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## Modulation of the Endocannabinoid System in Cardiovascular Disease:

### Therapeutic Potential and Limitations

**Pál Pacher, Partha Mukhopadhyay, Rajesh Mohanraj, Grzegorz Godlewski, Sándor Bátkai, and George Kunos**

*From the Sections on Oxidative Stress Tissue Injury (P.P., P.M., R.M.) and Neuroendocrinology (G.G., S.B., G.K.), Laboratory of Physiological Studies, National Institute on Alcohol Abuse and Alcoholism, National Institutes of Health, Bethesda, Md.*

Endocannabinoids, endogenous lipid mediators generated by virtually all cell types both in the brain and peripheral tissues, elicit a broad range of biological effects similar to those of marijuana. The endocannabinoid system (ECS) comprises the endocannabinoids, the enzymes involved in their biosynthesis and degradation, putative membrane transporter(s) involved in their cellular uptake and (possibly) release, and the G protein-coupled receptors that mediate their effects, including CB<sub>1</sub> and CB<sub>2</sub> as well as additional, as yet unidentified, receptors.<sup>1-4</sup> GPR55 has recently been proposed to be a cannabinoid receptor,<sup>5</sup> although its *in vivo* biological functions have not yet been identified. Arachidonoyl ethanolamide (anandamide [AEA]) and 2-arachidonoylglycerol are the 2 best characterized endocannabinoids. AEA can be a full or partial agonist at CB<sub>1</sub> receptors, depending on the system, and has low efficacy at CB<sub>2</sub> receptors, whereas 2-arachidonoylglycerol is a full agonist at both CB<sub>1</sub> and CB<sub>2</sub> receptors. AEA also binds to vanilloid VR<sub>1</sub> receptors, although with an affinity an order of magnitude lower than its affinity for CB<sub>1</sub> receptors.<sup>3</sup> Both AEA and 2-arachidonoylglycerol are generated in the cell membrane from membrane phospholipid precursors, and the synthesis of both ligands involves multiple, parallel biosynthetic pathways.<sup>4,6,7</sup> In contrast, their enzymatic degradation occurs through unique pathways, AEA being degraded primarily by fatty acid amidohydrolase (FAAH),<sup>8</sup> whereas 2-arachidonoylglycerol is degraded mainly by monoglyceride lipase, although additional enzymes may make a minor contribution to the *in vivo* degradation of both ligands.<sup>9</sup> In the absence of a cellular storage mechanism for endocannabinoids, their tissue levels are determined by the balance between the rate of their “on-demand” synthesis and their enzymatic degradation.

The CB<sub>1</sub> receptor is predominantly expressed in the central nervous system,<sup>3</sup> but is also present at much lower, yet functionally relevant, levels in various peripheral tissues, including the myocardium,<sup>10-12</sup> postganglionic autonomic nerve terminals,<sup>3</sup> and vascular endothelial and smooth muscle cells<sup>13,14</sup> as well as the adipose tissue,<sup>15,16</sup> liver,<sup>16-19</sup> and skeletal muscle.<sup>20</sup> Expression of CB<sub>2</sub> receptors was thought to be limited to hematopoietic and immune cells, but they have recently been identified in the brain,<sup>21</sup> liver,<sup>19</sup> myocardium,<sup>12</sup> and human coronary endothelial and smooth muscle cells.<sup>13,14</sup> In addition to G<sub>i/o</sub>-dependent pathways, CB<sub>1</sub> and CB<sub>2</sub> receptors may also signal through other G proteins as well as through G protein-independent pathways.<sup>22</sup>

Correspondence to Pál Pacher, National Institute on Alcohol Abuse and Alcoholism, 5625 Fishers Lane, MSC-9413, Bethesda, MD 20892-9413. E-mail pacher@mail.nih.gov.

Disclosures  
None.

Activation of the ECS has been implicated in CB<sub>1</sub>-mediated hypotension associated with hemorrhagic, septic, cardiogenic shock, advanced liver cirrhosis (reviewed in <sup>3</sup>), and doxorubicin-induced heart failure.<sup>12</sup> Increased ECS activity also contributes to the generation of cardiovascular risk factors in obesity/metabolic syndrome and diabetes such as plasma lipid alterations, abdominal obesity, hepatic steatosis, and insulin and leptin resistance.<sup>23-28</sup> However, the ECS may also be activated as a compensatory mechanism in various forms of hypertension where it counteracts not only the increase in arterial pressure, but also the inappropriately increased cardiac contractility through activation of CB<sub>1</sub> receptors.<sup>11,29</sup> In addition, the activation of CB<sub>2</sub> receptors in endothelial and inflammatory cells by endogenous or exogenous ligands was found to limit the endothelial inflammatory response, chemotaxis, and adhesion of inflammatory cells to the activated endothelium with the consequent release of various proinflammatory mediators, which are key processes in the initiation and progression of atherosclerosis and reperfusion injury<sup>13,30-33</sup> as well as smooth muscle proliferation.<sup>14</sup> Therefore, depending on the underlying pathology, selective activation of CB<sub>1</sub> or CB<sub>2</sub> receptors or inhibition of CB<sub>1</sub> receptors may offer therapeutic benefits (Figures 1 and 2).

## Cardiovascular Effects of Cannabinoids

In humans, acute use of marijuana commonly causes isolated tachycardia, whereas chronic use may lead to hypotension and bradycardia.<sup>3,34</sup> In anesthetized mice and rats, AEA, Δ<sup>9</sup>-tetrahydrocannabinol as well as potent synthetic cannabinoid ligands evoke CB<sub>1</sub> receptor-mediated bradycardia, hypotension, and depressed cardiac contractility, which are less pronounced or absent in conscious normotensive animals, but are amplified in hypertension.<sup>11,29,35,36</sup> The mechanisms underlying these latter effects are complex and involve a decrease in sympathetic outflow by inhibition of norepinephrine release from sympathetic nerve terminals through pre-synaptic CB<sub>1</sub> receptors<sup>37,38</sup> as well as direct effects on the vasculature and myocardium.<sup>39</sup> Concerning endocannabinoids, these effects are also complicated by their rapid metabolism to arachidonic acid that can be further metabolized into vasoactive prostanoids.<sup>3,40,41</sup>

CB<sub>1</sub> receptors are expressed in the myocardium where they mediate negative inotropy<sup>10,11,42</sup> both in vitro and in vivo and in the coronary and cerebral vasculature where they mediate vasodilation.<sup>43,44</sup> As mentioned previously, the negative inotropic effect of synthetic cannabinoids and AEA is potentiated in various forms of experimental hypertension. CB<sub>2</sub> receptors are present in cardiomyocytes<sup>12,45,46</sup> and in coronary artery endothelial and smooth muscle cells,<sup>13,14</sup> but their cardiac functions have not been extensively studied. Recent evidence indicates that activation of CB<sub>2</sub> receptors contributes to ischemic preconditioning and protects against ischemia/reperfusion injury in the myocardium<sup>47,48</sup> as well as in other tissues.<sup>32,33</sup> In isolated vascular preparations, both synthetic and endogenous cannabinoids elicit complex vasodilatory effects showing interspecies and tissue differences.<sup>39</sup> In addition to CB<sub>1</sub>, a number of other receptors have been implicated in these effects, including vanilloid VR<sub>1</sub> and peroxisome proliferator-activated receptor-γ and as yet undefined endothelial/vascular site(s) of action.<sup>1,34,49</sup> Furthermore, there are examples of both nitric oxide-mediated and nitric oxide-independent mechanisms of cannabinoid-mediated vasodilation (reviewed in References 1, 34, 39, and 41).

The majority of findings suggest that the ECS plays a limited, if any, role in cardiovascular regulation under normal conditions. However, it may emerge as an important player under various pathological conditions, ranging from hypertension and the metabolic syndrome to various forms of circulatory shock, atherosclerosis and restenosis, myocardial infarction, and heart failure, as discussed in more detail subsequently.

## Hypertension

The possible use of cannabinoid ligands as antihypertensive agents was first contemplated in the early 1970s based on the long-lasting decreases in blood pressure associated with chronic use of cannabis in humans and in response to acute or chronic administration of  $\Delta^9$ -tetrahydrocannabinol in experimental animals. However, this idea was later abandoned because of the inability to separate the neurobehavioral and cardiovascular effects of cannabinergic ligands.<sup>3</sup> The possible antihypertensive potential of cannabinoids has reemerged as a result of findings demonstrating their greater hypotensive efficacy in hypertensive compared with normotensive animals<sup>11,29,35,50</sup> and recent evidence for the tonic activation of the ECS in various experimental models of hypertension as a possible compensatory mechanism.<sup>11</sup> Surprisingly, this hypotensive “tone” could be attributed predominantly to a CB<sub>1</sub> receptor-mediated decrease in cardiac contractility rather than a decrease in vascular resistance.<sup>3,11,29</sup> Accordingly, preventing the degradation of endogenous AEA by pharmacological inhibition of FAAH increased myocardial levels of AEA and reduced blood pressure and the inappropriately increased cardiac contractility in hypertensive but not in normotensive rats.<sup>11</sup> Because blocking FAAH activity in rats does not elicit behavioral effects suggestive of addictive potential,<sup>51</sup> FAAH may be a therapeutic target in hypertension where its inhibition may decrease not only blood pressure, but could also limit/prevent the development of cardiac hypertrophy.

## Metabolic Syndrome and Cardiometabolic Risk

There is growing evidence for a key role for the ECS in obesity and related metabolic and cardiovascular disorders. Chronic treatment with the CB<sub>1</sub> antagonist rimonabant causes a transient reduction in food intake and a sustained weight loss in rodent models of diet-induced obesity, suggesting direct effects on peripheral fat metabolism (reviewed in<sup>3,52,53</sup>). Indeed, CB<sub>1</sub> receptors are expressed on both adipocytes and hepatocytes, and their activation can result in increased lipogenesis and decreased fatty acid oxidation in adipose tissue<sup>15</sup> and the liver,<sup>17</sup> whereas CB<sub>1</sub> blockade can reverse the increased adiposity and hepatic steatosis.<sup>54</sup> Interestingly, CB<sub>1</sub> blockade also improves glucose tolerance, insulin and leptin sensitivity, and the plasma lipid profile in diet-induced or genetically obese animals.<sup>55</sup> Recent findings using hepatocyte-specific CB<sub>1</sub> knockout mice suggest that hepatic CB<sub>1</sub> receptors play a key role in these effects as well as in the hepatic steatosis, but not in the increased adiposity induced by a high-fat diet.<sup>56</sup>

Four large multicenter clinical trials with rimonabant involving obese individuals with the metabolic syndrome and/or type 2 diabetes suggest multiple beneficial effects of chronic CB<sub>1</sub> blockade not only in reducing body weight and waist circumference, but also in correcting the associated dyslipidemia and improving glucose tolerance.<sup>23-28</sup> Furthermore, rimonabant attenuated markers of inflammation and reduced plasma leptin and insulin levels while increasing plasma adiponectin.<sup>23-28</sup> These beneficial metabolic effects were at least partially weight loss-independent, further suggesting a direct effect of the ECS on various hormonal/metabolic parameters.<sup>23-26,28</sup> The recently reported results of a fifth multicenter study,<sup>57</sup> the Stradivarius clinical trial, examined the effect of 18 months of rimonabant treatment on coronary disease progression in subjects with abdominal obesity/metabolic syndrome followed by intravascular ultra-sound. In these subjects, the favorable effects of rimonabant on body weight and hormonal/metabolic parameters were similar to those in the previous 4 large-scale trials. Regarding coronary disease progression, rimonabant had no effect on the primary end point, percent atheroma volume, but significantly decreased normalized total atheroma volume, which was the secondary end point.<sup>57</sup>

Despite these promising findings, there are some important caveats regarding the therapeutic exploitation of chronic CB<sub>1</sub> blockade. The abnormalities associated with abdominal obesity include hypertension, but only one of the above 5 clinical trials documented a statistically significant, yet modest, decline in blood pressure.<sup>23</sup> A meta-analysis of the blood pressure changes in the first 4 studies found that in the subgroup of patients with hypertension, there was a modest hypotensive effect of rimonabant over placebo (-7.5 versus -4.7 mm Hg systolic and -5.2 versus -3.0 diastolic, rimonabant versus placebo).<sup>28</sup> There was no weight-independent effect of rimonabant on blood pressure, and the degree of reduction was in fact less than what would be expected based on the weight loss.<sup>58</sup> It also remains to be seen whether this modest change remains statistically significant once the negative results in the Stradivarius study are incorporated in such a meta-analysis. This could suggest that a subthreshold pressor effect of rimonabant may counteract, and thus limit, the blood pressure reduction achieved through weight loss. In hypertensive rats, rimonabant has an acute pressor effect, but this is seen only in anesthetized animals and at a dose approximately 10 times higher than the dose used in the human clinical trials,<sup>11</sup> so these findings cannot be directly extrapolated to human studies. Nevertheless, such a mechanism may also operate in untreated hypertensive subjects and may partly account for the relative inefficacy of rimonabant in reducing blood pressure in obese individuals. A variant of the polymorphic FAAH gene, that results in low FAAH activity, predisposes young normotensive individuals to hypotension (presumably through increased anandamide “tone”) but does not affect blood pressure in obese hypertensive subjects,<sup>59</sup> in whom such an effect may be counteracted and thus masked by pressor stimuli. It remains to be seen whether CB<sub>1</sub> blockade in such individuals may result in a further increase in their blood pressure.

The hemodynamic consequences of CB<sub>1</sub> blockade also warrant further study. In several forms of cardiomyopathies, CB<sub>1</sub> antagonists were found to reverse the decreased cardiac contractility, suggesting their therapeutic potential.<sup>12,45</sup> Obesity is often associated with decreased cardiac contractility,<sup>60,61</sup> the effects of which could be masked by an increase in sympathetic tone with a net result of moderate hypertension, often seen in the metabolic syndrome. Indeed, cardiac norepinephrine spillover was more than doubled in obese hypertensive compared with normotensive obese individuals.<sup>62</sup> In such cases, the net effect of CB<sub>1</sub> blockade on blood pressure will depend on whether the pressor effect of increasing cardiac contractility and cardiac output or the depressor effect of the resulting reflex decrease in sympathetic tone dominates. The observed lack of a significant effect of rimonabant on blood pressure may well reflect such opposing mechanisms at play.

A more important concern is the increased incidence of psychiatric side effects, notably anxiety and depression, in patients on rimonabant versus placebo, although subjects with a history of psychiatric illness had been excluded from the first 4 clinical trials.<sup>23-26</sup> These side effects may have contributed to the relatively high rate of attrition in these studies and were largely responsible for an US Food and Drug Administration Advisory Panel not recommending approval for rimonabant's use in the United States in 2007. To further examine this issue, the Stradivarius trial did not exclude subjects with pre-existing anxiety and depression and confirmed the higher incidence of psychiatric adverse events in patients treated with 20 mg rimonabant daily compared with placebo.<sup>57</sup> However, the incidence of serious adverse events such as attempted or completed suicide was low and was not different between the rimonabant and placebo groups.<sup>57</sup> Also, when the RIO-Europe study was extended to a second year of treatment, the incidence of psychiatric adverse events declined and the difference between the placebo and rimonabant groups was no longer present.<sup>27</sup> This suggests that psychiatric adverse reactions to rimonabant appear early during treatment and may be self-limiting. Indeed, rapid tachyphylaxis to other centrally mediated effects of rimonabant such as appetite reduction<sup>63,64</sup> or inhibition of relapse to alcohol drinking<sup>65</sup> has been noted in animal studies. Whether

tachyphylaxis also develops to the anxiogenic and depressive effects of rimonabant remains to be determined.

## Myocardial Ischemia/Reperfusion and Preconditioning

As pointed out previously, endocannabinoids are overproduced during various forms of ischemia/reperfusion injury such as whole body ischemia/reperfusion associated with hemorrhagic shock and acute myocardial infarction and may contribute to the cardiovascular depressive state associated with these pathologies.<sup>3,48</sup> On the other hand, endocannabinoids have been proposed to protect against myocardial ischemia/reperfusion injury and to contribute to the ischemic preconditioning effect of endotoxin, heat stress, or brief periods of ischemia.<sup>47,48</sup> In some of the earlier studies, the weight of the evidence for endocannabinoid involvement was limited by the lack of use of selective ligands and/or cannabinoid receptor-deficient animal models and by the absence of direct quantification of tissue endocannabinoid levels.<sup>48</sup> In addition, a majority of these studies used *ex vivo* models of isolated perfused hearts, in which the immunomodulatory effects of cannabinoids could not be assessed.<sup>48</sup> This is particularly important, because the increased formation of reactive oxygen and nitrogen species on reperfusion as well as the rapid transcriptional activation of an array of proinflammatory genes immediately leads to the local sequestration and activation of polymorphonuclear leukocytes, resulting in a rapid amplification of the initial inflammatory response, reactive oxygen species generation (respiratory burst), and consequent tissue injury. Recent studies highlight the role of endocannabinoids acting through CB<sub>2</sub> receptors in protecting against myocardial, cerebral, and hepatic ischemia/reperfusion injury.<sup>48</sup> The protective effect of CB<sub>2</sub> receptor activation could be attributed to decreased endothelial cell activation and suppression of the inflammatory response as reflected in decreased expression of adhesion molecules, secretion of chemokines, leukocyte chemotaxis, rolling, adhesion to endothelium, activation and transendothelial migration, and interrelated oxidative/nitrosative damage.<sup>13,33,66,67</sup> Thus, endocannabinoids protect against myocardial ischemia/reperfusion injury most likely through activation of CB<sub>2</sub> receptors located both on endothelial and inflammatory cells and perhaps also on cardiomyocytes (Figure 2).

## Atherosclerosis and Restenosis

It has been well established that proinflammatory cytokines such as tumor necrosis factor- $\alpha$  and bacterial endotoxin(s) are key mediators in the development of atherosclerosis. They induce NF- $\kappa$ B-dependent upregulation of adhesion molecules and chemokines (eg, monocyte chemoattractant protein) in endothelial cells leading to the recruitment and increased adhesion of monocytes to the activated endothelium followed by their transendothelial migration.<sup>68</sup> The activated endothelium, in concert with monocytes, also releases a variety of factors that promote smooth muscle cell proliferation and migration from the media into the intima, and these cells then synthesize and deposit extracellular matrix<sup>66</sup> (Figure 2). Furthermore, there is an interplay of these processes with the increased generation of reactive oxygen and nitrogen species and lipid signaling during the vascular inflammation associated with atherosclerosis.<sup>68,69</sup> Endothelial activation and smooth muscle cell migration and proliferation also play important roles in the development of vascular remodeling that occurs during restenosis in patients who undergo balloon angioplasty.

The possible role endocannabinoids in inflammatory and other cell migration has been explored with both increases and decreases of cell migration being reported depending on the experimental conditions, the type of endocannabinoid or synthetic agonist, and the cell type used.<sup>70</sup> In an elegant recent study, Steffens and colleagues have demonstrated that orally administered  $\Delta^9$ -tetrahydrocannabinol (a mixed weak CB<sub>1/2</sub> receptor agonist) significantly attenuated atherosclerosis progression in an apolipoprotein E knockout mouse model of the

disease, an effect that could be blocked by a selective CB<sub>2</sub> receptor antagonist.<sup>30</sup> CB<sub>2</sub> receptors expressing immune cells were found to be present both in human and mouse atherosclerotic plaques, and Δ<sup>9</sup>-tetrahydrocannabinol treatment reduced the proliferative capacity and interferon-γ production of lymphoid cells and inhibited macrophage chemotaxis and migration in vitro in a CB<sub>2</sub>-dependent manner.<sup>30,31</sup>

Δ<sup>9</sup>-tetrahydrocannabinol itself would not be an acceptable treatment for slowing the progression of atherosclerosis due to its CB<sub>1</sub>-mediated psychoactive effects. This problem may be overcome, however, through the use of selective CB<sub>2</sub> receptor agonists. Indeed, 2 such compounds, JWH133 and HU308, were found to dose-dependently attenuate tumor necrosis factor-α induced NF-κB and RhoA activation, upregulation of adhesion molecules intercellular adhesion molecule-1 and vascular cell adhesion molecule-1, increased expression of monocyte chemoattractant protein, enhanced transendothelial migration of monocytes, and augmented monocyte–endothelial adhesion in human coronary artery endothelial cells.<sup>13</sup> In human coronary artery smooth muscle cells, CB<sub>2</sub> agonists attenuated tumor necrosis factor-α signaling through Ras, p38 MAPK, extracellular signal regulated kinase 1/2, SAPK/JNK, and Akt as well as tumor necrosis factor-α induced cell proliferation and migration.<sup>14</sup>

## Perspectives

The multiple beneficial effects of rimonabant on obesity and the associated hormonal/metabolic abnormalities have generated widespread interest in this class of compounds, but psychiatric adverse effects represent a major concern. Recent evidence indicates that endocannabinoid activation of peripheral CB<sub>1</sub> receptors plays a major role in diet-induced abdominal obesity, hepatic steatosis, dyslipidemia, and insulin and leptin resistance.<sup>17,56,71,72</sup> This suggests that the risk/benefit ratio of CB<sub>1</sub> blockade in the treatment of abdominal obesity/metabolic syndrome may be substantially improved if peripherally restricted CB<sub>1</sub> antagonists became available.

As for the progression of coronary artery disease, further prospective studies appear to be warranted to test whether CB<sub>1</sub> antagonist treatment results in the reduction of clinical events related to coronary disease. It would be also interesting to explore whether there is an added benefit in combining statins, which primarily reduce low-density lipoprotein cholesterol, with rimonabant, which increases high-density lipoprotein cholesterol without affecting low-density lipoprotein cholesterol, and also reduces plasma triglycerides. Also, the insulin-sensitizing action of rimonabant<sup>23-26</sup> would make it a suitable candidate for combination therapy with other antidiabetics that usually cause weight gain, which would be counteracted by the weight-reducing effect of rimonabant.

The potential antihypertensive effect of FAAH antagonism also deserves further exploration, including the use of novel, more potent and selective inhibitors of FAAH and addressing the issues of the potential development of tolerance to the antihypertensive effects of FAAH antagonists and their effects on the development and progression of cardiac hypertrophy.

Selective CB<sub>2</sub> agonists may have therapeutic value in myocardial infarction, atherosclerosis, and restenosis (Figure 2), which should be confirmed in additional animal models and, possibly, in humans. It would also be interesting to see if chronic therapy with the CB<sub>1</sub> antagonist rimonabant is associated with a compensatory increase in endocannabinoid activation of CB<sub>2</sub> receptors and whether such a mechanism may contribute to the therapeutic benefits of CB<sub>1</sub> blockade.

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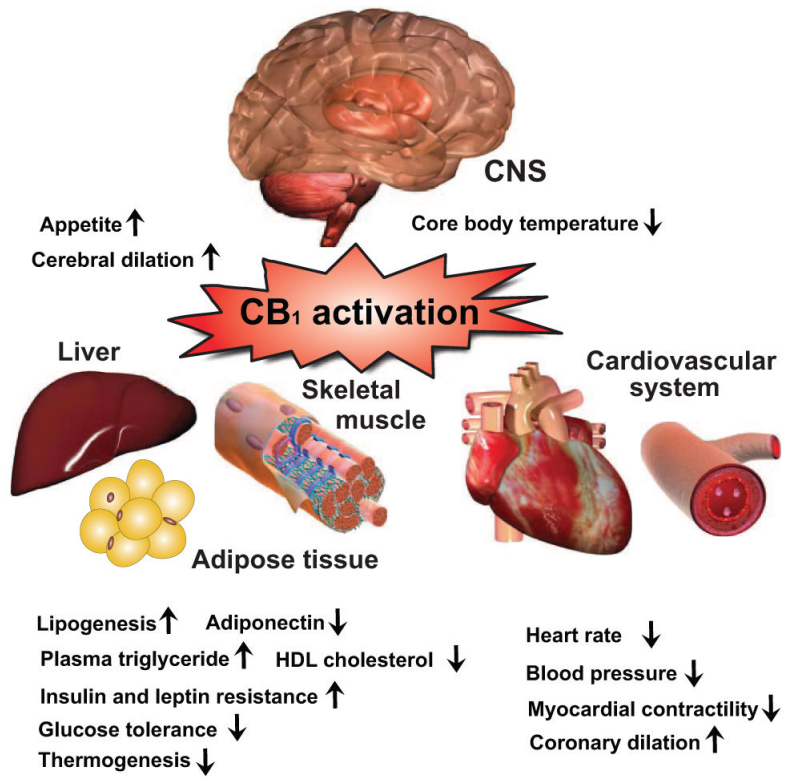
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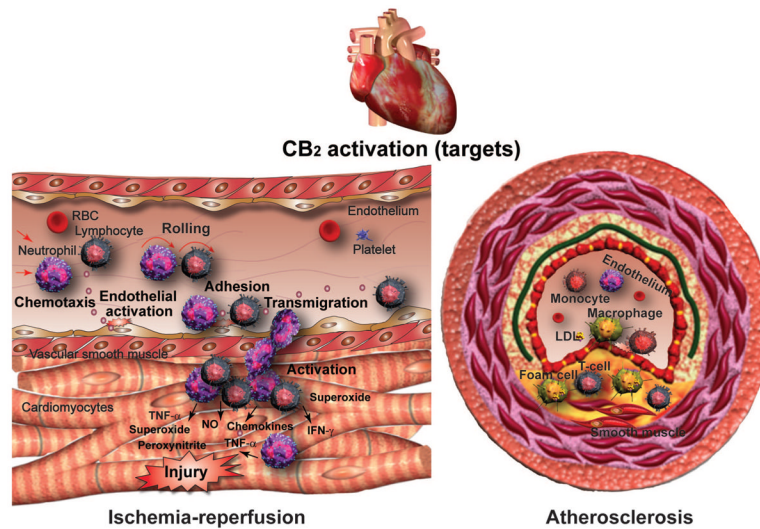


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**Figure 1.** Effects of CB<sub>1</sub> receptor activation with potential relevance to cardiovascular diseases/risk.



**Figure 2.** Therapeutic targets of CB<sub>2</sub> receptor stimulation in ischemia/reperfusion injury and atherosclerosis. CB<sub>2</sub> agonist decrease endothelial cell activation and inflammatory response, chemotaxis, and adhesion of inflammatory cells (lymphocytes, neutrophils, and/or monocytes) to activated endothelium, transendothelial migration of inflammatory cells, attachment to parenchymal cells and activation, and smooth muscle cell proliferation/migration.