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Mild Hypothermia Markedly Reduces Ischemia Related Coronary t-PA Release

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Background: In experimentally induced myocardial ischemia, mild hypothermia (33–35°C) has a robust cardioprotective effect. Tissue plasminogen activator (t-PA) is a profibrinolytic enzyme that is released from the vascular endothelial cells in response to ischemia and other injurious stimuli. t-PA has also been found to have proinflammatory properties that could contribute to reperfusion injury. We postulated that hypothermia could attenuate t-PA release in the setting of myocardial ischemia.

Methods: Sixteen 25–30 kg pigs were anesthetized and a temperature of 37°C was established using an intravascular cooling/warming catheter. The pigs were then randomized to hypothermia (34°C) or control (37°C). A doppler flow wire was placed distal to a percutaneous coronary intervention balloon positioned immediately distal to the first diagonal branch of the left anterior descending artery (LAD). The LAD was then occluded for ten minutes in all pigs. Coronary blood flow and t-PA was measured before, during and after ischemia/reperfusion. t-PA was measured in peripheral arterial blood and locally in the venous blood from the coronary sinus. Net t-PA release over the coronary bed was calculated by subtraction of arterial values from coronary sinus values. An estimate of differences in total t-PA release was calculated by multiplying net t-PA release with the relative increase in flow compared to baseline, measured in relative units consisting of ((ng/ml – ng/ml) x (cm/s / cm/s)).

Results: There was no observed difference in t-PA levels in peripheral arterial samples. As shown previously, net t-PA release increased during reperfusion. Hypothermia significantly inhibited the increase in t-PA release during reperfusion (peak value 9.44±4.34 ng/ml vs 0.79±0.45 ng/ml, p=0.02). The effect was even more prominent when an estimation of total t-PA release was performed with mean peak value in the control group 26 fold higher than in the hypothermia group (69.74±33.86 units vs 2.62±1.10 units, p=0.01).

Conclusion: Mild hypothermia markedly reduces ischemia related coronary tissue plasminogen activator release. The reduction of t-PA release may contribute to the cardioprotective effect of hypothermia.

Key words: t-PA, ischemia, hypothermia

Introduction

In experimentally induced myocardial ischemia, mild hypothermia (33–35°C) has a robust cardioprotective effect [1-4]. The clinical applicability of therapeutic hypothermia in the setting of ischemic heart disease is currently being investigated (RAPID MI-ICE pilot and CHIPAHA) [5, 6]. Moreover, the neurologic outcome and survival in cardiac arrest victims can be improved with mild resuscitative hypothermia [7]. This has led to a revision of guidelines to incorporate cooling of cardiac arrest victims at a class IIb level [8].

Tissue plasminogen activator (t-PA) is a protease that initiates endogenous fibrinolysis in the vascular compartment via conversion of plasminogen to plasmin, and is important in controlling the coagulation process due to its thrombolytic properties. It is synthesized and stored in endothelial cells and vascular neurons [9, 10], and is released in response to ischemia and other injurious stimuli [11-18]. Recombinant t-PA is the active substance in several thrombolytic drugs that are used to treat thromboembolic disease states such as myocardial infarction, stroke and pulmonary embolization.

Even though reperfusion of ischemic tissue is a prerequisite for salvage, reperfusion in itself may lead to accelerated and additional tissue injury beyond that generated by ischemia alone, a phenomenon referred to as “reperfusion injury” [19, 20]. The molecular basis for reperfusion damage has not been fully elucidated, but there is evidence for several possible mechanisms of damage including oxidative stress, calcium overload, mitochondrial damage, complement activation and an inflammatory reaction [21-26]. Interestingly, t-PA has been found to have proinflammatory properties that could contribute to reperfusion injury [27, 28]. The cardioprotective mechanisms of hypothermia are not fully understood and it is possible that a part of the protective effect could be mediated by attenuation of t-PA release. Our aim

was to evaluate if hypothermia could attenuate t-PA release in the setting of myocardial ischemia.

Methods

Experimental preparation

16 healthy domestic male and female 25-30 kg pigs were fasted overnight with free access to water. Premedication was administered with azaperone (Stresnil Vet., Leo; Helsingborg, Sweden), 2 mg/kg intramuscularly, 30 minutes prior to the procedure. After induction of anesthesia with thiopental (Pentothal, Abbott, Stockholm, Sweden) 5-25 mg/kg, the animals were orally intubated with cuffed endotracheal tubes. Thereafter, a slow infusion of 1.25 μl/ml fentanyl (Fentanyl, Pharmalink AB, Stockholm, Sweden) in buffered glucose (25 mg/ml) was started at a rate of 1.5 ml/min and adjusted as needed. During balanced anesthesia meprobamat (Mebumal, DAK, Copenhagen, Denmark) and thiopental (Pentothal, Abbott, Stockholm, Sweden), were titrated against animal requirements with small bolus doses. Mechanical ventilation was established with a Siemens-Elema 900B ventilator in the volume-controlled mode, adjusted in order to obtain normocapnia. Initial settings were: respiratory rate of 15/min, tidal volume of 10 ml/kg and positive end-expiratory pressure of 5 cm H_2O. The animals were ventilated with a mixture of dinitrous oxide (70%) and oxygen (30%).

Heparin (200 IU/kg) was given intravenously at the start of the catheterization. A 12 F introducer sheath (Boston Scientific SciMed, Maple Grove, MN, USA) was inserted into the surgically exposed left femoral vein. A 10.7 F Celsius Control™ cooling catheter (Innercool Therapies Inc, San Diego, CA, USA) was inserted through the sheath and positioned in the inferior vena cava with the tip of the catheter at the level of the diaphragm. Body temperature

was measured with a temperature probe (TYCO Healthcare Norden AB, Solna, Sweden) placed in the distal part of the esophagus. The catheter and the temperature probe were connected to the Celsius Control unit.

A 12 F introducer sheath (Boston Scientific Scimed, Maple Grove, MN, USA) was inserted into the surgically exposed left External Jugular Vein. A short 10 F special catheter of our own design was used to catheterize the Azygos Vein. (In pigs the coronary sinus ends in the Azygos vein.) Then a 6 F MPA coronary catheter (Boston Scientific Scimed, Maple Grove, MN, USA) was passed through the catheter with the tip in the Azygos Vein, into the Coronary Sinus, often with the help of a PT choice guide wire, (Boston Scientific Scimed, Maple Grove, MN, USA).

A 6 F introducer sheath (Boston Scientific Scimed, Maple Grove, MN, USA) was inserted into the surgically exposed left carotid artery upon which a 6F JL 3.5 Wiseguide™ (Boston Scientific Scimed, Maple Grove, MN, USA) was inserted into the left main coronary artery. An angiogram was obtained using 8–10 ml of the contrast medium Omnipaque™ 300 mg I^- /ml (Nycomed, Oslo, Norway) to ensure correct positioning of the catheter. The catheter was used to place a 0.014-inch, 12 MHz pulsed Doppler flow velocity transducer (Jometrics Flowire, Jomed NV) into the mid-portion of the left anterior descending artery (LAD) and a 0.014-inch PT choice™ guidewire (Boston Scientific Scimed, Maple Grove, MN, USA) into the distal portion of the LAD. A 3.0 × 20 mm over the wire Maverick™ angioplasty balloon (Boston Scientific Scimed, Maple Grove, MN, USA) was then positioned in the mid portion of the LAD, proximal to the flow velocity transducer but distal to the first diagonal branch, followed by the withdrawal of the PT choice guidewire. Continuous coronary velocity flow

profiles were displayed and recorded using the Doppler flow wire connected to a FloMap monitor (Cardiometrics, Mountain View, CA). All radiological procedures were performed in an experimental catheterization laboratory, (Shimadzu Corp., Kyoto, Japan).

Experimental protocol

Regardless of initial temperature, all pigs were cooled or warmed (as needed) to a baseline temperature of 37°C, which was maintained for 30 minutes. The pigs were then randomized to the hypothermia group or to the control group. The pigs randomized to hypothermia were cooled with the cooling/warming catheter to a temperature of 34.0°C, prior to balloon inflation, which was then maintained until sacrifice. The pigs randomized to the control group were actively maintained at 37°C using the endovascular cooling/warming catheter until sacrifice. In all pigs, the LAD was occluded distal to the first diagonal branch by inflation of the angioplasty balloon for a period of 10 min.

t-PA was measured at baseline, one minute before reperfusion (=9 min ischemia) and one, five and 10 minutes after reperfusion. Samples were collected from a peripheral artery and in the venous blood from the coronary sinus. Blood pressure, heart rate and coronary artery flow in the LAD was measured continuously. Flow was measured in average peak velocity (APV) in cm/sec. In a closed chest pig model it is not possible to measure vessel diameter and doppler flow at the same time. However, the diameter of the LAD was measured in separate pigs from both the hypothermic- and control group during baseline and during reperfusion at the same angle, and was found not to increase or decrease more than 10% even during maximum reactive hyperemia. Compensation for this only resulted in very minor changes of the results and was therefore not performed. A blood gas analysis was performed at baseline and at 1 and 10 min post-reperfusion.

t-PA measurements

Plasma concentrations of t-PA was determined by commercial ELISA kits (TintElize t-PA, Biopool AB, Umeå , Sweden and COALIZA PAI, Chromogenix, Haemochrom Diagnostica AB, Mölndal, Sweden). All samples from one experiment were assayed in duplicate on the same microtest plate. Intra-assay variation coefficients were 2.7% and 3.1% for respective assay. Net t-PA release over the coronary bed was calculated by subtraction of arterial values from coronary sinus values. An estimate of differences in total t-PA release was calculated by multiplying net t-PA release with the relative increase in flow compared to baseline, measured in relative units consisting of ((ng/ml – ng/ml) x (cm/s / cm/s)).

Calculation and statistics

Calculations and statistics were performed using the GraphPad Prism 4.0 software. Values are presented as mean ± SEM. Statistical significance was accepted when $P < 0.05$ (Mann-Whitney test).

Ethics

The study conforms to the Guide for the Care and Use of Laboratory Animals, US National Institute of Health (NIH Publication No. 85-23, revised 1996) and was approved by the Ethics Committee of Lund University, Sweden.

Results

There were no observed differences in basal t-PA levels in peripheral arterial or coronary sinus samples, figure 1. As shown previously, net t-PA release increased during reperfusion [29]. Hypothermia significantly inhibited the increase in net t-PA release during reperfusion (peak value 9.437±4.34 ng/ml vs 0.79±0.45 ng/ml, p=0.02), figure 2. The effect was even

more prominent when an estimation of total t-PA release was performed (factorial correction for blood flow, see above) with mean peak value in the control group 26 fold higher than in the hypothermia group (69.74±33.86 units vs 2.62±1.10 units, p=0.01), figure 3. Arterial blood-gas data were similar between the groups, table 1. As shown in a previous publication, coronary blood flow in the LAD increased dramatically during the early reperfusion phase [30]. The peak flow observed during post ischemic reactive hyperemia was significantly reduced by 43% in the hypothermia group compared to the control group ($p<0.01$). Peak flow occurred 2.5 min after reperfusion and was 83,6±7.8 cm/s in the normothermic group and 50,6±7.2 cm/s in the hypothermic group. There was no observed difference in coronary flow between the groups during baseline or 7 minutes after reperfusion [30]. There was a reduction in heart rate observed among the pigs randomized to the hypothermia group during the entire period of hypothermia, figure 4. The difference in heart rate was maintained at the same level during baseline, ischemia and reperfusion, and unaffected by the increased coronary flow measured during reactive hyperemia. The mean arterial blood pressure (MAP) was similar or even slightly increased in the reperfusion phase compared to the control group, figure 4 [30].

Discussion

The aim of this study was to evaluate the effect of mild hypothermia on ischemia related coronary t-PA release. The main finding was that mild hypothermia markedly reduces coronary t-PA release during the reperfusion phase, with mean peak value of total t-PA release 26 fold higher in the control group than in the hypothermia group.

A percutaneous catheter-based approach was chosen in this study, in order to induce ischemia with minimum trauma, operation-induced stress and secondary changes in circulatory physiology. Furthermore, it is important to reduce trauma to a minimal extent in order to

reduce tissue damage related t-PA release with unnatural influence on background t-PA levels. The use of angioplasty balloons allowed for precision in attaining an accurate and localized induction of ischemia as well as a reproducible area at risk in the myocardium [4]. The ischemic time of 10 minutes is relatively short, but it is an established time period for studying ischemia/reperfusion related t-PA release, and was therefore chosen [29]. The differences that were noted between the groups concerning heart rate and MAP translate into coronary artery flow and are thus taken into account in the analysis.

Ten minutes of ischemia does not cause any major myocardial necrosis, but it causes hypoxia and accumulation of acidic metabolites. The most prominent source of t-PA is the endothelial cells and the effects of hypothermia are probably mediated either directly on the endothelial cells or by attenuating the accumulation of stimulators of the endothelium, such as low pH, ATP, ADP, bradykinin etc. We have previously shown that hypothermia reduces reactive hyperemia by 43% [30]. Interestingly, hypothermia abolished t-PA release above basal levels completely, indicating a more selective effect on the endothelial t-PA release. This discrepancy also rules out blood flow mediated shear stress as the only factor for t-PA release. It also indicates that other factors besides shear stress that release t-PA during ischemia are affected by hypothermia, such ADP, bradykinin or substance P.

A direct effect of hypothermia on the endothelium seems plausible when the well documented tissue protective effect of mild hypothermia is taken into account. The exact mechanism through which therapeutic hypothermia exerts it’s tissue protective effect is not known, but it is thought to reduce the metabolic demand of the cells [31-34]. However, reduced oxygen demand does not fully explain the positive effects of hypothermia and several additive effects have been suggested [35]. It has been shown that mild hypothermia can prevent ischemic cells

from entering apoptosis through prevention of mitochondrial dysfunction and inhibition of caspase release [36-38]. Moreover, therapeutic hypothermia has been shown to improve ion homeostasis, suppress ischemia induced inflammatory reactions, decrease free radical formation, stabilize cellular membranes and prevent intracellular acidosis [39]. The fact that the animals were subject to a relatively short period of ischemia and were hypothermic during the entire ischemic time supports this conclusion, as hypothermia is known to have an especially robust tissue-protective effect under these conditions [40]..

Under physiologic circumstances, t-PA is important in keeping vessel walls free of thrombi formation and during pathologic thromboembolic disease states t-PA is a vital component of the endogenous thrombolytic system. Thrombolytic treatment also utilize t-PA as the active component and such therapy has been shown to increase survival and functional outcome in stroke and myocardial infarct patients [41, 42]. However t-PA appear to be a double-edged sword in the respect that it also has been found to have proinflammatory properties that could contribute to reperfusion injury [28]. t-PA is known to induce matrix degradation via activation of matrix metallopeptidase 9, and increase oxidative stress and inflammation via upregulation of inducible nitric oxide synthase [43, 44]. Furthermore, it is associated with activation and degranulation of mast cells with subsequent proinflammatory effects [27]. t-PA has also been shown to increase release of norepinephrine from sympathetic neurons and thereby contribute to cardiac arrythmias in ischemia/reperfusion [45]. Norepinephrine also shifts the metabolic balance in an unfavourable direction by increasing oxygen demand via increased heart rate and inotropy, and decreasing oxygen availability by constriction of coronary arteries, and may thus aggravate the primary ischemia [46, 47]. Consequently, decreased norepinephrine release per se would also be expected to protect the ischemic myocardium. Taken together, it is possible that the hypothermia related reduction in t-PA

release during ischemia/reperfusion may contribute to the tissue protective effects of hypothermia. It may also be part of the explanation to why hypothermia has been found to increase defibrillation success and resuscitation outcome at cardiac arrest in a porcine model [48].

Limitations

The model that was used in this study is based on experimentation on young, healthy animals subject to a short duration of ischemia that does not cause any substantial necrosis of the myocardium. Ischemia is brought about by the inflation of a balloon in a vessel that is free from atherosclerotic disease. It is possible that these circumstances inadequately mimic the pathophysiology of the clinical situation with thrombotic occlusion and development of necrosis. It is also possible that a profound reduction of t-PA release in the setting of thrombotic occlusion could be detrimental to reperfusion.

Conclusion

Mild hypothermia markedly reduces ischemia related coronary tissue plasminogen activator release. The reduction of t-PA release may contribute to the cardioprotective effect of hypothermia.

Acknowledgements

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donations of catheters and guide wires for use in animal research and Innercool therapies Inc, San Diego, CA, USA for unrestricted loan of the Celsius Control™ cooling consol.

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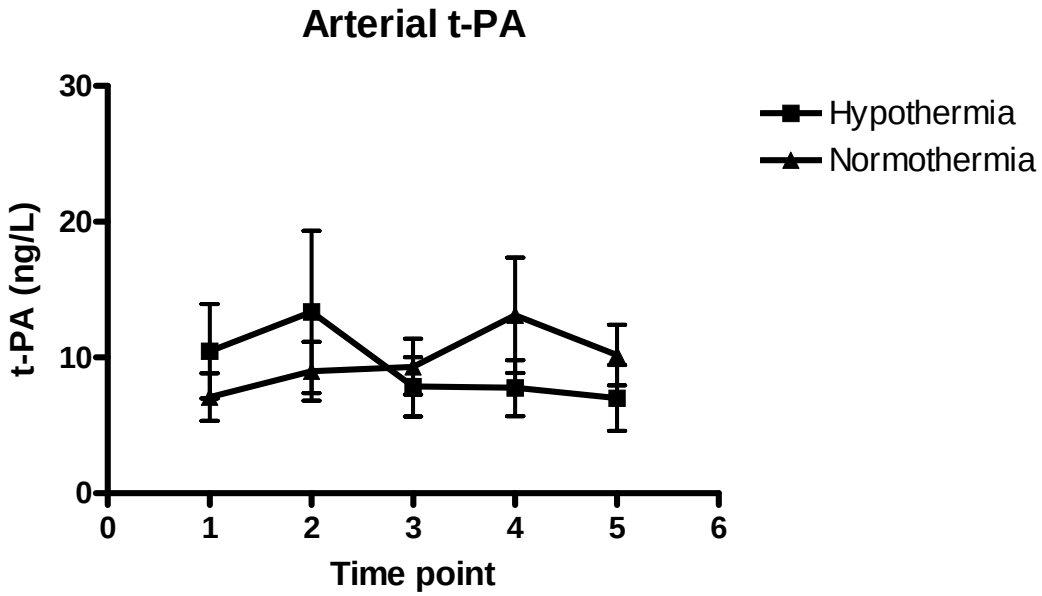
Arterial t-PA
30
20
10
0
t-PA (ng/L)
0
1
2
3
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Time point
Hypothermia
Normothermia

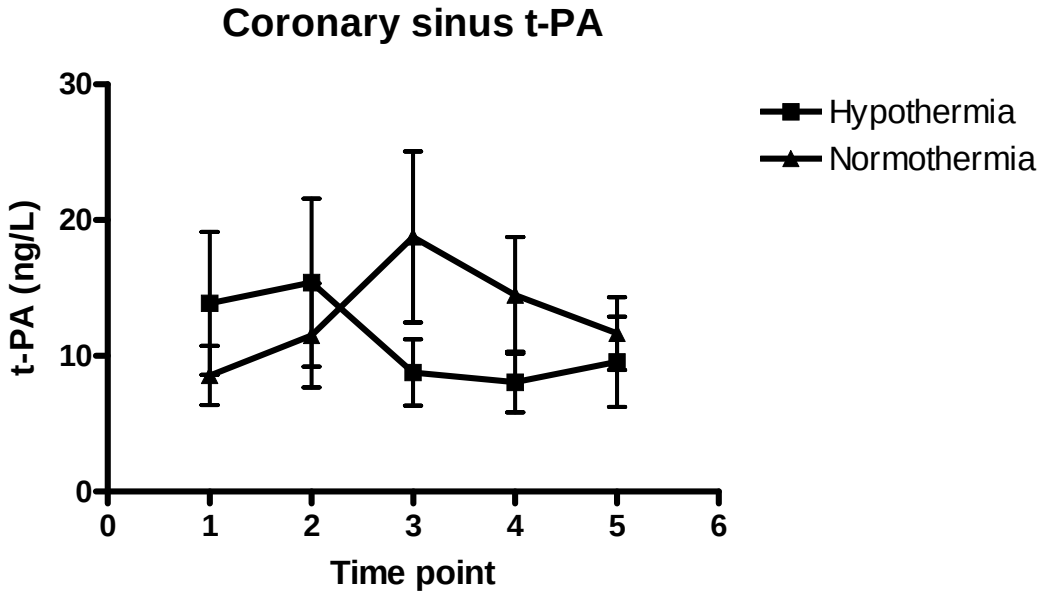
Coronary sinus t-PA
30
20
10
0
t-PA (ng/L)
0
1
2
3
4
5
6
Time point
Hypothermia
Normothermia

Figure 1 There were no statistically significant differences in basal t-PA levels in peripheral arterial or coronary sinus samples. Samples were collected at baseline, one minute before reperfusion, one minute after reperfusion, five minutes after reperfusion and 10 minutes after reperfusion.

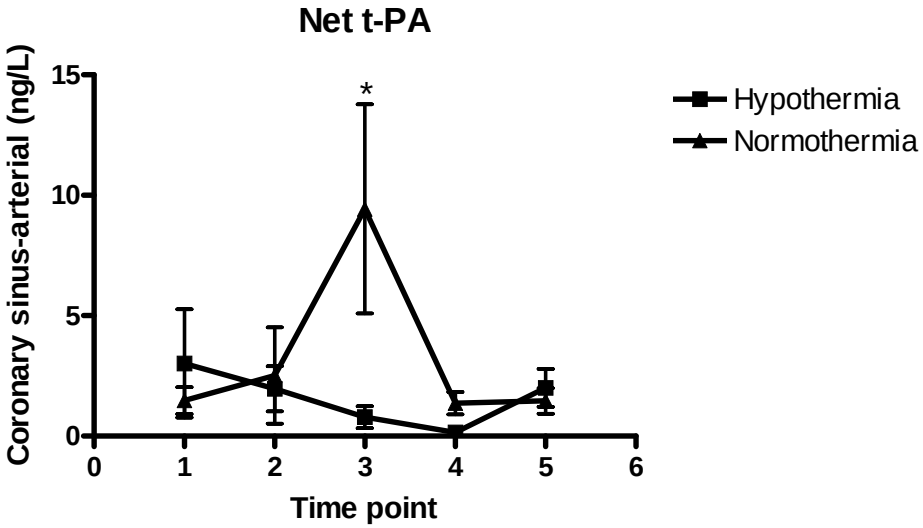

Figure 2 Net t-PA release over the coronary bed was inhibited my mild hypothermia during reperfusion (peak value 9.437±4.34ng/ml vs 0.79±0.45ng/ml, p=0.02). t-PA was simultaneously measured in the coronary sinus and in a peripheral artery at baseline, one minute before reperfusion, one minute after reperfusion, five minutes after reperfusion and 10 minutes after reperfusion.

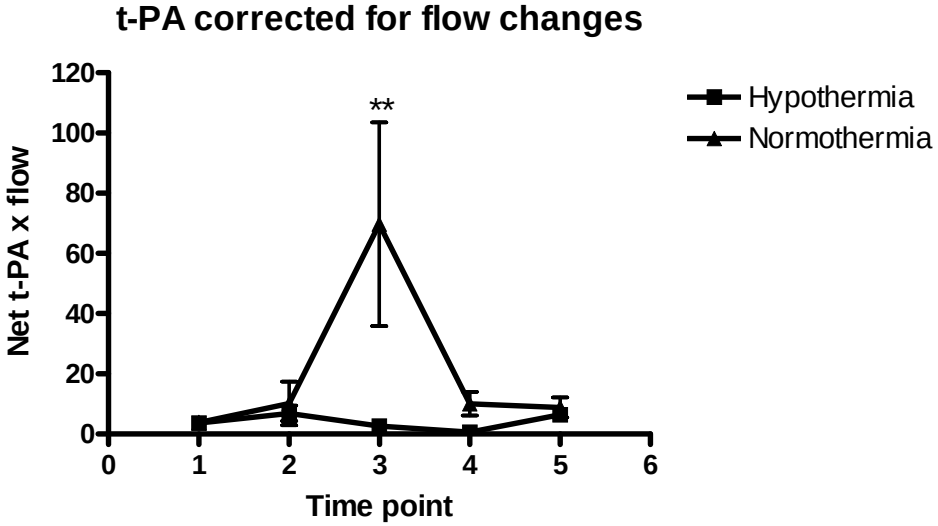

Figure 3 Illustrates an estimate of differences in total t-PA release: Mild hypothermia reduced flow in the LAD significantly as measured with the FloMap Doppler wire, as described in reference 30. The estimate of differences in total t-PA release was calculated by multiplying net t-PA release with the relative increase in flow compare to baseline. Hypothermia significantly inhibited the increase in total t-PA release during reperfusion (69.74±33.86 units vs 2.62±1.10 units, p=0.01). t-PA was simultaneously measured in the coronary sinus and in a

peripheral artery at baseline, one minute before reperfusion, one minute after reperfusion, five minutes after reperfusion and 10 minutes after reperfusion.

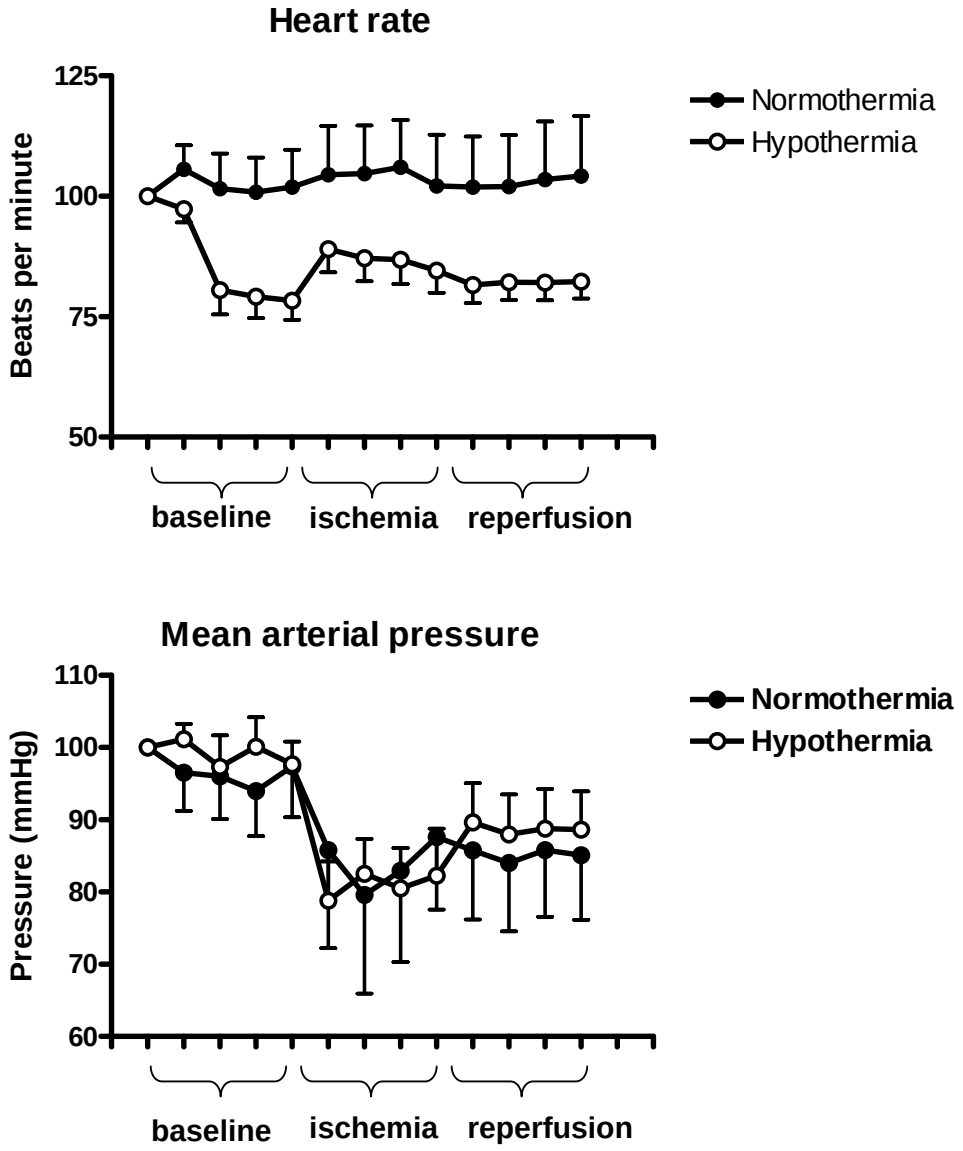

Figure 4 Heart rate (HR) was measured during baseline, ischemia, and reperfusion in both the normothermic and the hypothermic pigs. During the entire period of hypothermia HR was lower in the hypothermic group. Mean arterial pressure was similar, and reduced in both groups during ischemia and reperfusion compared to baseline.

Table1 Arterial blood-gas analysis

	Baseline		1 min reperfusion		10 min reperfusion	
	Control	Hypo	Control	Hypo	Control	Hypo
pH	7.46(±0.03)	7.43(±0.04)	7.47(±0.02)	7.44(±0.02)	7.42(±0.02)	7.43(±0.01)
PO2	22.2(±1.7)	19.6(±1.5)	21.7(±1.8)	20.9(±2.9)	21.1(±2.1)	19.1(±3.0)
PCO2	6.5(±0.8)	5.7(±0.5)	6.2(±0.8)	5.6(±0.3)	6.4(±0.8)	5.7(±0.3)

Samples were collected at baseline and at 1 and 10 min following reperfusion. No statistically significant differences were found between the groups. Data are expressed as mean ± SEM.