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
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 \textit{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).

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

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

**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.
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
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\textsuperscript{-/-} cells under hypoxic conditions (2% O\textsubscript{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\textsubscript{2}) and even more when proinflammatory cytokine interferon (IFN)-\textgamma 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\textsuperscript{-/-} cardiomyocytes and found significantly higher amount of apoptotic cells among the Cnr2\textsuperscript{-/-} 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-\textgamma 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\textsuperscript{-/-} M1 macrophages towards the supernatant of hypoxic cardiomyocytes than in WT M1 macrophages. This finding indicated a more aggressive nature of Cnr2\textsuperscript{-/-} 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\textsuperscript{-/-} than with WT M1 macrophages. In addition, we found that production of TNF-\textalpha 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\textsuperscript{-/-} 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
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.

Acknowledgements

OD was supported by DFG FOR926 grants DE802/2-1 and 2-2. GDD was supported by a BONFOR grant from the Medical School, University of Bonn, Germany.

Author details

Oliver Dewald* and Georg D. Duerr

*Address all correspondence to: o.dewald@uni-bonn.de

Department of Cardiac Surgery, University Clinical Centre Bonn, Germany

References

[1] Zoratti C, Kipmen-Korgun D, Osibow K, Malli R, Graier WF: Anandamide initiates Ca(2+) signaling via CB2 receptor linked to phospholipase C in calf pulmonary endothelial cells. British Journal of Pharmacology. 2003; 140:1351-1362. DOI:10.1038/sj.bjp.0705529
[2] Bonz A, Laser M, Kullmer S, Kniesch S, Babin-Ebell J, Popp V, Ertl G, Wagner JA: Cannabinoids acting on CB1 receptors decrease contractile performance in human atrial muscle. Journal of Cardiovascular Pharmacology. 2003; 41:657-664.

[3] Deutsch DG, Goligorsky MS, Schmid PC, Krebsbach RJ, Schmid HH, Das SK, Dey SK, Arreaza G, Thorup C, Stefano G, Moore LC: Production and physiological actions of anandamide in the vasculature of the rat kidney. The Journal of Clinical Investigation. 1997; 100:1538-1546. DOI:10.1172/JCI119677

[4] Frangogiannis NG: The inflammatory response in myocardial injury, repair, and remodelling. Nature reviews. Cardiology. 2014; 11:255-265. DOI:10.1038/nrcardio.2014.28

[5] Ginks WR, Sybers HD, Maroko PR, Covell JW, Sobel BE, Ross J, Jr.: Coronary artery reperfusion. II. Reduction of myocardial infarct size at 1 week after the coronary occlusion. The Journal of Clinical Investigation. 1972; 51:2717-2723. DOI:10.1172/JCI107091

[6] Maroko PR, Libby P, Ginks WR, Bloor CM, Shell WE, Sobel BE, Ross J, Jr.: Coronary artery reperfusion. I. Early effects on local myocardial function and the extent of myocardial necrosis. The Journal of Clinical Investigation. 1972; 51:2710-2716. DOI: 10.1172/JCI107090

[7] Murry CE, Jennings RB, Reimer KA: Preconditioning with ischemia: a delay of lethal cell injury in ischemic myocardium. Circulation. 1986; 74:1124-1136.

[8] Heusch G: Molecular basis of cardioprotection: signal transduction in ischemic pre-, post-, and remote conditioning. Circulation Research. 2015; 116:674-699. DOI:10.1161/CIRCRESAHA.116.305348

[9] Przyklenk K, Bauer B, Ovize M, Kloner RA, Whittaker P: Regional ischemic 'preconditioning' protects remote virgin myocardium from subsequent sustained coronary occlusion. Circulation. 1993; 87:893-899.

[10] Whittaker P, Przyklenk K: Reduction of infarct size in vivo with ischemic preconditioning: mathematical evidence for protection via non-ischemic tissue. Basic Research in Cardiology. 1994; 89:6-15.

[11] Thielmann M, Kottenberg E, Kleinbongard P, Wendt D, Gedik N, Pasa S, Price V, Tsagakis K, Neuhauser M, Peters J, Jakob H, Heusch G: Cardioprotective and prognostic effects of remote ischaemic preconditioning in patients undergoing coronary artery bypass surgery: a single-centre randomised, double-blind, controlled trial. Lancet. 2013; 382:597-604. DOI:10.1016/S0140-6736(13)61450-6

[12] Joyeux M, Arnaud C, Godin-Ribuot D, Demenge P, Lamontagne D, Ribuot C: Endocannabinoids are implicated in the infarct size-reducing effect conferred by heat stress preconditioning in isolated rat hearts. Cardiovascular Research. 2002; 55:619-625.
[13] Roberts R, DeMello V, Sobel BE: Deleterious effects of methylprednisolone in patients with myocardial infarction. Circulation. 1976; 53:1204-206.

[14] Frangogiannis NG, Smith CW, Entman ML: The inflammatory response in myocardial infarction. Cardiovascular Research. 2002; 53:31-47.

[15] Frangogiannis NG, Shimoni S, Chang SM, Ren G, Dewald O, Gersch C, Shan K, Aggeli C, Reardon M, Letsou GV, Espada R, Ramchandani M, Entman ML, Zoghbi WA: Active interstitial remodeling: an important process in the hibernating human myocardium. Journal of the American College of Cardiology. 2002; 39:1468-1474.

[16] Kukielka GL, Smith CW, LaRosa GJ, Manning AM, Mendoza LH, Daly TJ, Hughes BJ, Youker KA, Hawkins HK, Michael LH, et al.: Interleukin-8 gene induction in the myocardium after ischemia and reperfusion in vivo. The Journal of Clinical Investigation. 1995; 95:89-103. DOI:10.1172/JCI117680

[17] Entman ML, Youker K, Shoji T, Kukielka G, Shappell SB, Taylor AA, Smith CW: Neutrophil induced oxidative injury of cardiac myocytes. A compartmented system requiring CD11b/CD18-ICAM-1 adherence. The Journal of Clinical Investigation. 1992; 90:1335-1345. DOI:10.1172/JCI115999

[18] Kloner RA, Bolli R, Marban E, Reinlib L, Braunwald E: Medical and cellular implications of stunning, hibernation, and preconditioning: an NHLBI workshop. Circulation. 1998; 97:1848-1867.

[19] Kumar AG, Ballantyne CM, Michael LH, Kukielka GL, Youker KA, Lindsey ML, Hawkins HK, Birdsall HH, MacKay CR, LaRosa GJ, Rossen RD, Smith CW, Entman ML: Induction of monocyte chemoattractant protein-1 in the small veins of the ischemic and reperfused canine myocardium. Circulation. 1997; 95:693-700.

[20] Nahrendorf M, Swirski FK, Aikawa E, Stangenberg L, Wurdinger T, Figueiredo JL, Libby P, Weissleder R, Pittet MJ: The healing myocardium sequentially mobilizes two monocyte subsets with divergent and complementary functions. The Journal of Experimental Medicine. 2007; 204:3037-3047. DOI:10.1084/jem.20070885

[21] de Couto G, Liu W, Tseliou E, Sun B, Makkar N, Kanazawa H, Arditi M, Marban E: Macrophages mediate cardioprotective cellular postconditioning in acute myocardial infarction. The Journal of Clinical Investigation. 2015; 125:3147-3162. DOI:10.1172/JCI81321

[22] Lacraz S, Nicod LP, Chicheportiche R, Welgus HG, Dayer JM: IL-10 inhibits metalloproteinase and stimulates TIMP-1 production in human mononuclear phagocytes. The Journal of Clinical Investigation. 1995; 96:2304-2310. DOI:10.1172/JCI18286

[23] Gerszten RE, Garcia-Zepeda EA, Lim YC, Yoshida M, Ding HA, Gimbrone MA, Jr., Luster AD, Lusciniskas FW, Rosenzweig A: MCP-1 and IL-8 trigger firm adhesion of monocytes to vascular endothelium under flow conditions. Nature. 1999; 398:718-723. DOI:10.1038/19546
[24] Weber C, Draude G, Weber KS, Wubert J, Lorenz RL, Weber PC: Downregulation by tumor necrosis factor-alpha of monocyte CCR2 expression and monocyte chemotactic protein-1-induced transendothelial migration is antagonized by oxidized low-density lipoprotein: a potential mechanism of monocyte retention in atherosclerotic lesions. Atherosclerosis. 1999; 145:115-123.

[25] Weber C, Erl W, Weber KS, Weber PC: Effects of oxidized low density lipoprotein, lipid mediators and statins on vascular cell interactions. Clinical Chemistry and Laboratory Medicine. 1999; 37:243-251. DOI:10.1515/CCLM.1999.043

[26] Frangogiannis NG, Youker KA, Rossen RD, Gwechenberger M, Lindsey MH, Mendoza LH, Michael LH, Ballantyne CM, Smith CW, Entman ML: Cytokines and the microcirculation in ischemia and reperfusion. Journal of Molecular and Cellular Cardiology. 1998; 30:2567-2576. DOI:10.1006/jmcc.1998.0829

[27] Dewald O, Zymek P, Winkelmann K, Koerting A, Ren G, Abou-Khamis T, Michael LH, Rollins BJ, Entman ML, Frangogiannis NG: CCL2/Monocyte Chemoattractant Protein-1 regulates inflammatory responses critical to healing myocardial infarcts. Circulation Research. 2005; 96:881-889. DOI:10.1161/01.RES.0000163017.13772.3a

[28] Frangogiannis NG, Dewald O, Xia Y, Ren G, Haudek S, Leucker T, Kraemer D, Taffet G, Rollins BJ, Entman ML: Critical role of monocyte chemoattractant protein-1/CC chemokine ligand 2 in the pathogenesis of ischemic cardiomyopathy. Circulation. 2007; 115:584-592. DOI:10.1161/CIRCULATIONAHA.106.646091

[29] Ford WR, Honan SA, White R, Hiley CR: Evidence of a novel site mediating anandamide-induced negative inotropic and coronary vasodilator responses in rat isolated hearts. British Journal of Pharmacology. 2002; 135:1191-1198. DOI:10.1038/sj.bjp.0704565

[30] Lake KD, Compton DR, Varga K, Martin BR, Kunos G: Cannabinoid-induced hypotension and bradycardia in rats mediated by CB1-like cannabinoid receptors. The Journal of Pharmacology and Experimental Therapeutics. 1997; 281:1030-1037.

[31] Jarai Z, Wagner JA, Varga K, Lake KD, Compton DR, Martin BR, Zimmer AM, Bonner TI, Buckley NE, Mezey E, Razdan RK, Zimmer A, Kunos G: Cannabinoid-induced mesenteric vasodilation through an endothelial site distinct from CB1 or CB2 receptors. Proceedings of the National Academy of Sciences of the United States of America. 1999; 96:14136-14141.

[32] Ishac EJ, Jiang L, Lake KD, Varga K, Abood ME, Kunos G: Inhibition of exocytotic noradrenaline release by presynaptic cannabinoid CB1 receptors on peripheral sympathetic nerves. British Journal of Pharmacology. 1996; 118:2023-2028.

[33] Pacher P, Batkai S, Kunos G: Haemodynamic profile and responsiveness to anandamide of TRPV1 receptor knock-out mice. The Journal of Physiology. 2004; 558:647-657. DOI:10.1113/jphysiol.2004.064824
[34] Steffens S, Veillard NR, Arnaud C, Pelli G, Burger F, Staub C, Karsak M, Zimmer A, Frossard JL, Mach F: Low dose oral cannabinoid therapy reduces progression of atherosclerosis in mice. Nature. 2005; 434:782-786. DOI:10.1038/nature03389

[35] Mittleman MA, Lewis RA, Maclure M, Sherwood JB, Muller JE: Triggering myocardial infarction by marijuana. Circulation. 2001; 103:2805-2809.

[36] Wagner JA, Hu K, Bauersachs J, Karcher J, Wiesler M, Goparaju SK, Kunos G, Ertl G: Endogenous cannabinoids mediate hypotension after experimental myocardial infarction. Journal of the American College of Cardiology. 2001; 38:2048-2054.

[37] Underdown NJ, Hiley CR, Ford WR: Anandamide reduces infarct size in rat isolated hearts subjected to ischaemia-reperfusion by a novel cannabinoid mechanism. British Journal of Pharmacology. 2005; 146:809-816. DOI:10.1038/sj.bjp.0706391

[38] Munro S, Thomas KL, Abu-Shaar M: Molecular characterization of a peripheral receptor for cannabinoids. Nature. 1993; 365:61-65. DOI:10.1038/365061a0

[39] Montecucco F, Lenglet S, Braunersreuther V, Burger F, Pelli G, Bertolotto M, Mach F, Steffens S: CB(2) cannabinoid receptor activation is cardioprotective in a mouse model of ischemia/reperfusion. Journal of Molecular and Cellular Cardiology. 2009; 46:612-620. DOI:10.1016/j.yjmcc.2008.12.014

[40] Defer N, Wan J, Souktani R, Escoubet B, Perier M, Caramelle P, Manin S, Deveaux V, Bourin MC, Zimmer A, Lotersztajn S, Pecker F, Pavoine C: The cannabinoid receptor type 2 promotes cardiac myocyte and fibroblast survival and protects against ischemia/reperfusion-induced cardiomyopathy. FASEB Journal: Official Publication of the Federation of American Societies for Experimental Biology. 2009; 23:2120-2130. DOI: 10.1096/fj.09-129478

[41] Di Filippo C, Rossi F, Rossi S, D'Amico M: Cannabinoid CB2 receptor activation reduces mouse myocardial ischemia-reperfusion injury: involvement of cytokine/chemokines and PMN. Journal of Leukocyte Biology. 2004; 75:453-459. DOI:10.1189/jlb.0703303

[42] Julien B, Grenard P, Teixeira-Clerc F, Van Nhieu JT, Li L, Karsak M, Zimmer A, Mallat A, Lotersztajn S: Antifibrogenic role of the cannabinoid receptor CB2 in the liver. Gastroenterology. 2005; 128:742-755.

[43] Dewald O, Frangogiannis NG, Zoerlein M, Duerr GD, Klemm C, Knuefermann P, Taffet G, Michael LH, Crapo JD, Welz A, Entman ML: Development of murine ischemic cardiomyopathy is associated with a transient inflammatory reaction and depends on reactive oxygen species. Proceedings of the National Academy of Sciences of the United States of America. 2003; 100:2700-2705. DOI:10.1073/pnas.0438035100

[44] Duerr GD, Heinemann JC, Suchan G, Kolobar E, Wenzel D, Geisen C, Matthey M, Passe-Tietjen K, Mahmud W, Ghanem A, Tiemann K, Alferink J, Burgdorf S, Buchalla R, Zimmer A, Lutz B, Welz A, Fleischmann BK, Dewald O: The endocannabinoid-
CB2 receptor axis protects the ischemic heart at the early stage of cardiomyopathy. Basic Research in Cardiology. 2014; 109:425. DOI:10.1007/s00395-014-0425-x

[45] Duerr GD, Heinemann JC, Gestrich C, Heuft T, Klaas T, Keppel K, Roell W, Klein A, Zimmer A, Velten M, Kilic A, Bindila L, Lutz B, Dewald O: Impaired border zone formation and adverse remodeling after reperfused myocardial infarction in cannabinoid CB2 receptor deficient mice. Life Sciences. 2015; 138:8-17. DOI:10.1016/j.lfs.2014.11.005

[46] Batkai S, Pacher P: Endocannabinoids and cardiac contractile function: pathophysiological implications. Pharmacological Research. 2009; 60:99-106.

[47] Batkai S, Mukhopadhyay P, Harvey-White J, Kechrid R, Pacher P, Kunos G: Endocannabinoids acting at CB1 receptors mediate the cardiac contractile dysfunction in vivo in cirrhotic rats. American journal of physiology. Heart and Circulatory Physiology. 2007; 293:H1689-1695. DOI:10.1152/ajpheart.00538.2007

[48] Walsh SK, Hepburn CY, Kane KA, Wainwright CL: Acute administration of cannabidiol in vivo suppresses ischaemia-induced cardiac arrhythmias and reduces infarct size when given at reperfusion. British Journal of Pharmacology. 2010; 160:1234-1242. DOI:10.1111/j.1476-5381.2010.00755.x

[49] Kolossov E, Bostani T, Roell W, Breitbach M, Pillekamp F, Nygren JM, Sasse P, Rubenchik O, Fries JW, Wenzel D, Geisen C, Xia Y, Lu Z, Duan Y, Kettenhofen R, Jovinge S, Bloch W, Bohlen H, Welz A, Hescheler J, Jacobsen SE, Fleischmann BK: Engraftment of engineered ES cell-derived cardiomyocytes but not BM cells restores contractile function to the infarcted myocardium. The Journal of Experimental Medicine. 2006; 203:2315-2327. DOI:10.1084/jem.20061469

[50] Heinemann JC, Duerr GD, Keppel K, Breitbach M, Fleischmann BK, Zimmer A, Wehner S, Welz A, Dewald O: CB2 receptor-mediated effects of pro-inflammatory macrophages influence survival of cardiomyocytes. Life Sciences. 2015; 138:18-28. DOI:10.1016/j.lfs.2014.11.027

[51] Dewald O, Ren G, Duerr GD, Zoerlein M, Klemm C, Gersch C, Tincey S, Michael LH, Entman ML, Frangogiannis NG: Of mice and dogs: species-specific differences in the inflammatory response following myocardial infarction. The American Journal of Pathology. 2004; 164:665-677. DOI:10.1016/S0002-9440(10)63154-9

[52] Weis F, Beiras-Fernandez A, Sodian R, Kaczmarek I, Reichart B, Beiras A, Schelling G, Kreth S: Substantially altered expression pattern of cannabinoid receptor 2 and activated endocannabinoid system in patients with severe heart failure. Journal of Molecular and Cellular Cardiology. 2010; 48:1187-1193. DOI:10.1016/j.yjmcc.2009.10.025

[53] Weis F, Beiras-Fernandez A, Hauer D, Hornuss C, Sodian R, Kreth S, Briegel J, Schelling G: Effect of anaesthesia and cardiopulmonary bypass on blood endocannabinoid
concentrations during cardiac surgery. British Journal of Anaesthesia. 2010; 105:139-144. DOI:10.1093/bja/aeq117

[54] Duerr GD, Heinemann JC, Dunkel S, Zimmer A, Lutz B, Lerner R, Roell W, Mellert F, Probst C, Esmailzadeh B, Welz A, Dewald O: Myocardial hypertrophy is associated with inflammation and activation of endocannabinoid system in patients with aortic valve stenosis. Life Sciences. 2013; 92:976-983. DOI:10.1016/j.lfs.2013.03.014

[55] Topol EJ, Bousser MG, Fox KA, Creager MA, Despres JP, Easton JD, Hamm CW, Montalescot G, Steg PG, Pearson TA, Cohen E, Gaudin C, Job B, Murphy JH, Bhatt DL, Investigators C: Rimonabant for prevention of cardiovascular events (CRESCENDO): a randomised, multicentre, placebo-controlled trial. Lancet. 2010; 376:517-523. DOI: 10.1016/S0140-6736(10)60935-X

[56] Nissen SE, Nicholls SJ, Wolski K, Rodes-Cabau J, Cannon CP, Deanfield JE, Despres JP, Kastelein JJ, Steinhubl SR, Kapadia S, Yasin M, Ruzyillo W, Gaudin C, Job B, Hu B, Bhatt DL, Lincoff AM, Tuzcu EM, Investigators S: Effect of rimonabant on progression of atherosclerosis in patients with abdominal obesity and coronary artery disease: the STRADIVARIUS randomized controlled trial. JAMA. 2008; 299:1547-1560. DOI: 10.1001/jama.299.13.1547
