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Novel approaches to protect the heart against postischemic failure

Nové přístupy k ochraně srdce před postischemickým selháním

Ph.D. thesis

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Praha, 2021

Declaration

I hereby declare that I completed this Ph.D. thesis independently, except when explicitly indicated otherwise. It documents my own work, carried out under the supervision of Ing. František Papoušek, CSc. Throughout, I have properly acknowledged and cited all sources used. Neither this thesis nor its substantial part under my authorship has been submitted to obtain any other academic degree.

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Prohlášení

Prohlašuji, že jsem tuto závěrečnou doktorskou práci zpracoval samostatně s vyjímkou částí, u kterých je explicitně uvedeno jinak. Tento dokument je výsledkem mé práce vykonávané pod vedením Ing. Františka Papouška, CSc. Všechny zdroje použité při vypracování této práce jsou v ní uvedeny a řádně citovány. Tato práce, ani její podstatná část nebyla předložena k získání jiného nebo stejného akademického titulu.

V Praze
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Declaration of co-authorship

On behalf of all co-authors, I hereby declare that Mgr. Jaroslav Hrdlička has substantially contributed to the formation of the articles, which represent an integral part of this PhD thesis. He performed most of the experiments, especially in the publications where he is the first author, and he actively participated in the setup of the experiments, in the interpretation of the results and the preparation of the manuscripts.

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Abstrakt

Ischemická choroba srdeční a následné srdeční selhání patří k nejzávažnějším příčinám úmrtí v rozvinutých zemích. Zlepšení klinického obrazu pacientů s infarktem myokardu a úspěšná prevence postischemického srdečního selhání vyžaduje použití nových léčebných postupů, které by ochránily srdce před zhoubnými důsledky ischemického poškození. Přenos experimentálních kardioprotektivních postupů do klinické praxe zatím není úspěšný; přetrvává proto potřeba hledat nové efektivní strategie pro prevenci a léčbu srdeční ischemie.

V naší práci jsme se proto pokusili vyzkoušet nové protektivní postupy, s cílem ochránit myokard před postischemickým srdečním selháním, vyvolaným u laboratorních potkanů podvazem koronární arterie. Sledovali jsme preventivní a terapeutický vliv adaptace na kontinuální normobarickou hypoxii (CNH, 12 % O_2), preventivní a terapeutický vliv fyzické zátěže (běhací pás) a vliv farmakologického ovlivnění hladiny epoxyeikosatrienových kyselin (EET) na průběh postischemického srdečního selhání. U prvních dvou přístupů byly již dříve prokázány kardioprotektivní účinky při akutním ischemicko/reperfuzním poškození, projevující se zmenšením rozsahu infarktu myokardu. EETs jsou známé pro své antihypertenzní účinky, a zdají se proto vhodné pro výzkum klinicky relevantních modelů kardioprotekce u hypertenzních zvířat.

Naše výsledky ukázaly, že:

- Preventivní adaptace na CNH měla významný antiarytmický efekt, což vedlo ke zlepšenému přežívání; srdeční funkce v průběhu postischemického srdečního selhání však ovlivněna nebyla. Kardioprotektivní vliv preventivní adaptace na fyzickou zátěž se v našem experimentálním uspořádání prokázat nepodařilo.
- Terapeutická adaptace na CNH zpomalila průběh postischemického srdečního selhání a zlepšila srdeční funkci. Obdobně jako u preventivní adaptace jsme nepozorovali ani u terapeutické adaptace na fyzickou zátěž kardioprotektivní vliv na postischemické srdeční selhání.
- Preventivní podávání EET-B (analog EET) mělo významný antiarytmický efekt, což vedlo ke zlepšenému přežívání spontánně hypertenzních potkanů; zlepšilo rovněž postischemickou funkci srdce.
- Současné terapeutické podání EET-A (analog EET) a *c*-AUCB (inhibitor solubilní epoxid hydrolázy) zlepšilo funkci levé komory u normotenzních potkanů kmene Hannover Sprague-Dawley s postischemickým srdečním selháním. Samostatné podávání těchto látek kardioprotektivní efekt nemělo. Terapeutické podání EET-A a *c*-AUCB nemělo vliv na postischemické srdeční selhání u transgenního kmene potkanů s angiotensin II dependentní hypertenzí.

Naše výsledky ukazují, že mechanismy, které zvyšují odolnost srdečního svalu k akutní ischemii, mohou protektivně ovlivnit rovněž jeho postischemickou funkci a remodelaci.

Klíčová slova: kardioprotekce, srdeční selhání, hypoxie, zvýšená fyzická zátěž, epoxyeikosatrienové kyseliny, hypertenze

Abstract

Ischemic heart disease and resulting heart failure (HF) belong to the leading causes of death in developed countries. In order to prevent HF and improve clinical outcome in patients with myocardial infarction, novel therapies are required to protect the heart against the detrimental effect of ischemic injury. Due to the failure to translate numerous available experimental cardioprotective strategies into clinical practice, the need for novel protective treatments persists.

We have, therefore, tried to apply a novel approach to cardiac protection against the postischemic HF induced in rats by ligation of the coronary artery. For this purpose, we have studied (i) the preventive and therapeutic effects of adaptation to continuous normobaric hypoxia (CNH; 12% O_2) and exercise training (ExT; treadmill running), and (ii) the possible cardioprotective potential of epoxyeicosatrienoic acid (EET)-based therapy in order to attenuate the postischemic HF in rats. Adaptation to CNH and ExT is known for their cardioprotection in acute ischemia/reperfusion (I/R) injury manifested as reduction of infarct size. EETs exert antihypertensive effects and thus seem to be perspective for the research in clinically relevant models of cardioprotection in hypertensive animals.

Our results have revealed that:

- CNH prior to the I/R insult improved survival bud did not affect cardiac function in postischemic HF in Wistar rats. ExT prior to the I/R insult had no significant effect on cardiac function in postischemic HF in our experimental setup.
- Therapeutic adaptation to CNH attenuated the progression of postischemic HF in Wistar rats. On the other hand, therapeutic ExT did not affect the postischemic HF.
- Preventive EET-B (EET analogue) treatment led to the increased survival in spontaneously hypertensive rats subjected to I/R insult and improved cardiac function in postischemic HF.
- Therapeutic administration of EET-A (EET analogue) combined with *c*-AUCB (inhibitor of soluble epoxide hydrolase) improved cardiac function in normotensive HanSD rats with postischemic HF. Single therapies did not provide a cardioprotective effect in normotensive HanSD rats. Postischemic HF was not affected by the therapeutic administration of either EET-A or *c*-AUCB treatment in hypertensive TGR.

Based on our results, we can conclude that protective mechanisms leading to increased cardiac tolerance to acute ischemia could play an important role also in postischemic cardiac remodelling and function.

Keywords: cardioprotection, heart failure, hypoxia, exercise training, epoxyeicosatrienoic acids, hypertension

CONTENT

LIST OF ABBREVIATIONS

$-(dP/dt)_{max}$	peak rate of pressure decline in LV
$+(dP/dt)_{max}$	peak rate of pressure development on LV
AT	acceleration time to Vpa_{max}
ATP	adenosine triphosphate
AWT_d	end-diastolic anterior wall thickness
AWT_s	end-systolic anterior wall thickness
BW	body weight
CAD	coronary artery disease
c-AUCB	inhibitor of soluble epoxide hydrolase (*cis*-4-[4-(3-adamantan-1-yl-ureido)-cyclohexyloxy]-benzoic acid)
CH	chronic hypoxia
CNH	continuous normobaric hypoxia
CVD	cardiovascular diseases
CYP	cytochrome P-450
DHET	dihydroxyeicosatrienoic acid
ECM	extracellular matrix
EET	epoxyeicosatrienoic acid
EET-A	EETs analogue (sodium (*S*)-2-(Z)-(13-(3-pentyl)ureido)-tridec-8(*Z*)-enamido)succinate)
EET-B	EETs analogue (N-(5-((2-cetamidobenzo[d]thiazol-4-yl)oxy) pentyl)-N-isopropylheptanamide)
ExT	exercise training
ET	ejection time
ETpa	ejection time in pulmonary artery
FS	fractional shortening
FT	filling time
HanSD	Hannover Sprague-Dawley
HETE	hydroxyeicosatrienoic acid
HF	heart failure
HIF-1	hypoxia-inducible factor 1
HR	heart rate
I/R	ischemia/reperfusion
IHH	intermittent hypobaric hypoxia
IS/AR	infarct size/area at risk
IVCT	isovolumic contraction time
IVRT	isovoumic relaxation time
LV	left ventricle
LVD_d	end-diastolic diameter of LV cavity
LVD_s	end-systolic diameter of LV cavity
MI	myocardial infarction
MMP	matrix metalloproteinase
MPTP	mitochondrial permeability transition pore
NO	nitric oxide
P_{dev}	developed pressure
P_{ed}	end-diastolic pressure
P_{es}	end-systolic pressure
PH	pulmonary hypertension
pO_2	partial pressure of oxygen

PWT_d	end-diastolic posterior wall thickness
PWT_s	end-systolic posterior wall thickness
ROS	reactive oxygen species
RV	right ventricle
RWT	relative wall thickness
sEH	soluble epoxide hydrolase
SEM	standard error of the mean
SHR	spontaneously hypertensive rat
SOD	superoxide dismutase
SR	sarcoplasmatic reticulum
TGR	Ren-2 transgenic rat
Vm_{max}	maximal velocity of blood flow at the mitral valve
VO_2max	maximum rate of oxygen consumption
Vpa_{max}	maximal velocity of blood flow in the pulmonary artery
Vpa_{mean}	mean velocity of blood flow in the pulmonary artery

1. INTRODUCTION

Ischemic heart disease and heart failure (HF) that often results, remain the leading causes of death and disability in Europe and worldwide (Braunwald, 1997). As such, in order to prevent HF and improve clinical outcomes in patients presenting with an acute ST-segment elevation myocardial infarction (MI) and patients undergoing coronary artery bypass graft surgery, novel therapies are required to protect the heart against the detrimental effects of ischemia/reperfusion (I/R) injury. Therefore, it is not surprising that cardiovascular research is focused on the theoretical and applied basis of rational prevention and therapy of the most severe cardiovascular problems, such as acute and chronic myocardial ischemia. Advances in methodology, particularly in molecular biology and genetics, have helped substantially in the search for a better understanding of the underlying mechanisms.

Although recent advances in treatment have improved survival in patients presenting with an acute MI, the number of patients developing HF, a medical condition that exerts a huge global burden on healthcare and economic resources, has increased. Despite timely reperfusion with primary percutaneous coronary intervention, mortality and morbidity following myocardial infarction remain significant, with 7% death and 22% HF hospitalization at one year in patients presenting with MI. As such, novel cardioprotective strategies are still required to attenuate the detrimental effects of myocardial I/R injury, to prevent left ventricular (LV) remodelling, and reduce HF in patients with ischemic heart disease.

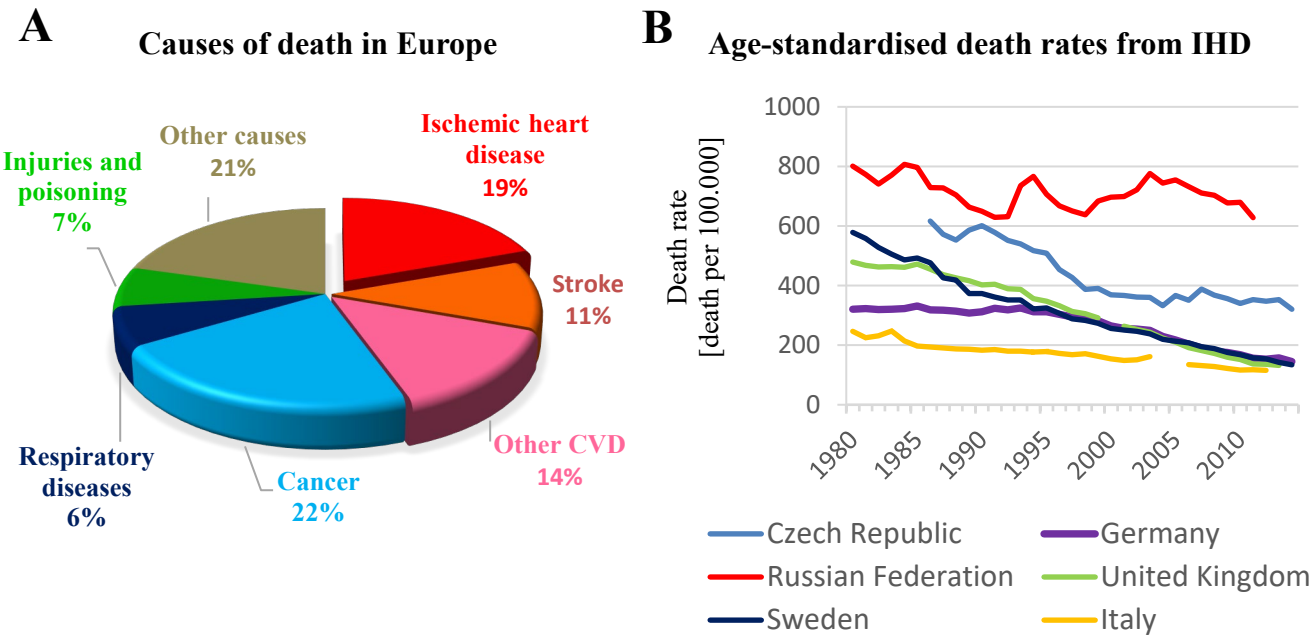

Figure 1 Causes of death (**A**) and Age-standardized death rates from ischemic heart disease (IHD) in selected countries since 1980 (**B**); Cardiovascular diseases (CVD). Wilkins *et al.*, 2017.

During the last three decades, a wide variety of protective strategies and pharmacological treatments have been tested in the clinic. However, their translation from experimental to clinical studies for improving patient outcomes has been both challenging and disappointing. The need for cardioprotective strategies for reducing I/R injury thus persists. Therefore, we tried to summarise current data on the possibilities of protecting the heart against ischemic injury (reviewed by Hausenloy *et al.*, 2017).

1.1. Myocardial ischemia

1.1.1. Definition

Ischemic states of the cardiovascular system originate from the disproportion between the amount of oxygen supplied to the cardiac cell and the amount required by the cell. However, the degree of ischemic injury depends not only on the intensity and duration of the ischemic stimulus but also on the level of cardiac tolerance to oxygen deprivation. Due to the high coronary arteriovenous difference, the myocardium cannot bring about a substantial improvement in oxygen supply by the increased extraction of oxygen from the blood; thus, the only way of meeting the higher oxygen demand is through the increased blood supply. Theoretically, any known mechanisms leading to tissue hypoxia can be responsible for reducing the oxygen supply of the myocardium. Still, the most common causes are undoubtedly (i) ischemic hypoxia (often described as "cardiac ischemia") induced by reduction or interruption of the coronary blood flow and (ii) systemic (hypoxic) hypoxia ("cardiac hypoxia") characterized by a drop in the partial pressure of oxygen (pO_2) in the arterial blood but adequate perfusion (pulmonary diseases, life at high altitude). For the sake of completeness, we would add (iii) anaemic hypoxia in which the arterial pO_2 is normal, but the oxygen transport capacity of the blood is decreased, and (iv) histotoxic hypoxia resulting from reduced intracellular utilization of oxygen in the presence of adequate saturation and an adequate blood flow (e.g. by inhibition of oxidative enzymes as a result of cyanide poisoning).

It should be emphasized that the terms "hypoxia" and "ischemia" are unfortunately used often interchangeably in the literature despite the fact the consequences of the two mechanisms at the cellular level are very different. In ischemia, there is a drop in the supply of oxygen and substrates and a significant reduction in the clearance of metabolites, particularly lactic acid and protons; the intracellular pH falls rapidly as the acid products of glycolysis accumulate. In contrast, in cardiac hypoxia, perfusion results in the washing out of glycolysis acid products, thereby retarding the rate of development of acidosis. Systemic hypoxia is usually a generalized phenomenon diffusely involving the whole myocardium, whereas ischemia is confined to the area supplied by the affected

coronary artery. Ischemic hypoxia is clinically manifested primarily in ischemic heart disease and its acute form, MI, while systemic hypoxia is associated with chronic cor pulmonale of varying origin, cyanosis due to hypoxemic congenital heart disease or changes induced in the cardiopulmonary system by a decrease in barometric pressure at high altitudes. In two cases, however, systemic hypoxia can be qualified as normal: (i) the fetal myocardium, which is exposed to hypoxia corresponding to an altitude of 8000 m ("Mount Everest *in utero"*) and (ii) myocardium of subjects living permanently at high altitudes. In both situations, the myocardium is significantly more resistant to acute oxygen deficiency

Another common confusion exists between the terms "ischemia" and "infarction". Myocardial ischemia does not only lead to changes in cardiovascular function and metabolism but also changes in homeostasis of electrolytes, neurohumoral regulations and myocardial ultrastructure. These changes can be seen within the first few minutes of ischemia and are reversible when perfusion is promptly (up to 30 to 40 min) restored. However, when ischemia is maintained, there is a gradual transition from reversible to irreversible injury as infarction develops, as described further. Infarction is thus synonymous with irreversible ischemic injury and cell death (reviewed by Ošťádal and Kolář, 1999).

Myocardial ischemia is caused by structural or functional alterations in coronary circulation such as coronary artery disease (CAD), blood clots or coronary artery spasm. This reduction in blood flow leads to oxygen and nutrient deprivation and results in metabolic, functional and morphological changes within the myocardium. The absence of oxygen prevents oxidative phosphorylation, leads to mitochondrial membrane depolarisation, adenosine triphosphate (ATP) depletion and disrupted myocardial contractile function (reviewed by Hausenloy and Yellon, 2013). Within tens of seconds, metabolism switches to anaerobic glycolysis that enables to cover the cardiomyocytes' basic energy demands, while the accumulation of lactate causes a decrease in intracellular pH (reviewed by Lopaschuk 2016). Depletion of energetic reserves leads to a gradual rise in diastolic tension of the ischemic area within 10-20 minutes resulting in contracture rigor in 60-90 minutes based on the experimental model (Katz and Tada, 1979; Jennings and Reimer, 1991). This could be dramatically accelerated by the inhibition of anaerobic glycolysis, as shown by Frank *et al*. (2012).

Anaerobic metabolism further leads to the intracellular accumulation of protons that activates the Na^+/H^+ antiporter. Exchange of H^+ for Na^+ activates Na^+/Ca^{2+} ion exchanger causing the intracellular Ca^{2+} overloading and cell death (reviewed by Avkiran and Marber, 2002). Adverse changes in the metabolism of ischemic cardiomyocytes can gradually result in autophagy, apoptosis and necrosis. Autophagy is a pro-survival lysosomal mechanism that can contribute to energy demands under stress condition and control the cell damage (Glick *et al*., 2010). Apoptosis is a highly regulated process of

cell death leading to the limited collateral damage activated by "death receptors" in the plasma membrane (extrinsic pathway) or by permeabilization of mitochondria (intrinsic pathway). Necrosis, on the other hand, is caused by physical or chemical trauma, and despite some level of regulation, leads to mitochondrial swelling, cell rupture and potential for further pathological consequences (reviewed by Chiong *et al*., 2011).

1.1.2. Ischemia/reperfusion injury

Early reperfusion remains the only effective strategy for restoring LV function and limiting MI size. However, reperfusion might lead to deterioration of myocardial injury, resulting in reperfusion-induced arrhythmias, myocardial stunning, microvascular obstruction, and death of cardiomyocytes (Jenning *et al*., 1960; Rona *et al*., 1979; reviewed by Piper *et al*., 1998).

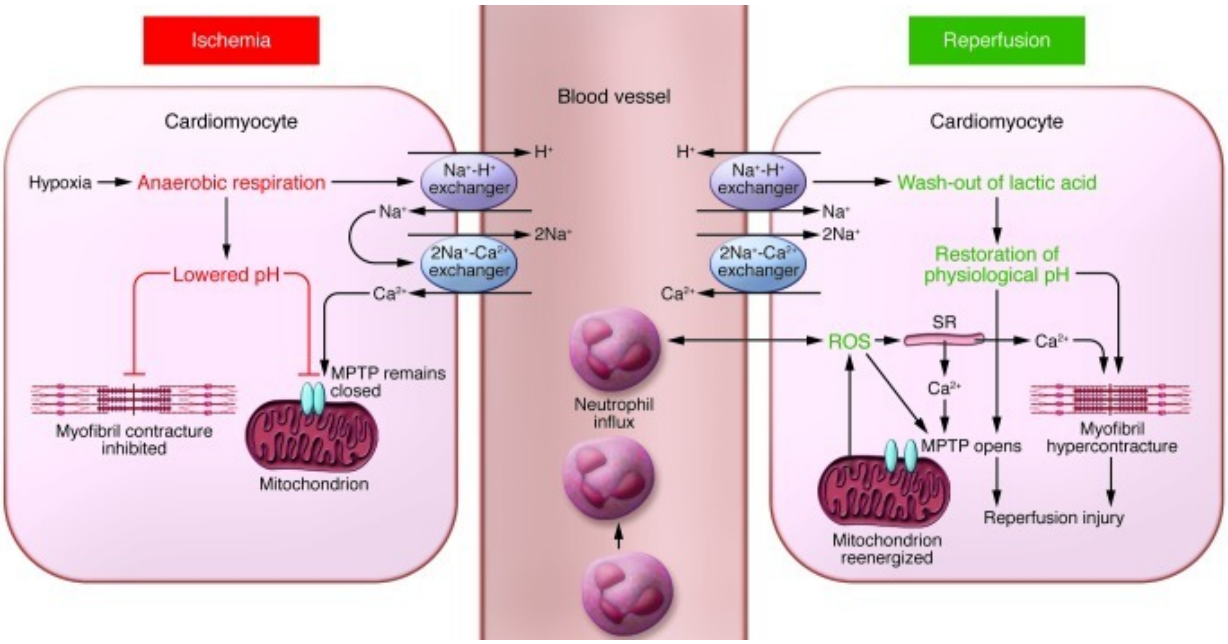

Figure 2 Principal mechanism of the ischemia/reperfusion injury. Hausenloy and Yellon, 2013.

Early after reperfusion, cardiomyocytes are exposed to a rapid increase of oxidative stress resulting in a burst of reactive oxygen species (ROS) inside the cell (Ferrari *et al*., 1992; Eefting *et al*., 2004). While ROS are produced by various sources, they have been considered the critical factor in reperfusion injury (reviewed by Granger and Kvietys, 2015). ROS can lead to disruption of the plasmatic membrane and sarcoplasmic reticulum (SR). Sudden mitochondrial re-energization exacerbates ischemia-induced Ca^{2+} overload and induce the opening of the mitochondrial permeability transition pore (MPTP). MPTP is a voltage-dependent, nonselective channel of the inner

mitochondrial membrane (Di Lisa *et al*., 2001). Intracellular pH decreased by ischemia is rapidly restored by the lactate washout and activation of Na^+/H^+ antiporters, which may lead to MPTP opening (Halestrap *et al*., 2004), results in mitochondrial membrane depolarization, uncoupling oxidative phosphorylation, the collapse of the mitochondrial membrane potential, ATP depletion and cell death (Heusch *et al*., 2010; Hausenloy and Yellon, 2013).

1.1.3. Myocardial infarction

Myocardial infarction is a focal irreversible myocardial injury induced by ischemia of the cardiac muscle. The clinical definition involves elevated cardiac troponin levels and the presence of at least one from the following conditions: chest pain, ST segment elevation on ECG, pathologic Q wave, regional disturbance of cardiac kinetic or presence of the intracoronary thrombosis (Thygesen *et al*., 2018). As described previously, MI is caused mainly by necrosis and apoptosis. Necrosis is dominant in the centre of MI and total myocardial injury, as demonstrated by Kajstura *et al*. (1998) in a rat model of coronary artery occlusion, where signs of necrosis were 30 - 50 times higher than signs of apoptosis. On the other hand, apoptosis is more frequent in the peri-infarct zone and plays a critical role in myocardial remodelling after I/R (reviewed by Konstantinidis *et al*., 2012).

Whereas MI is prevented by reperfusion, myocardial injury can be aggravated by physiological and pathological mechanisms. Myocardial injury is worsened by reperfusion insult, as mentioned previously. Moreover, I/R induced damage leads to an acute inflammatory response which plays a crucial role in the death or survival of damaged cardiomyocytes and myocardial scar healing (reviewed by Frangogiannis, 2014). Alongside the loss of contractile function, MI size and scar healing are detrimental for further clinical outcome and adverse myocardial remodelling, resulting in HF.

1.2. Postischemic myocardial remodelling

Acute loss of viable cardiomyocytes is followed by the gradual replacement of the dead cells by collagenous scar. This process, known as MI healing, starts immediately after MI and could be divided into the inflammatory, fibrotic, and remodelling phase (reviewed by Richardson *et al*., 2015 and Mouton *et al*., 2018).

The inflammatory phase of MI healing occurs during the first days in small animals (Fishbein *et al*., 1978; Yang *et al*., 2002) or the first weeks in large animals and humans (reviewed by Heusch *et al*., 2014). Myocardial necrosis is followed by a signalling cascade leading to the infiltration of neutrophils, macrophages, and lymphocytes within hours, peaks after several days, and lingers for

several weeks (Fishbein *et al*., 1978; Yang *et al*., 2002). Structural remodelling of extracellular matrix (ECM) is initiated by secretion and activation of matrix metalloproteinases (MMPs). These enzymes degrade cellular material and ECM, allowing phagocytosis of necrotic tissue by macrophages and contributing to further ECM accumulation by disrupting the present collagen fibres (reviewed by Lindsey, 2018).

Simultaneously, inflammatory cells release a plethora of signalling cytokines, growth factors and hormones, including tumor necrosis factor-α, interleukins 1, 2, 6 and 10, transforming growth factor β or interferon γ, that amplify and stabilize the pro-inflammatory environment in the infarcted area and regulate the inflammatory response (reviewed by Daskalopoulos *et al*., 2012 and Frangogiannis, 2014). These steps are essential for the recruitment and activation of fibroblasts resulting in the next stage of the MI healing process, fibrosis.

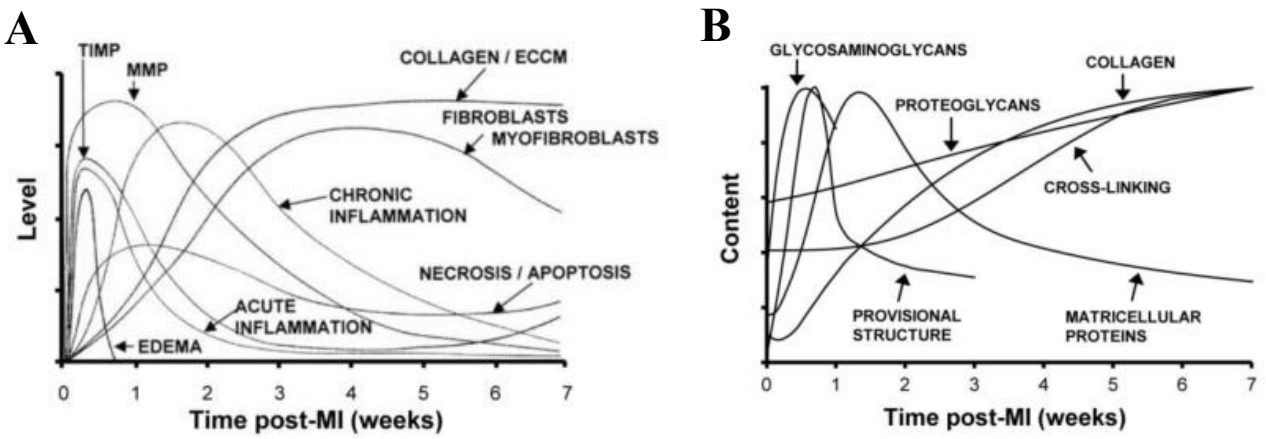

Figure 3 Time course of processes (**A**) responsible for wound healing after myocardial infarction and extracellular content changes during this period (**B**). Richardson *et al*. (2015)

The fibrotic phase of MI healing is characterized by the robust activation of myofibroblasts. This cell type is the most abundant in the healthy heart (Ma *et al*., 2014), and as a result of migration from the surrounding myocardium, proliferation and differentiation, the number of myofibroblasts can be markedly increased. Up to 20-fold higher presence of myofibroblasts within one week after MI in mouse myocardium was demonstrated by Fishbein *et al. (*1978). The role of myofibroblasts is in the intensive expression of pro-collagen (mainly type I and III) that peaks during the first week after MI and is accompanied by a waned expression of MMPs as shown by Cleutjens *et al*. (1995) and Zimmerman *et al*. (2001). The final composition of the ECM in healing myocardium is highly regulated by activation of the MMPs, the tissue inhibitors of MMPs and the collagen secretion. The total increase of the myocardial collagen content can be increased by up to 10-fold within weeks after MI (Gupta *et al*., 1994; Cleutjens *et al*., 1995). Whereas collagens represent the majority of newly

synthesized molecules in ECM of the healing myocardium, other matricellular proteins like fibronectin, thrombospondin, osteopontin, tenascin C or periostin are needed for proper scar formation. They have a structural and signalling role in developing a scar. This was demonstrated by Schroen *et al*. (2004) when thrombospondin knock-out mice exhibited worsened cardiac remodelling and increased incidence of cardiac rupture. Similarly, Konstandin *et al*. (2013) showed a dramatic progression of cardiac dysfunction after MI in fibronectin knock-out mice when compared to wild type animals.

Within weeks in small animals (mice and rats; Fishbein *et al*., 1978; Yang *et al*., 2002) or months in large animals (dogs and pigs) and humans (Dewald et al., 2004; Heusch *et al*., 2014), the fibrotic phase fluently transits into the remodelling/scar maturation phase. The myofibroblasts activity is attenuated, and their number is limited by apoptosis (reviewed by Sun and Weber, 2000). Collagen remains to be dominant in the scar composition and is stabilized by cross-linking. The stabilization is mediated by hydrolysylpyridium or lysyl oxidase and proteoglycans like decorin and biglycan that regulate the fibrinogenesis and fibre diameter (Dobaczevsky *et al*., 2010; Doi *et al*., 2000). During the maturation of the myocardial scar, the collagen content in various experimental models is stabilized. The final content of hydroxyproline is increased 5 to 15-fold when compared to pre-infarction myocardium (Gupta *et al*., 1994; Cleutjens *et al*., 1995; Marijianovski *et al*., 1997), and the density of the covalent cross-links is increased by 2-fold (McCormick *et al*., 1994; Fomovsky *et al*., 2010).

In conclusion, the postischemic myocardial remodelling leads to the elimination of necrotic tissue and ECM rearrangement. This secures the structural integrity of the injured myocardium and thus prevents fatal events like a myocardial rupture. On the other hand, increased collagen content in the myocardial scar and in the rest of the myocardium changes the biomechanical properties of the injured heart and may contribute to the adverse effect on the LV function (reviewed by Richardson *et al*., 2015).

1.3. Functional changes in MI heart

As shown by clinical and experimental studies, functional changes in LV are critically dependent on the MI size (Mathey *et al*., 1974; Fletcher *et al*., 1981). This reflects the disrupted cardiac contractility due to the loss of vital cardiomyocytes and alteration of mechanical properties of the heart due to the remodelling processes. At the same time, compensatory reflexes contribute to maintaining the perfusion of vital organs.

Even before the onset of the MI during ischemia, an acute drop of LV contractility is translated into a decrease of the systolic blood pressure, which triggers the reflexive response. This leads to activation of the sympathetic nervous system and increased contraction of systemic veins. As a result, diastolic pressure is increased, and LV filling is improved. The Frank-Starling mechanism consequently improves systolic contraction when increased diastolic pre-stretch results in increased systolic force (Gay *et al*., 1986; reviewed by Gronda *et al*., 2017). At the same time, sympathetic activation increases heart rate, which contributes to maintaining cardiac output. On the other hand, prolonged hyperactivity of the sympathetic nervous system together with reduced parasympathetic activity and activation of the renin-angiotensin-aldosterone system in patients with MI is a known risk factor leading to adverse remodelling, progressive LV dysfunction, and end-organ damage (Swedberg, 1988; La Rovere *et al*., 1998; Brunner-La Rocca *et al*., 2001). It was also observed that sympathetic/parasympathetic regulation of the cardiovascular system in postischemic HF becomes disrupted (Eckberg *et al.*, 1971; reviewed by Creager, 1992) and might lead to life-threatening ventricular arrhythmias (reviewed by Chakko *et al*., 1989).

Inadequate cardiac remodelling accompanied with large MI size can further lead to myocardial scar thinning and increased risk of its rupture (Jugdutt, 2010). Adverse fibrosis of non-infarcted myocardium is also associated with increased myocardial stiffness and relaxation abnormalities (reviewed by Richardson *et al.*, 2015). These changes consequently lead to worsened prognosis, excessive fibrosis and might aggravate the cardiac dysfunction by impairing the adequate oxidation of surviving cardiomyocytes. Finally, fibrosis alters the electrophysiological properties of a failing heart and increases the risk of life-threatening arrhythmias (Spach and Boineau, 1997).

1.4. Cardiac protection

As mentioned above, the degree of ischemic injury depends not only on the intensity and duration of the ischemic stimulus but also on the level of cardiac tolerance to oxygen deprivation. Therefore, it is not surprising that the interest of many experimental and clinical cardiologists during the past 50 years has been focused on the question of how cardiac tolerance to ischemia might be increased.

1.4.1. History and present status

In the late 1950s, the first observations appeared, showing that the incidence of myocardial infarction was lower in people living at high altitude. These epidemiological observations were later repeatedly confirmed in experimental studies using simulated hypoxia. In the early 1970s, the interest was focused on the possibility of limiting infarct size pharmacologically. This effort was, however,

not successful because it became increasingly obvious that clinical observations did not correspond to the optimism of experimental results. After the period of scepticism, the discovery of a short-term adaptation of the myocardium, so-called "ischemic preconditioning" by Murry *et al.* (1986), opened the door to the new era of cardiac protection. It has evolved from classical and delayed ischemic preconditioning (both of which are limited in their clinical application as they are invasive and need to be applied prior to ischemia) to ischemic post-conditioning (which allows the intervention to be applied at the time of reperfusion, but is still invasive), to remote ischemic conditioning (which has allowed the intervention to be applied non-invasively to the arm or leg, even during ongoing myocardial ischemia and at reperfusion) making it more clinically applicable (for review see Ošťádal 2009).

In the last few years, there has been an increasing number of neutral clinical cardioprotection studies; they have been extensively reviewed and discussed in the recent literature, and only an overview is provided here. To the endogenous cardioprotection, various strategies belong, e.g., administration of adenosine, atrial natriuretic peptide, or exenatide – a glucagon-like peptide analogue. They have shown a promise as a therapy for reducing infarct size, but whether they can improve clinical outcomes is not known and needs to be determined. The results with beta-blocker therapy (metoprolol) have had mixed results, in part due to the patient selection and the timing and dose used. Finally, an attempt was made to block the opening of mitochondrial permeability transition pore by cyclosporine. The results have been mostly neutral, which may have been due to patient selection and the dose of cyclosporine. Nevertheless, mitochondrial permeability transition pore remains a very promising possibility and should be further investigated (reviewed by Hausenloy *et al*., 2017)

Although many years of research on cardiac protection have provided important insights into the complex intracellular signalling pathways underlying cytoprotection at the cardiomyocyte level, the translation of protective strategies into the clinical setting for the benefit of patients has been largely disappointing. In this situation, the question arises how could this experimental failure be explained? The possible explanation is obviously multifactorial and includes, e.g. the use of healthy and young animals, absence of atherosclerosis, other comorbidities and co-medications in experimental animals, the effect of age and sex, and the lack of long-term follow-up of the benefits of various interventions.

Under such conditions, the selection of cardioprotective strategies for our experimental study was not simple. Our choice was, first of all, adaptation to chronic hypoxia, where our laboratory has more than 50 years of experience. It restricts infarct size, improves postischemic contractile dysfunction, reduces arrhythmias and provides robust and long-lasting protection. Similar cardioprotective effect manifested as limited infarct size, improved postischemic contractile dysfunction, and reduced arrhythmias can also be achieved by exercise training, which become a second potentially

cardioprotective approach in our study. Finally, the EETs represent a novel experimental approach with multipotent properties that qualify the EET-based therapies as a promising tool in the treatment of cardiovascular diseases (CVD).

1.4.2. Chronic hypoxia

Chronic hypoxia (CH) can be described as prolonged exposure to low levels of oxygen. As a natural environmental condition, CH can be found in high altitudes (more than2400 m above sea level), where the partial pressure of oxygen decreases below 15.4% (compared to 21% at the sea level). It is known that about 2% of the world population lives at high altitude (Hurtado *et al*., 2012). Their survival in this specific environment is determined by several adaptive mechanisms, including increased erythropoiesis, angiogenesis and metabolism remodelling resulting in more efficient O_2 utilization (reviewed by Essop, 2007). Epidemiological studies showed that chronic exposure to CH is associated with decreased prevalence of various disease states such as diabetes and obesity but mainly CVD. The first study targeting the prevalence of CVD in high altitude showed a lover incidence of MI in the Andean population (Hurtado, 1960). These observations were confirmed experimentally in Prague by Kopecký and Daum (1958). Their experiment demonstrated CH-induced cardioprotection manifested as improved cardiac function recovery after anoxia period in isolated cardiac muscle from animals periodically exposed high altitude hypoxia (mimicking 7000 m above sea level). The cardioprotective effects were later confirmed by Poupa *et al*. (1966) and Widimský *et al*. (1973).

Besides exposure to high altitude hypoxia, chronic hypoxia can be found in several disease states such as chronic ischemic heart disease, chronic obstructive lung disease, sleep apnea or hypoxemic congenital heart disease-induced cyanosis (reviewed by Ošťádal and Kolář, 2007). These conditions differ in intensity, duration and cumulative exposure to the hypoxia, but all seem to be capable of providing the protective effects in tissue oxygen deprivation.

As mentioned above, the most frequent physiological forms of hypoxia are ischemic hypoxia (induced by decreased tissue perfusion with blood), systemic hypoxia (caused by a drop in partial pressure of oxygen) and anaemic hypoxia (caused by the limited capacity of blood for oxygen transport; reviewed by Ošťádal and Kolář, 2007). Experimental models of hypoxia are based on mimicking these conditions. For example, exposure to hypobaric hypoxia, where barometric pressure and partial pressure of oxygen are decreased, simulates high altitude conditions. Normobaric hypoxia, where barometric pressure is unchanged and partial pressure of oxygen is limited by partial oxygen depletion.

Hypoxic experiments can be further divided according to the protocol of exposure as continuous or intermittent. Whereas continuous exposure is not interrupted by reoxygenation (normoxic) periods, intermittent hypoxia protocols can markedly vary in length of the hypoxic cycle, the number of repetition, hypoxia severity and the total duration of the protocol.

1.4.2.1. Cardioprotective effects of chronic hypoxia

Adaptation to chronic hypoxia in experimental conditions leads to increased tolerance to I/R injury. This was manifested as a reduction of I/R-induced MI size (Meerson *et al*., 1973; Turek *et al*., 1980; Cai *et al*., 2003), improved recovery of cardiac function during reperfusion (McGrath *et al*., 1973; Widimský *et al*., 1973; Tajima *et al*., 1994) and reduced occurrence and severity of I/R-induced arrhythmias (Meerson *et al*., 1987; Asemu *et al*., 2000).

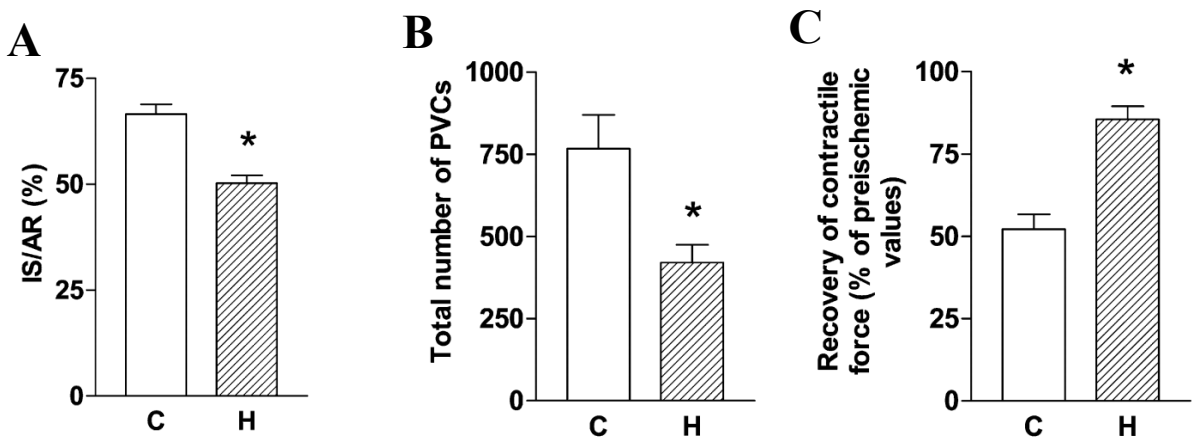

Figure 4 Examples of cardioprotective effects of adaptation to chronic intermittent hypoxia in rats. Reduction of infarct size, normalized to the area at risk (**A**; IS/AR, Neckář *et al*., 2002), decreased number of premature ventricular complexes during ischemia (**B**; PVCs, Asemu *et al*., 1999) and improved recovery of postischemic contractile function (**C**; Widimský *et al*., 1973). Control group (C), animals adapted to hypoxia (H).

Mallet *et al*. (2006) showed that one-day adaptation to intermittent normobaric hypoxia does not provide a cardioprotective effect in dogs one day after I/R, whereas prolonged adaptation can gradually reduce infarct size by up to 95%. Interestingly, this effect was abolished by omitting the reoxygenation periods. The importance of the balance between hypoxia intensity and reoxygenation period was also demonstrated by Kolář *et al*. (2008). They showed that the infarct size-limiting effect was induced by intermittent hypobaric hypoxia (IHH) after 6 weeks when adapted to 7000 m altitude for 8 hours per day, but not when adapted to 5000 m altitude for 6 hours per day. Infarct size-limiting effect was also observed in animals adapted to continuous normobaric hypoxia (CNH; Baker *et al*., 1997; Neckář *et al*., 2003).

Interestingly, chronic hypoxia-induced changes in cardiopulmonary structure and function can persist for several days to weeks (Ošťádal and Widimský, 1985; Faltová *et al*., 1987). This could preserve the cardioprotection after cessation of the hypoxic stimulus, as demonstrated by Neckář *et al*. (2004). They showed that the infarct size limiting effect of IHH can be detected 5 weeks after adaptation to hypoxia and is blunted within 12 weeks of normoxic recovery. On the other hand, the same study reported an absence of anti-arrhythmic effect within one week of normoxic recovery.

Even though the cardioprotective effects of hypoxia in acute I/R injury are intensively studied, much less is known about its role in MI healing and HF development. Xu *et al*. (2011) investigated the role of intermittent hypobaric hypoxia (IHH) on postischemic HF and showed therapeutic cardioprotection manifested as limited scar size, improved cardiac remodelling and improved cardiac dysfunction in rats. Naghshin *et al*. (2012) also showed a similar effect when sleep apnea-mimicking intermittent hypoxia improved LV contractility in a transgenic model of HF in mice. However, there are no experimental data showing the effect of CNH on postischemic HF.

1.4.2.2. Adverse effects of chronic hypoxia

Chronic exposure to hypoxia is associated with adaptive changes in the pulmonary circulation. Rotta *et al*. (1956) were the first to report that healthy adults settled at high altitude in Peru exhibited pulmonary hypertension (PH) and right ventricle (RV) hypertrophy. Their observations were confirmed by Vogel *et al*. (1962) and Sime *et al*. (1963), and a similar effect was also shown in children (Sime *et al*., 1963). The critical altitude for the development of PH and RV hypertrophy was set by Hurtado (1960) at the level of 3000 m above sea level, where the barometric pressure decreases by approximately 40% and oxygen fraction by 30%.

PH is induced by hypoxic pulmonary vasoconstriction, and this homeostatic mechanism contributes to blood oxygenation. Alveolar hypoxia leads to pulmonary artery smooth muscle contraction via ROS mediated alterations of cellular ion homeostasis. Local vasoconstriction then diverts the blood from the poorly ventilated part of the lungs. During sustained hypoxia, vasoconstriction is reinforced by Rho kinases and activation of hypoxia-inducible factor (HIF)-1α resulting in adverse pulmonary vascular remodelling and PH. Development of RV hypertrophy is then an adaptive response to increased RV afterload that allows maintaining the cardiac output (reviewed by Dunham-Snary *et al*., 2017). However, if untreated, these compensatory changes might result in progressive right HF and death.

Despite intensive research on this field, the detailed mechanisms of hypoxia-induced cardioprotection are not fully elucidated. However, several adaptive responses and key molecular components seem to be crucial for this phenomenon.

It is known that hypoxia stimulates erythropoiesis. This is mediated by (HIF)-1α (Semenza, 2004) and results in increased oxygen transport capacity of the blood. On the other hand, hypoxia-induced alterations of coronary circulation are inconclusive. The decrease in coronary blood flow was documented in humans (Grover and Alexander 1971; Moret *et al*., 1972), while the increase was observed in dogs and rats (Turek *et al*., 1975; Smith and Clark, 1979; Scheel *et al*., 1990). Furthermore, increased capillary density was reported in rats by Zhong *et al*. (2002), but not by Rakušan *et al*. (1981).

Hypoxia also induces a shift in energy substrate utilization leading to augmented carbohydrate metabolism, mitochondria respiratory capacity and thus to more effective ATP production (reviewed by Essop, 2007). This is associated with increased hexokinase activity and mitochondrial integrity (Wasková-Arnoštová *et al*., 2015). Since glycolysis is also the preferred energy source for intracellular Ca^{2+} management (Xu *et al*., 1995; Boehm *et al*., 2000), this metabolic adaptation can contribute to improved Ca^{+2} handling.

Cardiac function is critically dependent on Ca^{2+} release from the SR, its extracellular influx during systole and the reuptake and extracellular extrusion during diastole. During I/R, this mechanism is impaired by ATP shortage and ROS-induced disruption of ion transport and exchange (reviewed by Holmberg and Williams, 1992). It has been demonstrated that intermittent normobaric hypoxia improves SR Ca^{2+} handling and Na^{+}/Ca^{2+} exchange in rat cardiomyocytes (Yeung *et al*., 2007). Hypoxic preconditioning also preserved SR Ca^{2+} uptake during the late phase of ischemia (Wu *et al*., 2007). This effect then preserves cardiac function and prevents Ca^{2+} overload.

As mentioned previously, Ca^{2+} overload and high ROS production may lead to necrosis mediated by MPTP opening. Hypoxia-induced stabilization of Ca^{2+} homeostasis during I/R then contributes to improved cell survival. Interestingly, ROS seem to have a dual role in hypoxic cardioprotection. Excessive ROS production during I/R is cytotoxic, causes direct oxidative damage to proteins and membrane lipids, leading to disruption of cellular homeostasis and may result in cell death (reviewed by Zhou *et al*., 2015). On the other hand, experimental data show that low levels of ROS are necessary for hypoxia-induced cardioprotection. This was demonstrated by Kolář *et al*. (2007) when antioxidant N-acetylcysteine completely prevented hypoxia-induced cardioprotection in rats. A similar effect on N-acetylcysteine was also observed in dogs (Estrada *et al*., 2016).

To conclude, the adaptations to CNH and intermittent hypoxia show similar cardioprotective effects. Despite that exact mechanisms of these adaptations may vary, they share some key mediators. The beneficial effect of intermittent hypoxia was also reported in HF development, and we can speculate that the effect of CNH might be similar.

1.4.3. Exercise training

Physical activity is a natural stimulus with a plethora of predominantly positive effects on physiological functions. While the importance of physical activity for human health is documented since the dawn of civilization, the amount of physical work of individuals became limited for decades. This is considered a risk factor for CVD. When Morris and Crawford (1958) firstly proposed that physical activity protects from cardiovascular events, the beneficial potential of exercise training became intensively studied.

Epidemiological studies documented the major positive effects of exercise training (ExT) in both preventing CVD (Blair *et al*., 1989; Berlin and Colditz, 1990; Paffenbarger *et al*., 1993) and improving clinical outcome in patients with CVD (reviewed by O'Connor *et al*., 1989; Shephard and Balady, 1999). It was well established that ExT in experimental conditions limits the I/R injury, improves postischemic cardiac function and vascular tone regulation and attenuates inflammatory response and apoptosis. Yet, the role of ExT in postischemic HF remains unclear.

It is suggested that ExT can provide cardioprotective action in a biphasic manner similar to ischemic preconditioning (reviewed by Marongiu and Crisafulli, 2014). In rats, the first window of ExT-induced cardioprotection seems to be between 0.5 to 3 hours after a single bout of ExT. The second window of more persistent cardioprotection is achieved within 24 hours after ExT and lasts for up to several weeks (Yamashita *et al*., 1999; Hoshida *et al*., 2002). Whereas some studies confirmed single bout-induced cardioprotection in rats (Yamashita *et al*., 1999 and 2001; Hoshida *et al*., 2002), the focus is mostly on exercise protocols lasting for several days to weeks.

It is known that a certain level of exercise is necessary for achieving beneficial effects. However, the threshold level for ExT necessary for cardioprotection remains inconclusive. Some studies show cardioprotection induced by ExT at 60 to 70% of the maximum rate of oxygen consumption (VO_2max) (Demirel *et al*., 2001; French *et al*., 2008), whereas others use protocols with intensity at 70 to 80% of VO_2max (Powers *et al*., 1998; Lennon *et al*., 2004). Esposito *et al*. (2011) even demonstrated that the infarct size-limiting effect is proportional to exercise intensity when protocols with 60% and 80% of VO_2max were compared. On the other hand, Lennon *et al*. (2004) reported similar postischemic recovery of cardiac function in rats adapted to moderate (55% of VO_2max) and intensive (75% of VO_2max) training

As Pica and Brooks (1982) published, laboratory rats exhibit low individual variability of VO_2max (recently confirmed by Qin *et al*., 2020), and several weeks lasting endurance-based ExT does not markedly increase its value in adult male rats. On the other hand, ExT based on consecutive bouts of high-intensity activity can increase the VO_2max up to 1.7 fold compared to sedentary controls (Wisløff *et al*., 2001). Since most experiments are based on endurance training, specific assessment

of VO_2max during ExT protocols is not always reported. Reaching the sufficient level of ExT is then simply demonstrated by the presence of cardioprotective effects.

Among various types of ExT, swimming, treadmill running and voluntary running are used in the vast majority of experiments. All of these approaches have certain limitations associated with ExT intensity and level of stress during the procedure. Swimming is a simple ExT protocol with consistent results (McElroy *et al*., 1978; Freimann *et al*., 2005; Zhao *et al*., 2018), but it is associated with a high level of stress. This can be illustrated by using this protocol in experimental models of stress response regulation (Young *et al*., 1993; Wotjak *et al*., 2001). Forced treadmill running is considered a less stressful protocol with the benefit of precise control of the ExT intensity and provides consistent cardioprotective actions (Musch *et al*., 1989; Noble *et al*., 1999; Yamashita *et al*., 1999). Voluntary running is the most natural and the least stressful ExT protocol, but the experimental results regarding cardioprotective effects are inconclusive. The positive effect of voluntary running was reported by Budiono *et al*. (2012) when tolerance to myocardial ischemia was increased in mice. De Waard *et al*. (2009) showed improved postischemic cardiac function in voluntary running mice. Contrary to that, no effect of voluntary running on postischemic HF in rats was observed by Starnes *et al*. (2005). This is likely caused by individual differences in ExT intensity and volume, while the pattern of cardiovascular changes is similar to those in treadmill running (Yancey and Overton, 1993).

1.4.3.1. Cardioprotective effects of exercise training

Cardioprotective phenotypes in ExT are similar regardless of the ExT protocol. Infarct size-limiting effect can be acquired by forced swimming (McElroy *et al*., 1978; Freimann *et al*., 2005), treadmill running (Yamashita *et al*., 1999; Brown *et al*., 2003; French *et al*., 2008) and voluntary running (Budiono *et al*., 2012; Pósa *et al*., 2015). The occurrence of I/R arrhythmias can be reduced by treadmill running (Hoshida *et al*., 2002; Hamilton *et al*., 2004) or forced swimming (Bélichard *et al*., 1992).

While the mechanism of ExT-induced cardioprotection is not fully elucidated, it seems to be a result of several complex mechanisms rather than one molecular pathway. As reviewed by Laughlin *et al*. (2012), ExT leads to structural and functional changes in the coronary circulation exhibited as increased arteriolar density and diameter and by enhanced endothelium-dependent vasodilatation (Laughlin *et al*., 2012). Similar alterations were confirmed in various experimental models (McElroy *et al*., 1978; Freiman *et al*., 2005). But since the structural changes in coronary circulation do not occur within several days (Yamashita *et al*., 1999), this effect does not contribute to acute ExT-induced cardioprotection.

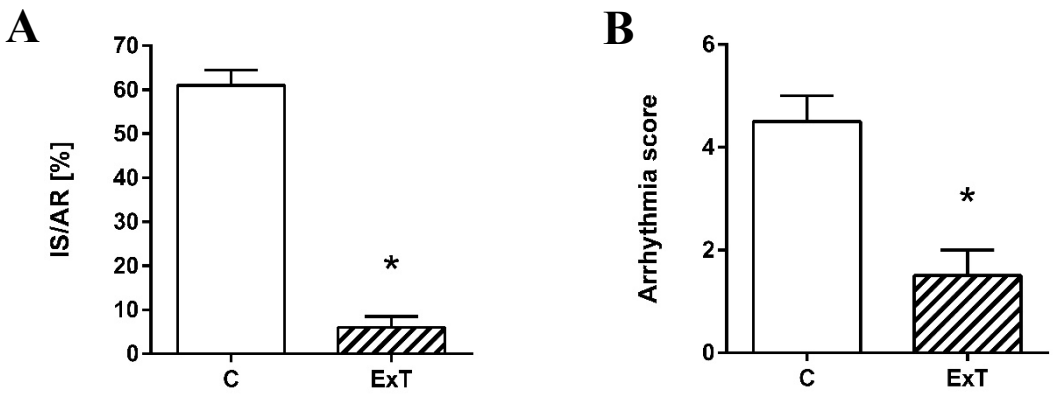

Figure 5 Examples of cardioprotective effects of exercise training. Reduction of infarct size, normalized to the area at risk (**A**; IS/AR, French *et al*., 2008) and decreased arrhythmia score (**B**; Hamilton *et al*., 2004). Control group (C), trained animals (ExT).

Several studies showed that ExT alters the nitric oxide (NO) signalling in both humans and animals (Davis *et al*., 2004; Green *et al*., 2004). It is suggested that increased NO levels contribute to attenuated apoptosis by enhanced S-nitrosylation of the cardiac proteins. NO can also reduce the mitochondrial ROS production during I/R by modifying the complex I (reviewed by Calvert and Lefer; 2013). As reviewed by Powers *et al*. (2008) and Frasier *et al*. (2011), the production of ROS in animals subjected to ExT is also reduced by an increase in synthesis and activation of superoxide dismutase (SOD), which is an enzyme that converts superoxide radicals to less reactive and further eliminated hydrogen peroxide. Several studies confirmed ExT-induced increase in SOD isoform SOD2 localized in the mitochondrial matrix (Hamilton *et al*., 2004; Yamashita *et al*., 1999; French *et al*., 2008; reviewed by Powers *et al*., 2008) and isoform SOD1 localized in the cytosol and mitochondrial intermembrane space (Lee *et al*., 2012). Limited ROS production then contributes to attenuated early postischemic apoptosis and necrosis as described previously. Furthermore, it has been observed that the cardioprotective effect of exercise may be at least partly due to the effect on the MPTP, similarly to some other protective phenomena (Kavazis *et al.*, 2008).

The previously mentioned mechanisms have the potential to contribute positively to the improvement of postischemic cardiac function and attenuation of postischemic cardiac remodelling. Despite that, the role of ExT in postischemic HF remains inconclusive. Freimann *et al*. (2005) demonstrated improved cardiac function and decreased level of LV fibrosis four weeks after MI in rats subjected to forced swimming prior to the I/R insult. De Waard *et al*. (2007) reported improved systolic function, but no changes in either MI size or LV remodelling in mice trained early after MI (voluntary running), suggesting the beneficial role of improved Ca^{2+} handling. Guizoni *et al*. (2016) reported improved LV systolic function even in rats trained (treadmill running) 3 months after MI, where the effect on early myocardial scar formation can be excluded. On the other hand, Musch *et al*.

(1989) reported improved hemodynamics but not cardiac function in rats subjected to treadmill running for 10 weeks after MI.

1.4.3.2. Adverse effects of exercise training

Despite the predominantly positive effects of ExT on the prevention and management of various diseases, certain adverse effects of ExT on cardiovascular health have been reported. O´Keefe *et al.* (2012) tried to identify risk factors of adverse cardiovascular changes in highly trained individuals. His work showed that individuals exposed to long-lasting intensive physical stress such as ultramarathons, distance triathlons and endurance cycling might exhibit signs of acute volume overload of the atria and RV with transient reduction of RV ejection fraction. These changes were associated with acutely elevated levels of biomarkers of cardiac injury. Chronic (months to years) exposure to such physical stress than in some individuals led to patchy myocardial fibrosis, creating a substrate for ventricular arrhythmias. It was also hypothesized that long-term excessive ExT might be associated with coronary artery calcification, diastolic dysfunction and large-artery wall stiffening.

Experimental models of prolonged endurance training (60 min/day, 16 weeks) showed LV and RV hypertrophy, diastolic dysfunction and dilatation of left and right atrium when compared to sedentary controls (Michaelides *et al.*, 2011; Benito *et al.*, 2011). These changes in cardiac function and geometry were accompanied by increased collagen deposition in all heart segments and markedly increased inducibility of ventricular tachycardia. Interestingly, the adverse effects of ExT were significantly regressed 8 weeks after its cessation. Therefore, it can be suggested that only prolonged and intensive ExT can induce adverse cardiovascular changes in healthy individuals, and these changes exhibit relatively high plasticity regarding the cessation of the exercise stimuli.

We can summarise that protocols of ExT with cardioprotective effect in acute I/R injury have a certain potential to exhibit beneficial action even in postischemic HF. On the other hand, the finding of adequate intensity and type of exercise seems to be a limiting factor for more conclusive results.

1.4.4. Epoxyeicosatrienoic acids

It has been shown that epoxyeicosatrienoic acids (EETs) affect numerous biological mechanisms associated with cardiovascular diseases, including regulation of vascular tone, modulation of inflammatory responses, reduction of I/R injury or lowering the blood pressure (reviewed by Oni-Orisan *et al*., 2014). These multipotent properties qualify EET-based therapies to be a promising tool in the treatment and management of CVD.

Eicosanoids are signalling molecules metabolized from arachidonic acid or similar polyunsaturated fatty acids with 20 carbon chain by enzymatic or non-enzymatic oxidation. Three metabolic pathways of eicosanoid synthesis are known. The cyclooxygenase pathway leads to prostanoids, mediators of inflammatory and anaphylactic reaction or vasoconstriction. The lipoxygenase pathway leads to leukotrienes, mediators of pro-inflammatory response. The third pathway is mediated by the cytochrome P-450 (CYP) that possess two distinct enzymatic activities, hydroxylase activity and epoxygenase activity. CYP hydroxylase activity leads to hydroxyeicosatrienoic acids (HETEs), whereas CYP epoxygenase produces EETs. Based on the epoxide group position, four isomers of EETs can be distinguished: 5,6-EETs, 8-9-EETs, 11,12-EETs and 14,15-EETs. Endogenous EETs are predominantly metabolized by soluble epoxide hydrolase (sEH) to their corresponding dihydroxyeicosatrienoic acids (DHETs; reviewed by Imig, 2012 and Yang *et al*., 2015b).

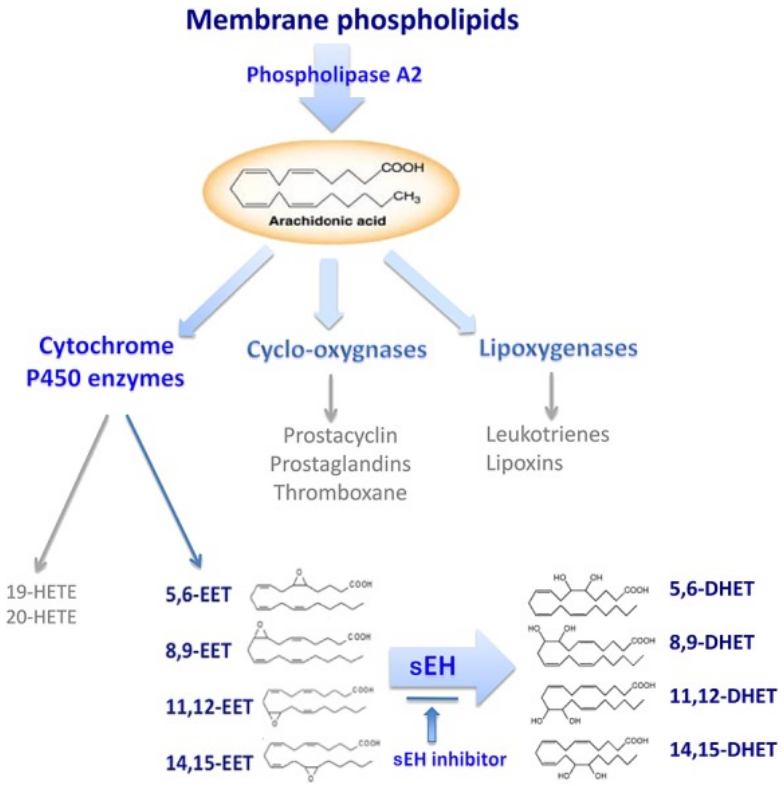

Figure 6 Metabolism of epoxyeicosatrienoic acids. Yang *et al*., 2015b.

Whereas the protective role of EETs was reported in numerous experimental setups, increased levels of EETs seem to be essential for achieving these effects (reviewed by Imig, 2018). Simultaneously, the biological actions of endogenous EETs are naturally limited by their rapid conversion to DHETs. Investigation of EETs actions is, therefore, associated with strategies that can increase tissue levels of EETs. These strategies are mostly based on the application of novel and more biologically stable exogenous EET analogues (reviewed by Campbell *et al*., 2017) and inhibitors of

sEH, which increased the bioavailability of the endogenous EETs (reviewed by Oni-Orisan *et al*., 2014).

1.4.4.1. Cardioprotective effects of EET-based therapies

The potential of the EET-based treatments to limit I/R injury was demonstrated by Nithipatikos *et al*. (2006) when pretreatment with 11,12-EET but not with 14,15-EET limited the infarct size in dogs. Similarly, Gross *et al*. (2008) showed that the administration of 11,12-EET and 14,15-EET but not 8,9-EET limited the infarct size in rats subjected to 30-min ischemia and 120-min reperfusion. Improved recovery of myocardial function after I/R insult was also observed in a transgenic mouse model with human cardiac CYP (CYP2J2) and in mice with a targeted disruption of the sEH-encoding gene (Ephx2; Seubert *et al*., 2004 and 2006, respectively).

Comparable cardioprotective effects were also observed for EET mimetics and analogues and for sEH inhibitors. For example, Batchu *et al*. (2012) reported improved LV function recovery in the Langendorf perfused mice heart subjected to I/R and treated with the EET mimetic UA-8. Neckář *et al*. (2018) showed the infarct size limiting effect of EET analogue EET-B that was comparable to the effect of 14,15-EET. Similar results were reported by Motoki *et al*. (2008) when the administration of 14,15-EET or sEH inhibitor AUDA provided the same infarct size-limiting effect. Interestingly, similar effects were acquired by applying EET-B, 14,15-EET or AUDA before or during ischemia (Neckář *et al*., 2018; Motoki *et al*., 2008).

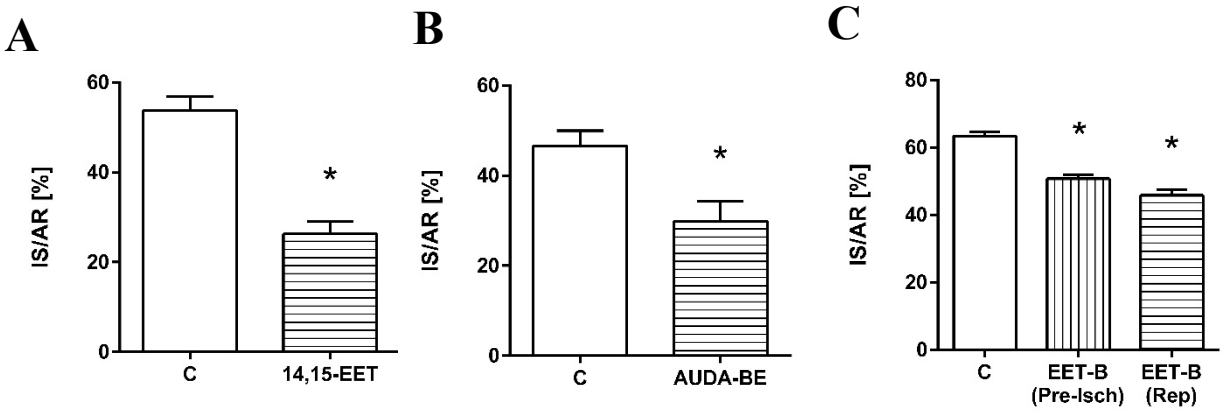

Figure 7 Examples of cardioprotective effects of EET-based treatments in rats. Reduction of infarct size in animals treated with 14,15-EET and AUDA-BE prior to the I/R insult (**A** and **B**; Motoki *et al.*, 2008) and in animals treated with EET-B before ischemia or during reperfusion (**C**, Neckář *et al.*, 2018) Infarct size/area at risk; IS/AR.

Beneficial effects on cardiac function are also reported in postischemic EET-based therapy. Improved LV function and absence of LV dilatation was observed in mice treated with EET agonist NUDSA after coronary ligation (Cao *et al*., 2015). Kompa *et al*. (2013) reported improved systolic

function and attenuated cardiac fibrosis in rats with therapeutic administration of sEH inhibitor GSK2188931B after MI. This was associated with prevented infiltration of macrophages into the peri-infarcted zone but not into the infarcted area. Merabet *et al*. (2012) showed improved LV function in rats treated with sEH inhibitor AUDA after MI, associated with decreased ROS levels. Another sEH inhibitor TPPU caused improved cardiac function and reduced migration and proliferation of fibroblasts in the heart after MI, as demonstrated by Sirish *et al*. (2013). Interestingly, these beneficial effects were achieved either by administration of sEH inhibitors immediately after coronary occlusion (Kompa *et al*., 2013), 8 and 47 days after (Merabet *et al*., 2012) or 7 days after (Sirish *et al*., 2013).

Pretreatment with EETs also attenuates the apoptotic signalling in cultured cells from neonatal rat heart exposed to hypoxia and reoxygenation (Dhanasekaran *et al*., 2008). Antiapoptotic properties were also shown in isolated human cardiomyocytes (Bodiga *et al*., 2009). It is suggested that these effects are associated with activation of sarcolemmal and mitochondrial K_{ATP} channels, while their inhibition blunted the cardioprotection.

Experimental results confirmed the cardioprotective potential of EET-based treatments in numerous models of CVD. Moreover, several studies showed an increased risk of CVD in individuals with CYP gene polymorphism (reviewed by Imig, 2018). It was also shown that increased activity of sEH in patients with established atherosclerotic CAD is associated with worsened prognosis (Schuck et al., 2013). On the other hand, increased plasma levels of EETs were found in patients with CAD (Wang *et al.*, 2010; Theken *et al.*, 2012) or after an ischemic stroke event (Ward *et al.*, 2011), suggesting the presence of a possible compensatory mechanism following the ischemic events. It is, therefore, hypothesised that EET-based treatment might provide clinically relevant beneficial effects in CVD patients, and several ongoing clinical trials aim to address this topic.

1.4.4.2. Antihypertensive effects of EET-based therapies

Hypertension is a severe medical condition and a frequent risk factor for CVD. Mechanisms responsible for development of hypertension are therefore studied in various experimental models. The spontaneously hypertensive rats (SHR), also used in our study, were first described by Okamoto and Aoki (1963) and are by far the most popular animal model of hypertension. This inbred rat strain exhibits a type of hypertension analogous to essential hypertension in humans (Adams *et al.*, 1989; Okamoto and Aoki, 1963). Increased blood pressure in SHR leads to compensated LV concentric hypertrophy even in the young animal in the pre-hypertensive stage (Sen *et al.*, 1974; Engelmann *et al.*, 1987; Dickhout and Lee, 1994). These adaptive changes in cardiac morphology are accompanied by myocardial fibrosis and a decrease in microvascular density. Chronic pressure overload then

results in the impairment of cardiac function. Old SHR animals exhibit signs of reinforced local fibrosis, focal ischemic myocardial lesions (Herrmann *et al.*, 1995), and the compensated LV hypertrophy transits into HF (Engelmann *et al.*, 1987; Conrad *et al.*, 1995). Similar adaptive changes in cardiac geometry and function were also observed in transgenic rats with overexpression of mouse Ren-2 (TGR) (Bachmann *et al.*, 1992), used as the second experimental model of hypertension in our study. Villareal *et al.* (1995) reported concentric hypertrophy in 16 weeks old TGR accompanied with perivascular but not diffuse fibrosis and increased LV stiffness

Antihypertensive actions of EET-based therapies were confirmed in several experimental models of hypertension. EET analogue NUDSA was the first to decrease blood pressure in spontaneously hypertensive rats (SHR; Imig *et al*., 2010). A similar effect was observed in the novel, orally active analogues EET-A and EET-B when administered for two weeks in SHR (Hye Khan *et al*., 2014) and EET-A lowered blood pressure in angiotensin II-dependent model of hypertension in Cyp1a1-Ren-2 transgenic rats (Jíchová *et al*., 2016). Antihypertensive effect was also observed in rats with angiotensin II infusion-induced hypertension when treated with sEH inhibitor NCND (Imig *et al*., 2002) or in mice with angiotensin II-induced hypertension treated with sEH inhibitor AUDA (Jung *et al*., 2005). On the other hand, antihypertensive effects were not seen with EET-A and EET-B treatment in Dahl salt-sensitive rats (Hye Khan *et al*., 2013), Goldblatt hypertensive rats (Alánová *et al*., 2015) or in Cyp1a1-Ren-2 transgenic rats (Jíchová *et al*., 2016).

As reviewed by Imig (2018), the antihypertensive effect of EET-based therapies seems to be robust, especially in angiotensin II-dependent models of hypertension. Moreover, they provide renoprotective effects and anti-inflammatory effect in a number of organs.

1.4.4.3. Adverse effects of EET-based therapies

Recent studies declare the potential adverse effect of sHE inhibitors associated with angiogenesis (Michaelis *et al*., 2003), tumorigenesis (Pozzi *et al*., 2010) and metastasis (Panigrahy *et al*., 2012; Wei *et al*., 2014). Whereas angiogenesis can be helpful in ischemic tissue revascularization, its overall effect might differ depending on specific cardiovascular or renal diseases (reviewed by Khurana *et al*., 2005). Angiogenetic and antiapoptotic actions might further contribute to negative outcomes in tumorigenesis and metastasis conditions. Panigrahy *et al*. (2012) demonstrated that sEH inhibitors enhanced tumorigenesis and metastasis in lung small cell carcinoma in mice, whereas Zhang *et al*. (2013) showed the opposite effect in dextran sulfate sodium-induced colitis in mice.

A negative effect of increased EET levels was also reported by Hutchens *et al*. (2008), where mice with sEH gene deletion or sEH inhibitors treatment exhibited prolonged recovery after cardiopulmonary resuscitation.

To summarise, experimental data show that EET-based therapies are a promising tool to target not only the adverse effects of acute I/R injury and following cardiac remodelling but also other cardiovascular comorbidities. Novel orally active EET analogues EET-A and EET-B showed antihypertensive actions similar to previously describe EET analogues with cardioprotective effects. It can be therefore hypothesized that their administration would lead to similar cardioprotection. Considering that, they could even manifest beneficial effects in postischemic HF in hypertensive animals.

2. AIMS OF THE THESIS

As mentioned previously, the failure to find a cardioprotective therapy despite 50 years of research should not put into doubt the ischemia/reperfusion injury as a viable target for protection. The novel protective strategies are still required to attenuate the detrimental effects of acute myocardial ischemic injury, prevent adverse left ventricle remodelling, and reduce the heart failure.

We have, therefore, tried to apply a novel approach to cardiac protection: we have studied (i) the preventive and therapeutic effects of adaptation to continuous normobaric hypoxia and exercise training on postischemic heart failure, and (ii) the cardioprotective potential of epoxyeicosatrienoic acid-based therapy to limit acute ischemia/reperfusion injury and to prevent the postischemic heart failure.

We tested, therefore, the hypothesis that the adaptation to continuous normobaric hypoxia or exercise training as well as the epoxyeicosatrienoic acid-based treatment are able to reduce the detrimental effects of postischemic heart failure.

The specific aims of this thesis were:

1. To evaluate the effect of continuous normobaric hypoxia and exercise training applied prior to the ischemia/reperfusion insult on postischemic heart failure in rats.

2. To evaluate the therapeutic effect of continuous normobaric hypoxia and exercise training on postischemic heart failure in rats.

3. To evaluate the effect of epoxyeicosatrienoic acid analogue EET-B on postischemic heart failure in spontaneously hypertensive rats.

4. To evaluate the effect of epoxyeicosatrienoic acid analogue EET-A and soluble epoxide hydrolase inhibitor *c*-AUCB on postischemic heart failure in normotensive rats and Ren-2 transgenic rats with angiotensin II-dependent hypertension.

3. MATERIAL AND METHODS

3.1. Animals

The experiments were conducted in accordance with the Guide for the Care and Use of Laboratory Animals published by the National Academy of Science, National Academy Press, Washington DC. The experimental protocols were approved by the Animal Care and Use Committee of the Institute of Physiology of the Czech Academy of Sciences.

Male Wistar rats and spontaneously hypertensive rats (SHR) were bred at the Institute of Physiology of the Czech Academy of Sciences. Male Hannover Sprague Dawley (HanSD) rats and heterozygous *Ren-2* transgenic (TGR) rats were bred at the Center of Experimental Medicine of the Institute for Clinical and Experimental Medicine. All animals were housed in the standard breeding box (2 or 3 animals per box) with a controlled environment (room temperature 22.5 °C – 23.5 °C; 12 h light-dark cycle, light from 6:00 AM) and access to standard chow and drinking water *at libitum* if not stated otherwise. Experiments were conducted with 10-week old rats (Wistar and SHR) or 12-week old rats (HanSD and TGR).

3.2. Experimental protocols of continuous normobaric hypoxia

Wistar rats were exposed to continuous normobaric hypoxia (CNH) (inspired O_2 fraction 0.12) in the normobaric chamber (6 m^3) equipped with hypoxic generators (Everest Summit, Hypoxico, New York, New York, United States of America). This exposure lasted for 3 weeks prior to the MI with one reoxygenation during this period in CNH + MI group (60 min – echocardiography examination). In MI + CNH animals, hypoxia exposure lasted for 3 weeks starting 7 days after MI with one reoxygenation event (60 min – echocardiographic examination). Protocols of CNH experimental groups are summarised in **Figure 8A**.

3.3. Experimental protocols of exercise training

Wistar rats assigned to exercise training (ExT) prior to the MI were habituated to forced treadmill running (Columbus Instrument, Columbus, Ohio, United States of America) by increasing speed (from 15 to 30 m/min) and duration (from 10 to 50 min/day) during the first 5 days of the exercise protocol. After 2 days of rest, the protocol continued with 5 more exercise sessions (30 m/min, 60 min/day, treadmill inclination 0°) and 2 days of rest before MI.

Wistar rats assigned to exercise training after MI started the habituation 7 days after MI with a less intensive protocol (speed from 10 to 15 m/min and duration from 10 to 50 min/day) in the first 5 days, followed by 2 days of rest. Exercise training then continued for 2 more weeks in a similar regime (15 m/min, 60 min/day; 5 days of exercise followed by 2 days of rest). Protocols of ExT are summarized in **Figure 8B**.

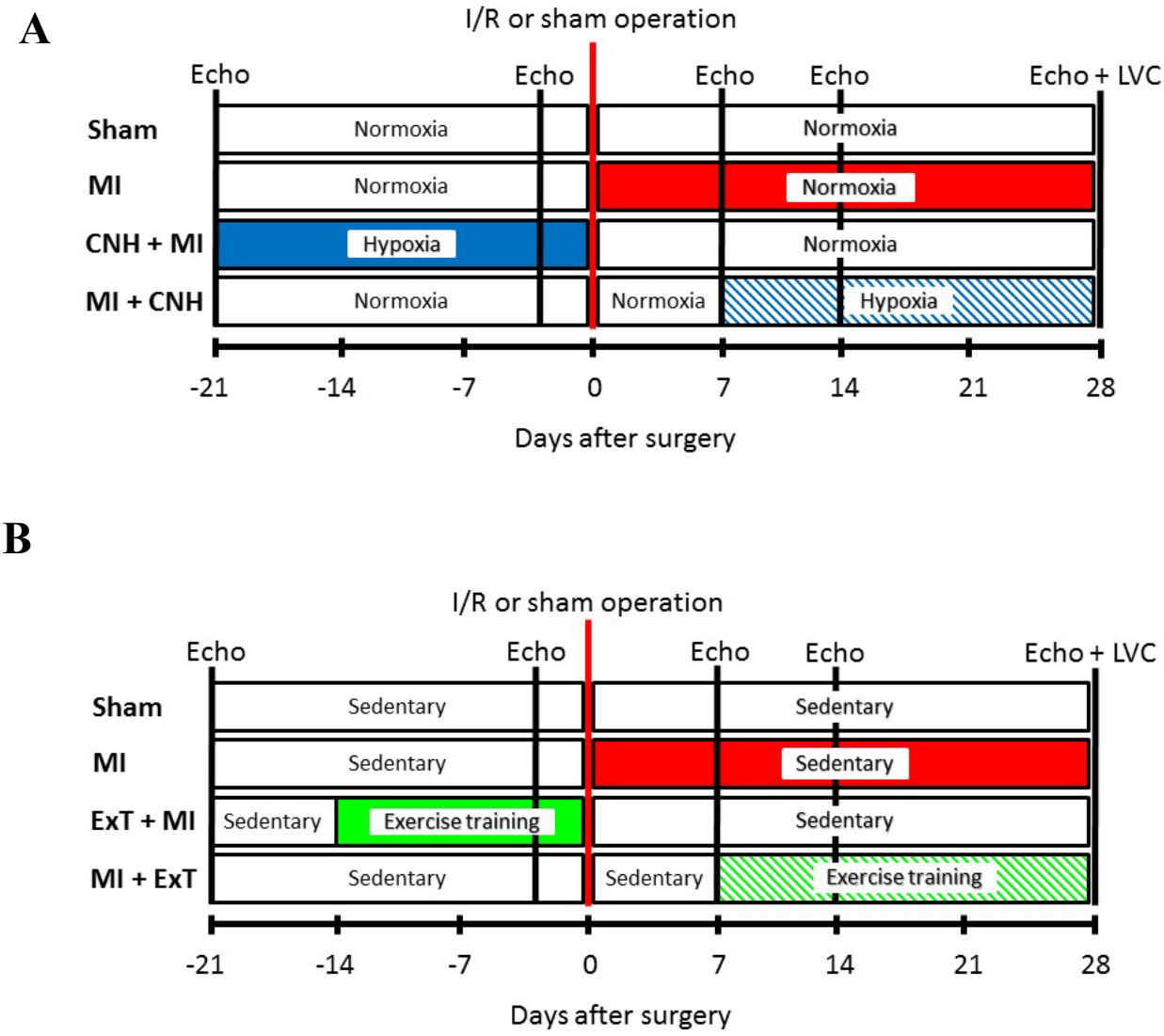

Figure 8 Experimental protocols of continuous normobaric hypoxia (CNH; **A**) and exercise training (ET; **B**). Sham-operated animals (Sham), animals with myocardial infarction (MI), animals adapted to CNH before MI (CNH + MI), animals adapted to CNH after MI (MI + CNH), animals trained before myocardial infarction (ExT + MI), and trained after MI (MI + ExT), ischemia/reperfusion (I/R), echocardiographic examination (Echo), left ventricle catheterization (LVC).

3.4. Experimental protocols of EET-based treatment

SHR were treated with N-(5-((2-cetamidobenzo[d]thiazol-4-yl)oxy)pentyl)-N-isopropylheptanamide (EET-B; 10 mg/kg/day, p.o.) for 2 weeks prior to I/R and 7 weeks thereafter.

Control groups received vehicle (0.05 % ethanol and 0.10 % polyethylene glycol 400). Experimental protocols of EET-B are summarized in **Figure 9A**.

HanSD and TGR were treated with sodium (*S*)-2-(Z)-(13-(3-pentyl)ureido)-tridec-8(*Z*)-enamido)succinate (EET-A; 10 mg/kg/day, p.o.) and/or *cis*-4-[4-(3-adamantan-1-yl-ureido)-cyclohexyloxy]-benzoic acid (*c*-AUCB; 1 mg/kg/day, p.o.) since 24 hours after I/R in drinking water. Experimental protocols of EET-A and/or *c*-AUCB are summarized in **Figure 9B**.

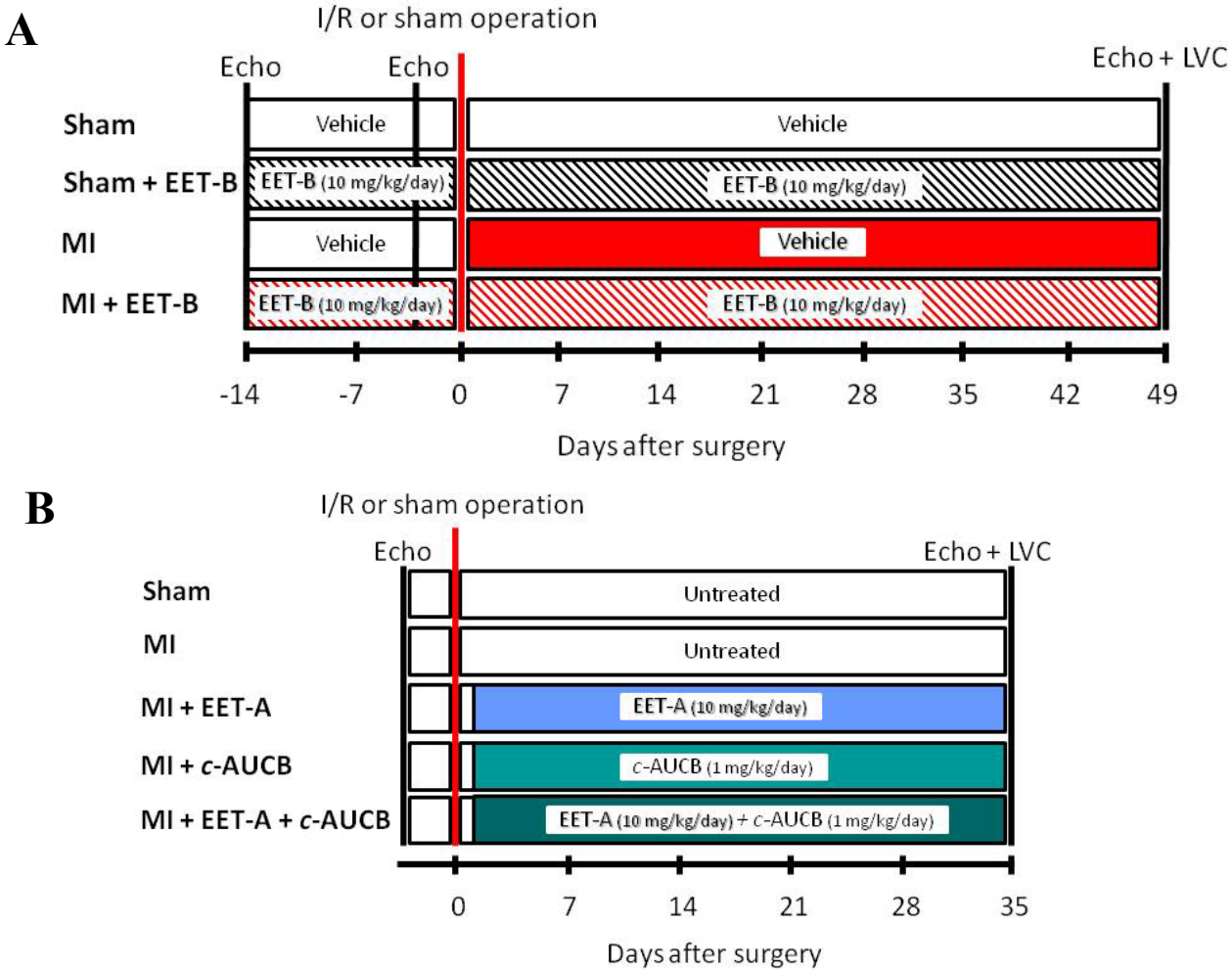

Figure 9 Experimental protocols of EET-based treatment. Untreated sham-operated animals (Sham), sham-operated animals with EET-B treatment (Sham+ EET-B), untreated animals with MI (MI), MI animals treated with EET-B (MI + EET-B), MI animals treated with EET-A (MI + EET-A), MI animals treated with *c*-AUCB (MI + *c*-AUCB), MI animals treated with EET-A and *c*-AUCB (MI + EET-A + *c*-AUCB), ischemia/reperfusion (I/R), echocardiographic examination (Echo), left ventricle catheterization (LVC).

3.5. Model of postischemic heart failure

Adult male rats were anaesthetized with sodium pentobarbital (60 mg/kg, i.p., Sigma Aldrich, St. Louis, Missouri, United States of America). Rats were intubated by endotracheal insertion of

polyethylene 12 gauge, connected to a respiratory device (Ugo Basile, Gemonia, Italy), and ventilated with room air at 68 strokes/min (individual tidal volume 1.2 ml per 100 g of body weight). Rectal temperature was monitored and maintained between 36.5 °C and 37.5 °C with a heated operating table. The left thoracotomy was performed, and the pericardium was removed. Silk ligature (6/0 silk, Chirmax, Prague, Czech Republic) was inserted 1-2 mm distal to the origin of the left anterior descending coronary artery, and the occlusive snare was placed around it. The ends of the suture were threaded through a 12-gauge polyethylene tube. After that, ECG was attached, and animals were stabilized for 10 minutes before the suture was tightened. In sham-surgery, animals were treated the same way without tightening the suture. Ischemic insult was confirmed by the presence of ischemic arrhythmias in ECG and characteristic change in local myocardial pigmentation. The 60-min occlusion in Wistar rats, HanSD and TGR or 30-min occlusion in SHR ended by releasing the snare and was followed by 5-min reperfusion. After the reperfusion period, ECG recording was terminated, the chest was closed by 2-3 intercostal stitches, remaining air or blood was extracted from the chest cavity, and the wound was closed by stitches (3/0 silk, Chirmax, Prague, Czech Republic). Animals were given analgesia (Ibuprofen, 6 mg p.o.; Reckitt Benckiser, Prague, Czech Republic) and monitored until partially awaken. After that, rats were extubated, housed in separate cages for 24 hours, and analgesia was administered for 2 more consecutive days (Ibuprofen, 6 mg/day p.o., Reckitt Benckiser, Slough, United Kingdom).

3.6. Echocardiographic assessment of left ventricle geometry and function

Geometry and function of the left ventricle were assessed using GE Vivid 7 Dimension and (GE Vingmed Ultrasound, Horten, Norway) and GE M12L linear probe (GE Vingmed Ultrasound, Horten, Norway). Conscious animals were initially anaesthetized with 3% isoflurane anaesthesia (Forane, Abbott Laboratories, Queenborough, United Kingdom), then placed on the heating pad in the supine position and further anaesthetized with 2% isoflurane. The rectal temperature was maintained between 36.5 °C and 37.5°C, and heart rate was monitored. The left chest area was shaved during 4-minute stabilization before the echocardiographic examination. Echocardiographic examination in CNH and ExT experiments was performed at the beginning of the experiment, 3 days prior to the surgery, and 7, 14, and 28 days after surgery. Echocardiographic examination in the EET-B experiment was performed at the beginning of the experiment, 3 days prior to the surgery and 49 days after surgery. Echocardiographic examination in EET-A and/or *c*-AUCB experiments was performed 3 days prior to the surgery and 35 days after surgery.

Basic left ventricle geometry was assessed in 2-D mode and M-mode in long and short axis. Following parameters of LV geometry were assessed: end-diastolic and end-systolic LV cavity

diameter (LVD_d, LVD_s), anterior wall thickness (AWT_d, AWT_s), posterior wall thickness (PWT_d, PWT_s). Fractional shortening (FS; 1.1) and relative wall thickness (RWT; 1.2) were derived as indicated below. Heart rate (HR) was assessed from 5 to 10 consecutive cardiac cycles.

$$FS = 100 \times \frac{LVD_d - LVD_s}{LVD_d} \quad (1.1)$$

$$RWT = 100 \times \frac{AWT_d + PWT_d}{LVD_d} \quad (1.2)$$

Mitral valve flow was assessed in the 4-chamber view, and pulmonary artery flow was assessed in the 2-D mode in long and short axis, all using pulse Doppler measurement. Following parameters of blood flow at the mitral valve were measured (as demonstrated in **Figure 10A**): maximal velocity of blood flow (Vm_{max}), filling time (FT), isovolumic contraction time (IVCT), ejection time (ET), and isovolumic relaxation time (IVRT).

For pulmonary artery blood flow following parameters were measured (as demonstrated in **Figure 10B**): maximal blood flow velocity (Vpa_{max}), mean blood flow velocity (Vpa_{mean}), acceleration time to Vpa_{max} (AT), and ejection time (ETpa) were measured. All echocardiographic records were analysed in EchoPAC PC 112 (GE Healthcare, Horten, Norway).

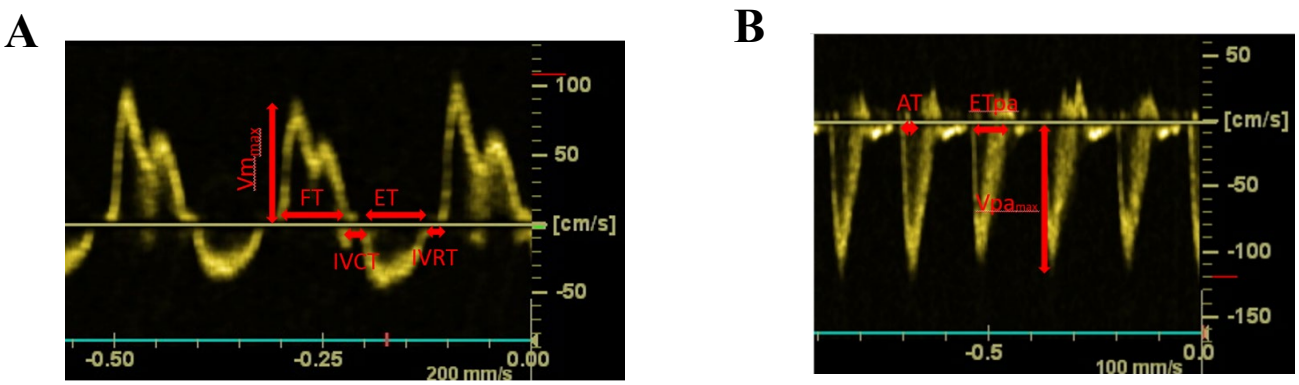

Figure 10 Assessment of echocardiographic parameters of blood flow at the mitral valve (**A**) and in the pulmonary artery (**B**).

3.7. Heart catheterisation

At the end of the study, the echocardiographic examination was immediately followed by LV catheterisation. Rats were kept in 2% isoflurane anaesthesia. Right carotid was exposed by neck incision, and SPR-407 microtip pressure catheter (Millar, Houston, Texas, United States of America) was introduced to LV. After adjusting the proper position of the catheter, the animals were stabilised for 10 minutes. Data were acquired by MPVS 300 (Millar, Houston, Texas, United States of America) and PowerLab 8/30 transducers (ADInstruments, Oxford, United Kingdom) and LabChart Pro

software (ADInstruments, Oxford, United Kingdom).

All data were acquired by repeated measuring of 5 consecutive pressure cycles. Following parameters were evaluated: heart rate (HR), end-diastolic pressure (P_{ed}), end-systolic pressure (P_{es}), developed pressure (P_{dev}), peak rate of pressure development ($+(dP/dt)_{max}$), peak rate of pressure decline ($-(dP/dt)_{max}$).

3.8. Scar circumference

At the end of the study, hearts were excised, washed with Tyrode´s solution, perfusion fixed, and stored in 4% paraformaldehyde for 2 days at 4°C. Hearts were cut perpendicularly to the long axis at the largest circumference, embedded in paraffin, sectioned (9-µm slices), and stained with Picrosirius Red (Sigma-Aldrich, St. Louis, Missouri, United States of America). Slices were recorded with Olympus VS 100-S1microscope (lens magnification 20x; Olympus, Hamburg, Germany) traced with computer planimetry (OlyVIA 2.4, Olympus, MIS Elements 4.11, Laboratory Imaging, Prague Czech Republic). The scar circumference (SC) was derived, as indicated below (1.3). Final SC was averaged from one mid-cavity and one apical histological slice.

$$\mathrm{SC} = 100 \times \frac{\text{scar midwall lenght}}{\text{LV midwall circumferrence}} \tag{1.3}$$

3.9. Statistical analysis

All data are expressed as mean ± standard error of the mean (SEM). Statistical analysis of the data was performed using GraphPad Prism 6 (Graph Pad Software, San Diego, California, United States of America). For the incidence of mortality, Fisher´s exact test was used. For multiple comparisons and differences between groups, one-way analysis of variance (ANOVA) and Holm-Šidák´s multiple comparison *post hoc* test were used. Values were considered statistically significant if exceeding 95% probability limits ($P < 0.05$).

4. RESULTS

4.1. Effect of continuous normobaric hypoxia and exercise training prior to ischemia/reperfusion insult on postischemic heart failure

In this study, we tried to determine the effect of two protocols with cardioprotective potential on postischemic heart failure. Animals were adapted to CNH for 3 weeks (CNH + MI) or exercise training for 2 weeks (ExT + MI). After that, 60-min coronary artery occlusion was performed, and cardiac function was assessed 1 and 4 weeks later. Two additional normoxic untrained groups were subjected to 60-min occlusion (MI) or sham-operation (Sham). Results of this were not published yet.

As shown in **Figure 11,** myocardial infarction led to 55 % mortality in the MI group (55/100) and 0 % mortality in the Sham group (0/12). Mortality in ExT + MI group was 44.4 % (8/18) and decreased to 26.3 % (5/19; significant) in CNH + MI group. Major causes of mortality in all groups were sustained ventricle fibrillation during the I/R period and sudden death within the following 48 hours (specific causes were not determined). The mortality did not change until the end of the experiment (4 weeks after MI) in any experimental group, with one exception in the MI group.

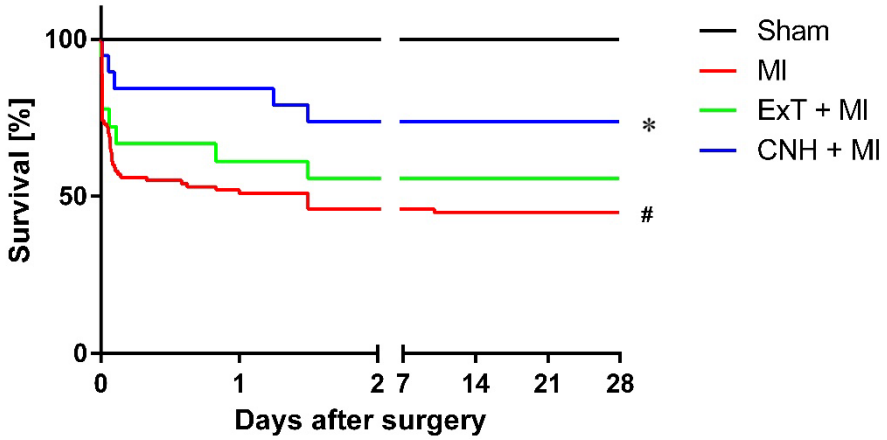

Figure 11 Survival of sham-operated animals (Sham), animals with myocardial infarction (MI), animals trained before myocardial infarction (ExT + MI) and animals adapted to continuous normobaric hypoxia before myocardial infarction (CNH + MI). # $p < 0.05$ MI vs. Sham, * $p < 0.05$ vs. MI.

Systolic function expressed as a fractional shortening (FS) did not differ between experimental groups at the beginning of the study (3 weeks before MI; data not shown) and were altered by neither CNH nor ExT compared to the MI group (**Figure 12**; 41.68 ± 0.72 % and 41.77 ± 0.48 %, respectively compared to 42.30 ± 0.58 %). As summarized in **Figure 12**, FS was not changed in the Sham group during the experiment. MI resulted in a major decrease of FS in the MI group (to 17.17 ± 0.89 %, 7 days after MI) and tended to further decrease until the end of the experiment. A similar effect was

observed in CNH + MI and ExT + MI groups, and we did not detect any significant differences in FS during the experiment compared to the MI group (28 days after MI FS: 15.06 ± 0.87 % and 14.18 ± 1.03 % compared to 15.02 ± 0.91 %).

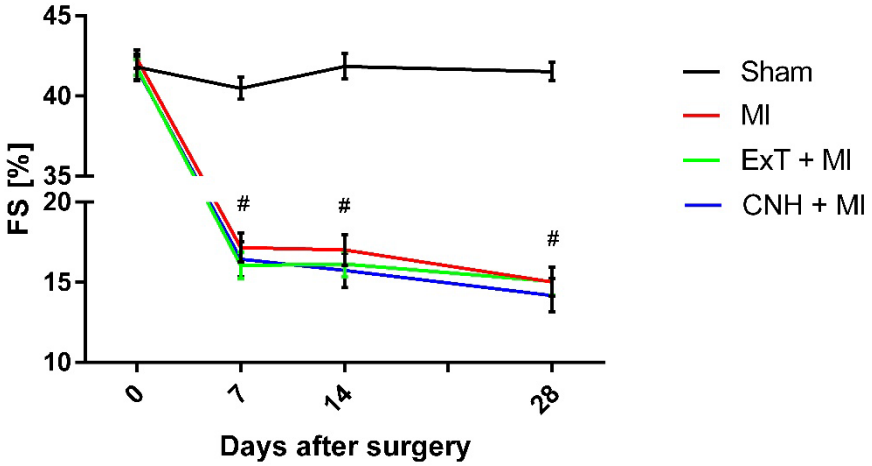

Figure 12 Fractional shortening (FS) in sham-operated animals (Sham), animals with myocardial infarction (MI), animals trained before myocardial infarction (ET + MI) and animals adapted to continuous normobaric hypoxia before myocardial infarction (CNH + MI). Values are expressed as mean ± SEM. # $p < 0.05$ MI vs. Sham.

Left ventricle cavity diameter in diastole (LVD_d) did not differ between experimental groups at the beginning of the study (data not shown). As shown in **Figure 13,** CNH but not ExT tended to decrease LVD_d before surgery compared to the MI group (7.39 ± 0.09 mm and 7.85 ± 0.10 mm compared to 7.83 ± 0.13 mm), but the effect was not significant. MI resulted in a progressive increase of LVD_d in all experimental groups, and neither CNH nor ExT had any significant effect compared to the MI group (LVD_d 4 weeks after MI: 11.31 ± 0.19 mm and 10.75 ± 0.27 mm compared to 11.17 ± 0.27 mm).

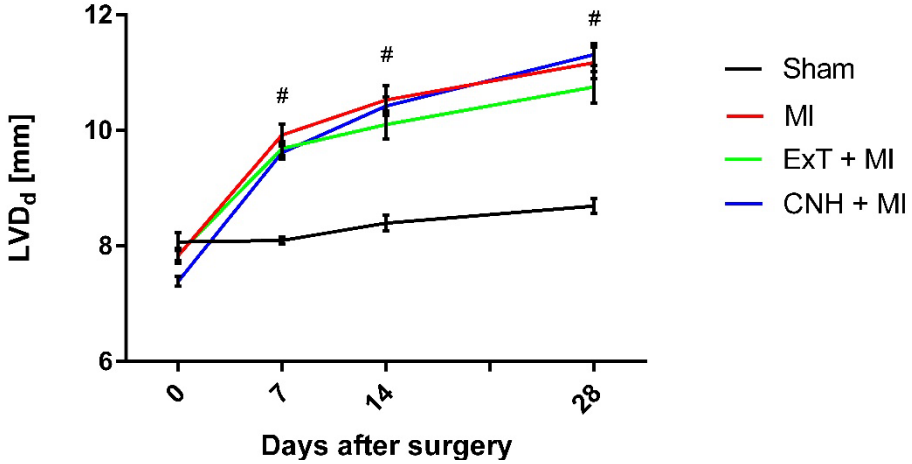

Figure 13 Left ventricle cavity diameter in diastole (LVD_d) in sham-operated animals (Sham), animals with myocardial infarction (MI), animals trained before myocardial infarction (ET + MI) and animals adapted to continuous normobaric hypoxia before myocardial infarction (CNH + MI). Values are expressed as mean ± SEM. # $p < 0.05$ MI vs. Sham.

As summarized in **Table 1**, CNH tended to increase both anterior and posterior wall thickness (AWT_d, PWT_d; before MI), but relative wall thickness (RWT) was not altered compared to the MI group (55.9 ± 1.3 % vs. 52.1 ± 1.6 %). Exercise training had no significant effect on the parameters of LV geometry before MI compared to the MI group.

MI resulted in a progressive increase of LV systolic diameter (LVD_s) that was altered by neither CNH nor ExT. In all experimental groups, MI led to a decrease in diastolic anterior wall thickness (AWT_d) compared to the Sham group (1.80 - 1.88 mm vs. 2.23 mm) and severe hypokinesis of the anterior wall that was exhibited as similar values of AWT_d and AWT_s (1.86 ± 0.03 mm and 1.86 ± 0.03 in MI group, respectively compared to 2.23 ± 0.08 mm and 3.60 ± 0.10 mm, respectively in Sham group). Systolic and diastolic posterior wall thicknesses (PWT) were affected by neither MI nor CNH and ExT. Relative wall thickness (RWT) was decreased in the MI group compared to the Sham group with any effect of neither ExT nor CNH.

Table 1 Parameters of LV geometry in sham-operated animals (Sham), animals with myocardial infarction (MI), animals trained before myocardial infarction (ExT + MI) and animals adapted to continuous normobaric hypoxia before myocardial infarction (CNH + MI). LVD_s, end-systolic LV diameter; AWT_s, end-systolic anterior wall thickness; AWT_d, end-diastolic anterior wall thickness; PWT_d, end-diastolic posterior wall thickness; PWT_s, end-systolic posterior wall thickness; RWT, relative wall thickness; HR, heart rate. Values are expressed as mean ± SEM. $^{\#}$ $p < 0.05$ MI vs. Sham.

	LVD_s [mm]	AWT_d [mm]	AWT_s [mm]	PWT_d [mm]	PWT_s [mm]	RWT [%]	HR [bpm]
3 days before MI							
Sham	4.74 ± 0.12	2.06 ± 0.03	3.19 ± 0.06	1.90 ± 0.03	2.84 ± 0.04	50.1 ± 0.7	372 ± 10
MI	4.57 ± 0.11	2.11 ± 0.04	3.24 ± 0.05	1.94 ± 0.04	2.98 ± 0.06	52.1 ± 1.6	382 ± 14
ExT + MI	4.61 ± 0.09	2.04 ± 0.05	3.26 ± 0.07	1.94 ± 0.09	2.92 ± 0.09	50.7 ± 0.7	384 ± 11
CNH + MI	4.28 ± 0.59	2.11 ± 0.04	3.36 ± 0.06	2.14 ± 0.07	3.21 ± 0.09	55.9 ± 1.3	369 ± 12
7 days after MI							
Sham	4.84 ± 0.05	2.26 ± 0.06	3.31 ± 0.09	1.92 ± 0.04	2.93 ± 0.06	51.7 ± 0.6	389 ± 8
MI	8.30 ± 0.21$^{\#}$	1.95 ± 0.07$^{\#}$	1.95 ± 0.06	1.93 ± 0.04	2.82 ± 0.06	38.8 ± 1.1$^{\#}$	378 ± 8
ExT + MI	8.14 ± 0.16	1.84 ± 0.06	1.89 ± 0.07	1.93 ± 0.06	2.66 ± 0.09	39.4 ± 1.8	378 ± 6
CNH + MI	7.94 ± 0.18	1.90 ± 0.08	1.86 ± 0.12	2.06 ± 0.05	2.92 ± 0.08	39.7 ± 1.0	385 ± 9
28 days after MI							
Sham	5.11 ± 0.07	2.23 ± 0.08	3.60 ± 0.10	2.02 ± 0.06	3.19 ± 0.07	46.7 ± 2.3	365 ± 5
MI	9.61 ± 0.30$^{\#}$	1.86 ± 0.03$^{\#}$	1.86 ± 0.03$^{\#}$	2.11 ± 0.04	2.95 ± 0.06	35.5 ± 0.8$^{\#}$	376 ± 7
ExT + MI	9.15 ± 0.30	1.80 ± 0.08	1.88 ± 0.07	2.14 ± 0.08	2.89 ± 0.10	38.6 ± 1.6	381 ± 11
CNH + MI	9.67 ± 0.24	1.88 ± 0.08	1.83 ± 0.04	2.29 ± 0.11	3.01 ± 0.18	36.3 ± 1.3	372 ± 10

Figure 14A illustrates the LV dilatation and MI scar size in the mid-cavity histological slices and a tendency for increased collagen content in the non-infarcted area in CNH + MI animals. As shown in **Figure 14B**, MI resulted in the scarification of 24.7 % of LV circumference, which was not affected

by CNH (25.0 ± 1.0 %) but increased in the ExT + MI group (37.0 ± 1.5 %). **Table 2** summarizes that body weight and relative kidney weight did not differ among experimental groups at the end of the study. Relative lung weight tended to increase in the MI group compared to the Sham group and was almost doubled in CNH + MI compared to the MI group.

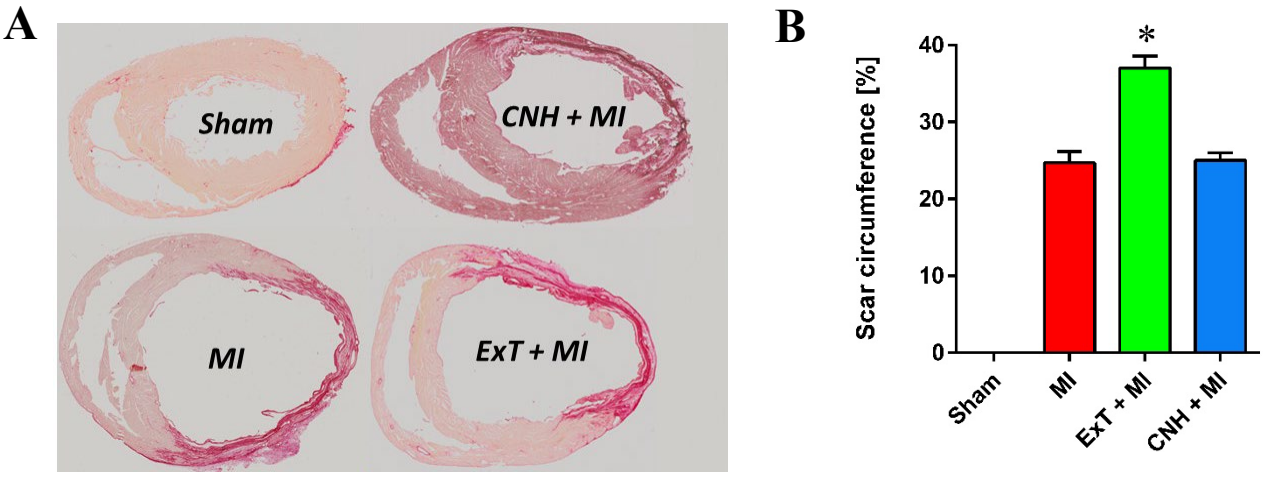

Figure 14 Histology of Picrosirius red-stained hearts at the mid-cavity level (**A**) and myocardial scar circumference (**B**) of sham-operated animals (Sham), animals with myocardial infarction (MI), animals trained before myocardial infarction (ExT + MI) and animals adapted to continuous normobaric hypoxia before myocardial infarction (CNH + MI). * $p < 0.05$ vs. MI.

Table 2 Body weight and relative organ weights at the end of the study in sham-operated animals (Sham), animals with myocardial infarction (MI), animals trained before myocardial infarction (ExT + MI) and animals adapted to continuous normobaric hypoxia before myocardial infarction (CNH + MI). BW, body weight. Values are expressed as mean ± SEM. * $p < 0.05$ vs. MI.

	BW [g]	Lungs/BW [mg/g]	Kidneys/BW [mg/g]
Sham	533 ± 44	3.01 ± 0.35	6.23 ± 0.53
MI	512 ± 40	3.82 ± 1.97	6.18 ± 0.72
ExT + MI	508 ± 65	3.30 ± 0.50	5.93 ± 0.41
CNH + MI	511 ± 55	6.42 ± 2.78*	6.01 ± 0.40

As summarized in **Figure 15**, MI resulted in decrease of maximal rate of pressure development $+(dP/dt)_{max}$ compared to Sham group (4359 ± 157 mmHg/s vs. 5202 ± 175 mmHg/s) and tended to increase maximal rate of pressure decrease $-(dP/dt)_{max}$ compared to Sham group (-3563 ± 142 mmHg/s vs. -4300 ± 196 mmHg/s). End-systolic pressure (P_e) was not altered in the MI group when compared to the Sham group (89.9 ± 3.3 mmHg vs. 93.9 ± 5.3 mmHg) and was increased by ExT (106.2 ± 5.3 mmHg). End-diastolic pressure (P_{ed}) tended to increase in the MI group when compared to the Sham group (14.86 ± 1.90 mmHg vs. 8.12 ± 1.19 mmHg, not significant), and CNH tended to amplify this tendency (20.9 ± 3.3 mmHg).

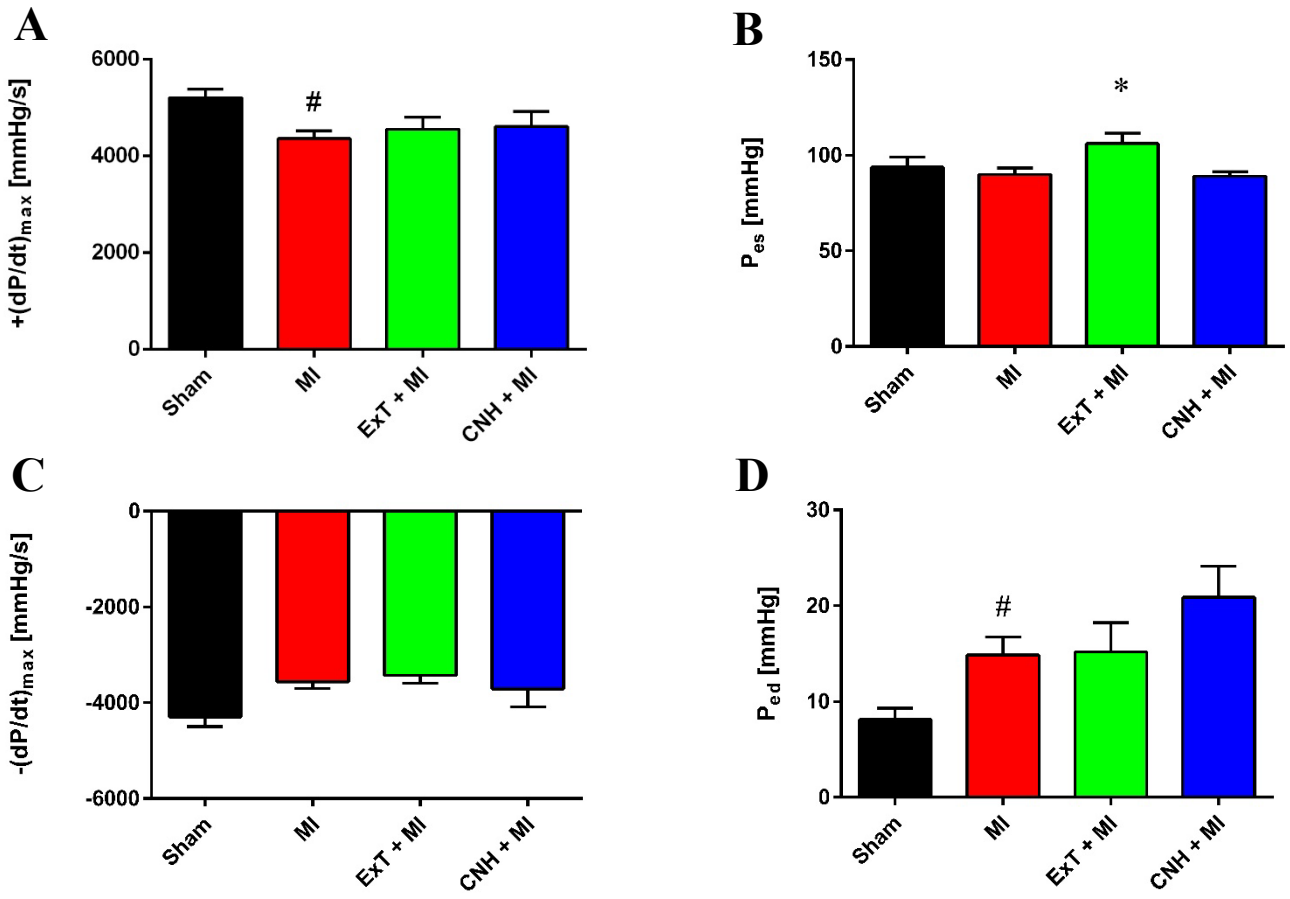

Figure 15 Peak rate of LV blood pressure development (**A**; $+(dP/dt)_{max}$), peak rate of LV blood pressure decrease (**C**; $-(dP/dt)_{max}$), end-systolic pressure (**B**; P_{es}) and end-diastolic pressure (**D**; P_{ed}) at the end of the study in sham-operated animals (Sham), animals with myocardial infarction (MI), animals trained before myocardial infarction (ExT + MI) and animals adapted to continuous normobaric hypoxia before myocardial infarction (CNH + MI). Values are expressed as mean ± SEM. $^{\#}$ $p < 0.05$ MI vs. Sham, * $p < 0.05$ vs. MI.

4.2. Therapeutic effect of continuous normobaric hypoxia and exercise training on postischemic heart failure

The aim of this study was to determine the therapeutic effect of two protocols with cardioprotective potential on postischemic heart failure. Therefore, animals underwent 60-min coronary artery occlusion and, after 1 week of recovery, were exposed to continuous normobaric hypoxia or exercise training for the next 3 weeks. Their cardiac function was assessed before ischemia/reperfusion injury and 1, 2 and 4 weeks later. Results of this study were published in Publication A (Hrdlička *et al.*, 2016; enclosed in full length in chapter **9. Supplements**).

The control MI group and the Sham group are identical to those in the previous study. Animals exposed to CNH and ExT after MI (MI + CNH and MI + ExT, respectively) were randomly selected from the MI group 1 week after surgery. As shown in **Figure 16**, survival was affected by postischemic exposure to neither CNH nor ExT, and no animal died in these two experimental groups.

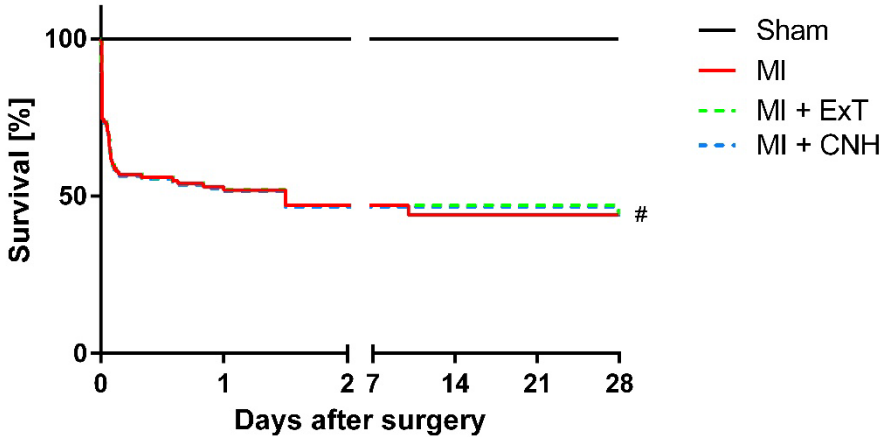

Figure 16 Survival of sham-operated animals (Sham), animals with myocardial infarction (MI), animals trained after myocardial infarction (MI + ET) and animals adapted to continuous normobaric hypoxia after myocardial infarction (MI + CNH). $^{\#}$ $p < 0.05$ MI vs. Sham.

As summarized in **Figure 17**, systolic function did not differ in experimental groups 7 days after MI (beginning of therapy), and contrary to the MI group, both CNH and ExT tended to attenuate the decrease of FS at the end of the study (FS: 16.85 ± 0.83 % and 17.45 ± 2.21 % compared to 15.02 ± 0.91 %), but the effect did not reach statistical significance.

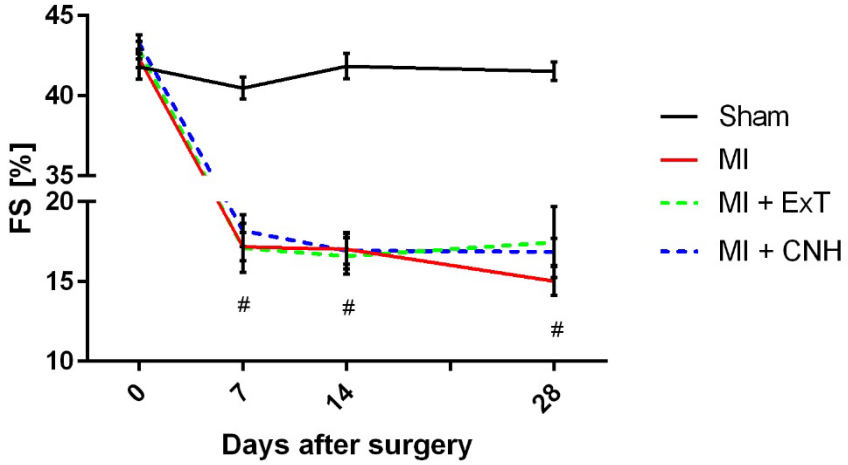

Figure 17 Fractional shortening (FS) in sham-operated animals (Sham), animals with myocardial infarction (MI), animals trained after myocardial infarction (MI + ExT) and animals adapted to continuous normobaric hypoxia after myocardial infarction (MI + CNH). Values are expressed as mean ± SEM. $^{\#}$ $p < 0.05$ MI vs. Sham.

As shown in **Figure 18**, the end-diastolic diameter of the left ventricle cavity (LVD_d) did not significantly differ among experimental groups with MI 7 days after surgery (9.92 ± 0.18 mm, 9.48 ± 0.20 mm and 9.73 ± 0.15 mm). Progressive postischemic dilatation of LV observed in MI was attenuated by CNH but not by ExT: between days 7 and 28, LVD_d rose by 13.7 %, 3.9 % and 15.5 % in MI, MI + CNH and MI + ExT, respectively.

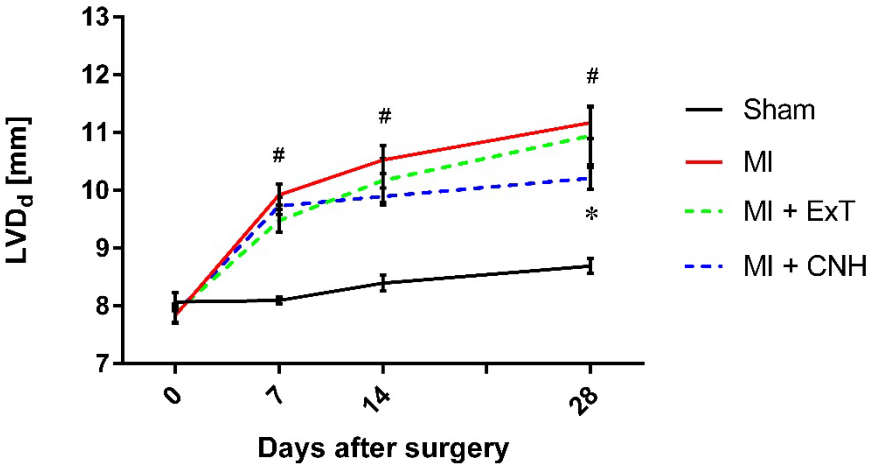

Figure 18 Left ventricle cavity diameter in diastole (LVD_d) in sham-operated animals (Sham), animals with myocardial infarction (MI), animals trained after myocardial infarction (MI + ExT) and animals adapted to continuous normobaric hypoxia after myocardial infarction (MI + CNH). Values are expressed as mean ± SEM. $^{\#}$ $p < 0.05$ MI vs. Sham, * $p < 0.05$ vs. MI.

As summarized in **Table 3,** LVD_s was increased, AWT_d, AWT_s and RWT were decreased, and PWT_d and PWT_d were not altered in the MI group compared to the Sham group 7 days after surgery. No differences in parameters of LV geometry were observed among experimental groups with MI 7 days after surgery (before the beginning of the ExT or CNH therapy). Parameters of LV geometry

were not altered in MI + ExT group compared to the MI group. Exposure to CNH decreased LVD_s and increased RWT compared to the MI group.

Table 3 Parameters of left ventricle geometry in sham-operated animals (Sham), animals with myocardial infarction (MI), animals trained after myocardial infarction (MI + ExT) and animals adapted to continuous normobaric hypoxia after myocardial infarction (MI + CNH). LVD_s, end-systolic LV diameter; AWT_s, end-systolic anterior wall thickness; AWT_d, end-diastolic anterior wall thickness; PWT_d, end-diastolic posterior wall thickness; PWT_s, end-systolic posterior wall thickness; RWT, relative wall thickness; HR, heart rate. Values are expressed as mean ± SEM. $^{\#}$ $p < 0.05$ MI vs. Sham, * $p < 0.05$ vs. MI.

	LVD_s [mm]	AWT_d [mm]	AWT_s [mm]	PWT_d [mm]	PWT_s [mm]	RWT [%]	HR [bpm]
3 days before MI							
am	4.74 ± 0.12	2.06 ± 0.03	3.19 ± 0.06	1.90 ± 0.03	2.84 ± 0.04	50.1 ± 0.7	372 ± 10
MI	4.57 ± 0.11	2.11 ± 0.04	3.24 ± 0.05	1.94 ± 0.04	2.98 ± 0.06	52.1 ± 1.6	382 ± 14
MI + ExT	4.42 ± 0.09	2.10 ± 0.04	3.23 ± 0.07	1.86 ± 0.03	2.97 ± 0.06	50.7 ± 1.6	393 ± 9
MI + CNH	4.48 ± 0.07	2.12 ± 0.04	3.25 ± 0.06	1.87 ± 0.05	2.94 ± 0.06	50.5 ± 1.2	376 ± 8
7 days after MI							
Sham	5.10 ± 0.06	2.26 ± 0.04	3.31 ± 0.09	1.92 ± 0.03	2.93 ± 0.06	51.9 ± 0.8	389 ± 8
MI	9.92 ± 0.18$^{\#}$	1.95 ± 0.05$^{\#}$	1.95 ± 0.06$^{\#}$	1.93 ± 0.04	2.82 ± 0.06	39.0 ± 0.9$^{\#}$	378 ± 8
MI + ExT	9.48 ± 0.20	2.00 ± 0.04	2.03 ± 0.08	2.02 ± 0.06	2.69 ± 0.10	42.0 ± 1.3	412 ± 6*
MI + CNH	9.42 ± 0.13	1.86 ± 0.07	1.99 ± 0.88	1.99 ± 0.06	2.98 ± 0.07	40.8 ± 0.8	364 ± 7
28 days after MI							
Sham	5.11 ± 0.07	2.23 ± 0.08	3.60 ± 0.10	2.02 ± 0.06	3.19 ± 0.07	46.7 ± 2.3	365 ± 5
MI	9.61 ± 0.30$^{\#}$	1.86 ± 0.03$^{\#}$	1.86 ± 0.03$^{\#}$	2.11 ± 0.04	2.95 ± 0.06	35.5 ± 0.8$^{\#}$	376 ± 7
MI + ExT	9.07 ± 0.20	1.85 ± 0.08	1.97 ± 0.07	2.31 ± 0.05$^{\#}$	3.19 ± 0.11	38.0 ± 1.0	392 ± 11
MI + CNH	8.18 ± 0.21*	1.83 ± 0.05	1.88 ± 0.05	2.32 ± 0.07$^{\#}$	3.26 ± 0.07	41.7 ± 0.8*	340 ± 12

Figure 19A illustrates the LV dilatation and MI scar size in the mid-cavity histological slices. As shown in **Figure 19B**, MI resulted in the scarification of 24.7 % of LV circumference. Neither ExT nor CNH affected the scar circumference (30.3 ± 2.7 % and 29.8 ± 2.5 %, respectively).

Table 4 shows that body weight was altered by neither MI nor ExT and CNH at the end of the study. Similarly, relative lung weight and relative kidney weight were not affected.

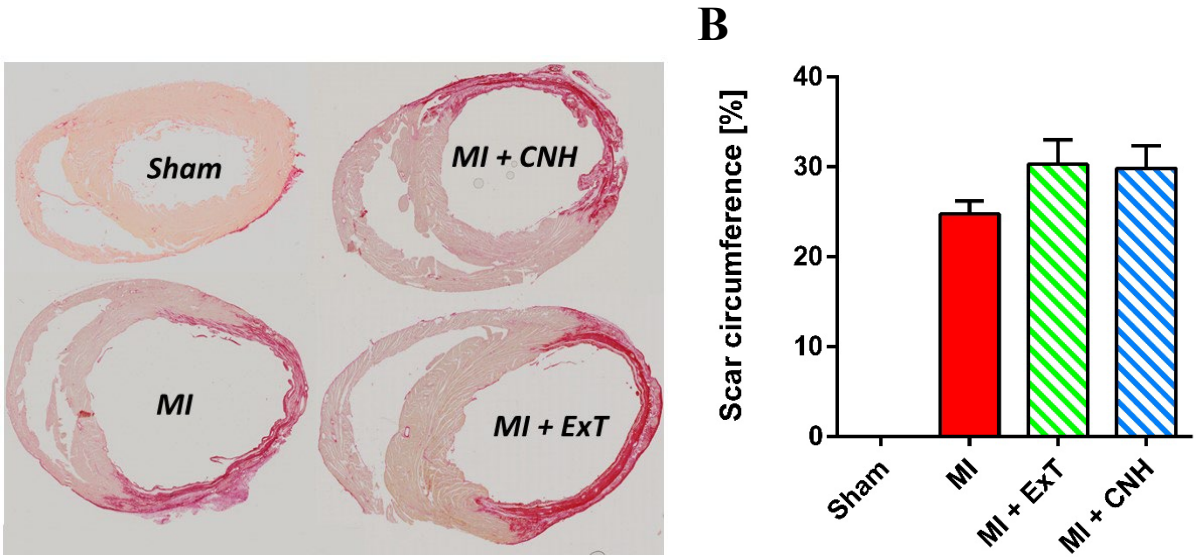

Figure 19 Histology of Picrosirius red-stained hearts at the mid-cavity level (**A**) and myocardial scar circumference (**B**) in sham-operated animals (Sham), animals with myocardial infarction (MI), animals trained after myocardial infarction (MI + ExT) and animals adapted to continuous normobaric hypoxia after myocardial infarction (MI + CNH).

Table 4 Body weight and relative organ weights at the end of the study in sham-operated animals (Sham), animals with myocardial infarction (MI), animals trained after myocardial infarction (MI + ExT) and animals adapted to continuous normobaric hypoxia after myocardial infarction (MI + CNH). BW, body weight. Values are expressed as mean ± SEM.

	BW [g]	Lung/BW [mg/g]	Kidneys/BW [mg/g]
Sham	533 ± 44	3.01 ± 0.35	6.23 ± 0.53
MI	512 ± 40	3.82 ± 1.97	6.18 ± 0.72
MI + ExT	466 ± 14	4.40 ± 2.06	6.18 ± 0.63
MI + CNH	450 ± 48	4.57 ± 0.61	6.41 ± 0.77

Compared to MI group, ExT tended to increase $+(dP/dt)_{max}$, but only CNH had significant effect (**Figure 20A**; 5353 ± 308 mmHg/s and 5057 ± 232 mmHg/s vs. 4359 ± 157 mmHg/s). Similar tendency was observed in $-(dP/dt)_{max}$ (**Figure 20B**; -4180 ± 279 mmHg/s and -4196 ± 242 mmHg/s vs. -3563 ± 142 mmHg/s). End-systolic pressure tended to be increased by ExT and was increased in MI + CNH compared to MI group (**Figure 20C**; 101.7 ± 3.5 mmHg and 106.9 ± 4.1 mmHg vs. 89.9 ± 3.3 mmHg). End-diastolic pressure was significantly affected by neither ExT nor CNH compared to MI group **(Figure 20D**; 16.8 ± 2.1 mmHg and 14.0 ± 1.3 mmHg vs. 14.9 ± 1.9 mmHg).

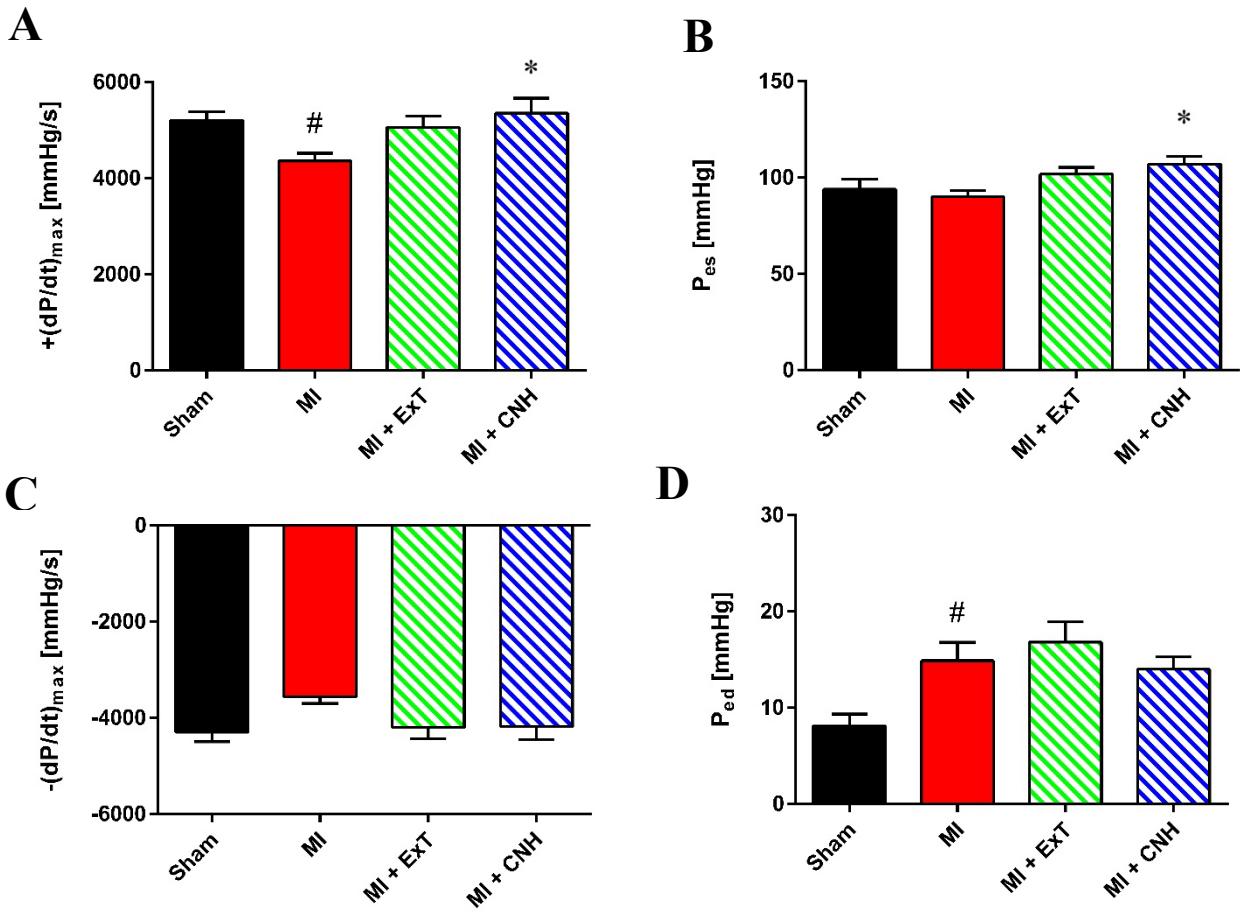

Figure 20 Peak rate of LV blood pressure development (**A**; $+(dP/dt)_{max}$), peak rate of LV blood pressure decrease (**C**; $-(dP/dt)_{max}$), end-systolic pressure (**B**; P_{es}) and end-diastolic pressure (**D**; P_{ed}) in sham-operated animals (Sham), control group (MI), animals trained after myocardial infarction (MI + ExT) and animals adapted to continuous normobaric hypoxia after myocardial infarction (MI + CNH) 28 days after surgery. Values are expressed as mean ± SEM. $^{\#}$ $p < 0.05$ MI vs. Sham, * $p < 0.05$ vs. MI.

4.3. Effect of epoxyeicosatrienoic acid analogue EET-B on postischemic heart failure in spontaneously hypertensive rats

The aim of this study was to determine possible cardioprotective effects of novel EET analogue EET-B on the progression of postischemic heart failure development in spontaneously hypertensive rats (SHR). For this purpose, animals were pretreated for 2 weeks before the 30-minute I/R insult, and treatment continued for another 7 weeks. Cardiac function was assessed at the beginning and end of the study and before surgery. Results of this study were published in Publication B (Neckář *et al.*, 2019; enclosed in full length in chapter **9. Supplements).**

As shown in **Figure 21A**, animal weights did not differ between experimental groups at the beginning of the study and before surgery. Surgical insult led to weight drop in animals with MI but not in sham-operated animals. This effect was compensated within 2 weeks after surgery, and animal weights did not differ between experimental groups at the end of the study. **Figure 21B** demonstrates that there was zero mortality in sham-operated groups (0/6 in untreated and 0/6 in treated animals), 29.4 % in the MI group (5/17) and mortality was significantly reduced to 9.1 % by EET-B pretreatment (1/11).

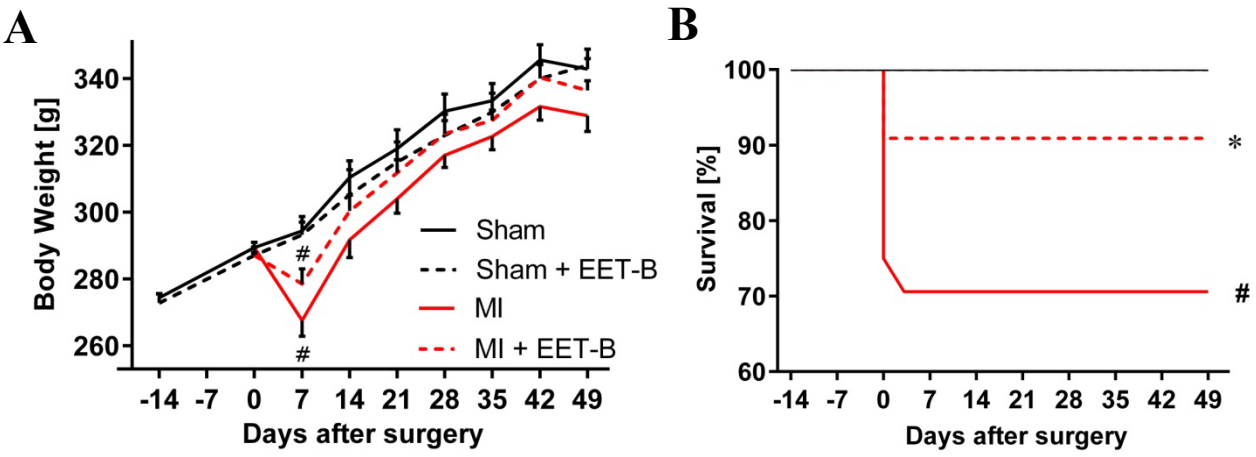

Figure 21 Body weight (**A**) and survival (**B**) in untreated sham-operated animals (Sham), EET-B treated sham-operated animals (Sham + EET-B), untreated animals with myocardial infarction (MI) and EET-B treated animals with myocardial infarction (MI + EET-B). Values are expressed as mean ± SEM. $^{\#}$ $p < 0.05$ MI vs. Sham, * $p < 0.05$ vs. MI.

As summarized in **Figure 22, Figure 23** and **Table 5**, LV systolic function and geometry did not differ among experimental groups at the beginning of the study and was not altered by EET-B pretreatment prior to the MI.

There was a significant increase of LVD_d and LVD_s in Sham groups (both untreated and EET-B treated) at the end of the study compared to the beginning of the study (**Figure 23** and **Table 5**). This growth-related change was further translated into a significant decrease of FS (by 16.3 % and 14.2 %, respectively) compared to baseline values (**Figure 22**).

At the end of the study, LVD_d and LVD_s were significantly increased in both experimental groups with MI compared to corresponding Sham groups (**Figure 23** and **Table 5**). In untreated animals, MI resulted in systolic dysfunction (**Figure 22**, FS: 18.4 ± 1.0 % vs. 30.6 ± 1.0 %) compared to the corresponding Sham group. EET-B treatment attenuated MI associated change in LVD_s but not in LVD_d (**Figure 23**), which resulted in improvement of systolic function (FS: 23.7 ± 0.7 %).

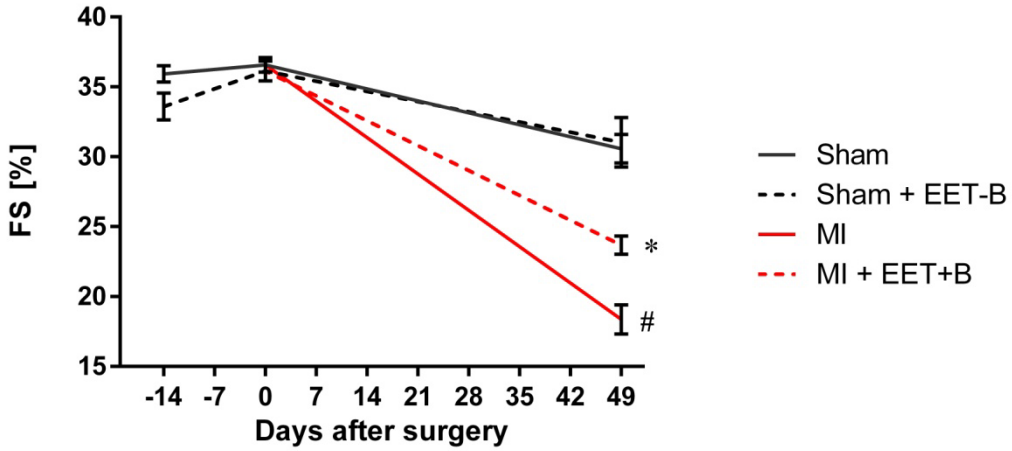

Figure 22 Fractional shortening (FS) in untreated sham-operated animals (Sham), EET-B treated sham-operated animals (Sham + EET-B), untreated animals with myocardial infarction (MI) and EET-B treated animals with myocardial infarction (MI + EET-B). Values are expressed as mean ± SEM. # $p < 0.05$ MI vs. Sham, * $p < 0.05$ vs. MI.

Table 5 shows that diastolic thicknesses of LV walls (AWT_d and PWT_d) were affected by neither MI nor EET-B treatment. While systolic thicknesses of LV walls (AWT_s and PWT_s) were decreased in untreated animals with MI (compared to the corresponding Sham group), this difference was not observed in EET-B treated animals (compared to the corresponding Sham group). Despite that, a significant effect of EET-B on systolic wall thickness in MI animals was not confirmed (MI vs. MI + EET-B). An increase in LVD_d but not in AWT_d and PWT_d in animals with MI was further translated into decreased RWT that was not altered by EET-B treatment.

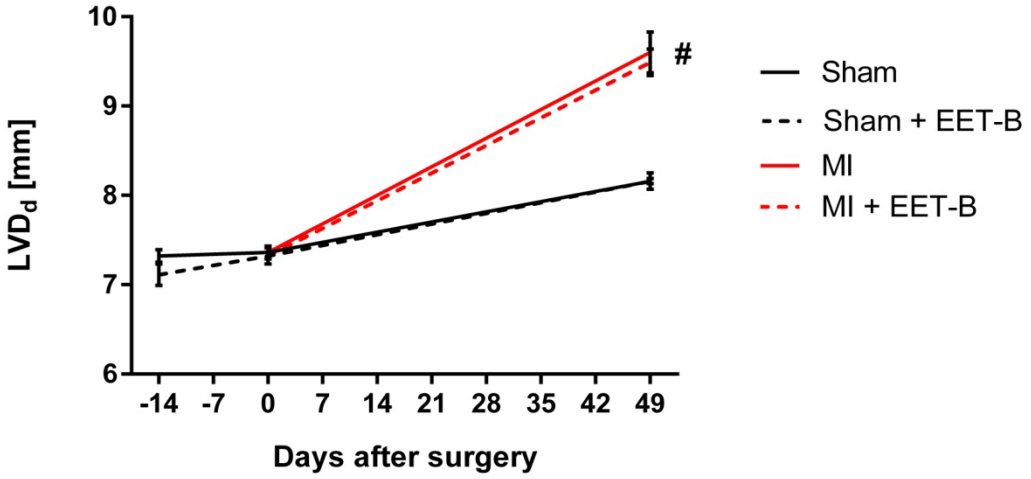

Figure 23 Left ventricle cavity diameter (LVD_d) in untreated sham-operated animals (Sham), EET-B treated sham-operated animals (Sham + EET-B), untreated animals with myocardial infarction (MI) and EET-B treated animals with myocardial infarction (MI + EET-B). Values are expressed as mean ± SEM. # $p < 0.05$ MI vs. Sham.

Table 5 Echocardiographic parameters of left ventricle geometry during the experiment in untreated sham-operated animals (Sham), EET-B treated sham-operated animals (Sham + EET-B), untreated animals with myocardial infarction (MI) and EET-B treated animals with myocardial infarction (MI + EET-B). LVD_s, end-systolic LV diameter; AWT_s, end-systolic anterior wall thickness; AWT_d, end-diastolic anterior wall thickness; PWT_d, end-diastolic posterior wall thickness; PWT_s, end-systolic posterior wall thickness; RWT, relative wall thickness. Values are expressed as mean ± SEM. # $p < 0.05$ MI vs. corresponding Sham group, † $p < 0.05$ vs. corresponding group before MI, * $p < 0.05$ vs. MI.

	LVD_s [mm]	AWT_d [mm]	AWT_s [mm]	PWT_d [mm]	PWT_s [mm]	RWT [%]	HR [bpm]
14 days before MI							
Untreated	4.67 ± 0.07	1.78 ± 0.04	2.90 ± 0.05	1.83 ± 0.02	2.73 ± 0.04	49.4 ± 1.0	338 ± 7
EET-B	4.74 ± 0.12	1.93 ± 0.06	2.94 ± 0.05	1.82 ± 0.05	2.66 ± 0.07	53.0 ± 1.1	308 ± 8
3 days before MI							
Untreated	4.67 ± 0.07	1.88 ± 0.05	3.02 ± 0.06	1.91 ± 0.04	2.86 ± 0.06	51.6 ± 1.5	339 ± 9
EET-B	4.67 ± 0.11	1.98 ± 0.06	3.11 ± 0.07	1.91 ± 0.05	2.85 ± 0.06	53.4 ± 1.6	350 ± 5
49 days after MI							
Sham	5.67 ± 0.10†	1.88 ± 0.09	2.96 ± 0.09	1.99 ± 0.07	2.86 ± 0.06	47.4 ± 1.9	355 ± 7
Sham + EET-B	5.77 ± 0.12†	2.02 ± 0.07	3.12 ± 0.06	1.98 ± 0.04	2.87 ± 0.11	48.9 ± 1.1	337 ± 7
MI	7.85 ± 0.26#	1.85 ± 0.14	2.34 ± 0.23#	1.94 ± 0.08	2.56 ± 0.07#	40.1 ± 3.1#	318 ± 7
MI + EET-B	7.25 ± 0.15*	1.94 ± 0.18	2.72 ± 0.30	2.06 ± 0.10	2.81 ± 0.14	42.1 ± 2.2	332 ± 11

As summarized in **Table 6**, relative lung weight was markedly increased in both MI groups (compared to the corresponding Sham group), and EET-B treatment attenuated this increase from 75.4 % to 31.1 %. Similarly, relative heart weight was increased by 10% and 13%, respectively (compared to a corresponding Sham group), but the effect of EET-B was not observed.

Table 6 Relative heart weight and lung weight in untreated sham-operated animals (Sham), EET-B treated sham-operated animals (Sham + EET-B), untreated animals with myocardial infarction (MI) and EET-B treated animals with myocardial infarction (MI + EET-B). Values are expressed as mean ± SEM. # $p < 0.05$ MI vs. Sham, * $p < 0.05$ vs. MI.

	Heart/BW [mg/g]	Lung/BW [mg/g]
Sham	3.57 ± 0.02	3.58 ± 0.14
Sham + EET-B	3.47 ± 0.08	3.64 ± 0.09
MI	3.94 ± 0.07#	6.28 ± 0.49#
MI + EET-B	3.93 ± 0.12	4.81 ± 0.49*

4.4. Therapeutic effect of EET-A and *c*-AUCB on postischemic heart failure in normotensive HanSD rats and hypertensive *Ren-2* transgenic rats

The aim of this study was to determine the effect of orally active epoxyeicosatrienoic acid analogue EET-A and soluble epoxide hydrolase inhibitor *c*-AUCB on the progression of postischemic heart failure in a well-established model of angiotensin II-dependent hypertension (*Ren-2* transgenic rats) and normotensive HanSD rats.

Animals were subjected to 60-min coronary artery occlusion or sham operation. After 24-hour recovery, rats were randomly assigned to 5 experimental groups: i) Sham-operated untreated (Sham), ii) post-MI untreated (MI), iii) post-MI treated with EET-A (10 mg/kg/day, p.o.; MI + EET-A), iv) post-MI treated with *c*-AUCB (1 mg/kg/day, p.o.; MI + *c*-AUCB) and iv) post-MI treated with the combination of EET-A and *c*-AUCB (10mg/kg/day and 1 mg/kg/day, respectively, p.o.; MI + EET-A + *c*-AUCB). Cardiac function was assessed at the beginning, 1 week after surgery and end of the study by echocardiography and by left ventricle catheterisation at the end of the study. Results of this study were published in Publication C (Hrdlička *et al.* 2019; enclosed in full length in chapter **9. Supplements**).

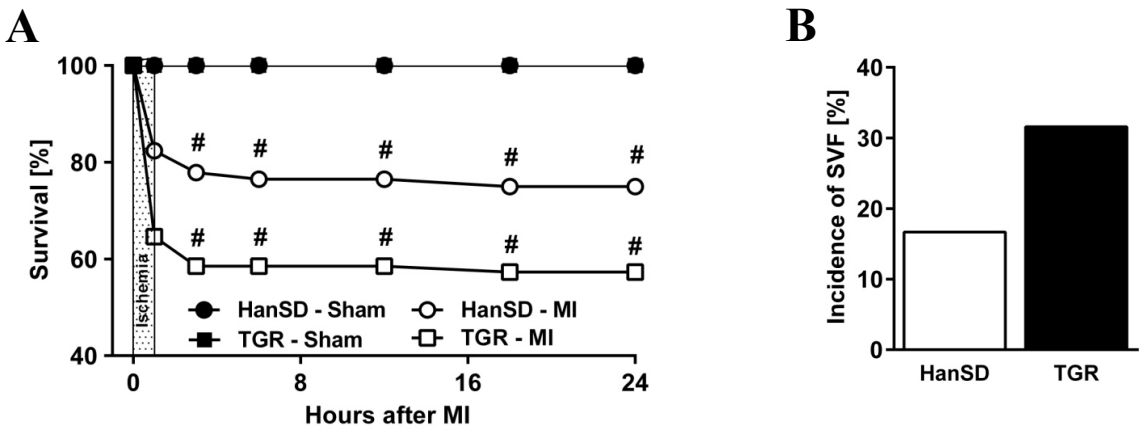

Figure 24 Survival rate in first 24 hours after ischemia (**A**) and incidence of sustained ventricular fibrillation (**B**) in Hannover Sprague Dawley (HanSD) and *Ren-2* transgenic rats (TGR) experimental group: sham untreated (Sham), MI untreated (MI) Values are expressed as mean ± SEM. $^{\#}$ $P < 0.05$ MI vs. corresponding Sham group.

Ischemia/reperfusion insult led to significant mortality in both strains. As demonstrated in **Figure 24**, early mortality could be divided into three periods. The highest mortality in both HanSD and TGR was during the ischemic period (17.7 % and 35.4 %, respectively) and was caused by sustained ventricular fibrillation, as shown in **Figure 24B**. The mortality was further increasing during the first few hours of recovery and stabilized within 24 hours after ischemia **(Figure 24A**). Total mortality after 24 hours was significantly lower in HanSD compared to TGR (25.0 % and 42.7 %, respectively).

Since 24 hours after I/R insult additional two untreated HanSD rats, four untreated TGR rats a three TGR rats in every treated group died until the end of the study resulting in total mortality of 27.9 % (19 out of 68) in HanSD and 61 % (50 out of 82) in TGR. No animal died in sham-operated groups.

The baseline values (before MI) of AWT_d, PWT_d and LVD_d in Sham HanSD were 2.10 ± 0.07 mm, 2.09 ± 0.04 mm and 8.04 ± 0.16 mm, respectively and did not differ among experimental groups. Baseline systolic function (expressed as FS) in all HanSD experimental groups ranged from 39.9 % to 42.1 % and did not differ among groups.

Baseline values of AWT_d, PWT_d and LVD_d in TGR (2.60 ± 0.09 mm, 2.61 ± 0.07 mm and 7.89 ± 0.14 mm, respectively) showed concentric LV hypertrophy and mild systolic dysfunction (FS: 36.9 ± 1.6 %) compared to corresponding HanSD. As shown in **Figure 25**, **Figure 26** and **Table 8**, similar differences between Sham HanSD and Sham TGR were observed at the end of the study.

Systolic function at the end of the study (**Figure 25**) was markedly decreased in untreated HanSD and TGR compared to corresponding sham group (11.1 ± 1.0 % vs. 40.8 ± 0.5 % and 11.0 ± 0.9 % vs. 36.9 ± 1.6 %, respectively). Treatment with EET-A and *c*-AUCB tended to attenuate this systolic dysfunction and their combination showed significant improvement in HanSD (FS: 13.5 ± 0.7 %, 13.7 ± 1.0 % and 14.4 ± 0.9 %, respectively) but no effect of treatment was observed in TGR (FS: 11.7 ± 1.1 %, 10.9 ± 1.0 % and 10.2 ± 0.4 %, respectively).

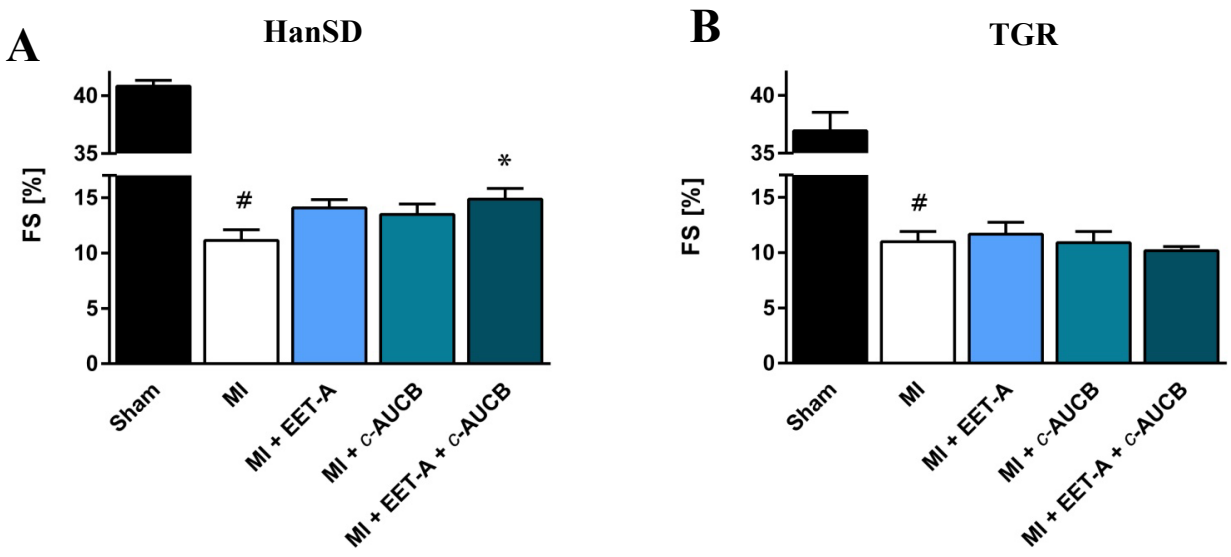

Figure 25 Fractional shortening (FS) in Hannover Sprague-Dawley (HanSD, **A**) and *Ren-2* transgenic rats (TGR, **B**) subjected to Sham operation (Sham) or Myocardial infarction (MI) and treated with epoxyeicosatrienoic acid analogue (EET-A) and soluble epoxide hydrolase inhibitor (*c*-AUCB) for 5 weeks since 24 hours after MI. Values are expressed as mean ± SEM. $^{\#}$ $P < 0.05$ MI vs. corresponding Sham group, * $P < 0.05$ vs. corresponding MI group.

Figure 26 shows a major increase of LVD_d at the end of the study in animals with MI in both untreated HanSD and TGR compared to a corresponding Sham group (11.70 ± 0.17 mm vs. 8.82 ± 0.20 mm and 11.65 ± 0.29 mm vs. 8.52 ± 0.17 mm, respectively) and tendency (not significant) to attenuate this effect with EET-A, *c*-AUCB and their combination (11.24 ± 0.17 mm, 11.13 ± 0.16 mm and 11.13 ± 0.24 mm in HanSD and 11.19 ± 0.30 mm, 11.54 ± 0.17 mm and 11.47 ± 0.16 mm in TGR).

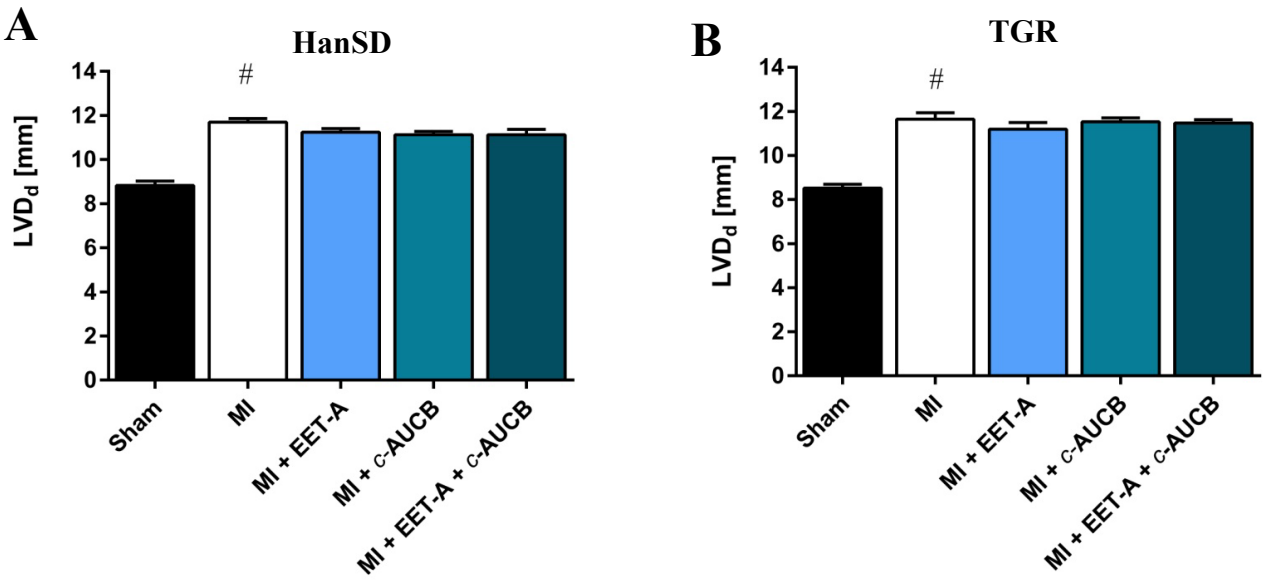

Figure 26 Diastolic diameter of left ventricle cavity (LVD_d) in Hannover Sprague-Dawley (HanSD, **A**) and *Ren-2* transgenic rats (TGR, **B**) subjected to Sham operation (Sham) or Myocardial infarction (MI) and treated with epoxyeicosatrienoic acid analogue (EET-A) and soluble epoxide hydrolase inhibitor (*c*-AUCB) for 5 weeks since 24 hours after MI. Values are expressed as mean ± SEM. $^{\#}$ P < 0.05 MI vs. corresponding Sham group.

Results summarized in **Table 8** further show that untreated animals with MI have decreased diastolic and systolic AWT and systolic LVD compared to the corresponding Sham group. The decrease in AWT_d and increase in LVD_d was then translated into a decrease of RWT (by 31 % in HanSD and 48 % in TGR). The increase of LVD_s tended to be attenuated by EET-A and *c*-AUCB treatments and was significantly lower in animals with combined treatment in HanSD (compared to untreated MI group), but no effect of treatment on LVD_s was observed in TGR. Other parameters of LV geometry were not affected by the treatment in any strain.

As shown in **Figure 27**, animal BW did not differ between experimental groups of the same strain at the begging of the study. Average BW in all experimental groups tended to increase during the whole experimental period with the exception of the first week, which is due to stress caused by the surgical insult. At the end of the experiment, no significant changes of BW were observed between experimental groups.

Table 8 Echocardiographic parameters of left ventricle geometry in Hannover Sprague-Dawley (HanSD) and *Ren-2* transgenic rats (TGR) 5 weeks after MI. Animals were subjected to sham operation (Sham) or Myocardial infarction (MI) and treated with epoxyeicosatrienoic acid analogue (EET-A) and soluble epoxide hydrolase inhibitor (*c*-AUCB) for 5 weeks since 24 hours after MI. LVD_s, end-systolic LV diameter; AWT_d, end-diastolic anterior wall thickness; AWT_s, end-systolic anterior wall thickness; PWT_d, end-diastolic posterior wall thickness; PWTs, end-systolic posterior wall thickness; RWT, relative wall thickness; HR, heart rate; bpm, beats per minute. Values are expressed as mean ± SEM. $^{\#}$ $P < 0.05$ MI vs. corresponding Sham group.

	LVD_s [mm]	AWT_d [mm]	AWT_s [mm]	PWT_d [mm]	PWT_s [mm]	RWT [%]	HR [bpm]
HanSD							
Sham	5.27 ± 0.16	2.21 ± 0.09	3.44 ± 0.11	2.28 ± 0.09	3.39 ± 0.10	51.1 ± 2.1	360 ± 14
MI	10.37 ± 0.21$^{\#}$	1.69 ± 0.08$^{\#}$	1.66 ± 0.07$^{\#}$	2.41 ± 0.10	2.88 ± 0.13$^{\#}$	35.1 ± 0.9$^{\#}$	327 ± 11
MI + EET-A	9.65 ± 0.17	1.61 ± 0.05	1.62 ± 0.06	2.26 ± 0.07	2.92 ± 0.09	34.6 ± 0.7	347 ± 8
MI + *c*-AUCB	9.62 ± 0.20	1.61 ± 0.05	1.62 ± 0.05	2.34 ± 0.10	2.95 ± 0.11	35.6 ± 1.2	355 ± 7
MI + EET-A + *c*-AUCB	9.50 ± 0.29*	1.60 ± 0.07	1.70 ± 0.12	2.16 ± 0.05	2.83 ± 0.07	34.0 ± 1.1	356 ± 7
TGR							
Sham	5.44 ± 0.13	2.64 ± 0.10	3.66 ± 0.14	2.57 ± 0.10	3.42 ± 0.10	65.1 ± 2.8	373 ± 13
MI	10.35 ± 0.33$^{\#}$	1.95 ± 0.08$^{\#}$	2.03 ± 0.15$^{\#}$	2.41 ± 0.94	3.16 ± 0.11	35.0 ± 1.6$^{\#}$	353 ± 11
MI + EET-A	9.90 ± 0.35	1.88 ± 0.08	1.93 ± 0.13	2.41 ± 0.05	3.17 ± 0.06	38.1 ± 2.2	351 ± 15
MI + *c*-AUCB	10.28 ± 0.24	1.84 ± 0.06	1.96 ± 0.14	2.40 ± 0.09	3.00 ± 0.09	35.3 ± 1.0	343 ± 10
MI + EET-A + *c*-AUCB	10.30 ± 0.16	1.85 ± 0.08	1.94 ± 0.18	2.43 ± 0.11	3.10 ± 0.14	35.4 ± 1.7	321 ± 16

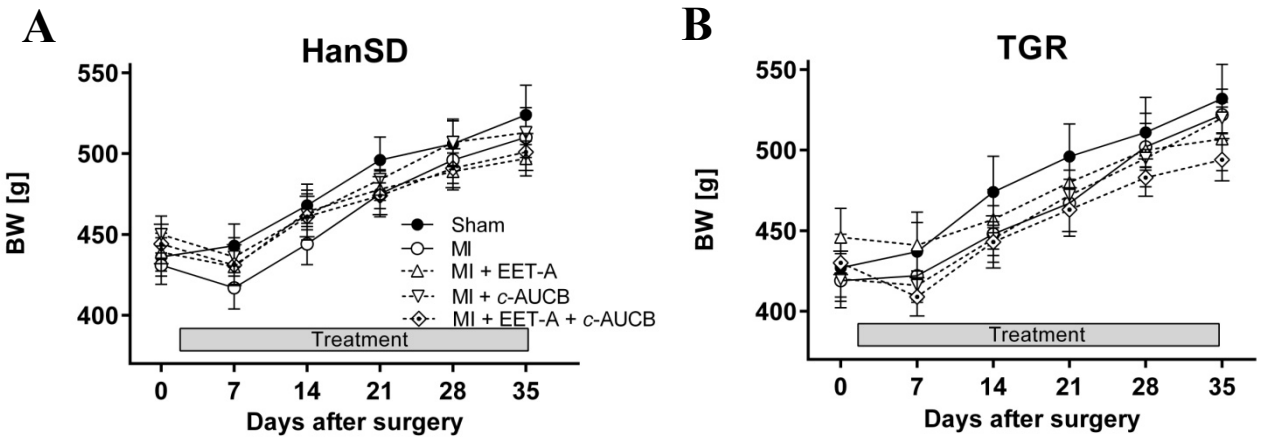

Figure 27 Body weight in Hannover Sprague Dawley (HanSD; **A**) and *Ren-2* transgenic rats (TGR; **B**) in Hannover Sprague-Dawley (HanSD, **A**) and *Ren-2* transgenic rats (TGR, **B**) subjected to Sham operation (Sham) or Myocardial infarction (MI) and treated with epoxyeicosatrienoic acid analogue (EET-A) and soluble epoxide hydrolase inhibitor (*c*-AUCB) for 5 weeks since 24 hours after MI. Values are expressed as mean ± SEM.

As summarized in **Table 7**, relative heart weight in HanSD was significantly increased in untreated animals with MI compared to the corresponding Sham group with no significant effect of treatment. MI also resulted in a relative lung weight increase by 139 % compared to the Sham group. Treatment with EET-A alone or combined with *c*-AUCB tended to prevent this increase in lung weight (to 65 % and 63 % compared to the Sham group), but the effect did not reach statistical significance. Relative kidney weight was significantly altered by neither MI nor any treatment in HanSD.

In TGR, relative heart weight was affected by neither MI nor treatments. Relative lung weight was increased by 124 % in untreated MI animals compared to the Sham group, and treatments with EET-A, *c*-AUCB and their combination had no effect (increase by 112 %, 127 % and 148 %, respectively). Relative kidney weight was lowered by 13 % (significant) in the untreated MI group compared to the corresponding Sham group and was not affected by treatment.

Table 7 Relative heart weight, relative lungs weight and relative kidney weight in Hannover Sprague-Dawley (HanSD, **A**) and *Ren-2* transgenic rats (TGR, **B**) subjected to Sham operation (Sham) or Myocardial infarction (MI) and treated with epoxyeicosatrienoic acid analogue (EET-A) and soluble epoxide hydrolase inhibitor (*c*-AUCB) for 5 weeks since 24 hours after MI. Values are expressed as mean ± SEM. # $p < 0.05$ MI vs. corresponding Sham group.

	Heart/BW [mg/g]	Lung/BW [mg/g]	Kidneys/BW [mg/g]
HanSD			
Sham	2.71 ± 0.14	2.94 ± 0.07	3.20 ± 0.07
MI	3.23 ± 0.14#	7.04 ± 0.82#	3.17 ± 0.15
MI + EET-A	3.03 ± 0.09	4.84 ± 0.64	3.43 ± 0.06
MI + *c*-AUCB	3.10 ± 0.10	6.63 ± 1.28	3.44 ± 0.09
MI + EET-A + *c*-AUCB	3.00 ± 0.05	4.78 ± 0.75	3.46 ± 0.04
TGR			
Sham	3.22 ± 0.11	3.05 ± 0.13	3.57 ± 0.10
MI	3.44 ± 0.11	6.82 ± 0.59#	3.11 ± 0.09#
MI + EET-A	3.19 ± 0.08	6.46 ± 0.97	3.19 ± 0.04
MI + *c*-AUCB	3.19 ± 0.14	6.93 ± 0.74	3.14 ± 0.09
MI + EET-A + *c*-AUCB	3.11 ± 0.11	7.55 ± 1.29	3.07 ± 0.04

Progression of postischemic HF was associated with several changes of mitral flow time parameters. In both untreated HanSD and TGR animals, MI led to a significant increase of LV filling peak velocity, prolongation of isovolumic contraction time and reduction of filling time, but did not affect ejection time and isovolumic relaxation time (**Table 9**). In HanSD rats, a single administration of *c*-AUCB significantly reduced the prolongation of isovolumic contraction time. Combined therapy shortened the isovolumic contraction time and prolonged the filling time compared to untreated controls. No effect of neither single nor combined EET-based therapy on HF associated changes in mitral flow time parameters was observed in TGR.

Table 9 Parameters of mitral valve flow in Hannover Sprague-Dawley (HanSD) and *Ren-2* transgenic rats (TGR) 5 weeks after MI. Animals were subjected to sham operation (Sham) or Myocardial infarction (MI) and treated with epoxyeicosatrienoic acid analogue (EET-A) and soluble epoxide hydrolase inhibitor (*c*-AUCB) for 5 weeks since 24 hours after MI. Vm_{max}, left ventricle filling peak velocity; FT, left ventricle filling time; IVCT, isovolumic contraction time; ET, left ventricle ejection time; IVRT, isovolumic relaxation time. Values are expressed as mean ± SEM. $^{\#}$ $P < 0.05$ MI vs. corresponding Sham group, * $P < 0.05$ vs. corresponding MI group.

	Vm_{max} [m/s]	FT [ms]	IVCT [ms]	ET [ms]	IVRT [ms]
HanSD					
Sham	1.11 ± 0.04	64.1 ± 3.6	13.9 ± 1.2	67.9 ± 4.4	23.7 ± 1.8
MI	1.32 ± 0.04$^{\#}$	45.2 ± 1.7$^{\#}$	49.2 ± 5.3$^{\#}$	61.5 ± 2.0	29.5 ± 1.8
MI + EET-A	1.29 ± 0.04	49.6 ± 1.6	40.0 ± 5.5	63.7 ± 1.8	25.5 ± 2.2
MI + *c*-AUCB	1.31 ± 0.06	46.6 ± 1.8	30.5 ± 3.2*	59.9 ± 3.5	30.6 ± 2.7
MI + EET-A + *c*-AUCB	1.17 ± 0.05	54.0 ± 2.0*	31.4 ± 3.7*	57.9 ± 1.9	26.3 ± 1.4
TGR					
Sham	1.20 ± 0.04	59.5 ± 3.0	14.6 ± 1.6	63.8 ± 2.0	21.4 ± 2.6
MI	1.51 ± 0.06$^{\#}$	44.8 ± 1.9$^{\#}$	35.9 ± 4.7$^{\#}$	59.1 ± 3.8	32.0 ± 4.3
MI + EET-A	1.36 ± 0.06	47.0 ± 1.3	41.3 ± 7.3	61.2 ± 4.8	31.3 ± 7.2
MI + *c*-AUCB	1.38 ± 0.06	47.0 ± 2.5	46.1 ± 5.8	64.8 ± 2.9	20.7 ± 1.7
MI + EET-A + *c*-AUCB	1.27 ± 0.09	43.1 ± 3.5	56.2 ± 9.4	61.0 ± 4.7	29.0 ± 3.8

MI resulted in a reduction of pulmonary artery (PA) peak and mean blood flow velocities, but did not alter the PA ejection time and acceleration time in both untreated groups (**Table 10**). In HanSD rats, the PA mean velocity was significantly increased in animals with single *c*-AUCB as well as combined treatment. Any effect of either single or combined therapy on HF associated changes in PA blood flow was observed in TGR.

Table 10 Parameters of pulmonary artery flow in Hannover Sprague-Dawley (HanSD, A) and *Ren-2* transgenic rats (TGR, B) subjected to Sham operation (Sham) or Myocardial infarction (MI) and treated with epoxyeicosatrienoic acid analogue (EET-A) and soluble epoxide hydrolase inhibitor (*c*-AUCB) for 5 weeks since 24 hours after MI. Vpa_{max}, pulmonary artery peak velocity; Vpa_{mean}, pulmonary artery mean velocity; AT, acceleration time; ETpa, pulmonary artery ejection time. Values are expressed as mean ± SEM. $^{\#}$ $P < 0.05$ MI vs. corresponding Sham group, * $P < 0.05$ vs. corresponding MI group.

	Vpa_{max} [m/s]	Vpa_{mean} [m/s]	AT [ms]	ETpa [ms]
HanSD				
Sham	1.06 ± 0.05	0.46 ± 0.02	27.5 ± 1.5	95.1 ± 3.0
MI	0.77 ± 0.05$^{\#}$	0.31 ± 0.02$^{\#}$	25.4 ± 1.6	92.1 ± 2.2
MI + EET-A	0.92 ± 0.04	0.39 ± 0.02*	28.0 ± 1.4	91.1 ± 1.2
MI + *c*-AUCB	0.89 ± 0.04	0.37 ± 0.02	27.5 ± 1.9	89.5 ± 2.4
MI + EET-A + *c*-AUCB	0.92 ± 0.04	0.39 ± 0.02*	27.1 ± 1.4	90.7 ± 1.3
TGR				
Sham	1.08 ± 0.3	0.47 ± 0.02	28.2 ± 1.3	89.8 ± 1.7
MI	0.80 ± 0.06$^{\#}$	0.32 ± 0.03$^{\#}$	25.2 ± 1.4	88.1 ± 1.5
MI + EET-A	0.89 ± 0.05	0.37 ± 0.03	25.4 ± 0.7	87.7 ± 1.5
MI + *c*-AUCB	0.83 ± 0.04	0.34 ± 0.03	26.3 ± 1.3	90.4 ± 2.0
MI + EET-A + *c*-AUCB	0.75 ± 0.04	0.30 ± 0.03	26.7 ± 1.7	91.2 ± 1.3

As demonstrated in **Figure 28**, the progression of postischemic HF led to impairment of contractile function. In untreated animals with MI, a marked decrease of peak rate of pressure development $+(dP/dt)_{max}$ was observed in both HanSD rats and TGR (compared to the corresponding sham group; 3890 ± 291 mmHg/s vs. 5947 ± 301mmHg/s and 3485 ± 417mmHg/s vs. 6910 ± 462mmHg/s, respectively). In HanSD, single therapy with EET-A and *c*-AUCB tended to improve $+(dP/dt)_{max}$ to 4596 ± 297mmHg/s and 4442 ± 287 mmHg/s, respectively, though the effect was not significant. Combined treatment of EET-A and *c*-AUCB resulted in a stronger cardioprotective effect increasing the $+(dP/dt)_{max}$ to 5278 ± 255 mmHg in HanSD (compared to untreated HanSD controls). Single EET-A treatment increased $+(dP/dt)_{max}$ in TGR to 4259 ± 501 mmHg/s, but the effect did not reach statistical significance. Similar effects of treatments were observed on the peak value of pressure decline rate ($-(dP/dt)_{max}$; **Figure 28C and 28D**).

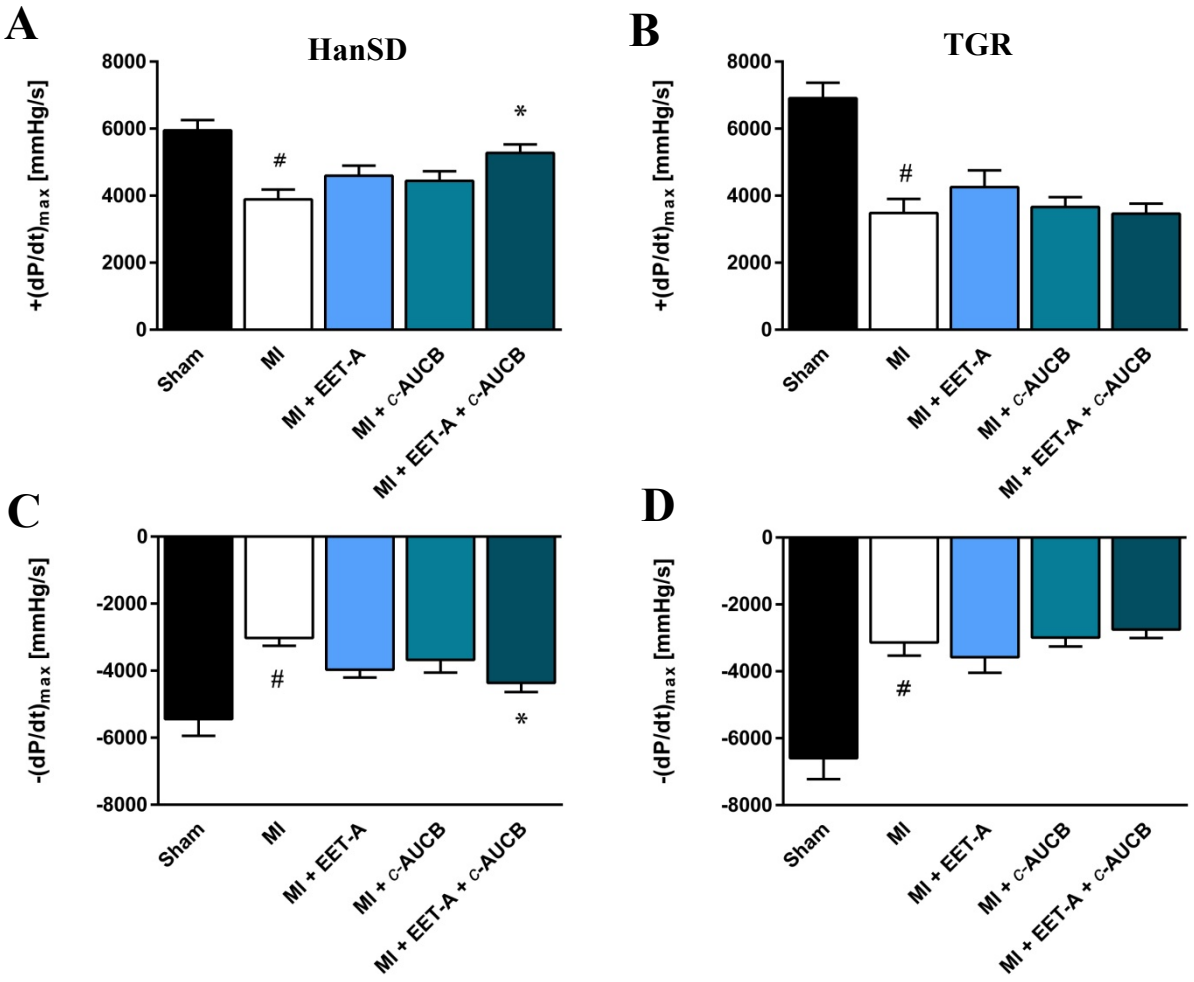

Figure 28 Peak rates of left ventricle pressure development ($+(dP/dt)_{max}$; **A, B**), and decline ($-(dP/dt)_{max}$; **C, D**) in Hannover Sprague-Dawley (HanSD, **A, C**) and *Ren-2* transgenic rats (TGR, **B, D**) subjected to Sham operation (Sham) or Myocardial infarction (MI) and treated with epoxyeicosatrienoic acid analogue (EET-A) and soluble epoxide hydrolase inhibitor (*c*-AUCB) for 5 weeks since 24 hours after MI. Values are expressed as mean ± SEM. $^{\#}$ $P < 0.05$ MI vs. corresponding Sham group, * $P < 0.05$ vs. corresponding MI group.

In HanSD and TGR, MI resulted in a significant reduction of systolic blood pressure at the end of the study (to 104.7 ± 2.6 mmHg and 97 ± 6.4 mmHg, respectively) compared to corresponding Sham animals (117.7 ± 3.7 mmHg and 139.6 ± 4.4 mmHg, respectively). Systolic blood pressure in all treated groups varied between 102.1 and 108.3 mmHg in HanSD and between 93.4 and 99.2 in TGR with no significant differences. EET-based therapy showed no effect on LV developed pressure in neither HanSD nor TGR (**Figure 29**).

MI led to an increase of the end-diastolic pressure in both HanSD and TGR (**Figure 29A and B**: 30.5 ± 3.3 mmHg and 23.0 ± 2.5 mmHg) compared to the corresponding Sham group (9.7 ± 1.3 mmHg and 14.9 ± 3.2 mmHg). EET-A given alone or combined with *c*-AUCB, unlike single *c*-AUCB therapy, was able to significantly attenuate this increase in HanSD (P_{ed}: 20.0 ± 2.6 mmHg, 21.6 ± 2.2 mmHg and 24.7 ± 3.3 mmHg, respectively). No effect of treatment on P_{ed} was observed in TGR.

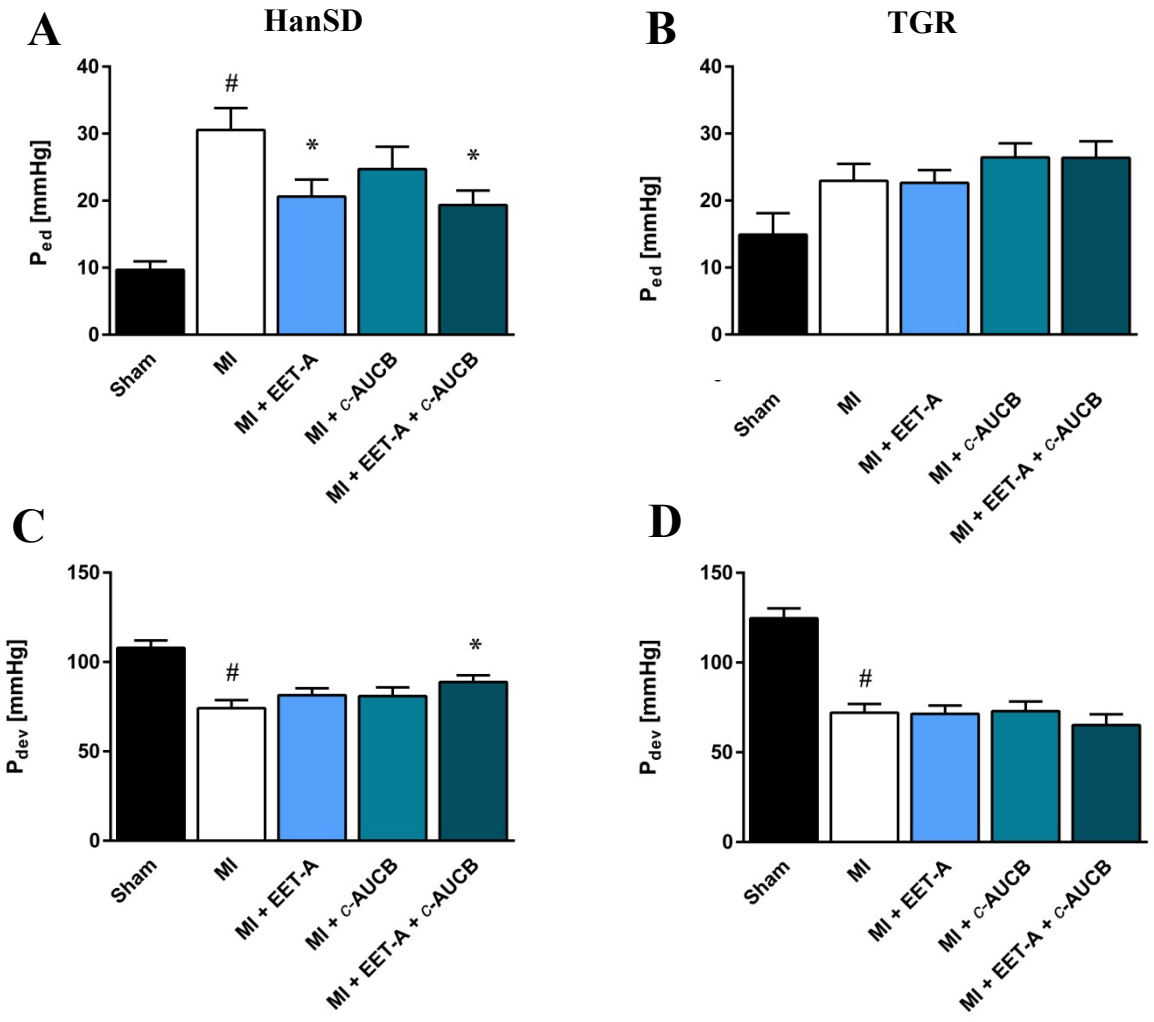

Figure 29 – End-diastolic pressure (P_{ed}; **A, B**) and developed pressure (P_{dev}; **C, D**) in Hannover Sprague-Dawley (HanSD, **A, C**) and *Ren-2* transgenic rats (TGR, **B, D**) subjected to Sham operation (Sham) or Myocardial infarction (MI) and treated with epoxyeicosatrienoic acid analogue (EET-A) and soluble epoxide hydrolase inhibitor (*c*-AUCB) for 5 weeks since 24 hours after MI. Values are expressed as mean ± SEM. $^{\#}$ $P < 0.05$ MI vs. corresponding Sham group, * $P < 0.05$ vs. corresponding MI group.

Overall results summary:

Continuous normobaric hypoxia prior to the ischemia/reperfucsion insult improved survival but did not affect postischemic heart failure in Wistar rats. Exercise training prior to the ischemia/reperfusion insult had no significant effect on cardiac function in our experimental setup.

Therapeutic adaptation to continuous normobaric hypoxia attenuated the progression of postischemic heart failure in Wistar rats. On the other hand, therapeutic adaptation to exercise training did not affect the postischemic heart failure in Wistar rats

EET-B (epoxyeicosatrienoic acid analogue) treatment led to increased survival in spontaneously hypertensive rats subjected to ischemia/reperfusion insult and improved their postischemic cardiac function.

Therapeutic administration of EET-A (epoxyeicosatrienoic acid analogue) combined with *c*-AUCB (inhibitor of soluble epoxide hydrolase) improved cardiac function in normotensive Hannover Sprague-Dawley rats (HanSD) with postischemic HF. Single therapies did not provide a cardioprotective effect in normotensive HanSD rats. Postischemic heart failure was not affected by the therapeutic administration of either EET-A or *c*-AUCB treatment in hypertensive Ren-2 transgenic rats.

5. DISCUSSION

We tried to characterize the cardioprotective potential of different approaches in order to prevent or at least attenuate postischemic HF progression in normotensive and hypertensive animals.

For this purpose, in the first part of this thesis, we used adaptation to CNH and ExT prior to or after the I/R insult in normotensive animals. The second part of this thesis is dedicated to the role of novel epoxyeicosatrienoic acid analogues EET-A and EET-B and sEH inhibitor *c*-AUCB in one strain of normotensive and two strains of hypertensive rats. The discussed effectiveness of these protocols and treatments is based on a precise assessment of LV geometry and function using mostly echocardiography and catheterisation.

5.1. Effect of continuous normobaric hypoxia and exercise training prior to ischemia/reperfusion insult on postischemic heart failure

The main finding of this part of our study is that the adaptation to CNH but not ExT improves survival during acute I/R insult, and they do not attenuate the progression of postischemic HF in Wistar rats.

It is well established that animals adapted to both CNH and ExT exhibit cardioprotective phenotypes if subjected to acute I/R insult. As natural and clinically relevant strategies for increasing resistance to myocardial I/R injury, both of these approaches are intensively studied. While the underlying mechanisms of their actions are not entirely understood, it is well established that their actions are closely related to inflammatory response, redox state regulation and apoptotic signalling. These mechanisms are associated with acute I/R injury and the following postischemic myocardial remodelling and HF development. Yet, the effect of CNH and ExT on postischemic HF remains unclear.

The beneficial role of chronic hypoxia in cardiovascular diseases in humans was confirmed by several epidemiological studies (Hurtado 1960; Mortimer *et al.*, 1977; Anderson and Honigman, 2011). The positive effect of adaptation to chronic hypoxia can be manifested as limited MI (Meerson *et al.*, 1973; Turek *et al.*, 1980), improved postischemic recovery of cardiac contractile function (Tajima *et al.*, 1994; Naghshin *et al.*, 2009) or decreased incidence and severity of ischemic and reperfusion arrhythmias (Meerson *et al.*, 1987; Asemu *et al.*, 2000). Moreover, some studies reported that hypoxia-induced cardioprotective phenomenon could persist up to several weeks after cessation of the hypoxic stimulus (Ošťádal and Widimský, 1985; Faltová *et al., 1987*; Neckář *et al.*, 2004).

Therefore, we hypothesised that some of these effects of adaptation to hypoxia might be manifested in the experimental model of postischemic HF. This was confirmed in mice with HF(transgenic animals with cardiac overexpression of tumor necrosis factor α) adapted to intermittent hypoxia (Naghshin *et al.*, 2012), but the role of adaptation to CNH in postischemic HF is unknown.

Our results confirmed that CNH-adapted animals are less susceptible to sustained ventricle fibrillation during the I/R period as previously reported by Asemu *et al*. (1999) or Alánová *et al.* (2017). This was further translated into improved survival, while mortality in the MI group was comparable to similar models of large MI (Pfeffer *et al.*, 1985; Bech *et al.*,1990).

In our experimental setup, CNH adaptation did not alter LV scar size, whereas some studies reported a decrease in early postischemic MI size in animals adapted to CNH (Maslov *et al.*, 2013; Chytilová *et al.*, 2015; Alánová *et al.*, 2017). Two aspects could explain this inconsistency. First, the ischemic period in our experiment was prolonged to 60 minutes. This likely exceeded the improved ability of hypoxia-adapted cardiomyocytes to survive the I/R insult, resulting in no reduction of MI size and limited potential to attenuate HF development. Second, unlike acute studies, we assessed the extent of myocardial injury in our experiment one week (echocardiography) or four weeks (echocardiography, catheterisation, immunohistochemistry) after the insult, which does not allow us to distinguish between initial infarct size and further myocardial remodelling. Considering these limitations, we did not observe the improvement of either progressive dilatation or cardiac dysfunction in CNH-adapted animals compared to normoxic ones.

It is known that the exposure to a hypoxic environment leads to compensatory changes in the pulmonary circulation. If this exposure is prolonged, it might result in pulmonary hypertension, right ventricle HF and lung oedema (Herger *et al.*, 1999). Postischemic LV HF may also lead to lung oedema caused by progressive LV diastolic dysfunction (Waters and Smith, 1951; Luisada, 1953; recently reviewed by King and Goldstein, 2020). Our data show that these adverse effects of CNH and postischemic HF can interfere even after cessation of the hypoxic stimulus. This was demonstrated by a dramatic increase in relative lung weight in CNH + MI compared to the MI group.

As the signs of postischemic HF progression strongly correlate with scar size (Pfeffer *et al.*, 1979; Fletcher *et al.*, 1981), we can conclude that in our experimental setup, CNH prior to the I/R insult had no beneficial effect on postischemic HF development in Wistar rats, but worsened the lung oedema.

In exercise-trained animals, our result did not show significant improvement in survival. Despite that, we observed a tendency to ExT-induced anti-arrhythmic effect as previously reported by Hamilton *et al.* (2004) or Dor-Haim *et al.* (2017). A similar tendency was also observed by Alánová *et al.* (2017).

Whereas Barboza *et al.* (2016) and Bozi *et al.* (2013) showed improved LV function in rats trained prior to the MI, we did not confirm this effect in our study. As discussed previously, a possible explanation could be based on different size of initial infarction. Bozi *et al.* (2013) reported smaller scar circumference and improved function. On the other hand, our data show increased scar circumference and unchanged function compared to sedentary animals with MI. Considering the strong correlation of infarct size and cardiac dysfunction (Baily *et al.*, 1993 and dos Santos *et al.*, 2008), we can speculate that worsened LV remodelling in our experiment was partially compensated by improved LV function resulting in a neutral effect.

Still, the increased scar size in our ExT animals is contradictory to previous results of Yamashita *et al.* (1999), Demirel *et al.* (2001), Freimann *et al.* (2005) or Budiono *et al.* (2012). The different protocol of MI induction might partially explain this inconsistency. Whereas Budiono *et al*. (2012) used 20-min ischemia followed by reperfusion and Demirel *et al.* (2001) used 30-min ischemia followed by reperfusion, Yamashita *et al.* (1999) and Freimann *et al.* (2005) used permanent ligation of the coronary artery. Therefore, we could hypothesise that the anti-inflammatory effect of ExT (reviewed by Petersen and Pedersen, 2005) can disrupt the balance in inflammatory signalling during myocardial remodelling and thus lead to different outcomes based on the intensity and duration of the ischemic event and previous ExT regimen. This idea is supported by the observation of Hammerman *et al.* (1983), that the myocardial scar was thinner in rats exposed to forced swimming before MI compared to sedentary controls. On the other hand, Tang *et al.* (2011) did not observe a similar effect in treadmill running rats 7 days after moderate MI. Finally, we cannot exclude that endurance training provides the infarct size-limiting effect in a time-dependent manner similar to that demonstrated by Yamashita *et al.* (1999). He showed that a single bound of exercise lowers the infarct size when assessed 30 minutes after reperfusion but not 3, 24 and 72 hours after reperfusion.

To conclude, our ExT regimen prior to the I/R insult did not alter postischemic HF progression in HanSD rats.

5.2. Therapeutic effect of continuous normobaric hypoxia and exercise training on postischemic heart failure

The main finding of this part of our study is that adaptation to CNH but not to ExT after ischemia/reperfusion insult attenuates the development of postischemic HF in Wistar rats (Hrdlička *et al.*, 2016).

Potential problems associated with variable initial infarct size, as discussed in the previous chapter, were eliminated by assessing cardiac function prior to randomising the experimental groups after MI. The adaptation to CNH or ExT began 7 days after MI when the early MI wound healing phase shifts towards ECM reconstruction (reviewed by Richardson *et al.*, 2015). Based on echocardiographic data, no differences in heart geometry and function among experimental groups occurred at this time point.

Several studies reported improved survival after MI associated with therapeutic intermittent hypoxia (Xu *et al.*, 2011) or ExT (Lundeberg *et al.*, 1998). We did not observe significant mortality in any experimental group, and our study thus did not show any differences in survival among experimental groups.

Therapeutic effects of intermittent hypobaric hypoxia in HF were addressed by several studies (del Pilar *et al.*, 2006; Xu *et al.*, 2011; Naghshin *et al.*, 2012). On the other hand, the role of CNH remains unknown. Our study was the first one to demonstrate that postischemic adaptation to CNH attenuates progressive LV dilatation in a way comparable to IHH (Xu *et al.*, 2011). Since CNH was associated with lower body weight than the MI control group, some could argue that it is an effect of growth retardation (Litwin *et al.*, 1994). Comparison of LVD_d to BW relationship for MI and MI + CNH groups (Figure 30) demonstrates that growth retardation may contribute to this difference but does not fully explain it. We could also exclude the effect of different scar size since they did not differ among experimental groups at the end of the study.

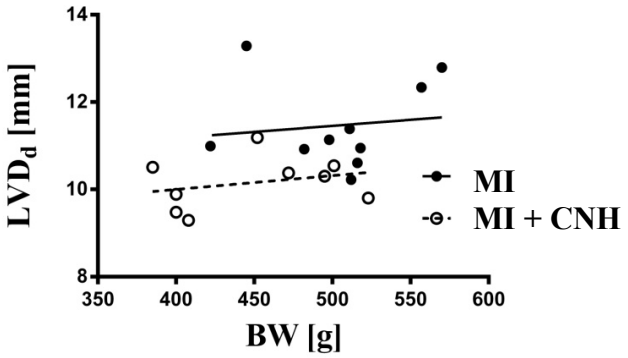

Figure 30 LVDd to BW relationship in animals adapted to continuous normobaric hypoxia after myocardial infarction (MI+ CNH) compared to MI controls.

Regarding the LV function, we observed a positive effect of CNH on postischemic LV contractility and systolic pressure. A similar effect was also reported by Xu *et al.* (2011) in animals adapted to IHH after MI. Postischemic HF progression is associated with diastolic dysfunction, which may lead to disruption of the pulmonary circulation and pulmonary oedema, as discussed previously. Similar dysfunction and signs of pulmonary oedema were observed in our experiment. It is known that adaptation to CNH is also associated with an adverse effect on pulmonary circulation due to inducing pulmonary hypertension (Rotta *et al.*, 1956, Sime *et al.*, 1963, Herget *et al.*, 1999). Interestingly, postischemic exposure to CNH did neither amplify the diastolic dysfunction nor affect the lung oedema in animals with HF.

In ExT animals, neither scar size nor LVD_d differed compared to the MI group. Therefore, we could conclude that contrary to previous reports (Musch *et al.,* 1989; Ait Mou *et al.,* 2009; Bito *et al.,* 2010), ExT in our experimental setup did not affect progressive LV dilatation. Physical activity exhibited some tendencies to improve functional parameters like $+(dP/dt)_{max}$, $-(dP/dt)_{max}$ or RWT, but these changes were not significant. We can mention two potential limitations of our study to explain these diverse results. First, as discussed previously, the large size of MI may limit the potential protective effect by reducing the mass of surviving cardiomyocytes. Second, several studies showed that certain intensity of physical load is needed to trigger the ExT-associated cardioprotection (Noble *et al.*, 1999; Lennon *et al.*, 2004; Starnes *et al.*, 2005). For treadmill running, this minimal level of exercise intensity is usually reported to be between 60 and 70% of VO_2max (or treadmill speed of 25 to 30 m/min and duration of 60 min/day: Lennon *et al.*, 2004; Starnes *et al.*, 2005). In our experiment, the exercise capacity of animals with MI limited the treadmill protocol to the speed of 15 m/min and a duration of 60 min/day. Therefore, it is likely that animals with large MI and hence limited tolerance to physical work cannot reach the threshold exercise intensity needed for significant improvement of LV function and remodelling.

To conclude, we did not observe the therapeutic effect of ExT on postischemic HF in our experimental setup.

5.3. Effect of epoxyeicosatrienoic acid analogue EET-B on postischemic heart failure in spontaneously hypertensive rats

The main finding of this chapter is that novel orally active EET analogue EET-B reduces I/R associated mortality and attenuates postischemic LV systolic dysfunction in spontaneously hypertensive rats without affecting blood pressure. This is accompanied by the reduction of pulmonary oedema (Neckář *et al.*, 2019).

It has been well established that EETs play a beneficial role in a variety of biological mechanisms associated with cardiovascular diseases and their risk factors, including vasodilatation (Fulton *et al.*, 1998; Ceresa *et al.*, 1999), anti-inflammatory effect (Node *et al.*, 1999; Falck *et al.*, 2003; Levick *et al.*, 2007; Thomson *et al.*, 2012), anti-fibrotic effect (Levick *et al.*, 2007; Li *et al.*, 2014) as well as lipid metabolism and insulin sensitivity (Luo *et al.*, 2010; Roche *et al.*, 2015; He *et al.*, 2016). Acute administration of EET analogues or sEH inhibitors (two most common strategies to increase EETs bioavailability) prior to ischemia or at the beginning of reperfusion are associated with increased

cardiac resistance to I/R insult in multiple experimental models (Batchu *et al.*, 2012; Motoki *et al*., 2008; Neckář *et al.*, 2012; Imig and Hammock 2009; Luo *et al.*, 2010; Roche *et al.*, 2015).

While there is ample evidence that EETs play a role even in arrhythmogenesis (Westphal *et al.*, 2013; Bukhari *et al.*, 2018; Li *et al.*, 2009), the effect of known EET analogues on I/R arrhythmias remains unclear. Several results show that EET-A (orally active analogue of EET similar to EET-B; Campbel *et al.*, 2017) and sEH inhibitor *c*-AUCB have the potential to significantly decrease the incidence of ischemic arrhythmias including life-threatening sustained ventricle fibrillation (Červenka *et al.*, 2018). A similar effect of EET-B was observed in our experiment.

It has been reported that EET-A improved tolerance to acute I/R insult in normotensive HanSD rats (Alánová *et al.*, 2015). Infarct size-limiting effect was also observed when EET-B was administered to HanSD rats at the beginning of reperfusion (Neckář *et al*., 2018). On the other hand, Červenka *et al*. (2018) demonstrated that EET-A or sEH inhibitor treatment did not affect infarct size in either normotensive HanSD or hypertensive TGR animals. This inconsistency seems to be based on different experimental models. Therefore, we can only speculate whether the cardioprotective effect of EET-B showed in our study was associated with increased resistance to acute I/R injury or rather with alteration of postischemic myocardial remodelling.

Interestingly, the cardioprotective effect of EET-B was independent of the blood pressure. The antihypertensive effect of EET-based treatment may vary depending on the experimental model of hypertension. While blood pressure was decreased by EET-based treatment in Ang-II-dependent models of hypertension (Neckář *et al.*, 2012; Hye Khan *et al.*, 2014; Červenka *et al.*, 2018), no effect of either EET-A or EET-B on hypertension was observed in Dahl salt-sensitive rats (Hye Khan *et al.*, 2013), Goldblatt hypertensive rats (Alánová *et al.*, 2015) and Cyp1a1-Ren-2 transgenic rats (Jíchová *et al.*, 2016). Detailed reports stress out that this antihypertensive effect is dependent not only on the aetiology of hypertension but also on the timing of treatment and alterations in EET metabolism. Jíchová *et al.* (2016) demonstrated that EET-A treatment attenuated blood pressure increase when applied simultaneously with indol-3-carbinol (hypertension-inducing xenobiotic), but not when hypertension was already established. On the other hand, Xiao *et al.* (2010) reported that hypertension in SHR could be prevented by cytochrome P450 overexpression. Taken together, our results show the cardioprotective effect of EET-based treatment in experimental models of hypertension can be manifested regardless of its antihypertensive actions.

As demonstrated previously, EET-based treatment has a certain potential to attenuate the progression of postischemic HF. It has been shown that EET analogue NUDSA improved cardiac function in mice after MI (Cao *et al*., 2015), and sEH inhibitors GSK2188931B or AUDA attenuated post-MI remodelling (Kompa *et al.*, 2013 and Merabet *et al.*, 2012). Our results are thus in line with previous reports.

While the detailed mechanism of complex cardioprotective actions of EET-based treatment is still to be elucidated, several studies suggest that cardiac anti-fibrotic and anti-inflammatory effects might play a key role. Both inflammation and fibrosis are crucial for postischemic cardiac remodelling. It was clearly demonstrated that cardiac fibrosis associated with MI (Kompa *et al.*, 2013; Merabet *et al.*, 2012; Sirish *et al.*, 2013) or pressure overload (Li *et al.*, 2014; He *et al.*, 2016; Xiao *et al.*, 2010) could be attenuated by EET-based treatment. A similar effect was observed in the inflammatory response after MI (Kompa *et al.*, 2013; Li *et al.*, 2014). We can hypothesise that both anti-inflammatory and anti-fibrotic effects of EET-B were responsible for the attenuation of postischemic HF development observed in our study. This view is further supported by our follow-up study results showing EET-B-induced reduction in myocardial collagen deposition and reduced monocyte/macrophage infiltration (Neckář *et al.*, 2019). However, further research will be needed to determine the role of anti-inflammatory and anti-fibrotic effects of EET-based therapies on postischemic HF.

5.4. Therapeutic effect of EET-A and *c*-AUCB on postischemic heart failure in normotensive HanSD rats and hypertensive *Ren-2* transgenic rats

The main finding of the final part of our study is that EET-based therapy using EET-A and *c*-AUCB can attenuate the progression of postischemic HF in normotensive HanSD rats but not in transgenic rats with Ang II-dependent hypertension (TGR) (Hrdlička *et al.*, 2019).

The first result of this study was that TGR were more susceptible to I/R-induced sustained ventricle fibrillation than HanSD. This is in agreement with previous reports of Červenka *et al.* (2018) and Ferdinandy *et al.* (2007). However, the total incidence of sustained ventricle fibrillation in our experiment was lower than that reported by Červenka *et al.* (2018), although the ischemia was three times longer (60 vs 20 min). Taken into account that ischemic fibrillation generally occurs in the early (5th to 7th minute) and the late (20th to 30th minute) phase of ischemia (Cascio *et al.*, 2005), a similar or higher incidence of fibrillations could be expected in our experiments. The reason for this difference is unclear, although the different size of area at risk can play a role.

Our data confirmed concentric LV hypertrophy in TGR as reported earlier (Bachman *et al.* 1992; Lee *et al.*, 1996; Langheinrich *et al.*, 1996) and mild systolic dysfunction (Whaley-Connell *et al.,*2007; De Mello *et al.*, 2013). On the other hand, systolic dysfunction in TGR was not reported by Habibi *et al.* (2011), Ma *et al.* (2012) or Neckář *et al.* (2012). This inconsistency could be explained

by different methods and parameters of LV systolic function assessment, where the sensitivity of specific parameters may vary.

I/R insult resulted in MI and the development of HF with a similar degree of systolic dysfunction in HanSD and TGR. Based on the low variability of evaluated parameters (mainly FS, LVD_s, AWT_s and PWT_s) and the previously mentioned strong correlation between MI size and echocardiographic parameters of cardiac function, we can conclude that the extent of MI was highly consistent in all experimental groups. However, we observed individual variability in HF progression expressed as increasing mortality during the experiment and increased variability of relative lung weight or end-diastolic pressure at the end of the study.

As reported previously, EET-A exhibited an antihypertensive effect in various forms of ANG II-dependent hypertension (Neckář *et al.*, 2012; Hye Khan *et al.*, 2014; Červenka *et al.*, 2018), and a similar effect was described for *c*-AUCB in various experimental models of experimental hypertension (Neckář *et al.*, 2012; Honetschlägerová *et al.*, 2013; Kopkan *et al.*, 2012). We could not confirm these effects in our experiments due to the postischemic LV dysfunction-induced decrease in blood pressure. This pressure decrease in hypertensive animals with postischemic cardiac dysfunction is consistent with previous reports of Nishikimi *et al.* (1995), Mori *et al.* (1998) or Wiemer *et al.* (2001).

Increased bioavailability of EETs by their exogenous administration or by inhibition of sEH attenuates lung injury caused by acute I/R (Townsley *et al.*, 2010; Chen *et al.*, 2015), lipopolysaccharides (Tao *et al.*, 2016) and bleomycin (Zhou *et al.*, 2016; Dong *et al.*, 2017). It also seems to have a protective effect on lung diseases associated with inflammation and oxidative stress like asthma (Yang *et al.*, 2015a and 2017). These protective effects are mostly associated with either attenuation of increased endothelial cell permeability (Townsley *et al.*, 2010; Alvarez *et al.*, 2004) or attenuation of inflammatory reaction (Yang *et al.*, 2015a and 2017; Chen *et al.*, 2015). We can speculate that both these protective mechanisms are likely to reduce lung oedema and improve pulmonary artery flow, as observed in HanSD animals in our study. On the other hand, improved LV systolic function and delayed diastolic dysfunction may also contribute to the reduction of lung oedema by delaying its onset.

Our results showed improved systolic function in HanSD rats with EET-based treatment, which is in agreement with previous studies of Li *et al.* (2009), Merabet *et al.* (2012), Kompa *et al.* (2013), Sirish *et al.* (2013) and Akhnokh *et al.* (2016). These beneficial effects are usually accompanied by increased tissue levels of EETs (Li *et al.*, 2009; Sirish *et al.*, 2013; Červenka *et al.*; 2018) and can be abolished by coadministration of cytochrome P450 epoxygenase inhibitors (Merabet *et al.*, 2012), a key enzyme in EETs production. It is, therefore, likely that EETs play an important beneficial role in the progression of postischemic HF.

Nevertheless, the data from other experimental models of HF remain inconsistent. For example, Xu *et al.* (2006) and Sirish *et al.* (2013) report positive effects of sEH inhibitors AEPU, AUDA and TPPU on adverse cardiac remodelling in mice with pressure overload due to thoracic aortic constriction. On the other hand, Morgan *et al.* (2013) reported increased EETs/DHETs ratio but no effect on LV dysfunction in GSK2256294 (sEH inhibitor) treated rats and mice with pressure overload. Similarly, Červenka *et al.* (2015a, 2015b) observed an increase of EETs/DHETs ratio but no effect on LV dysfunction in HanSD, TGR and Fawn-hooded rats with volume overload (aortocaval fistula) treated with *c*-AUCB. It seems, therefore, that EETs/DEHTs ratio is not a reliable marker for the prediction of cardioprotective effects of EET-based therapies.

Experimental models of heart failure and hypertension are usually associated with a further end-organ injury. Regarding their role in regulating blood pressure and circulation, these reports are often focused on kidneys. Renoprotective effects can be independent of antihypertensive actions. Combination of antihypertensive and renoprotective actions of EET-based treatment was observed in cyclosporin-induced hypertension with PVPA (EET analogue) administration (Yeboah *et al.*, 2016), radiation-induced nephropathy and EET-A treatment (Hye Khan *et al.*, 2016), Ang II-dependent hypertension and treatment with EET-A or sEH inhibitor *c*-AUCB (Červenka *et al.*, 2018) or in SHR with combined treatment of EET-A and AAA (antagonist of 20-HETE receptors; Gawrys *et al.*, 2020). On the other hand, renoprotection independent of antihypertensive effect was reported by Hye Khan *et al.* (2013) in Dahl salt-sensitive rats treated by EET-B or Cyp1a1-Ren-2 transgenic rats with established hypertension (Jíchová *et al.*, 2016). The renoprotective effect of EET-A without antihypertensive actions was also observed in the study parallel to our experiment (Hrdlička *et al.*, 2019). Therefore, we can conclude that the renoprotective actions of EET-based treatments might contribute to their cardioprotective effects and vice versa.

5.5. Conclusions

Despite intensive research on the field of cardioprotection against ischemia, the struggle to translate the promising experimental results into clinical practice fails to provide significant improvement in long-term clinical outcomes. This has resulted in overall scepticism regarding the applicability of such experimental protocols of cardioprotection. However, we believe that several cardioprotective protocols used in this thesis, e.g. adaptation to CNH, ExT and EET-based treatment, have certain potential not only to improve the tolerance to acute ischemic insults but also to attenuate the progression of the postischemic HF. Our results have revealed that all these approaches are associated with some beneficial effect; in the case of adaptation to CNH and EET-based therapies,

the differences were highly significant. In addition, the EET-induced cardioprotective effects seem to be dependent on animal strains

Based on our results, we can conclude that mechanisms leading to increased cardiac tolerance to acute ischemia could play an important role also in postischemic cardiac remodelling and function.

6. SUMMARY

1. Preventive adaptation to continuous normobaric hypoxia led to an antiarrhythmic effect and resulted in improved survival. We showed the beneficial effect of preventive adaptation to continuous normobaric hypoxia in animals with postischemic heart failure. On the other hand, we did not observe the cardioprotective effect of preventive exercise training.

2. Therapeutic adaptation to continuous normobaric hypoxia attenuated the dilatation of the left ventricle and improved its function in rats with postischemic heart failure. Again, we showed the beneficial effect of therapeutic adaptation to continuous normobaric hypoxia in rats with postischemic heart failure. Similarly to preventive exercise training, we did not observe the cardioprotective effect of exercise training after myocardial infarction.

3. Preventive administration of EET-B (analogue of epoxyeicosatrienoic acids) led to an antiarrhythmic effect during the ischemia/reperfusion insult and improved survival in spontaneously hypertensive rats. EET-B treatment improved postischemic cardiac function without effect on left ventricle dilatation. We showed the beneficial effect of EET-B treatment in spontaneously hypertensive rats with postischemic heart failure.

4. Therapeutic administration of EET-A (analogue of epoxyeicosatrienoic acids) combined with *c*-AUCB (inhibitor of soluble epoxide hydrolase) improved left ventricle function but did not attenuate left ventricle dilatation in normotensive Hannover Sprague-Dawley rats (HanSD) with postischemic heart failure. We showed the beneficial effects of combined treatment with EET-A and *c*-AUCB in normotensive HanSD rats with postischemic heart failure. The beneficial effects of this treatment were, however, not significant in HanSD with single therapy and were not observed in hypertensive Ren-2 transgenic rats in either single or combined therapy.

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8. LIST OF PUBLICATIONS

8.1. Publications enclosed in full length.

Publication A

Hrdlička, J., Neckář, J., Papoušek, F., Vašinová, J., Alánová, P., Kolář, F. (2016) **Beneficial effect of continuous normobaric hypoxia on ventricular dilatation in rats with post-infarction heart failure.** *Physiol Res.* 65(5): 867-870. (IF = 1.461)

Publication B

Neckář, J., Hye Khan, M.A., Gross, G.J., Cyprová, M., Hrdlička, J., Kvasilová, A., Falck, J.R., Campbell, W.B., Sedláková, L., Škutová, Š., Olejníčková, V., Gregorovičová, M., Sedmera, D., Kolář, F., Imig, J.D. (2019) **Epoxyeicosatrienoic acid analog EET-B attenuates post-myocardial infarction remodeling in spontaneously hypertensive rats.** *Clin. Sci. (Lond.)*. 133(8): 939-951. (IF = 5.223)

Publication C

Hrdlička, J., Neckář, J., Papoušek, F., Husková, Z., Kikerlová, S., Vaňourková, Z., Vernerová, Z., Akat, F., Vašinová, J., Hammock, B.D., Hwang, S.H., Imig, J.D., Falck, J.R., Červenka, L., Kolář, F. (2019**) Epoxyeicosatrienoic Acid-Based Therapy Attenuates the Progression of Postischemic Heart Failure in Normotensive Sprague-Dawley but Not in Hypertensive Ren-2 Transgenic Rats.** *Front. Pharmacol.* 10:159. (IF = 4.468)

8.2. Other publications

Alánová, P., Chytilová, A., Neckář, J., Hrdlička, J., Míčová, P., Holzerová, K., Hlaváčková, M., Macháčková, K., Papoušek, F., Vašinová, J., Benák, D., Nováková, O., Kolář, F. (2017) **Myocardial ischemic tolerance in rats subjected to endurance exercise training during adaptation to chronic hypoxia.** *J. Appl. Physiol.* (1985) 122(6): 1452-1461. (IF = 3.256)

Adameová, A., Hrdlička, J., Szobi, A., Farkašová, V., Kopásková, K., Muráriková, M., Neckář, J., Kolář, F., Ravingerová, T., Dhalla, N.S. (2017) **Evidence of necroptosis in hearts subjected to various forms of ischemic insults**. *Can. J. Physiol. Pharmacol.* 95(10): 1163-1169. (IF = 2.210)

Lichý, M., Szobi, A., Hrdlička, J., Horváth, C., Kormanová, V., Rajtík, T., Neckář, J., Kolář, F., Adameová, A. (2019) **Different signalling in infarcted and non-infarcted areas of rat failing hearts: A role of necroptosis and inflammation.** *J. Cell. Mol. Med.* 23(9): 6429-6441. (IF = 4.658)

Lichý, M., Szobi, A., Hrdlička, J., Neckář, J., Kolář, F., Adameová, A. (2020) **Programmed Cell Death in the Left and Right Ventricle of the Late Phase of Post-Infarction Heart Failure.** *Int. J. Mol. Sci.* 21(20): 7782. (IF = 4.653)

9. Supplements

Publication A

Hrdlička, J., Neckář, J., Papoušek, F., Vašinová, J., Alánová, P., Kolář, F. (2016) **Beneficial effect of continuous normobaric hypoxia on ventricular dilatation in rats with post-infarction heart failure.** *Physiol Res.* 65(5): 867-870.

Publication B

Neckář, J., Hye Khan, M.A., Gross, G.J., Cyprová, M., Hrdlička, J., Kvasilová, A., Falck, J.R., Campbell, W.B., Sedláková, L., Škutová, Š., Olejníčková, V., Gregorovičová, M., Sedmera, D., Kolář, F., Imig, J.D. (2019) **Epoxyeicosatrienoic acid analog EET-B attenuates post-myocardial infarction remodeling in spontaneously hypertensive rats.** *Clin. Sci. (Lond.)*. 133(8): 939-951.

Publication C

Hrdlička, J., Neckář, J., Papoušek, F., Husková, Z., Kikerlová, S., Vaňourková, Z., Vernerová, Z., Akat, F., Vašinová, J., Hammock, B.D., Hwang, S.H., Imig, J.D., Falck, J.R., Červenka, L., Kolář, F. (2019**) Epoxyeicosatrienoic Acid-Based Therapy Attenuates the Progression of Postischemic Heart Failure in Normotensive Sprague-Dawley but Not in Hypertensive Ren-2 Transgenic Rats.** *Front. Pharmacol.* 10:159.