INVESTIGATION OF NOVEL LIPID-DEPENDENT CARDIOVASCULAR PROCESSES

PhD thesis book

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I. Introduction

Besides their well-known functions as sources of stored energy or components of cell membranes, several groups of lipid molecules function as signalling agents and mediate various (patho)physiological processes. Two representative groups of the most significant lipid mediators are endocannabinoids and sphingolipids.

The investigation of the endocannabinoid system began with the discovery of *Cannabis Sativa.* Its main active ingredient the delta-9-tetrahydrocannabinol (THC) has been attributed psychotropic properties. The research of THC binding sites led to the discovery of two types of cannabinoid receptors (CB_1R and CB_2R), both coupling through $G\alpha_{i/o}$ proteins. CB_1Rs exist primarily on central and peripheral neurons and one of their main functions is to inhibit neurotransmitter release. They are located peripherally as well regulating basic physiological processes. CB_2Rs are expressed in immune and haematopoietic cells and modulate a broad spectrum of immune effects. 2-arachydonoylglycerol (2-AG) is an endogenous ligand of these receptors. It is synthetized “on demand” near its site of action in response to increased intracellular calcium.

Cardiovascular effects of endocannabinoids are complex and may involve central and peripheral compounds. They elicit bradycardia and a triphasic blood pressure response in *in vivo* experiments. Cannabinoids produce vasodilation in various vascular beds in which both extracellular (CB_1R, CB_2R, yet unidentified CBRs, vanilloid receptors) and intracellular (NO release, arachidonic metabolites, EDHF release) mechanisms can be involved. Endocannabinoids and CB_1Rs are present in cardiomyocytes and they play a notable role in the modulation of cardiac functions. Endocannabinoids seem to have cardiodepressant and vasodilator activities in the heart and these are mediated mainly by CB_1Rs. It has been reported that angiotensin II (Ang II), a strong vasoconstrictive agent with positive inotropic effects, induces the release of 2-AG via activation of AT_1Rs.

Sphingosine-1-phosphate (S1P) is another lipid mediator of great importance and it is present in all types of eukaryotic tissues. S1P concentration in blood plasma is relatively high (200-1000 nM) and it can be found in its free form or bound to its carriers (albumin, HDL). Carrier binding can fundamentally determine its effect *in vivo*. Plasma S1P is derived mainly from erythrocytes and endothelial cells,

however, large amounts of S1P are stored in platelets and released upon activation. During acute coronary syndrome (ACS), when the rupture of an atherosclerotic plaque leads to platelet activation, the normally small free S1P fraction is multiplied. This may contribute to pathological changes in coronary circulation and cardiac function.

S1P creates its diversified effects through its specific G protein-coupled receptors ($S1P_{1\text{-}5}R$). Whereas $S1P_{4,5}Rs$ are found primarily in the lymphatic and nervous systems, $S1P_{1\text{-}3}Rs$ are commonly found throughout the body. They are expressed in the vascular endothelium, vascular smooth muscle cells, and cardiomyocytes. Through $S1P_{1\text{-}3}Rs$ S1P mediates its cardiovascular effects. S1P's vascular tone regulating effect is controversial: both vasoconstrictive and endothelium-dependent vasodilator effects have been attributed to S1P. Furthermore, S1P enhances myocardial survival under hypoxic conditions, is involved in the process of ischemic pre- and post-conditioning, and reduces the rate of ischaemia/reperfusion (I/R) injury. Interestingly, $S1P_3Rs$ seem to be involved both in vasoconstrictive and cardioprotective – two opposing – effects of S1P.

II. Objectives

Cardiac AT_1R activation has utmost relevance in heart (patho)physiology, however, it is yet unknown whether concomitant 2-AG production and CB_1R activation modulate these effects. The main aim of our "endocannabinoid study" was to reveal the participation of paracrine endocannabinoid mechanisms in Ang II signalling in the heart. For this purpose, we investigated how inhibition of 2-AG formation and CB_1R activation modify the effects of Ang II administration in isolated Langendorff-perfused rat hearts.

The cardiac effects of S1P reported in I/R injury are controversial. Activation of S1PRs seems to be cardioprotective, whereas the acute effects of S1P to reduce coronary flow (CF) and cardiac contractility are expected to interfere with successful post-ischemic recovery. In ACS, when S1P is released in large amounts from activated platelets, its favourable and potentially deleterious effects might clash with one another. In the present study, we aimed to delineate how these opposing S1P actions actually affect postischemic cardiac injury after a non-fatal ischemic insult.

III. Methods

Isolated adult male Sprague-Dawley rat and WT, $S1P_2$-KO and $S1P_3$-KO C57BL/6 murine hearts were perfused at constant pressure in the Langendorff system with modified Krebs-Hensleit buffer. After cannulation and stabilization of the heart, baseline data were recorded and the applied pharmacons (2-AG (10^{-6} M), WIN55,212-2 (10^{-6} M, synthetic CB agonist), Ang II (10^{-7} M), O2050 (10^{-6} M, CB_1R inhibitor), Orlistat (10^{-6} M, DAGL inhibitor)), S1P (10^{-6} M) or their vehicles were infused to the perfusion line. In the I/R-injury protocol, the 5-min S1P infusion was followed by a 20-min global ischemia Then, perfusion was restarted and reperfusion was maintained for 2 h. CF and left ventricular pressure changes were continuously monitored during the entire experiments. Results are expressed as mean ± SEM.

IV. Results

Effects of CB receptor agonists and antagonist on CF and contractile function

To better understand the effects of cannabinoids on isolated Langendorff-perfused rat hearts we examined the effects of CB receptor endogenous and exogenous agonists and antagonist on CF and contractile function. Both CB receptor agonists enhanced CF. 2-AG administration had no significant effect on contractile performance; however, WIN55,212-2 produced a marked decline in left ventricular developed pressure (LVDevP). 2-AG was also applied in the presence of CB_1R neutral antagonist O2050. The CF-increasing effect of 2-AG was prevented by O2050.

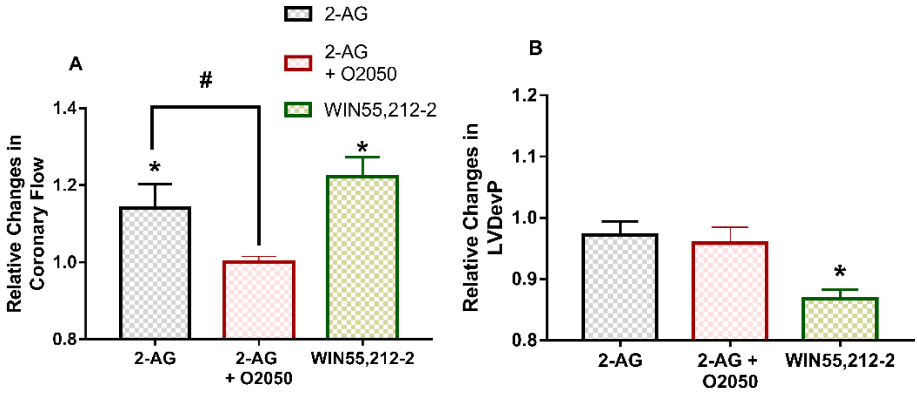


Figure 1. Peak effects of 2-arachydonoylglycerol (2-AG) and synthetic cannabinoid receptor agonist WIN55,212-2 on coronary flow and left ventricular developed pressure (LVDevP) of isolated rat hearts. Data are presented as relative values compared to pre-infusion control data. n = 9 (2-AG and 2-AG + O2050), n = 5 (WIN55,212-2); * $p < 0.05$ vs. pre-infusion value; # $p < 0.05$ vs. 2-AG peak effect, paired t-test.

The influence of CB_1R antagonist O2050 and DAGL inhibitor Orlistat on Ang II effects

To test how 2-AG-release during AT_1R—$G\alpha_{q/11}$ signalling may modify the primary Ang II responses in the heart via potential transactivation of CB_1Rs, Ang II was also infused in the presence of CB_1R antagonist O2050 and DAGL inhibitor Orlistat. The presence of O2050 in the perfusate substantially altered the effects of Ang II. The deep decline in CF was attenuated by O2050. In addition, the temporary decrease in inotropic and lusitropic function was completely abolished when CB_1Rs were blocked by O2050.

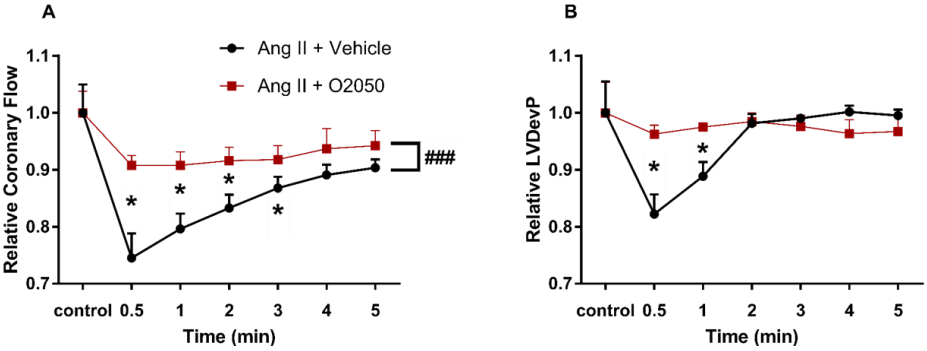


Figure 2. Influence of O2050 on the effects of angiotensin II (Ang II) on coronary flow and left ventricular developed pressure (LVDevP) of isolated rat hearts. The presented data are expressed as relative values compared to pre-infusion control data. n = 7 (Ang II + vehicle), n = 8 (Ang II + O2050); * $p < 0.05$ vs. control; ### $p < 0.001$ vs. vehicle; two-way repeated measurement ANOVA and Dunnett's post-hoc test.

Inhibition of DAGL, the enzyme which is supposed to produce 2-AG from diacylglycerol during $G\alpha_{q/11}$-coupled signalling of Ang II, moderated the Ang II-induced peak reduction in CF and the significant decrease in LVDevP was not observable.

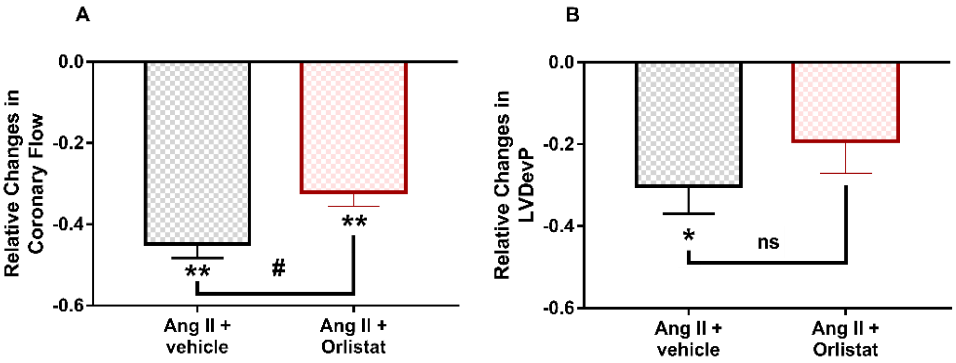


Figure 3. Influence of diacylglycerol lipase inhibitor Orlistat on the peak effects of angiotensin II (Ang II) on coronary flow and left ventricular developed pressure (LVDevP) of isolated rat hearts. The presented data are expressed as relative values compared to pre-infusion control data. n = 4 (Ang II + vehicle), n = 6 (Ang II + Orlistat); * $p < 0.05$ and ** $p < 0.01$ vs. control (pre-infusion value) paired t-test; # $p < 0.05$ vs. Ang II +vehicle; t-test. ns: non-significant.

Dose-dependent effects of intravascular S1P on CF administered with or without S1P-chaperon albumin

To characterize the effect of intravascular S1P on CF, we carried out concentration-response experiments in isolated

murine hearts either with and without its important chaperone, albumin. When administered without a carrier, S1P elicited a concentration-dependent CF reduction in isolated hearts with an ED50 value of 1.17×10^{-6} M. Therefore, the S1P in further experiments was applied at 1 microM - a dose close to its ED50 value. The coronary effect of S1P was similar in the presence of albumin, however the ED50 value slightly shifted to a smaller concentration range (1.85×10^{-7} M) though it was not statistically significant.

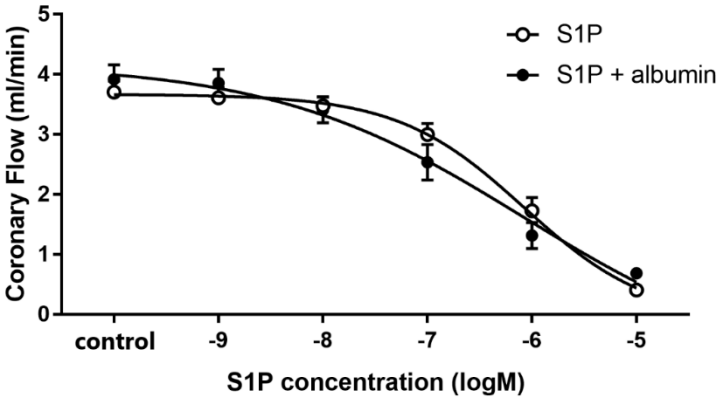


Figure 4. Dose-dependent effects of sphingosine-1-phosphate (S1P) on coronary flow of isolated murine hearts infused alone or in the presence of S1P-carrier albumin. n=9 (S1P), n = 8 (S1P + albumin). Non-linear regression analysis and comparison of Fits.

Effects of intravascular S1P exposition on CF and heart function in WT mice

Administration of S1P reduced CF by 44 ± 3%. This remarkable decrease started at the beginning of the S1P infusion and continued progressively during the 5 min. During the 20-min wash-out period, CF did not return to the baseline level. CF reduction induced by S1P coexisted with compromised left ventricular contractile performance, which is indicated by a 54 ± 9% drop in LVDevP.

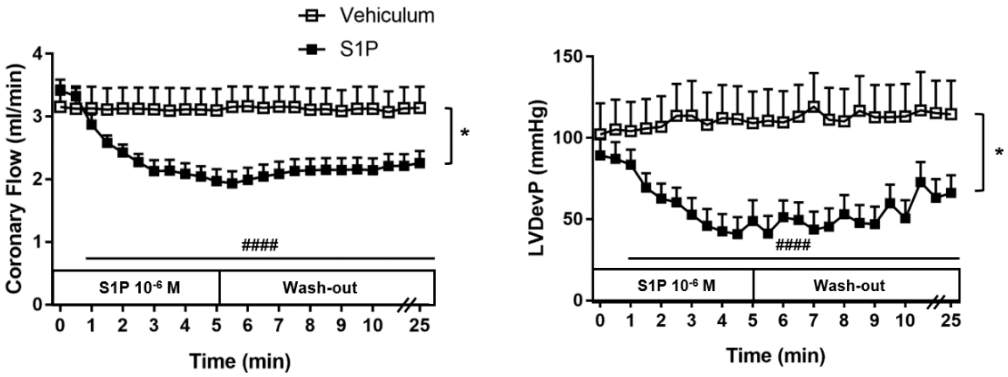


Figure 5. Effects of sphingosine-1-phosphate (S1P) on coronary flow (A) and left ventricular developed pressure (LVDevP) (B) of isolated mouse hearts. n=6 (vehicle), n = 9 (S1P); ####$p < 0.0001$ vs. baseline (pre-infusion value), *$p < 0.05$ vs. vehicle; two-way repeated measurement ANOVA and Dunnett's post hoc test.

Effects of intravascular S1P exposition on CF and heart function in WT mice

The CF-reducing effect of S1P developing in $S1P_2$-KO mice was similar to that of WT littermates. The drop of the LVDevP was also similar in the two groups, with no statistically significant difference.

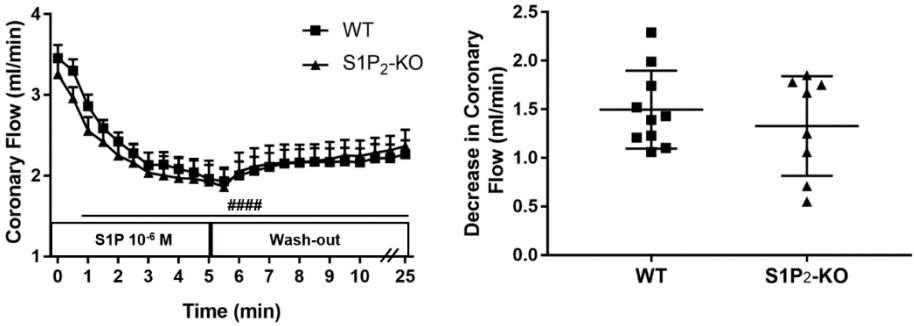


Figure 6. Effects of sphingosine-1-phosphate on coronary flow of hearts isolated from WT and $S1P_2$R-KO mice. n=10 (WT), n = 8 ($S1P_2$R-KO); #### $p < 0.0001$ vs. baseline in both groups, two-way repeated measurement ANOVA followed by Dunnett's post hoc test.

In $S1P_3$-KO hearts, the CF-reducing effect of S1P was markedly diminished compared to WT mice. There was a significant difference in the maximal effects: CF was dropped by 1.95 ± 0.33 mL/min in WT and only by 0.93 ± 0.10 mL/min in $S1P_3$-KO mice. The decrease in left ventricular contractile performance upon S1P infusion was also

attenuated in S1P$_3$-KO mice. The maximal drop in LVDevP was significantly reduced compared to WT controls.

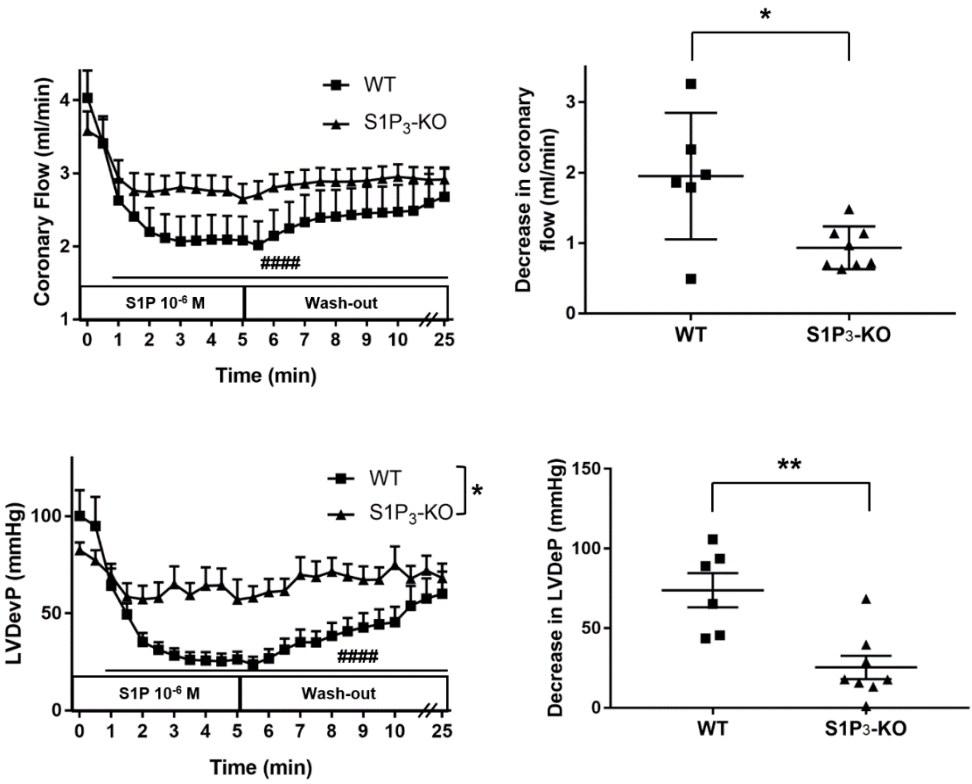
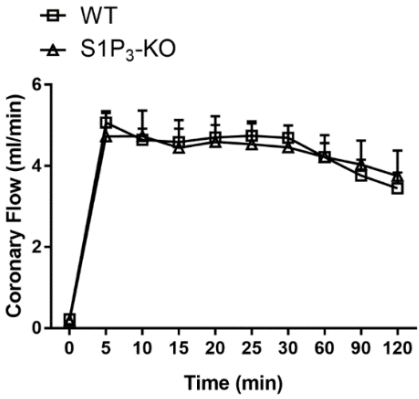
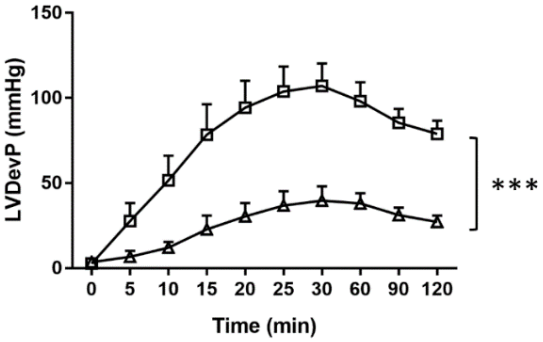


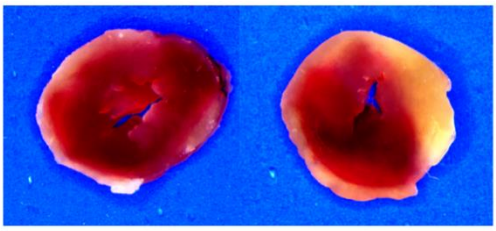
Figure 7. Effects of sphingosine-1-phosphate on coronary flow (CF) and left ventricular developed pressure (LVDevP) of hearts isolated from WT and S1P$_3$R-KO mice. CF and LVDevP are shown in the left panels. Maximal decrease in CF and LVDevP compared to preinfusion baseline are shown in the right panels. n=6 (WT), n = 8 (S1P$_3$R-KO); #### $p < 0.0001$ vs. baseline; * $p < 0.05$, ** $p < 0.01$ vs. WT; two-way repeated measurement ANOVA and Dunnett's post hoc test and unpaired t-test.

Role of myocardial S1P$_3$R activation in I/R Injury

To better understand the apparent contradiction between the widely reported cardioprotective and observed cardiosuppressive effects of S1P, we aimed to separate the myocardial and coronary actions of S1P in a model of I/R injury. First, we investigated the effects of potential S1P$_3$R activation during I/R in the absence of intravascularly administered S1P. WT and S1P$_3$-KO hearts were exposed to an I/R protocol, CF and myocardial function were monitored during reperfusion.







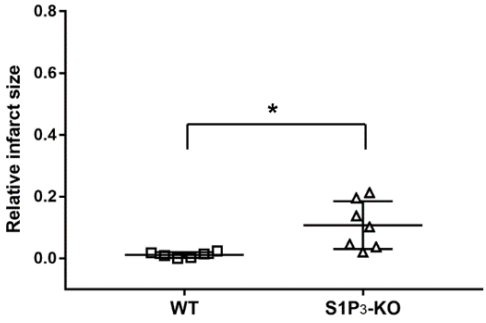


Figure 8. Postischemic coronary flow and left ventricular developed pressure (LVDevP) in isolated WT and $S1P_3$R-KO mouse hearts without sphingosine-1-phosphate (S1P) administration. Relative infarct size and representative sections from the hearts. n=6 (WT), n = 8 ($S1P_3$R-KO); * $p < 0.05$, *** $p < 0.001$, with two-way repeated measurement ANOVA and Dunnett's post hoc test, unpaired t-test.

CF did not differ significantly between WT and $S1P_3$-KO mice. In contrast, parameters describing myocardial performance showed marked differences. The lack of $S1P_3$ resulted in a far worse postischemic functional recovery as evidenced by the drop of the LVDevP.

Effects of preischemic intravascular S1P exposure on I/R injury

Next, we investigated the role of intravascular S1P by administering S1P to the perfusion solution before ischaemia at a concentration of 10^{-6} M for 5 min. Under these conditions, CF returned to a significantly higher value during reperfusion in the $S1P_3$-KO hearts indicating $S1P_3$-mediated coronary vasoconstriction. Postischemic myocardial function failed to return during the reperfusion without any difference between the two groups.

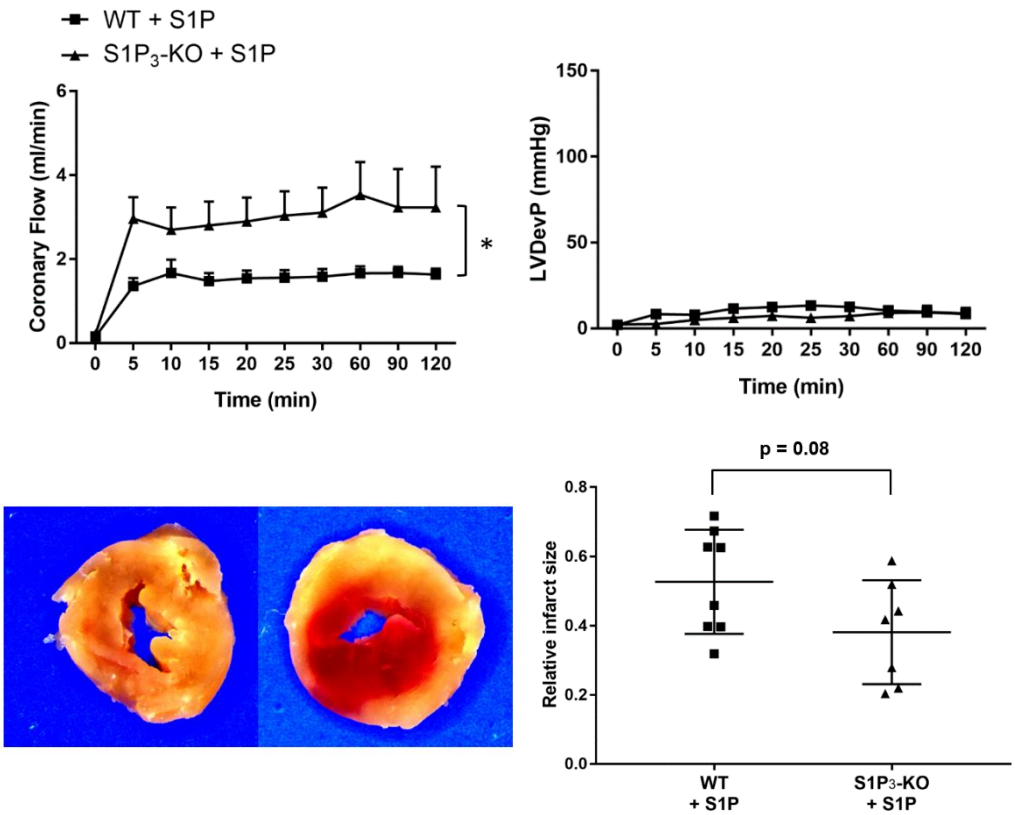


Figure 9. Postischemic coronary flow and left ventricular developed pressure (LVDevP) in isolated WT and $S1P_3$R-KO mouse hearts with S1P administration. Relative infarct size and representative sections from the hearts. n=6 (WT + S1P), n = 8 ($S1P_3$R-KO + S1P); * $p < 0.05$, with two-way repeated measurement ANOVA and Dunnett's post hoc test, unpaired t-test.

V. Conclusions

In the first part of our study, we reported that the short-term effects of Ang II in an isolated heart preparation with intact circulation are modulated by DAGL activation and CB_1 receptor transactivation, indicating a significant role of Ang II-signalling-induced release of the endocannabinoid 2-AG. We propose that the short-term cardiac effects of Ang II are exhibited as the summation of the simultaneous cardiac and vascular effects of Ang II and 2-AG. Whether this holds for long-term effects of Ang II, when the local renin-angiotensin system is upregulated in the heart under pathological conditions, and whether these potential effects are detrimental or beneficial needs further investigation.

In the second part of our study, using isolated perfused murine hearts, we designed experimental models to simulate and explore the actions of S1P release in ACS. Our results suggest that in clinical situations, when thrombotic coronary occlusion causes cardiac ischemia, the released S1P might compromise postischemic recovery due to its unfavourable coronary effects, which might outweigh the presumed cardioprotective effects of S1P produced by the ischemic myocardium.

VI. Bibliography of the candidate's publications

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Nyilvános idéző összesen: 2 | Független: 1 | Függő: 1 | Nem jelölt: 0 | WoS jelölt: 1 | Scopus jelölt: 2 | WoS/Scopus jelölt: 2 | DOI jelölt: 2
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