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 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 protein-coupled receptors CB1R and CB2R. Endocannabi-
TABLE 1

CB₁R and CB₂R expression changes in disease

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

<table>
<thead>
<tr>
<th>Disease</th>
<th>CB₁R/CB₂R Regulation</th>
<th>Proposed Role of CB₁R/ CB₂R in Disease</th>
<th>Therapeutic Implications</th>
</tr>
</thead>
<tbody>
<tr>
<td>Neuropathic pain</td>
<td>CB₁R and CB₂R 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; Mittrirattanakul et al., 2006).</td>
<td>Inhibition of neurotransmitter release decreases hyperexcitability of sensory pathways (CB₁R); inhibition of inflammation (CB₂R) (for review, see Costigan et al., 2009).</td>
<td>CB₂R up-regulation enhances analgesic response to exogenous cannabinoids in animal model of neuropathic pain (Lim et al., 2003).</td>
</tr>
<tr>
<td>Neuroinflammation and brain injury</td>
<td>CB₂R 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).</td>
<td>CB₂R expressed on T cells reduces inflammation in MS (Maresz et al., 2007).</td>
<td>T-cell CB₂R is already highly activated by endocannabinoids in MS; increasing receptor number is thus important for increasing efficacy of CB₂R agonist (Maresz et al., 2007).</td>
</tr>
<tr>
<td>Cancer</td>
<td>Increased CB₁R and CB₂R expression in multiple human cancers (for review, see Sarfaraz et al., 2008).</td>
<td>Cannabinoid receptor activation leads to apoptosis via cell cycle regulation and ER stress (for review, see Sarfaraz et al., 2008).</td>
<td>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 CB₁R and CB₂R (Sarfaraz et al., 2005), whereas Δ⁹-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).</td>
</tr>
<tr>
<td>Gastrointestinal</td>
<td>CB₁R up-regulated in mouse model of diarrhea (Izzo et al., 2001); CB₁R and CB₂R up-regulated in multiple models of colitis (Massa et al., 2004; Kimball et al., 2006).</td>
<td>CB₁R up-regulated in rat brain after mild concussive head injury and NMDAR blockade (Hansen et al., 2001).</td>
<td>Neuroprotection by decreasing glutamate release/excitotoxicity, among other possible mechanisms (for review, see Mechoulam et al., 2002).</td>
</tr>
<tr>
<td>Liver</td>
<td>Hepatic CB₁R and CB₂R 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).</td>
<td>Down-regulated CB₁R in human colorectal tumors (Wang et al., 2008).</td>
<td>Loss of CB₁R leads to enhanced colorectal tumor proliferation in mouse model of colorectal cancer (Wang et al., 2008).</td>
</tr>
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<td></td>
<td>Hepatic CB₁R 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).</td>
<td></td>
<td>Up-regulation of CB₁R probably accounts for increased efficacy and potency of agonists in slowing intestinal transit in mouse model of diarrhea (Izzo et al., 2001).</td>
</tr>
<tr>
<td>Metabolic</td>
<td>Adipose CB₁R up-regulated in Zucker rat model of obesity (Bensaid et al., 2003) and mice fed high-fat diet (Jourdan et al., 2010).</td>
<td>CB₂R up-regulation enhances adipocyte fatty acid synthesis in mice fed high-fat diet (Osei-Hyiaman et al., 2005).</td>
<td>CB₁R antagonism also prevents high-fat diet-induced increases in hepatic CB₂R expression (Jourdan et al., 2010).</td>
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<td></td>
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<td>CB₂R 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).</td>
<td>Antagonism of CB₂R decreases hepatic fatty acid synthesis in mice fed high-fat diet (Osei-Hyiaman et al., 2005).</td>
</tr>
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<td></td>
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<td>CB₁R antagonism also prevents high-fat diet-induced increases in hepatic CB₁R expression (Jourdan et al., 2010).</td>
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<td></td>
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<td></td>
<td>CB₂R up-regulation in adipocytes may account for increased efficacy of CB₁R antagonist in reducing weight in obese compared with lean Zucker rats (Vickers et al., 2003).</td>
</tr>
</tbody>
</table>
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 CB1R expression is highest, stimulation of presynaptic CB1R inhibits neurotransmitter release by stimulating potassium channels and inhibiting calcium channels (for review, see Howlett et al., 2002). In the liver, where CB1R is normally expressed at low levels, stimulation of CB1R leads to enhanced expression of acetyl-CoA carboxylase-1 and fatty acid synthase and thus increases lipogenesis (Osei-Hyman et al., 2005). CB2R expression is highest in immune cells and thus increases lipogenesis (Osei-Hyman et al., 2005). In the liver, where CB1R is normally expressed at low levels, stimulation of CB1R leads to enhanced expression of acetyl-CoA carboxylase-1 and fatty acid synthase and thus increases lipogenesis (Osei-Hyman et al., 2005).

### Cardiovascular
- **CB1R** up-regulated in myocardium and aorta in rat model of hypertension (Batkai et al., 2004).
- **CB1R** up-regulated in immune cells in atherosclerotic plaques in humans and mouse model (Steffens et al., 2005).
- **CB1R** up-regulated in myocardium and aorta in rat model of hypertension (Batkai et al., 2004).

### Psychiatric
- **CB1R** up-regulated in prefrontal cortex of depressed suicide victims (Hungund et al., 2004) and patients with schizophrenia (Dean et al., 2001).
- **CB1R** has both excitatory and inhibitory effects on synaptic transmission in the prefrontal cortex (LaFurcade et al., 2007; Chiu et al., 2010); the effect of its up-regulation in these diseases is not known.

### Table 1

<table>
<thead>
<tr>
<th>Disease</th>
<th>CB1R/CB2R Regulation</th>
<th>Proposed Role of CB1R/CB2R in Disease</th>
<th>Therapeutic Implications</th>
</tr>
</thead>
<tbody>
<tr>
<td>Skeletal muscle CB1R up-regulated in mice fed high-fat diet (Pagotto et al., 2006).</td>
<td>CB1R up-regulation in skeletal muscle may contribute to insulin resistance in obesity (for review, see Kunos et al., 2008).</td>
<td>CB1R antagonism prevents high-fat diet-induced increases in adipose CB1R expression (Jourdan et al., 2010).</td>
<td></td>
</tr>
<tr>
<td>Cardiovascular</td>
<td>CB1R 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).</td>
<td>CB1R up-regulation in the heart and vasculature may account for increased potency and efficacy of agonists in hypertensive animals (Batkai et al., 2004).</td>
<td></td>
</tr>
<tr>
<td>Psychiatric</td>
<td>CB1R decreases plaque progression, possibly by reducing infiltration of immune cells and cytokine release (Steffens et al., 2005).</td>
<td>Targeting the endocannabinoid system in these diseases will require better understanding of its role.</td>
<td></td>
</tr>
</tbody>
</table>

### Notes
- **MS**, multiple sclerosis; **ALS**, amyotrophic lateral sclerosis; **EAE**, experimental autoimmune encephalitis; **NMDAR**, N-methyl-D-aspartate receptor; **ER**, endoplasmic reticulum; **Δ9-THC**, tetrahydrocannabinol; **aHSC**, activated hepatic stellate cell.

Other diseases, alterations in receptor expression are maladaptive, examples being CB1R 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 CB1R 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 CB1R up-regulation reduced the analgesic effects of cannabinoids (Lim et al., 2004). Regional up-regulation of CB1R 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 Δ9-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
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$_2$R 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$_2$R expression (Lim et al., 2003). Spinal cord CB$_2$R 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$_2$R up-regulation is probably made possible by local increases in spinal glucocorticoid receptors (GR) (Wang et al., 2004). Localized mechanisms of CB$_2$R 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$_2$R 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>
<thead>
<tr>
<th>Disease/System</th>
<th>Mechanism</th>
<th>Cell Type / Animal Model</th>
<th>Implications</th>
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</thead>
<tbody>
<tr>
<td>Liver steatosis</td>
<td>RAR-γ binds the $–500$ to $+50$ region of the CB$_1$R promoter and increases its expression (Mukhopadhyay et al., 2010).</td>
<td>Primary cultured mouse hepatocytes</td>
<td>Retinoic acid released from hepatic stellate cells induces CB$_1$R expression in hepatocytes, which in turn induces lipogenesis (Jeong et al., 2008).</td>
</tr>
<tr>
<td>Inflammation</td>
<td>Cannabinoids induce CB$_1$R mRNA expression in T cells by an IL-4- and CB$_2$R-dependent mechanism (Borner et al., 2007).</td>
<td>Jurkat T cells</td>
<td>CB$_1$R 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$_1$R, and IL-4 in turn increases CB$_2$R transcription (Borner et al., 2008).</td>
</tr>
<tr>
<td>Neurodegenerative</td>
<td>CB$_2$R protein up-regulation in the spinal cord is mediated by Trk and MAPK (Lim et al., 2003) and GR (Wang et al., 2007).</td>
<td>Human colorectal cancer cells</td>
<td>Loss of CB$_2$R 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$_2$R regulation.</td>
</tr>
<tr>
<td>Cancer</td>
<td>Increased DNA methylation of CB$_2$R promoter leads to decreased expression of CB$_2$R (Wang et al., 2008).</td>
<td>Human colorectal cancer cells</td>
<td>Loss of CB$_2$R 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$_2$R regulation.</td>
</tr>
<tr>
<td>Feeding behaviors and energy homeostasis</td>
<td>CNS-specific knockout of SF-1 leads to loss of CB$_2$R expression in the ventromedial hypothalamus. SF-1 directly increases CB$_2$R expression via SF-1 element at $–101$ within the mouse promoter (Kim et al., 2008).</td>
<td>CNS-specific SF-1 knockout mouse and various cell lines</td>
<td>CNS-specific SF-1 knockouts do not show the appetite-stimulating effects of CB$_2$R agonists. SF-1 regulation of CB$_2$R expression in the VMH is thus required for cannabinoid effects on food intake (Kim et al., 2008).</td>
</tr>
<tr>
<td>Neurodegenerative</td>
<td>Decreased striatal CB$_2$R levels in HD are due to decreased transcription (McCaw et al., 2004).</td>
<td>Mouse HD model</td>
<td>Striatal CB$_2$R modulates dopamine transmission, which is dysregulated in HD (see refs within McCaw et al., 2004).</td>
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</tbody>
</table>
cated in up-regulation of both CB₁R and CB₂R 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 CB₂R in human colorectal cancer cells has been attributed to methylation of the CB₂R 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 CB₁R up-regulation in hepatocytes and T cells, respectively (Börner et al., 2007; Mukhopadhyay et al., 2010), and long-term CB₁R antagonism has been found to counter increases in hepatic and adipose CB₁R expression in response to high-fat diet (Jourdan et al., 2010). In addition, administration of a mixed CB₁R/CB₂R agonist increases expression of the CB₂A isoform (see section IV.B) in mouse cerebellum (Liu et al., 2009). Because endocannabinoids are often increased along with cannabinoid receptors in disease (Mitirirattanakul et al., 2006; Jeong et al., 2008), such autoreinduction 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 CB₁R 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 CB₁R 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 (Mitirirattanakul 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 CB₂R 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 CB₂R 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, CB₁R antagonists have been proposed for treatment of liver fibrosis, which is marked by maladaptive up-regulation of CB₁R (Teixeira-Clerc et al., 2006). However, systemic use of the CB₁R 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 CB₁R up-regulation; the retinoic acid system has already been implicated and could thus be a possible target (Mukhopadhyay et al., 2010). Given that CB₁R 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 CB₁R and CB₂R are summarized here and in Fig. 1.

A. Cannabinoid Receptor-1. In human, rat, and mouse, the CB₁R 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 CB₁R using hippocampal RNA, giving a large (approximately 20 kb) 5′ UTR characteristic of neuronally expressed genes. This 5′ UTR can be alternatively spliced (CB₁A–D) or transcribed at different sites (CB₁A–D versus CB₁E) 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 CB₁R-expressing neuroblastoma cell lines. The more active promoter upstream of exon1 was further investigated in T cells, which normally express low levels of CB₁R (Börner et al., 2008). It is noteworthy that positive and negative regulatory regions mediating basal expression of CB₁R in resting T cells differed from findings in neuronal lines, suggesting cell-type specific CB₁R 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 CB₁R expression.

Mouse CB₁R gene structure was studied by McCaw et al. (2004) using RNA from striatum. In contrast to human CB₁R (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 CB₁R promoter (Kim et al., 2008; Mukhopadhyay et al., 2010); however, these studies did not specify which putative promoter was examined.

The originally cloned CB₁R 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 CB₂R (see next section).

B. Cannabinoid Receptor-2. Like CB₁R, the CB₂R coding region is contained in a single exon and is flanked by upstream noncoding exons in human, mouse, and, debatably, rat. Human CB₂R consists of three exons alternatively transcribed and spliced to yield isoforms CB₂A and -B (Liu et al., 2009). CB₂B, the first cloned cDNA, is transcribed from a promoter proximal to exon2.
and expressed most highly in immune cells and tissues. The more recently identified CB2A contains exon 1 and exon 3 and is generated from a promoter 5’ proximal to exon 1. In contrast to CB2B, CB2A is most highly expressed in testis and shows some expression in the brain.

Mouse CB2R similarly consists of three exons alternatively transcribed by two promoters (Onaivi et al., 2006; Liu et al., 2009). In contrast to humans, however, both CB2A and CB2B are expressed predominantly in the spleen (Liu et al., 2009). Rat CB2R 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 CB2A and -B isoforms transcribed from promoters flanking exon 1 and 2, like human and mouse CB2R. Rat CB2A and -B also showed expression patterns similar to those of mouse. In contrast, Brown et al. (2002) identified three coding exons in rat CB2R using the same species and tissue (spleen).

Table 3

<table>
<thead>
<tr>
<th>Transcription Factor</th>
<th>References</th>
<th>Cell line</th>
<th>Function</th>
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</thead>
<tbody>
<tr>
<td>CB1R AR (androgen receptor)</td>
<td>Lin et al., 2009</td>
<td>Human prostate cancer PC3 cells</td>
<td>Activates transcription of androgen responsive genes to affect cell proliferation and differentiation.</td>
</tr>
<tr>
<td>SUZ12 (suppressor of zeste 12)</td>
<td>Boyer et al., 2006; Ku et al., 2008; Marson et al., 2008</td>
<td>Mouse ES cells</td>
<td>Component of the PRC2/EED-EZH2 complex, which represses target genes by methylating lysine residues on histone H3.</td>
</tr>
<tr>
<td>EED (embryonic ectoderm development)</td>
<td>Boyer et al., 2006</td>
<td>Mouse ES cells</td>
<td>Also a component of the PRC2/EED-EZH2 complex (see above).</td>
</tr>
<tr>
<td>CREM (cAMP responsive element modulator)</td>
<td>Martinov et al., 2010</td>
<td>Mouse germ cells</td>
<td>Binds the CRE; isoforms are either activators or repressors.</td>
</tr>
<tr>
<td>JARID2 (jumonji, AT rich interactive domain 2)</td>
<td>Peng et al., 2009; Pasini et al., 2010</td>
<td>Mouse ES cells</td>
<td>Regulates histone methyltransferase complexes.</td>
</tr>
<tr>
<td>REST/NRSF (RE1-silencing transcription factor/ neuron restrictive silencer factor)</td>
<td>Abrajano et al., 2009</td>
<td>Mouse neurons</td>
<td>Binds NRSFs and represses neuronal gene expression via recruitment of the BHC (see below).</td>
</tr>
<tr>
<td>RCor1/CoREST (REST corepressor 1)</td>
<td>Abrajano et al., 2009</td>
<td>Mouse neurons</td>
<td>Component of the BHC that is recruited to NRSF sites by REST, where it deacetylates and demethylates histones and thus represses neuronal gene expression.</td>
</tr>
<tr>
<td>RNF2 (ring finger protein 2)</td>
<td>Boyer et al., 2006</td>
<td>Mouse ES cells</td>
<td>E3 ubiquitin-protein ligase that regulates monoubiquitination of histone H2A lysine residues, a repressive mark.</td>
</tr>
<tr>
<td>CUX-1 (cut-like homeobox 1)</td>
<td>Kedinger et al., 2009</td>
<td>Several human cancer cell lines</td>
<td>Part of the homeodomain family of DNA binding proteins; may regulate differentiation and cell cycle progression.</td>
</tr>
<tr>
<td>CB2R EP300 (E1A binding protein p300)</td>
<td>Blow et al., 2010</td>
<td>Mouse embryonic heart tissue</td>
<td>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.</td>
</tr>
<tr>
<td>ERG (v-ets erythroblastosis virus E26 oncogene homolog)</td>
<td>Wilson et al., 2010</td>
<td>Mouse HPC7 hematopoietic progenitor cells</td>
<td>Recruits SETDB1 histone methyltransferase to target genes.</td>
</tr>
<tr>
<td>STAT3 (signal transducer and activator of transcription 3)</td>
<td>Kwon et al., 2009</td>
<td>Mouse CD4 Ts</td>
<td>Activated by phosphorylation upon cell stimulation by cytokines and growth factors. Has broad functions, including cell growth and apoptosis.</td>
</tr>
<tr>
<td>GATA3 (GATA binding protein 3)</td>
<td>Kidder and Palmer, 2010</td>
<td>Mouse trophoblast stem cells</td>
<td>Regulates T-cell development.</td>
</tr>
</tbody>
</table>

CRE, cAMP response element; NRSF, 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 CB2A and CB2B isoforms in humans versus rodents (with the 2A isoform appearing in the brain only in humans) suggests differential CB2R promoter regulation across species. Such differences in rodent and human CB2R isoform expression should also be kept in mind when interpreting results from animal models. For example, the human CB2A 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 CB1R and CB2R 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 CB1R 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 CB1R and CB2R 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 CB1R and CB2R (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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