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The hemodynamic aspects of pharmacological manipulation of cardiac contractility and vascular resistance in rewarming from hypothermia

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My greatest admirations and respect goes to the reindeer herders in the mountains and the fishermen at sea. They are the true masters of *the cold*.

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1 Acknowledgement

This thesis and experimental work was carried out as a medical student participating in the research program (Forskerlinja) (2010 - 2015) and PhD student (2015 – 2017), at UiT, Arctic University of Norway and The Mayo Clinic (2010-2011), Dept of physiology and Biomedical Engineering with our collaborator Prof. Gary Sieck. Much of the thesis has been written and finalized while working as a medical doctor at the University Hospital of Northern-Norway (2017 – 2018).

I would firstly thank Prof. Torkjel Tveita for opening the doors to the wonderful world of experimental physiology, by allowing me to participate in his project on accidental hypothermia. Looking back at the now eight years long journey I am amazed of the trust and confidence you have had in me. Allowing me to travel to The Mayo Clinic at 22, alone, with little experimental experience. In Rochester I was lucky to have Prof. Gary Sieck and Dr. Arteaga as mentors and supervisors in the lab. I always felt welcome in the SieckLab and learned more about experimental research than I could ever imagine.

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2 Abstract

Paper I

To investigate the hemodynamic response of calcium-sensitization and PDE3-inhibitions during hypothermia and rewarming we administered levosimendan in an experimental model of accidental hypothermia.

Paper II

In an identical experimental model of hypothermia we aimed to investigate the hemodynamic effects of systemic vascular resistance reduction during hypothermia and rewarming. This was achieved by administration of nitroprusside.

Paper III

To further investigate the hemodynamic effects of levosimendan and nitroprusside in the rewarming scenario, a modified version of the experimental model from paper I and II was applied. Organ perfusion was measured during and after rewarming.

Main results and conclusions

Hypothermia and rewarming depressed hemodynamic function in all control groups. Levosimendan improved cardiac output more than nitroprusside, while nitroprusside reduced vascular resistance. Nitroprusside demonstrated to superiorly improve organ blood flow.

The hemodynamic state after rewarming seems to be a combination of reduced cardiac function and unwanted systemic vasoconstriction. Organ perfusion is not limited only by available CO, but also possibly altered autoregulation and vascular pathology.

3 Abbreviations - sorted by occurrence

HCD: Hypothermia-induced cardiac dysfunction
CO: Cardiac output
SV: Stroke volume
FFA: Free fatty acid
cTnI: Cardiac troponin I
PKA: Protein kinase A
BV: Blood volume
SVR: Systemic vascular resistance
MAP: Mean arterial pressure
RAAS: Renin-angiotensin-aldosterone system
LV: Left ventricle
CPB: Cardiopulmonary bypass
PV: Plasma volume
PCOP: Plasma colloid pressure
ICOP: Interstitial colloid pressure
CBF: Cerebral blood flow
OBF: Organ blood flow
MBF: Myocardial blood flow
RBF: Renal blood flow
SBF: Splanchnic blood flow
CPR: Cardio pulmonary resuscitation
ECMO: Extra corporal membrane oxygenation
COMT: catechol-O-metyl transferase
E_{max}: Maximum response
EC_{50}: Half maximal effective concentration
HR: Heart rate
CI: Cardiac index
PDE3: Phosphodiesterase III
cAMP: cyclic AMP
LS: Levosimendan
SNP: Sodium nitroprusside

PRSW: Preload recruitable stroke work
ESP: End-systolic pressure
dP/dt_{max}: Maximum rate of LV pressure change
dP/dt_{min}: Minimum rate of LV pressure change
Tau: The isovolumic relaxation constant
SW: Stroke work
D_{O2}: Oxygen delivery
D_{O2}: Oxygen consumption
ESPVR: End systolic-pressure volume relationship
PDM: Disintegration per minute
SO_2: oxygen saturation
Hb: Hemoglobin
Hct: Hematocrit
HCO_3: Bicarbonate
CaO_2: Arterial blood oxygen content
CvO_2: Venous blood oxygen content
$CMRO_2$: Cerebral metabolic rate of oxygen
MVO_2: Cardiac oxygen consumption
GFR: Glomeruli filtration rate

4 Publications included

The studies in this thesis were carried out between 2010-2017 at the Anesthesia and Critical Care Research Group at the Department of Clinical Medicine, The Arctic University of Norway in Tromsø. Part of the biochemical analysis was performed at the Department of physiology and biomedical engineering at The Mayo Clinic, Rochester, MN, USA. The included papers in this thesis are listed below and will be referred to by their numerals.

4.1 **Paper I**

Cardiovascular effects of levosimendan during rewarming from hypothermia in rat. Cryobiology, Volume 69, Issue 3, Desember 2014, Pages 402-410, https://doi.org/10.1016/j.crybiol.2014.09.007
Erik Sveberg Dietrichs, Brage Håheim, Timofei Kondratiev, Gary Sieck, Torkjel Tveita

4.2 **Paper II**

The beneficial hemodynamic effects of nitroprusside during experimental hypothermia and rewarming. Cryobiology, Volume 77, August 2017, Pages 75-81, https://doi.org.10.1016/j.cryobiol.2017 .05.002
Brage Håheim, Timofei Kondratiev, Erik Sveberg Dietrichs, Torkjel Tveita

4.3 **Paper III**

The beneficial effects of sodium nitroprusside and levosimendan on organ perfusion during rewarming from experimental hypothermia – Manuscript
Brage Håheim, Timofei Kondratiev, Erik Sveberg Dietrichs, Torkjel Tveita

5 Introduction

5.1 Grading of hypothermia

The dangers of hypothermia correlate to temperature depth and duration[1]. To aid health professionals and scientists, grading systems have been developed to simplify clinical decision making and treatment procedures. As in many areas of medicine, there has been a gradual development, and the grading of hypothermia have been changed to coincide with available knowledge. In 1974 Popovic defined hypothermia as a core temperature <35°C, and the international community still agrees on this definition[2]. Further, Popovic sub-classified hypothermia as mild (35-32°C), moderate (32-22°C), deep (22-8°C) or profound (<8°C). In 1994 Danzl classified hypothermia as mild (35-32°C), moderate (32- 28°C), severe (28-20°C), or profound (<20°C)[1]. The European Resuscitation Council's guidelines for treating hypothermic patients use a modified version of this system, without a profound group[3]. A more simplified scaling was proposed by Polderman in 2009[4]. Mild hypothermia (35-34°C), moderate (34-30°C), and severe (<30°C). This grading is used by the American Heart Association and in University Hospital of Northern-Norway 2014 guidelines for treating accidental hypothermia[5].

5.2 Epidemiology of hypothermia

The yearly incidence and mortality of isolated hypothermia is low[6]. However, it is associated with a high lethality and is a common complication in surgery and trauma situations. Epidemiological studies performed in 1970s show 30-80% lethality among hypothermic patients. In more recent studies, lethality has fallen to around 30%[7-10]. It is important to stress that the hypothermic patient population is heterogenic, and total lethality rate hide the complexity surrounding chance of survival[7,8,10].

5.2.1 Hypothermic patients

Most epidemiological studies on hypothermia are small, single-center and with few patients. In the urban situation, Roeggla et al, (80 patients, 1991-1998 in Vienna), Megarbane et al (81 patients, 1981 – 1998 in Paris) and van der Ploeg et al (84 patients, 2000 – 2008 in Amsterdam) have described the hypothermic epidemiology. The results are consistent between the three studies[7,8,10]. Based on autopsy data from 63 hypothermia deaths in Alabama, USA, Taylor et al concluded that death due to hypothermia can be divided in two

main groups; “the old and comorbid” and “the young intoxicated”[11]. This is consistent with the findings of Roeggla, Megarbane and van der Ploeg were 50 – 85 % were male, the average age was 47 - 65 years, 22–35 % were found indoors and the total mortality was 28 – 35 %.

These studies show a discrepancy in demographics, comorbidity and mortality between the patients found indoor and those found outside. The average age of patients found indoor was 67 – 69 years of age and 42 – 50 in the outdoor groups. Megarbane et al investigated the difference in comorbidity between the two groups and found that the patients found indoors had higher rate of chronic illness. Van der Ploeg et al made no such analysis, but their survival data show a higher median age in non-survivors (68 years) than the survivor group (38 years)[7,8,10].

Variable	Found inside	Found outside
Age (years)	67 – 69	42 – 50
Intoxicated (%)	19	76
Septicemia (%)	30	6
Neuropsychiatric (%)	27	12
Hypo-glycemia/thyroidism (%)	13	6
Cardiac arrhythmias (%)	5	-
Unspecified pathology (%)	-	6
Survival (%)	44 - 81	6 - 11

5.2.2 Trauma patients and hypothermia:

Hypothermia is an independent mortality risk-factor in trauma patents[12,13], and 9.7% of hypothermia patients was traumatically injured[14]. Unconsciousness, hemorrhage, hypoxia and hypoperfusion are all important factors predisposing trauma patients to hypothermia[15]. In the US National Trauma Data Bank, 1.9 % of patients where hypothermic and the mortality rate was 25 % versus 3 % compared to patients with a core temperature >35°C[16]. Wang et al. showed a linear relationship between degree of hypothermia and mortality in trauma patients, with 100 % mortality at core temperature <32°C independent of injury severity score[14]. In Melbourne, Australia, Ireland et al. found that 13.25% of 737 trauma patients were

hypothermic upon hospital admission[12]. A mortality of 29.9 % was found in these patients versus 5.98 % in non-hypothermic patients. In addition, hypothermic trauma patients stay longer in hospital and have a lower chance of being discharged to their home[12].

5.3 *The cardiovascular pathophysiology of hypothermia and rewarming shock*

From the clinical perspective, the cardiovascular-, respiratory- and cerebral depression during hypothermia are critical: All hypothermic patients stand in risk of cardiac arrest, apnea or loss of consciousness[1,17,18]. Depending on the degree and prolongation of the hypothermic period the pathological changes of cooling manifest as acute and prolonged organ damage or failure[1]. One clinical consequence of hypothermia-induced hemodynamic pathology is cardiovascular dysfunction; rewarming shock, at low core temperatures and during rewarming. Investigators have demonstrated that hypothermia and rewarming is associated with changes in circulating blood volume, cardiac contractility and vascular resistance[19-23].

5.3.1 Hypothermia-induced cardiac dysfunction (HCD)

HCD is evident both from clinical and preclinical data[20,21]. Clinically, this is manifest as a lowered blood pressure and evidence of depressed organ perfusion[1,21]. In experimental models, investigators have demonstrated lowered cardiac output (CO), stroke volume (SV) and cardiac contractile force during and after rewarming[20,24,25]. Further, investigators have attempted to describe and explain the physiological and cellular changes caused by hypothermia that depress the contractile properties of the heart. Findings show that cardiomyocyte ATP-availability, electrochemical homeostasis, endocrine control and cross-bridge formation all seems to be altered during hypothermia, and possibly contribute to the development of HCD. The following subsection aim to present a detailed description of these changes and their possible contribution to HCD.

5.3.1.1 Cardiac metabolism and ATP production

A balance between production and usage of ATP is an absolute necessity to cellular function and depressed production is associated with cellular dysfunction and cell death[26]. The heart depends on a great production of ATP to drive the actin-myosin cross-bridge complex and maintain electrochemical homeostasis[27]. Bui-Mong-Hung et al. investigated the cardiac tissue content of high-energy phosphates during hypothermia in vitro and found normal levels after cooling to 13°C[28]. These findings were supported by Tveita et al. who also demonstrated that

high-energy phosphate levels are preserved during hypothermia at 25°C, and after rewarming in an in vivo dog model[24]. Contrary to these findings, Tveita later showed reduced ATP after four hours of hypothermia at 15°C and after rewarming, in an in vivo rat model showing significant reduction in cardiac functional variables[29].

Production of high-energy phosphates depend on oxygen availability, metabolic substrate and mitochondrial integrity[30]. Cardiomyocyte damage and stress cause a shift in metabolic substrate from free fatty acids (FFA) to glucose and FFA oxidation has been seen as an indicator for cardiomyocyte health and effective metabolism[31]. In an attempt to investigate the effect of hypothermia and rewarming on myocardial substrate selection, Steigen et al. described a shift towards FFA oxidation, with reduction in both substrates, at 15°C. After rewarming, only FFA returned to normal levels[32]. Steigen et al. theorized that the shift towards FFA might explain the myocardial oxygen wastage described after rewarming [24,33]. In a second study on rat hearts they demonstrated lower cardiac performance and elevated post-hypothermic intracellular Ca^{2+} in isolated hearts that were provided exogenous FFA in addition to glucose[34]. Aasum et al, demonstrated improved cardiac performance if carbohydrate metabolism was pharmacologically induced during rewarming[35]. However, in dogs cooled to 25°C and rewarmed, Steigen et al. found conflicting results to that of the rat. Like in the rats, FFA and glucose uptake and usage is decreased during hypothermia, however rewarming normalized glucose oxidation while FFA oxidation was reduced[25]. Tveita et al also found similar results in a second study on dogs cooled to 25°C[24].

5.3.1.2 Intracellular ion balance and pH

8-10 % of cardiac metabolism is utilized to uphold intracellular ion balance[27]. In hypothermia, Na^+/K^+-ATPase activity is reduced[36]. In isolated cardiomyocytes, hypothermia have demonstrated to elevate intracellular Na^+[37,38], and potentiate hypoxia-induced Na^+ accumulation[39]. In vivo, Tveita et al. found elevated cardiomyocyte cytosolic volume after 4 hours at 15°C in rats. This was further elevated upon rewarming, also including intracellular and mitochondrial volume. The investigators interpreted this as evidence of Na^+ accumulation during hypothermia and rewarming[40].

As with Na^+, cooling causes a disruption of cellular Ca^{2+} homeostasis[41]. In isolated cardiomyocytes Liu et al showed Ca^{2+} accumulation due to reversal of the Na^+/Ca^{2+} exchanger[42]. In isolated hearts, Aasum et al made the same discoveries during hypothermia,

and also showed elevated Ca^{2+} after rewarming[35]. These findings where later confirmed by Kondratiev and Wold using an in vivo model[43,44]. Cardiac Ca^{2+} homeostasis is in synchronization with the bi-phasic nature of the cardiac cycle and hypothermia-induced bradycardia affect this balance[45]. Hypothermia prolong action potential duration and the opening of, and flux through, the L/T-type Ca^{2+} channels, which has been proposed as an important mechanism of Ca^{2+} accumulation, in addition to reversal of the Na^{+}/Ca^{2+} exchanger [46]. However, Bjørnstad et. al proposed that Ca^{2+}-accumulation caused the prolongation of action potential duration, not the other way around[47]. In addition to altered sarcolemmal Ca^{2+}-fluxes, hypothermia seems to disrupt intracellular Ca^{2+} handling by the sarcoplasmatic reticulum and the mitochondria[42,48]. The elevated cellular Ca^{2+} has been proposed to be buffered by the mitochondria, and that this cause mitochondrial Ca^{2+}-overload[49]. Ca^{2+} is an important regulator of mitochondrial function and Ca^{2+} is known to trigger cellular apoptosis[50,51].

In ischemia, the anaerobic metabolic production of lactate and accumulation of H^{+} is thought be a key mechanism underlying cellular acidosis[52]. This may cause cellular swelling as the H^{+}/Na^{+}-exchanger contribute to further influx of Na^{+} when H^{+} is expelled in exchange for Na^{+}. Elevated levels of H^{+} may cause depressed ATP production, reduced contractility and Ca^{2+} buffering[53]. In hypothermia, Ellis et al. demonstrated that cooling to 20°C did not alter cellular pH[54] and Kusuoka et al. later reproduced these findings at 30°C[55]. Using magnetic resonance imaging in vivo, Swain et al. documented elevated pH (7.0 to 7.2) in intact sheep hearts cooled to 26°C[56]. Boutillier argued that the H^{+}/Na^{+}-exchanger is affected by hypothermia[52]. This has been confirmed by Hoshino, who demonstrated that the H^{+}/Na^{+}-exchanger activity is reduced by 50% when subjected to a given H^{+} load at 25°C, this reduction in H^{+}/Na^{+}-exchanger activity would stand in contrast to the findings of Ellis and Kusuoka, as accumulated H^{+} would lead to reduced pH.
However, Hoshino also demonstrated that cellular buffering capacity was significantly elevated at 25°C[57]. This might explain why pH remain unchanged during hypothermia.

5.3.1.3 Sympathetic control:

The hormonal response to cooling and hypothermia have been investigated in multiple species, with conflicting findings. In rats, cooling to 22°C elevated aldosterone, angiotensin II, epinephrine, norepinephrine and dopamine[58,59]. In humans, perioperative hypothermia at 35°C has shown to increase blood levels of epinephrine and norepinephrine, which do not

normalize after rewarming[60]. In unanaesthetized men, Hiramatsu made the same discoveries[61]. Kuroda et al. demonstrated that RAAS hormones also are increased in humans after cooling to 30°C. Unlike epinephrine and norepinephrine, all hormones, expect aldosterone, is normalized after rewarming[62]. On the other hand, Turley et al. found no alteration in catecholamine levels in 2-week-old lambs after cooling to 14°C[63]. This resonates with Chernow, who argued that hypothermia causes a sympathetic nervous system "switch-off". They showed that, while cooling from 37°C to 30°C elevated epinephrine and norepinephrine, continued cooling below 29°C reduced catecholamine levels to prehypothermic values[64]. They attributed the hypotensive state observed in hypothermic patients to this "switch-off". In an recent study from our lab, we show that the reduced influence of the autonomic system continues throughout rewarming[65].

5.3.1.4 Altered myofilament Ca^{2+}sensitivity

Regulation of myofilament cross-bridge formation and contractile force is multifaceted. Ca^{2+} fluxes, post-translational regulation by myofilament phosphorylation, pH and ATP availability all regulate the speed and effectiveness of the cross-bridge cycle[53]. Phosphorylation of myofilaments such as troponin I, troponin T, myosin binding protein C and myosin light chain kinase is tightly regulated to match the physiological demand of cardiac contractility and relaxation[66]. Phosphorylation regulate cross-bridge rate, Mg-ATPase, myofilament sliding velocity and most notably myofilament Ca^{2+}sensitivity[67,68]. Han et al investigated the effects on hypothermia on myofilament Ca^{2+}sensitivity in isolated papillary muscle and found significant reduction during hypothermia. This resonates with findings from other investigators[42,53]. Han further showed that the hypothermia-induced reduction in Ca^{2+}-sensitivity continues after rewarming, and is associated with increased phosphorylation of the cardiac troponin I main site, Ser23/24[20]. In a second study, Schaible et al. showed progressive increase in Ser23/24 phosphorylation and active protein kinase A (PKA), its main activator, during hypothermia. Upon rewarming, both was reduced, however not to pre-hypothermic levels[69]. In a recent study, Scahible et al. also proposed the importance of reactive oxygen species as a contributing factor to the reduced Ca^{2+}-sensitivity[70].

5.3.2 Vascular function and rheology in rewarming shock

Arterial perfusion pressure and CO depends on cardiac contractility, blood volume (BV), vascular resistance (SVR), and vascular compliance[71]. Like cardiac contractility, BV, SVR

and compliance are all altered by hypothermia and rewarming[19,22,72]. The following section aim to present a brief introduction to these changes and focus on how vascular physiology is affected by hypothermia and rewarming, and possibly contribute to rewarming shock.

5.3.2.1 Systemic vascular resistance

In fluid dynamics, resistance to flow is calculated with an adaptation of Ohm's law, and can be calculated from pressure decline and flow ($\text{SVR} = \frac{\text{MAP}-\text{CVP}}{CO}$)[73]. Regulation of SVR depends on vessel radius (r) and length (L), viscosity (μ) and blood flow (Q). The Hagen-Poiseullie equation ($\Delta P = \frac{8\mu LQ}{\pi r^4}$) attempts to portrait the determining factors underlying vascular resistance and it is readily used in physiological and medical education to illustrate the regulatory mechanisms of flow of blood in the vasculature[74,75]. However, while an useful illustration, it assumes multiple unphysiological conditions to be true, most notably that blood is a Newtonian liquid and that large artery flow is laminar, both not true[74].

The relationship between SVR, CO, MAP and central venous pressure show that changes in one variable affect the others. If no compensation, therapeutic or physiological, occurs, a lowered SVR, normal MAP and SVP necessitate improved CO. Further, as the heart pump against the aortic blood pressure, elevated SVR and afterload, forces the heart to generate greater force to maintain CO[73]. Pathological elevation in SVR, such as a hypertensive crisis or administration of exogenous vasoconstrictors are known to depress CO[76]

Elevation of SVR was described in early hemodynamic research of hypothermia, and has been documented in different species and experimental models in later works[22,77]. Bullard et al. concluded that SVR elevation during hypothermia is caused by increased blood viscosity[22,78]. As blood is a non-Newtonian liquid, the viscosity-sheer rate relationship is non-linear, has sheer-thinning properties, and viscosity increases as flow and pressure is reduced, as during hypothermia[79,80]. Hypothermia is associated with hematological changes associated with elevated viscosity, hemoconcentration, altered erythrocyte morphology and sludging of blood[81-85]. Although few investigations have been conducted, rewarming seems to normalize blood viscosity[86]. Despite this, multiple studies have demonstrated a continued increase in SVR during and after rewarming[24,77,87]. If this elevated SVR is a physiological mechanism to compensate for lowered CO or the result of vascular pathology or a result of autonomic

dysregulation is a topic in need of further investigation, as this, to my best understanding, is not well described.

In classic physiology teaching, regulation of SVR is achieved by neurohormonal and endothelial manipulation of vessel radius by smooth muscle vasoconstriction or dilatation[73]. As highlighted in the previous section, hypothermia and rewarming is associated with changes in endogenous catecholamine and renin-angiotensin-aldosterone-system (RAAS) levels. While many studies indicate an elevation in RAAS levels, the data seems inconsistent and dependent on depth and prolongation of the hypothermic period, as well as the investigated species[58,62,64]. In addition to these changes, Fuhrman et al. demonstrated reduced enzymatic breakdown of epinephrine at low temperatures[88].

While the endogenous levels of vasoactive substances and the activity of autonomic nervous system during hypothermia is not fully understood, investigators have demonstrated changes in arterial and venous function and responsiveness to α and β-receptor agonists, as well as other vasoactive substances at low temperatures. In veins, Webb-Peploe et al. found that cooling dogs caused venoconstriction[89]. Shepherd and Vanhoutte found increased sensitivity to multiple vasoactive substances and reduced threshold for contraction in saphenous, cutaneous, mesenteric and muscular vein from dog, after cooling[90,91]. This resonate with findings from other investigations and in other species[92-95]. In arteries, Bandick et al. found increased effects of norepinephrine in rabbit femoral artery[96]. In rat, Harker et al showed the same elevation in tail arteries, but not in the femoral artery or the aorta[97]. In addition, in rat, Dietrichs et al. found elevated MAP and reduced CO after epinephrine infusion[98]. Moreover, while the vascular sensitivity to vasoactive substances is increased, the contractile force of vascular smooth muscle is decreased with falling temperature [99].

Lastly, the integrity of the endothelial layer of vascular smooth muscle cells are important in regulation of vessel vasoconstriction and dilatation. Hypothermia is associated with impaired endothelial-*dependent* vasodilatation in coronary arteries of dogs, while endothelial independent vasodilatation is maintained. After rewarming this impairment was normalized[100]. Later, Cooper et al. showed that hypothermic circulatory arrest affected endothelial-*dependent* vasodilatation in the large renal arteries and pulmonary veins, but not in the cerebral vasculature, in vivo. This was, however, found in vitro. Cooper attributed this to damage to, or dysfunction of vascular endothelial cells[101] while smooth muscle function is

preserved. This is in agreement with a review by Hansen, who show that the literature strongly suggest hypothermia to cause major changes to endothelial structure and function[102].

5.3.2.2 Vascular compliance

Vascular compliance is the innate ability of a vessel to distend and expand its volume with increased transmural pressure. Vessel compliance is an important determinant of blood pressure and organ perfusion[71]. The elastic properties of the large vessels provide a Windkessel-effect: Transforming the pulsatile flow of the aorta to a linear flow in the arterioles and capillaries. Further, this elastic system provides a shock absorbent, alleviating late systolic afterload[103,104]. There is a strong association between chronic increase in arterial stiffness, reduced vascular compliance and cardiovascular disease[105,106]. However, rapid changes in compliance and stiffness of arteries do occur in response to autonomic regulation, physical stress and acute vascular pathology, and has negative effects on CO[107-109]. Multiple investigators have demonstrated that vascular compliance is reduced during hypothermia[72,110,111]. In a study by Deal et al. on vascular compliance in sheep lung during cooling on cardiopulmonary-bypass (CPB), a decreased compliance was found that normalized upon rewarming[110].

5.3.2.3 Circulating blood volume and composition:

The importance of blood volume (BV) as a factor for stroke volume and organ perfusion in circulatory physiology is well established[71,73]. Hypothermia-induced changes in plasma and blood volume (PV) was described by Rodbard et al. already in 1951, reporting a reduction in PV and BV after cooling rabbits to 29°C[23]. Later, several investigators have demonstrated similar findings in different species and protocols of hypothermia[19,112-114]. It seems, to be a discrepancy between the investigators in their understanding of the mechanisms underlying PV and BV loss during hypothermia. Based on his 1951 study, Rodbard advocated that the loss of PV and BV resulted from vascular trapping of circulating BV. This was rationalized with findings of lowered erythrocyte volume, followed by a theoretical deduction of fluid displacement[23]. The findings were later supported by D`Amato[115]. Later, Chen and Chein disputed this theory as they found reduced PV, increased thoracic lymphatic duct flow, elevated plasma colloid osmotic pressure (PCOP) and hematocrit in dogs cooled to 25°C. They contributed the loss of volume to transcapillary leakage of fluid due to hypothermia-induced elevations in blood pressure[114]. Finally, in an attempt to determine the strength of

these theories Nose et al. aimed to elucidate on the relationship between interstitial fluid space and the vascular system during cooling to 30°C. Nose et al found reduced PV and increased PCOP, but no change in hematocrit or BV. Based on this they concluded that the primary force of PV loss is hypothermia-induced redistribution of circulating blood to circulatory beds of high compliance[116]. Nose explained the findings of Chen and Chein by referring to Green and Jackman who concluded that plasma loss could be explained by redistribution of blood to organs with high transvascular filtration coefficient for water and low reflection coefficient for protein, such as the splanchnic vascular bed[111,112].

After rewarming from 2 hours at 25°C in dogs, Fedor and Fisher found a normalization of PV and BV[113]. When Tveita et al. extended the hypothermic period to 4 hours at 15°C, more pronounced changes of plasma protein leakage were indicated. This persisted after rewarming accompanied by increased interstitial colloid osmotic pressure (ICOP), as well as reduced PV[19]. They concluded that extended hypothermia is associated with significant plasma fluid and protein loss, which is not mitigated by rewarming. These results resonate with Nose`s theory of fluid and protein leakage due to redistribution[116].

5.3.3 Organ perfusion in hypothermia and rewarming

The previous section has described the consequence of hypothermia and rewarming on cardiac and vascular function. These changes are important in regulation of organ perfusion, and oxygenation. The following section will explore how such changes affect organ perfusion and in turn oxygen delivery. It will also look at how oxygen consumption is affected during hypothermia.

5.3.3.1 Organ perfusion, hypothermia and rewarming

Under normothermic conditions, vital organ blood flow is tightly regulated to maintain constant flow over a wide physiological range[73]. Lassen demonstrated this effectiveness in a classic study investigating cerebral blood flow (CBF) while changing MAP in intervals between 50 – 150 mmHg[117]. Further, in healthy humans, a 30% reduction in CO result in a 10% reduction in CBF[118]. This has been attributed to organ specific autoregulation to secure local blood flow, and has been identified to all organs[73,117,118].

In states of hemodynamic shock, reduced organ perfusion is associated with elevated mortality[119]. Hypothermia is associated with depressed vital organ blood flow (OBF). In dogs,

cooling to 21ºC reduces CBF, myocardial (MBF), renal (RBF) and splanchnic blood flow (SBF) with 75, 60, 72 and 84 %, respectively[81]. 4 hours of hypothermia at 15ºC in rats, reduced CBF, MBF, RBF and SBF by 82, 91, 98 and 91% respectively[19]. In these two experiments, CO is reduced by 80 and 91%, respectively. After rewarming, total OBF was reduced by 3 % in the dogs, two hours after rewarming, while the total OBF the rats significantly reduced by an average of 67 % after rewarming. Moreover, CO was reduced by 67 % in rats and only 14% in the dogs[19,81].

The discrepancy in OBF between these studies might be explained by the difference in experimental protocol. The rats suffered 4 hours at 15ºC – 13ºC against 30 minutes at 21ºC in the dog model. While CO was depressed in both protocols after rewarming, OBF was only depressed in the rats. The increased discrepancy between post-hypothermic CO and OBF in the rats might indicate failure of organ autoregulation of blood flow in combination with HCD.

5.4 Treatment of hypothermia and rewarming shock

Treatment of victims of accidental hypothermia has improved significantly in quality as medical knowledge and life-supporting procedures have progressed. This is evident as mortality of hypothermic patients has fallen from 80 %, in earlier reports, to ≈ 30 % in modern studies[7,10,120-122]. Treatment of severely hypothermic patients is multidisciplinary and rely on appropriate rewarming techniques, including respiratory and hemodynamic support, both invasive and non-invasive.

5.4.1 Clinical guidelines of treatment of hypothermic patients:

Multiple clinical guidelines to treat hypothermic patients exist[1,3,5,18,123-125]. First and foremost, hypothermic patients should be transported to a medical center capable of providing intensive care treatment and expertise in applying comprehensive rewarming techniques. Further, patients with low core temperature and hemodynamic instability, the availability of invasive rewarming and advanced hemodynamic support is needed. The regional guidelines at The University Hospital of Northern-Norway dictates that patients with a core temperature <28°C and/or an unstable hemodynamic situation should be transported directly to, the University Hospital of Northern-Norway, and not attempted to be rewarmed at smaller local hospitals[5].

This mirrors the recommendations from Brown et al. 2012 New England Journal of Medicine review on treatment of accidental hypothermia[18]

In the ICAR MEDCOM guidelines for avalanche rescue, patients with a core temperature <32°C and spontaneous circulation and respiration should be treated at a hospital with active heating. Victims with cardiac standstill treated with prehospital cardio-pulmonary resuscitation (CPR) and transported to a hospital providing extra corporal membrane oxygenation (ECMO) rewarming technique[125].

The safety and effectiveness of vasoactive substances in hypothermic patients have been questioned[1,3,18]. This skepticism might be well be reasoned, as little clinical evidence exists on the effects of hypothermia on pharmacodynamics- and pharmacokinetic changes. The international guidelines reflect this: The European Guidelines on cardiac resuscitation withstand the use of all pharmacological treatment <30°C[3]. This is mirrored in the new Norwegian national guidelines for treatment of accidental hypothermia, and highlighted by Brown et al[18]. The following section aim to focus at the experimental and clinical data available on the effect of hypothermia and rewarming on the pharmacodynamic and pharmacokinetic properties of common vasoactive substances.

5.4.2 Pharmacological support:

5.4.2.1 Pharmacokinetics of vasoactive drugs in hypothermia:

Hypothermia is associated with changes in enzymatic activity, renal filtration rate, hemodynamics and fluid balance. Such alterations affect the pharmacokinetic properties of many vasoactive substances. In a review from 2010, van den Broek et. al. summarized findings from 39 studies investigating changes in the pharmacological properties of different therapeutic substances during hypothermia[126]. This data show that hypothermia is associated with decreased clearance and volume of distribution of important drugs used in the intensive care setting. Fentanyl, pentobarbital, atropine, ethanol, benzodiazepines, morphine, propofol and muscle relaxants all had decreased clearance, while verapamil and propranolol had increased clearance below 30°C[127-139]. Distribution volume was also significantly altered by hypothermia, and pharmacologic agents like theophylline, curare and morphine had decreased[136-138,140], midazolam increased[141], and barbiturates were subjected to both increased and decreased volume of distribution during hypothermia[142,143].

Lastly, van den Broek et al. demonstrated altered pharmacodynamics of vasoactive substances important in acute and intensive care of hemodynamically unstable patients[126]. In their review, they present changed pharmacodynamic properties of epinephrine, isopenaline and dobutamine during hypothermia[144,145]. Following is a more detailed description on the documented changes, as well as other available studies.

5.4.2.2 β and α-adrenergic agonists

Hypothermia is associated with temperature- dependent changes in adrenergic receptors. Williams and Broadley demonstrated β_1-supersensitivity after cooling to 30°C and 25°C[146]. This is supported by Dietrichs et al. who made similar findings in cardiomyocytes after cooling to 15°C in vitro, and in vivo showed that epinephrine elevated cardiac cAMP four-fold at 15°C compared to 37°C[98,145]. On isolated rabbit hearts Riishede and Nielsen-Kudsk also demonstrated a supersensitivity of epinephrine at all temperatures during cooling to 22°C[144]. Like the β-receptor, vascular α receptor sensitivity is also significantly elevated after cooling. Flavahan and Vanhoutte, and Gomez et al. independently demonstrated this in both human and canine arteries and veins[147,148].

In addition to receptor specific changes, hypothermia seems to alter the pharmacokinetic properties of monoamines that are dependent on their removal from the synaptic cleft by catechol-O-metyl transferase (COMT)[149]. Cooling reduces enzymatic activity of COMT and increase the concentration of epinephrine and norepinephrine in the adrenergic-receptor vicinity[88,149].

5.4.2.2.1 Epinephrine

Administration of exogenous epinephrine, and thus stimulation of cardiac and vascular α_2, β_1 and β_2 receptors, causes increased cardiac contractility, elevated cardiac output and peripheral vasodilatation during normothermia. In hypothermic cardiac tissue, Riishede and Nielsen-Kudsk demonstrated an elevated contractile velocity and frequency, but reduced maximal contractile amplitude with epinephrine[144]. Reduced contractile force was also found by Schiffmann et al, who showed depressed effects of epinephrine on isolated hearts at 28°C[150]. They reported that the negative effects of epinephrine during hypothermia was in close relation to intracellular Ca^{2+} concentrations[150]. In vivo studies on epinephrine administration

during hypothermia, demonstrated a possible negative effect on global hemodynamics[98,145,151]. Tveita and Sieck investigated the effects of hypothermia on dose-response of epinephrine in rat[151]. They demonstrated that a high dose of epinephrine (1.25 μg/min) significantly elevated CO at 37°C, but the effects was diminished upon cooling to 33°C. This was not the case with a lowered dose, as 0.125 μg/min improved CO and SV down to 30°C. Kondratiev et al. also found a depressive effect on CO and SV with 1 μg bolus of epinephrine during rewarming from 15°C, while 0.1 μg significantly elevated CO during and after rewarming[145]. Dietrichs et al. had similar findings during hypothermia and rewarming, but epinephrine elevated CO after successful rewarming, when animals went through the hypothermia and rewarming protocol without receiving epinephrine or other cardiovascular support[98]. In contrast to the highlighted studies, Cotten and Brown found a preserved contractile response to epinephrine in dogs cooled to 22°C. A 1 μg/kg bolus injection at 22°C caused an 136% increase in cardiac contractile parameters[152].

The vascular response to epinephrine seems to be altered by hypothermia. Kondratiev et al, Tveita and Sieck, and Dietrichs et al. showed elevated MAP and SVR with low-dose epinephrine during hypothermia[98,151]. This is in sharp contrast to the vascular effects seen in normothermia, where such low doses of epinephrine reduce SVR through β-mediated vasodilatation.

5.4.2.2.2 Norepinephrine

The elevated α-receptor sensitivity associated with hypothermia have significant physiological and hemodynamic consequences. Norepinephrine, is a potent α-receptor agonist, commonly used as a vasopressor to maintain perfusion pressure in hemodynamic unstable patients during intensive care treatment. Cotten et. al demonstrated in an in vivo dog model that norepinephrine potentiated the contractile properties of the heart at 29°C. As with epinephrine, the beneficial effects diminished at higher concentrations. Further, both the negative and positive effects of norepinephrine on cardiac contractility are abolished by β-blockade, indicating that norepinephrine bind β-receptors during hypothermia[153].

While norepinephrine has biphasic effects on cardiac contractility in dogs, Weiss et al. found a graded response to 0.2-5.0 μg/kg/min of norepinephrine at 30°C, and although not significant, CO was depressed and SVR and MAP, elevated. After rewarming, norepinephrine

had a more beneficial profile, and, although not significant, provided graded elevation in CO and MAP, with little change in SVR[154]. In a similar model, Weiss et al., failed to demonstrate the same beneficial effects after rewarming. In more detail: 0.2 and 1.0 μg/kg/min did improve CO insignificantly, 5 μg/kg/min depressed CO significantly and increased MAP with no change in SVR[155].

It seems that the effects of norepinephrine during hypothermia is closely related to changes in β-receptor affinity to norepinephrine and cause reduced CO and SVR, possibly through the negative effects on β-receptor stimulation during hypothermia.

5.4.2.2.3 Isoprenaline

Riishede and Nielsen-Kudsk investigated the effects of isoprenaline down to 22°C. Here they demonstrated elevated contraction velocity (E_{max} and EC_{50}), and heart rate (HR), in vitro. At low doses at 22°C isoprenaline provided elevated contraction amplitude, but high concentrations depressed contraction[144]. In isolated hearts cooled to 27°C Nakae et al. demonstrated that 1 nM isoprenaline improved contractile function as measured by dP/dT_{max} and developed LV pressure[156].

The improved contractile force by isoprenaline demonstrated in vitro, is not reproduced in hemodynamic in vivo studies of hypothermia and rewarming. Lauri fond no benefit of isoprenaline on CO at 25°C, a finding repeated after rewarming[157]. These findings resonated with data from Han et al. from hypothermic rats where only a high dose isoprenaline produced a small elevation in CO, with no alleviation of SVR. After rewarming the low doses of isoprenaline depressed CO, while the highest dose only improved CO by 10 %. Isoprenaline increased SVR at all concentrations after rewarming[158].

5.4.2.2.4 Dopamine and dobutamine

Dopamine has been demonstrated to improve CO, also during hypothermia[159]. The findings resonate with Filseth et al. who found improved cardiac index (CI), HR, and reduced SVR with 8 and 16 μg of dopamine at 32°C. At 25°C the same dopamine doses elevated HR, SVR improved, but caused no change in CI. Contrary to these findings, in a similar study, Roscher et al. found little benefit of dopamine (5μg/12μg) on CO and SVR at 32°C, despite a reduction in MAP and elevated HR. Riishede and Nielsen-Kudsk also investigated effects of

dobutamine on isolated rabbit hearts during cooling to 22°C. They found that dobutamine reduced amplitude of left ventricular force at 27°C, increased it at 22°C, but never to normothermic values. The same pattern was present for contraction velocity. As with epinephrine, high concentrations of dobutamine produced depression in force production[144].

In isolated guinea pig hearts, Rieg et al. found no significant change in inotropic force in response to increased concentration of dobutamine given at 34°C and 31°C[160]. In larger animal models English et al. demonstrated improved effects of dobutamine as dopamine in their study[159]: Improved hemodynamics during hypothermia.

5.4.2.3 Milrinone

Different from β-receptor agonists, milrinone influences the pathway downstream from the β-receptor by inhibiting phsophodeesterase-3 (PDE3), an enzyme responsible for cAMP breakdown[161]. In normothermia, milrinone is demonstrated to reduce SVR by vasodilatation and elevate CO through increased cardiac contractility. During cooling, Tveita and Sieck demonstrated elevated CO and reduced SVR in rats pre-treated with milrinone[162]. Dietrichs et al. demonstrated the same beneficial effect when rats were treated with milrinone during rewarming from 3 hours of hypothermia[163]. The beneficial effects of milrinone on cardiac contractility during hypothermia has been questioned by investigators[160]. As neither of the experimental studies mentioned have measured cardiac contractility, it is possible that the beneficial effects of milrinone on post-hypothermic CO originates from LV afterload reduction.

5.4.2.4 Levosimendan (LS)

Unlike milrinone and β-receptor agonists, LS works by elevating myofilament Ca^{2+} sensitivity in cardiomyocytes. In addition, it opens K^{+}-channels in vascular smooth muscle, promoting vasodilatation. Lastly, at high cellular concentrations, LS also exhibit PDE3-inhibitior properties, which together improve cardiomyocyte contractility and cause vasodilatation. The beneficial effects of LS on cardiac contractility seems to be preserved at 31 and 34°C[160]. Further, in rat models of hypothermic cardioplegia and extracorporeal rewarming under simultaneous administration of LS, it seems to improve cardiac function after weaning from extracorporeal circulation[164]

5.4.2.5 Sodium nitroprusside (SNP)

SNP, a nitric oxide donor, impose vasodilatation by promoting smooth muscle cGMP activation. In intensive care medicine SNP is used to ameliorate high blood pressure and afterload in critically ill heart failure patients[165,166]. While hemodynamic effects of SNP have been investigated extensively in the normothermic setting, few studies are available on its hemodynamic effects during hypothermia. In dogs cooled to 29°C, Morray et al. found improved CO and reduced SVR after SNP infusion following rewarming[167].

6 Aims of thesis

This thesis is motivated by an ambition to better understand the cardiovascular pathophysiology underlying HCD by adding new experimental research. Even more ambitious: to improve patient treatment and outcome based on this new information. If hypothermia exposure time is sufficiently long and the temperature deep enough, the cardiovascular state imposed is not reversed by rewarming, and the following physiological consequences are hemodynamic instability and reduced organ perfusion, and in turn organ damage and dysfunction. The hemodynamic instability calls upon challenging intensive care treatment, prolonged hospitalization, which unfortunately ends up in high patient mortality.

This thesis will focus on and explore two key aspects associated with accidental hypothermia and rewarming; *depressed cardiac contractility* and *elevated systemic vascular resistance*. The thesis aims to answer three questions related to these two aspects:

- Is hypothermia-induced contractile dysfunction ameliorated by increasing myocardial Ca^{2+}-sensitization pharmacologically by LS?
- Is the elevated vascular resistance during and after hypothermia a physiological compensation to the lowered cardiac output and perfusion pressure, or part of a hypothermia-induced pathophysiologic process on its own, leading to depressed cardiac function?
- Is organ perfusion affected by pharmacological amelioration of HCD, or reducing systemic vascular resistance, or the combined impact of cardiac support and vasodilation during hypothermia and rewarming?

To answer these questions, we utilized a rat model of accidental hypothermia designed to induce and study HCD. This thesis aimed at studying the combined impact of HCD and vascular function on organ blood flow. To do this we manipulated cardiac contractility and/or vascular resistance with either LS or SNP.

6.1 Paper I

The aim of this paper was to investigate how HCD is affected by the combined impact of increased Ca^{2+}-sensitivity and PDE3-inhibition during hypothermia and rewarming. This was achieved with the administration of a high-dose LS in our experimental hypothermia rat

model of after 3 hours of exposure to 15°C. In addition, the study aspired to investigate the effects of PDE3-inhibition on cardiac troponin I phosphorylation, a known cellular mechanism associated with hypothermia-induced contractile dysfunction.

6.2 Paper II

The aim was to investigate if the elevated SVR associated with hypothermia and rewarming depresses cardiac function or not. An identical experimental rat model, as in paper I was utilized. To manipulate SVR in isolation, SNP was administered during the last hour of hypothermia and during rewarming.

6.3 Paper III

Paper III aimed to explore whether the improved cardiac function demonstrated by both LS and SNP after rewarming results in improved organ perfusion, oxygen delivery and oxygen consumption during rewarming from hypothermia. To achieve these aims we used a modified version of the experimental model of Paper I and II, introducing a method to measure organ blood flow.

7 Summary of results

7.1 Paper I

This study focused on hemodynamic effects of elevating myofilament Ca^{2+} sensitization during rewarming to improve cardiac contractility and possibly ameliorate HCD. A high dose LS was used to achieve the proposed aim. Animals treated with LS regained pre-hypothermic contractility (PRSW) and CO. Further analysis showed that both increased SV and HR contributed to the elevated CO at 37°C after rewarming. LS also reduced SVR during hypothermia and rewarming. Placebo treated animals had depressed CO and PRSW, while SVR was significantly increased after rewarming at 37°C.

7.1.1 Cooling

When cooled from 37°C to 15°C, both hypothermic groups suffered a significant depression of hemodynamic function. MAP, CI, CO, dP/dT_{max}, end systolic pressure (ESP) and HR were all significantly depressed at 15°C, while SV, minimum rate of LV pressure change (dP/dT_{min}), SVR and the isovolumic relaxation constant (Tau) all increased.

7.1.2 Rewarming

After rewarming, under continuous LS or placebo infusion, hemodynamic function differed between the two groups. Treated with LS, the animals had improved CO, PRSW, dP/dT_{max} ESP, CI, SV, HR and SW after at 37°C after rewarming compared to placebo. In addition, while the placebo group presented evidence of rewarming shock (depressed CO, PRSW, CI, SV and SW) the LS treated animals regained pre-hypothermic baseline values for most hemodynamic variables.

7.1.3 Normothermic control

After 5 hours of normothermia, with the last 2 hours with continuous LS infusion, CO, HR, dP/dT_{max}, SV and SW were all increased compared to baseline.

7.1.4 Phosphorylation of cTnI

Western Blot data showed a significant increase in cTnI S23/24 phosphorylation in the LS treated group (0.28 ± 0.03) compared to placebo (0.16 ± 0.03) after rewarming.

7.1.5 Release of cTnI

After rewarming at 37°C plasma cTnI was significantly different between the hypothermic groups and the normothermic control group (1.4 ± 0.4 ng/mL). No difference was, however, found between the two hypothermic groups receiving LS (8.2 ± 2.1 ng/mL) or placebo (14.6 ± 4.6 ng/mL).

7.2 Paper II

This study aimed at separating the hemodynamic effects of pharmacologic vasodilatation during H/R by use of SNP infusion during rewarming. After rewarming, SNP-treated animals had lower SVR, MAP and EDP, and elevated CO compared to saline treated animals.

7.2.1 Baseline and cooling

At baseline no differences in hemodynamics were found between groups. After cooling to 15°C all groups showed depression in CO, MAP, HR, EDP, and dP/dT_{max}, while SV, dP/dT_{min} and Tau were increased.

7.2.2 After rewarming

Within group comparisons:

Both groups demonstrated depressed SV, CO and PRSW compared to baseline. The SNP treated group also had a reduced MAP and ESP, while the control group had elevated SVR.

Between-group comparisons

After rewarming SNP improved CO and SV, and reduced SVR, ESP and MAP compared to the control group. No difference in PRSW was found between the groups.

7.3 Paper III

This study aimed to investigate if the beneficial effects of LS and SNP on CO and SVR also affect OBF and oxygen delivery during rewarming. LS and SNP treated animals demonstrated elevated CBF in addition to CO and SV after rewarming. SNP also elevated myocardial and stomach blood flow, effects not seen in the LS group.

7.3.1 Baseline and cooling

No difference was found in hemodynamic or blood-gas data between the three hypothermic groups at baseline.

7.3.2 After rewarming

With-group comparisons

After rewarming, all three groups demonstrated reduced CO and MAP, while SV was reduced only in the control group when compared to their prehypothermic baseline values. Compared to normothermic baseline, SBG and KBF was reduced in all groups at both 30°C and 37°C Further, CBF in the control group and left side CBF in the control and LS groups was reduced as 30°C compared to normothermic baseline. After rewarming to 37°C SNP and

Between-group comparisons

After rewarming CO and SV were significantly increased and SVR reduced in the LS and SNP groups compared to control. LS also improved CO compared to SNP-treatment. At 30°C, no significant difference was found in organ perfusion between the three groups. Following rewarming, SNP improved CBF, MBF, SBF and total OBF, compared to the control group, while LS only improved CBF. Lastly, LS elevated DO_2 and VO_2 compared to SNP and the control group after rewarming.

8 Methodological description and considerations

8.1 Experimental animal model (I, II, III)

All experiments utilized an in-vivo animal model. Male Wistar rats (250 – 350g) was obtained from Charles River, Inc (USA). The animals were quarantined for one week before use. Housing was in accordance with guidelines for accommodation and care of animals (article 5 of the European Convention for the Protection of Vertebrate Animals used for Experimental and Other Scientific Purposes, Strasbourg, 18.III.1986). The rats had a microbiological status according to the recommendation of the Federation of European Laboratory Animal Science Associations. The animals had ad libitum access to water and food and provided with toys and gnawing sticks. The experimental protocol was approved by the Norwegian Animal Research Authority and conducted accordingly.

8.2 Anesthesia and analgesia (I, II, III)

Pharmacological anesthesia and analgesia was used in all experiments. The animals received an intraperitoneal injection of pentobarbital (50 mg/kg) and fentanyl (0.05 mg/kg) to induce surgical anesthesia and analgesia. Depth of anesthesia was determined by level of arousal, reaction to toe-pinch method and sound. Once deeply sedated, intravenous access was attained by introduction of a small pediatric CVC gently placed in the right jugular vein and a continuous infusion of pentobarbital (7.5 mg/kg/h) and fentanyl (0.05 mg/kg/h) was started. Once hemodynamic parameters are available, anesthesia was adjusted based also on heart rate and mean arterial pressure. During hypothermia <30°C pharmacological anesthesia was suspended as cold-narcosis commence.

It is well known that warm-blooded animals lose consciousness when cooled to lower temperatures[168,169]. The effectiveness of cold-narcosis has been compared to pharmacological anesthesia and indicators of cerebral metabolism and arousal is shown to be equally reduced[170]. *Further*, the pharmacodynamics and pharmacokinetic properties of anesthetics are affected by hypothermia. Clearance of pentobarbital is reduced by 50 % at 30°C and 67% at 25°C, while pentobarbital effectiveness is only reduced with 25 %. Also, fentanyl clearance is reduced by hypothermia. However, no data exists of fentanyl effectiveness[126]. *Thirdly*, the depressive effects of pentobarbital combined with the negative effects of hypothermia on cardiovascular function prohibit the experiments to investigate spontaneous circulation at deep hypothermia. The sum total of these concerns presented, support the use of cold-narcosis

<30°C. During rewarming at 30°C, pharmacological anesthetics and analgesics was reinstated throughout all experiments.

8.3 Temperature, cooling, hypothermia and rewarming (I, II, III)

All animals where immobilized on a hollow aluminum surgical table. The table was circulated with hot or cold water. Core temperature was monitored with an esophageal (I, II) or aortic (III) thermocouple. Cooling and rewarming was achieved by adding ice or warm water and regulating the water bath thermostat. Once the animal reached 15°C core temperature, cooling was stopped and 4 (I, II) or 3 hours (III) hypothermia was initiated. Cooling and rewarming took on average 60 and 90 minutes, respectively.

8.4 Respiratory support (I, II, III)

Airways where secured by tracheostomy in all animals. None of the animals achieved apnea by fentanyl or pentobarbital and all had spontaneous respiration at the start of the experiments. In rats, hypothermia is associated with depressed respiratory function <24°C. When apnea occurred in the hypothermic animals, a rodent ventilator was connected, and mechanical ventilation commenced. All animals where ventilated with a frequency of 16-18 breaths/min, a peak inspiratory pressure of 18 mmH_2O and an inspiratory/expiratory rate of 45%.

8.4.1 Regulation of respiration by α or pH-stat in hypothermia

Blood gas management in hypothermia is a much-debated topic as alterations in blood rheology increase the solubility of gases, and in turn affecting the acid-base equilibrium[56]. Blood pH increase with about 0.016 points for every °C of reduced temperature[56,171]. Two main schools have emerged management of ventilation and blood gas interpretation: α-stat and pH-stat methods. The pH-stat method aims to adjust arterial pCO_2 and pH to temperature corrected values. This usually entail increased pCO_2 and reduced pH by hypoventilation. α-stat ignores the need for temperature correction of blood gas values, and ventilation is adjusted to pCO_2 of 5.3 kPa and pH 7.4[172-174]. The physiological and clinical consequence of α and pH stat is that the relative hypoventilation caused by pH stat cause cerebral vasodilatation and increase CBF during hypothermia[172]. This has been confirmed in randomized human studies undergoing hypothermia during CPB[175]. The benefit of this

vasodilatation has been questioned during CPB as studies have demonstrated poor neurological outcome in pH-stat treated humans after hypothermic CPB[175,176].

In the included studies mechanical ventilation was adjusted just after cooling to 15°C. An arterial blood gas was obtained and analyzed with the α-stat method and ventilation adjusted accordingly. The α-stat method is the preferred method in clinical practice[175,176]. Further, paper III aim to investigate the effects of SNP and LS on CBF during hypothermia and rewarming. pH-stat influence CBF by hypercapnic vasodilatation during hypothermia, this could interfere with the wanted effects of SNP and LS on vascular resistance and changes in CBF.

8.5 Pharmacological intervention with LS and SNP

In all three papers pharmacological intervention was initiated 1 hour prior to rewarming from 15°C.

In Papers I and III LS was used in similar doses as in clinical studies [177]. A bolus dose of 24 μg/kg/min was given the first 10 minutes, and then a continuing infusion of 0.6 μg/kg/min during the remaining hypothermic and rewarming period. The rationale behind a high dose of LS was to make use of both the Ca^{2+} sensitizing and PDE3-inhibiting effects of LS.

As the aim of Paper II was to investigate the effects of SVR on cardiac function, SNP was administered aimed at lowering SVR. As SVR was calculated in the post-experimental analysis, MAP was utilized as a surrogate. During rewarming, SNP was administered with the aim of reducing MAP by 30 %, compared to historic controls from Paper I and from a previous study from Dietrichs et al, in an identical model[163]. In a dose-finding study, it was identified that the standard 10 μg/kg/min normothermic dose resulted in cardiac standstill. Accordingly, infusion was started with 0.6126 μg/kg/min at 15°C. Upon rewarming the dose of SNP was continually elevated to meet target MAP. In Paper II the average dose after rewarming was 14 μg/kg/min. In paper III SNP was applied in the same manner as in Paper II, however the average dose after rewarming was 8 μg/kg/min.

The effects of SNP have been demonstrated to be in close correlation to the hemodynamic situation and it was found that elevated CO and preload reduce the effect of a stable SNP

infusion [178]. The fact that rewarming from hypothermia is a state of regained hemodynamic function might explain the need for a continued elevation of SNP during rewarming. Further, the discrepancy between SNP treatment in Papers II and III might be explained by the effects of microspheres on the general circulation. It is evident from the results that the hypothermic control group suffers hemodynamically after microsphere injection at 30°C, compared to its counterparts in Papers I and II. The depressed hemodynamic state might affect the needed SNP to maintain a lowered MAP during rewarming.

8.6 Hemodynamic measurements

8.6.1 Vascular pressure, pressure derivatives and heart rate

8.6.1.1 Paper I, II

Arterial pressure was obtained with a 22G fluid filled catheter in the left femoral artery. Mean arterial pressure (MAP) was calculated as (1/3 systolic pressure) + (2/3 diastolic pressure). LV pressure was acquired with a Millar conductance catheter (SPR-838, Millar Instruments Inc., Texas, USA).

8.6.1.2 Paper III

Arterial and LV pressure was both obtained with 22G fluid filled catheters. Arterial pressure was obtained from the left femoral artery, while LV pressure was provided through right carotid artery catheterization. MAP was calculated as (1/3 systolic pressure) + (2/3 diastolic pressure).

8.6.2 Stroke volume (SV) and cardiac output (CO)

8.6.2.1 Paper I, II

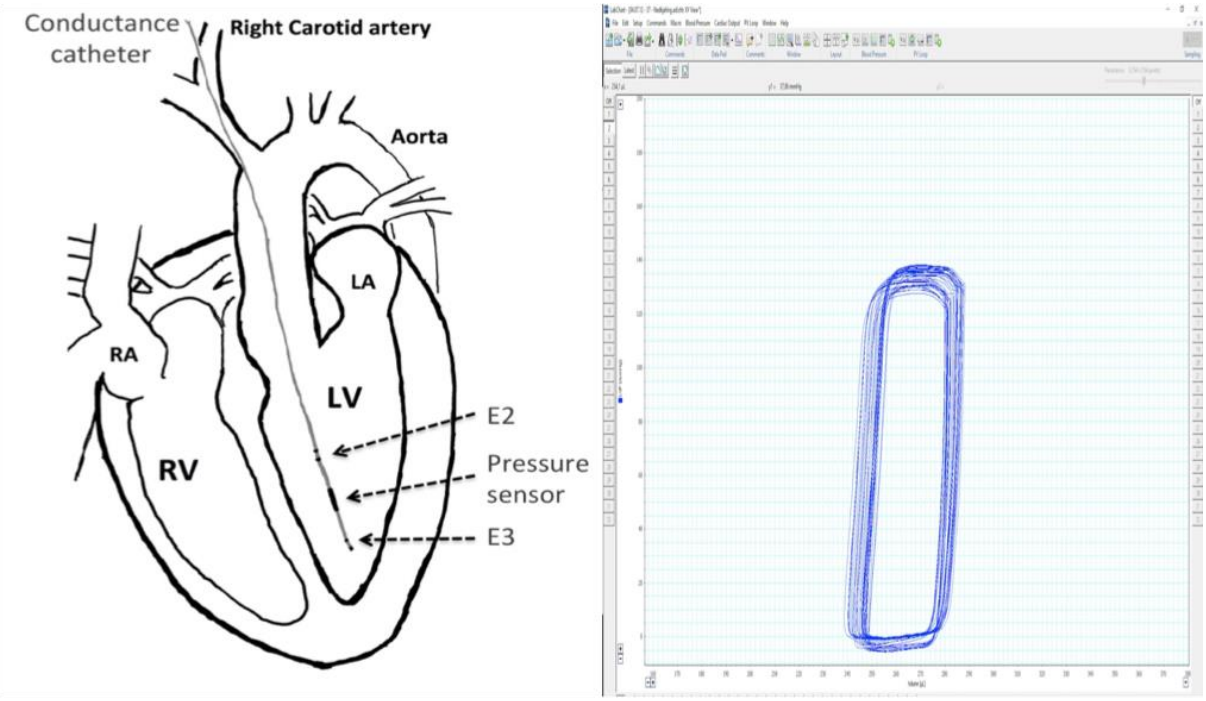
Real-time LV volume was obtained with a miniaturized 2.0F conductance catheter placed in the left ventricle, through the right carotid artery (SPR-838, Millar Instruments Inc., Texas)[179]. The catheter is placed as shown in Fig 1 and consists of one manometer and four electrodes (E1-E4). A constant current of low-amplitude high frequency is applied to the outer most electrodes, creating a small electrical field. The difference is voltage measured between E2-E3 depend on the conducting properties of the medium the electrical field passes through and is proportional to the volume of the same medium. As the electrical field is larger than the ventricular space during contraction, both the ventricular volume and the overlapping cardiac

muscle is included in the volume calculation. The result is an overestimated end-diastolic and end-systolic volume. To identify the ventricular volume, calibration is necessary[179].

Calibration can be achieved by a hypertonic saline (30 %) method (parallel conduction). This is done with 2-3, 40 μL, saline injections before recording[179,180]. In the presented rat model, recordings were performed at 37, 32, 28, 24, 20 and 15°C during cooling and rewarming. Hypothermia is associated with large rheological and cardiac changes, affecting conductance and ventricular volume. This entail that hypertonic saline calibration has to be performed prior to all recordings. This would cause an unphysiological sodium load, possibly harmful to the hypothermic rat.

We used the second option of ex-vivo calibration with cuvettes with known volumes[179]. Temperature calibration was preformed ex-vivo in an isolated cuvette with known diameters (2–7 mm) filled with heparin-treated blood[20,181]. The four-electrode Millar catheter (SPR-838) was inserted in the cuvette and volume was calculated using the following formula: $V = \pi r^2 L$, where r is the radius of the cuvette and L is the distance between catheter electrodes (E2 – E3). The cuvette was then lowered in a temperature regulated water and cooled to 15°C. The temperature specific measurements (*37, 32, 28, 24, 20 and 15°C)* were corrected using the cuvette calibration method described. CO was automatically calculated by LabChart 8. Calibration was applied just prior to analysis.

While cuvette calibration helps to identify the linear relationship between conductance and volume at different temperatures, there is still difficulty to accurately true EDV and ESV. While a disadvantage, it does not prevent the studies to determine stroke volume as this is calculated by subtracting EDV with ESV. Because of the linear relationship between resistance and volume any change in blood volume during the heart cycle can be determined[179]. Further, the methodological review from Burkhoff et al. referred to a CO of 41 – 60 in normal rats. While 10 mL/min higher, the animals described are up to 150g heavier. These comparisons give confidence to trust that the SV and CO data provided by the method are reliable enough to answer the proposed hypothesizes[179].



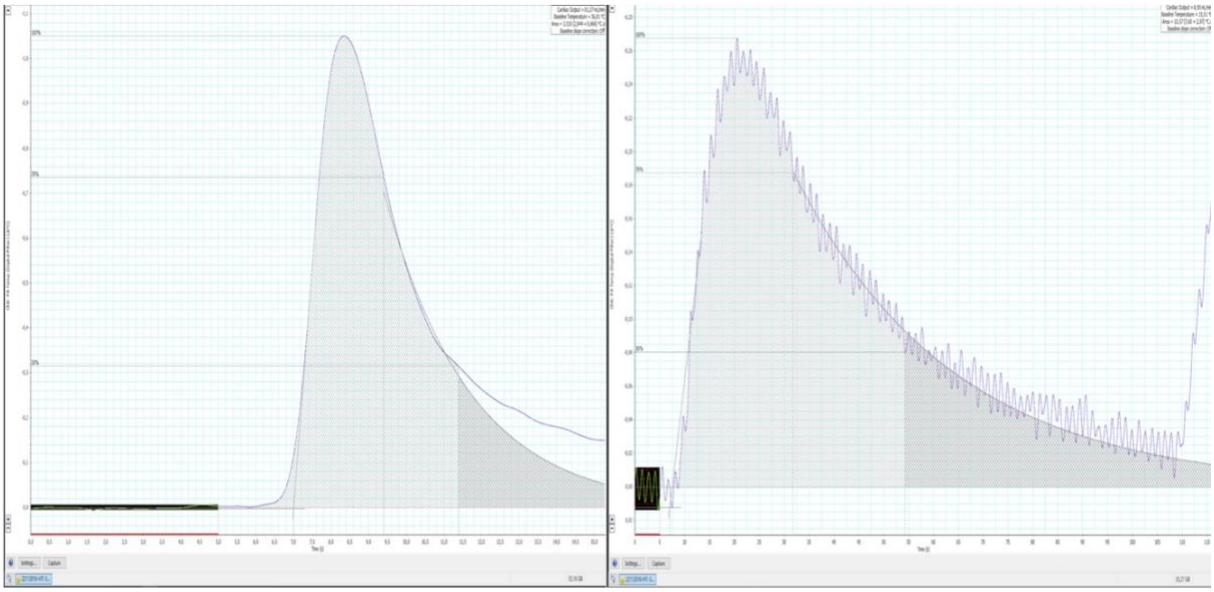
Left: Cross-section with placement of conductance catheter
Right: Pressure-volume view of LV during multiple cardiac cycles. LA: Left atrium, LV: Left ventricle, RA: Right atrium, RV: Right ventricle, E2 and E3: Conductance electrodes
Original figure by Erik Dietrichs - modified

8.6.2.2 Paper III

The aim of Paper III was to investigate how regional blood flow during rewarming is affected by pharmacological manipulation of vascular resistance. To achieve this a microsphere technique was used to measure regional blood flow. Since the microspheres had to be introduced directly in the LV, the conductance catheter method was unsuitable. Therefore, CO was with thermodilution method. Thermodilusion is well studied in small rodent models and have been utilized in an identical rat model[43,145]. Due to technical difficulties, Kondratiev, was not able to produce viable measurements below 20°C. In the presented paper (III), improved equipment and analyzing tools provided reliable measurements even at 15°C. It should be noted that thermodilution in small animals, and at low flow, could overestimate CO due to a higher heat diffusion[182,183].

At 37, 30, 22 and 15°C, injections were performed in triplets and an average of all three was calculated. Before the measurements were performed, cooling or rewarming was halted to the point where the animals core temperature stabilized. 0.15 mL precooled (5°C) saline was rapidly injected directly in the right atrium. Changes in temperature was detected by a thermocouple placed in the aortic arch and thermodilution curves (fig 2) was analyzed and

recorded with LabChart 8. This provided direct CO measurements. CO and HR were used to calculate SV at each time-point (SV = CO / HR).



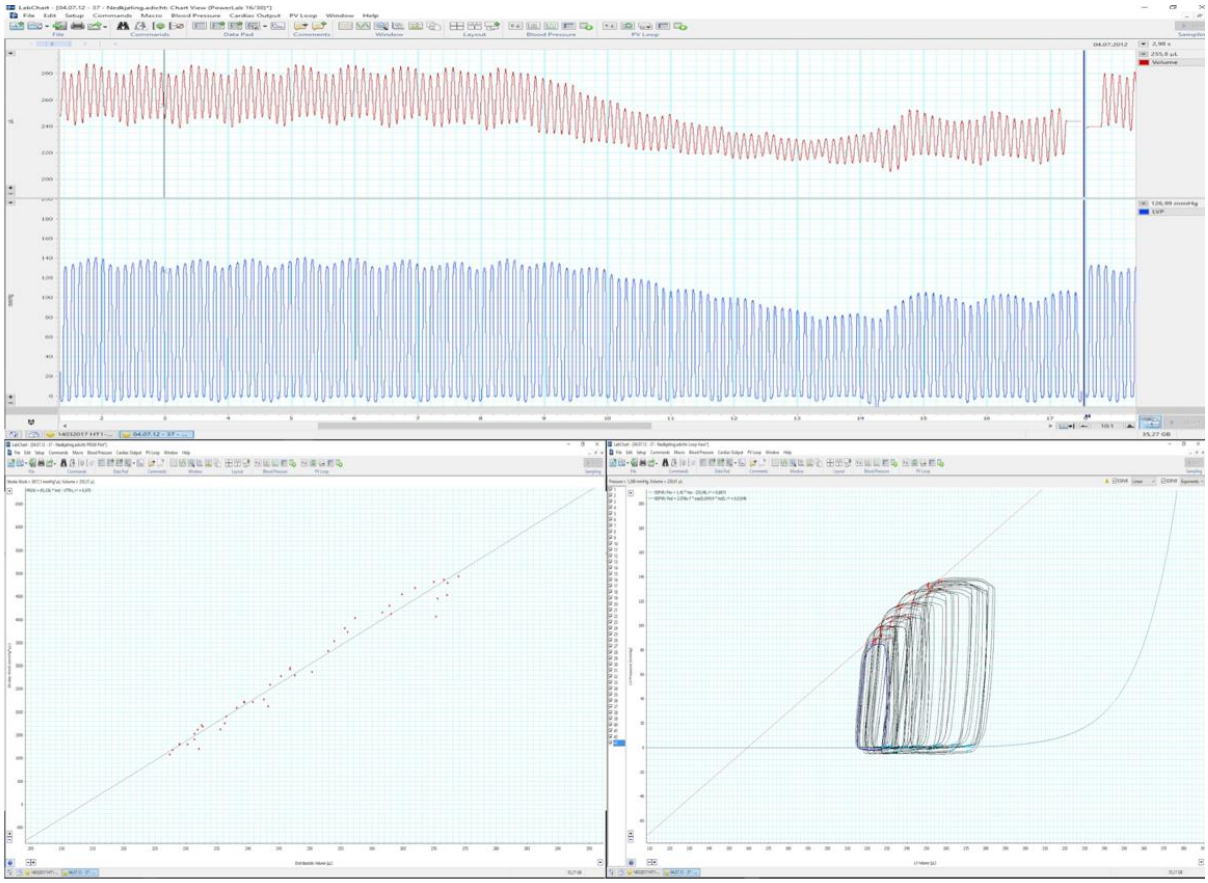
Left: Thermodilution curve at 37°C after injection of 0.15mL 4°C saline
Right: Thermodilution curve at 15°C after injection of 0.15mL 4°C saline

8.6.3 Contractility (I, II)

Cardiac contractility is the hearts ability to produce force. Contractility is difficult to measure, as many hemodynamic variables is influenced by the physiological state, such as preload, afterload and heart rate. Investigators have aimed to find variables reflecting contractility independent of extra cardiac influences. In vitro, total force produced, maximum tension and time to peak is used as contractile measurement in isolated muscle strips. In vivo, these measurements are impossible to acquire. Max ventricular pressure, dP/dT_{max} and SW are all dependent on either heart rate, preload and afterload[179]. To account for this, preload independent measurement has been developed. End systolic-pressure volume relationship (ESPVR) was first described by Sagawa et al. in 1977 and PRSW was proposed as a stronger indicatory of cardiac contractility by Glower et al. in 1985[184,185]. Glower et al. demonstrated that the relationship between SW and EDV is more linear and afterload independent than ESPVR. Lastly, a mathematical consequence of PRSW is that it independent of cardiac size, and direct comparison between species is therefore possible[179].

In Paper I and II PRSW was used as a preload-independent measurement of contractility to assess cardiac function before and after hypothermia. PRSW was obtained by external vena cava occlusions in triplets at baseline and after successful rewarming as compromised hemodynamics prohibits vena cava occlusions during hypothermia. PRSW was calculated by LabChart 8. The process is exemplified and illustrated in the figure below.

PRSW was chosen as a favorable contractile parameter mainly as Gower et al discussed in their paper, as the relationship between SW and EDV is more linear than the ESPVR. But also due to limitations with our model to acquire a reliable ESP. A reoccurring challenge is that that the manometer is touching the ventricular wall during end systole. This create a "devil horn" on the cyclic pressure volume loops. During stable hemodynamic recording, these artifacts are ameliorated by catheter manipulation. However, the dynamic changes during vena cava occlusions can displace the catheter and the manometer in some cases touch the ventricle wall and create "devil horns". Therefore, ESP and ESPVR is unreliable in the presented models. PRSW is however calculated independent of ESP by LabChart 8, as SW is calculated by the geometrical areal of each pressure-volume loop. The development of "devil horns" do potentially affect this calculation, but only minimally. PRSW is therefore a much more suitable measurement for contractility in the presented models.

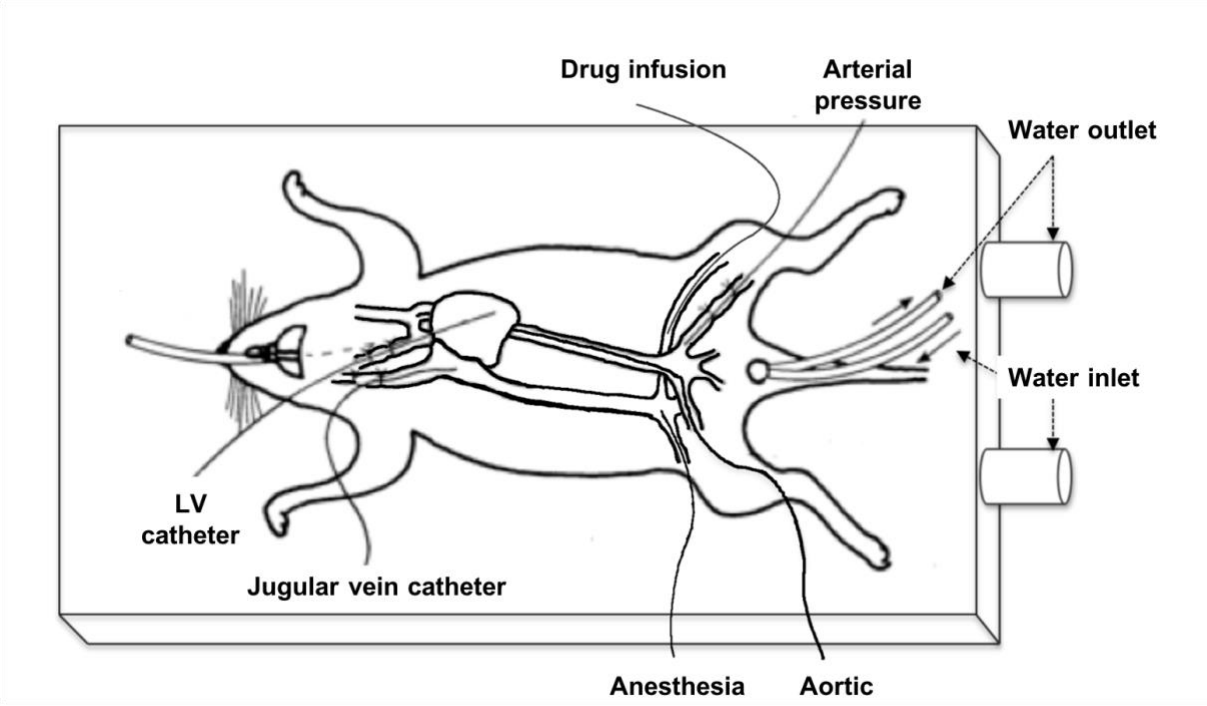


Above: Recording of LV pressure and volume during external vena cava occlusion (VCO)
Below: Right: PV-loop view of VCO with ESPVR analysis
Left: PRSW analysis of EDV vs SW

8.6.4 Regional blood flow (III)

A stable-isotope labeled microspheres (Biopal, Inc) technique was applied to investigate regional blood flow[186]. 0.5 ml of 125 000 microspheres (250 000/mL) was injected in to the LV. (BioPal, Mi, USA) Simultaneously 0.5mL arterial blood was sampled from the femoral artery at a rate of 0.5 mL/min[19,187]. Data was obtained at 30°C and 37°C during rewarming from hypothermia and at 37°C baseline, and at experimental endpoint in the normothermic animals. After ended experiments biopsies was collected from the brain, heart, liver, kidneys, ventricle, intestine, muscle and skin and weighed. The samples were then freeze-dried and shipped to BioPal (MI, USA) for analysis. Organ blood flow was later calculated by normalizing organ microsphere concentration [disintegration per minute (dpm) / g] with the microsphere concentration of the 0.5 ml/min reference sample (dpm / mL / min)[186]. Total measured blood flow was calculated as the sum blood flow to all organs in each individual

animal.



Original figure by Erik Dietrichs – modified

8.7 Biochemical analysis

8.7.1 Blood gas analysis and calculation of oxygen content (III)

In paper III arterial blood gas was obtained at 37°C baseline, 15°C at the start of hypothermia, and at 30°C and 37°C during rewarming. A venous blood gas was obtained only after rewarming at 37°C. The blood was analyzed with no temperature correction applied (Rapidlab 800, Chiron Diagnostics, USA). The blood was analyzed for pO_2, pCO_2, oxygen saturation (SO_2), pH, Hemoglobin (Hb), Hematocrit (Hct) and lactate. HCO_3^- and base excess was automatically calculated based on the measured data. Arterial and venous blood oxygen content (CaO_2 and CvO_2), oxygen delivery (D_{O2}) and oxygen consumption (V_{O2}) was calculated with the following formulas: CaO_2 and CvO_2 = (Hb x 1,34 x SO_2 / 100) + (pO_2 x 0.0031 x 7.5), D_{O2} = CaO_2 x CO and V_{O2} = CO x (CaO_2-CvO_2)[188].

8.7.2 Cardiac troponin I phosphorylation (I)

After successful rewarming, the hearts were isolated and flash-frozen in liquid nitrogen. Phosphorylation levels on cTnI sites Ser23/24 were investigated using Western blot[20]. Antibodies targeting either total cTnI (Fitzgerald) or phosphorylated cTnI at Ser23/24 (Cell

Signal) was used. The amount of phosphorylation is measured as phosphorylation rate (density of phosphorylated Ser23/24-cTnI divided by total cTnI).

8.7.3 Cardiac troponin I release (I)

After experiments were completed, arterial blood was sampled from the left femoral artery. Blood was centrifuged, and the plasma extracted from the tubes. Plasma-cTnI was then analyzed, using a high sensitivity rat cTnI ELISA kit (Life Diagnostics, Inc., West Chester, PA, USA.)

8.8 Statistical models

8.8.1 Hemodynamic data

All statistical analyses were done in SigmaPlot 13 (USA). Before any statistical test were run, data distribution was investigated for normality using Shapiro-Wilks test, if rejected (< 0.05) non-parametric ANOVA on ranks was performed. Hemodynamic development between within each group was compared with a repeated measurement one-way ANOVA. If the H_0 was rejected ($p< 0.05$) a Dunnett's post-hoc test was performed as against pre-hypothermic control-levels (I, II, III). In-group change in PRSW was analyzed with a paired T-test (I, II). Between-group comparison at each time-point was done with a student T-test was preformed (I, II). In paper III, between-group comparison was done with a one-way ANOVA, if the H_0 was rejected, a Tukey post-hoc test was performed. Statistical significance was accepted if $p <0.05$. All results are presented as mean (I, II, II) +/- SEM (I, II) and SD (III).

8.8.2 Biochemical data

The cTnI-release data failed the Shapiro-Wilks test (I) between groups were analyzed using one-way repeated measures ANOVA on ranks.

8.8.3 Western Blot analysis

Data was analyzed with a one-way ANOVA, with a Tukey post hoc test.

9 General discussion

9.1 Hypothermia, cardiac dysfunction and elevation of vascular resistance

The presented studies (I, II, III) demonstrate a continuously depressed cardiac function and elevated SVR during hypothermia. In the hypothermia control groups, across the studies, the continued depression of SV, CO and contractility and elevation of SVR was present after rewarming. This resonates with previous investigations on the hemodynamic consequence of hypothermia and rewarming[20,163,188,189]. The intervention studies (I, II, III) further show the beneficial effects of both LS and SNP on SV, CO and SVR. Further, LS improved CO and SV superiorly in all three studies, while SNP lowered SVR more than LS, independently of measuring technique. Lastly, only LS elevated cardiac contractility after rewarming, as shown by improved PRSW and LV dP/dT_{max} (I, II).

Multiple studies have attempted to improve cardiac contractility and hemodynamic function during hypothermia by stimulating the β-receptor[98,145,181,190]. These studies all show little beneficial effects on the hemodynamic situation and a narrowed therapeutic window of β-agonists at low temperatures. Unlike direct adrenergic agonists, LS promote cardiac contractility independent of the β-adrenergic axis. LS increase contractility by elevation of Ca^{2+}-sensitivity of troponin C, promoting actin-myosin binding[191,192]. We here demonstrate that the therapeutic window of LS is preserved at low temperatures and improve CO and dP/dT_{max} during rewarming from 20°C. This resonate with clinical and experimental animal studies, with CPB, on the effects of LS at hypothermic temperatures[164,193].

At elevated concentrations, LS also promote PDE3-inhibition[192], preventing cAMP breakdown and indirectly promoting the β-adrenergic pathway. The cAMP-pathway is important in regulation of Ca^{2+}-handling by phosphorylation of phospholamban, regulating the sarcoplasmic reticulum Ca^{2+}-ATPase[53]. It is possible that the beneficial effects of milrinone on cardiac function in the rewarming scenario, like also in part by LS, is due to improved cardiac Ca^{2+}-handling. In Paper I we show elevated troponin I phosphorylation with LS. This is an indication that LS elevate activity in the cAMP and PKA pathway[67]. It has been speculated that elevated Ser23/24 phosphorylation is contributing to contractile dysfunction during hypothermia as Ser23/24 phosphorylation is associated with lowered Ca^{2+} sensitivity [20,69]. However, it is likely that the beneficial effects of LS on Ca^{2+}-sensitivity by directly binding to the c-terminal end of troponin I override the negative effects of Ser23/24 on Ca^{2+}

sensitivity. In separate studies, Dietrichs and Tveita demonstrated the beneficial effects of milrinone, a selective PDE3-inhibitor on cardiac function during hypothermia and rewarming[162,163].

Elevation of SVR during and after hypothermia have been described in early investigations[22,77]. The presented studies (I, II, III) show that both LS and SNP lower SVR during and after rewarming. SNP lowered SVR superiorly to LS in all three studies. SNP has no documented direct effects on inotropic function, as also supported by finding a reduced PRSW after rewarming in our study. The likely explanation to our findings is that SNP ameliorated CO by reducing LV afterload, by systemic vasodilatation.

In early investigations of hypothermia and rewarming the elevated SVR was explained by increased viscosity[22,77]. From our experiment (III) we observe that the post-hypothermic heart has to struggles against an elevated afterload, but by introducing SNP during rewarming systemic vasodilatation is achieved and provides relief to the failing heart. The presented studies do not provide an answer to the mechanisms underlying the elevated SVR but indicate that a vascular myogenic component, responsive to SNP, is of significant importance. As highlighted in the introduction, investigators have identified changes in RAAS and catecholamine levels[58,62,64,88], vascular β-receptor super-sensitization and altered receptor composition[90,98,144,145,147,148], endothelial dysfunction and smooth muscle cell dysregulation[101,102].

9.2 Organ blood perfusion, cardiac output and cardiovascular autoregulation in hypothermia

Hypothermia depress organ perfusion. Depending on the depth and length of hypothermia, this continues even after rewarming[19,81]. In this study (III) we show a reduction in CBF, MBF, SBF and KBF in non-treated animals during and after rewarming from 3 hours at 15°C. These findings are consistent with similar experimental models[19]. At the same time, we demonstrate a reduction in CO and elevated SVR. In concordance to Paper I and II, LS elevated CO superiorly to SNP in Paper III. While SNP elevated CBF, MBF, SBF *and* total OBF compared to the control group, LS only elevated CBF. These findings are interesting as it seems that the available increase in CO produced by LS do no translate into elevated OBF. In contrast, SNP improved organ perfusion with only a minor elevation in CO. This indicates

that other mechanisms than just the availability of blood flow limits organ perfusion in the rewarming scenario.

9.2.1 Cerebral blood flow (CBF)

In animal experiments with spontaneous circulation and CPB, cerebral oxygen consumption ($CMRO_2$) has been demonstrated to be reduced during hypothermia[194-196]. Further, down to 28°C CBF/$CMRO_2$ ratio is unchanged as CBF is decreased proportionally to $CMRO_2$. In pigs cooled on CPB, Ehrlich et al. found the ratio to be doubled after cooling to 18°C[195], this finding is mirrored in dog experiments[175,197,198]. This increased mismatch between CBF and $CMRO_2$ has been interpreted as a failure in cerebral autoregulation[172]. The investigation by Ehrlich also indicted that cerebral metabolism is not limited by the lowered CBF, as hypercapnia-induced increase in CBF failed to elevate $CMRO_2$ at 18°C[195].

After rewarming pigs following 2,5 hours hypothermic CPB, Steen et al. found a continued reduction in $CMRO_2$, as well as reduced CBF[199]. On the contrary, Michenfelder, using an almost identical model, restored $CMRO_2$ to baseline values, with a continuation in CBF depression after rewarming[197,198]. Michenfelder argued that the findings of Steen et al. were the result of pharmacologic depression of cerebral metabolism caused by high doses of thiopental. Like Michenfelder, also Murkin found normalization of $CMRO_2$ in humans, after rewarming from hypothermic CPB[175].

It is evident that the highlighted findings are not directly comparable to findings in our model, as all studies are based on using CPB in large animals and in man. However, these data show that the effects of temperature on cerebral metabolism are fairly linear and that $CMRO_2$ is normalized after rewarming. Studies show the detrimental effects of hypothermia and rewarming on CBF [19,197,198]. It is therefore likely that CBF/$CMRO_2$ ratio is lowered after hypothermia, like Michenfelder and Steen demonstrated in their dogs, irrespective if on CPB or with spontaneous circulation[87,197,198].

Clinical studies on the neurological outcome in patients treated with hypothermic CPB applying the pH-stat vs. the α-stat strategy have shown that pH-stat may induce cerebral vasodilatation and elevation of CBF during hypothermia. However, it causes a worse neurological outcome than α -stat. The investigators attributed this to a higher load of microemboli from particles and gas bubbles during CPB[200,201]. The pH-stat strategy aims at

regulating pCO_2 during hypothermia, and as rewarming progress, the relative hypercapnia induced cerebrovascular vasodilatation lost. At the same time as the $CBF/CMRO_2$ ratio is shifted. The investigations are therefore not able to detect a potentially beneficial effect of continued cerebrovascular vasodilatation after rewarming.

Study III is limited to investigate whether the elevated CBF induced by SNP or LS has benefits to the animal outside the measured hemodynamic improvements. However, the presented body of evidence suggest that the rewarmed brain is vulnerable to low CBF and might indicate that SNP and LS may secure cerebral oxygen delivery to prevent further neurological damage.

Autonomic regulation of cerebral flow seems to be maintained at temperatures down to 28°C. Below this, a state of hyperperfusion occur, and investigators have interpreted this as failed autoregulation[172,195]. During rewarming, however, the growing discrepancy between $CRMO_2$ and CBF indicate hypoperfusion and a continued failure in autoregulation. This is supported by findings of elevated cerebral vascular resistance after rewarming, as well as a lowered CBF, despite normal perfusion pressure given by the CPB[202,203]. In models of spontaneous circulation, as the one presented (III), the reduction in CO cannot explain the reduced CBF, and an elevation of CO by LS do not result in fully restored CBF. This might indicate that the cerebral vascular regulatory system is affected by hypothermia and rewarming, and the beneficial effects of SNP suggest cerebrovascular vasoconstriction.

9.2.2 Myocardial blood flow

Coronary perfusion pressure depends on aortic pressure and coronary vascular resistance[204]. During systole, the intraventricular pressure prevents perfusion of the myocardium, as this constricts the coronary capillaries. Further, the coronary vasculature is sensitive to the metabolic demand of the heart, and a strong relationship has been found between MBF, cardiac oxygen consumption (MVO_2) and tissue oxygen content. More minor regulatory mechanisms are neurohormonal control and vasoactive responses to changes in local blood biochemistry. Berne approximated that changes in MVO_2 may cause five to ten-fold changes in MBF, while the latter only produce changes between 40-50%[73,204,205].

Investigators have demonstrated a reduction in MBF during hypothermia with spontaneous circulation[19,81,205-207]. Further, Berne showed that the coronary regulation of flow is altered in

hypothermia. As the relative reduction in MBF is lower that the change in aortic perfusion pressure, he argued that the coronary vessel is relatively vasodilated[205,208]. He further stated that the effects of hypothermia on coronary smooth muscle is relaxation, and that this is the main explanation to the vasodilatation and high MBF/MVO_2-ratio[205,208].

In concordance with previous studies, study III also shows a reduced MBF during and after rewarming[19]. MBF regulation after rewarming is not fully elucidated. In an in vivo study on coronary response to vasodilatation during hypothermia, Tveita et al. found that the coronary vasculature has reduced sensitivity to endothelium *dependent* and *independent* vasodilatation, which was normalized after rewarming[100]. This is a possible indication of functioning vascular regulation. While the actual study (III) made no attempts to investigate endothelium *dependent* vasodilatation, it however showed that SNP induced extensive vasodilation of the coronary circulation. Like during normothermia, LS turned out unable to elevate MBF in the rewarming scenario, despite a higher aortic perfusion pressure and higher CO[209]. As (for) with the cerebral circulation, this indicate that coronary resistance is an important factor in post-hypothermic organ hypoperfusion.

Further, the data show that LS improved cardiac contractility with no elevation in MBF. This is important as the increased contractility should come at an cost in O_2 expenditure[210,211]. As this model was not equipped to measure MVO_2 and cardiac oxygen extraction, this is only speculations. However, the findings indicate that the cardiac DO_2 sufficiently supplied MVO_2, also in the case of incrased contractility. This mirrors the ideas of Kondratiev et al. that oxygen supply is not a limiting factor for cardiac function in the rewarming scenario[188].

9.2.3 Renal blood flow

Regulation of RBF is controlled by two main vascular mechanisms, a fast-myogenic response and a slowed feedback mechanism dependent on glomerular filtration rate (GFR) and urine production. Both mechanisms regulate RBF by afferent vasoconstriction/dilatation, while the slowed feedback mechanisms also regulate RBF indirectly through changes in blood volume and perfusion pressure through the RAAS. The slowed feedback mechanism is also connected to renal function and metabolism[73,212].

In rats with spontaneous circulation, RBF is reduced by 40% at 28°C[213]. Identical findings were made by Hong, Tveita, Anzai and Levy in different models of experimental hypothermia

investigating renal blood flow[19,81,214,215]. Broman and Levy independently argued that the reduced RBF was a consequence of elevated renal vascular resistance. Renal metabolism and function also demise during cooling. GFR is reduced, and both renal reabsorption of sodium, and active excretion are reduced. The consequence is increased water loss, recognized as the well documented elevated urine production during hypothermia[213,215,216].

After rewarming from 28°C, Broman found complete restoration of RBF and renal function[213]. This stand in contrast to the findings in paper III, as well as multiple other investigators[19,81,217]. Bromans experiment did not prolong the hypothermic period, and the discrepancy in temperature, might explain the findings[213]. In dogs, Karim and Patton independently demonstrated depressed GFR and RBF after rewarming from 25°C[216,217].

Investigators argue that the reduced RBF and GFR after rewarming is due to maintained vasoconstriction. In a second study, Broman concluded that circulating catecholamines or efferent renal sympathetic activation did not play a role and pointed to other unidentified mechanisms. Karim highlight the elevated RAAS hormones have been demonstrated to increase during hypothermia[217,218].

The presented data show that neither SNP, nor LS improved RBF after rewarming, despite elevation of CO in response to both these substances. Interestingly, Broome et al. investigated the effects of SNP on RBF and SBF during angiotensin II-induced vasoconstriction[219]. They found that SNP failed to alleviate RBF, but normalized SBF. They interpreted this as evidence of a powerful renal vasoconstrictive effect of ATII. In paper III, the same discoveries where made. While not conclusive, this might indicate that RAAS, and especially angiotensin II, plays an important part in vascular dysfunction during and after rewarming. However, in a previous experiment exposing the same model to a similar H/R protocol as in experiment III, we reported changes in renal morphology after rewarming similar to those of acute tubular necrosis. This finding may well correspond with the present finding of a reduced RBF after rewarming[220].

9.3 Translational value, and future research

The use of small animal models to demonstrate and investigate physiological and pathophysiological processes has provided much new knowledge and expanded medical and

physiological research. However, direct translation between species and implementation to clinical practice must be done with great caution.

The presented model is designed to induce and investigate effects of deep hypothermia on cardiovascular function. To do this the rat model was chosen as this species is able to maintain spontaneous circulation at temperatures down to 13˚C. Some of the methodological limitations are previously highlighted, but the physiological differences between species has not been discussed. In light of the obvious limitations and difference in species I do believe that the presented and applied model is able to answer the proposed questions and produce new reproducible knowledge about physiology under hypothermic condition. This model has demonstrated to reproduce similar pathophysiological changes of hypothermia and rewarming as described in other species, including humans. I therefore believe that the pathophysiological and pharmaceutical discoveries presented here are relevant and can be applied in larger models exposed to experimental hypothermia and rewarming, also in combination with cardiac arrest or active rewarming techniques.

To further explore the therapeutic possibilities of LS and SNP in the rewarming scenario the next natural project is to move to a large animal model. As severely hypothermic and hemodynamically unstable patents are rewarmed by CPB or ECMO, a study on the effects of SNP and LS co-treatment on cardiac function and organ perfusion during experimental hypothermia and ECMO rewarming would be very interesting, especially with focus on weaning from extracorporeal circulatory support.

9.4 Final conclusions

The overreaching objective of this thesis was to investigate the relationship between cardiac - and vascular function in a model of accidental hypothermia and rewarming. We therefor studied effects of levosimendan and sodium nitroprusside on cardiac function and on organ blood flow. The main conclusions based on the experimental results are:

- Levosimendan improved CO after rewarming from hypothermia. This is attributable to increased cardiac contractility as demonstrated by elevated PRSW, and possibly to systemic vasodilatation. This demonstrates that hypothermia-induced cardiac dysfunction is partly reversible by means of a Ca^{2+} sensitizer.

- Having no effect on cardiac contractility after rewarming, SNP reduced SVR and elevated CO. We interpret this as follows; the post-hypothermic elevation of SVR limits cardiac performance, a mechanism which appears to be an important pathophysiologic element in the ensuing hypothermia-induced cardiac dysfunction

- Organ perfusion was improved both by LS and the vasodilator SNP. SNP improved organ blood flow more than LS, despite a more modest elevation of CO. We interpret this as follows: Hypothermia-induced persistent elevation of vascular resistance leads to a reduced organ blood flow and a compromised cardiac function during rewarming. Further, the literature and presented studies suggest impaired autoregulation of organ blood flow after rewarming. The amelioration by SNP suggest unwanted pathological vasoconstriction.

10 References

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Cardiovascular effects of levosimendan during rewarming from hypothermia in rat ☆

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ABSTRACT

Background: Previous research aimed at ameliorating hypothermia-induced cardiac dysfunction has shown that inotropic drugs, that stimulate the cAMP, – PKA pathway via the sarcolemmal β-receptor, have a decreased inotropic effect during hypothermia. We therefore wanted to test whether levosimendan, a calcium sensitizer and dose-dependent phosphodiesterase 3 (PDE3) inhibitor, is able to elevate stroke volume during rewarming from experimental hypothermia.
Methods: A rat model designed for circulatory studies during experimental hypothermia (4 h at 15 °C) and rewarming was used. The following three groups were included: (1) A normothermic group receiving levosimendan, (2) a hypothermic group receiving levosimendan the last hour of stable hypothermia and during rewarming, and (3) a hypothermic placebo control group. Hemodynamic variables were monitored using a Millar conductance catheter in the left ventricle (LV), and a pressure transducer connected to the left femoral artery. In order to investigate the level of PKA stimulation by PDE3 inhibition, myocardial Ser23/24-cTnI phosphorylation was measured using Western-blot.
Results: After rewarming, stroke volume (SV), cardiac output (CO) and preload recruitable stroke work (PRSW) were restored to within pre-hypothermic values in the levosimendan-treated animals. Compared to the placebo group after rewarming, SV, CO, PRSW, as well as levels of Ser23/24-cTnI phosphorylation, were significantly higher in the levosimendan-treated animals.
Conclusion: The present data shows that levosimendan ameliorates hypothermia-induced systolic dysfunction by elevating SV during rewarming from 15 °C. Inotropic treatment during rewarming from hypothermia in the present rat model is therefore better achieved through calcium sensitizing and PDE3 inhibition, than β-receptor stimulation.

Introduction

Case-reports show that the human body can survive core temperatures down to 13.7 °C and up to 7 h of hypothermic cardiac arrest [6,18]. Although survival is reported in such extreme situations, the mortality rate of accidental hypothermia is still described to be between 29% [30] and 80% [17]. A report from Melbourne showed that 13% of patients admitted to the emergency department had a core temperature below 35 °C. This patient group had a threefold independent risk of death [11]. The complications related to hypothermia is also acknowledged in surgical procedures, as use of therapeutic hypothermia during aortic surgery is related to increased need for inotropic support [2]. Thus, finding optimal strategies for treatment of patients subjected to therapeutic hypothermia and victims of accidental hypothermia is essential.

Abbreviations: PDE3, phosphodiesterase III; CO, cardiac output; LV, left ventricle; cTnC, cardiac troponin C; cTnI, cardiac troponin I; cAMP, cyclic adenosine monophosphate; PKA, protein kinase A; PVDF, polyvinylidene difluoride; MAP, mean arterial pressure; SV, stroke volume; TPR, total peripheral resistance; SR, sarcoplasmic reticulum; LVEDV, left ventricular end-diastolic volume; LVESV, left ventricular end-systolic volume; LVEDP, left ventricular end-diastolic pressure; CI, cardiac index; SW, Stroke work; $LVdp/dt_{max}$, maximum rate of left ventricular pressure change; PRSW, preload recruitable stroke work.

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Contributing to the high mortality of accidental hypothermia, rewarming is often complicated by cardiac dysfunction. Although this condition [29] was described as early as in 1826 by the French surgeon Moricheau-Beaupré [7], the pathophysiology behind it is not yet completely understood. Animal studies show that hypothermia-induced cardiac dysfunction is related to calcium overload [33], but not oxygen deficiency [13]. Further studies have tested inotropic drugs in order to counteract development of this condition. Among tested drugs are the β-agonists epinephrine and isoproterenol, which at normal core temperatures mediate positive inotropic effects by stimulating the cyclic AMP (cAMP), – protein kinase A (PKA) pathway. Remarkably, preclinical studies demonstrate diminished or adverse cardiovascular effects of these drugs when applied to treat hypothermia-induced cardiac dysfunction. Rather than giving positive inotropic effect, increased cardiac afterload, and lack of elevated SV dominated the hemodynamic response to β-agonists in hypothermic animals [14,12,8,28].

The calcium sensitizer levosimendan has potential in this setting. Acting through binding of cardiac troponin C (cTnC), levosimendan provides inotropic effect by stabilizing the calcium–cTnC–cTnI complex. In this way, levosimendan accelerates the cross-binding between actin and myosin [20]. In high concentrations levosimendan also function as a PDE3 inhibitor [25,3]. Inhibition of PDE3 will however increase cAMP and PKA, and thus induce Ser23/24-phosphorylation of cTnI. In a previous study carried out in our lab, we showed that contractile dysfunction after rewarming was related to increase of PKA-induced Ser23/24-cTnI phosphorylation [9], known to reduce myofilament calcium-sensitivity [19]. In contrast to epinephrine [28], which only had positive inotropic effect above 28 °C, administration of the PDE3 inhibitor milrinone demonstrated positive inotropic effect also during cooling below 28 °C [27]. Thus, in spite of the assumed increase in PKA-mediated Ser23/24-cTnI phosphorylation, PDE3 inhibition shows favorable effects on LV cardiac function at low core temperatures. According to clinical studies [16] we therefore wanted to test a high dose of levosimendan (bolus: 24 μg/kg, continuing infusion: 0.6 μg/kg/min) to make use of the combined effect of calcium sensitizing and PDE3 inhibition and explore whether this has potential to ameliorate hypothermia-induced cardiac dysfunction. To achieve this, we tested the effect of levosimendan on cardiac function during rewarming from 15 °C, using our rat model designed for hemodynamic measurements where spontaneous cardiac activity is maintained at all temperatures [33,13,14,12,8,28].

Materials and methods

Male Wistar rats (270–346 g) were used. The animals were provided by Charles River and quarantined for 1 week on arrival. Housing was provided in accordance with guidelines for accommodation and care of animals (article 5 of the European Convention for the Protection of Vertebrate Animals used for Experimental and Other Scientific Purposes, Strasbourg, 18.III.1986). The rats had a microbiological status according to the recommendation of the Federation of European Laboratory Animal Science Associations. Free access to food and water was permitted at all times. The experimental protocol was approved by the Norwegian Animal Research Authority and conducted accordingly.

Anesthesia

Anesthesia was introduced intraperitoneally by pentobarbital sodium (55 mg/kg) and fentanyl (50 μg/kg), followed by a continuous infusion of 7.5 mg/kg/h pentobarbital sodium and 50 μg/kg/h fentanyl through an intravenous line in the right jugular vein, extended to the right auricle. The anesthesia infusion was maintained at all hours in normothermic animals. In hypothermic animals, infusion was terminated at 30 °C during cooling and restarted at the same temperature during rewarming, due to hypothermia-induced anesthesia and reduced drug metabolism. The animals were monitored by toe-pinch for any sign of discomfort so that additional anesthesia could be provided if necessary. This is a well-established method for testing the effects of analgesic drugs in rodents and has been extensively tested in rats [4]. Toe pinch has been used for this purpose in all studies in the present model [33,13,14,12,8,28].

Respiratory support

Animals were placed on the operating table in a supine position. The trachea was opened, and a tracheal tube inserted. All animals had spontaneous and sufficient ventilation at core temperatures >20 °C. Below 20 °C, ventilation was achieved by a volume-controlled small-animal respirator (New England rodent ventilator, model 141, New England Instruments, Medway, MA) using room air. Normoventilation was achieved through adjusting ventilation in accordance with blood gas analyzes (ABL 800 blood gas analyzer, Bergmann diagnostika). During controlled ventilation, the alpha-stat strategy was followed.

Core cooling and rewarming

Animals were cooled and rewarmed by circulating cold or warm water (Thermo stated water bath type RTE-110, Neslab Instruments, Newington, NH) through an U-shaped polyethylene tube placed in the lower bowel. The tube was inserted gently to avoid harm of the intestine. In addition, the double-layered operating table made of hollow aluminum was circulated by temperature-adjusted water. Core temperature was continuously monitored using a thermocouple wire, positioned in the lowest part of the esophagus connected to a thermocouple controller (Thermalert Th-5, Bailey Instruments). Cooling and rewarming of the animals each lasted 1 1/2 h, while the hypothermic period (15 °C) lasted 4 h (Fig. 1).

Hemodynamic measurements

Hemodynamic variables were obtained using a pressure–volume conductance catheter (SPR-838, Millar Instruments Inc.,

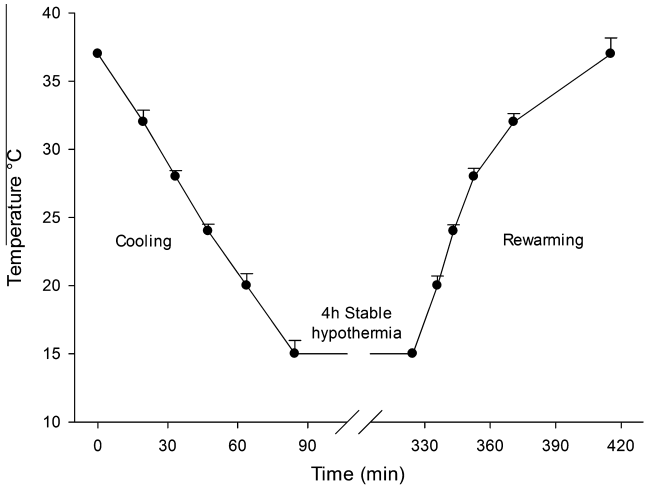

Fig. 1. Temperature profile of the experiments in rats assigned to either of the hypothermic groups, showing the cooling (37–15 °C) and rewarming (15–37 °C) rates and the stable hypothermic period (15 °C): Values are mean ± SEM.

Texas). The miniaturized 2.0 French pressure–volume conductance catheter allowed for assessment of *in vivo* left ventricular (LV) mechanical function in rats [1]. A constant sinusoidal alternating current (0.02 mA root means square at 20 kHz) was applied to drive the conductance catheter, through which changing conductance was used for the measurement of blood volume. Volume measurements in this study included parallel conductance (G_p). Further description of this method and temperature calibration of the catheter is described in detail in a previous report [8]. The load-independent LV-contractility index preload recruitable stroke work (PRSW) was measured by occluding the inferior vena cava transiently under the diaphragm. According to Filseth et al. this method was applied only prior to cooling and after rewarming, as caval occlusions at low temperatures does not induce changes in PV loops that can be applied to calculate PRSW [5]. To monitor peripheral vascular responses during cooling and rewarming, mean arterial pressure (MAP) was measured using a pressure transducer connected to a fluid-filled catheter (22G) inserted into the left femoral artery. During experiments, hemodynamic measurements were recorded at the following temperatures: 37, 32, 28, 24, 20 and 15 °C.

Experimental design

After surgery, the animals were given 1 ml saline and allowed to rest for 1 h before start of experiments. Levosimendan or placebo-levosimendan (placebo) was administered through an i.v. line in the left femoral vein, extended to the inferior caval vein. Infusion was started after 3 h of normothermia or hypothermia. The content of the placebo drug is identical to levosimendan except for absence of the active substance. Animals in hypothermic groups were core cooled to 15 °C and maintained at this temperature for 4 h, before rewarming to 37 °C. In the normothermic group, animals were held at 37 °C for 5 h.

Normothermic levosimendan group (n = 6)

After 3 h, animals received a bolus dose of 24 μg/kg of levosimendan infused over a period of 10 min, followed by a continuous 0.6 μg/kg/min infusion during the last 2 h of experiments.

Hypothermic levosimendan group (n = 7)

After 3 h at stable hypothermia (15 °C), animals were infused with a bolus dose of 24 μg/kg of levosimendan over 10 min, followed by a continuous infusion of 0.6 μg/kg/min during the last hour of hypothermia and till rewarming was completed.

Hypothermic placebo group (n = 7)

After 3 h of stable hypothermia (15 °C), animals were infused with a bolus dose of 24 μg/kg placebo for 10 min, followed by a continuous infusion of 0.6 μg/kg/min during the last hour of hypothermia and till rewarming was completed.

Measurement of cTnI phosphorylation

After successful rewarming, blood was removed by rapid flushing of sterile saline through the jugular catheter as described earlier [26]. The heart was quickly isolated, the LV dissected out and flash-frozen in liquid nitrogen. Measurements of the cTnI phosphorylation level on PKA associated sites Ser23/24 were performed using Western blot. The tissue was homogenized with a standard cell lysis buffer (Cell signaling) with 1 mM PMSF (Sigma). The protein level was measured with Lowry assay (Bio-Rad DC protein Assay). 45 μg of protein was loaded in each well separated with SDS–PAGE and transferred to a polyvinylidene difluoride (PVDF) membrane (Bio-Rad). Transferred proteins on PVDF membranes were detected with specific antibodies for either total cTnI (Fitzgerald) or phosphorylated cTnI at Ser23/24 (Cell Signal) and visualized by a chemo luminescent for detecting horseradish peroxidase (Bio-Rad). The bands were quantitatively analyzed using molecular imaging software (v. 4.0.1 Kodak). The amount of phosphorylation is measured as phosphorylation rate (density of phosphorylated Ser23/24-cTnI divided by total cTnI).

Measurement of cTnI release

After experiments were completed, arterial blood was sampled from the left femoral artery. Blood was centrifuged and the plasma extracted from the tubes. Plasma-cTnI was then analyzed, using a high sensitivity rat cTnI ELISA kit, Life Diagnostics, Inc., West Chester, PA, USA.

Statistics

Changes from baseline in hemodynamic variables were compared by One-way repeated measures ANOVA. When significant differences were found, Dunnett's method was used to compare values within group vs. baseline. Differences in cTnI release between groups were analyzed using one-way ANOVA on ranks. When significant differences were found, Dunn's method was used to compare values between groups. PRSW data within each group were compared using a paired *t*-test. Differences between hypothermic groups at same temperatures and cTnI phosphorylation between the hypothermic groups after rewarming were measured using a two-tailed, unpaired Student's *t*-test. Differences were considered significant at $p < 0.05$.

Results

Hemodynamics

Normothermia (Fig. 2)

Hemodynamic effects of levosimendan infusion in normothermic controls (37 °C). Compared to values measured at start of experiment (baseline), levosimendan infusion (120 min) caused significant elevation of stroke volume (SV), cardiac output (CO), heart rate (HR), maximum rate of LV pressure change ($LVdp/dt_{max}$) and stroke work (SW), whereas total peripheral resistance (TPR) decreased. Hemodynamic stability, up to 5 h at 37 °C, has been documented in numerous other studies using the present intact rat model [33,12].

Stable hypothermia

Levosimendan and placebo groups (15 °C). At the end of the 4 h stable hypothermia period, 1 h after start of placebo or levosimendan infusions in the two groups, most indexes of hemodynamic function were significantly reduced from their prehypothermic baseline values. Exceptions were left ventricular end-diastolic pressure (LVEDP), left ventricular end-diastolic volume (LVEDV), left ventricular end-systolic volume (LVESV) and SV, which remained unchanged. TPR was increased only in the placebo group (Figs. 3 and 4).

At the end of 4 h stable hypothermia and 1 h of levosimendan or placebo infusion, TPR was significantly lower and HR significantly higher in the levosimendan group compared to the placebo group.

Rewarming (Figs. 3, 4 and 7)

Placebo group (37 °C). After rewarming from 15 °C, SV, CO, SW and PRSW were all significantly reduced when compared to prehypothermic baseline values. TPR remained significantly elevated, when compared to prehypothermic baseline.

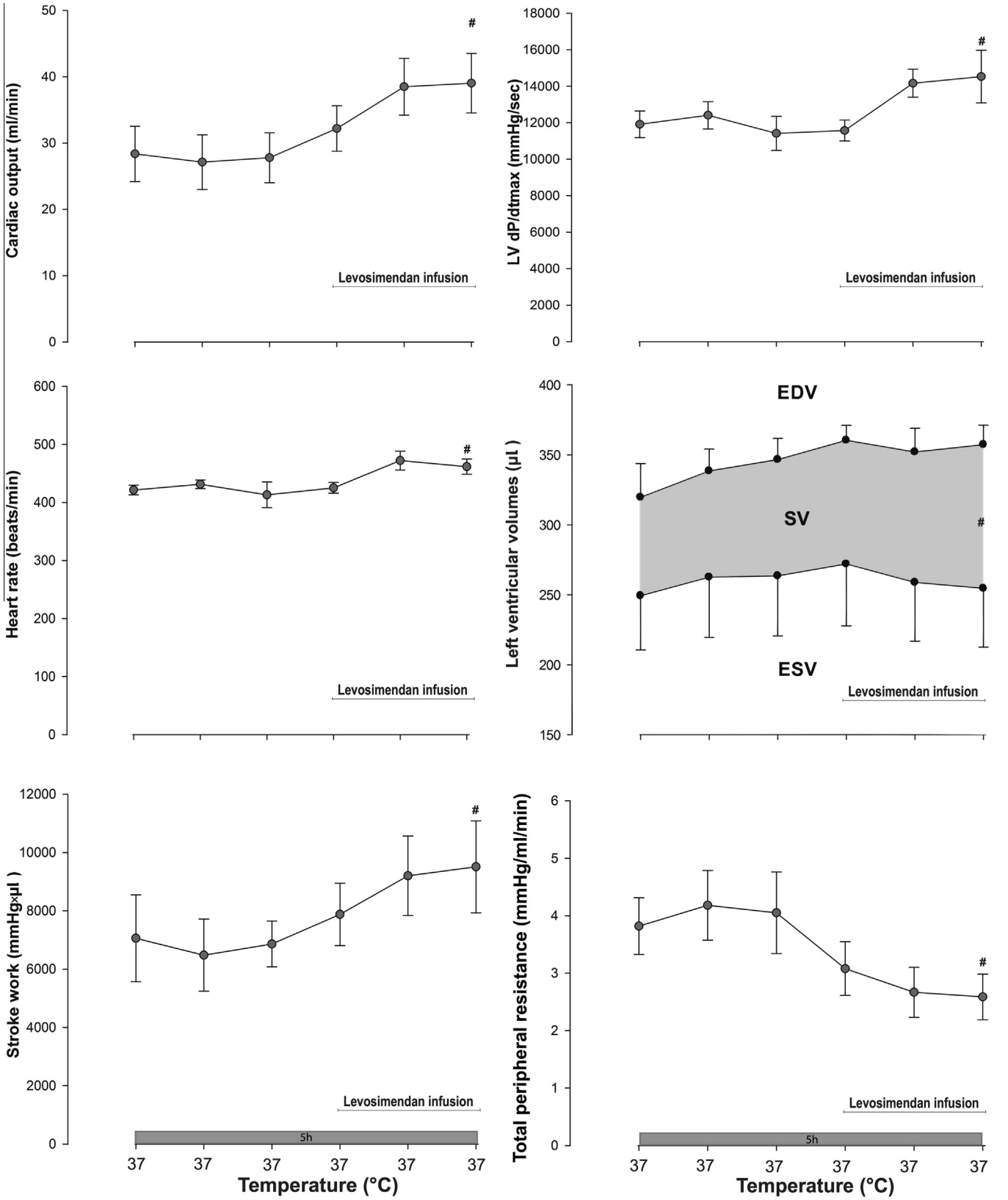

Fig. 2. Hemodynamic parameters in the normothermic control group, receiving levosimendan (bolus dose: 24 µg/kg, continuous infusion: 0.6 µg/kg/min) during the last 2 h of experiments. EDV: End-diastolic volume, SV: Stroke volume, ESV: End-systolic volume. Values are mean ± SEM. #Significantly different from within group baseline ($p < 0.05$). *Significant difference between groups ($p < 0.05$).

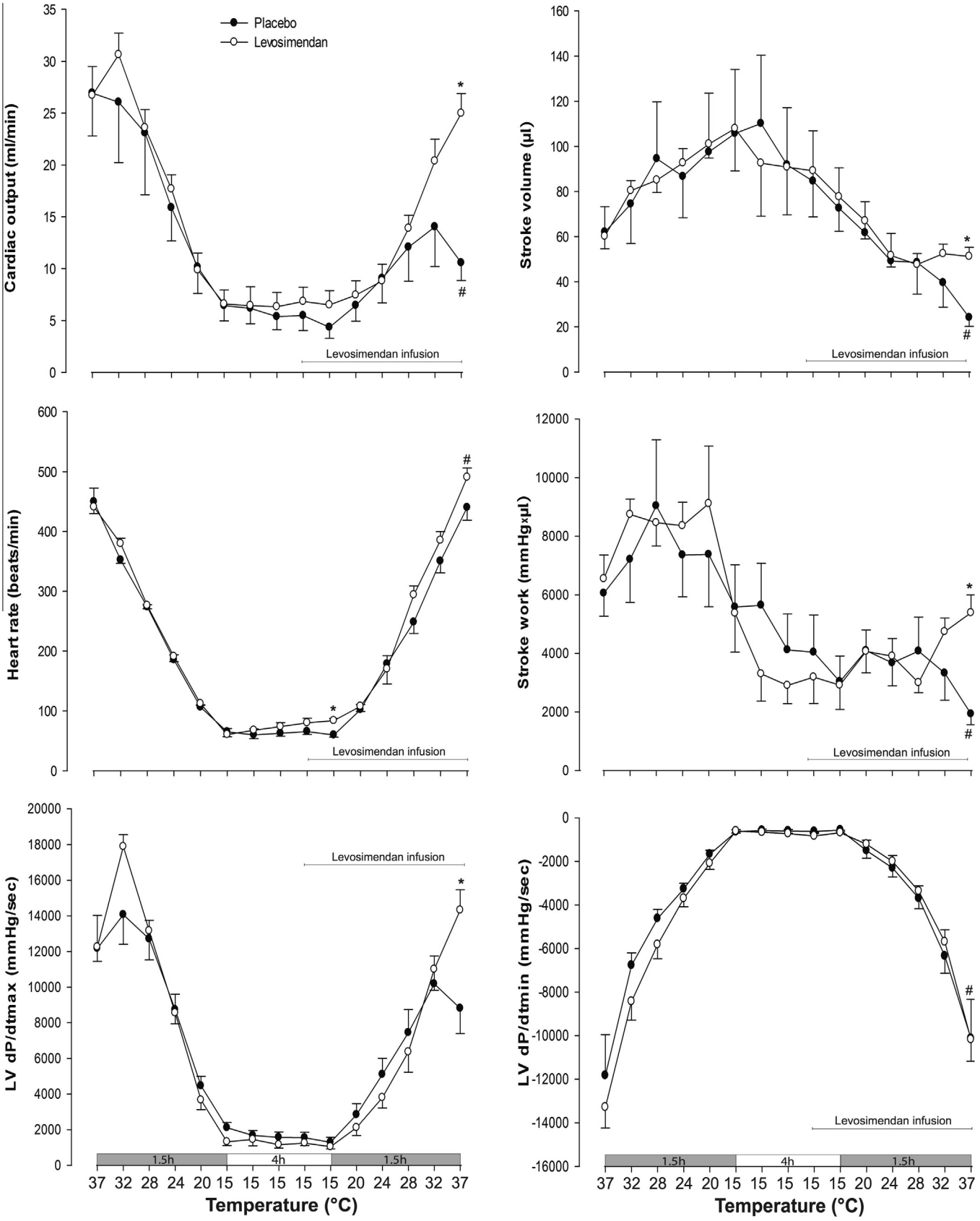

Fig. 3. Hemodynamic parameters in the two hypothermic groups, receiving either levosimendan or placebo (bolus dose: 24 µg/kg, continuous infusion: 0.6 µg/kg/min) during the last hour of hypothermia and during rewarming. Values are mean ± SEM. #Significantly different from within group baseline ($p < 0.05$). *Significant difference between groups ($p < 0.05$).

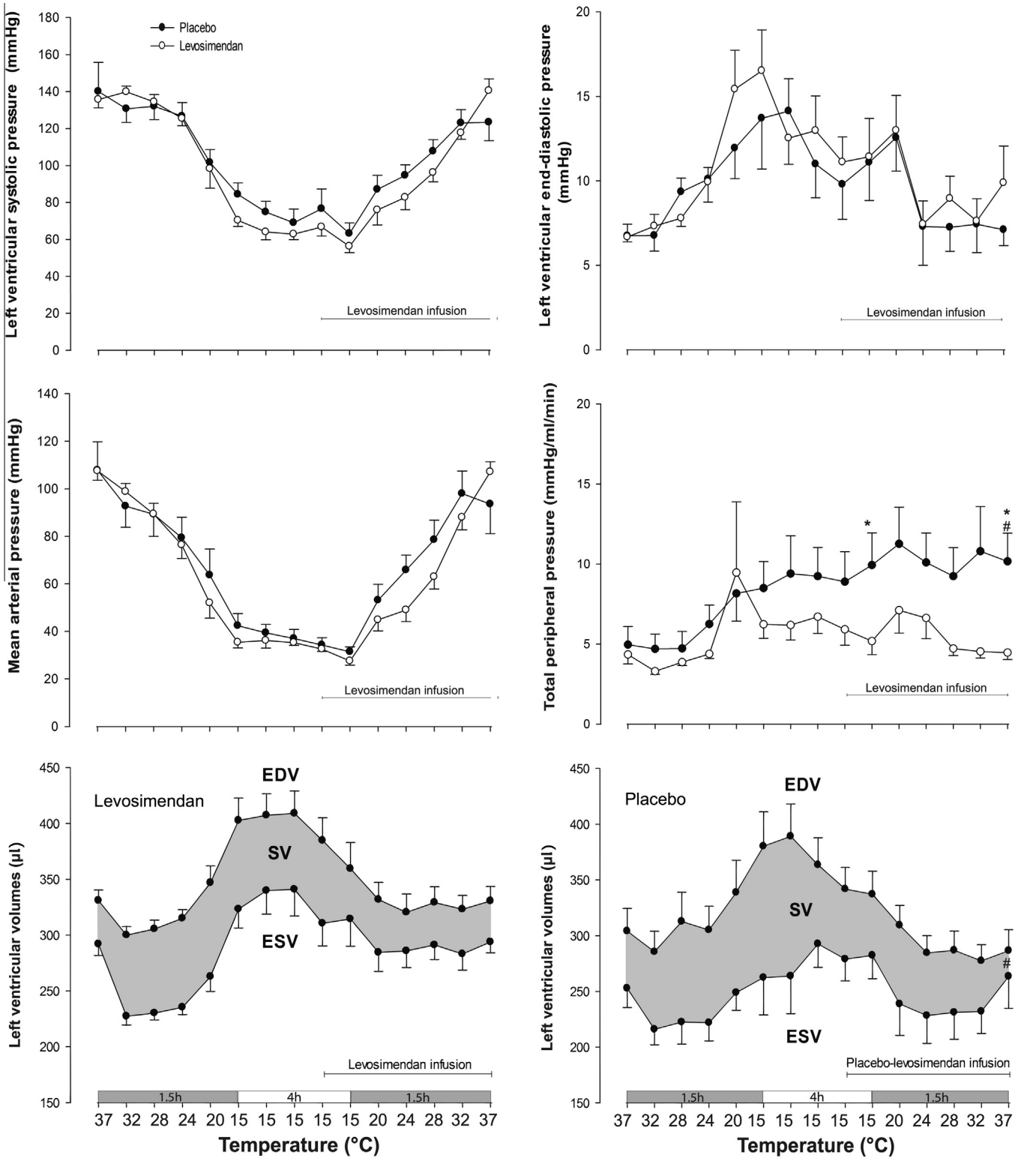

Fig. 4. Hemodynamic parameters in the two hypothermic groups, receiving either levosimendan or placebo (bolus dose: 24 µg/kg, continuous infusion: 0.6 µg/kg/min) during the last hour of hypothermia and during rewarming. Values are mean ± SEM. #Significantly different from within group baseline ($p < 0.05$). *Significant difference between groups ($p < 0.05$).

Levosimendan group (37 °C). In the levosimendan group rewarming caused a return to within pre-hypothermic baseline values of most cardiac variables. Minimum rate of LV pressure change ($LVdp/dt_{min}$) was decreased after rewarming, while HR was increased.

Differences between rewarmed levosimendan and placebo groups (37 °C). After rewarming SV, CO, $LVdp/dt_{max}$, SW and PRSW were significantly higher in the levosimendan group compared to the placebo group. TPR was significantly lower in the levosimendan group when compared to the placebo group.

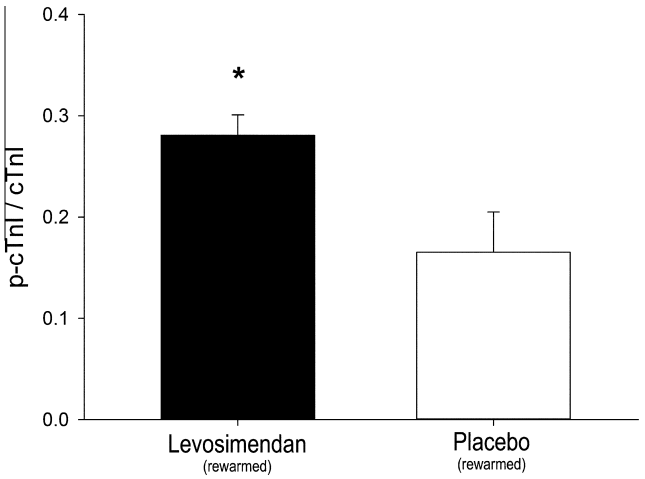

Fig. 5. cTnI phosphorylated at the Ser23/24 site in fraction of total cTnI, measured in left-ventricular tissue from excised hearts after rewarming in the two hypothermic groups, receiving either levosimendan or placebo (bolus dose: 24 μg/kg, continuous infusion: 0.6 μg/kg/min) during the last hour of hypothermia and during rewarming: Values are mean ± SEM. *Significant difference between groups ($p < 0.05$).

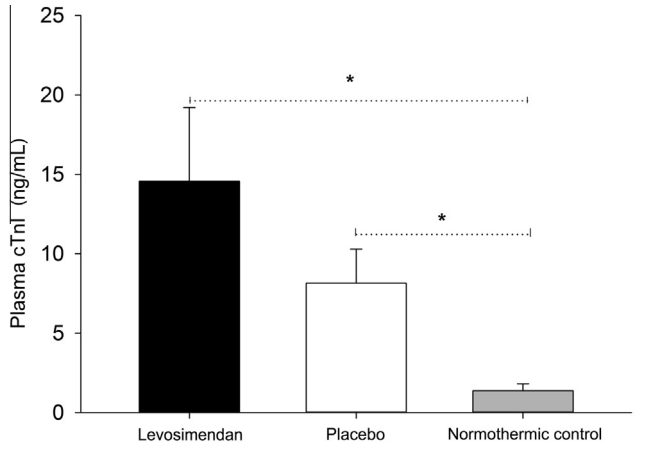

Fig. 6. Plasma-cTnI levels in the normothermic control group, receiving levosimendan (bolus dose: 24 μg/kg, continuous infusion: 0.6 μg/kg/min) during the last 2 h of experiments and in the two hypothermic groups, receiving either levosimendan or placebo (bolus dose: 24 μg/kg, continuous infusion: 0.6 μg/kg/min) during the last hour of hypothermia and during rewarming: Values are mean ± SEM. *Significant difference between groups ($p < 0.05$).

Phosphorylation of cTnI (Fig. 5)

After rewarming, Ser23/24-cTnI phosphorylation was significantly increased in the levosimendan group when compared to the placebo group. Phosphorylation results are displayed as a ratio between Ser23/24-cTnI phosphorylation and total cTnI protein.

cTnI release (Fig. 6)

Level of plasma-cTnI release at the end of experiments showed significant differences when comparing the normothermic group with both the rewarmed levosimendan group, and rewarmed placebo group. No significant differences were found between the rewarmed groups.

Discussion

Levosimendan administered during the last hour of hypothermia (4 h, 15 °C) and throughout rewarming to 37 °C caused a

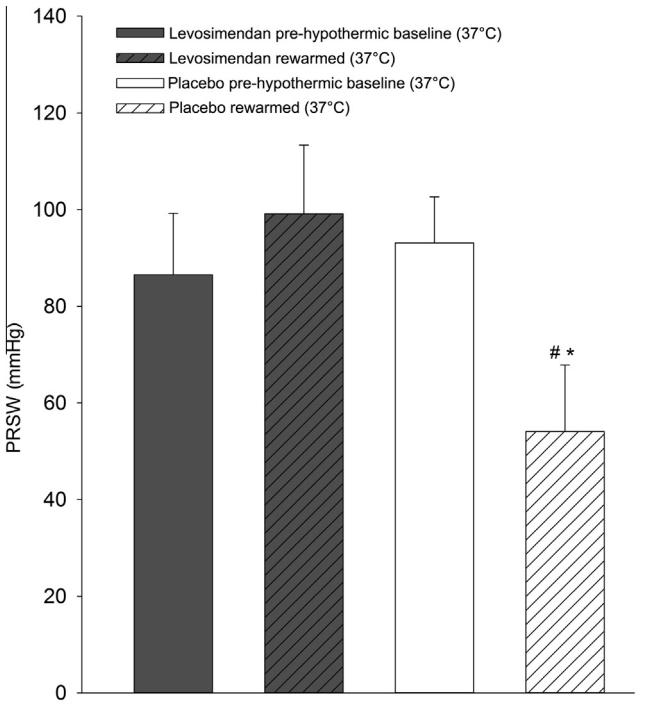

Fig. 7. Preload recruitable stroke work before cooling and after rewarming in the two hypothermic groups, receiving either levosimendan or placebo (bolus dose: 24 μg/kg, continuous infusion: 0.6 μg/kg/min) during the last hour of hypothermia and during rewarming: Values are mean ± SEM. #Significantly different from within group baseline ($p < 0.05$). *Significant difference between groups ($p < 0.05$).

return to within prehypothermic levels of SV, CO and PRSW. This is essentially different from the significant reduction of these variables after rewarming in the placebo group, demonstrating the positive inotropic effect of levosimendan during both hypothermia and rewarming.

Several inotropic drugs have been tested in the pursuit to pharmacologically elevate SV during rewarming from hypothermia. The present study demonstrates that levosimendan ameliorates hypothermia-induced cardiac dysfunction by elevating SV to prehypothermic levels after rewarming. This stand in stark contrast to our previous studies on β-receptor agonists, demonstrating that the inotropic properties of such drugs are altered during hypothermia and rewarming [14,12,8,28]. In more detail, only low-dose (0.125 μg/min) epinephrine showed beneficial hemodynamic effects when administered throughout rewarming from 15 °C. Elevation of SV was not achieved by higher epinephrine doses like in normothermia and vasoconstriction was induced [12], This shows that low core temperatures narrow the therapeutic window of inotropic treatment with β-agonists. The apparent challenges in providing cardiovascular pharmacological support during rewarming of hypothermic patients is reflected by recommendations in both the European [23] and American [31] guidelines, where administration of drugs is recommended only at core temperatures above 30 °C. Our findings indicate that use of inotropic support during hypothermia as well as rewarming from low core temperatures can provide favorable hemodynamic effects and ameliorate hypothermia-induced cardiac dysfunction. In the present study, we have demonstrated these favorable effects during rewarming after 4 h exposure to 15 °C, when applying a levosimendan-dose equivalent to what is defined as a high dose in clinical medicine. Others have also reported positive effects of lower doses of levosimendan during 15 min exposure to core-temperatures at 13–15 °C in preclinical studies of thoracic surgery [21,22]. These findings indicate

that the positive inotropic effect of levosimendan may appear at a wider therapeutic window than for β-receptor agonists during hypothermia and rewarming.

The present experiment shows that levosimendan facilitates Ser23/24-cTnI phosphorylation when administered during rewarming from 15 °C. Phosphorylation of cTnI is well documented and three main phosphorylation sites are explored: Ser23/24, Ser43/45 and Thr144, which are targeted by various kinases. PKA, a downstream kinase from cAMP in the pathway stimulated by β-receptor agonists and PDE3 inhibition, has shown great specificity for Ser23/24 [24]. Phosphorylation of this site is associated with reduced myofilament calcium-sensitivity [19]. Interestingly, Han et al. found that site-specific cTnI phosphorylation at Ser23/24 was increased after rewarming from 15 °C in rat papillary muscle and related this to contractile dysfunction [9]. The ability of levosimendan to support cardiac function and avoid stimulation of the cAMP–PKA pathway, thereby preventing further Ser23/24-cTnI phosphorylation, has earlier been used to describe the positive inotropic effect of this drug during rewarming after a short exposure to hypothermia [22]. However, positive inotropic effect of stimulating the cAMP, – PKA pathway through PDE3-inhibition during hypothermia, have already been observed in our rat model, using milrinone [27]. Levosimendan is also known to inhibit PDE3 in high doses as used in the present study [25,3], and we found increased Ser23/24-cTnI phosphorylation when compared to the placebo group after rewarming. This indicates that levosimendan facilitated cAMP, – PKA mediated Ser23/24-phosphorylation of cTnI via PDE3 inhibition and was observed in the presence of restored contractile function (PRSW) in the levosimendan group after rewarming. We therefore suggest that the combined PDE3 inhibition and calcium sensitizing mediated by levosimendan overcomes the potential negative inotropic effects of increased Ser23/24-cTnI phosphorylation [9]. Furthermore, at low core temperatures the importance of PDE3 inhibition might be enhanced due to hypothermia-induced cTnI phosphorylation [9], which is reported to decrease the calcium-sensitizing effect of levosimendan [10]. Despite offering positive inotropic effect, levosimendan-treatment did not protect against release of the myocardial injury marker plasma-cTnI. Increased release of this injury marker after rewarming also in the hypothermic placebo group, but not in the normothermic controls, shows that hypothermia caused myocardial tissue injury.

The positive inotropic effect of PDE3 inhibition reported in the present and previous studies [27], strongly indicates that stimulation of the cAMP, – PKA pathway through inhibition of cAMP degradation is favorable during rewarming. Efforts to stimulate this pathway through β-receptor agonists however, have demonstrated impaired inotropic properties of such drugs already during cooling to moderate hypothermia (34–33 °C) [8,28]. One mechanism facilitating this could be hypothermia-induced changes in β-receptor function. Interestingly, both increased and decreased β-receptor sensitivity is reported at low temperatures [15,32] and underlying mechanisms for diminished effect of β-agonists during hypothermia still remains unclear. Thus, more knowledge about the effects of hypothermia on β-receptor function is needed.

Conclusion

The present experiment shows positive inotropic effect of levosimendan during rewarming, when combining the calcium sensitizing and the PDE3 inhibitory effects offered by a high-dose of this drug. We therefore suggest that at low core temperatures, pharmacologic efforts to elevate cardiac function are better achieved by stabilization of the cTnC–cTnI complex and prevention of cAMP breakdown, rather than by attempting to increase cAMP formation via β-receptor stimulation.

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The beneficial hemodynamic effects of afterload reduction by sodium nitroprusside during rewarming from experimental hypothermia

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ABSTRACT

Background: Rewarming from hypothermia is associated with depressed cardiac function, known as hypothermia-induced cardiac dysfunction (HCD), and increased systemic vascular resistance (SVR). Previous studies on pharmacological treatment of HCD have demonstrated beneficial effects when using drugs with the combined effects; cardiac inotropic support and peripheral vasodilation. The presented study aims to investigate the isolated effects of arterial dilatation on cardiac functional variables during rewarming from hypothermia using sodium nitroprusside (SNP).
Methods: We utilized a rat model designed to induce HCD following 4 h at 15 °C and rewarming. To study effects on left ventricular (LV) functional variables in response to afterload reduction by SNP during rewarming a conductance catheter was used. Index of LV contractility, preload recruitable stroke work (PRSW), was obtained with inferior vena cava occlusions at 37 °C before and after hypothermia. Pressure signals from a catheter in the left femoral artery was used to pharmacologically adjust SVR.
Results: After rewarming both animal groups showed significant reduction in both SV and CO as a manifestation of HCD. However, compared to saline controls, SV and CO in SNP-treated animals increased significantly during rewarming in response to afterload reduction displayed as reduced SVR, mean arterial- and end-systolic pressures. The cardiac contractility variable PRSW was equally reduced after rewarming in both groups.
Conclusion: When rewarming the present model of HCD a significant increase in SVR takes place. In this context, pharmacologic intervention aimed at reducing SVR show clear positive results on CO and SV. However, a reduction in SVR alone is not sufficient to fully alleviate CO during HCD, and indicate the need of additional inotropic support.

1. Background

Hypothermia-induced cardiac dysfunction (HCD) is a well-known and life-threatening complication that may occur during treatment of accidental hypothermia victims [5,28,38]. In experimental models of hypothermia and rewarming, HCD has been identified as a key mechanism underlying rewarming shock [35,39]. Being a feared complication, rewarming shock develops as the cardiovascular system fails to provide adequate perfusion during rewarming and contributes to the high lethality in accidental hypothermia victims [5,28], reported to be between 28 and 35% [22,31,42]. Clinically, patients present with symptoms of low cardiac output (CO) accompanied by a rapid drop in blood pressure, despite restoration of core temperature [5].

Previous experiments have reported positive effects of milrinone and levosimendan on stroke volume (SV) and CO and when applied during rewarming in attempts to alleviate HCD [6,7]. The documented increase in cardiac function of these drugs is mainly attributed their direct positive inotropic effects. However, the

Abbreviations: LV, Left ventricle; MAP, Mean arterial pressure; HR, Heart rate; ESP, LV end-systolic pressure; dP/dt max, Maximum rate of LV pressure change; dP/dt min, Minimum rate of LV pressure change; SV, Stroke volume; CO, Cardiac output; SVR, Systemic vascular resistance; SW, Stroke work; PRSW, Preload recruitable stroke work; SNP, Sodium Nitroprusside; HCD, Hypothermia-induced cardiac dysfunction; CPC, Cardiac pumping capacity.

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beneficial effects were accompanied by a reduction in mean arterial pressure (MAP) and systemic vascular resistance (SVR) explained by the well documented vasodilating effects of these drugs [26]. In normal physiology, vasodilatation reduces SVR and alleviate cardiac work and afterload, elevating CO [11]. This has also been demonstrated in patients with ischemic heart failure and cardiogenic shock where SVR reduction improved left-ventricular function and CO [3]. The beneficial effects of levosimendan and milrinone on HCD stand in contrast to the effects of adrenaline documented in the same experimental model. Unlike levosimendan or milrinone, adrenaline increased SVR and failed to alleviate CO during hypothermia and rewarming [8,12,19].

Cardiovascular physiology research has demonstrated that the relationship between CO and MAP is determined by four principal factors; SVR, cardiac pumping capacity (CPC), blood volume, and vascular compliance [11]. The beneficial effects of levosimendan and milrinone to elevate CO take place by simultaneously increasing CPC by Ca^{2+}-sensitization and phosphodiesterase-3 inhibition and by reducing SVR via peripheral vasodilation through opening of smooth muscle K^{+}_{ATP}-channels and phosphodiesterase-3 inhibition [26]. As both SV and SVR are altered in HCD, the combined pharmacological effects of levosimendan and milrinone, to support cardiac inotropy and induce vasodilatation, complicate the interpretation of the hemodynamic data. In a quest to ameliorate and understand HCD and rewarming shock, it is important to determine the separated effects of vasodilatation and positive inotropy.

Different from milrinone and levosimendan, sodium nitroprusside (SNP) is a potent vascular vasodilator with no inotropic effects [33]. It has been meticulously studied and are readily used in both clinical practice and in basic research and has demonstrated to improve cardiac function during cardiogenic shock and when used *after* rewarming from hypothermia in dog [3,24,32,34]. SNP relax smooth muscle and reduce SVR by nitric oxide activation of the cGMP-pathway. In vitro studies report possible inotropic support of SNP on cardiomyocytes [18,23]. However, *in vivo* experiments finds no clinical relevant changes in contractile parameters in healthy and failing hearts [33].

The aim of this study is to investigate if reduction in SVR during rewarming from experimental hypothermia may improve cardiac function. To test this we applied SNP in comparison with non-treated saline controls in a well-established *in vivo* rat model of HCD equipped for invasive hemodynamic monitoring [6,7,19].

2. Materials and methods

2.1. Animals

Male Wistar rats (n = 14, mean weight 313.8 g ± 7.18) were used. Animals were obtained from Charles River and quarantined for 1 week before experiment. Housing was provided in accordance with guidelines for accommodation and care of animals (article 5 of the European Convention for the Protection of Vertebrate Animals used for Experimental and Other Scientific Purposes, Strasbourg, 18.III.1986). The rats had a microbiological status per the recommendation of the Federation of European Laboratory Animal Science Associations. The animals had ad libitum access to water and food. The experimental protocol was approved by the Norwegian Animal Research Authority and conducted accordingly.

2.2. Anesthesia

Before vivisection, the animals were anesthetized with 50 mg/kg sodium pentobarbital and 0.05 mg/kg fentanyl with an intraperitoneal bolus injection. The animals received 7.5 mg/kg/h sodium pentobarbital and 0.05 mg/kg/h fentanyl through a pediatric central venous catheter placed in the right jugular vein. Cold narcosis occurs <30 °C, pharmacological anesthesia was therefore discontinued <30 °C and continued upon rewarming [27]. Pain and discomfort was monitored with the toe-pinch method as well as hemodynamic markers of stress. Toe-pinch is a well-known and established method to determine the depth of anesthesia in rodents [4,7,19].

2.3. Respiratory support

Airways were secured by a tracheostomy followed by insertion of a tracheal tube. In this model apnea occurred at around 20 °C, whereby mechanical ventilation with a small rodent ventilator is started (Kent Scientific, USA). Mechanical ventilation was discontinued once spontaneous respiration returned upon rewarming.

2.4. Hemodynamic measurements

Continuous cardiac volume and pressure measurements were provided through cannulation of the left carotid artery with a 2.0 French conductance catheter (SPR-838, Millar Instruments Inc., USA) placed in the left ventricle [2]. The conductance catheter technique and calibration is previously described in detail in earlier reports [6,7,12]. A fluid filled 22G catheter placed in the left femoral artery provided systemic arterial pressure measurement. Both catheters were connected to a PowerLab 16/30 transducer (ADInstruments, New Zealand), and the data was recorded and analyzed using LabChart 7 (ADInstruments, New Zealand). Hemodynamic data was recorded at 37 °C, 32 °C, 28 °C, 24 °C, 20 °C, and hourly for 4 h at 15 °C. To assess cardiac contractility, preload recruitable stroke work (PRSW) was obtained by occluding the inferior vena cava at 37 °C baseline and after rewarming. The reduced hemodynamic function during hypothermia <20 °C prohibited vena cava occlusions and therefore PRSW values was not obtainable during the hypothermia protocol [6,10].

2.5. Cooling and rewarming

Temperature control was obtained with a water bath (Thermostat water bath type RTE-110, Neslab Instruments, USA) circulating cold or warm water through a U-shaped polyethylene tube gently inserted into the intestine. In addition, the water bath was connected to a hollow, double walled, aluminum surgical table providing external heating and cooling. Temperature was continuously monitored with the use of an esophageal thermocouple (T-type) connected to a transducer (Colombia Instruments, USA). Both cooling and rewarming of the animals lasted 90 min, while the hypothermic period lasted 4 h (Fig. 1).

2.6. Sodium nitroprusside and saline

Sodium nitroprusside (Nitropress®) from Hospira (IL, USA) (25 mg/mL) was used. On the day of experiment, the sodium nitroprusside stock was diluted to 0.125 mg/ml (1:200) in 5% glucose [17]. As placebo in the saline control group 0.9% NaCl (Fresenius Kabi, Germany) was used.

2.7. Experimental design and pharmacological intervention

Two experimental groups were included in this study. Group 1 – Saline and Group 2 – Nitroprusside. After surgery, all animals received 1 mL saline with 10 IE Heparin/mL and were allowed to stabilize for 1 h before cooling. Both groups were subjected to 4 h of hypothermia at 15 °C with subsequent rewarming. Pharmacological

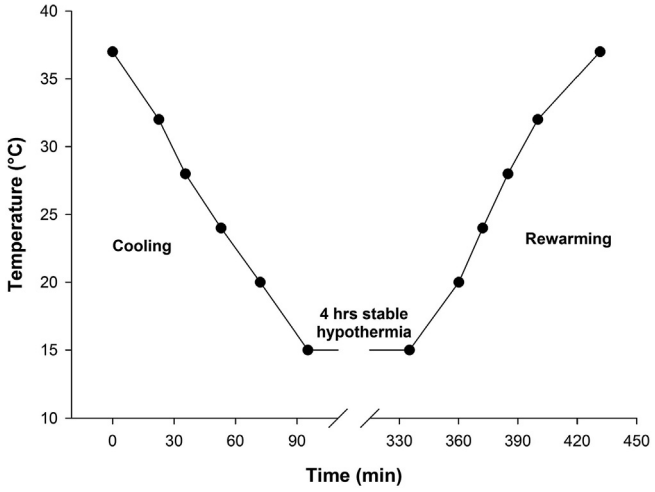

Fig. 1. Temperature profile of all 14 hypothermic rats, showing cooling (37-15°C), 4 h stable hypothermia (15°C) and rewarming (15–37°C). Values are mean.

intervention was started after 3 h at 15 °C (15°C_3) and continued throughout rewarming. Saline (Group 1) was infused with a bolus dose of 0.83 ml 0.9% saline over 20 min, followed by a continuous infusion of 0.5 mL/h. Nitroprusside (Group 2) infusion started at 0.625 μg/kg/min and was titrated to reduce MAP by 30% during rewarming.

2.8. Statistical analysis

The hemodynamic progression in each group was analyzed with a repeated measurement one-way ANOVA. Data distribution was tested with Shapiro-Wilks test, and if failed ($p < 0.05$) an ANOVA on ranks test was utilized. If the null-hypothesis (H_0) was rejected ($p < 0.05$), a post-hoc Dunnett's test was performed to analyze differences from pre-hypothermic baseline values. Between-groups comparisons at each time-point were done with a two-tailed Students t-test. Data distribution was tested with Shapiro-Wilks test, and if failed ($p < 0.05$) a Mann-Whitney *U* test was utilized. A paired *t*-test was used to compare PRSW at 37 °C before cooling vs. after rewarming. Statistical significance was accepted at $p < 0.05$. All results are presented as mean ± SEM.

3. Results

3.1. Dose-finding study

We used MAP as a surrogate for SVR during drug administration in the dose-finding study. To mimic the vascular vasodilator effects of milrinone during hypothermia and rewarming we determined a 30% reduction in MAP as target [6,7]. When using the same dose SNP (10 μg/kg/min) as normothermia during 15 °C, severe hypotension and cardiac standstill occurred [16]. Therefore, from a dose-finding study we found that at 15 °C a 20-fold dose-reduction of SNP was required to achieve the target reduction of MAP which at the same time gave an acceptable hemodynamic function. Accordingly, SNP infusion started with 0.6125 μg/kg/min at 15°C_3. Once rewarming was started the vasodilatory effects of nitroprusside diminished and infusion rate was dynamically increased to achieve target MAP.

3.1.1. Within-group comparisons (Table 1)

3.1.1.1. Group 1 – saline. Compared to baseline (37°C_{BL}) a significant reduction was found in heart rate (HR), mean arterial pressure (MAP), LV end-systolic pressure (ESP), cardiac output (CO), and LV peak rate of pressure rise (dP/dT_{max}) after cooling to 15 °C (15°C_0). Moreover, LV peak rate of pressure decline (dP/dT_{min}) and Tau were increased. After rewarming (37°C_{RW}) a significant reduction in MAP, SV, CO, dP/dT_{max}, SW and preload recruitable stroke work (PRSW) was found when compared to 37°C_{BL}. Also, a significant increase in dP/dT_{min} and SVR was found (Table 1, Fig. 3).

3.1.1.2. Group 2 – sodium nitroprusside (SNP). After cooling to 15°C_0 a significant reduction in HR, MAP, ESP, CO, dP/dT_{max} and SW was found compared to 37°C_{BL}. Tau, dP/dT_{min}, and SVR was significantly incased. After rewarming to 37°C_{RW} there was a significant reduction in MAP, ESP, SV, CO dP/dT_{max}, SW and PRSW, whereas a significant increase in dP/dT_{min} was found when compared to 37°C_{BL} (Table 1, Fig. 3).

3.1.2. Between-group comparisons (Figs. 2 and 3)

3.1.2.1. Baseline, cooling and hypothermia (37°C_{BL} - 15°C_3). No significant difference was found in any hemodynamic parameters between groups at 37°C_{BL}, after cooling to 15°C_0 or after 3 h of hypothermia (15°C_3) prior to pharmacological intervention.

3.2. Pharmacological intervention and rewarming (15°C_3 - 32°C_{RW})

Throughout rewarming from 15°C_4 a significant reduction in MAP and ESP was present at all time-points in SNP treated animals compared to saline. Further, SV was significantly elevated at 32°C_{RW} and SW significantly depressed at 20°C_{RW}, 24°C_{RW} and 28°C_{RW} in the SNP treated animals. No difference was found between groups in HR, dP/dT_{max}, CO, SVR or Tau throughout rewarming to 32°C_{RW}.

3.3. After rewarming (37°C_{RW})

After rewarming to 37 °C, MAP, ESP and SVR were significantly lowered, and SV and CO significantly elevated in animals treated with SNP compared to saline. No difference was found in HR, dP/dT_{max}, PRSW and SW.

4. Discussion

This study demonstrates that dynamic reduction of SVR, enabled by peripheral arteriolar vasodilatation with sodium nitroprusside (SNP), alleviated cardiac workload and elevated post-hypothermic SV and CO when compared to non-treated controls. However, the vasodilator effects achieved by SNP were not able to restore cardiac function to pre-hypothermic levels.

4.1. Nitroprusside in hypothermia

This study is the first to show maintained vasodilator effects of SNP also at temperatures down to 15 °C. Further, we found that by reducing MAP, to achieve the wanted reduction in SVR, a simultaneous increase in CO and SV took place. This was not a result of altered cardiac contractility as dP/dT_{max}, SW and PRSW was identical between the groups at 37°C_{RW}. This is in concordance with studies on the inotropic effects of SNP as they show unaltered contractility in both healthy and failing hearts [33]. Interestingly, SNP infusion rate had to be rapidly increased throughout rewarming to achieve target MAP. Boon et al. showed that the dose-response of SNP is strongly dependent on intra-vascular fluid volume and CO [1]. Pharmacodynamical effects of SNP is increased in the presence of elevated preload and CO, while it is reduced in a setting with low preload and reduced CO [1]. Treatment of hypothermia entails significant changes in circulating blood volume and a dynamic increase in CO during the rewarming phase [20,40].

Table 1
Hemodynamic parameters, and systolic and diastolic indicators at four staple time points: 37°C_{BL}, 15°C_0, 15°C_4 and 37°C_{RW}. Values are mean ± SEM.* indicate significate difference from 37°C_{BL} within groups ($p < 0.05$).

Parameter	Group	37°C_{BL}	15°C_0	15°C_4	37°C_{RW}
Heart rate, beats/min	Saline	435 ± 15	50 ± 2*	48 ± 2*	458 ± 16
	Nitroprusside	443 ± 14	48 ± 2*	51 ± 3*	462 ± 12
MAP, mmHg	Saline	120.7 ± 7.6	36.5 ± 2.9*	31.2 ± 2.0*	106.1 ± 2.0*
	Nitroprusside	112.5 ± 2.8	42.8 ± 4.7*	21.2 ± 1.0*	79.3 ± 6.5*
ESP, mmHg	Saline	135.1 ± 6.7	73.4 ± 6.4*	56.3 ± 4.4*	125.9 ± 6.0
	Nitroprusside	129.1 ± 2.9	72.1 ± 5.9*	37.2 ± 2.0C*	87.6 ± 5.4*
SV, μL	Saline	63.6 ± 10.5	141.6 ± 14.6*	82.8 ± 9.4	21.5 ± 3.3*
	Nitroprusside	64.6 ± 7.4	113.7 ± 14.4*	68.7 ± 6.4*	36.1 ± 4.8*
CO, mL/min	Saline	27.78 ± 4.76	7.71 ± 0.73*	3.91 ± 0.39*	9.74 ± 1.35*
	Nitroprusside	28.56 ± 3.28	6.35 ± 0.99*	3.63 ± 0.47*	16.45 ± 1.83*
SVR, mmHg/mL/min	Saline	5.19 ± 0.96	5.03 ± 0.63	8.3 ± 0.68	12.8 ± 2.51*
	Nitroprusside	4.22 ± 0.45	8.27 ± 1.77*	6.87 ± 1.46	5.36 ± 0.86
Systolic indicators					
dP/dT_{max}, mmHg/s	Saline	10630 ± 864	1668 ± 132*	1139 ± 146*	8158 ± 68*
	Nitroprusside	10753 ± 454	1973 ± 215*	784 ± 103*	7781 ± 791*
SW, mmHg*mL	Saline	7.65 ± 1.20	7.25 ± 0.71	3.36 ± 0.45	1.82 ± 0.23*
	Nitroprusside	7.54 ± 0.97	6.00 ± 0.78	1.72 ± 0.17*	2.42 ± 0.22*
PRSW, mmHg	Saline	109.10 ± 7.38	–	–	63.40 ± 15.21*
	Nitroprusside	102.99 ± 9.45	–	–	62.14 ± 8.94*
Diastolic indicators					
dP/dT_{min}, mmHg/s	Saline	−11031 ± 720	−560 ± 49*	−480 ± 48*	−9079 ± 514*
	Nitroprusside	−12961 ± 565	−596 ± 95*	−327 ± 39*	−7778 ± 681*
Tau, ms	Saline	7.70 ± 0.47	72.62 ± 4.37*	77.25 ± 5.97*	8.42 ± 0.74
	Nitroprusside	9.13 ± 0.17	96.60 ± 12.81*	41.81 ± 3.49*	6.66 ± .35

These changes would explain the need for rapid dose adjustments during rewarming to maintain a reduced MAP. The dynamic and easily reversed effects of SNP during rewarming provide an opportunity to titrate infusion rate to prevent hemodynamic instability. As SNP breakdown occurs via a reaction inside erythrocytes involving hemoglobin the half-life is independent of liver and kidney metabolism [17]. This prevents an increase in half-life of SNP at low temperatures, as seen with many other vasoactive substances during hypothermia [41]. This enables an easy reversibility of SNP and offers a clinical advantage over drugs with temperature-dependent prolongation of half-life when applied during rewarming from hypothermia.

4.2. Hypothermia, vascular resistance and rewarming shock

Elevated SVR during hypothermia has previously been claimed to result from temperature-induced increase in blood viscosity [25]. As demonstrated in the present study, SVR continued to increase in the saline group, despite normalized blood viscosity upon rewarming, as demonstrated by Poulos [29]. This indicates involvement of other mechanisms underlying the elevated post-hypothermic SVR. Changes in SVR depend on vascular smooth muscle tone and endothelial function [15]. So far, no descriptive literature is available on smooth muscle function during hypothermia and rewarming. Investigators have, however, documented that hypothermia and rewarming may cause endothelial damage and dysfunction [14,30,43]. The lowered SVR by SNP, milrinone and levosimendan demonstrate that endothelium-independent vasodilatation is achieved also during hypothermia. In more detail, this indicates that vascular smooth muscle responds to nitric oxide and elevated cyclic AMP in a similar way both in hypothermic and normothermic conditions, as already suggested [32,36]. These findings may indicate that hypothermic and post-hypothermic elevation of SVR is not a compensatory mechanism or solely the result of increased blood viscosity, but possibly a consequence of hypothermia-induced vascular dysfunction because of endothelial damage. Endothelial damage may well be a result of exposure to long-lasting and profound hypothermia as in the present experiment.

The presented study finds that pharmacologic elevation of CO can be achieved during HCD in the absence of specific cardiac inotropic support. These findings provide nuance and increase emphasis on the heart-vasculature relationship during hypothermia and rewarming. Further, the present data provide new insight to why inodilatators such as milrinone, levosimendan and dopamine may elevate CO to pre-hypothermic values after rewarming [6,7,9], whereas the inoconstrictor adrenaline caused a significant reduction of CO and SV by elevating SVR [8,19]. While SNP showed beneficial effect on CO after rewarming, the absence of additional inotropic support explains why SNP as monotherapy failed to return CO to pre-hypothermic levels. This shows that the key to proper pharmacological treatment of HCD and rewarming shock is the combination of cardiac inotropic support and appropriate SVR regulation. Finally, as this is an animal study it is important not to translate the presented findings to clinical recommendations without careful consideration and awareness of animal model limitations. However, the model used has demonstrated to be reproducible in multiple species and believed to accurately represent the human condition of rewarming shock [10,13,21,37]. Further, SNP is a well-tested and used drug in clinical practice and research, with demonstrated beneficial effects in human cardiac disease [3,32,34]. This strengthens our conviction that the presented findings are relevant to the clinical situation and that this is a track worth pursuing in further animal studies.

5. Conclusion

This experiment shows that CO is elevated if SVR is dynamically reduced by SNP-induced vasodilatation during rewarming from hypothermia. The elevated SVR found in untreated animals during rewarming seems to have negative hemodynamic effects, rather than functioning as a compensatory mechanism, especially when heart function suffers from HCD. However, the SNP-induced reduction of SVR alone is not sufficient to fully alleviate CO during HCD, and indicate the need for supplemental inotropic support during rewarming of hypothermic patients.

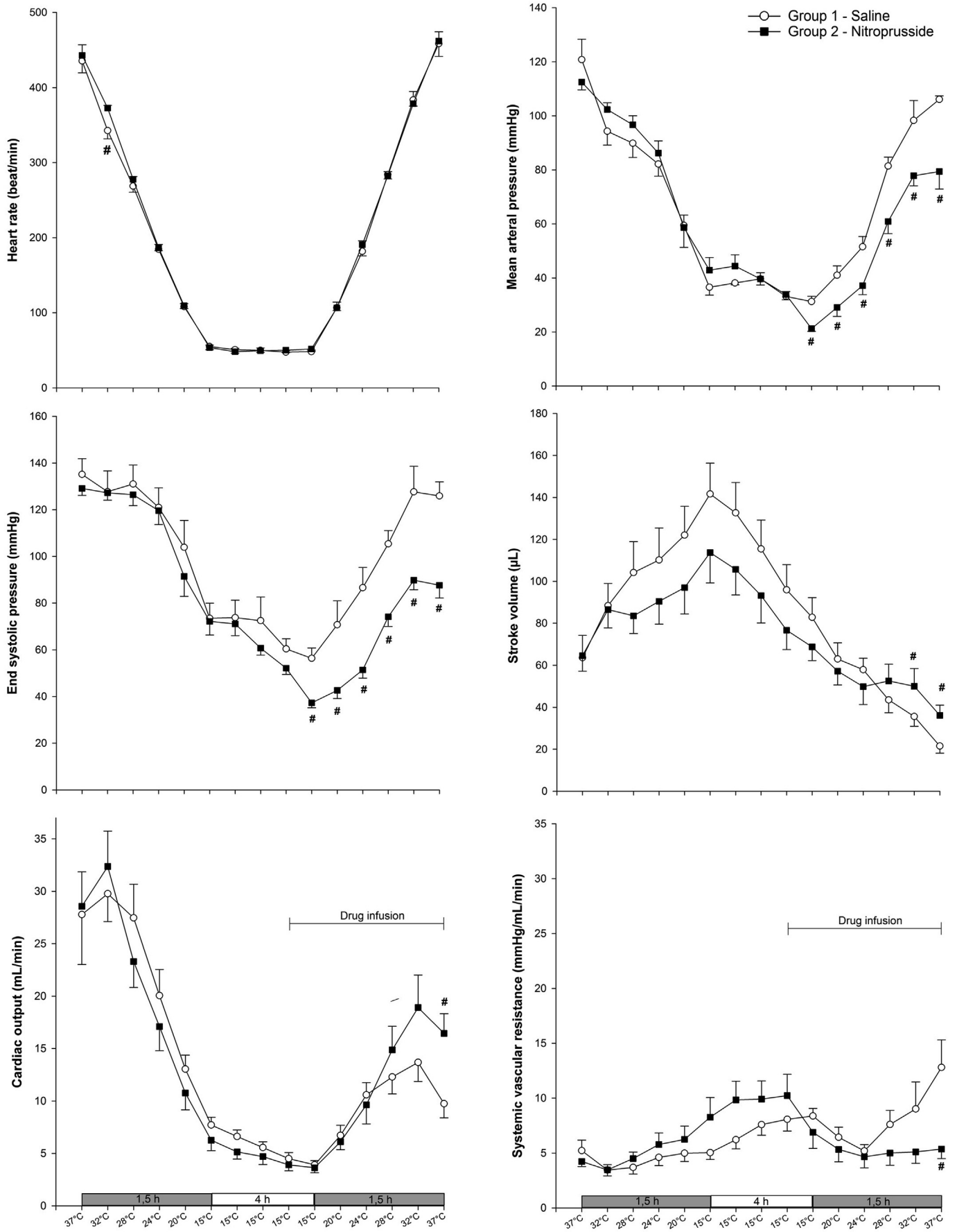

Fig. 2. Hemodynamic variables during cooling, hypothermia and rewarming in both experimental groups. Values are mean ± SEM. # indicate Group 2 - Nitroprusside is significantly different from Group 1 - Saline ($p < 0.05$).

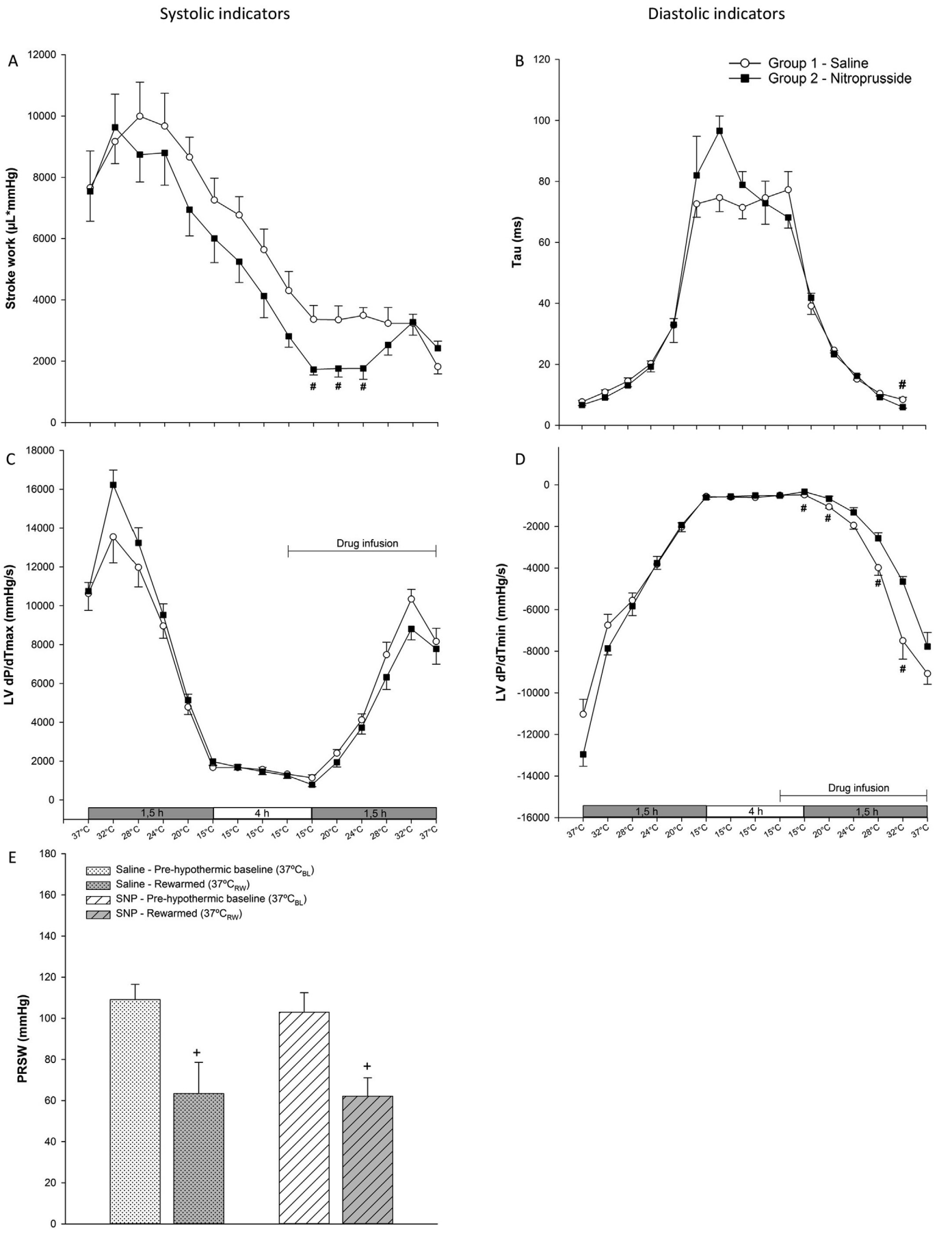

Fig. 3. Hemodynamic variables during cooling, hypothermia and rewarming in both experimental groups. Values are mean ± SEM. # indicate Group 2 - Nitroprusside is significantly different from Group 1 - Saline ($p < 0.05$). + indicate 37°C$_{RW}$ significantly different from 37°C$_{BL}$ ($p < 0.05$).

Ethics approval and consent to participate

The animal experimental protocol and housing conditions was approved by Norwegian Animal Research Authority and in accordance to the ARRIVE guidelines.

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Conflicts of interests

None.

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