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Perspective

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The Highs and Lows of Cannabinoid Receptor Expression in Disease: Mechanisms and Their Therapeutic Implications

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***Abstract*——Alterations in the endogenous cannabinoid system have been described in almost every category of disease. These changes can alternatively be protective or maladaptive, such as producing antinociception in neuropathic pain or fibrogenesis in liver disease, making the system an attractive therapeutic target. However, the challenge remains to selectively target the site of disease while sparing other areas, particularly mood and cognitive centers of the brain. Identifying regional changes in cannabinoid receptor-1 and -2 (CB_1R and CB_2R) expression is particularly important when considering endocannabinoid system-based therapies, because regional increases in cannabinoid receptor expression have been shown to increase potency and efficacy of exogenous agonists at sites of disease. Although there have been extensive descriptive studies of cannabinoid receptor expression changes in disease, the underlying mechanisms are only just beginning to unfold. Understanding these mechanisms is important and potentially relevant to therapeutics. In diseases for which cannabinoid receptors are protective, knowledge of the mechanisms of receptor up-regulation could be used to design therapies to regionally increase receptor expression and thus increase efficacy of an agonist. Alternatively, inhibition of harmful cannabinoid up-regulation could be an attractive alternative to global antagonism of the system. Here we review current findings on the mechanisms of cannabinoid receptor regulation in disease and discuss their therapeutic implications.**

I. Introduction: The Cannabinoid Receptors and Their Response to Disease

The endocannabinoid system is uniquely poised to respond locally to disease. Endocannabinoids are synthesized "on demand" from membrane phospholipids in response to increases in intracellular calcium (as occurs with neuronal activation or cell stress) and immediately released to act in paracrine fashion on nearby $G_{i/o}$-protein coupled receptors CB_1R[1] and CB_2R. Endocannabi-

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TABLE 1
CB_1R and CB_2R expression changes in disease

Major findings in several disease categories have been selected; for more exhaustive review, see references cited in text.

Disease	CB_1R/CB_2R Regulation	Proposed Role of CB_1R/CB_2R in Disease	Therapeutic Implications
Neuropathic pain	CB_1R and CB_2R up-regulated in peripheral and central sensory pathways in animal models of neuropathic pain (Siegling et al., 2001; Lim et al., 2003; Zhang et al., 2003; Walczak et al., 2005; Mitrirattanakul et al., 2006).	Inhibition of neurotransmitter release decreases hyperexcitability of sensory pathways (CB_1R); inhibition of inflammation (CB_2R) (for review, see Costigan et al., 2009).	CB_1R up-regulation enhances analgesic response to exogenous cannabinoids in animal model of neuropathic pain (Lim et al., 2003).
Neuroinflammation and brain injury	CB_2R up-regulated in microglia/ macrophage-like cells of patients with MS or ALS (Yiangou et al., 2006) and in microglia of EAE mouse model of MS (Maresz et al., 2005).	CB_2R expressed on T cells reduces inflammation in MS (Maresz et al., 2007).	T-cell CB_2R is already highly activated by endocannabinoids in MS; increasing receptor number is thus important for increasing efficacy of CB_2R agonist (Maresz et al., 2007).
	CB_1R up-regulated in rat brain after mild concussive head injury and NMDAR blockade (Hansen et al., 2001).	Neuroprotection by decreasing glutamate release/excitotoxicity, among other possible mechanisms (for review, see Mechoulam et al., 2002).	
Cancer	Increased CB_1R and CB_2R expression in multiple human cancers (for review, see Sarfaraz et al., 2008).	Cannabinoid receptor activation leads to apoptosis via cell cycle regulation and ER stress (for review, see Sarfaraz et al., 2008).	Up-regulation of receptors may be crucial for antitumor effects of cannabinoids. For example, a nonselective agonist inhibits growth of prostate cancer cells with high expression of CB_1R and CB_2R (Sarfaraz et al., 2005), whereas Δ^9-THC stimulates growth in breast cancer cells with low expression (McKallip et al., 2005). Increasing receptor expression where it is lost could be a novel therapeutic option (Wang et al., 2008).
	Down-regulated CB_1R in human colorectal tumors (Wang et al., 2008).	Loss of CB_1R leads to enhanced colorectal tumor proliferation in mouse model of colorectal cancer (Wang et al., 2008).	
Gastrointestinal	CB_1R up-regulated in mouse model of diarrhea (Izzo et al., 2001); CB_1R and CB_2R up-regulated in multiple models of colitis (Massa et al., 2004; Kimball et al., 2006).	CB_1R expressed on enteric neurons decreases intestinal motility, secretion, and visceral pain and promotes wound healing. CB_2R expressed on gut immune cells decreases inflammation (for review, see Izzo and Camilleri, 2008).	Up-regulation of CB_1R probably accounts for increased efficacy and potency of agonists in slowing intestinal transit in mouse model of diarrhea (Izzo et al., 2001).
Liver	Hepatic CB_1R and CB_2R up-regulated in cirrhosis in humans and rodent activated hepatic stellate cells (aHSCs) (Julien et al., 2005; Teixeira-Clerc et al., 2006; Jeong et al., 2008).	Fibrogenic stimuli up-regulate hepatic CB_1R, which stimulates lipogenesis in hepatocytes (Osei-Hyiaman et al., 2005) and promotes fibrogenesis through aHSCs and myofibroblasts (Teixeira-Clerc et al., 2006). CB_2R activation leads to apoptosis of profibrogenic aHSCs and myofibroblasts (Julien et al., 2005).	Antagonism of CB_1R and agonism of CB_2R reduces proliferation of fibrogenic cells (Julien et al., 2005; Teixeira-Clerc et al., 2006).
	Hepatic CB_1R also up-regulated in mice fed high-fat (Osei-Hyiaman et al., 2005; Jourdan et al., 2010; Quarta et al., 2010) and alcohol diets (Jeong et al., 2008).		Antagonism of CB_1R decreases hepatic fatty acid synthesis in mice fed high-fat diet (Osei-Hyiaman et al., 2005). CB_1R antagonism also prevents high-fat diet-induced increases in hepatic CB_1R expression (Jourdan et al., 2010).
Metabolic	Adipose CB_1R up-regulated in Zucker rat model of obesity (Bensaid et al., 2003) and mice fed high-fat diet (Jourdan et al., 2010).	CB_1R stimulates lipogenesis and inhibits fatty acid oxidation in adipose cells and may increase insulin release from the pancreas (Matias et al., 2006; for review, see Kunos et al., 2008).	CB_1R up-regulation in adipocytes may account for increased efficacy of CB_1R antagonist in reducing weight in obese compared with lean Zucker rats (Vickers et al., 2003).

TABLE 1
Continued

Major findings in several disease categories have been selected; for more exhaustive review, see references cited in text.

Disease	CB_1R/CB_2R Regulation	Proposed Role of CB_1R/ CB_2R in Disease	Therapeutic Implications
	Skeletal muscle CB_1R up-regulated in mice fed high-fat diet (Pagotto et al., 2006).	CB_1R up-regulation in skeletal muscle may contribute to insulin resistance in obesity (for review, see Kunos et al., 2008).	CB_1R antagonism prevents high-fat diet-induced increases in adipose CB_1R expression (Jourdan et al., 2010).
Cardiovascular	CB_1R up-regulated in myocardium and aorta in rat model of hypertension (Batkai et al., 2004).	CB_1R lowers blood pressure by decreasing cardiac contractility and vascular resistance, but this effect is only seen in hypertensive, not normotensive animals (Batkai et al., 2004).	CB_1R up-regulation in the heart and vasculature may account for increased potency and efficacy of agonists in hypertensive animals (Batkai et al., 2004).
	CB_2R up-regulated in immune cells in atherosclerotic plaques in humans and mouse model (Steffens et al., 2005).	CB_2R decreases plaque progression, possibly by reducing infiltration of immune cells and cytokine release (Steffens et al., 2005).	
Psychiatric	CB_1R up-regulated in prefrontal cortex of depressed suicide victims (Hungund et al., 2004) and patients with schizophrenia (Dean et al., 2001).	CB_1R has both excitatory and inhibitory effects on synaptic transmission in the prefrontal cortex (Lafourcade et al., 2007; Chiu et al., 2010); the effect of its up-regulation in these diseases is not known.	Targeting the endocannabinoid system in these diseases will require better understanding of its role.

MS, multiple sclerosis; ALS, amyotrophic lateral sclerosis; EAE, experimental autoimmune encephalitis; NMDAR, *N*-methyl-D-aspartate receptor; ER, endoplasmic reticulum; Δ^9-THC, tetrahydrocannabinol; aHSC, activated hepatic stellate cell.

noids are then rapidly cleared by cellular uptake and enzymatic degradation. Cannabinoid receptors work through a variety of signaling mechanisms to exert physiological and pathophysiological effects in different tissues. In neurons, where CB_1R expression is highest, stimulation of presynaptic CB_1R inhibits neurotransmitter release by stimulating potassium channels and inhibiting calcium channels (for review, see Howlett et al., 2002). In the liver, where CB_1R is normally expressed at low levels, stimulation of CB_1R leads to enhanced expression of acetyl-CoA carboxylase-1 and fatty acid synthase and thus increases lipogenesis (Osei-Hyiaman et al., 2005). CB_2R expression is highest in immune cells, where it seems to have several immunosuppressive effects, including inhibition of proinflammatory cytokine production (Maresz et al., 2007). Cannabinoid receptor signaling is discussed in detail in several recent reviews (Cabral and Griffin-Thomas, 2009; Turu and Hunyady, 2010).

In addition to increasing levels of endocannabinoids, the system often responds to stress by altering the expression of CB_1R and/or CB_2R. In some diseases, such as neuropathic pain and multiple sclerosis, increases in cannabinoid receptor expression are thought to reduce symptoms and/or inhibit progression of disease and thus serve a protective role (for review, see Pertwee, 2009). In other diseases, alterations in receptor expression are maladaptive, examples being CB_1R up-regulation in liver fibrosis and down-regulation in colorectal cancer (Teixeira-Clerc et al., 2006; Wang et al., 2008). In both cases, regulation of cannabinoid receptor expression is of interest from a therapeutics perspective. Regional up-regulation of CB_1R correlates with enhanced potency and efficacy of agonists at sites of disease in several animal models, including intestinal inflammation and hypertension (Izzo et al., 2001; Bátkai et al., 2004); a more causal relationship was suggested in neuropathic pain, where inhibition of CB_1R up-regulation reduced the analgesic effects of cannabinoids (Lim et al., 2003). Regional up-regulation of CB_1R is similarly thought to enhance efficacy of systemic antagonists in models of obesity (for review, see Vickers et al., 2003; Kunos et al., 2008). Such up-regulation should therefore increase the benefit-to-side-effect ratio of systemic agonists (for review, see Pertwee, 2009) and antagonists. In addition, identifying the lack of cannabinoid receptor up-regulation could be important. For example, prostate cancer cells that highly express cannabinoid receptors respond favorably to agonists (Sarfaraz et al., 2005), whereas breast cancer cells that express low levels of cannabinoid receptors show increased proliferation in response to Δ^9-tetrahydrocannabinol (McKallip et al., 2005). These alterations in cannabinoid receptor expression have been extensively reviewed elsewhere (Di Marzo et al., 2004; Pertwee, 2005, 2009; Pacher et

[1]Abbreviations: CB_1R, cannabinoid receptor-1; CB_2R, cannabinoid receptor-2; ChIP, chromatin immunoprecipitation; GR, glucocorticoid receptor; kb, kilobase(s); MAPK, mitogen-activated protein kinase; Trk, receptor tyrosine kinase; UTR, untranslated region.

al., 2006; Di Marzo, 2008; Izzo and Camilleri, 2008) and are briefly summarized in Table 1 to emphasize the global nature of these alterations and their therapeutic implications.

II. Mechanisms of Cannabinoid Receptor Regulation

Despite the growing list of diseases that show cannabinoid receptor expression changes, relatively little is known about the mechanisms underlying these changes. In Table 2, we summarize all current findings of which we are aware. These studies of mechanism span a number of diseases and vary in the level of detail, from investigating changes in cannabinoid receptor protein levels to promoter occupancy, but several themes are apparent across the disease models examined so far. For one, up-regulation is induced by factors that are released locally in response to disease, in accordance with the region-specific nature of these expression changes. For example, spinal cord CB_1R protein increases were found to be mediated in part by the Trk/MAPK pathway in a rat model of neuropathic pain, suggesting a mechanism by which neurotrophic factors released locally by nerve injury (Ha et al., 2001) could modulate CB_1R expression (Lim et al., 2003). Spinal cord CB_1R increases in neuropathic pain were also found to be mediated by glucocorticoids (Wang et al., 2007). Although corticosteroids are increased systemically in the same model (Wang et al., 2004), localized CB_1R up-regulation is probably made possible by local increases in spinal glucocorticoid receptors (GR) (Wang et al., 2004). Localized mechanisms of CB_1R regulation were also observed in liver and immune cells. Retinoic acid, which is synthesized and stored by hepatic stellate cells, was found to increase CB_1R transcription in hepatocytes through retinoic acid receptor-γ (Mukhopadhyay et al., 2010). Cytokines, which are released locally in inflammation to regulate neighboring immune cells, have been impli-

TABLE 2
Mechanisms of CB_1R and CB_2R regulation in disease

Disease/System	Mechanism	Cell Type/ Animal Model	Implications
Liver steatosis	RAR-γ binds the −500 to +50 region of the CB_1R promoter and increases its expression (Mukhopadhyay et al., 2010).	Primary cultured mouse hepatocytes	Retinoic acid released from hepatic stellate cells induces CB_1R expression in hepatocytes, which in turn induces lipogenesis (Jeong et al., 2008).
Inflammation	Cannabinoids induce CB_1R mRNA expression in T cells by an IL-4- and CB_2R-dependent mechanism (Borner et al., 2007).	Jurkat T cells	CB_1R is expressed at very low levels in resting T cells; when induced, it seems to have anti-inflammatory effects (Molina-Holgado et al., 2003; Nakajima et al., 2006). These studies together suggest a feedback mechanism in T cells whereby cannabinoids induce expression of anti-inflammatory IL-4 via CB_2R, and IL-4 in turn increases CB_1R transcription (Borner et al., 2008).
	IL-4 directly increases CB_1R mRNA expression in T cells via STAT6 binding to a STAT6 element at nt −2769 upstream from human exon1 TSS (Borner et al., 2008).	Primary human T cells and Jurkat T cells	
	IFNγ and GM-CSF synergize to increase CB_2R mRNA expression in microglia (Maresz et al., 2005).	Primary cultured mouse microglial cells	Increases in proinflammatory IFNγ and GM-CSF could mediate the increased microglial expression of CB_2R seen in EAE model of MS (Maresz et al., 2005).
Neuropathic Pain	CB_1R protein up-regulation in the spinal cord is mediated by Trk and MAPK (Lim et al., 2003) and GR (Wang et al., 2007).	Rat chronic constriction injury model of neuropathic pain	Neurotrophic factors and corticosteroids released with nerve injury may mediate CB_1R up-regulation via the Trk/MAPK pathway and GR, respectively (Lim et al., 2003; Wang et al., 2007).
Cancer	Increased DNA methylation of CB_1R promoter leads to decreased expression of CB_1R (Wang et al., 2008).	Human colorectal cancer cells	Loss of CB_1R expression leads to enhanced tumor proliferation in a mouse model of colorectal cancer (Wang et al., 2008); first evidence of a role for epigenetics in CB_1R regulation.
Feeding behaviors and energy homeostasis	CNS-specific knockout of SF-1 leads to loss of CB_1R expression in the ventromedial hypothalamus. SF-1 directly increases CB_1R expression via SF-1 element at nt −101 within the mouse promoter (Kim et al., 2008).	CNS-specific SF-1 knockout mouse and various cell lines	CNS-specific SF-1 knockouts do not show the appetite-stimulating effects of CB_1R agonists. SF-1 regulation of CB_1R expression in the VMH is thus required for cannabinoid effects on food intake (Kim et al., 2008).
Neurodegenerative	Decreased striatal CB_1R levels in HD are due to decreased transcription (McCaw et al., 2004).	Mouse HD model	Striatal CB_1R modulates dopamine transmission, which is dysregulated in HD (see refs within McCaw et al., 2004).

RAR-γ, retinoic acid receptor-γ; IL, interleukin; STAT, signal transducer and activator of transcription; TSS, transcription start site; IFN, interferon; GM-CSF, granulocyte-macrophage–colony-stimulating factor; EAE, experimental autoimmune encephalitis; MS, multiple sclerosis; CNS, central nervous system; SF-1, steroidogenic factor-1; nt, nucleotide(s); HD, Huntington's disease.

cated in up-regulation of both CB_1R and CB_2R in immune cells (Maresz et al., 2005; Börner et al., 2008).

Second, the regulatory factors identified so far are common to many physiological and pathophysiological processes and could thus be starting points for investigation of cannabinoid receptor regulation in other disease models. For example, retinoic acid has been implicated in cell survival, differentiation, axonal outgrowth, and immune regulation (for review, see Maden, 2007; Montrone et al., 2009; Noy, 2010), whereas neurotrophic factors have been implicated in neuronal survival and synaptic plasticity (for review, see Huang and Reichardt, 2001). A role for epigenetics, a widespread mechanism by which diseases cause long-lasting changes in gene expression by DNA and histone modifications, is also emerging. Down-regulation of CB_1R in human colorectal cancer cells has been attributed to methylation of the CB_1R promoter and leads to enhanced tumor proliferation in animal models (Wang et al., 2008).

Finally, autoregulation is an emerging and intriguing mechanism of cannabinoid receptor regulation. Endocannabinoids and exogenous cannabinoids have been implicated in CB_1R up-regulation in hepatocytes and T cells, respectively (Börner et al., 2007; Mukhopadhyay et al., 2010), and long-term CB_1R antagonism has been found to counter increases in hepatic and adipose CB_1R expression in response to high-fat diet (Jourdan et al., 2010). In addition, administration of a mixed CB_1R/CB_2R agonist increases expression of the CB_2A isoform (see section IV.B) in mouse cerebellum (Liu et al., 2009). Because endocannabinoids are often increased along with cannabinoid receptors in disease (Mitrirattanakul et al., 2006; Jeong et al., 2008), such autoinduction is probably common to many diseases. Understanding this autoregulation is also important from a therapeutics standpoint. Cannabinoid receptor antagonists could produce their effects through down-regulation of cannabinoid receptors in addition to blockade of cannabinoid receptor signaling pathways, whereas agonists could increase receptor expression in addition to stimulation of signaling pathways and thus amplify response to treatment.

III. Therapeutic Implications

The mechanisms of cannabinoid receptor regulation not only shed light on the pathophysiology of a disease but are also of interest from a therapeutics perspective. The challenge remains to selectively target the endocannabinoid system while sparing other areas, particularly mood and cognitive centers of the brain. As mentioned in section I, regional increases in cannabinoid receptor expression are thought to selectively enhance the effects of agonists at sites of disease, thus increasing their benefit-to-side-effect ratio (for review, see Pertwee, 2009). In diseases for which cannabinoid receptors are protective, knowledge of the mechanisms of this receptor up-regulation could be used to design therapies to regionally enhance receptor expression and thus further optimize benefit-to-side effect ratio or eliminate the need for systemic agonists altogether. In neuropathic pain, for example, enhanced analgesic effect of cannabinoids has been linked to increases in spinal cord CB_1R expression (Lim et al., 2003). As mentioned in section I, this up-regulation is mediated in part by Trk/MAPK pathways and glucocorticoid receptors (Lim et al., 2003; Wang et al., 2007). Increasing spinal cord CB_1R expression by local administration of Trk agonists or glucocorticoids could further increase the efficacy and potency of exogenous cannabinoids and thus allow for lower, nonpsychotropic doses. Moreover, increases in endocannabinoid production in neuropathic pain (Mitrirattanakul et al., 2006) could have a saturating effect on cannabinoid receptors. Increasing receptor number could boost responsiveness to these increased endocannabinoids and thus bypass the need for exogenous agonists. Such a rationale has already been proposed for treatment of multiple sclerosis, in which CB_2R on T cells was found to be highly activated in the experimental autoimmune encephalomyelitis model, presumably by increased levels of endocannabinoids (Maresz et al., 2007). In other diseases, increasing cannabinoid receptor expression where it is lost could be an attractive therapeutic option. Loss of CB_1R as a result of promoter methylation in colorectal cancer, as seen in human cell lines, could be rescued using a demethylating agent in the hopes of decreasing tumor proliferation (Wang et al., 2008).

Alternatively, inhibition of cannabinoid receptor up-regulation where it is harmful could be an attractive alternative to systemic antagonism. For example, CB_1R antagonists have been proposed for treatment of liver fibrosis, which is marked by maladaptive up-regulation of CB_1R (Teixeira-Clerc et al., 2006). However, systemic use of the CB_1R antagonist rimonabant for "prevention of cardiovascular events" (CRESCENDO trial) was terminated because of adverse neuropsychiatric effects (Topol et al., 2010). A better alternative might be blockade of hepatic CB_1R up-regulation; the retinoic acid system has already been implicated and could thus be a possible target (Mukhopadhyay et al., 2010). Given that CB_1R is normally expressed at very low levels in the liver (Teixeira-Clerc et al., 2006) and thus probably does not serve much of a role in normal liver physiology, inhibition of its up-regulation in the liver is likely to be safe.

IV. Cannabinoid Receptor Gene Structure

Our understanding of cannabinoid receptor gene structure, essential for studying its direct regulation, is relatively recent. Experimentally determined gene

structures and promoters of CB_1R and CB_2R are summarized here and in Fig. 1.

A. Cannabinoid Receptor-1. In human, rat, and mouse, the CB_1R coding region is contained within one exon and shows significant homology across species; however, there seems to be considerable variation in the length of 5′ untranslated region (UTR). Zhang et al. (2004) identified three additional upstream exons in human CB_1R using hippocampal RNA, giving a large (approximately 20 kb) 5′ UTR characteristic of neuronally expressed genes. This 5′UTR can be alternatively spliced (CB_1A–D) or transcribed at different sites (CB_1A–D versus CB_1E) to yield five possible transcripts with region-specific expression in the brain. The 3-kb sequence upstream from the exon1 transcription start site showed significant promoter activity in various CB_1R-expressing neuroblastoma cell lines. 5′ Flanking sequences of exon 3 yielded much lower promoter activity, consistent with the lower expression of transcript CB_1E. The more active promoter upstream of exon1 was further investigated in T cells, which normally express low levels of CB_1R (Börner et al., 2008). It is noteworthy that positive and negative regulatory regions mediating basal expression of CB_1R in resting T cells differed from findings in neuronal lines, suggesting cell-type specific CB_1R promoter regulation. A functional signal transducer and activator of transcription-6 (STAT6) element located −2769 upstream from the exon1 start site was also found to mediate interleukin-4-inducible CB_1R expression.

Mouse CB_1R gene structure was studied by McCaw et al. (2004) using RNA from striatum. In contrast to human CB_1R (namely the striatal transcripts isolated by Zhang et al., 2004), the mouse gene contains a shorter 5′UTR, with only one additional exon located upstream of the coding exon. This exon contains multiple transcription start sites (with the major sites at the beginning of exon1); however, the promoter activities of these regions were not examined. Later studies identified direct interactions of retinoic acid receptor-γ and steroidogenic factor-1 with the mouse CB_1R promoter (Kim et al., 2008; Mukhopadhyay et al., 2010); however, these studies did not specify which putative promoter was examined.

The originally cloned CB_1R from a rat cerebral cortex cDNA library (Matsuda et al., 1990) similarly consists of two exons; however, additional 5′ exons have not been investigated. Homology between human and rodent 5′UTR has not been examined; in general, species comparison is more detailed for CB_2R (see next section).

B. Cannabinoid Receptor-2. Like CB_1R, the CB_2R coding region is contained in a single exon and is flanked by upstream noncoding exons in human, mouse, and, debatably, rat. Human CB_2R consists of three exons alternatively transcribed and spliced to yield isoforms CB_2A and -B (Liu et al., 2009). CB_2B, the first cloned cDNA, is transcribed from a promoter proximal to exon2

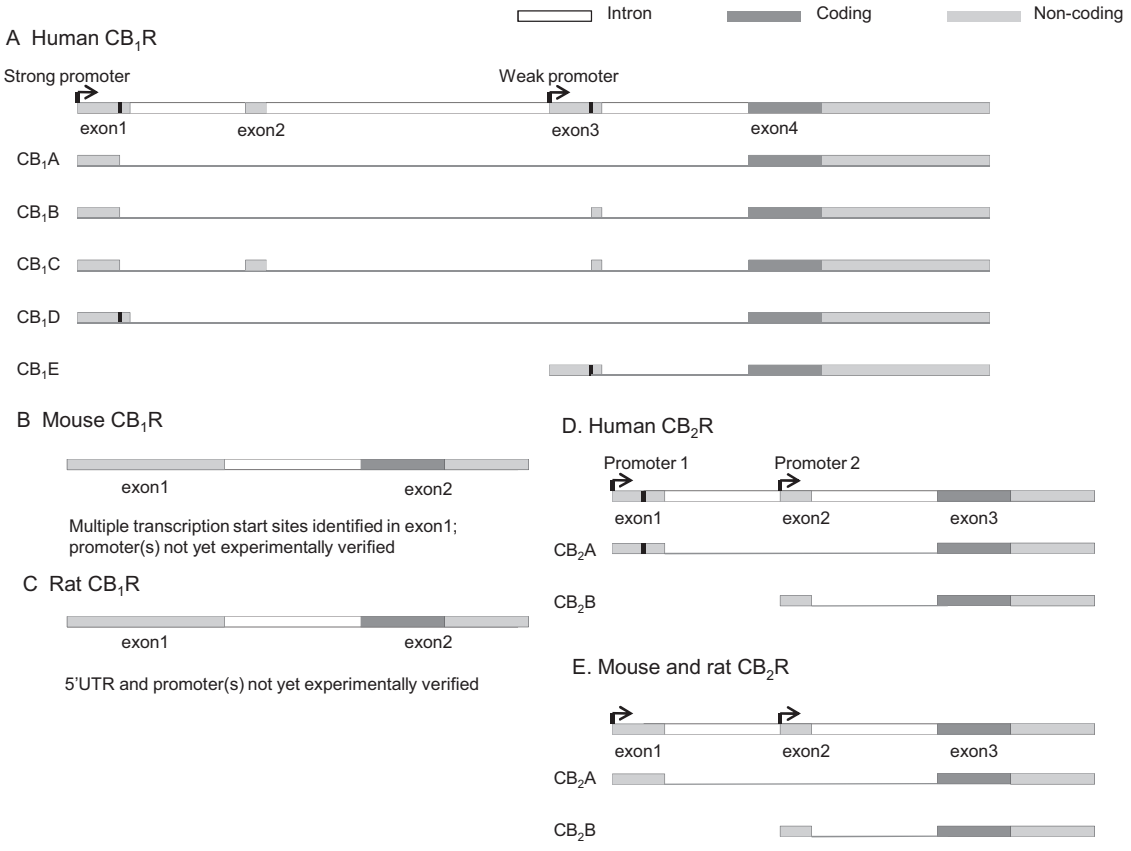

FIG. 1. CB_1R and CB_2R gene structures, promoters, and alternative transcripts. Transcripts are depicted below the complete gene structures and are not drawn to scale. Data from Zhang et al. (2004), human CB_1R; McCaw et al. (2004), mouse CB_1R; Matsuda et al. (1990), rat CB_1R; Liu et al. (2009), human, rat, and mouse CB_2R.

and expressed most highly in immune cells and tissues. The more recently identified CB_2A contains exon1 and exon3 and is generated from a promoter 5′ proximal to exon1. In contrast to CB_2B, CB_2A is most highly expressed in testis and shows some expression in the brain.

Mouse CB_2R similarly consists of three exons alternatively transcribed by two promoters (Onaivi et al., 2006; Liu et al., 2009). In contrast to humans, however, both CB_2A and CB_2B are expressed predominantly in the spleen (Liu et al., 2009). Rat CB_2R gene structure seems more complex than that of human and mouse, although findings are conflicting. In Fig. 1, we present findings by Liu et al. (2009) of CB_2A and -B isoforms transcribed from promoters flanking exon 1 and 2, like human and mouse CB_2R. Rat CB_2A and -B also showed expression patterns similar to those of mouse. In contrast, Brown et al. (2002) identified three coding exons in rat CB_2R using the same species and tissue (spleen).

Like CB_1R, human and rodent CB_2R differ in length of 5′UTR, with human CB_2R again being considerably longer. Moreover, the CB_2R 5′UTR showed little alignment between human-rodent (although rat-mouse 5′UTR aligned well, and there was significant homology in coding region across all species) (Liu et al., 2009). The lack of

TABLE 3

Transcription factors found to interact with CB_1R and CB_2R promoters using high-throughput ChIP (ChIP-Chip or ChIP-Seq) experiments

The selection of studies was found through the Chromatin Enrichment Analysis database (ChEA) (http://amp.pharm.mssm.edu/lib/chea.jsp) (Lachmann et al., 2010). Transcription factor functions are adapted from MetaCore database (http://www.genego.com/metacore.php).

Transcription Factor	References	Cell line	Function
CB_1R			
AR (androgen receptor)	Lin et al., 2009	Human prostate cancer PC3 cells	Activates transcription of androgen responsive genes to affect cell proliferation and differentiation.
SUZ12 (suppressor of zeste 12)	Boyer et al., 2006; Ku et al., 2008; Marson et al., 2008	Mouse ES cells	Component of the PRC2/EED-EZH2 complex, which represses target genes by methylating lysine residues on histone H3. Complex may also repress transcription by recruiting DNA methyltransferases.
EED (embryonic ectoderm development)	Boyer et al., 2006	Mouse ES cells	Also a component of the PRC2/EED-EZH2 complex (see above).
CREM (cAMP responsive element modulator)	Martianov et al., 2010	Mouse germ cells	Binds the CRE; isoforms are either activators or repressors.
JARID2 (jumonji, AT rich interactive domain 2)	Peng et al., 2009; Pasini et al., 2010	Mouse ES cells	Regulates histone methyltransferase complexes.
REST/NRSF (RE1-silencing transcription factor/ neuron restrictive silencer factor)	Abrajano et al., 2009	Mouse neurons	Binds NRSEs and represses neuronal gene expression via recruitment of the BHC (see below).
RCor1/CoREST (REST corepressor 1)	Abrajano et al., 2009	Mouse neurons	Component of the BHC that is recruited to NRSE sites by REST, where it deacetylates and demethylates histones and thus represses neuronal gene expression.
RNF2 (ring finger protein 2)	Boyer et al., 2006	Mouse ES cells	E3 ubiquitin-protein ligase that regulates monoubiquitination of histone H2A lysine residues, a repressive mark.
CUX-1 (cut-like homeobox 1)	Kedinger et al., 2009	Several human cancer cell lines	Part of the homeodomain family of DNA binding proteins; may regulate differentiation and cell cycle progression.
CB_2R			
EP300 (E1A binding protein p300)	Blow et al., 2010	Mouse embryonic heart tissue	Binds phosphorylated CREB and functions as a histone acetyltransferase. Important in cell proliferation and differentiation, and thought to have a role in the stimulation of hypoxia-induced genes.
ERG (v-ets erythroblastosis virus E26 oncogene homolog)	Wilson et al., 2010	Mouse HPC7 hematopoietic progenitor cells	Recruits SETDB1 histone methyltransferase to target genes.
STAT3 (signal transducer and activator of transcription 3)	Kwon et al., 2009	Mouse CD4 Ts	Activated by phosphorylation upon cell stimulation by cytokines and growth factors. Has broad functions, including cell growth and apoptosis.
GATA3 (GATA binding protein 3)	Kidder and Palmer, 2010	Mouse trophoblast stem cells	Regulates T-cell development.

CRE, cAMP response element; NRSE, neuron-restrictive silencer elements; BHC, BRAF-HDAC complex; REST, RE1-silencing transcription factor; CREB, cAMP response element-binding protein.

significant homology in 5′UTR and different tissue distribution of CB_2A and CB_2B isoforms in humans versus rodents (with the 2A isoform appearing in the brain only in humans) suggests differential CB_2R promoter regulation across species. Such differences in rodent and human CB_2R isoform expression should also be kept in mind when interpreting results from animal models. For example, the human CB_2A isoform could produce unwanted central nervous system effects because of its low expression in the brain; these effects would not be seen in mice (for review, see Campbell et al., 2001; Liu et al., 2009).

The production of these alternative CB_1R and CB_2R transcripts differing in 5′UTR in disease is worth investigating. These transcripts have already been shown to have region-specific expression; they could also differ in mRNA stability, subcellular localization and translational efficacies (see references in Zhang et al., 2004). The importance of the CB_1R 5′UTR in disease is suggested by the presence of single-nucleotide polymorphisms in 5′ UTR introns and exons (TAG haplotype) that are associated with lower mRNA levels and with substance abuse (Zhang et al., 2004). It will also be important to identify the use of alternative promoters in disease when studying direct mechanisms of cannabinoid receptor regulation.

V. High-Throughput Studies of Transcription Factor Interactions with Cannabinoid Receptors 1 and 2

In addition to the directed studies of cannabinoid receptor promoter regulation (Table 2), high-throughput chromatin immunoprecipitation (ChIP) studies using various cell lines have identified a number of transcription factors that interact with the CB_1R and CB_2R promoters. We used the Chromatin Enrichment Analysis database of such ChIP experiments (Lachmann et al., 2010) to compile a list of transcription factors that interact with CB_1R and CB_2R (Table 3). Many of these transcription factors are implicated in DNA methylation and histone post-translational modifications, further supporting a role for epigenetic mechanisms in cannabinoid receptor regulation. These studies vary in their methods of defining target genes; often, regulatory regions are assigned based on a given distance (e.g., 50 kb) from a locus. Moreover, the binding of a transcription factor does not always mean that it has functional effects on transcription at a given locus (Chen et al., 2008).

In addition to identifying transcription factors that interact with the cannabinoid receptor promoters through such ChIP studies, identifying transcription factors downstream of cannabinoid receptor signaling will be important for further studies of cannabinoid receptor autoregulation. Along these lines, Bromberg et al. (2008) identified 33 transcription factors activated in Neuro2A neuroblastoma cells after stimulation with cannabinoid receptor agonist using an array of transcription factor-binding site oligonucleotides. Taken together, transcription factors identified by these high-throughput studies can be starting points for further directed investigations of cannabinoid receptor regulation in disease.

VI. Conclusions and Future Directions

Alteration in cannabinoid receptor expression is appearing more and more to be a widespread response to disease. As investigation of the mechanisms underlying these changes continues, we will probably begin to see commonalities across diseases. Cytokines, growth factors, hormones, and other factors released in response to tissue injury and inflammation are thus rational starting points for further investigations of mechanism. Autoregulation of cannabinoid receptor expression will also likely be identified in other models. Findings of specific signaling pathways and transcription factors will hopefully be accompanied by identification of the epigenetic modifications ultimately underlying these expression changes.

Understanding these mechanisms of cannabinoid receptor regulation will hopefully expand our options for therapeutically targeting the endocannabinoid system. Selectively enhancing receptor expression could allow for lower doses of systemic agonists or eliminate the need for them altogether; selectively inhibiting expression could likewise avoid systemic antagonism. These improved endocannabinoid system-based therapies could have a lot to offer medicine.

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