both phases).[135,136] There is good evidence that CB_2 receptor activation can induce antinociception in the second phase of the formalin paw test (see below). Even so, it is unlikely that the signs of hypoalgesia that are exhibited by $FAAH^{-/-}$ mice in this pain model are mediated by CB_2 receptors as the ability of SR141716A to eliminate these signs in such animals is not shared by SR144528.[136]

The concept that cannabinoid CB_1 receptors can mediate antinociception produced by inhibitors of FAAH or endocannabinoid cellular uptake or by genetic deletion of FAAH is consistent with several findings that have been extensively reviewed elsewhere.[20,138-140] Briefly, these findings are first, that CB_1 receptors are located on pain pathways in the brain and spinal cord and on the central and peripheral terminals of primary afferent neurons that mediate both neuropathic and nonneuropathic pain, and second, that cannabinoid receptor agonists can induce signs of antinociception when injected either systemically or directly onto a CB_1 receptor-expressing region of a pain pathway. CB_1 receptor agonists exhibit antinociceptive activity in a wide range of experimental pain models and this antinociception has often been found to be antagonized by SR141716A, albeit sometimes in models in which this antagonist produces signs of hyperalgesia when administered by itself. This hyperalgesia could reflect either antagonism of endocannabinoids released onto CB_1 receptors or an ability of SR141716A to reduce the extent to which CB_1 receptors couple spontaneously to their effector mechanisms (*Exogenous Ligands* section) or both of these actions. Since SR141716A-induced inverse agonism is expected to occur particularly in tissues in which there is a relatively high expression of CB_1 receptors, it is noteworthy that CB_1 receptor expression levels in the spinal cord increase in a rat model of neuropathic pain in which SR141716A has been found to be hyperalgesic[99,141] (see also Table 2).

That CB_1 receptors have a role in pain perception is also supported by the results from experiments in which antisense methods have been used to achieve a "knockdown" of CB_1 receptors in the brain or spinal cord.[142-144] Additional support comes from findings that mice from which the CB_1 receptor has been genetically deleted ($CB_1^{-/-}$ mice) exhibit reduced antinociception following a forced swim in water at

34°C[145] and increased tactile sensitivity.[146] Unexpectedly though, there are also reports that compared with wild-type animals, $CB_1^{-/-}$ mice exhibit either no differences in nociceptive behavior in hot-plate, tail-immersion, tail-flick, tail-pressure, or abdominal-stretch tests[146-148] or even signs of hypoalgesia in the hot-plate test and the first phase of the formalin paw test.[148]

There is evidence that CB_2 receptors can also mediate analgesia. Thus, for example, when administered systemically to rats or mice the CB_2-selective agonists HU308, GW405833, and AM1241 have all been found to exhibit antinociceptive activity in various pain models in a manner that is opposed by a CB_2-selective antagonist. Such apparent CB_2-mediated antinociception has been observed for HU308 in the second phase of the mouse formalin paw test,[149] for GW405833 in rat carrageenan and rat and mouse Freund's complete adjuvant models of inflammatory pain and in rat models of neuropathic and incisional pain,[150,151] and for AM1241 in rat models of allodynia and of thermal and inflammatory pain and in mouse and rat models of neuropathic pain.[146,152-155] It is unlikely that these CB_2-selective agonists were also activating CB_1 receptors, as AM1241 was no less effective in reducing signs of neuropathic pain in $CB_1^{-/-}$ mice than in wild-type animals[146] and as HU-308 and AM1241 were not antagonized by CB_1-selective antagonists. It is possible that CB_2 receptors that mediate antinociception are located on nonneuronal cells in the skin and that, when activated, these receptors modulate the endogenous release of molecules that target peripheral nociceptors.[152] Indeed, evidence has recently emerged that suggests that one mechanism by which CB_2 selective agonists such as AM1241 produce antinociception, at least in a rat model of thermal pain, may be by stimulating the release of β-endorphin from CB_2-expressing cells in the skin such as keratinocytes onto μ-opioid receptors located on the terminals of primary afferent neurons.[156] However, it would be premature to exclude other possibilities, for example, that CB_2 receptors can also be expressed by sensory neurons[157] and that such CB_2 receptors, or indeed microglial CB_2 receptors that appear in the spinal cord after nerve injury[100] (see also Table 2), can mediate antinociceptive effects. It also remains possible that CB_2-selective agonists can produce antinociception in at least some pain models by activating "CB_2-like" receptors, putative receptors at which the endogenous fatty acid amide palmitoylethanolamide may induce hypoalgesia (reviewed elsewhere[7,139]).

In some pain models, signs of hypoalgesia that seem to be produced by endogenously released endocannabinoids appear to be entirely CB_1 receptor mediated. However, there is also some evidence that antinociception may sometimes be induced instead by endogenous activation of CB_2 or (CB_2-like) receptors. Thus, in $FAAH^{-/-}$ mice, there is an elevation in the tissue levels of palmitoylethanolamide,[135] which may produce antinociception by acting on a CB_2-like receptor, and in the carrageenan model of inflammatory pain, although not in the formalin paw test (see preceding paragraph), these knockout mice exhibit signs of hypoalgesia that are not reduced by SR141716A but are lessened, albeit only partially, by SR144528.[136] It has also been reported that URB597 decreases carrageenan-induced mouse paw edema in an SR144528-sensitive manner.[158] Moreover, when administered by itself, this CB_2-selective antagonist has been found to produce signs of hyperalgesia in rats both in the carrageenan model[150] and in the first phase of the formalin paw test.[159] However, there are also several reports that CB_2 receptor antagonists are not hyperalgesic in these rat models of inflammatory pain or in models of allodynia or thermal pain.[152-155,160] A CB_1- or CB_2 receptor antagonist may of course sometimes fail to enhance signs of pain in an experimental model because the pain score is already maximal. Conversely, it remains possible that in experiments in which SR144528 was hyperalgesic, it was acting as an inverse agonist rather than as an antagonist of endogenously released endocannabinoids.

There is evidence that cannabinoid receptor agonists can also relieve pain in humans, for example, in patients with neuropathic pain[161] and in some patients with cancer, multiple sclerosis, spinal cord injury, blepharospasm, brachial plexus damage, or pain from limb amputation.[161-169] Whether inhibitors of endocannabinoid metabolism or cellular uptake will also be able to induce analgesia in the clinic has still to be established.

Finally, there is evidence that CB_2 receptor *inverse* agonists have therapeutic potential as antiinflammatory agents. Thus, although there is little doubt that CB_2 receptor agonists can produce antinociception in experimental models of various kinds of pain that include inflammatory pain, it has also been found that the CB_2 receptor inverse agonists, SR144528 and JTE-907, can inhibit carrageenan-induced mouse paw edema,[170] indicating them to be antiinflammatory agents, and that a third CB_2 receptor inverse agonist, Schering-Plough's compound 4j, inhibits immune cell migration both in vitro and in vivo.[171,172] There is also some evidence that although FAAH inhibitors may be effective against thermal and inflammatory pain, they lack potential for the management of neuropathic pain. Thus, in experiments with sciatic nerve ligation models, it has been observed that $FAAH^{-/-}$ mice do not show signs of hypoalgesia[136] and that rats exhibit an antinociceptive response to URB597 only when this is administered at a dose well above its threshold dose for FAAH inhibition.[173]

Multiple Sclerosis

There are 2 reports that in a mouse model of multiple sclerosis, spasticity can be ameliorated both by the FAAH inhibitor,

AM374, and by the inhibitors of endocannabinoid cellular uptake, AM404, VDM11, OMDM-1, and OMDM-2.[77,133] The model used, the chronic relapsing experimental allergic encephalomyelitis (CREAE) model, is one in which levels of anandamide and 2-arachidonoyl glycerol in both brain and spinal cord are higher than in unlesioned animals (Table 2). In another model of multiple sclerosis in which mice are inoculated intracerebrally with Theiler's murine encephalomyelitis virus (TMEV), OMDM-1 has been found to oppose the impaired rotarod performance and reductions in spontaneous motor activity exhibited by the lesioned animals and to enhance levels of anandamide although not 2-arachidonoyl glycerol in the spinal cords of these animals.[174]

There are several reasons for believing that the amelioration of spasticity induced in CREAE mice by inhibitors of FAAH or endocannabinoid cellular uptake is mediated at least in part by CB_1 and possibly also by CB_2 receptors. First, the antispastic effect of the FAAH inhibitor, AM374, has been found to be blocked by SR141716A and SR144528.[77] As AM374 is not itself expected to bind to cannabinoid receptors at the dose used, this finding suggests that it produced its inhibitory effect on spasticity indirectly by enhancing endocannabinoid concentrations at these receptors. Second, Pryce et al[175] have found that compared with wild-type CREAE mice, $CB_1^{-/-}$ CREAE mice exhibit an earlier onset of spasticity, more immobility, residual paresis, spinal cord axonal loss and spinal neurodegeneration, and greater mortality. Third, there have been several reports that the exogenous administration to lesioned rodents of cannabinoid receptor agonists, including anandamide and 2-arachidonoyl glycerol, *R*-(+)-WIN55212 and Δ^9-THC, the CB_1-selective agonists, *R*-(+)-methanandamide and ACEA, and the CB_2-selective agonists, JWH-133 and JWH-015, can reduce spasticity or other signs of neurological damage such as tremor and spasm[77,176-178] or ameliorate atonia, ataxia, gait abnormalities, paralysis, moribundity, and mortality[179-181] or improve rotarod performance.[182] These experiments were performed with rodent models of multiple sclerosis in which demyelination was induced by inoculation either with TMEV[181,182] or with mixtures containing CNS tissue or myelin basic protein (CREAE/EAE models). Finally, the CB_1/CB_2 receptor agonist *R*-(+)-WIN55212 has been found to ameliorate clinical signs of demyelination in mice in a manner that is both stereoselective (CREAE and TMEV mice) and susceptible to antagonism by SR141716A and SR144528 (CREAE mice).[176,177,181]

Evidence that cannabinoids can reduce the spasms, spasticity, or tremor of multiple sclerosis has also been obtained in clinical trials with multiple sclerosis patients[165,166,183,184] (also reviewed elsewhere[164]). The degree of spasm or spasticity was either scored by the investigators using an objective measure or assessed subjectively by the patients themselves. The negative results sometimes obtained in such experiments when spasticity has been scored objectively[166,184,185] may well be a reflection of the low sensitivity of the available methods.

Further support for the hypothesis that CREAE mice release endocannabinoids onto cannabinoid receptors and that the resultant activation of these receptors reduces spasticity comes from the finding that CREAE mice with mild spasticity became significantly more spastic when injected with SR141716A alone or in combination with SR144528.[176] However, since these agents are both inverse agonists, it is also possible that they acted in an endocannabinoid-independent manner by reducing the extent to which cannabinoid receptors were coupling spontaneously to their effector mechanisms. Because SR141716A and SR144528 increase spasticity in CREAE mice, the finding that these cannabinoid receptor antagonists oppose *R*-(+)-WIN55212-induced reductions in the spasticity of CREAE mice should be interpreted with caution.

Results obtained with CREAE/EAE or TMEV models of multiple sclerosis also suggest that cannabinoid CB_1 or CB_2 receptor activation by exogenously administered or endogenously released agonists may oppose the progression of multiple sclerosis by slowing the neurodegenerative process,[175] reducing inflammation,[174,179,181,182] and promoting remyelination.[182]

Cancer

Evidence is emerging that certain types of cancer cells overproduce anandamide and/or 2-arachidonoyl glycerol (Table 1), that these endocannabinoids act through cannabinoid receptors to inhibit cancer cell proliferation or invasion, and that this inhibitory effect can be enhanced by inhibitors of endocannabinoid metabolism or cellular uptake.

Ligresti et al[41] have detected both these endocannabinoids in biopsy specimens from human CB_1-, CB_2-, and FAAH-expressing colorectal tissue and found that the levels of anandamide and 2-arachidonoyl glycerol were highest in precancerous adenomatous polyps, lower in carcinomas, and lowest in normal tissue. Experiments were also performed with cultured colorectal cancer cells (CaCo-2 cells) that express CB_1 receptors and FAAH but not CB_2 receptors and contain anandamide and 2-arachidonoyl glycerol. The proliferation of these cells was inhibited by anandamide, by 2-arachidonoyl glycerol, by certain synthetic CB_1 receptor agonists, by the FAAH inhibitor *N*-arachidonoyl serotonin, and by the endocannabinoid cellular uptake inhibitors, VDM11 and VDM13, in a manner that was sensitive to antagonism by SR141716A. Of interest, anandamide did not inhibit the proliferation of CaCo-2 cells that had differentiated into enterocytes. These differentiated cells exhibit much lower malignancy and invasiveness, have a lower

endocannabinoid content, and express more FAAH than undifferentiated CaCo-2 cells.

More recently, Bifulco et al[186] showed that in vivo growth of rat thyroid tumors in athymic mice could be inhibited by intratumoral injections of VDM11 or *N*-arachidonoyl serotonin. The tumor content of 2-arachidonoyl glycerol was increased by both these drugs, whereas the tumor content of anandamide (and palmitoylethanolamide) was increased only by *N*-arachidonoyl serotonin. It was also found that in vitro proliferation of these tumor cells was inhibited both by VDM11 and by the cannabinoid receptor agonist, 2-methyl-arachidonyl-2'-fluoro-ethylamide, and that this inhibition was blocked by SR141716A but not by SR144528. 2-Arachidonoyl glycerol and *N*-arachidonoyl serotonin, which also inhibited the in vitro proliferation of these cells, were less susceptible to antagonism by SR141716A. These findings are in line with evidence from previous investigations that both cancer cell proliferation in vitro and angiogenesis, tumor growth, and metastatic spreading of cancer cells in vivo can be decreased by CB_1 receptor activation.[187-191] Evidence that CB_2 receptors mediate antitumor effects also exists.[188,190,191] Intriguingly, it is likely that as well as acting through CB_1 receptors to inhibit the growth of tumors in vivo and cancer cell proliferation in vitro, the anandamide analog, 2-methyl-arachidonyl-2'-fluoro-ethylamide, increases expression of CB_1 receptors in these tumors and cancer cells but decreases the levels of these receptors in healthy, noncancerous cells.[187,189] Such an action would presumably be shared by other cannabinoid receptor agonists when these are exogenously administered or endogenously released.

In another recent investigation, Nithipatikom et al[36] found first, that certain CB_1 and CB_2-expressing human cultured androgen-independent prostate cancer cells produce 2-arachidonoyl glycerol at high concentrations, and second, that in vitro invasion of these cells could be inhibited by 2-arachidonoyl glycerol, noladin ether, *R*-(+)-WIN55212 and *R*-(+)-methanandamide, and also by 2 inhibitors of 2-arachidonoyl glycerol metabolism, MAFP and DAK. SR141716A but not SR144528 increased invasion of the prostate cancer cells and reversed the inhibitory effect of MAFP. It was also found that MAFP-induced inhibition of invasion of these cells could be enhanced by 2-arachidonoyl glycerol. Evidence has also recently been obtained from experiments with human cultured androgen-sensitive prostate cancer cells for a CB_1 and CB_2 receptor-mediated induction of apoptosis and inhibition of cell growth by *R*-(+)-WIN55212 and for the reduction by this cannabinoid receptor agonist of angiogenesis.[75]

Intestinal Disorders

There is evidence first, that certain disorders characterized by inflammation of the gastrointestinal tract or by diarrhea may be associated with an increase in intestinal endocannabinoid levels and/or in the expression of CB_1 receptors by myenteric neurons (Table 3), second, that the resultant hyperactivity of the endocannabinoid system ameliorates at least some of the symptoms of these diseases and, third, that this amelioration can be mimicked by CB_1 receptor agonists or enhanced by inhibitors of endocannabinoid metabolism.[192]

Izzo et al[119] have obtained evidence that the endocannabinoid system acts through overexpressed CB_1 receptors to oppose cholera toxin-induced accumulation of intestinal fluid in mice. Thus, levels of anandamide, although not of 2-arachidonoyl glycerol or palmitoylethanolamide, increased in the small intestine in response to cholera toxin as did the expression of CB_1 receptors. The intestinal fluid accumulation induced by cholera toxin was increased by SR141716A but not by SR144528 and was reduced by CP55940 and ACEA pretreatment in a manner that was sensitive to antagonism by SR141716A but not SR144528. Intestinal fluid accumulation was also prevented by VDM11 in an SR141716A-sensitive manner but was unaffected by the CB_2-selective agonist JWH-015 or by the TRPV1 antagonist capsazepine.

Results obtained by Massa et al[118] from experiments with $CB_1^{-/-}$, $FAAH^{-/-}$, and wild-type mice suggest that the CB_1 component of the endogenous cannabinoid system decreases colonic inflammation induced by intrarectal administration of 2,4-dinitrobenzene sulphonic acid (DNBS). DNBS-induced colitis was more marked in $CB_1^{-/-}$ mice and less marked in $FAAH^{-/-}$ mice than in wild-type mice. Moreover, in wild-type mice, DNBS-induced colitis was reduced by HU-210 and enhanced by SR141716A and provoked an increase in the number of CB_1-expressing cells in mouse colonic myenteric plexus. There is also a report that intestinal inflammation and hypermotility induced by oral croton oil is associated with an increase in CB_1 expression level in mouse jejunum.[117] The sensitivity of croton oil-treated mice to CP55940 and cannabinol-induced inhibition of gastrointestinal transit of an orally administered charcoal suspension increased as well. High levels of anandamide and 2-arachidonoyl glycerol were detected in the small intestines of both croton oil-treated and control mice, even though FAAH activity was 2-fold *higher* in inflamed small intestine than in control tissue.

Mental Disorders

Giuffrida et al[54] have found that levels of anandamide but not of palmitoylethanolamide or oleoylethanolamide are markedly higher in the cerebrospinal fluid of antipsychotic-naïve first-episode paranoid schizophrenics and of schizophrenics taking "atypical" antipsychotics than in the cerebrospinal fluid of healthy controls. In contrast, anandamide levels were not elevated in schizophrenics taking

"typical" antipsychotics or in patients with dementia or affective disorders. In the antipsychotic-naïve schizophrenics there was a negative correlation between anandamide levels and severity of symptoms, a finding that is at least consistent with the hypothesis that anandamide has a *protective* role in schizophrenia. These findings raise the possibility that in schizophrenia, exaggerated dopamine release onto postsynaptic D_2-like receptors triggers release of anandamide, which then acts as a retrograde messenger to induce a CB_1 receptor-mediated attenuation of dopamine release. This chain of events would most likely be prevented by typical antipsychotics as these block D_2-like receptors but be unaffected by atypical antipsychotics as these act mainly on $5\text{-}HT_{2A}$ receptors. Giuffrida et al[54] have also suggested that heavy cannabis use may promote psychotic episodes in vulnerable individuals by desensitizing CB_1 receptors such that the ability of endogenously released anandamide to ameliorate signs and symptoms of schizophrenia is compromised.

Marsicano et al[108] performed experiments with an aversive memory model in which mice are trained to associate a tone with a foot-shock such that once they are conditioned, they freeze when re-exposed just to the tone. Their main findings were that this tone provoked an increase in anandamide and 2-arachidonoyl glycerol levels in the basolateral amygdala complex but not the medial prefrontal cortex of conditioned mice, that extinction of tone-induced freezing behavior was more pronounced in wild-type than $CB_1^{-/-}$ mice, and that extinction was also impaired by SR141716A in wild-type mice. These results prompted the suggestion that the endocannabinoid system could represent a therapeutic target for the treatment of diseases associated with inappropriate retention of aversive memories or with inadequate responses to aversive situations such as post-traumatic stress disorders or phobias.

Excitotoxicity and Traumatic Brain Injury

There is some evidence from experiments with mice that anandamide is released onto CB_1 receptors during excitotoxicity (Table 4) and that this release has a protective role.[128] More specifically, it has been found that kainic acid elevates anandamide but not 2-arachidonoyl glycerol or palmitoylethanolamide in the hippocampus, that this excitotoxin induces more severe seizures when the CB_1 receptor is genetically deleted or blocked with SR141716A, and that CB_1 receptor-expressing mice can be protected from kainic acid-induced seizures by the endocannabinoid cellular uptake inhibitor, UCM707. In addition, the data obtained suggest that the CB_1 receptors mediating this protective effect are located on principal forebrain neurons rather than on GABAergic interneurons, the likely end result of anandamide-induced activation of these receptors being inhibition of glutamate release.

There is also evidence that the endocannabinoid system may protect against the consequences of traumatic brain injury, although in this case through the release of 2-arachidonoyl glycerol. Thus, results obtained using a mouse model of closed head injury suggest that brain levels of 2-arachidonoyl glycerol increase in response to traumatic brain injury (Table 4) and that when administered exogenously, this endocannabinoid can act through CB_1 receptors to reduce brain edema and improve neurobehavioral function and clinical recovery.[126,193]

Parkinson's Disease

It has been postulated that it may prove possible to alleviate symptoms of Parkinson's disease by using inhibitors of the metabolism or cellular uptake of anandamide to raise endogenous levels of this fatty acid amide so that its inhibitory effect on glutamate release from corticostriatal neurons is enhanced.[84,85] This hypothesis was prompted by results obtained in experiments using a rat model of Parkinson's disease in which nigrostriatal dopaminergic neurons are destroyed unilaterally by injecting 6-hydroxydopamine close to the substantia nigra on one side of the brain. These experiments showed that the frequency of glutamatergic spontaneous excitatory postsynaptic potentials (sEPSPs) was greater in corticostriatal slices of lesioned than of control (sham-operated) animals and that this increased rate of firing could be reduced by the fatty acid amide hydrolase inhibitors, PMSF and MAFP, and by the anandamide cellular uptake inhibitors, AM404 and VDM11. The direct CB_1 receptor agonist, HU-210, was found to mimic this effect of these inhibitors, while the CB_1-selective antagonist, SR141716A prevented the effects of AM404 and PMSF. It was also found that 6-hydroxydopamine elevates levels of anandamide in the striatum but not in the cerebellum. Striatal and cerebellar levels of 2-arachidonoyl glycerol were unaffected. That inhibitors of anandamide metabolism or cellular uptake may alleviate symptoms of Parkinson's disease is also supported by a report that intraperitoneally administered AM404 ameliorates akinesia and sensorimotor orientation and reduces amphetamine-induced turning behavior in 6-hydroxydopamine-lesioned rats.[86] The effect of AM404 on amphetamine-induced turning behavior was abolished by the CB_1-selective antagonist, AM251. There is also evidence from experiments with 6-hydroxydopamine-lesioned rats that drugs that enhance the activity of the endocannabinoid system may have the capacity to suppress or prevent unwanted dyskinesias that are often induced in parkinsonian patients by the therapeutic agent, L-dihydroxyphenylalanine (L-DOPA).[87]

Results from other investigations have raised the possibility that symptoms of Parkinson's disease could be alleviated by CB_1 receptor antagonists. Thus there are 2 reports from the

same laboratory that the akinesia, sensorimotor orientation, and asymmetric motor behavior exhibited by rats with 6-hydroxydopamine-induced unilateral nigral lesions can be significantly attenuated by SR141716A or AM251 when these are given either systemically or unilaterally into the striatum or globus pallidus on the lesioned side of the brain.[194,195] An additional finding, that inhibitory effects of systemic SR141716A or AM251 on amphetamine-induced asymmetric motor behavior could be prevented by AM404, was taken as evidence for the presence of a CB_1 receptor-mediated modulation of nigrostriatal dopaminergic tone in the lesioned animals and also suggests that these antagonists were acting to reduce this tone. SR141716A and AM251 were most effective when given to rats exhibiting particularly severe behavioral signs of nigral degeneration, suggesting that cannabinoid CB_1 receptor antagonists might be useful for treating advanced stages of Parkinson's disease in humans.[195] That CB_1 receptor antagonists could be used for the management of Parkinson's disease has also been proposed by Lastres-Becker et al.[61] This suggestion was prompted by results they obtained in experiments with tissue both from parkinsonian patients and from marmosets lesioned with 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP). More specifically, they found that CB_1 receptors in parkinsonian and MPTP-lesioned basal ganglia bind more [^{3}H]CP55940 and exhibit greater coupling efficiency than control tissue and that these changes were less marked in the basal ganglia of MPTP-treated animals that had been exposed to L-DOPA. It is noteworthy, however, that Meschler et al[196] have reported that SR141716A does not alleviate motor deficits induced by MPTP in cynomolgus monkeys.

Clearly no firm conclusions can yet be drawn about the role of the endocannabinoid system in Parkinson's disease or about the extent to which modulation of this system with drugs could alleviate undesirable symptoms associated either with the disease itself or with the established anti-parkinsonian drug, L-DOPA.

Cardiovascular Disorders

There is evidence that anandamide and 2-arachidonoyl glycerol are major contributors to hypotension associated with hemorrhagic, septic, or cardiogenic shock. Thus first, it has been found that hypotension induced in anaesthetized rats by bleeding, by acute myocardial infarction, or by intravenous administration of bacterial lipopolysaccharide (LPS), a major pathogenic factor in septic shock, can be opposed by SR141716A or AM281, whereas exogenous 2-arachidonoyl glycerol administration induces hypotension in anaesthetized normotensive animals.[113,116,197,198] Second, there are reports that macrophages obtained from the blood of rats that are normotensive or in hemorrhagic shock can synthesize anandamide[197] and that in vitro administration of LPS elevates levels of 2-arachidonoyl glycerol in rat platelets and of anandamide in rat and mouse macrophages and human lymphocytes[113-115] (Table 3). There is also a report that anandamide and 2-arachidonoyl glycerol are detectable in monocytes and platelets isolated from rats after acute myocardial infarction but not in cells obtained from control animals[116] (Table 3). Third, blood from hemorrhaged rats and cells isolated from rat blood after acute myocardial infarction or onset of LPS-induced hypotension can cause prolonged hypotension in anaesthetized normotensive rats.[113,116,197] This hypotension was found to be considerably attenuated or completely abolished by SR141716A, pointing to an involvement of CB_1 receptors. Moreover the ability of LPS to increase anandamide levels in mouse macrophages in vitro can be enhanced by the nonselective FAAH inhibitor, PMSF, and a much greater decrease in blood pressure can be elicited by LPS-treated peritoneal macrophages when these cells are obtained from $FAAH^{-/-}$ rather than from $FAAH^{+/+}$ mice.[114]

It is possible that CB_1-mediated hypotension may aid survival in hemorrhagic and cardiogenic shock. Thus, as well as attenuating hypotension induced in urethane-anaesthetized rats by bleeding or acute myocardial infarction, SR141716A has been found to reduce the survival time of these animals.[116,197] Conversely, survival of hemorrhaged rats is significantly increased by the cannabinoid receptor agonists, Δ^9-THC and HU-210.[197] In LPS-treated rats, however, survival can be improved both by Δ^9-THC and by SR141716A or AM281.[113,198]

There is also evidence that CB_1 receptors may mediate a protective hypotensive effect in spontaneously hypertensive rats.[111] These are animals in which it has been found that blood pressure is elevated by SR141716A and reduced by the FAAH inhibitor, URB597, and by 2 inhibitors of endocannabinoid cellular uptake, AM404 and OMDM-2. The blood pressure of normotensive rats was not affected by any of these drugs. Compared with normotensive rats, spontaneously hypertensive rats also express more CB_1 receptors in the myocardium and aorta (Table 3) and show greater sensitivity to anandamide- and HU-210-induced hypotension.[111] However, FAAH expression is higher and myocardial levels of anandamide (but not of 2-arachidonoyl glycerol) are lower in spontaneously hypertensive rats than in normotensive animals (Table 3), raising the possibility that the endocannabinoid system may protect against hypertension in this animal model by increasing "target organ sensitivity" rather than by increasing endocannabinoid levels.[111]

Finally, it is possible that the endocannabinoid system may protect against atherosclerosis as first, CB_2 receptors are expressed by macrophages and T-lymphocytes within atherosclerotic plaques of human and mouse diseased arteries (Table 1), and second, there is evidence that when

administered orally at 1 mg/kg per day, Δ^9-THC can act through CB_2 receptors to produce a significant inhibition of atherosclerosis progression in mice.[71]

THERAPEUTIC STRATEGIES

It is clear from the preceding 2 sections of this review that the endocannabinoid system is thought to upregulate in some disorders and that one consequence of this upregulation may be the alleviation of symptoms or even the removal of the underlying causes of some of these symptoms. This section begins by considering how direct agonists might best be used in the clinic to mimic protective effects of the endocannabinoid system. It then goes on to discuss some alternative potential strategies that would augment these protective effects indirectly by potentiating endogenously released endocannabinoids through effects on their metabolism, cellular uptake, or receptors.

Direct Cannabinoid Receptor Agonists

Two CB_1/CB_2 cannabinoid receptor agonists have been licensed for clinical use in some countries for several years. These are dronabinol (Marinol; Δ^9-THC) and the synthetic Δ^9-THC analog, nabilone, which can be prescribed as antiemetics (both drugs) and to stimulate appetite (dronabinol).[199] Both these drugs are administered orally. In addition, Sativex, a medicine that contains Δ^9-THC and cannabidiol, was recently licensed for the management of neuropathic pain associated with multiple sclerosis.[199,200] A significant number of patients also claim to obtain relief from symptoms of multiple sclerosis and, indeed, from various kinds of chronic pain, from arthritis and/or from neuropathy by self-medicating with cannabis.[201,202] As to novel synthetic CB_1/CB_2 or CB_2 cannabinoid receptor agonists that exhibit antinociceptive activity in animal models of pain, there are as yet no clinical data, although several of these have been synthesized and investigated preclinically by drug companies (eg, by GlaxoSmithKlein[150,151] and by Bayer[203]).

A strong case can be made for using cannabinoid receptor direct agonists in the clinic now, particularly to relieve neuropathic pain and to ameliorate the spasms and spasticity of multiple sclerosis. However, a strong argument can also be made for conducting research directed at establishing how in the longer term the benefit-to-risk ratio of drugs that rely on the activation of cannabinoid receptors for their sought-after clinical effects can be improved. Some potential strategies for meeting this objective are listed below.

- Exploit the ability of cannabinoid CB_1 receptors expressed outside the central nervous system to mediate sought-after effects such as pain relief (reviewed elsewhere[139,140]), the inhibition of cancer cell proliferation and spread (*Cancer* section) and the amelioration of certain intestinal and cardiovascular disorders (*Intestinal Disorders* and *Cardiovascular Disorders* sections). One possibility would be to administer CB_1 receptor agonists transdermally and, indeed, there is already evidence that this strategy is effective in human subjects for reducing experimental pain.[204,205] A second possibility would be to develop CB_1 receptor agonists that do not readily cross the blood-brain barrier.
- Exploit the ability of cannabinoid CB_1 receptors expressed in the spinal cord to mediate pain relief by administering CB_1 receptor agonists intrathecally (reviewed elsewhere[139,140]). This is already an accepted procedure that is, for example, used for the self-administration of baclofen by some multiple sclerosis patients.
- Exploit the ability of cannabinoid CB_2 receptors to mediate pain relief by administering a CB_2-selective agonist (*Pain* section). This would of course avoid all unwanted consequences of CB_1 receptor activation, provided the dose of agonist used was not excessive.
- Exploit the ability of a low dose of a cannabinoid receptor agonist such as Δ^9-THC both to interact synergistically with a low dose of an opioid such as morphine or codeine for the production of analgesia and to oppose onset of opioid tolerance (reviewed elsewhere[139,206]). The success of this approach will depend on the extent to which these drugs interact synergistically in the clinic to produce undesirable effects. It is worth noting that there is already a report that orally-administered Δ^9-THC had a "morphine-sparing effect" for a patient with severe abdominal pain.[207]
- Exploit the apparent ability of drugs such as the nonpsychotropic cannabinoid, cannabidiol, to reduce some of the undesirable effects of CB_1 receptor activation and perhaps also provide additional beneficial effects.[208] Indeed, the licensed medicine, Sativex, contains both cannabidiol and Δ^9-THC.[199,200]

For some clinical applications it may be advantageous to combine 2 or more of these potential strategies. For example, neuropathic pain might sometimes best be relieved by targeting both CB_1 and CB_2 receptors, perhaps by administering a CB_2 receptor agonist together with a CB_1 agonist that does not readily cross the blood-brain barrier.

Any agonist that possesses CB_1 or CB_2 selectivity is expected to target CB_1 or CB_2 receptors selectively only

within a finite dose range and to activate both receptor types when administered at a dose that lies above this range. Such a finding has, for example, been made with the CB_2-selective agonist GW405833.[151] For any particular agonist, the width of its CB_1 or CB_2 selectivity window will of course be affected by the CB_1 to CB_2 receptor ratios of the tissues in which sought-after (or unwanted) effects are induced by CB_1 or CB_2 receptor activation. It is noteworthy, therefore, that not all tissues express CB_1 and CB_2 receptors in equal numbers in health and that there is also evidence that disparate changes in CB_1 and CB_2 expression levels may be induced in some cells or tissues either pathologically or pharmacologically. Consequently, when an agonist that binds more readily either to CB_1 or to CB_2 receptors is administered to patients, it is likely that the CB_1 or CB_2 selectivity of this ligand will not be the same in all tissues that express both receptor types and also that any selectivity will be lost when a certain dose level is exceeded. It is also important to bear in mind that there is evidence that some cannabinoid receptor agonists can activate non-CB_1, non-CB_2 pharmacological targets. Since cannabinoid receptor agonists differ in the extent to which they appear to interact with each of these proposed additional targets, it follows that some ligands with apparently similar abilities to activate CB_1 and/or CB_2 receptors are likely to possess different pharmacological profiles.

Indirect Cannabinoid Receptor Agonists

Existing indirect cannabinoid receptor agonists are all drugs that delay the removal of endocannabinoid molecules from their sites of action. They can be subdivided into FAAH inhibitors that inhibit the metabolism of anandamide and certain other fatty acid amides, MAG-lipase inhibitors that inhibit the metabolism of 2-arachidonoyl glycerol and related compounds, and drugs that inhibit endocannabinoid cellular uptake (*Inhibitors of Endocannabinoid Cellular Uptake and Intracellular Metabolism* section). Following the discovery of allosteric sites on CB_1 receptors, it may also prove possible to develop a second category of indirect cannabinoid receptor agonists that act by allosterically enhancing the ability of endogenously released (and exogenously administered) ligands to activate cannabinoid CB_1 receptors. The argument for using indirect agonists as therapeutic agents is that there are disorders in which endocannabinoids appear to be released selectively onto just certain populations of cannabinoid receptors, some or all of which mediate symptom relief. Consequently, drugs that potentiate endocannabinoids are expected to augment the relief of symptoms produced by such disorders without eliciting as many unwanted cannabinoid receptor-mediated responses as direct agonists that of course will activate all accessible CB_1 and/or CB_2 receptors.

Currently, much attention is being directed at the possibility of developing FAAH inhibitors as medicines. As a result FAAH inhibitors with some degree of selectivity have been developed, and the ability of these compounds to ameliorate symptoms in animal models of pain, multiple sclerosis, excitotoxicity, Parkinson's disease, and hypertension and to reduce cancer cell proliferation has been demonstrated. There is also evidence from animal experiments that FAAH inhibitors are more selective than direct CB_1 receptor agonists, at least after single administration. Thus, for example, in contrast to direct CB_1 agonists, FAAH inhibitors can produce antinociception in mice at doses that do not also induce hypomotility, hypothermia, or catalepsy.[20,37,38] It is noteworthy, however, that there are as yet no published clinical data for this group of compounds. Also, although FAAH inhibitors can induce signs of analgesia in animal models of acute and inflammatory pain, there is some doubt as to whether they would be effective against neuropathic pain. Some important pharmacological considerations that relate to FAAH inhibitors are listed below.

- Inhibition of FAAH is likely to augment anandamide levels not only at cannabinoid receptors but also at its other targets, for example the vanilloid TRPV1 receptor and the putative abnormal cannabidiol receptor, if this endocannabinoid is being released onto these other targets.
- Inhibition of FAAH is also likely to augment levels of certain other endogenous fatty acid amides when these are undergoing release. These amides include 2 cannabinoid receptor ligands, oleamide which might well increase drowsiness if its levels in the brain are elevated,[209] and *N*-arachidonoyl dopamine. They also include ligands that do not readily bind to cannabinoid receptors such as palmitoylethanolamide, which produces antinociception possibly by acting on peripheral "CB_2-like" receptors (*Pain* section), oleoylethanolamide, which is thought to stimulate fat utilization through activation of PPAR-α receptors, and *N*-arachidonoyl glycine, which is itself a FAAH inhibitor that can induce antinociception in animals when administered exogenously.
- A FAAH inhibitor is not expected to enhance the ability all endocannabinoids to induce symptom relief. Thus, some putative endocannabinoids are metabolically stable (eg, noladin ether) or are metabolized mainly by enzymes other than FAAH (eg, 2-arachidonoyl glycerol).
- Inhibition of FAAH may increase the extent to which the fatty acid amide substrates of this enzyme are degraded by other enzymes. For example, anandamide is known also to be metabolized by PAA, by cyclo-oxygenase-2, by lipoxygenases, and by cytochrome P450. Moreover, as there is evidence

that the lipoxygenase metabolites of anandamide are more potent than their parent compound as TRPV1 agonists,[210] it is possible that some patients receiving a FAAH inhibitor would experience either hyperalgesia due to TRPV1 activation or analgesia resulting from TRPV1 desensitization.

Turning now to inhibitors of endocannabinoid cellular uptake, there is evidence that these too have therapeutic potential. This comes from experiments in which such inhibitors have been found to decrease cancer cell proliferation and tumor growth and to ameliorate symptoms in animal models of pain, multiple sclerosis, excitotoxicity, Parkinson's disease, cholera toxin-induced diarrhea, and hypertension. As to MAG lipase inhibitors, any assessment of their therapeutic potential must await the development of suitable compounds, there being a need for compounds that inhibit MAG lipase at doses that do not also inhibit FAAH or interact directly with cannabinoid receptors or with other pharmacological targets that might compromise their selectivity. There is also an urgent need for an allosteric enhancer of the CB_1 receptor that exhibits appropriate efficacy and selectivity. Such an enhancer will most likely differ from a FAAH inhibitor in the following ways:

- by possessing the ability to produce greater symptom alleviation in at least some disorders through the augmentation of CB_1 receptor-mediated responses to all endocannabinoids no matter how their actions are terminated and
- by lacking the ability to augment the activation by anandamide or other fatty acid amides of targets other than CB_1 receptors.

Cannabinoid Receptor Expression Levels and Signaling

There is evidence that upregulation of the endocannabinoid system leading to an alleviation of symptoms can take the form not only of enhanced endocannabinoid release but also of an increase in cannabinoid receptor density or coupling efficiency in tissues in which these receptors mediate symptom relief or inhibition of disease progression when activated by endogenously released endocannabinoids or by exogenously administered cannabinoid receptor agonists. Indeed, results from rat experiments suggest that the endocannabinoid system may protect against hypertension through an increase in CB_1 receptor density/sensitivity rather than through an increase in endocannabinoid release (*Cardiovascular Disorders* section). Other seemingly protective increases in CB_1 and/or CB_2 receptor density or coupling efficiency have been detected in human and mouse atherosclerotic plaques, in human prostate cancer cells, and in animal models of Parkinson's disease, neuropathic pain, diarrhea, intestinal inflammation, and colitis.

It has been reported that cannabinoid receptor expression is higher in some cancer cells than in normal cells[75,187,189] and also that it sometimes correlates with the degree of cancer cell malignancy.[131] It will be important to identify other disorders in which cannabinoid receptor upregulation is confined entirely or mainly to tissues in which activation of cannabinoid receptors is expected to alleviate symptoms or to inhibit disease progression. This is because such a pattern of receptor upregulation may well improve the selectivity of cannabinoid receptor agonists by enhancing their potency for the production of sought-after effects more than their potency for the production of unwanted effects. Selectivity improvements resulting from an uneven cannabinoid receptor upregulation of this kind are expected to be most evident for partial agonists such as Δ^9-THC and cannabinol. Thus although an increase in receptor density will augment the potencies of both full and partial agonists, it will often also increase the size of the maximal response to a partial agonist without affecting the maximal response to a full agonist. This difference between the pharmacology of full and partial agonists can be seen in results from experiments with cannabinol and CP55940 in which an increase in the intestinal expression of CB_1 receptors (and intestinal inflammation) had been induced in mice by oral croton oil and in which the measured response was cannabinoid-induced CB_1 receptor-mediated inhibition of upper gastrointestinal transit of a charcoal suspension.[117] Thus, it was found that this increase in CB_1 expression level was accompanied not only by a leftward shift in the log dose-response curve of cannabinol but also by an increase in the size of the maximal effect of this partial agonist. In contrast, the higher efficacy agonist, CP55940, exhibited an increase in its potency but no change in its maximal effect.

The upregulation of a subpopulation of cannabinoid receptors that alleviates symptoms or inhibits disease progression when activated should of course also augment protective effects of endogenously released endocannabinoids and hence of FAAH inhibitors and other types of indirect agonist. For anandamide, cannabinoid receptor upregulation is likely to produce an increase in maximal effect as well as potency as this endocannabinoid is a CB_1 and CB_2 receptor partial agonist. On the other hand, 2-arachidonoyl glycerol would be expected to exhibit only an increase in potency as it has been found in several investigations to exhibit much higher CB_1 and CB_2 receptor efficacy than anandamide (*Endocannabinoids* section).

Tolerance can develop to CB_1 receptor agonists and there is evidence that this is often caused by a reduction in CB_1 receptor density or signaling.[211] Consequently, it is tempting to speculate that if a particular disease causes an increase

in cannabinoid receptor density or signaling this receptor upregulation may oppose the ability of endogenously released or exogenously administered cannabinoid receptor agonists to induce tolerance. If such receptor upregulation is restricted largely to receptors that mediate an alleviation of symptoms or an inhibition of disease progression, then patients would be protected from the development of tolerance to sought-after but not to unwanted effects of a cannabinoid receptor agonist in a manner that would lead to a broadening of the agonist's therapeutic window in response to its repeated administration. There is already good evidence that tolerance develops less readily to some effects of cannabinoid receptor agonists than to others (reviewed elsewhere[212]) and, indeed, that some sought-after therapeutic effects of a CB_1 receptor agonist may be more resistant to tolerance development than some of its unwanted effects.[213] CB_1 receptor agonists may also sometimes widen their own therapeutic windows by inducing a selective increase in the density of CB_1 receptors that are mediating a sought-after effect such as inhibition of the growth and proliferation of cancer cells.

Damaging Upregulation of the Endocannabinoid System?

There are some disorders in which the endocannabinoid system appears to upregulate to induce *undesirable* symptoms, an indication that this system may sometimes malfunction. For example, it has been postulated that upregulation of the endocannabinoid system can

- impair fertility in some women as there are reports (1) that FAAH activity and expression fall in the lymphocytes of some women who miscarry or who fail to become pregnant after in vitro fertilization (IVF) and embryo transfer, (2) that this fall is accompanied in the IVF-embryo transfer group of women by a corresponding rise in blood anandamide[65,66] (see also Table 1), and (3) that anandamide can impair embryo implantation and development in mice[214];
- contribute toward obesity in some individuals as, for example, it has been found that hypothalamic levels of anandamide and/or 2-arachidonoyl glycerol are raised in obese *db/db* and *ob/ob* mice and *fa/fa* rats (Table 2), that CB_1 receptor expression levels are elevated in the adipose tissue of obese *fa/fa* rats (Table 2), and that SR141716A reduces the food intake of *db/db* and *ob/ob* mice, reduces the body weight of *db/db* mice, and decreases the food intake, body weight, and waist circumference of obese humans[102,215];
- contribute toward osteoporosis and other diseases in which there is bone loss, as bone mineral density is greater in $CB_1^{-/-}$ mice than in wild type animals, as ovariectomy does not induce bone loss in $CB_1^{-/-}$ mice, as AM251 and SR144528 protect against ovariectomy-induced bone resorption, as CB_1 and CB_2 receptor antagonists/inverse agonists significantly inhibit evoked osteoclast formation in CB_1 and CB_2 receptor-expressing mouse bone marrow cultures, and as anandamide and CP55940 can stimulate osteoclast formation in these cultures[216];
- contribute toward the production of cerebral injury in stroke as unilateral ischemia/reperfusion injury induced in rats by transient middle cerebral artery occlusion has been reported to be associated with an elevation of whole brain anandamide and as infarct volume and neurological impairment in lesioned rats were both found to be less after pretreatment with selective CB_1 receptor antagonists[123] (see also Table 4);
- contribute toward the life-threatening excessive hypotension of endotoxaemic shock triggered by advanced liver cirrhosis[68,112] (Table 3) or by the cemented hip arthroplasty procedure[76] (Table 1) (but may aid survival in hemorrhagic, septic, and cardiogenic shock);
- contribute toward the development of hyperreflexia and hyperalgesia during cystitis as it has been found that urinary bladder levels of anandamide increase in a rat model of this disorder (Table 2) and that reflex activity increased both when anandamide was applied to healthy bladders and when the FAAH/anandamide cellular uptake inhibitor, palmitoylisopropylamide, was applied to healthy or inflamed bladders[101];
- contribute toward intestinal inflammation in ileitis as there is a report that ileal levels of anandamide (and 2-arachidonoyl glycerol) increase in a rat model of this disorder (Table 3), as inflammation and fluid accumulation in the rat ileum increase in response to intraluminal injection of either of these endocannabinoids, and as intraluminal injections of the FAAH/MAG lipase inhibitors, PMSF or MAFP, augment intra-ileal fluid accumulation induced by *Clostridium difficile* toxin A[120];
- contribute toward intestinal hypomotility in paralytic ileus as reductions in mouse intestinal motility induced by peritoneal irritation with acetic acid has been shown to be counteracted by SR141716A (although not by SR144528), to be exacerbated by the anandamide cellular uptake inhibitor, VDM11, in an SR141716A-sensitive manner, and to be accompanied in the small intestine by increases

in anandamide levels and in the neural density of CB_1 receptor immunoreactivity[121] (see also Table 3).

Endocannabinoids appear to produce many of their unwanted effects by interacting with cannabinoid CB_1 receptors. Thus, results from animal experiments suggest that endocannabinoids act on these receptors to induce (1) impairment of embryo implantation and development (reviewed elsewhere[214]), (2) increases in food intake and body weight associated with obesity,[102] (3) cerebral injury in stroke,[123] (4) hypotension in liver cirrhosis,[68] and (5) intestinal hypomotility in paralytic ileus.[121] As to the CB_2 component of the endocannabinoid system, evidence is emerging that this too may sometimes induce unwanted effects by promoting inflammation in a manner that can be counteracted by CB_2 receptor inverse agonism. There are also unwanted effects of the endocannabinoids that appear to be mediated by TRPV1 rather than CB_1 or CB_2 receptors. These are urinary bladder hyperreflexia in cystitis, and intestinal inflammation and fluid accumulation in ileitis.[101,120] In these instances the undesirable effects can be attenuated by TRPV1 receptor antagonism but not CB_1 receptor antagonism which, indeed, was found to exacerbate anandamide-induced bladder hyperreflexia. Clearly then, there may be a place in the clinic for CB_1 and TRPV1 receptor antagonists and possibly also for CB_2 receptor inverse antagonists. Indeed, the CB_1 receptor antagonist, SR14171A (rimonabant; Acomplia), is already in the final stages of development as an anti-obesity agent.[215] Selective inhibitors of endocannabinoid biosynthesis, activators of endocannabinoid-metabolizing enzymes, and allosteric antagonists of the CB_1 receptor also have therapeutic potential for the management of some disorders with symptoms that are induced by endogenously released cannabinoids. However, such drugs have yet to be developed.

Future Directions

There is no doubt that the endocannabinoid system upregulates both in response to certain pathological changes or to stress and during normal physiological events such as aging, feeding, fasting, and exercise. There is also good evidence that this upregulation can take the form of an increase both in the release of endocannabinoid molecules onto their receptors and in the density or coupling efficiency of some of these receptors. Often, this upregulation appears to protect the organism from unwanted symptoms or even to slow the progression of a disease. However, there is also evidence that upregulation of the endocannabinoid system can sometimes trigger unwanted symptoms, an indication that this system has its own pathology and possibly also that it is sometimes influenced detrimentally by pathological events taking place in some other system from which it receives input.

As to the future, it will be important to obtain a more complete list of pathological processes that are modulated by the endocannabinoid system and to determine for each of these processes whether this modulation has desirable or undesirable consequences. It will also be important to establish both the nature of this modulation and the extent to which it is confined to tissues in which cannabinoid receptor activation affects symptoms or disease progression. This in turn will help identify the best pharmacological strategy for the management of any particular disorder in which the endocannabinoid system has upregulated, be this to administer a CB_1 and/or CB_2 receptor agonist or antagonist that does or does not readily cross the blood-brain barrier, a CB_1 and/or CB_2 receptor agonist intrathecally or directly to some other site outside the brain, a partial CB_1 and/or CB_2 receptor agonist rather than a full agonist, a CB_1 and/or CB_2 receptor agonist together with a noncannabinoid, an inhibitor or activator of endocannabinoid biosynthesis, cellular uptake or metabolism, an allosteric modulator of the CB_1 receptor, or a CB_2 receptor inverse agonist. Other important objectives for future research are

- to obtain a more complete understanding of the processes that determine the biosynthesis, release, and fate of endocannabinoids,
- to identify those drugs that are best at modulating these processes selectively or at producing selective allosteric enhancement or antagonism of cannabinoid receptors,
- to characterize the pharmacology of these drugs and to test them in the clinic,
- to investigate the extent to which tolerance develops over time to protective effects of endocannabinoids when these are released by themselves or in the presence of drugs that augment the tissue levels of released endocannabinoids,
- to establish in greater detail the part played by non-CB_1, non-CB_2 targets in both the protective and the undesirable consequences of endogenous cannabinoid release, and
- to gain a better understanding of the part played by spinal or peripheral CB_1 and CB_2 receptors in modulating the symptoms and progression of particular diseases.

Finally, since the completion of this review, evidence has emerged that the orphan G protein-coupled receptor, GPR55, is a cannabinoid receptor,[217,218] prompting a need for research that will identify the physiological and/or pathological roles of this receptor and characterize its pharmacology. The discovery of a selective MAG lipase inhibitor, URB602, has also just been announced.[219]

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