



## Negative inotropic effects of epinephrine in the presence of increased $\beta$ -adrenoceptor sensitivity during hypothermia in a rat model <sup>☆</sup>



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### ABSTRACT

**Background:** Animal studies show reduced inotropic effects of cardiac  $\beta$ -adrenoceptor agonists like epinephrine (Epi) during hypothermia and rewarming, while drugs targeting other pharmacological mechanisms have positive effects. This study therefore aimed to determine  $\beta$ -adrenoceptor sensitivity in isolated cardiomyocytes and investigate hemodynamic effects of Epi and its ability to stimulate cardiac  $\beta$ -adrenoceptors at different temperatures in vivo.

**Methods:** Isolated rat myocardial cells were incubated with the radioactive  $\beta$ -adrenoceptor ligand [<sup>3</sup>H]-CGP12177 and propranolol, used as a displacer. Cells were subjected to normothermia (37 °C) or hypothermia (15 °C). After incubation, radioactivity was measured to estimate  $\beta$ -adrenoceptor affinity for propranolol (IC<sub>50</sub>), as a measure of  $\beta$ -adrenoceptor sensitivity. In separate in vivo experiments, Epi (1.25  $\mu$ g/min) was administered the last 5 min of experiments in normothermic (37 °C, 5 h), hypothermic (4 h at 15 °C) and rewarmed rats (4 h at 15 °C, and subsequently rewarmed to 37 °C). Hemodynamic parameters were monitored during infusion. Hearts were thereafter freeze-clamped and tissue cAMP was measured.

**Results:** In vitro measurements of IC<sub>50</sub> for propranolol showed a hypothermia-induced increase in  $\beta$ -adrenoceptor sensitivity at 15 °C. Corresponding in vivo experiments at 15 °C showed decreased cardiac output and stroke volume, whereas total peripheral resistance (TPR) increased during Epi infusion, simultaneous with a 4-fold cAMP increase.

**Conclusions:** This experiment shows a hypothermia-induced in vivo and in vitro increase of cardiac  $\beta$ -adrenoceptor sensitivity, and simultaneous lack of inotropic effects of Epi in the presence of increased TPR. Our findings therefore indicate that hypothermia-induced reduction in inotropic effects of Epi is due to substantial elevation of TPR, rather than  $\beta$ -adrenoceptor dysfunction.

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**Abbreviations:** LV, left ventricle; CO, cardiac output; SV, stroke volume; cAMP, cyclic adenosine monophosphate; TPR, total peripheral resistance; PDE3, phosphodiesterase 3; IC<sub>50</sub>, the half maximal inhibitory concentration; G<sub>p</sub>, parallel conductance; MAP, mean arterial pressure; dP/dt<sub>max</sub>, the maximum rate of pressure change in the ventricle; dP/dt<sub>min</sub>, the minimum rate of pressure change in the ventricle; LVESV, LV end-systolic volume; LVEDP, LV end-diastolic pressure; P<sub>min</sub>, the minimum LV pressure; LVEDV, LV end-diastolic volume; Tau, the isovolumic relaxation constant; cTnI, cardiac troponin I.

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### Introduction

Rewarming from accidental hypothermia is associated with cardiac dysfunction, recognized by depressed cardiac output (CO) [33]. In order to elevate CO and ameliorate acute cardiac dysfunction during normothermic conditions, guidelines recommend use of inotropic drugs [22]. Induced hypothermia is also associated with increased need for such drugs, as more than 50% of cardiac arrest survivors who are eligible for therapeutic hypothermia are in need of inotropic support to maintain adequate circulation [2]. Both the American [34] and European guidelines [27] for cardiac support of patients during rewarming from accidental hypothermia do however state that use of drugs like epinephrine

(Epi) only should occur after reaching a core temperature of 30 °C. Numerous preclinical studies support this careful approach to pharmacologic treatment of hypothermic patients: They report hypothermia-induced changes in pharmacodynamics and pharmacokinetics of potent drugs like Epi and isoproterenol [30,12,7,14]. This is supported by studies showing that reduced core temperature induces a decline in cytochrome P450 activity and other important enzymes for drug elimination [37].

During normal core temperatures, the  $\beta$ -adrenoceptor agonists Epi and isoproterenol mediate inotropic effects via the sarcolemmal G-protein – protein kinase A (PKA) signaling pathway, by increasing intracellular cyclic adenosine monophosphate (cAMP) levels [7,10]. During cooling below 34 °C, the inotropic effect of Epi has been reported to decrease [30]. Vascular pharmacologic responses do however appear increased during hypothermia, as Epi infusion elevates total peripheral resistance (TPR) substantially, due to  $\alpha$ -receptor stimulating properties of this ligand [30]. Studies on inotropic drugs, which mediate their effects through strategies other than stimulating the  $\beta$ -adrenoceptor complex, show different cardiovascular responses during hypothermia, including better effects on cardiac contractility at low temperatures. Examples are the phosphodiesterase 3 (PDE3) inhibitor milrinone and the calcium sensitizer levosimendan [25,29].

Reduced inotropic effects of  $\beta$ -adrenoceptor agonists and the maintained effect of drugs increasing cAMP through PDE3 inhibition during hypothermia, indicate altered responses to  $\beta$ -adrenoceptor stimulation in hypothermic hearts. Several theories have been proposed to explain this. A study on temperature-dependent effects on cardiac adrenoceptors suggested a shift from  $\beta$ - to  $\alpha$ -adrenoceptor properties during cooling [16]. Another possible explanation is that low temperatures cause decreased coupling between  $\beta$ -adrenoceptors and adenylate cyclase, thereby impairing the ability of  $\beta$ -adrenoceptor agonists to increase cAMP, as observed in turkey erythrocytes [3]. This would also explain why Jones et al. found increased cAMP in isolated hamster hearts under isoproterenol stimulation at 37 °C, but not at 22 or 7 °C [10]. However, both normal cardiac  $\beta$ -adrenoceptor activity and  $\beta$ -adrenoceptor super-sensitivity have been reported during hypothermia [1,35] and consequently the underlying mechanisms for diminished inotropic effects of  $\beta$ -agonists during hypothermia remain unclear. To investigate whether this reduction of inotropic effects could be due to mechanisms like temperature-induced alterations in  $\beta$ -adrenoceptor binding and function, or due to altered pharmacodynamic changes in the vascular bed, we combined two experiments to: (1) estimate the affinity of  $\beta$ -adrenoceptors for propranolol in hypothermic rat cardiomyocytes, serving as a measure of  $\beta$ -adrenoceptor sensitivity to ligand-binding, and (2) measure cardiac tissue cAMP level and hemodynamic effects in response to administering Epi during in vivo hypothermia.

## Materials and methods

Male Wistar rats ( $n = 49$ ) were used in the experiment. The rats (Charles River, Germany) had a microbiological status according to the recommendation of the Federation of European Laboratory Animal Science Associations. The animals were quarantined for 1 week on arrival. During the experiment, housing was provided in accordance with guidelines for accommodation and care of animals (article 5 of European Convention for the Protection of Vertebrate Animals used for Experimental and Other Scientific Purposes, Strasbourg, 18.III.1986). Free water and food access were permitted. The experimental protocol was approved by the Norwegian Animal Research Authority and conducted accordingly.

## *In vitro beta-adrenoceptor assay*

### *Isolation of cardiomyocytes*

Rats ( $n = 8$ ) were sacrificed by an intraperitoneal injection of pentobarbital sodium (220 mg/kg) and fentanyl (50  $\mu$ g/kg). Hearts were excised and put in ice-cooled, oxygenated Krebs solution before tied to a Langendorff perfusion system. Perfusion rate was 7 ml/min with initial 5 min pure Krebs solution perfusion, followed by addition of 0.6 mg/ml collagenase for 20 min. All solutions were kept at 37 °C. Hearts were removed from the Langendorff system and cut into smaller pieces, before suspended in Krebs solution containing 10 mg/ml albumin and 0.6 mg/ml calcium (calcium solution), kept on a shaking plate for 10 min and minced in collagenase solution. Preparations were continually gassed with medical air (with added 5% CO<sub>2</sub>). Following centrifugation, collagenase solution was gradually replaced with calcium solution. In the final cell suspension, rod-shaped cells were counted as fraction of total cell number. This cell suspension was divided in 2 groups, 1 hypothermic group (15 °C) and 1 normothermic group (37 °C) (Table 1).

### *$\beta$ -Adrenoceptor labeling*

$\beta$ -Adrenoceptors were equilibrated with 1 nM of the hydrophilic radioactive marker [<sup>3</sup>H]-CGP12177 (Perkin Elmer, Massachusetts, USA), binding  $\beta$ -adrenoceptors on the external surface of cell membranes. Addition of the lipophilic, non-selective  $\beta$ -adrenoceptor blocker propranolol (Actavis, Ireland) in increasing doses from 10<sup>-8</sup> M to 10<sup>-5</sup> M was carried out in both groups for displacement of [<sup>3</sup>H]-CGP12177. As we aimed to examine binding properties of a  $\beta$ -adrenoceptor ligand in the in vitro experiment, not the cellular effects of  $\beta$ -adrenoceptor agonism or antagonism, propranolol was chosen according to earlier reports demonstrating its suitability when studying ligand-binding of  $\beta$ -adrenoceptors [26]. Incubation lasted 1/2 h, based on a pilot study not showing any differences with incubation over 1/2–2 h at 37 °C or 15 °C.

### *Measurement of $\beta$ -adrenoceptor sensitivity*

After incubation, cells were isolated from the buffer solution, by washing the suspension through a 0.67 mm thick (pore size 2.7  $\mu$ m) glass microfiber filter (Whatman, UK). Protein level in cell suspensions was determined using a Bradford protein assay (Bio-Rad Laboratories, California, USA). Radioactivity in filters was measured using a 1900 TR liquid scintillation spectrometer (Packard Instrument Company, Illinois, USA). Protein-corrected radioactivity was used to plot displacement of [<sup>3</sup>H]-CGP12177 with increasing concentrations of propranolol.  $\beta$ -Adrenoceptor binding of propranolol was measured by the half maximal inhibitory concentration (IC<sub>50</sub>), calculated according to Chou [5] and used as a measure of  $\beta$ -adrenoceptor sensitivity for ligand-binding.

### *In vivo hemodynamic monitoring and cardiac tissue cAMP measurements*

#### *Experimental protocol*

After surgery, animals were allowed to rest for 45 min before start of experiments. 3 different temperature protocols were used: Animals in the hypothermic Epi group ( $n = 8$ ) were core cooled to 15 °C and maintained at this temperature for 4 h, before a 5 min infusion of 1.25  $\mu$ g/min Epi was administered through a catheter in the femoral vein at the end of experiments. This dose of Epi was selected according to other studies in the same model [30,12]. Rewarmed ( $n = 6$ ) Epi animals underwent the same protocol as the hypothermic Epi group but were rewarmed to 37 °C prior to 5 min infusion of 1.25  $\mu$ g/min Epi. In the normothermic ( $n = 7$ ) Epi group animals were held at 37 °C for 5 h, followed by 5 min, 1.25  $\mu$ g/min Epi infusion. Control animals were assigned to

**Table 1**

Protocol for the in vitro and in vivo experiment: The table shows the time, temperature and procedure protocol of both the in vitro and in vivo experiment. In vitro groups: Normothermia (NT) and hypothermia (HT). In vivo groups: Normothermic control (NT), normothermic epinephrine group (NT Epi), hypothermic control group (HT), hypothermic epinephrine group (HT Epi), rewarmed control group (HTRW) and rewarmed epinephrine group (HTRW Epi).

Group	Protocol			
<i>In vitro experiment</i>				
NT	Cell isolation	[ <sup>3</sup> H]-CGP12177 labeling of $\beta$ -adrenoceptors	0.5 h incubation at 37 °C	Radioactivity measurement
HT	Cell isolation	[ <sup>3</sup> H]-CGP12177 labeling of $\beta$ -adrenoceptors	0.5 h incubation at 15 °C	Radioactivity measurement
<i>In vivo experiment</i>				
NT	5 h Stable normothermia 37 °C			Excision of hearts for cAMP measurements
NT Epi	5 h Stable normothermia 37 °C		5 min Epi infusion	
HT	1.5 h Cooling: 37–15 °C	4 h Stable hypothermia 15 °C		
HT Epi	1.5 h Cooling: 37–15 °C	4 h Stable hypothermia 15 °C	5 min Epi infusion	
HTRW	1.5 h Cooling: 37–15 °C	4 h Stable Hypothermia 15 °C	1.5 h Rewarming: 15–37 °C	
HTRW Epi	1.5 h Cooling: 37–15 °C	4 h Stable hypothermia 15 °C	1.5 h Rewarming: 15–37 °C	5 min Epi infusion

identical hypothermic ( $n = 7$ ), rewarmed ( $n = 7$ ), or normothermic ( $n = 6$ ) temperature protocols and underwent the same surgical procedure as the Epi groups, without 5 min Epi infusion at the end of experiments.

#### Anesthesia

Anesthesia was introduced intraperitoneally by 55 mg/kg pentobarbital sodium and 50  $\mu$ g/kg fentanyl, followed by a continuous infusion of 7.5 mg/kg/h pentobarbital sodium and 50  $\mu$ g/kg/h fentanyl through an intravenous line in the right jugular vein, extended to the right auricle. The infusion was maintained at all hours in normothermic animals. Infusion in hypothermic animals 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.

#### 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 New England rodent ventilator, model 141 (New England Instruments, Massachusetts, USA), using room air. Normoventilation was achieved through adjusting ventilation in accordance with blood gas analyzes from an ABL 800 blood gas analyzer (Radiometer, Denmark). During controlled ventilation, the alpha-stat strategy was followed.

#### Core cooling and rewarming

Animals were cooled and rewarmed using a RTE-110 thermo stated water bath (Neslab Instruments, New Hampshire, USA) by circulating cold or warm water through an U-shaped polyethylene tube, which was inserted gently into the lower bowel 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 esophagus, connected to a Thermalert Th-5 thermocouple controller (Bailey Instruments, UK). Cooling and rewarming each lasted 1.5 h and the hypothermic period (15 °C) lasted 4 h in both the rewarmed and the hypothermic groups. Normothermic animals were kept at 37 °C for 5 h. The rate of core rewarming was chosen based on clinical practice in our university hospital, where fast rewarming has proven successful in hypothermic patients after nearly 7 h of hypothermic cardiac arrest [20].

#### Hemodynamic measurements

Hemodynamic variables were obtained using a SPR-838 Millar pressure–volume conductance catheter (Millar Instruments Inc., Texas, USA). The miniaturized 2.0 french pressure–volume conductance catheter allowed for the assessment of in vivo left ventricular (LV) mechanical function in rats [4]. 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 calibration of the catheter is described in detail in a previous report [7]. In addition, mean arterial pressure (MAP) was measured using a pressure transducer connected to a fluid-filled catheter (22 G) inserted into the left femoral artery.

#### Determination of cardiac tissue cAMP levels

After finishing the temperature protocol, Epi groups were subjected to a 1.25  $\mu$ g/min Epi infusion. After 5 min, rats were euthanized and hearts were excised during ongoing infusion of Epi. Control animals underwent the same procedure without Epi infusion. The left ventricle was dissected free, freeze-clamped on liquid nitrogen and stored at –70 °C. For measuring cardiac tissue cAMP levels in left ventricle samples, the tissue was pulverized and 50–100 mg was diluted in 1 ml, 0, 1 M HCl and centrifuged. The supernatant was analyzed using a rat cAMP ELISA kit (Enzo Life Sciences, New York, USA). Protein concentration in the supernatant was determined using a Bradford protein assay (Bio-Rad Laboratories, California, USA). Cardiac tissue cAMP levels were corrected according to protein level in each sample.

#### Statistics

Results are presented as mean  $\pm$  SEM. For within group comparisons of hemodynamics before and after Epi infusion, data were assessed by a paired *t*-test. IC<sub>50</sub> values for  $\beta$ -adrenoceptor binding of propranolol were compared using Mann–Whitney rank sum test, rather than an unpaired two-tailed *t*-test, as values were not normally distributed (Shapiro–Wilk test). Comparison of cAMP levels was analyzed by One-Way Analysis of Variance test followed by an all-pairwise multiple comparisons using Tukey's test. Differences were considered significant at  $p < 0.05$ .

#### Results

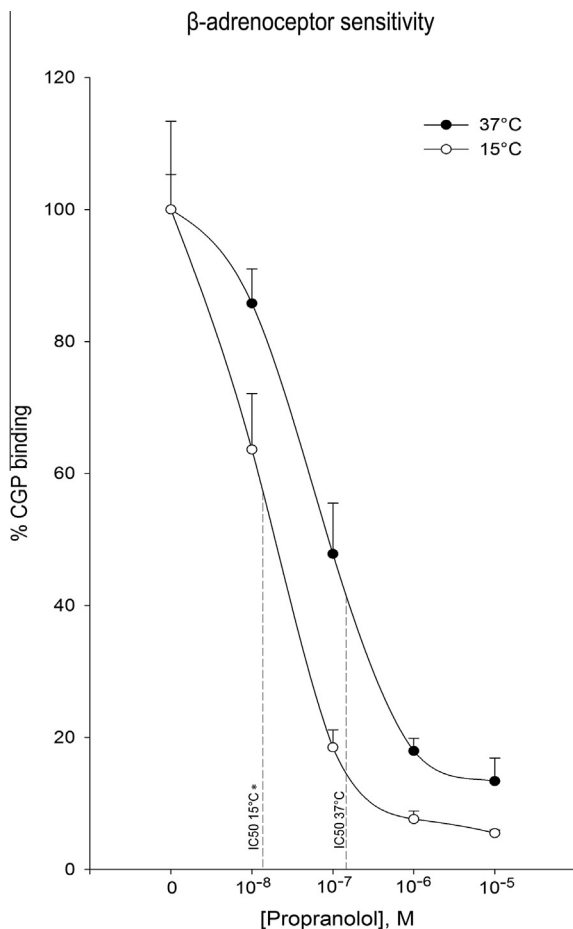
##### $\beta$ -Adrenoceptor binding of propranolol

Propranolol caused a concentration-dependent displacement of [<sup>3</sup>H]-CGP12177 in both groups. Calculation of displacement values

showed that the  $IC_{50}$  concentration of propranolol was 9 times higher in normothermic ( $1.2 \cdot 10^{-7}$  M) than in hypothermic cells ( $1.4 \cdot 10^{-8}$  M). Thus, in normothermic heart cells a larger concentration of propranolol was needed to reduce the receptor specific binding of [ $^3$ H]-CGP12177, showing higher  $\beta$ -adrenoceptor sensitivity for ligand binding in hypothermic cells (Fig. 1).

### Hemodynamics

During 5 min infusion of Epi (1.25  $\mu$ g/min), hemodynamic responses were recorded at 15 °C in the hypothermic group, and at 37 °C in the rewarmed and normothermic groups. In the hypothermic group, CO was significantly reduced during Epi infusion, while increased in the rewarmed and normothermic groups. Heart rate and MAP were increased in all groups. Stroke volume (SV) was reduced in the hypothermic group and increased in the rewarmed group. The maximum rate of pressure change in the ventricle ( $dP/dt_{max}$ ) was reduced in the hypothermic group and increased in both the normothermic and rewarmed groups, while  $dP/dt_{min}$  was reduced in all groups. TPR, LV end-systolic volume (LVESV) and LV end-diastolic pressure (LVEDP) were increased only in the hypothermic group. The minimum LV pressure ( $P_{min}$ ) was increased in the hypothermic group and decreased in the rewarmed group. LV end-diastolic volume (LVEDV) and the isovolumic relaxation constant ( $\tau$ ) remained unchanged during Epi infusion in all groups (Figs. 2 and 3).



**Fig. 1.**  $IC_{50}$  values for  $\beta$ -adrenoceptor binding of propranolol:  $IC_{50}$  values for  $\beta$ -adrenoceptor binding of propranolol in hypothermic (15 °C) and normothermic (37 °C) isolated rat cardiomyocytes, \*Significant difference between groups ( $p < 0.05$ ).

### cAMP measurements

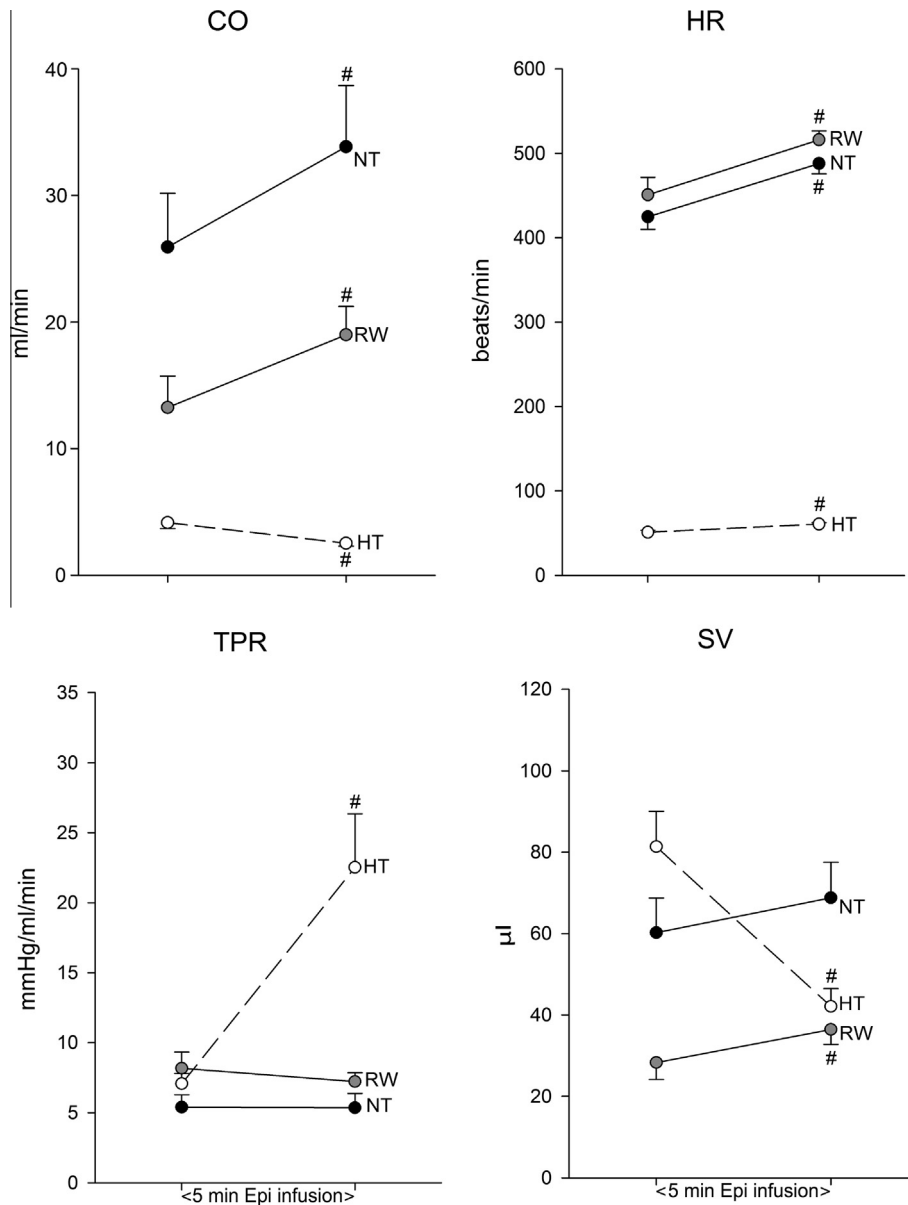
After the end of the temperature protocols, with additional 5 min (1.25  $\mu$ g/min) infusion of Epi in the Epi groups, protein corrected cardiac cAMP level in freeze-clamped LV heart tissue was measured in all groups and compared. cAMP was statistically higher in the normothermic and hypothermic Epi groups, compared to untreated normothermic and hypothermic control rats. There was no statistical difference between cAMP levels in rewarmed rats receiving Epi and rewarmed control rats. cAMP levels were significantly higher in Epi treated hypothermic rats compared to both normothermic and rewarmed Epi treated rats (Fig. 4).

### Discussion

The present study demonstrates that cardiac  $\beta$ -adrenoceptor sensitivity is elevated both in vitro and in vivo during hypothermia, concurrent with intact vascular effects of Epi and lack of positive inotropic effects. These findings show that decreased  $\beta$ -adrenoceptor sensitivity is not the mechanism behind reduced inotropic effects of Epi during hypothermia.

Williams and Broadley [35] described hypothermia-induced super-sensitivity of the  $\beta$ -adrenoceptor in guinea pig atria, which correlates with the in vivo 4-fold increase of cardiac tissue cAMP observed at 15 °C following Epi infusion in the present study. At low core temperatures the activity of drug-metabolizing enzymes is reduced [28]. Hypothermia-induced reduction in catechol-O-methyl transferase activity has therefore been proposed as an explanation for the hypersensitivity to  $\beta$ -adrenoceptor agonists [23]. During the 5 min Epi infusion in the present experiment, increased Epi half-life ( $T_{1/2}$ ) might have contributed to elevated stimulation of  $\beta$ -adrenoceptors as the normothermic  $T_{1/2}$  is 2 min [24]. Further, decreased enzymatic breakdown of cAMP through reduced PDE3 function, or reduced extracellular release of cAMP as observed in cold fibroblasts [11], might also have enhanced cAMP increase in hypothermic hearts. The present  $IC_{50}$  values of propranolol do however show a 9-fold increase in  $\beta$ -adrenoceptor sensitivity in isolated cardiomyocytes at 15 °C. We therefore see the 4-fold increase of cAMP during 5 min Epi infusion in vivo at 15 °C as an effect mainly caused by a hypothermia-induced increase in  $\beta$ -adrenoceptor sensitivity.

However, hypothermia-induced  $\beta$ -adrenoceptor hypersensitivity is disputed, as several studies have observed reduced inotropic effects of  $\beta$ -adrenoceptor agonists [30,12,7,14]. Kunos and Nickerson proposed a hypothermia-induced reduction in receptor sensitivity caused by conversion from  $\beta$ - to  $\alpha$ -properties of adrenoceptors [16]. Benfey, who found that the inotropic response to isoproterenol was intact during hypothermia, opposed this finding. He found sustained inotropic effects to be dependent on reduced calcium concentration in the experimental solution [1], different from the calcium overload found in hypothermic in vivo rat hearts [13,36]. In the present study, we found that Epi had negative inotropic effects at 15 °C, recognized by decreased CO, despite giving a 4-fold increase of cAMP in cardiac tissue. Different from at 37 °C, we also found a pronounced increase in TPR at 15 °C while MAP was increased to the same level in all three groups. In the hypothermic rats, we observed a 3-fold increase in LVEDP during the 5 min Epi infusion. However, neither LV  $dP/dt_{min}$  nor  $\tau$  values in our experiment indicate impaired myocardial relaxation or diastolic dysfunction. The rapid increase in LVEDP is therefore more likely a consequence of the sudden increase in TPR, resulting in a pronounced slowing of LV pressure fall [17]. Thus, diastolic filling starts at a higher ventricular pressure ( $P_{min}$ ) causing decreased compliance [6], with similarities to vascular congestion during acute hypertensive crisis [17]. This is different from in the normothermic and rewarmed groups where LVEDP was unchanged and

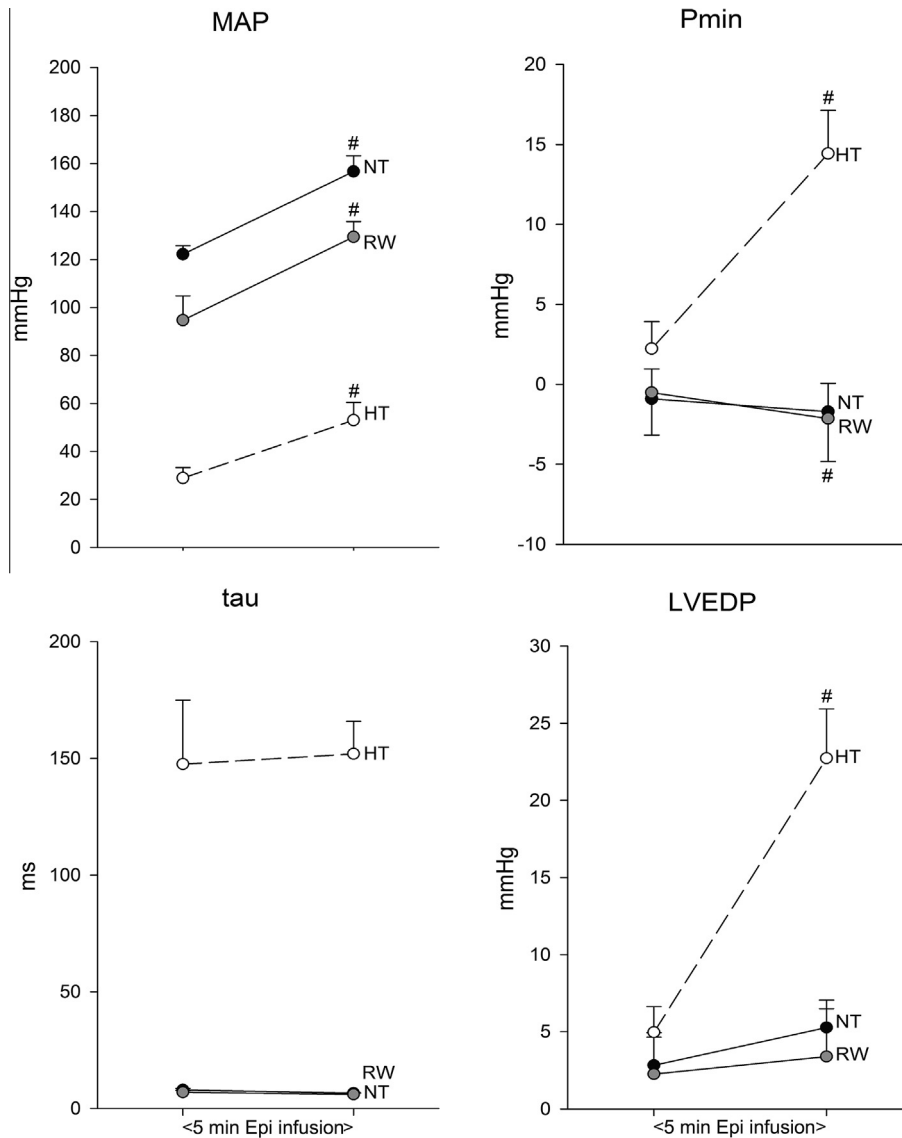


**Fig. 2.** Hemodynamic changes during 5 min Epi infusion: Changes in cardiac output (CO), heart rate (HR), total peripheral resistance (TPR) and stroke volume (SV) during 5 min epinephrine (Epi) infusion in normothermic (NT), rewarmed (HTRW) and hypothermic (HT) rats. #Significant difference within the group after Epi infusion.

$P_{\min}$  was unaffected or reduced during Epi infusion. Thus, both normothermic and rewarmed hearts were able to adjust to the sudden increase in MAP during Epi infusion. In hypothermic animals however, Epi infusion gave a significant increase in TPR, causing acute congestion with depressing effect on cardiac ejection that resulted in acute reduction of CO.

Isoproterenol, a selective  $\beta$ -adrenoceptor agonist, also has been reported to lack inotropic effects during hypothermic conditions [7]. It is therefore less likely that the absent inotropic effects of Epi in the present study are solely dependent on a sudden increase in TPR. In an extensive study of the effects of adrenergic stimuli on the biology of cardiomyocytes in several species, Mann et al. [19] found high concentrations of cAMP to be cardiotoxic. This negative effect of increased cAMP was found to be associated with high influx of calcium from the extracellular space, thus inducing cytosolic calcium overload and hypercontracture. The latter morphological finding is also seen after hypothermic cardioplegia in man

[18], but not in spontaneously beating rat hearts exposed to prolonged hypothermia in vivo [32]. However, in rats exposed to 4 h hypothermia (15 °C), cardiac calcium overload is present [13,36]. In the present study, the 4-fold increase in cardiac tissue cAMP during Epi infusion after 4 h at 15 °C might therefore aggravate a hypothermia-induced calcium overload through high influx of calcium via L-type calcium channels. Calcium overload is interpreted to be an important mechanism for development of hypothermia-induced cardiac dysfunction [36]. Increased cAMP is also known to mediate increased phosphorylation of cardiac troponin I (cTnI) at sites Ser23/24 causing decreased myofilament calcium sensitivity [15]. Han et al. showed that such cTnI phosphorylation takes place due to hypothermia and rewarming and gives the expected reduction of calcium sensitivity and force of contraction [8]. The negative effects we observed in response to the 4-fold cAMP increase after Epi infusion at 15 °C, and positive effects observed in response to a smaller increase in cAMP at 37 °C, indicate that



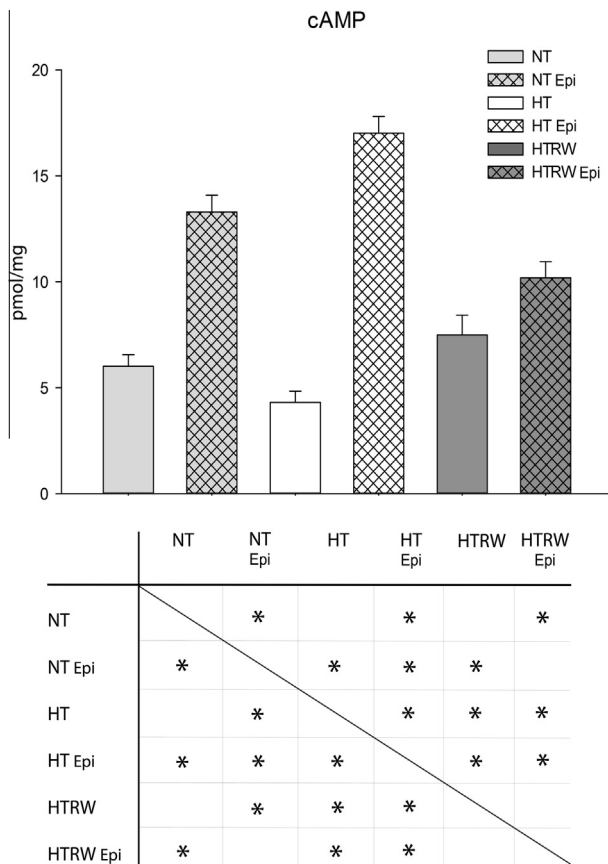
**Fig. 3.** Hemodynamic changes during 5 min Epi infusion: Changes in mean arterial pressure (MAP), minimum left ventricular pressure ( $P_{\min}$ ), the left ventricular isovolumic relaxation constant ( $\tau$ ) and left ventricular end-diastolic pressure (LVEDP) during 5 min epinephrine (Epi) infusion in normothermic (NT), rewarmed (HTRW) and hypothermic (HT) rats. #Significant difference within the group after Epi infusion.

increased cAMP production within a narrow range might be crucial to elevate cardiac function during hypothermia.

cAMP levels in rewarmed control rats were higher than in hypothermic controls. This rewarming-induced increase of cAMP is consistent with earlier findings from our group, which show that the high-energy phosphates ATP and ADP also increased in rat hearts after rewarming from 13 to 15 °C [31]. Different from before cooling and during hypothermia, 5 min Epi infusion after rewarming did not mediate a significant increase of cardiac tissue cAMP. Rewarming rats from hypothermia (20 °C) is reported to increase the level of endogenous plasma catecholamines substantially [9] and high levels of such plasma catecholamines reduce  $\beta$ -adrenoceptor response to further stimulation [21], which will affect cardiac cAMP levels. These reports are therefore supported by our findings of high cAMP levels in untreated animals, and no significant cAMP increase during Epi infusion in the 2 rewarmed groups. The response to Epi infusion after rewarming was not absent, as increased CO was observed during Epi infusion, demonstrating that

the non-significant increase of cAMP gave a positive inotropic effect in this group.

We have previously demonstrated positive inotropic effects of low-dose Epi (0.125  $\mu\text{g}/\text{min}$ ), in contrast to a high-dose (1.25  $\mu\text{g}/\text{min}$ ), during rewarming of rats from 15 °C [12]. Along with the present results, this indicates a reduced “therapeutic window” of Epi during hypothermia and rewarming, where elevated TPR and lack of positive inotropic effects dominate the hemodynamic response. Inotropic treatment through inhibition of cardiac tissue cAMP breakdown, using the PDE3 inhibitor milrinone, has demonstrated more favorable effects when tested in the present rat model [29]. As well as elevating CO, milrinone possess the ability to reduce, rather than increase MAP and thus have a better potential for cardiac support during hypothermia. Due to an apparent reduced therapeutic window and dose-dependent adverse effects of combined  $\alpha$ - and  $\beta$ -agonists, such as Epi, efforts to give inotropic support during hypothermia and rewarming should be provided through other mechanisms like PDE3 inhibition.



**Fig. 4.** Levels of cAMP in freeze-clamped heart tissue: Levels of cAMP in freeze-clamped heart tissue in groups given epinephrine (Epi) and untreated controls groups. HTRW: 4 h hypothermia (15 °C) and subsequent rewarming to 37 °C, HT: 4 h hypothermia (15 °C) without subsequent rewarming, NT: 5 h normothermia (37 °C). In control animals, hearts were excised after the end of the temperature protocol, with no additional treatment. Epi groups were treated with 5 min Epi infusion after the temperature protocol was completed, before excision of hearts. \*Significant difference between groups ( $p < 0.05$ ).

## Conclusion

Hypothermia (15 °C) elevates *in vitro*  $\beta$ -adrenoceptor sensitivity in isolated rat cardiomyocytes, reproduced *in vivo* by substantial increase of cardiac tissue cAMP in rats given Epi. Based on the present data we therefore suggest that the negative inotropic effects of Epi during hypothermia are related to elevated vascular effects and un-physiological increase of cAMP, rather than hypothermia-induced  $\beta$ -adrenoceptor dysfunction.

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