

*Review***Targeting the endocannabinoid system
with cannabinoid receptor agonists:
pharmacological strategies and
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Human tissues express cannabinoid CB₁ and CB₂ receptors that can be activated by endogenously released ‘endocannabinoids’ or exogenously administered compounds in a manner that reduces the symptoms or opposes the underlying causes of several disorders in need of effective therapy. Three medicines that activate cannabinoid CB₁/CB₂ receptors are now in the clinic: Cesamet (nabilone), Marinol (dronabinol; Δ⁹-tetrahydrocannabinol (Δ⁹-THC)) and Sativex (Δ⁹-THC with cannabidiol). These can be prescribed for the amelioration of chemotherapy-induced nausea and vomiting (Cesamet and Marinol), stimulation of appetite (Marinol) and symptomatic relief of cancer pain and/or management of neuropathic pain and spasticity in adults with multiple sclerosis (Sativex). This review mentions several possible additional therapeutic targets for cannabinoid receptor agonists. These include other kinds of pain, epilepsy, anxiety, depression, Parkinson’s and Huntington’s diseases, amyotrophic lateral sclerosis, stroke, cancer, drug dependence, glaucoma, autoimmune uveitis, osteoporosis, sepsis, and hepatic, renal, intestinal and cardiovascular disorders. It also describes potential strategies for improving the efficacy and/or benefit-to-risk ratio of these agonists in the clinic. These are strategies that involve (i) targeting cannabinoid receptors located outside the blood-brain barrier, (ii) targeting cannabinoid receptors expressed by a particular tissue, (iii) targeting upregulated cannabinoid receptors, (iv) selectively targeting cannabinoid CB₂ receptors, and/or (v) adjunctive ‘multi-targeting’.

Keywords: Δ⁹-tetrahydrocannabinol; cannabinoid CB₁ and CB₂ receptors; cannabinoid receptor agonists; therapeutic applications and strategies; blood-brain barrier

1. INTRODUCTION

The endocannabinoid system consists of at least two types of G-protein-coupled receptor, cannabinoid CB₁ and CB₂ receptors, of endogenous agonists for these receptors that are known as ‘endocannabinoids’ and include anandamide and 2-arachidonoyl glycerol, and of the processes responsible for endocannabinoid biosynthesis, cellular uptake and degradative metabolism [1]. Importantly, there is convincing evidence that there are some disorders in which the endocannabinoid system upregulates in a manner that induces or exacerbates certain disorders, including obesity [1]. The discovery of the link between obesity and the endocannabinoid system prompted the development of the CB₁ receptor antagonist/inverse agonist, rimonabant (SR141716A; Acomplia) as an anti-obesity agent. This drug entered European clinics in 2006 for the management of obesity, but was withdrawn in 2008 because of safety concerns about its adverse effects,

particularly an increased incidence of depression, anxiety and suicidality [2]. As a result, major pharmaceutical companies appear to have lost interest entirely in all drugs that block CB₁ receptors. This has prompted a need for a strategy that would significantly improve the benefit-to-risk ratios of rimonabant-like drugs, just one possibility being to develop a medicine from a CB₁ receptor antagonist or antagonist/inverse agonist that does not readily cross the blood-brain barrier [2,3].

There is also convincing evidence, however, that there are a number of serious disorders that are ameliorated by ‘autoprotective’ increases in the release of endocannabinoids onto subpopulations of their receptors and/or in the expression or coupling efficiency of cannabinoid receptors in certain locations. Such increases have, for example, been observed in human cancer and in animal models of neuropathic and inflammatory pain, multiple sclerosis, intestinal disorders, post-traumatic stress disorder, traumatic brain injury, haemorrhagic, septic and cardiogenic shock, hypertension, atherosclerosis and Parkinson’s disease [1].

Licensed medicines that exploit beneficial effects of direct cannabinoid receptor activation have already

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been developed [3,4]. Two of these, the CB₁/CB₂ receptor agonist, Δ⁹-tetrahydrocannabinol (Δ⁹-THC; dronabinol; Marinol) and its synthetic analogue, Nabilone (Cesamet), were approved over 25 years ago as medicines for suppressing nausea and vomiting produced by chemotherapy. Subsequently, the use of dronabinol as an appetite stimulant, for example in AIDS patients experiencing excessive loss of body weight, was also approved. One other medicine that contains Δ⁹-THC, in this case together with the non-psychoactive plant cannabinoid, cannabidiol, is Sativex. This was licensed in Canada in 2005 for the symptomatic relief of neuropathic pain in multiple sclerosis and as an adjunctive analgesic treatment for adult patients with advanced cancer. In 2010, it was also licensed in the UK and Canada for the treatment of spasticity due to multiple sclerosis and has more recently become an approved medicine in several other countries. Although these medicines do of course all display a favourable benefit-to-risk ratio, they can give rise to unwanted side effects [3–5].

There is currently a lot of interest in the possibility of developing medicines from compounds that inhibit the cellular uptake and/or metabolism of endocannabinoids when these are being released in an autoprotective manner [1,6]. However, also attracting considerable interest is the idea of exploiting one or other of a wide range of pharmacological strategies expected to maximize the beneficial therapeutic effects and/or minimize the unwanted effects of drugs that activate cannabinoid receptors directly. It is these strategies that form the subject of this review.

2. DIRECT ACTIVATION OF CANNABINOID RECEPTORS LOCATED OUTSIDE THE BLOOD-BRAIN BARRIER

It is now generally accepted, first, that many of the unwanted effects of cannabinoid receptor agonists are caused by their activation of CB₁ receptors located within the brain and, second, that beneficial effects such as pain relief, amelioration of certain intestinal and cardiovascular disorders, and inhibition of cancer cell proliferation and spread can be induced by selectively activating CB₁ and/or CB₂ receptors expressed outside the central nervous system [1]. This raises the possibility of developing a peripherally restricted medicine that selectively activates cannabinoid receptors located outside the blood-brain barrier. Attention is focused particularly on the possibility of developing such medicines for pain relief.

One peripherally restricted cannabinoid receptor agonist that possesses antinociceptive activity is naphthalen-1-yl-(4-pentylxynaphthalen-1-yl)methanone. This is a potent, high-efficacy, orally bioavailable CB₁/CB₂ receptor agonist that displays significant anti-hyperalgesic activity in a rat sciatic nerve partial ligation model of neuropathic pain and that appears to act by targeting peripheral CB₁ receptors [7]. Thus, its antihyperalgesic effect can be attenuated by a CB₁-selective antagonist (SR141716A), but not by a CB₂-selective antagonist (SR144528); it can produce this antinociceptive effect without inducing a behavioural effect thought to be mediated by central CB₁ receptors (catalepsy), and it does not readily enter the

brain. The peripherally restricted potent, orally active CB₁/CB₂ receptor agonist, 'compound A', has also been reported to display anti-hyperalgesic activity at sub-cataleptic doses in a rat spinal nerve ligation model of neuropathic pain and to produce signs of anti-hyperalgesia in the mouse formalin paw model of inflammatory pain [8]. Three other such compounds are AZD1940, AZD1704 and AZ11713908, each of which seems to produce signs of analgesia in rodent models of acute, inflammatory and/or neuropathic pain through the activation of peripheral cannabinoid CB₁ receptors when administered orally [9,10]. It is also noteworthy that AZ11713908 generates fewer signs of CNS side effects in a rat Irwin test than the CB₁/CB₂ receptor agonist, *R*-(+)-WIN55212. There is evidence too that a synthetic analogue of Δ⁸-THC, ajulemic acid (CT-3), may ameliorate neuropathic pain mainly by targeting cannabinoid receptors located outside the blood-brain barrier [3]. Five further examples of peripherally restricted cannabinoids that can induce antinociception in animal models are the cannabillactone, AM1710 [11]; the 1-(4-(pyridin-2-yl)benzyl)imidazolidine-2,4-dione derivative, compound 44 [12]; the 5-sulphonyl-benzimidazole derivative, compound 49 [13]; the γ-carboline, compound 29 [14]; and the thiaziazole, compound LBP1 [15]. AM1710 reduces signs of pain elicited by thermal (but not mechanical) stimulation of the rat hind paw at doses that do not produce signs of unwanted CNS side-effects [11]. Similar results were obtained with LBP1 in a rat model of neuropathic pain [15]. AM1710 and compounds 44 and 49 are CB₂-selective cannabinoid receptor agonists, whereas compounds 29 and LBP1 are dual CB₁/CB₂ receptor ligands.

Finally, although orally administered AZD1940 displays antinociceptive activity in rat models of acute and neuropathic pain [9], results obtained in single-dose phase-II studies indicated that it was ineffective against acute pain induced in human subjects by capsaicin or by molar tooth extraction [16].

3. DIRECT ACTIVATION OF CANNABINOID RECEPTORS EXPRESSED BY A PARTICULAR TISSUE

There is a strong possibility that the benefit-to-risk ratio of a cannabinoid CB₁ or CB₁/CB₂ receptor agonist could be markedly increased by restricting the distribution of active concentrations of this agonist to a tissue that expresses cannabinoid receptors, which, when activated, would mediate relief from the unwanted effects of one or more particular disorders. Two such tissues may be skin and spinal cord, there being good evidence that these both contain cells that express CB₁ and CB₂ receptors, the activation of which can produce signs of analgesia or anti-hyperalgesia in animal models of acute, inflammatory or neuropathic pain [3]. There is evidence too that when administered intrathecally, the CB₁/CB₂ receptor agonist, *R*-(+)-WIN55212, can induce spinal CB₁ and CB₂ receptor-mediated signs of relief from bone-tumour-related pain [17], and also antinociception in a rat formalin paw model of inflammatory pain, although not in the rat hot plate model of acute pain [18].

In addition, results obtained from experiments with mice indicate that *R*-(+)-WIN55212 can act through CB₁ and CB₂ receptors to reduce signs of hyperalgesia without also inducing catalepsy when it is injected into tumour-bearing hind paws [19], and that the CB₂-selective agonist, JWH-015, can induce a CB₂-receptor-mediated reduction in bone-cancer-related pain caused by implantation of NCTC2472 fibrosarcoma cells into the femur [20]. It has been found too that intraplantar injection of 2-arachidonoyl glycerol can induce CB₂ receptor-mediated relief from hyperalgesia in a murine model of human metastatic bone cancer pain in which fibrosarcoma cells are injected into and around the calcaneus bone of the left hind paw of each animal, and in which CB₂ receptor expression increases in non-neuronal cells in the plantar skin of the tumour-bearing paw [21]. Other cannabinoid receptor agonists that have been found to reduce signs of hyperalgesia in this experimental model include AM1241, which is CB₂-selective and was antagonized by the CB₂-selective antagonist, AM630, but not by the CB₁-selective antagonist, AM281, and arachidonylcyclopropylamide, which is CB₁-selective and was antagonized by AM281, but not by AM630 [22]. Experiments with mice have also shown that *R*-(+)-WIN55212 can reduce nociception in the radiant heat tail-flick test when it is applied topically to the tail at a dose that did not impair rotarod performance [23,24]. It could well be, therefore, that by applying a cannabinoid receptor agonist directly to the skin, it would be possible to relieve pain that is restricted to one or more specific regions of the body surface without also provoking major off-target cannabinoid receptor-mediated effects. Further support for this possibility comes from experiments performed with human volunteers, which showed that hyperalgesia induced by capsaicin application to the skin, and the perception of itch induced by cutaneous administration of histamine, could both be decreased by pretreatment with the CB₁/CB₂ receptor agonist, HU-210, when this was administered by skin patch or dermal microdialysis at a dose that did not produce psychological side effects [25,26]. Also meriting further investigation is the possibility that topical application of a cannabinoid CB₁ receptor agonist to one or more areas of the skin might be an effective way of treating (or even preventing) melanoma induced by ultraviolet irradiation [27].

4. ACTIVATING UPREGULATED CANNABINOID RECEPTORS

Some disorders seem to trigger a 'protective' upregulation of certain cannabinoid CB₁ or CB₂ receptors that, when activated, can slow the progression of these disorders or ameliorate their symptoms [1,3]. As discussed in greater detail elsewhere [3], the occurrence of such protective upregulation raises the possibility that for the treatment of at least some disorders, a partial cannabinoid receptor agonist—for example, Δ⁹-THC or cannabiniol—might display a greater benefit-to-risk ratio than a higher efficacy agonist such as CP55940. This is because the extent to which the size the maximal effect of an agonist increases in response to any upregulation of its receptors is inversely related to the efficacy of that agonist.

5. ACTIVATING CANNABINOID CB₂ RECEPTORS

Significant attention is currently being directed at the possibility of developing medicines from compounds that can activate CB₂ receptors at doses that induce little or no CB₁ receptor activation. This has been triggered by the evidence that many of the adverse effects induced by mixed CB₁/CB₂ receptor agonists result from CB₁ rather than from CB₂ receptor activation, and that CB₂-selective agonists have a number of important potential therapeutic applications. These include the relief of various kinds of pain and the treatment of pruritus, of certain types of cancer, of cough and of some neurodegenerative, immunological, inflammatory, cardiovascular, hepatic, renal and bone disorders (table 1). There is also evidence, first, that CB₂ receptor activation can ameliorate neuroinflammation by protecting the blood-brain and blood-spinal cord barriers [67,68]), and second that activation of these receptors can reduce inflammation following spinal cord injury by lowering the expression of toll-like receptors [68]).

Importantly, none of the CB₂-selective agonists that have been developed to-date are completely CB₂-specific. As a result, they are expected to display CB₂-selectivity only within a finite dose range and to target CB₁ receptors as well when administered at a dose that lies above this range. Indeed, there is evidence from experiments with CB₁ wild-type and knockout mice that although some CB₂-selective agonists can reduce spasticity in an autoimmune encephalomyelitis model of multiple sclerosis, this depends on their ability to activate CB₁ receptors at doses above those at which they activate CB₂ receptors [69]. Evidence has also been obtained first, that cannabinoid receptor-dependent alleviation of mechanical allodynia that is induced in mice by brachial plexus avulsion appears to be mainly CB₂-mediated in the initial phase but both CB₁- and CB₂-mediated in the late phase [32], and second, that in a mouse collagen-induced arthritis model, although signs of arthritis are reduced by prolonged CB₂ but not prolonged CB₁ receptor activation, thermal hyperalgesia is reduced by acute CB₁ but not by acute CB₂ receptor activation [70]. In addition, it is likely that pharmacological targets other than CB₂ or CB₁ receptors contribute to sought-after or unwanted effects of CB₂-selective agonists, there being evidence, for example, that some but not all such agonists can activate GPR55 and/or modulate activation of this deorphanized receptor by L-α-lysophosphatidylinositol [71]. It is noteworthy too that CB₂ receptors seem to increase survival rate in a model of mild sepsis but to reduce survival rate in a model of more severe sepsis, that CB₂ receptor activation appears both to exaggerate and to block inflammatory responses in a model of allergic contact dermatitis, and that some inflammatory responses that seem to be aggravated by CB₂ receptor agonists are alleviated by CB₂ receptor inverse agonists [41].

6. POTENTIAL ADJUNCTIVE STRATEGIES FOR CANNABINOID RECEPTOR ACTIVATION

There is good evidence that it may be possible to improve the benefit-to-risk ratio of a cannabinoid receptor agonist such as Δ⁹-THC, CP55940, *R*-(+)-WIN55212 or HU-210 for the management of pain by administering it together with a second drug.

Table 1. Examples of potential therapeutic targets for selective CB₂ receptor agonists.

disorder or symptom	references
acute or post-operative pain	[28,29] ^a
persistent inflammatory pain	[28 ^a ,29 ^a ,30]
neuropathic pain	[12,13,28 ^a ,29 ^a ,30–32]
cancer pain including bone cancer pain	[20,22,28 ^a ,33 ^a ,34 ^a]
pruritus	[35]
Parkinson's disease	[36,37] ^a
Huntington's disease	[37] ^a
amyotrophic lateral sclerosis	[38,39]
multiple sclerosis	[4 ^a ,13,40 ^a]
autoimmune uveitis	[41] ^a
HIV-1 brain infection	[42] ^a
alcohol-induced neuroinflammation/neurodegeneration	[42] ^a
anxiety-related disorders	[43]
impulsivity: e.g. in bipolar disorder, personality disorders, attention-deficit hyperactivity disorder and substance use disorders	[44]
cocaine dependence ^b	[45]
traumatic brain injury	[46]
stroke	[47,48 ^a]
atherosclerosis	[49,50]
systemic sclerosis	[41] ^a
inflammatory bowel disease	[41 ^a ,51 ^a ,52 ^a ,53]
chronic liver diseases; alcoholic liver disease	[52 ^a ,54–57 ^a ,58]
diabetic nephropathy	[59]
osteoporosis	[60] ^a
cough	[61] ^a
breast, prostate, skin, pancreatic, colorectal, hepatocellular and bone cancer; lymphoma/leukaemia and gliomas	[34 ^a ,51 ^a ,52 ^a ,62 ^a ,63 ^a ,64,65]

^aReview article.^bNicotine self-administration and reinstatement of nicotine-seeking behaviour have been found to be unaffected by selective CB₂ receptor agonism or antagonism in rats [66].

Thus, for example, additive or synergistic interactions resulting in antinociception have been reported to occur in the rat formalin paw model of inflammatory pain between

- intraperitoneal Δ^9 -THC and morphine [72];
- intrathecal *R*-(+)-WIN55212 and an intrathecally administered α_2 -adrenoceptor agonist (clonidine), cholinesterase inhibitor (neostigmine) or local anaesthetic (bupivacaine) [73,74];
- anandamide and the cyclooxygenase inhibitor, ibuprofen, administered by intraplantar injection [75]; and
- HU-210 and the non-steroidal anti-inflammatory drug, acetylsalicylic acid, co-administered systemically [76].

Additive or synergistic interactions resulting in antinociception have also been found to occur between

- low-dose *R*-(+)-WIN55212 and a cyclooxygenase-2 inhibitor, NS-398, co-administered intracisternally, for the attenuation of nociceptive scratching behaviour induced in rats by formalin injection into the temporomandibular joint of the jaw [77];
- *R*-(+)-WIN55212 and the non-steroidal anti-inflammatory drug, ketorolac, co-administered systemically, for the attenuation of nociception in a mouse model of inflammatory visceral pain, although not in the mouse tail flick model of acute pain [78];

- HU-210 and the non-steroidal anti-inflammatory drug, acetylsalicylic acid, co-administered systemically, in the rat hot plate model of acute pain [76];
- Δ^9 -THC and an opioid such as morphine, codeine or fentanyl in mouse, rat, guinea pig and monkey models of acute or arthritic pain [79–89];
- CP55940 and the α_2 -adrenoceptor agonist, dexmedetomidine, in the mouse hot plate and tail flick models of acute pain [83];
- CP55940 and the *N*-methyl-D-aspartate (NMDA) receptor antagonist, (–)-6-phosphonomethyl-decahydroisoquinoline-3-carboxylic acid (LY235959), in the mouse hot plate test [90]; and
- *R*-(+)-WIN55212, given by intracerebroventricular or intraplantar injection, and a selective agonist for the neuropeptide FF₁ or FF₂ receptor, injected intracerebroventricularly, in mouse models of acute pain [91].

Importantly, evidence has been obtained through the construction of isobolograms that of the above interactions, those between *R*-(+)-WIN55212 and clonidine, neostigmine or bupivacaine [73,74] as well as those between anandamide and ibuprofen [75], Δ^9 -THC and an opioid [81,83,86], CP55940 and dexmedetomidine [83] and CP55940 and LY235959 [90], are all synergistic rather than just additive in nature. A synergistic antinociceptive interaction has also been reported to occur between the CB₁-selective agonist, arachidonylcyclopropylamide, and the

Table 2. Examples of additive or synergistic interactions observed *in vivo* between cannabinoid receptor agonists and non-cannabinoid receptor ligands in animal models of certain disorders.^a ACEA, arachidonyl-2'-chloroethylamide; 8-OH-DPAT, 8-hydroxy-2-(di-*n*-propylamino) tetralin hydrobromide; i.p., intraperitoneal; i.v., intravenous; s.c., subcutaneous.

disorder and measured effect	cannabinoid receptor agonist	co-administered compound	reference
<i>anxiety or depression</i>			
anxiolytic effects in mouse elevated plus-maze ^c and mouse hole-board test	<i>R</i> -(+)-WIN55212 (i.p.)	diazepam (i.p.)	[98]
anxiolytic effects in mouse light-dark box, open-field test and elevated plus-maze	low-dose Δ^9 -THC (i.p.)	low-dose nicotine (s.c.)	[99,100]
anxiolytic effect in rat elevated plus-maze	low-dose Δ^9 -THC (i.p.)	low dose of the 5-HT _{1A} receptor-selective agonist, 8-OH-DPAT (i.p.)	[101]
antidepressant effect in rat forced swim test	low-dose CP55940 (i.p.)	low-dose imipramine (i.p.)	[102]
<i>epilepsy</i>			
anticonvulsant effect on mouse pentylenetetrazole-induced clonic or tonic-clonic seizures	low-dose of the CB ₁ -selective agonist, ACEA (i.p.)	low-dose naltrexone (i.p.)	[103]
anticonvulsant effect on mouse pentylenetetrazole-induced clonic seizures	low-dose <i>R</i> -(+)-WIN 55212 (i.p.)	ethosuximide, phenobarbital or valproate (i.p.)	[104]
anticonvulsant effect on mouse maximal electroshock-induced seizures	low-dose <i>R</i> -(+)-WIN55212 (i.p.)	carbamazepine, phenytoin, phenobarbital or valproate (i.p.)	[105]
anticonvulsant effect on mouse maximal electroshock-induced seizures	low-dose of the CB ₁ -selective agonist, ACEA (i.p.)	phenobarbital (i.p.)	[106]
anticonvulsant effect on mouse electroshock-induced seizures	low-dose <i>R</i> -(+)-WIN55212 (i.p.)	diazepam (i.p.)	[107]
<i>haemorrhagic shock or glaucoma</i>			
increased survival time in a rat model of haemorrhagic shock	Δ^8 -THC (i.v.)	cyclooxygenase-2 inhibitor, NS-398 (i.v.)	[108]
reduction of rat intraocular pressure	low-dose <i>R</i> -(+)-WIN55212 (i.p.)	low-dose abnormal-cannabidiol or cannabigerol-dimethyl heptyl (topical)	[109]
<i>cancer or chemotherapy-induced vomiting</i>			
reduction of glioma xenograft growth in nude mice	low-dose Δ^9 -THC (peritumorally)	low-dose temozolomide (peritumorally) ^b	[110]
inhibition of vomiting and retching induced by cisplatin in house musk shrews	low-dose Δ^9 -THC (i.p.)	low dose of the 5-HT ₃ receptor antagonist, ondansetron (i.p.)	[111]

^aSee introduction to this section for antinociceptive interactions.

^bLow-dose temozolomide also exerted a strong anti-tumoral effect in combination with a low-dose mixture of Δ^9 -THC and the non-psychoactive phytocannabinoid, cannabidiol.

^cIsobolographic analysis indicated this interaction to be synergistic.

CB₂-selective agonist, AM1241, in a mouse model of cancer pain following intraplantar coadministration of these two compounds [22]. This is of interest since it raises the possibility that, for at least some kinds of pain, a mixed CB₁/CB₂ agonist may be more effective as an analgesic medicine than a CB₁- or CB₂-selective agonist.

Results from clinical studies with patients experiencing chronic non-cancer pain have also provided evidence that cannabinoid receptor agonists can enhance opioid-induced analgesia [92,93] and that inhaled vapourized cannabis can augment the analgesic effect of the opioids, morphine and oxycodone in patients experiencing various kinds of chronic pain without inducing any unacceptable adverse events [94]. In contrast, no synergistic or additive antinociceptive interaction has been detected between Δ^9 -THC and

the μ -opioid receptor agonist, piritramide, in patients suffering from acute post-operative pain [95] or between Δ^9 -THC and morphine in human volunteers subjected to noxious electrical or thermal stimulation of the skin or to painful digital pressure [96,97]. However, together (but not separately), these drugs did reduce the affective response to cutaneous thermal stimulation [97].

Evidence that certain potentially beneficial effects of a cannabinoid receptor agonist other than pain relief can be enhanced by administering it together with one or other of a set of non-cannabinoid receptor ligands has also emerged from *in vivo* animal experiments (table 2). It should be noted that the anticonvulsant interactions between *R*-(+)-WIN55212 and ethosuximide or valproate that are referred to in table 2 were probably at least partly pharmacokinetic in nature [104], whereas those between *R*-(+)-WIN55212 or

arachidonyl-2'-chloroethylamide and phenobarbital were most likely pharmacodynamic in nature [104, 106]. It is noteworthy too that additive or synergistic interactions have also been observed to occur *in vitro* between cannabinoid receptor agonists and anti-cancer drugs for the production of apoptosis or anti-proliferative effects in certain cancer cell lines [110,112].

One other adjunctive strategy for a cannabinoid receptor agonist may be to administer it together with a CB₁ receptor antagonist/inverse agonist. Thus, for example, it has been found that

- an ultra-low dose of SR141716A can prolong *R*-(+)-WIN55212-induced antinociception in a rat model of acute pain [113];
- an ultra-low dose of AM251 can enhance the ability of the CB₁-selective agonist, arachidonyl-2'-chloroethylamide, to protect mice from pentylenetetrazole-induced seizures [114]; and
- administration of a selective CB₁ receptor antagonist/inverse agonist together with a CB₂-selective agonist may be particularly effective for the treatment of hepatic ischaemia/reperfusion injury caused by liver transplantation [115] and of disorders, such as Parkinson's disease [116], systemic sclerosis [117], chronic liver diseases, including alcohol-induced liver injury [56] and stroke [118], and perhaps also for the management of cocaine dependence [45,119].

It is possible that the last of these three potential adjunctive strategies could be exploited using Δ^9 -tetrahydrocannabivarin, because this plant cannabinoid can both block CB₁ receptors and activate CB₂ receptors [120,121]. Indeed, there is already evidence from experiments using animal models of Parkinson's disease and hepatic ischaemia/reperfusion injury, that Δ^9 -tetrahydrocannabivarin would display efficacy as a medicine against both of these disorders [115,116].

Ideally, a multi-targeting strategy should of course be one that enhances sought-after effects to a greater extent than unwanted effects. It is noteworthy, therefore, that there is already evidence from experiments performed with mice or rats that the risk of developing dependence to opioids [122,123] and nicotine [99] increases when such a compound is co-administered with a cannabinoid CB₁/CB₂ receptor agonist. There is evidence too that Δ^9 -THC can undergo additive or synergistic interactions with a range of non-cannabinoids to disrupt motor function and thermoregulation, as indicated by the production of catalepsy, hypokinesia or hypothermia in mice or rats. These non-cannabinoids include opioids, nicotine, benzodiazepines, prostaglandins, reserpine and ligands that activate or block muscarinic cholinceptors or some types of dopamine, noradrenaline, 5-hydroxytryptamine or γ -aminobutyric acid receptors [72,99,124,125]. There is also evidence that *R*-(+)-WIN55212 enhances not only the anticonvulsant effects of carbamazepine, phenytoin, phenobarbital, valproate and ethosuximide in mice (table 2), but also the impairment of skeletal muscle strength by all these compounds, the impairment of motor co-ordination by phenobarbital, valproate and ethosuximide, and the impairment of long-term

memory by phenytoin, phenobarbital, valproate and ethosuximide [104,105]. In contrast, however, the CB₁-selective agonist, arachidonyl-2'-chloroethylamide, enhanced the anticonvulsant effect of phenobarbital in mice (table 2) without augmenting impairment by this barbiturate of skeletal muscle strength, motor co-ordination or long-term memory [106]. It is also noteworthy that administration of a cannabinoid receptor agonist, together with morphine, seems to oppose the development of tolerance to the antinociceptive effects of these compounds. Thus, for example, chronic systemic administration of a low-dose combination of Δ^9 -THC and morphine to rats has been reported to induce antinociception without also producing tolerance in a rat paw pressure model of acute pain in which tolerance did develop when morphine or Δ^9 -THC was administered chronically by itself at a higher dose [87]. Furthermore, it has been found first, that chronic systemic co-administration of CP55940 with morphine can attenuate the tolerance that develops to the antinociceptive effect of morphine in the mouse hot plate test when it is administered repeatedly by itself [90], and second, that an ultra-low dose of SR141716A that prolongs *R*-(+)-WIN55212-induced antinociception in the rat tail flick test also opposes the development of tolerance to this CB₁/CB₂ receptor agonist [113]. There is evidence too that the CB₂-selective agonist, AM1241, can prevent the neuroinflammatory consequences of sustained morphine treatment [126].

7. MIXING STRATEGIES

There may be therapeutic benefits to be gained from combining some of the strategies that have been mentioned in this review. One possibility for pain relief would be to administer a CB₂-selective agonist intrathecally instead of orally. Thus, there have been reports that JWH-015 can reduce signs of post-operative pain in rats [127], and that signs of neuropathic pain can be reduced by JWH-133 in mice [128], and by AM1710 in rats [129] when these three CB₂-selective agonists are injected intrathecally. There is evidence too that signs of analgesia induced in models of acute pain by transdermal administration of an opioid can be enhanced by transdermal or intrathecal co-administration of a low dose of a CB₁/CB₂ receptor agonist [24,84]. It is also noteworthy that antinociceptive synergy has been detected in the mouse tail flick test between low-doses of *R*-(+)-WIN55212 co-administered topically and intrathecally [23].

8. CONCLUSIONS AND FUTURE DIRECTIONS

This review has focused on preclinical findings described in papers published up to April 2012 that together provide an indication of the likely strengths and weaknesses of a number of potential strategies for improving the therapeutic efficacy and/or minimizing the adverse effects of cannabinoid receptor agonists in the clinic.

The available published information about each of these strategies suggests that, for many of them, their strengths significantly outweigh any of their identified weaknesses. This information has, however,

come almost entirely from preclinical research. Consequently there is now an urgent need, first, unless they have already been performed, for phase I clinical trials with healthy human subjects that test the safety of each drug that is selected to implement one or other of these potential strategies and, second, for phase II trials with patients. When planning such clinical trials, it will be important to construct a short-list of disorders that are in need of better medicines, and whose signs, symptoms and/or progression are most likely to be managed effectively in the clinic by one or other of the strategies described in this review. For each of these disorders, it will also be important to select the strategy that would be the one most likely to produce the greatest benefit-to-risk ratio in patients.

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