Chapter 9

The Role for the Endocannabinoid System in Cardioprotection and Myocardial Adaptation

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Abstract

Results from different studies showing CB2 receptor-associated cardioprotective action are still fairly controversial and no single specific mechanism could be identified. Several groups investigated the involvement of the endocannabinoid system in cellular systems and function of cardiomyocytes, fibroblasts, macrophages and endothelial cells. While some studies are limited in their translational relevance, a few recent studies describe a myocardial ischemia and reperfusion scenario in a fashion comparable to the clinical situation. Recent studies provided evidence for involvement of the CB2 receptor–endocannabinoid axis in prevention of cardiomyocyte apoptosis including modulation of antioxidative enzymes and contractile elements expression. CB2 receptor has further been shown to specifically modulate the inflammatory response and macrophage function after myocardial ischemia. These effects have an impact on the subsequent myocardial remodeling, where the CB2 receptor modulates function of myofibroblasts, collagen production and limitation of myocardial infarction size. Recent experimental and clinical data showed the association of the endocannabinoid system in myocardial hypertrophy. In conclusion, increasing amount of evidence supports a crucial role of the endocannabinoid system in cardioprotection and myocardial remodeling, while some of them even suggest model-independent systemic effects in adaptation of cardiomyocytes or components of the extracellular matrix.

Keywords: endocannabinoids, myocardial ischemia, reperfusion, cardioprotection, remodeling

1. Introduction

Cannabinoids have been described as potent regulators of a variety of neurological functions influencing pain control, behaviour and memory. The discovery of the cannabinoid receptors

CB1 and CB2 led to initial description of CB1 receptor to be restricted to neurons while CB2 receptor was found on immunological cells. Later studies reported these receptors being also localized on vascular cells [1] and in the heart [2]. Furthermore, production of ligands to the cannabinoid receptors—endogenous cannabinoids—was reported in endothelial cells [3]. Experiments performed *in vitro* and *in vivo* showed that the effects of endocannabinoids on the cardiovascular system are pleiotropic and only partially understood to date. Due to the socioeconomic impact of cardiovascular diseases, a better understanding of the pathology and associated mechanisms is needed for development of novel therapeutic strategies. Since modern therapies are aiming to disease prevention with early treatment, the mechanisms of cardioprotection gained a significant attention and have been investigated more deeply. The cardioprotective mechanisms provide limitation of the myocardial damage after injury and are very complex. Growing evidence supporting the role of inflammation in cardioprotection [4] and modulation of inflammatory response by endocannabinoids led to investigations of the endocannabinoids in myocardial injury and protection.

2. Mechanisms of cardioprotection

Myocardial protection is a very complex system involving not only intracellular mechanisms in cardiomyocytes, but also bearing a large contribution of cells within the local microenvironment in the heart. The contradictions in the experimental evidence for specific mechanisms in the cardiomyocytes are not only related to differences in experimental setup, but also most probably associated to variations in mediators and cells within the local microenvironment. These factors make it difficult to draw clear conclusions from experimental data which will lead to new targets for therapy. Therefore, significant efforts have been made to enlighten the complexity of cardioprotection.

A number of signalling cascades and systems are involved in cardioprotection. Based on strong experimental and clinical evidence, the first line of intervention is aiming at the earliest possible restoration of the blood flow, i.e., reperfusion. The very early observation of timely onset of reperfusion leading to preservation of myocardial function [5, 6] provided ground for the clinical introduction of early percutaneous coronary intervention. Subsequently, Murry introduced the concept of ischemic preconditioning based upon four episodes of five minutes ischemia interrupted by each five minutes of reperfusion before a myocardial infarction was induced (40 minutes ischemia) and thereby resulting in decreased infarct size [7]. Interestingly, this effect was not found after a three-hour ischemia period underlining the ultimate goal of early reperfusion. This concept of myocardial conditioning was first applied in temporal relation to the myocardial injury, thereby defining preconditioning and postconditioning [8]. Studies extended this concept by introduction of spatial component in remote preconditioning, where short, repetitive limb occlusions provide protection to the following longer episode of myocardial ischemia [9, 10]. The latter concept was clinically implemented and proved to be beneficial to the patients [11]. Numerous studies described a wide range of molecules and signalling cascades involved utilizing different models, species, and pharmacological or genetic manipulation. So far there are only scattered reports investigating the role

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of endocannabinoids in ischemic preconditioning. One of the studies applied heat stress preconditioning 24 hours before isolation of the hearts, which then underwent 30 min ischemia and 120 minutes reperfusion *ex vivo* using Langendorff system [12]. The application of selective CB2 receptor antagonist SR144528 reduced the protective effects of heart preconditioning on infarct size. The authors therefore suggested a potential protective role of CB2 receptor in ischemia and reperfusion (I/R).

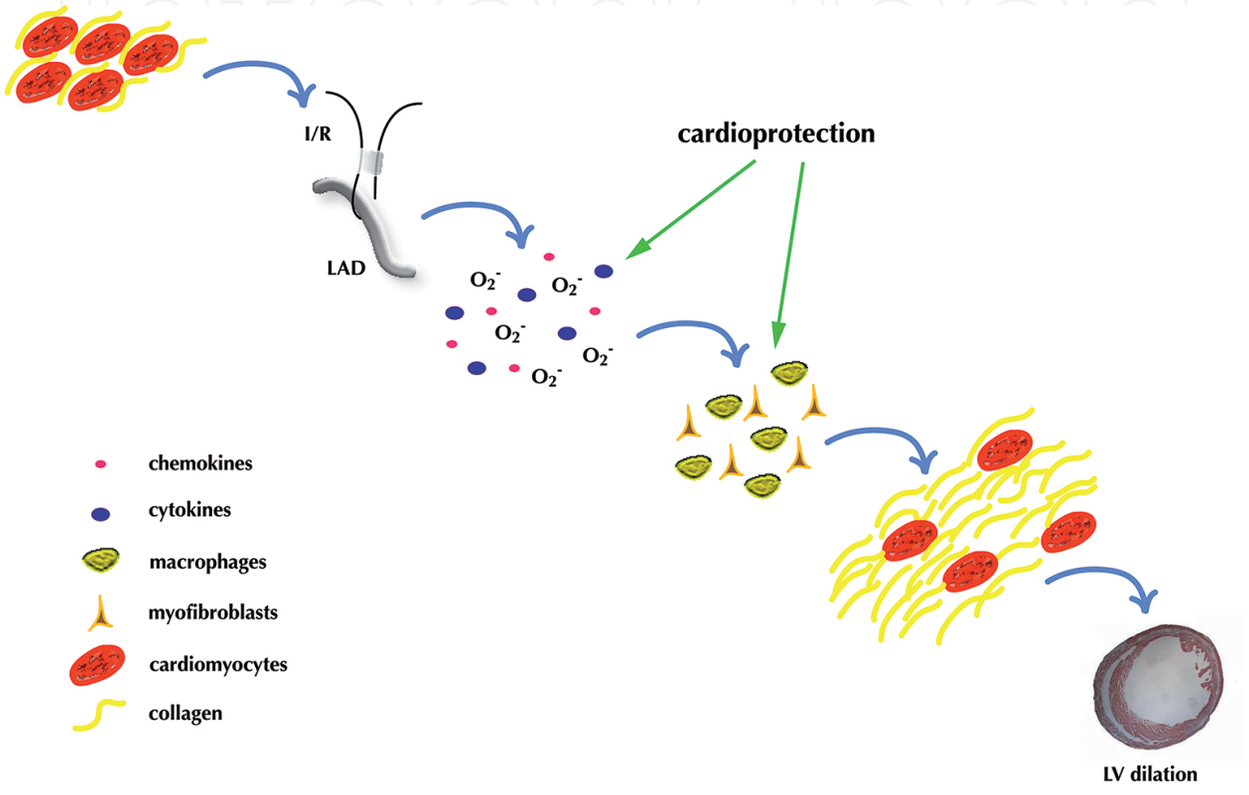

Figure 1. Cascade of events after reperfusion of ischemic myocardium. I/R, ischemia and reperfusion; LAD, left anterior descending artery; LV, left ventricular.

Another important area of cardioprotection originated in studies describing effects of modulation of inflammatory response during reperfusion injury. The very early studies reported detrimental outcome in patients treated with methylprednisolone after reperfusion of myocardial infarction [13]. Despite this drawback, it was the experimental work in subsequent years which provided solid evidence for beneficial effects of reperfusion [14]. The effects of inflammation in reperfusion must also be differentiated in a temporal and spatial context, because reperfusion initially induces a strong inflammatory response. In short-term (few days), this leads to a stronger functional impairment of the heart than without reperfusion, but the long-term effects of reperfusion have been proven to preserve the myocardial function and could even prevent development of dysfunction. The ischemia of myocardial tissue leads to accumulation of free radicals and toxic metabolic products while the adenosine triphosphate storages are depleted and cellular homeostasis is increasingly impaired. The reperfusion of ischemic myocardium is associated with activation of the complement system and a

strong increase in reactive oxygen species (ROS). The subsequent response includes activation of tumour necrosis factor α (*TNF-α*) and initiation of a cytokine response [15] leading to a cascade of events (**Figure 1**). Activation of *TNF-α* leads to induction of interleukin (*IL-*)*8* and CC chemokine ligand (*CCL*)*2*, which – in combination with complement factor C5a activation —attracts neutrophil granulocytes to the ischemic myocardium [16]. The extravasation of neutrophils and the expression of intercellular adhesion molecule (*ICAM-*)*1* lead to direct adhesion of neutrophils on cardiomyocytes with damaging effects involving ROS [17]. The ROS cause an oxidative burst leading to irreversible cellular damage [18] and is counteracted by different scavenger enzymes, e.g., peroxidases, superoxide dismutases (SODs) or catalase. The damaged cardiomyocytes release chemokine *CCL2* and cytokine transforming growth factor (*TGF-*)*β* and thereby promote invasion of mononuclear cells [19].

Differentiation of monocytes to macrophages in myocardium leads to even further increased production of inflammatory cytokines, while macrophages initiate their production of growth factors, e.g., basic fibroblast growth factor or vascular endothelial growth factor. As a result, proliferation of fibroblasts, differentiation of myofibroblasts and neoangiogenesis are initiated and all aiming at granulation tissue formation and tissue remodeling. These events involve different macrophage subpopulations, which are differentiated upon polarization of the lymphocytes response from *Th1* to *Th2* type [20]. While previous studies described classical proinflammatory M1 and alternative anti-inflammatory M2 subtype of macrophages, novel studies provide evidence of even more subtypes of these crucial cells in tissue repair. The application of so called cardiosphere-derived cells led to differentiation of a unique cardioprotective subtype of macrophages in infarcted rat hearts not bearing M1 or M2 markers and resulting in reduction of infarct size [21]. The inflammatory response has to be deactivated at the certain point of granulation tissue formation in order to provide rapid tissue remodeling and formation of a stable scar. This resolution of inflammatory response is mediated by anti-inflammatory cytokines, e.g., *IL-10*, which also inhibit matrix metalloproteinases (MMP) and stimulate their counter actors, tissue inhibitors of MMP (TIMP) [22]. Therefore, the regulation of macrophage function during myocardial remodeling gained a strong attention in recent years.

Among other factors, specific chemokines have been associated with modulation of macrophage function. Chemokines are a subgroup of cytokines having distinct effects on mononuclear cells and macrophages, but also on neutrophils and endothelial cells. One of the most potent monocyte chemoattractants is the chemokine *CCL2*, which is associated with transendothelial migration and differentiation of monocytes into macrophages [23–25]. *CCL2* is induced by proinflammatory cytokines *TNF-α* and *IL-1β* [24] and mediates mononuclear cell migration into reperfused myocardial infarction [26]. It has also been associated with differentiation of myofibroblasts and collagen production. Reperfusion of myocardial infarction in *CCL*-deficient ($CCL2^{-/-}$) mice was associated with prolonged inflammatory response and delayed formation of granulation tissue resulting in attenuated myocardial remodeling [27]. This was accompanied by decreased differentiation of myofibroblasts and significantly larger left ventricular diameter when compared with wild-type (WT) mice. Another study provided additional evidence for a crucial role of chemokine *CCL2* in the ischemic heart. In a model

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of repetitive brief I/R there was a significant reduction in collagen deposition and fibrosis associated with no ventricular dysfunction in *CCL2*$^{-/-}$ mice when compared to interstitial fibrosis and moderate dysfunction in WT animals [28]. Therefore, modulation of macrophage function *via* pharmacological manipulation of chemokine expression profile could be a promising target in development of novel clinical strategies.

3. Experimental evidence for involvement of endocannabinoids in cardioprotection

One of the first publications reported a CB1 receptor-mediated decrease in contractility of human atrial muscle [2]. Cannabinoids also led to a reduction of left ventricular systolic pressure [29]. There is a certain variability in results between *in vivo* and *ex vivo* effects of cannabinoids reported in the myocardium [30], the vasculature [31], the peripheral [32] and the central nervous system. The alterations in vascular tone were accompanied by changes in myocardial contractility and chronotropy and were associated to both CB1 receptor as well as vanilloid receptor [33]. In regard to pathophysiology, beneficial effects were reported for the experimental treatment of atherosclerosis using Δ-9-tetrahydrocannabinol (THC) [34]. In contrast, the results of endocannabinoid effects in the heart were heterogenous. One group reported triggering of heart attacks after use of marijuana [35], while other groups described protective effects in ischemic heart disease upon a decrease in mortality after experimental myocardial infarction [36], or anandamide-induced reduction of infarction size [37]. Still, the underlying mechanisms are not well understood and many investigations aim to shed more light into this clinically important filed. Myocardial I/R is always associated with inflammatory response and it is therefore likely that the endocannabinoid system may act in this process *via* the CB2 receptor as it modulates the function of macrophages [38]. A cardioprotective role has been postulated upon induction of *extracellular signal-regulated kinases (ERK)1/2* after 30 minutes of myocardial ischemia and 10 minutes reperfusion in mice [39]. Another study provided *in vitro* evidence of CB2 receptor-related cardioprotection *in vitro* using hydrogen peroxide treatment leading to increased apoptosis of cardiomyocytes and higher differentiation potential of myofibroblasts [40]. The same report described CB2 receptor-dependent down regulation of *caspase 3* after one hour ischemia and three days reperfusion, but provided surprising results in WT mice with normal left ventricular function after four weeks of reperfusion accompanied by infarct size of only 4% of left ventricular area. Other studies aimed to provide more insights into CB2 receptor mediated mechanisms in cardioprotection.

Application of a non-specific (acting on CB1 and CB2 receptor) agonist WIN55212-2 was shown to reduce infarct size in a mouse model of coronary occlusion without reperfusion, while it decreased myeloperoxidase activity of neutrophils [41]. CB2 receptor can influence the *Th1*/*Th2*-polarization of lymphocytes *in vitro*, which is an important step in differentiation of macrophage subpopulations. This is relevant for cardiac repair since macrophage subpopulations are involved in granulation tissue formation [20], remodeling and scar formation *via* modulation of fibroblasts and differentiation of myofibroblasts. The myofibroblasts are the major source of extracelullar matrix components and thereby play a crucial role in tissue

remodeling. In this context, CB2 receptor has been associated with regulation of myofibroblast differentiation in a murine liver fibrosis model [42].

Recent work from our group investigated the role of endocannabinoids and CB2 receptor in a mouse model of non-infarcted ischemic cardiomyopathy induced by brief repetitive I/R. Repetitive daily episode of 15 minute ischemia followed by reperfusion until the next day lead to a transient inflammatory reaction, development of interstitial fibrosis and left ventricular dysfunction [43]. We could show that fibrosis and dysfunction are reversible after 60 days of recovery after the last episode of I/R, where normal left ventricular myocardium is found. This is of clinical interest since: (a) repetitive episodes of ischemia are the hallmark of angina pectoris in patients and (b) these functional and morphological characteristics are also found in human hibernating myocardium with restoration of normal function after revascularization [8]. Mice with overexpression of SOD showed significantly less inflammation and fibrotic depositions associated with almost normal left ventricular function in this model and thereby revealed the importance of ROS in development of fibrosis and left ventricular dysfunction [43]. Another study in the same mouse model revealed a crucial role for the chemokine *CCL2* in development of interstitial fibrosis and left ventricular dysfunction [28]. It was therefore a logical next step to utilize CB2-deficient ($Cnr2^{-/-}$) mice in model of repetitive, brief I/R [44]. In an initial set of experiments, we found persistent induction of CB2 receptor in WT hearts upon repetitive I/R. Since there is no reliable CB2 antibody for histological detection in mice available we isolated cardiomyocytes using Langendorff apparatus and after their purification we could demonstrate induction of *Cnr2* mRNA selectively in cardiomyocytes. $Cnr2^{-/-}$ mice underwent the repetitive I/R protocol and presented with small infarcted areas—microinfarctions—indicating irreversible loss of cardiomyocytes already after three days I/R. The discontinuation of the I/R protocol led to no restoration of the left ventricular function in $Cnr2^{-/-}$ mice after 60 days, in contrast to full recovery in WT mice. WT hearts showed a transient increase in production of anandamide in parallel to the inflammatory reaction, whereas 2-arachidonoylglycerol (2-AG) level was elevated only after 7 days I/R. These data clearly showed not only the involvement of CB2 receptor and endocannabinoids in ischemic myocardium, but also provided a time course of their expression. The study revealed increased apoptosis and ROS production in $Cnr2^{-/-}$ hearts when compared to the WT mice. The investigation of mechanisms associated to the increased apoptosis in $Cnr2^{-/-}$ hearts revealed a CB2 receptor-associated regulation in expression of contractile elements and antioxidative enzymes (**Figure 2**). Analysis of inflammatory response revealed a CB2 receptor dependent induction of the cytokine *IL-1β* and the chemokines *CCL2*, *CCL3* and *CCL4* in this model. Interestingly, $Cnr2^{-/-}$ mice were able to induce the inflammatory response by a stronger induction of monocyte-colony stimulating factor (*M-CSF)* and *TNF-α* than the WT mice. This led to persistent macrophage infiltration of the ischemic myocardium in $Cnr2^{-/-}$ mice, while they were also unable to induce the anti-inflammatory cytokine *IL-10* and thereby resolve the inflammatory response. Magnetic sorting of macrophages using flow cytometry and their mRNA expression profile provided evidence for a delayed initiation of the anti-inflammatory M2a subpopulation of macrophages in $Cnr2^{-/-}$ mice. Additional experiments using reconstituted chimeric mice provided additional evidence for the pivotal role of macrophages in the irreversible loss of cardiomyocytes in $Cnr2^{-/-}$ mice.

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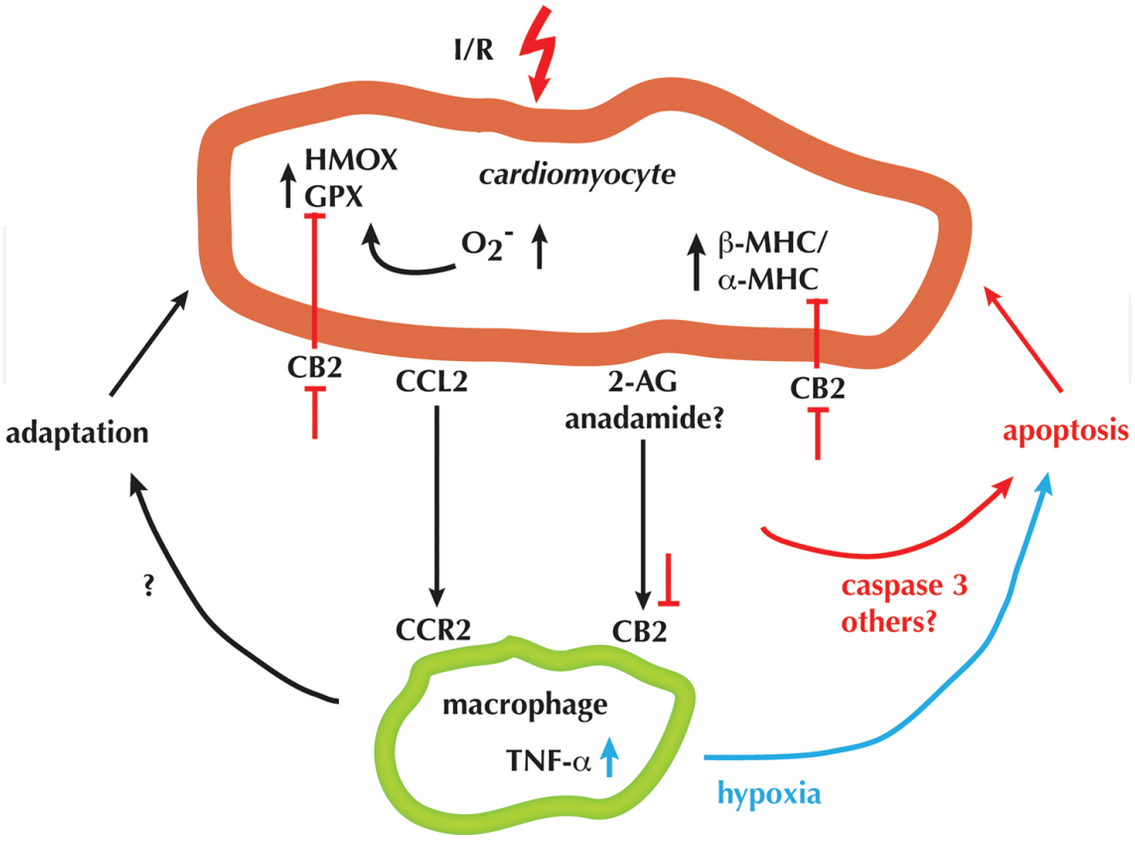

Figure 2. CB2 receptor-dependent mechanisms of cardioprotection in ischemic non-infarcted murine myocardium. I/R, ischemia and reperfusion; HMOX, heme oxygenase; GPX, glutathione peroxidase; MHC, myosin heavy chain; 2-AG, 2-arachidonoylglycerol; TNF-α, tumour necrosis factor α.

The consequences of prolonged inflammatory response were not limited to cardiomyocyte loss, but also involved adverse remodeling process in *Cnr2*$^{-/-}$ hearts. A morphological differentiation of collagen deposition revealed a comparable collagen area between the two genotypes, but significantly less interstitial fibrosis and concentration of collagen in microinfarctions in *Cnr2*$^{-/-}$ hearts [44]. At the molecular level this was associated with significantly less mRNA expression of *collagen III*, which is the reversible form of the deposited collagen isoforms. The significantly lower differentiation of myofibroblasts was associated with a low expression or early remodeling marker *tenascin C in vivo* and *in vitro*. Taken together, the survival of non-infarcted ischemic myocardium is dependent on a complex involvement of endocannabinoids and CB2 receptor in molecular and cellular mechanisms of cardioprotection.

Based on these findings we utilized *Cnr2*$^{-/-}$ mice in a model of reperfused myocardial infarction. One hour of ischemia was followed by reperfusion for different time periods up to seven days and led to a significantly worse left ventricular function in *Cnr2*$^{-/-}$ mice when compared to the WT mice [45]. Histological analysis showed expansion of the infarcted area as a transmural lesion in *Cnr2*$^{-/-}$ when compared to the non-transmural scar formation in WT mice. Myocardial infarction was associated with increased production of anandamide and 2-AG, but also of their associated lipids oleoyl ethanolamine and palmitoyl ethanolamide in both

genotypes. The molecular analysis showed a similar pattern as in non-infarcted repetitive I/R, with an impaired induction of antioxidative enzymes and unfavourable expression of contractile elements in *Cnr2*$^{-/-}$ mice. Molecular analysis revealed an *IL-1β*- and *TNF-α*-associated induction of inflammatory response with only low-level chemokine response after six hours of reperfusion in *Cnr2*$^{-/-}$ mice. In contrast WT mice showed a regular pattern with a significant increase in expression of *TNF-α* and chemokines *CCL2*, *CCL3* and *CCL4*. The significantly higher density of macrophages was associated with their prolonged action in infarction until seven days reperfusion and their completely transmural involvement in *Cnr2*$^{-/-}$ mice [45]. The analysis of myocardial remodeling revealed significantly less myofibroblasts and a lower induction of *tenascin C* in *Cnr2*$^{-/-}$ hearts. The most important finding was the lack of *thrombospondin 1* induction in *Cnr2*$^{-/-}$ hearts, which was responsible for the impaired formation of the infarction border zone and thereby failed limitation of the myocardial injury.

In summary, our *in vivo* studies showed substantial involvement of endocannabinoids and CB2 receptor in cardioprotective mechanisms and subsequent myocardial remodeling in the murine heart. While their clinical relevance still remains to be investigated, it is even more important to better understand the molecular mechanisms and the impact of cellular interactions mediated by this system.

4. Endocannabinoids in cellular mechanisms of myocardial adaptation

Several studies investigated the effects of cannabinoid receptors in regulation of cellular homeostasis and pathology, but their methodological differences and model-related problems do not allow to drawn clear and direct conclusions. A number of pharmacological studies investigated the impact of cannabinoid receptors on blood pressure *in vivo* and *ex vivo* [46] and some of these studies showed associated negative inotropic effects mediated by CB1 receptor. This was further supported by the evidence for CB1 receptor-mediated contractile dysfunction in experimental models of hepatic cirrhosis [47]. Still, none of these studies provided insights into specific cell actions of CB1 receptor. A study using 30 min left anterior descending artery (LAD) occlusion and 2.5 hours of reperfusion thereafter showed CB2 receptor effects by using non-specific agonist WIN55212-2 and reversal of its action with CB2 receptor antagonist AM630 [41]. Thereby, the authors described CB2 receptor-mediated effects on inflammatory response and myeloperoxidase activity indicating general leukocyte involvement. Another study utilized selective CB2 receptor agonist JWH-133 and showed cardioprotective effects based on activation of *ERK1/2* and the signal transducer and activator of transcription *(STAT)3*-mediated pathway [39]. The same study described also attenuated neutrophil recruitment towards inflammatory cytokine *TNF-α in vitro*.

Potential cardioprotective effects were described for cannabidiol based on the lower incidence of arrhythmia in rat hearts after ischemia and reperfusion, but the authors only speculated about involvement of cardiac current and channels [48]. The already above mentioned study suggested CB2 receptor-related cardioprotection after hydrogen peroxide treatment leading to increased apoptosis of cardiomyocytes and higher differentiation

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potential of myofibroblasts *in vitro* [40]. Our own work provided evidence for increased mRNA expression of *Cnr2* in purified cardiomyocytes after three days of brief repetitive I/R [44]. Cardiomyocytes were isolated using enzyme digestion in Langendorff apparatus and subsequent separation of them from fibroblasts was achieved using a short stay in cell culture where fibroblasts attach rapidly to the dish. We also used embryonic cardiomyocyte cell culture (having a large proportion of concomitant fibroblasts needed for survival) and were able to show a lower induction of antioxidative enzyme *heme oxygenase 1* and chemokine *CCL2* in *Cnr2*$^{-/-}$ cells under hypoxic conditions (2% O_2). In order to eliminate the confounding effects of fibroblasts, we utilized puromycin-purified embryonic stem cell-derived cardiomyocytes (97%-pure cardiomyocyte cell culture) [49]. This pure cardiomyocyte culture confirmed our findings on *heme oxygenase 1* and *CCL2*, and in addition provided evidence for hypoxia-dependent up regulation of CB2 receptor [44]. These data clearly showed specific CB2 receptor-related effects in cardiomyocytes.

Based on our data from cardiomyocytes *in vitro* and macrophage modulation *in vivo* we further investigated cellular interactions between cardiomyocytes and macrophages [50]. Initially we demonstrated the topical expression of CB2 receptor on WT cardiomyocytes and both WT macrophage subtypes M1 and M2 in cell culture, which increased under hypoxic conditions (2% O_2) and even more when proinflammatory cytokine interferon *(IFN)-γ* was added into the culture medium [50]. In order to exclude methodological problems of cell culture we quantified the number of vital WT cardiomyocytes in the culture after 12 and 24 hours cultivation under normoxia and hypoxia and found comparable cell numbers in both conditions, while the number was slightly lower after 24 hours indicating only minor loss due to apoptosis. Next, we compared apoptosis in WT vs. *Cnr2*$^{-/-}$ cardiomyocytes and found significantly higher amount of apoptotic cells among the *Cnr2*$^{-/-}$ cardiomyocytes. This raised the question whether the increased apoptosis alone is solely responsible for the loss of cardiomyocytes observed in our *in vivo* model, and we therefore investigated the function of macrophages in the next step. We stimulated the macrophages with *IFN-γ* in order to stimulate the differentiation into M1 subtype [50]. In order to measure the migration potential of this subtype we used either supernatant from the cardiomyocytes cell culture after 24 hours hypoxia or potent chemoattractants *CCL2* and *M-CSF* in a Boyden chamber, which are both strongly induced after myocardial infarction in mice [51]. We found a significantly stronger migration potential of *Cnr2*$^{-/-}$ M1 macrophages towards the supernatant of hypoxic cardiomyocytes than in WT M1 macrophages. This finding indicated a more aggressive nature of *Cnr2*$^{-/-}$ M1 macrophages and we subsequently utilized them in co-culture with cardiomyocytes. The co-culture experiments revealed significantly higher loss of embryonic cardiomyocytes and their apoptosis when combined with *Cnr2*$^{-/-}$ than with WT M1 macrophages. In addition, we found that production of *TNF-α* in M1 macrophages was dependent on stimulation of CB2 receptor by anandamide [50]. In summary, we were able to identify at least some of the mechanisms behind the aggressive nature of macrophages in *Cnr2*$^{-/-}$ mice and their interaction with cardiomyocytes under conditions, which are comparable to the *in vivo* situation. Still, it remains to be elucidated in future studies which molecular pathways are involved in this cellular interaction and expand it towards other cells in the heart.

5. Clinical perspective for endocannabinoids in myocardial adaptation

A number of clinical studies described the involvement of endocannabinoids in human cardiac conditions. One study described an increased level of endocannabinoids in the blood stream and higher expression of CB2 receptor in the heart of patients with terminal heart failure [52]. Another study from the same group described significant reduction of plasma anandamide concentration after induction of general anaesthesia using isoflurane [53]. In the same patient population they reported a significant increase in 2-AG after onset of cardiopulmonary bypass during heart surgery, but remained only speculative on the clinical relevance of these findings by suggesting association with inflammatory response. A recent study from our group showed activation of the endocannabinoid system and up regulation of its receptors in myocardial hypertrophy in patients with aortic valve stenosis [54]. We were able to identify expression of CB2 receptor predominantly on cardiomyocytes, but also on myofibroblasts and mononuclear cells in hypertrophic myocardium. The same study revealed a persistent low-grade inflammation and active remodeling in hypertrophied hearts and this shows parallels to our experimental findings discussed above.

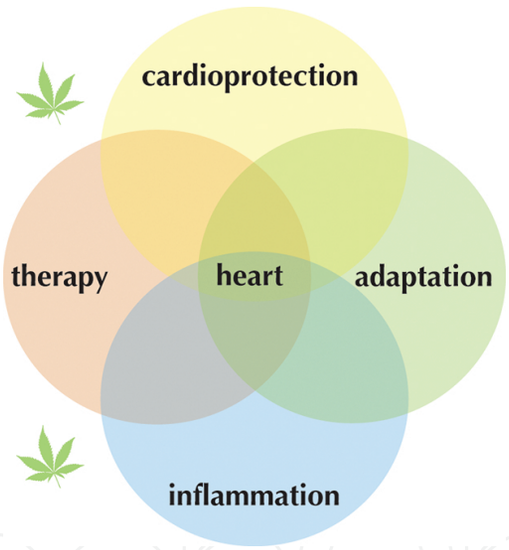

Figure 3. Complex relations in a clinical scenario targeting endocannabinoid system.

The endocannabinoid system gained clinical relevance in the last few years because of a CB1-receptor antagonist based therapy (rimonabant) being approved for clinical use in severely obese patients, but then disappeared rather early due to unwanted and detrimental side effects [55]. Still, one study investigated the effect of rimonabant on progression of atherosclerosis in patients with abdominal obesity and coronary artery disease (STRADIVARIUS randomized controlled trial). The results were disappointing for the primary endpoint, since no effect could be identified on the disease progression [56]. Still, the secondary endpoint of normalized total atheroma volume was met and this could be the basis for future studies. In the light of our results on the role of the chemokine *CCL2* and the CB2 receptor in myocardial remodeling and adaptation to injury [27, 43–45], it has to be emphasised, that we need to

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expand our knowledge of cellular interactions and mechanisms in other disease models. The complexity of this system and its interaction are shown in **Figure 3**. The next step will be the investigation of highly specific compounds acting on cannabinoid receptors. Nevertheless, the modulation of inflammatory response remains to be a potential therapeutical target in cardioprotection.

6. Conclusions

Growing amount of evidence supports the role of the endocannabinoid system and cannabinoid receptors in cardioprotection and myocardial adaptation. Several mechanisms have been described in specific cells *in vitro* and some of these show parallels with the *in vivo* data. The data on CB2 receptor-mediated adaptation of injured myocardium show a spatiotemporal resolution of its actions on different cells in the heart and shed more light into the finely balanced system of cardioprotection. Therefore, an even better mechanistic understanding of the cannabinoid system and its action on the cardiovascular system in the healthy and the diseased state are needed than the present one we have. This will eventually allow the identification of promising new pathways and/or targets for the treatment of cardiovascular diseases.

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