PERSISTENT MYOCARDIAL DEPRESSION DURING HYPOTHERMIA AND REWARMING FOLLOWING SYMPATHETIC STIMULATION DURING COOLING

5-års oppgave Stadium IV, Embetsstudiet Medisin, Universitetet i Tromsø

av Stina Therese Sollid

Veileder dr. med. Torkjell Tveita, anestesiavdelingen, UNN

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Abstract

Conscious victims of accidental hypothermia exert serious shivering during the first phase of cooling in order to prevent a further drop in core temperature. A common opinion exist that this profound physiological effort may influence myocardial function during hypothermia as well during rewarming. This experimental study was performed in order to explore the hypothesis that sympathetic stimulation during cooling may cause depression of myocardial function.

Two groups of male Wistar rats were cooled to 15°C and remained at this temperature for one hour before rewarming. Group I received intravenous infusion with Epinephrine 1 μg/minute for 1 hour during cooling to 28. Group II received saline infusion. Heart rate (HR) and mean arterial pressure (MAP) were recorded continuously through a saline-filled catheter in a femoral artery, the left ventricle was catheterised via the right common carotid artery and parameters of left ventricular function were recorded (LVSP, LVEDP, and derivatives LV dP/dt_{max} and LV dP/dt_{min}). CO was measured by the thermodilution technique. The epinephrine given elevated the pre-hypothermic CO by approximately 20%, and thus simulates a real life "light" stress situation. Comparing pre-hypothermic hemodynamic control values (before epinephrine or saline infusions were started) with those after rewarming we found in Group II that these variables either returned to control: HR, MAP, CO, LVSP, LV dP/dt_{max} and LV dP/dt_{min}, or differed significantly from controls: TPR. After rewarming, in contrast to the saline infusions group, the animals that received epinephrine infusions showed a significant lowering of CO, and a significant increase in TPR compared to prehypothermic values.

The present study demonstrates that, in essential contrast to non-epinephrine stimulated control rats, following a moderate sympathetic stimulus during cooling a significant depression of cardiac function occur during hypothermia as well as after rewarming.

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A General Introduction to the Blood Circulatory System and Hypothermia

The Blood Circulatory System

There are three main blood circulatory systems, the systemic, the pulmonary and the portal system[1,12]. Two of these, the systemic and the pulmonary, depend directly upon the heart to function[1].

The principal functions of the blood circulatory system are 1) transport of oxygen and nutrients to the tissues, and 2) transport of carbon dioxide and other metabolic waste products from the tissues[ibid]. The blood circulatory system is also involved in temperature regulation and the distribution of molecules, hormones and cells belonging to the immune system[1,35].

The systemic circulatory system, among other functions, transfer the oxygenated blood from the left ventricle of the heart to the body-tissues and transport back again from the tissues to the right atrium of the heart the deoxygenated blood[1,12]. The pulmonary system and the lungs receive deoxygenated blood from the right ventricle of the heart, and the left atrium receives oxygenated blood from the pulmonary system[ibid].

Histology of blood vessels of the systemic circulatory system

The whole blood circulatory system has a common basic structure; beginning with the layers facing the lumen of the vessel.

- The tunica intima is the innermost layer facing the lumen, it is composed of a single layer of epithelial cells, named the endothelium[3,35]. The endothelium is supported by a basal lamina that is composed of the glycosaminoglycan heparan-sulphate, the fibrous protein collagen type IV, and the structural glycoproteins fibronectin, laminin and entactin[3]. The function of the basal lamina being that of giving structural support and function as an anchor for the overlying epithelium to the underlying tissues. Beneath the basal lamina is a layer of fibrocollagenous tissue[ibid].

- The tunica media lays outside the tunica intima, it is mainly composed of smooth musculature[3,35].

- The tunica adventitia is the outermost layer of the vessel, it is predominantly composed of collagen. In the thickest vessels there are small blood vessels, vasa vasorum, penetrating the tunica adventitia, these blood vessels may also send branches to the tunica media to supply it as well as the tunica adventitia with blood[ibid].

Components of the systemic circulatory system are the arterial system, the capillaries and post-capillary venules of the microcirculation, and the venous system[ibid].

The arterial system:

The arterial system is the arteries (elastic arteries and muscular arteries) and arterioles[ibid]. Elastic arteries, that being the aorta, the aa. carotis communis, aa. subclaviae, most of the aa. pulmonales and the aa. renales, have a high content of elastic tissue in the tunica media. The elastic tissue makes the elastic arteries capable of expansion and recoil, one may therefore say that elastic arteries are perfectly constructed to receive the systolic pressure wave created when blood is being pumped out of the left ventricle into the systemic circulation[1]. A muscular artery follows in the lengthening of elastic arteries and function mainly as a distributor of blood[3,35]. In muscular arteries the elastic tissue in the tunica media has been reduced to a well-defined fenestrated elastic sheet, the lamina elastica interna, separating the tunica intima and the tunica media, and a less definable elastic sheet, the lamina elastica externa, separating the tunica media and the tunica adventitia[ibid].

Arterioles form when the diameter of the lumen of the vessel decreases. The arterioles are the terminal branches of the arterial system, and they end up supplying the microcirculation. The tunica media is in arterioles even more than in muscular arteries composed of smooth musculature, in large arterioles the tunica media is composed of six layers of smooth musculature, while in the small arterioles the tunica media only consists of two layers. Contraction and relaxation of the circulatory arranged muscle fibers of the tunica media makes the arterioles very capable of changing the lumen diameter, and hence blood flow to the microcirculation as well as regulation of the systemic blood pressure. There is no lamina elastica externa in arterioles, and the tunica adventitia merges with the surrounding tissue[ibid].

The microcirculation:

Capillaries are the smallest of the vessels, the diameter varies between 3 um and up to 40 um. Capillaries have partly permeable thin walls, this walls being composed of a single layer of flattened endothelial cells line faces the lumen, the endothelium rests on a basal lamina. There is neither a tunica media nor a tunica adventitia. The capillary endothelium are of three different kind, it can be continuos, meaning that it is an uninterrupted lining, fenestrated, meaning the endothelium contain numerous pores or fenestration's or it can be discontinuous. Most common of these three is the continuos type, the fenestrated is found in tissues with

extensive molecular exchange with the blood, e.g. the kidney and the small intestine, and the discontinuous is found only in liver sinusoids. Sinusoids are capillaries of wide diameter found in the liver, spleen, lymph nodes, bone marrow and also some endocrine glands, in all the other but the liver the endothelium may be of either of the two other types[ibid].

The capillaries permit the transfer of some blood components between the lumen and the surrounding tissue, the different ways of transfer being; passive diffusion for gases, ions and low molecular weight metabolites, pinocytosis for proteins and lipids and intracellular transport[3].

The regulation of blood flow in the capillary bed is mediated both by the arterioles and pre-capillary spinchters, situated at the arteriolar-capillary junction, and also by arterio-venous. Arterio-venous shunts provide a direct connection between the arterial and the venous systems[3,35].

Post-capillary venules are formed by the union of several capillaries and drain the capillary bed. These vessels appear to be the main point at which the leucocytes leave and enter the circulation[ibid].

The venous system:

Collecting venules receives blood from the post-capillary venules, and then muscular venules and veins follow leading the blood to the right atrium. Veins in general have a larger lumen and a relatively thinner wall than arteries. The collecting venules are devoid of smooth musculature, when smooth muscle cell can be identified in their wall they are named muscular venules. Muscular venules have a diameter of 50-100 um, their tunica intima is devoid of elastic fibbers and the smooth muscles consist of one or two layers comprising the tunica media. Veins vary in diameter, the smallest have only two or three layers of circularly arranged smooth musculature in the tunica media, while the large veins have a relatively thick tunica media and the smooth muscle cells are separated by collagenous connective tissue. The tunica adventitia contain vasa vasorum and also lymphatic vessels, both of these are more numerous in veins than in arteries of the same size. In the venae cava the smooth musculature is arranged in a longitudinally pattern instead of the circulatory pattern seen in the other veins[ibid].

The blood flow in veins occurs passively down a pressure gradient toward the right atrium of the heart[1,11]. The skeletal musculature act as an exterior pump on the veins pushing the blood forward, also each time a human inspire a negative pressure is created inside the chest cavity and hence the right atrium and "draws" the blood to the heart[ibid]. The blood is prevented by running back again by valves, the valves being closed when blood tends to flood backwards. These veins are most often found in the extremities and have a diameter of more than 2 mm[3,34]. The valves of veins usually consist of two leaflets and are composed of fibro-elastic tissue lined on both sides by endothelium[ibid].

Body fluid compartments and the blood components

The body fluids are distributed among two compartments, the extracellular fluid and the intracellular fluid3[12]. The intracellular fluid comprises about 2/3 of total body fluid, and the extracellular 1/3. The extracellular fluid is further divided into two larger, the interstitial fluid and the blood plasma, and one small compartment, the transcellular fluid. The interstitial fluid accounts for ¾ of the extracellular fluid while the blood plasma for ¼. The blood plasma and the interstitial fluid are communicating through pores in the capillary membrane[ibid].

Blood contains both the noncellular blood plasma, accounting for nearly 60 % of total blood volume, and cells, accounting for 40%[3,12]. The cellular part of the blood can be divided into three groups, the erythrocytes, the leucocytes, and the trombocytes. The leucocytes are of two major groups, the granulocytes with three subgroups, and the mononuclear leucocytes with two major subgroups[ibid].

Starling Forces

The starling hypothesis explains the balance of hydrostatic forces and of osmotic forces across the capillary endothelium[1,12]. Normally in the capillary bed there is a net positive pressure on the atrial side, thus driving fluid outward to the interstitium, and a net negative pressure on the venous side, thus promoting absorption of fluid into the capillary from the interstitial fluid[ibid]. This is explained by the equation:

$$Q_f = k(P_c + \pi_{if}) - (P_{if} + \pi_p)$$

Q_f = fluid movement

k = capillary filtration coefficient

P_c = capillary hydrostatic pressure

$$P_{if} = \text{interstitial fluid hydrostatic pressure}$$

$$\pi_p = \text{plasma colloid osmotic pressure}$$

$$\pi_{if} = \text{interstitial fluid colloid osmotic pressure}$$

The P_c is the major force driving fluid outward on the atrial side of the capillary bed (a net positive pressure). The P_c falls on the way through the capillary bed thus making the π_p the dominating force (net negative pressure) driving fluid into the capillaries again on the venous side[ibid].

Regulation of peripheral blood flow

There is both an intrinsic control and an extrinsic control of peripheral blood flow; the intrinsic is local control while the extrinsic is central nervous control[1].

Intrinsic regulation:

At least three different mechanisms; auto-regulation, endothelium-mediated control, and metabolic control are regulating the intrinsic control of peripheral blood flow[ibid].

By auto-regulation one means the ability to maintain a relatively constant blood flow during changes in perfusion pressure. With constant tissue metabolism changes in perfusion pressure will lead to changes in vascular resistance in such a way that constant flow is being maintained. This is explained by the myogenic mechanism; when the pressure difference across the wall of a blood vessel, the transmural pressure, increases it causes the vascular smooth muscle to contract causing vasoconstriction, and when the transmural pressure decreases the vascular smooth muscle relaxes causing vasodilatation. This myogenic response is independent of the endothelium only relaying of the musculature in the vessel. It has been proposed that when the vessel wall is being stretched it causes activation of membrane bound Ca^{2+}-channels and thus elevates the intracellular concentration of Ca^{2+}[ibid].

The endothelium-mediated regulations depend upon mechanisms lying within it, and are most potent in large vessels. When the transmural pressure is constant an increase in shear stress, as a result of increased flow in the vessel, causes vasodilatation. The mechanism for vasodilatation is thought to be that increased shear stress stimulates the endothelium cells to paracrine nitric oxide secretion, and it is the nitric oxide that causes vasodilatation[1,24]. It is also thought that other substances, such as prostacyclin causing vasodilatation and endothelin

causing vasoconstriction, are being secreted by the endothelium as a response to shear stress, but also in response to a variety of circulatory substances and inflammatory mediators[ibid].

Metabolic regulation is a result of the tissues metabolic activity effect and its effect on blood flow. When the tissue substrate, O_2, decreases metabolites are synthesized and secreted locally causing vasodilatation. One is not sure of what kind of metabolites these are, but some suggestions are; CO_2, H^+, lactate, K^+, phosphate, interstitial osmolarity, adenosine, and prostaglandins[ibid].

Extrinsic regulation:

The extrinsic regulation of peripheral blood flow, as the intrinsic, works in several ways; the symphatetic neural system, the parasymphatetic neural system, the adrenal medullas humoral metabolites, and the vascular reflexes[1,2].

In the dorsal lateral medulla oblongata of the central nervous system a pressor-region is located, in the areas caudal and ventro-medial for the pressor region a less definable depressor region is located. Stimulation of the pressor region causes increased symphatetic activity that again causes vasoconstriction, increased heart rate, and enhanced inotropic effect of myocardium. When the depressor region is being stimulated the symphatetic activity is decreased and this causes reversed responses; vasodilatation, decreased heart rate, and decreased inotropic effect of the myocardium. The pressor area are tonically active, upon stimulation "information" descends in efferent preganglionic fibers to the intermediolateral gray matter of the spinal cord in the areas Th1 to L_2/L_3 and then to the paravertebral symphatetic chain. From these paravertebral ganglion postganglionic efferent fibers reaches the vessel wall, and norepinephrine is released from the nerve ending causing vasoconstriction. All vessels are more or less innervated by sympathetic noradrenergic efferent fibers acting upon α_1-receptors causing vasoconstriction. Some blood vessels in skeletal musculature also have cholinergic sympathetic innervation that stimulating muscle relaxation leading to vasodilatation through β_2-receptors. Inhibition of the pressor area reduces their tonic activity and hence diminishes the frequency of impulses in the efferent nerve fibers, which again causes vasodilatation. Neural regulation of peripheral flow is mainly controlled through a change in the frequency of impulses from the pressor region via the efferent sympathetic fibers to the vessels. The parasympathetic nervous system innervates

the vessels of the viscera, bladder, intestines and genitalia system, but has no main influence on the peripheral flow[ibid].

The adrenal medulla secretes epinephrine and nor-epinephrine upon stimulation, but one ought to remember though that under normal physiological circumstances the sympathetic nervous system is by far the most important. Norepinephrine produces vasoconstriction in most organs via α_1-receptors, epinephrine causes vasoconstriction in all organs, except skeletal muscle and liver, via β_2-receptors[1,2,24].

The vascular reflex system aims to provide information about the lumenal pressure (baroreceptive information) and the bloodgas levels, e.g. the oxygen and carbon dioxide levels (chemoreceptive information) to the central nervous system via afferent nervous fibers[1].

The arterial baro-receptors are located in the carotid sinuses of the internal carotid artery and in the aortic arch[ibid]. The most sensitive of these receptors is the carotid ones. Afferent fibers ascend respectively in the nervus glossopharyngeus and the nervus vagus to the nucleus tractus solitarii, the frequency of firing is enhanced in both by increased blood pressure and diminished by a decreasing blood pressure[1,2]. Upon stimulation, meaning the frequency of firing is being enhanced, the pressor area already mentioned is inhibited the result being vasodilatation. The baroreceptor system play a key role in short term adjustments of blood pressure in response to relatively abrupt changes in blood volume, cardiac output and peripheral resistance. Baro-receptors sensitivity decreases in hypertension because the carotid sinuses become stiffer and less deformable as a result of high intra-arterial pressure, this raises the set set point for baro-receptors[ibid].

The cardiopulmonary baro-receptors are located in the atriums, the ventricles, and in the pulmonary vessels[1]. Vagal (parasympathetic) and sympathetic afferent fibers innervate all of these[1,2]. The receptors in the atriums are of two different kinds; A-receptors are activated by the tension developed during atrial contraction, while the B-receptors are activated by stretch of the atriums during atrial filling[ibid]. The receptors in the ventricles and in the pulmonary vessels are being activated by changes in intracardial, venous, or pulmonary pressure. When activated impulses ascend via the vagal nerve to the vagal center in the medulla, the result upon activation of this center being inhibition of sympathetic activity to the kidney, and

stimulatory impulses on the sympathetic activity to the sinus node. End results of these actions are enhanced renal blood flow, enhanced urinary flow, and cardiac acceleration[ibid].

The chemoreceptors of the periphery are located in the aortic arch (the aortic bodies) and in the carotid sinuses (carotid bodies)[1]. These receptors are sensitive to changes in pH, pO_2, and pCO_2. A low pH, a high pCO_2, or a low pO_2 activate the receptors causing increased impulses in the afferent fibers stimulating the pressor area causing an increased resistance in arterioles and in veins so that blood are pooled to the organs most in need of it[ibid].

The central chemoreceptors are located in the medulla oblongata, they are activated by high levels of pCO_2 and by low pH[1,2]. The result of this activation again being that of pooling blood to the most essential organs[1].

The brain and the heart for the most depend upon the intrinsic regulation, the skin solely on the extrinsic regulation, while the skeletal-muscular system depend on both[ibid].

Hemodynamics

In a closed system as the circulatory system pressure equals flow times resistance:

$$BP = CO * R$$

BP = Blood pressure

CO = cardiac output

R = resistance

Thus the blood pressure will increase when either the cardiac output or the resistance increases[1,12]. The resistance increases when the diameter of the vessels decreases[ibid].

Blood flow in human vessels can be looked upon as laminar[1]. Laminar flow implicates that an infinitely thin layer of blood closest to the vessel wall does not move, then the next layer moves slowly, the next even less slowly, and with the fastest blood flow in the center of the vessel lumen[ibid]. Blood flow is determined by; pressure difference between the two ends of a vessel, the radius of the vessel, and the viscosity of the blood. The relation between these are expressed by the Poiseuille-Hagen formula:

$$Q = (P_i-P_o) * (\pi/ () *(1/\eta) * (r^4/L)$$

Q = blood flow

P_i-P_o = pressure difference

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$$\eta = \text{viscosity}$$
$$r = \text{radius of the vessel}$$
$$L = \text{length of the vessel}$$

Since flow is equal to pressure difference divided by resistance (R):

$$R = 8\eta L/\pi r^4$$

From this formula one can sees that flow varies directly and pressure inversely with the fourth power of the radius of the vessel[ibid].

The major site of resistance is the arterioles, the blood pressure take a sharp drop and gets even lower in the capillaries where it is about 37 mmHg in the beginning and 17 mmHg at the end and the pulse pressure has completely disappeared. The pressure continuos to all steadily, and in the venous system it is about 5 mmHg at the entrance of the vena cavas into the right atrium[ibid].

Another law, the law of Laplace, shows the relation between distending pressure and wall tension. In a distensible hollow object, here a vessel, the distending pressure is equal at equilibrium to the wall tension divided by the two principal radii of the curvature of the object, in a vessel that have a cylindrical form the radius is infinite:

$$P = T/r$$

$$P = \text{distending pressure}$$
$$T = \text{tension}$$
$$r = \text{radius}$$

From the formula one read that as the radius of the vessel decreases the balancing wall tension necessary to balance the distending pressure is lowered[ibid].

Velocity of the blood refers to the rate of displacement of fluid with respect to time, expressed as cm/sec. Velocity can mathematically be expressed by:

$$V = Q/A$$

$$V = \text{velocity}$$
$$Q = \text{blood flow}$$
$$A = \text{cross sectional area}$$

From this formula one sees that velocity is directly related to the cross sectional area of vessels, as the cross sectional area increases the velocity falls causing the lowest velocity in the capillaries[ibid].

Body Temperature and Temperature Regulation

When considering the human temperature one must think of the human body as a core surrounded by a shell (the skin)[22]. The core temperature differs from individual to individual and also within the individual (but only by ± 0,6°C)[11]. Normal core temperature measured rectally lays between a little less than 36°C and 37,5°C[ibid]. From the core heat is transferred via the blood to the skin, the degree of heat loss being controlled the degree of vasoconstriction of arterioles and also by atrio-venous anastomes that supply blood to the venous plexus of the skin[12,22].

Heat gains and heat losses

Most of our heat is produced in the deep lying organs, such as the liver, brain, heart, and the skeletal muscles during exercise. Heat is generated in several ways, by all cells basal metabolism, by metabolism in active muscles, by cells under the influence of tyroxine, norepinephrine and/or epinephrine, and also by chemically active cells[ibid].

Other heat gains include short wave heat rays from the sun, long wave heat rays from the surroundings, and ingestion of hot food/drink[22]. The skin as well as subcutaneous tissues act as insulators, and high amounts of fat in the subcutis is shown to raise the limit to becoming hypothermic[20,22].

Shivering can increase body metabolism 2-5 times[20]. At the same time as shivering serves to conserve heat it is also lost because of increased convection because of body movement. A person can prevent hypothermia by behavioral mechanisms, both physical exercise and making up a fire-place serves to conserve heat. Through exercise heat is actually also lost because of increased peripheral blood flow. By wearing clothes which creates a layer of air trapped next to skin a person's heat loss by means of convection is reduced[20,22].

Heat is lost by four different mechanisms[12,22].

Radiation

This is loss of heat by means of long wave heat rays to the surroundings, this accounts for as much as 60% of the total heat loss.

Conduction

This form of heat loss accounts for approximately 15% of total loss. Heat is lost through contact between the skin and the surroundings, i.e. air or furniture's.

Convection

Heat loss to the air by means of conduction must have occurred for convection to happen, and the loss of heat is by means of convection currents driving the heat away from the person's body. Strong wind may increase convection to as much as 65% of total heat loss.

Evaporation

The last mechanism of human heat loss includes both the insensible evaporation trough skin and lungs, and also sweating which is controlled by the sympathetic nervous system[ibid].

Central and peripheral regulation of temperature

Thermal skin receptors

The receptors in the skin detecting "shell" temperature are slow adapting, meaning that skin temperature must change rapidly for the receptors to respond[2]. When skin temperature decreases cold receptors are stimulated, these receptors are A_δ-myelinated nerve endings transferring message to the central nervous system at a velocity of 20 m/s[2,12]. On the other hand, when skin temperature raises the warmth receptors are stimulated, these receptors are free nerve endings and information to the central nervous system travels at 0,4-2,0 m/s. Pain receptors also respond to temperature when becoming extreme enough. Information travels in the spinothalamic tract on its way to the central nervous system[2].

There are also deep body temperature receptors, these receptors detect "core" temperature and mainly detect cold. These receptors are located in the spinal cord, the abdominal viscera, and around great veins[2,12].

Hypothalamus

Located in the preoptic area of the anterior hypothalamus are heat sensitive neurons and cold sensitive neurons, these neurons are believed to function as temperature sensors for controlling the body temperature. An increase in temperature of the blood flowing through

this area is detected an increases the activity of the temperature sensitive neurons, vice versa will a decreased blood temperature lower the neurons activity[ibid].

In the posterior hypothalamus signals from the preoptic area and information from the peripheral receptors are combined to control the bodies heat production and heat conservation in the most favorable way for the person involved. When skin is chilled signals are emitted causing; shivering, increased tyroxine production, increased blood concentration levels of epinephrine and norepinephrine, inhibition of sweating, and vasoconstriction of vessels in the skin. All the above mentioned favors heat conservation and promotes heat production. The opposite happens when skin is heated; sweating is promoted, signals causing vasodilatation and skin vessels, and inhibition of excess heat production are emitted[ibid].

Hypothermia

Hypothermia is defined as having a body core temperature below normal[20,37]. Hypothermia may either be induced or accidental[22]. Induced hypothermia involves a technique where a persons core temperature is lowered to between 26°C and 32,5°C, the purpose being to reduce the oxygen need of tissue[ibid]. This technique is especially used during cardiovascular and neurological surgeries[14]. Accidental hypothermia on the other hand does not occur by purpose as the name applies[22]. Accidental hypothermia may be acute or chronic, the latter evolves over weeks[ibid]. Cooling is also used in on a hurt limb or joint after traumatic accidents, i.e. in sports, the effect of the cooling is thought to antagonize that of histamine thus preventing infalammation[29].

Staging of hypothermia

There are quit a few definitions of hypothermia, most divides it into three stages:

Mild hypothermia

The core temperature is above 33°C (35,0-32,2°C)[20,22]. The person exposed to hypothermia will have a feeling of coldness, and the body will try to produce and to conserve heat in ways mentioned above. The blood pressure raises slightly, and the person will also begin to hyperventilate[ibid].

<u>Moderate hypothermia</u>

Core temperature between 33°C-23°C (32,2°C-24,0°C). The vasoconstriction as a method of heat conservation continuos, but the shivering will cease. The person will now also have a depressed tissue metabolism. Upon clinical examination the person will have a low or not recognizable pulse, and the blood pressure as well as a respiratory frequency is low[ibid].

<u>Severe hypothermia</u>

The core temperature is now below 23°C (24°C). The person's temperature regulation is completely loosed and therefor the mechanisms for heat conservation are inactive, and body heat is being lost passively to the surroundings[ibid].

The critical temperatures for man is 25°C if being exposed to hypothermia while being surrounded by air, this critical temperatures is only 33°C if the person is surrounded by water[22]. In water the conduction of heat increases compared to air[12]. Water can absorb up to 10 times as much heat from a person as do air, and this is why hypothermia is promoted. The thermal conductance of water is almost one thousand times greater than that of air at comparable temperatures[34].

The effect of hypothermia on major organ systems

<u>The central nervous system</u>

When the core temperature gets only about 2-5°C below a person's normal temperature mental distortion are seen and present themselves most often as confusions and delusions[20,22]. Mildly hypothermic patients are although at these temperatures most often alert and well oriented. Upon becoming moderate hypothermic dysarthria, difficulty in understanding, drowsiness, and difficulty in voluntary movement of limbs are symptoms seen in the patient. In the lower temperature range of moderate hypothermia the patient becomes stupourous, but he can still be aroused by others voices or touches. The tendon reflexes will also have disappeared. A deeply hypothermic patient will not respond to any external stimuli, his pupils will be miotic and the light reflex is absent[ibid].

The brains metabolic rate is lowered, and the utilization of glucose is decreased compared to in a normothermic person. The cerebral blood flow is also decreased in hypothermia[ibid].

Hypothermic effects on the symphatetic nervous system are reported in a scientific study performed on five adult baboons[5]. They documented a rise in the concentration of epinephrine and norepinephrine in plasma when baboons were cooled from 37°C to 33°C, at further lowering of the temperatures (31°C to 29°C) the values decreased to below basal levels. This might indicate that the symphatetic nervous system switches off at about 29°C[ibid], and at temperatures below this vasodilatation is seen further supporting the switch off theory[41].

There has also been documented an increased sensitivity of the postjunctional α_1- and α_2-adrenoreceptors in skin arteries in humans and a depressed contractility in the same arteries to α-adrenergic stimulation and direct activation of vascular smooth muscles during cooling[9].

The cardiovascular system

Initially when only the "shell" is cooled tachycardia and an increase in mean arterial blood pressure is seen, both these effect is a result of the sympathetic stimulation that occur in order to conserve body heat as earlier described[4,5,11,14,36,40]. The heart rate have been shown to rise to such an extend corresponding to a 300-400% increase in metabolism with a sudden whole-body immersion into cold water (15°C)[41,45]. In cardio pulmonary bypass (CPB) on piglets performed under hypothermic conditions MAP raised some 48% compared to the normothermic control group[14].

When the core temperature sinks further a decrease in CO and in BP or/and MAP are reported by several studies[4,5,18,23,27,32,33,36,40]. The CO may decrease as much as 30% with the core temperature laying in the upper part of the moderate hypothermic stage, and the MAP up to 27%[27].

Changes in the coronary blood temperature to hypothermic values increased the contractile strength of the muscle fibers in the left ventricle in a study on Mongrel dogs[36]. The explanation of the increased contractile strength may be associated with elevated intracellular Ca^{2+}-levels seen with hypothermia[41]. Ca^{2+} is essential for promoting sliding of the actin and myosin filaments in the heart thus causing muscle contraction[12], and therefor the increased Ca^{2+}-levels favor the muscle contraction. In the dog study mentioned above the blood were ejected more slowly, meaning the ejection time increased, and the left ventricle relaxed slower (prolonged isovolumetric phase) during hypothermia[35]. The left ventricle was also shown to

be more vulnerable to left ventricular failure and fibrillation. The cardiac oxygen consumption were also shown to decrease[ibid].

Total peripheral resistance (TPR) was shown to increase significantly in a study on Mongrel dogs[32]. Upon receiving continouos injections of epinephrine and norepinephrine, these hypothermic dogs (rectal temperature 24-25°C) showed both an increase in BP an TPR[ibid]. TPR also increased in a study on hypothermic rabbits, supporting these findings[36]. TPR increase is also shown in another Mongrel dog study[17]. In this latter study some of the heart preparations were denervated, and these showed a decrease in coronary vascular resistance when exposed to hypothermia, thus underlining the role of nervous regulation of resistance in vessels[ibid].

Atrial fibrillation is known to occur at temperatures in the moderate hypothermia interval, and at even lower temperatures (deep hypothermia) ventricle fibrillation is seen[22]. Asystoli has also been reported[ibid]. Both asystoli and ventricular fibrillations are difficult to manage, and both may be terminal in profound hypothermia[26].

The blood circulatory system an the body fluid compartments

Hypothermia, both induced and accidental, has been associated with changes in plasma volume and the clinical sign edema upon rewarming[14]. The mechanism behind this fluid shift is on the other hand not understood. Løfstrøm, in a rabbit study, showed a decrease in total plasma volume by 13% at 26°C, and a decrease by 20% at 20°C[19]. These findings are supported by others[4,6,40]. In an animal experiment using piglets a normothermic and hypothermic group were compared during CPB with respect to fluid extravasation[14]. A marked increase in fluid extravasation were found during hypothermic CPB compared to the normothermic CPB group, the total intravascular protein and albumin levels although remained constant in both groups meaning that the extravasated fluid mainly were composed of water and small solutes[ibid]. The theory of loss of fluid from the intravascular compartment to the interstitial fluid compartment during hypothermia is supported by a seven times higher fluid need in hypothermic compared to normothermic piglet during CPB[15].

A significant increase in the colloid osmotic pressure (π_p), with an average increase of 4,6% with cooling to 25°C, were shown in a study on 40 rabbits, the filtration coefficient showed no significant alteration in the same study[36]. Heltne, on the other hand, shows a significant

decrease in π_p in hypothermic CPB on piglets[14]. Heltne, in another study states that the filtration coefficient (k) increases moderately in hypothermia, and this together with a decreased π_p result in extravasation of fluid[13].

In a study on Wistar rats the scientist learned that the viscosity of blood increases with hypothermia[30], these findings being supported by others[7,18,36] and with a temperature lowering down to 12°C the viscosity increases at least 30%[30]. In this same study on Wistar rats mentioned above no significant changes where noticed in capillary permeability, a study including 40 rabbits supports this[42].

Reports on a decrease in total red blood cells[20] as well as no change in red blood cell volume[4,6] are reported. The suggested decrease in total red blood cells is believed to occur as a result of stagnation in peripheral vessels[20]. Margination of white blood cells is also documented[36]. There is also a disagreement around the plasma protein concentration, some report it decreasing[4,41] while other reporting it being unchanged[6]. Though not an agreement on the number of total red blood cells an agreement seems to be of an increase in hematocrit[4,6,20,36,41], the reason most likely being the decrease in total plasma volume[ibid].

The blood-vessels active transport mechanism of substances, such as albumin, seems to be depressed during hypothermia[30]. The plasma clearance of albumin is reduced 60% in a muscular vascular bed (in rats) when temperature is lowered to 15°C compared to normal temperature[ibid].

The respiratory system

There is initially, meaning when only the skin is cooled, a hyperventilating response[22]. The respiratory rate has then been reported slowing down as the core temperature decreases[36]. The metabolic need of the lungs is lowered in hypothermia as it does in other organs as well[22].

Rewarming from hypothermia

Upon rewarming death shall not be recalled until the person's temperature is above 32°C[16], this is because with deep hypothermia it is difficult to tell whether a person is alive or not[ibid]. The problem lies in difficulty of detection respiration, difficulty in palpation of pulse, difficulty in measuring blood pressure, stiffness of joints simulating rigor mortis, and off

course the person being unconscious[8,20]. All these results from the hypothermic effect on the respiratory- central nervous-, and cardiovascular system.

There is an agreement today that hypothermic persons should be rewarmed by extracorporeal methods[10,16], this way the temperature can be raised by 8-10°C/hour[16]. A too rapid rewarming of rabbits resulted in lung edema and heart failure, explained by a volume overload that the circulation can't handle so rapidly[20].

Rewarming from short-lasting induced hypothermia, as during a CPB, usually restores the hemodynamic values to prehypothermic values[41]. On the contrary people being rewarmed from accidental hypothermia may suffer from rewarming shock, a happening of which the cause is not known[22,41]. Both cardiac output and heart rate falls and the person may experience cardiac arrest[10]. A sudden fall in TPR after rewarming contributes to the development of shock[22]. Theories trying to explain the phenomena are cardiac insufficiency and changes in the symphatetic control on the peripheral vascular bed, which again causes the posthypothermic circulatory instability[41]. Rewarming from accidental hypothermia does not necessarily have to end in shock, but the phenomena observed during and after rewarming could when severe enough explain it. In an animal study BP and HR reached prehypothermic values, while CO only was 33% ± 7% of prehypothermic values[40]. Further the posthypothermic TPR was 4,3 times higher than prehypothermic values, leading one to believe that a disturbance has occurred in the symphatetic control, and the plasma volume were only 77% ± 3% of prehypothermic values. The blood flow in internal organs were lower and in peripheral organs the same as the prehypothermic values, opposite of what preservation of internal organs would give[ibid].

Macroscopic and microscopic findings with hypothermic death
There are few, if any, common macroscopic or microscopic characteristics observed in humans believed having died from hypothermia, thus making hypothermia an exclusion diagnosis[31]. The few unspecific signs are; pinkish colored death marks, the color of the blood being pale red, and petecchial bleedings on the serosa of the gastrointestinal tract[22,31]. The petecchial bleeding may be caused by stagnation of erythrocytes. Visceral congestion and tissue edema are also unspecific findings[ibid].

Ethanol and its Effects on the Human Body

Central nervous system symptoms of acute ethanol intoxication can present themselves in different ways such as slurred speech, impaired coordination, and decreased sensory, motor and intellectual discrimination[28]. The person having consumed alcohol will experience an increased self-confidence and a feeling of euphoria. Ethanol poisoning causes severe depression of consciousness and respiratory depression that may lead to coma and/or death. Coma occurs at ethanol consumption of 300 mg/ml and death from respiratory failure at 400-500 mg/ml. Ethanol's overall effect on the central nervous system is depressant and some of the mechanisms are; an increased fluidity of lipid membranes, an inhibition of transmitter release in response to depolarization of the nerve terminal, an inhibition of the opening of voltage-sensitive Ca^{2+}-channels in neurons, and an inhibition of glutamates excitatory effect[ibid].

Ethanol is known to cause cutaneous vasodilatation, thus causing a warm feeling in the person having consumed it[ibid].

Ethanol further inhibits antidiuretic hormone secretion from the posterior pituitary which leads to increased diuresis, ethanol also stimulate secretion of the anterior pituitary hormone ACTH the effect being increased levels of adrenal steroid hormones in the blood[12,28]. Ethanol consumption over weeks is also known to increase the concentration of HDL in blood, thus exerting a protective effect against atheroma creation in the vessel-wall. Ethanol also inhibits platelet-aggregation, and therefor exerts a protective effect against ischemic heart disease[ibid].

Ethanol is known to have an overall depressant effect on the central nervous system[28]. In a retrospective study evaluating accidental hypothermia in 39 humans, intoxicated where compared to non-intoxicated with a serious primary disease[43]. Alcohol was the reason for intoxication in 28 and heroin in 1. The mortality rates were 6,5% in the intoxicated group compared to 75% in the group with an underlying disease. From this one can not conclude either way, in favor or against alcohol as a protective against hypothermia, only that a primary disease lowers the survival rate, but it may support the idea of alcohol as being protective of hypothermic death[ibid]. In another study on humans no significant differences were observed with respect to respiratory rate, shivering or heat loss between a group having consumed and a

group not having consumed alcohol during cold water immersion[34]. The alcohol group did though report less discomfort to the cold than the non-alcohol group[ibid]. Vangard on the other hand showed with whole-body immersion of humans in cold water (15°C) that shivering was delayed 15-20 minutes in the group intoxicated with alcohol, while shivering instantaneously begun in the control group that not had consumed any alcohol[42]. In the same study the individuals not having ingested alcohol felt discomfort during immersion, while the alcohol-intoxicated group had a feeling of well-being[ibid].

Ingested alcohol does not impair thermoregulatory ability in situations of cold stress; this statement is based on no significant difference in the cold induced vasodilatation phenomena in skin in a group having a blood alcohol level compared to a group not having ingested alcohol[25]. These results are supported Vangard who initially showed a small rise in core temperature in both an alcohol and a non-alcohol group upon cold water immersion due to closure of the arterio-venous anastomoses in the skin, and the core temperature fell at the same rate in both groups[42]. MacGregor, in a study on dogs, did not either receive any difference between the alcohol-intoxicated group and a control group that had not received alcohol infusion, with respect to rate of cooling, neither did he find any significant difference with respect to BP or HR[21].

Part 2:
The Animal Study

Introduction

The basis of the study was a hypothesis that absence of adrenergic stress during hypothermia might have a protective effect on the heart and the cardiovascular system as a whole, and on the other side that the presence of adrenergic stress had a negative effect.

When a person is exposed to a cold environment he will try to conserve heat in order to avoid hypothermia. To produce heat shivering sets in, the levels of tyroxine is increased as well as the concentration of epinephrine and nor-epinephrine, and as a consequence the basal metabolism rate increases and heat is produced[2,12]. An increase in sympathetic activity also leads to vasoconstriction, increased HR, and an enhanced inotropic effect on the heart[1,2]. In hypothermic studies where both the sympathetic nervous system hormones, epinephrine and nor-epinephrine, are given MAP has shown to increase further compared to hypothermic controls not receiving these hormones[32].

In a study on fetal sheep the concentrations of epinephrine and nor-epinephrine were shown to increase during cooling, epinephrine levels increased almost six times and nor-epinephrine 7 times compared to pre-hypothermic values[11]. Gomez, in a study on human skin artery responses to tissue cooling, showed an increased sensitivity in both α_1- and α_2-postjunctional adrenoceptors to norepinephrine at 24°C than at 37°C[9]. He suggests that increased levels of epinephrine and nor-epinephrineibid can explain part of the increased sensitivity. Gomez results are supported by a study on isolated hamster arteries[46]. These studies showed an increase in the affinity for β-adrenoceptor at hypothermic temperatures compared to normothermia. On the other hand has in vivo studies on dogs shown a decreases adrenergic effect to isoproterenol during hypothermia with respect to both chrontropi and inotropi[45]. In hypothermic conditions intracellular Ca^{2+} is raised, and this can explain the weak effect of isoproterenol on contractility in deep hypothermia[ibid].

Clinical "tellings" have shown a lower core temperature in hypothermic survivors with a blood alcohol level compared to persons with no alcohol traces in their blood. Studies have shown that ethanol protects against hypothermia, and lowers the critical temperature 2-5°C[16], and it is possible that high blood alcohol levels favor survival in hypothermia[43]. Different alcohol's, such as ethanol, propanol-1, and butanol-1, all increases the tolerance to low body

temperatures in dogs by 4,2°C, 3,2°C, and 1,7°C respectively[21]. From animal studies it seems clear that intoxication with alcohol lowers the critical borders for lethal arrhythmia, such as ventricular fibrillation[21]. This raises the question of alcohol with its physical effects and responses on humans in some way act to protect our heart and brain, or does it simulate a low adrenergic situation and can intoxication with alcohol therefore be looked upon as a model of absence of adrenergic stimuli during hypothermia. The central nervous effects of ethanol on the other hand make us less critical in action, and hypothermia are more likely to happen both because of our actions and because of a feeling of warmth from the vasodilatation of skin vessels.

The aim of the study was to look at differences between the presence and the non-presence of adrenergic stimuli during hypothermia on some cardiovascular system parameters as CO, MAP, and HR during hypothermia affected by infusions of epinephrine compared to saline-infusions. By comparing a group of rats given epinephrine to promote stress reactions (BP only raised slightly compared what happens in real life hypothermia) with a group of rats not experiences this stress one wanted to see the outcome on both hypothermic and posthypothermic hemodynamic and vascular values.

Methods

Caring of animals before study

Wistar rats from B&K Universal, Sollentula, Sweden. Age 60 days, sex male, and weight 250 g. Microbiological status according to FELASA recommendation. Quarantined for one week. Housing during experiments: kept behind bars with Beekay bedding made in Meldal, Norway. Room's temperature and humidity regulated (21°C ± 1°C, 55% ± 10%), 17 air changes per hour. Fluorescent lightning c.250 Lux at 1 m above the floor from 08.00 to 20.00. Feeding: Rat & Mouse Standard Diet manufactured by B&K Universal ltd. (Grimston, Aldborrough, East Yorkshire, England, HU 114QE), made in Uppsala, Sweden. Drinking: free access to water. Experiments performed in March 2001 over a period of 1 day using 14 rats.

Anesthesia, care of animals during study, and method used in the experiments

The animals were anesthetized with sodium pentobarbital, 50 mg·kg⁻¹ body weight, i.p., followed by continuos i.v. infusion of 7,5 mg·kg⁻¹·h⁻¹. Since hypothermia itself acts as anesthetics, and also since drug metabolism would be expected to decrease in hypothermia, pentobarbital infusion was discontinued in animals undergoing hypothermia as soon as cooling were started. The animals were observed for signs of discomfort during hypothermia and rewarming in order to give supplementary anesthesia if needed. However, no additional pentobarbital was given because the animals appeared anesthetized, i.e. remained asleep and showed no signs of discomfort. The rats were placed on the operating table in a supine position. The trachea was opened, a tracheal tube inserted, and respiration supported below 20°C during cooling by a volume-controlled, small-animal respirator (New England Medical Instruments, medway, Mass.), using room air. Arterial blood pH and blood gases were determined by a Radiometer ABL 1 acid-base laboratory (Radiometer, Copenhagen, Denmark), and all blood samples were analyzed at 37°C and corrected actual core temperature.

The animals were cooled and rewarmed by circulating cold or warm water through U-shaped polyethylene tubes placed in the esophagus and lower bowels. In addition, the operating table, which was made of hollow aluminum, was circulated. A change in core temperature of ~1°C/2,7 min was achieved by this method. Core temperature was recorded by a thermocouple positioned in the aortic arch.

Two groups of male Wistar rats were cooled to 15°C and remained at this temperature for one hour before rewarming. Group I (n=7) received intravenous infusion with Epinephrine 1 µg/minute for 1 hour during cooling to 28, a dose that elevated cardiac output (CO) by approximately 20% at 37°C. Group II (n=7) received saline infusion. Heart rate (HR) and mean arterial pressure (MAP) were recorded continuously through a saline-filled catheter in a femoral artery, the left ventricle was catheterized via the right common carotid artery and parameters of left ventricular function were recorded (LVSP, LVEDP, and derivatives LV dP/dt$_{max}$ and LV dP/dt$_{min}$). CO was measured by the thermodilution technique. The epinephrine given elevated the pre-hypothermic CO by approximately 20%, and thus simulates a real life "light" stress situation. Results are presented as mean ± SEM.

Results

Compared to their control values all hemodynamic variables were measured lowered after 1 hour at 15°C in both groups. Interestingly, MAP, CO, left ventricular systolic pressure (LVSP), LV dP/dt$_{max}$ and LV dP/dt$_{min}$ (fig. 1A, C, E, F), all appeared lower in the Epinephrine group (Group I) compared to the saline group (Group II).

Comparing pre-hypothermic hemodynamic control values (before epinephrine or saline infusions were started) with those after rewarming we found in Group II that these variables either returned to control: HR, MAP, CO, LVSP, LV dP/dt$_{max}$ and LV dP/dt$_{min}$, or differed significantly from controls: TPR 0,84±0,07 vs. 0,67±0,03 mmHg/ml/min (fig. 1D and table 1). SV (fig. 1B) was slightly, but not significant, lowered from 0,38±0,02 to 0,46±0,03 ml/beat (17%).

After rewarming, in contrast to the saline infusions group, the animals that received epinephrine infusions showed a significant lowering of CO: 194±13 vs. 144±8 ml/min, and a significant increase in TPR:0,61±0,04 vs. 0,810,04 mmHg/ml/min compared to prehypothermic values. The following variables did not differ significantly from pre-hypothermic values, but showed a clear tendency towards decreasing: SV 0,48±0,01 vs. 0,34±0,02 ml/beat, LV dP/dt$_{max}$ 8061±630 vs. 6660±665 mmHg/s.

Discussion

The present study shows a reduction of the hemodynamic variables after rewarming of rats having been exposed to a modest adrenergic stimulus during the first phase of cooling to 15°C when compared to a control group of rats that not have been exposed to an adrenergic stimulus. In addition, in the group receiving adrenergic stimulus a tendency towards reduced hemodynamic functions during 1 hour at 15°C were seen when compared to the control animals.

This study was performed in order to explore the clinical opinion, although based on anecdotic tellings, that an absence of physical discomfort and stress during cooling might protect the victim of accidental hypothermia in a way that leads to a more successful recovery

during and after rewarming. It must be pointed out that in the present experiment only a small adrenergic stimulus was applied, and in order to mimic this adrenergic stimulus an epinephrine infusion was used which increased cardiac output by 25% at 37°C. This stimulus is in essential contrast to a spontaneous adrenergic stimulus during cold exposure, which brings about a 200 – 300% increase in CO.

When hypothermia is induced, as during CBP surgery, the hemodynamic values return to pre-hypothermic values after rewarming[41]. A person that is going to have surgery where hypothermia is used as organ protector receives anesthesia before surgery. The anesthesia prevents activation of the sympathetic nervous system, and therefore sympathetic activity such as an increased HR, vasoconstriction and enhanced inotropic effect of the myocardium is not seen[1,28]. Ethanol depressant effect may prevent activation of the sympathetic nervous when exposed to hypothermia, the lack of shivering supporting this. The anesthesia as well prevents sympathetic switch on. Can the reason for a raised critical temperature in ethanol-intoxicated persons and the return of hemodynamic values after rewarming in induced hypothermic patients simply be explained by the sympathetic nervous system not being switch on? One knows that ethanol inhibit the opening of voltage-sensitive Ca^{2+}-channels in neurons[28], whether it inhibits opening of other Ca^{2+}-channels is not known.

Epinephrine and nor-epinephrine produce their effects trough the plasma membrane receptors β_1, β_2, β_3, α_1, and α_2. The biological effects of all three β-receptors are exerted through the stimulation of adenylyl cyclase using cAMP as their second messenger, the β-receptors are coupled to a stimulatory G protein (G_s). The α_2-receptor is coupled to an inhibitory G protein (G_i) and binding will decrease the levels of camp and inhibit its effects. The α_1-receptor, which nor-epinephrine produces vasoconstriction in most organs through, is coupled to the phosphatidylinositol system where membrane phospholipids are broken down and serve as second messengers and act to mobilize calcium ions from the mitochondria and endoplasmatic reticulum thus raising the levels of intracellular calcium.

When a cardiac muscle cell is depolarized voltage-sensitive calcium channels in the cell membrane are activated and allows calcium to enter the cell, the channels are quickly inactivated thus preventing further influx of calcium[1,12,28]. Depolarization of cardiac muscle cells also causes release of calcium from the sarcoplasmatic reticulum. The calcium ions bind

to and transform the conformation of the troponin complex on the actin filament thus promoting cross-bridging of the actin- and myosin-filaments allowing muscle contraction to appear. Epinephrine, among other things, increases the numbers of calcium-channels in the cell membrane. For the muscle fibers to relax the intracellular calcium levels must be lowered. The most important mechanism for this is the Na^+-Ca^{2+} -exchanger in the plasma membrane where one calcium-ion is extruded while three sodium-ions are brought into the cytosol; this exchanger is directly dependent upon the Na^+-K^+-exchanger because the sodium ions themselves need to be extruded to maintain the membrane potensial[ibid].

In a study on fetal sheep in utero temperatures were lowered from approximately 41°C to 35°C and differences between a non-selective ß-agonist, a non-selective α-antagonist, and saline-infusions on HR and MAP were examined[11]. HR and MAP had increased 20% and 8% respectively before the drugs were administered some 30 minutes into cooling. The effect of the adrenergic blockade was a decrease in HR by approximately 33% in the ß-adrenergic blockade while remaining the same in the α-adrenergic blockade; the MAP fell 9% in the α-adrenergic blockade and was not affected by ß-adrenergic blockade[ibid]. The decrease in MAP, and also in HR and CO, with administration of adrenergic blocks is supported a study on Mongrel dogs[26].

As mentioned earlier, in the study on fetal sheep in-utero both plasma nor-epinephrine and plasma epinephrine rose during cooling[11]. These findings are supported by others that argues that increased levels of catecholamines is the reason for the increased sensitivity seen in both α-receptors at hypothermic temperatures[9], and also a reported increase in the affinity for ß-adrenoceptor can be explained by higher levels of the two hormones at low temperatures[46]. On the other hand has an in vivo studies on dogs during deep hypothermia (25°C) shown a decreased adrenergic effect to a β-agonist during hypothermia with respect to both chrontropi and inotropi[45], the already high levels of intracellular Ca^{2+} induced by hypothermia can explain the weak effect of the β-agonist at 25°C because no further in the calcium levels where possible[ibid].

We are unable to explain the exact pathophysiological effects of epinephrine in our experiments, but from other studies[41], we feel sure that an increase of intracellular Ca^{2+} of myocytes is essential in the pathogenesis of reduced hemodynamic function following

hypothermia and rewarming. At hypothermic temperatures increased contractile strength in the muscle fibers of the left ventricle of dogs[36] is explained by increased levels intracellular calcium ions of thus supporting other findings of raised Ca^{2+}-levels. The increased intracellular levels of Ca^{2+} in hypothermic conditions are explained by a delayed inactivation of the calcium-channels in the cell-membrane[45], studies have also shown that the efficacy of the Na^+-K^+-exchanger decreases during hypothermia thus promoting further increase in the intracellular levels of calcium.

Conclusion

The present study demonstrates that, in essential contrast to non-epinephrine stimulated control rats, following a moderate sympathetic stimulus during cooling a significant depression of cardiac function occur during hypothermia as well as after rewarming. Thus, following a moderate sympathetic stimulation during cooling, a direct depressive effect on myocardial contractile function is suggested.

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Attachment 1:

An schematic model of calcium-ion transport in the cardiomyocite
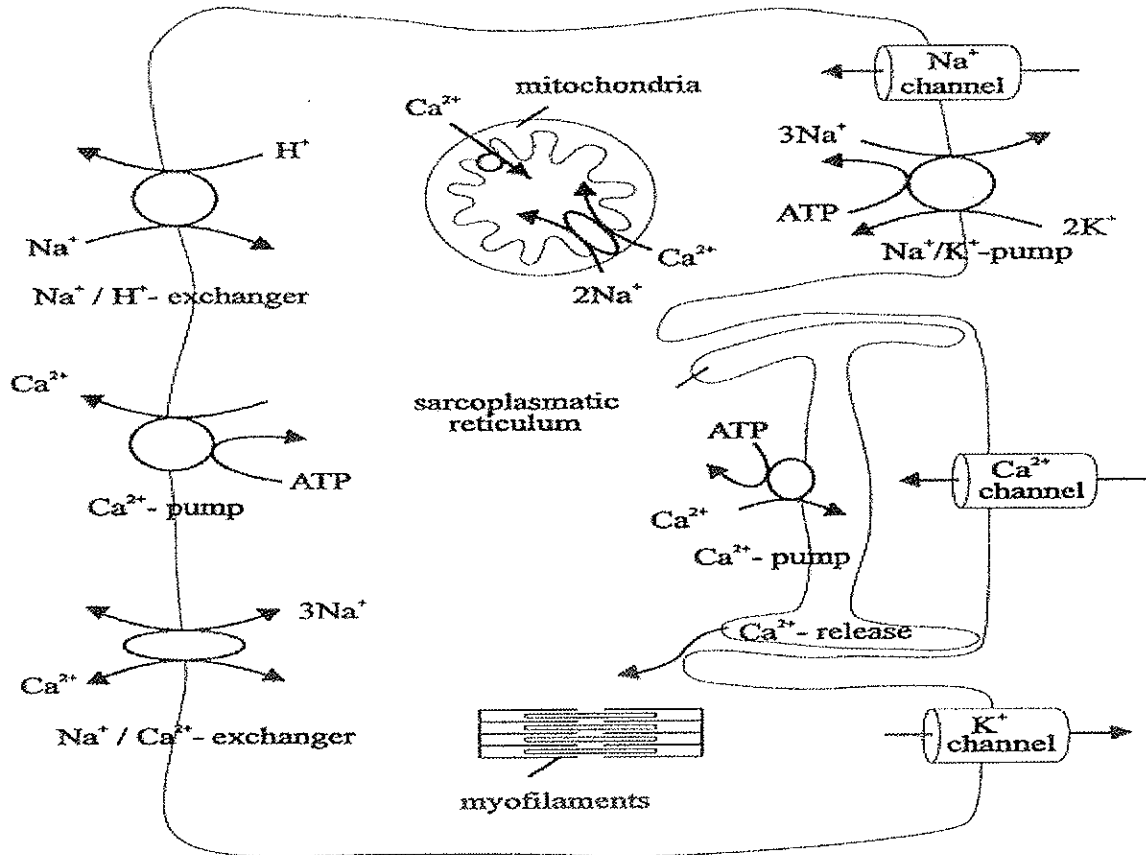
Attachment 2:

		Prehypothermic before epinephrine	Prehypothermic 1µg/min epinephrine	Hypothermic 15°C (1h)	Posthypothermic
HR, (beats/min)	Epi.	405±26‡	472±24	47±3*‡	418±23*†‡
	Placebo	406±13	-	49±2*	434±16†
MAP (mmHg)	Epi.	115±5‡	154±4	35±4*‡	115±4†‡
	Placebo	122±4	-	42±3*	136±7†
CO¹, (ml/min)	Epi.	194±13‡	244±16	124±8*‡	144±8*‡
	Placebo	185±8	-	124±14*	168±16
SV¹, (ml/beat)	Epi.	0,48±0,01	0,52±0,02	1,39±0,12*‡	0,34±0,02†
	Placebo	0,46±0,03	-	1,5±0,17*	0,38±0,02†
TPR¹, (mmHg/ml/min)	Epi.	0,61±0,04	0,65±0,05	0,34±0,03*‡	0,81±0,04*†‡
	Placebo	0,67±0,03	-	0,41±0,05*	0,84±0,07*†‡
LVSP, (mmHg)	Epi.	159±8‡	210±6	63±5*‡	144±4†‡
	Placebo	172±6	-	79±7*	175±4†
LV dP/dt max, (mmHg/s)	Epi.	8061±630‡	11638±858	1023±137*‡	6660±665†‡
	Placebo	9012±308	-	1132±117*	8967±483†
LV dP/dt min, (mmHg/s)	Epi.	-6612±342‡	-9374±448	-335±59*‡	-6209±520†‡
	Placebo	-8151±436	-	-389±66*	-7835±492†

Table 1. *Hemodynamic values under hypothermia and rewarming.*
Values are mean ± SEM (n=7). HR, heart rate; MAP, mean aortic pressure; CO, cardiac output; SV, stroke volume; TPR, total peripheral resistance; LVSP, left ventricular systolic pressure; LV dP/dt max, maximum rate of left ventricular pressure rise; LV dP/dt min, maximum rate of left ventricular pressure decline. *P<0,05 significantly different compared with prehypothermic values; †P<0,05 significantly different compared with hypothermia; ‡P<0,05 significantly different compared with epinephrine values. ¹ - for CO, SV, TPR values hypothermic at 20°C during rewarming.
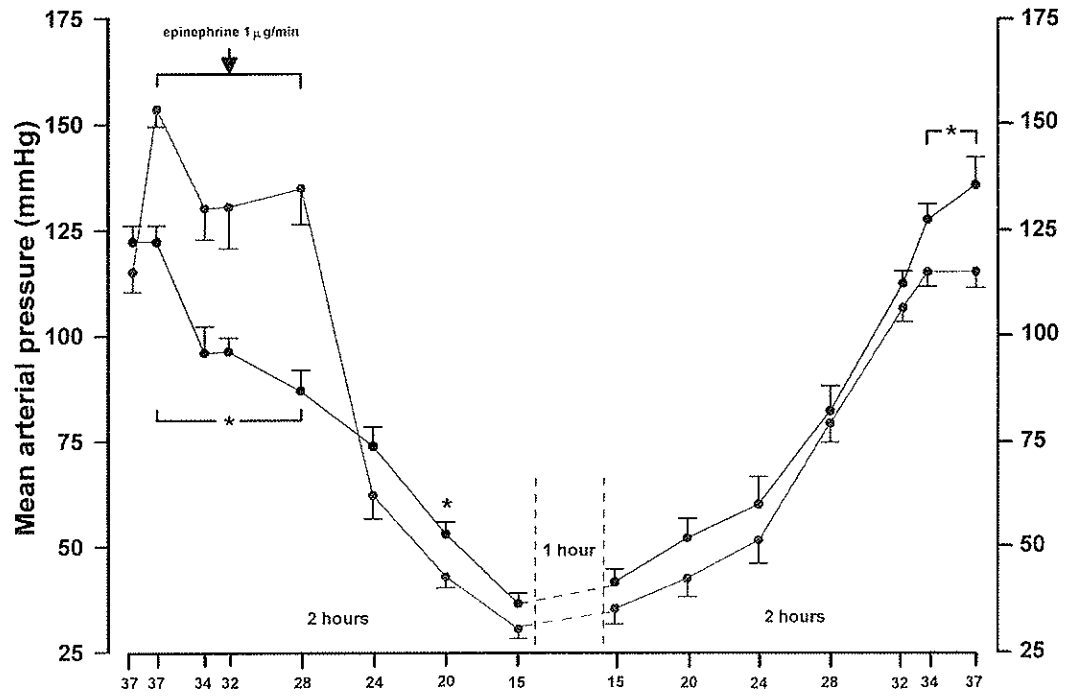
Attachment 3:

Explanation to graphs 1A, 1B, 1C, 1D, 1E, and 1F

Fig.1 Cardiac output, stroke volume, mean arterial pressure, total peripheral resistance (TPR); left ventricular systolic pressure (LVSP) and maximum rate of left ventricular pressure rise (LV dP/dt max) expressed as a function of temperature during cooling and rewarming without ● and with epinephrine infusion between 37 – 28 °C ● ; Values are mean ± SEM. *p<0.05 vs. epinephrine group; ⊥p<0.05 vs. prehypothermic control values.
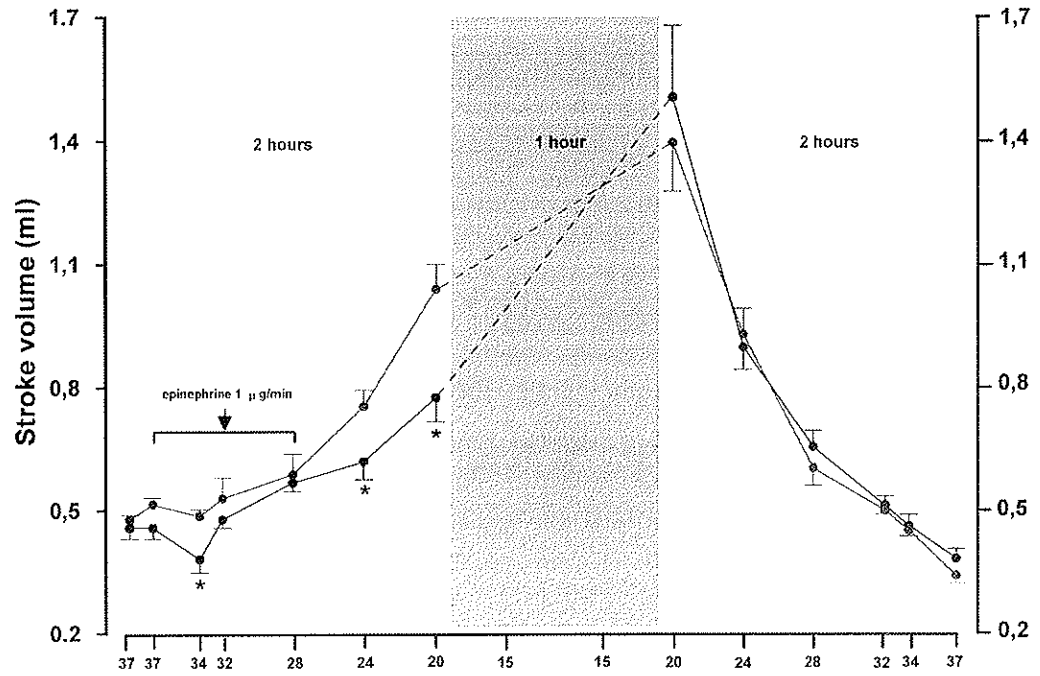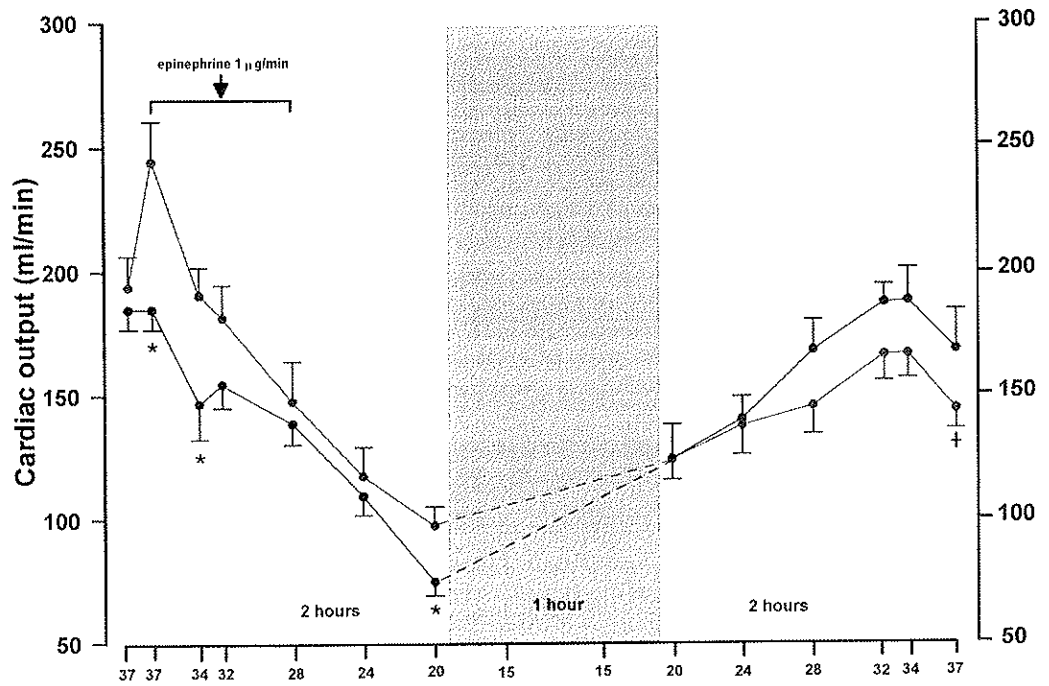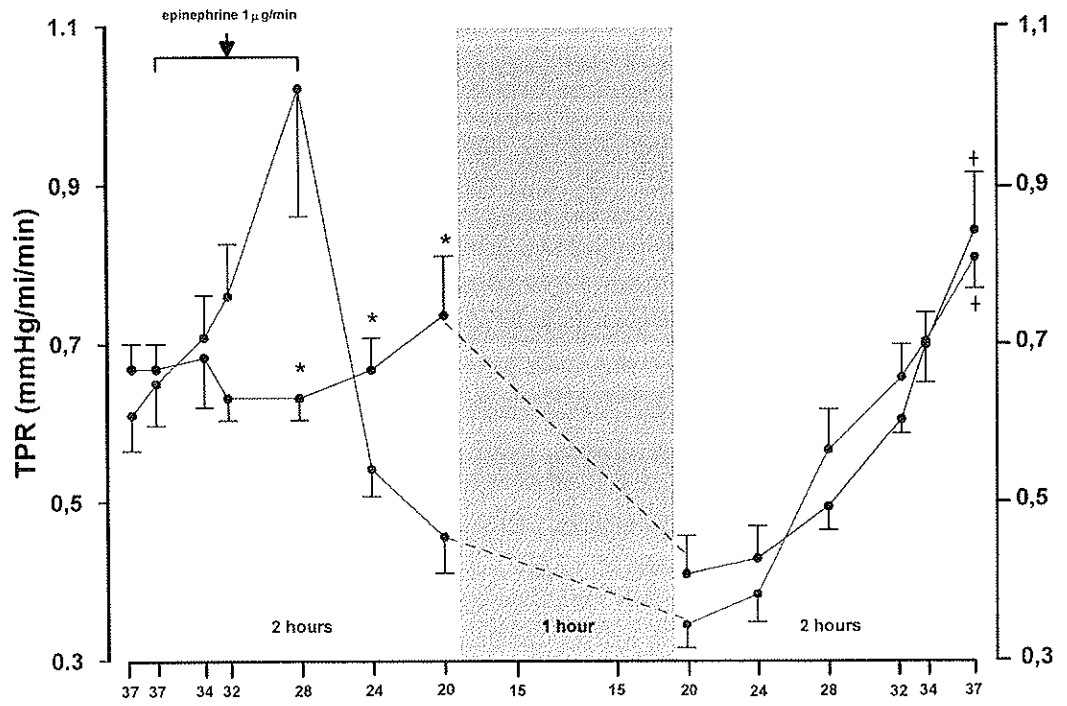
Attachment 4:

Fig. 1A

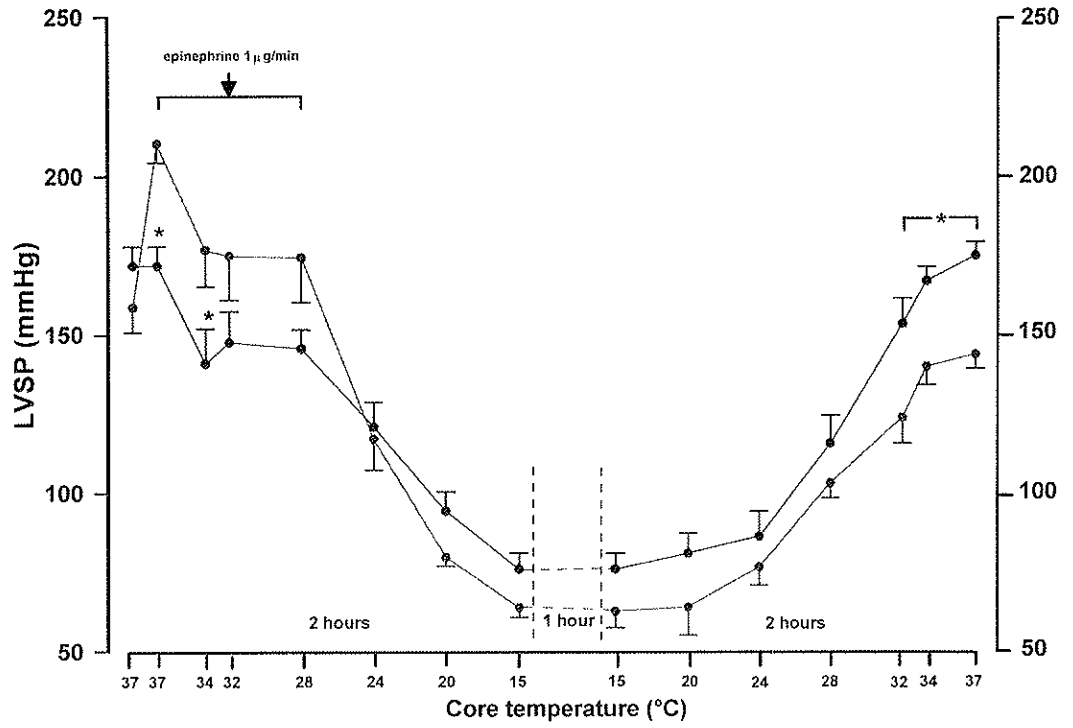Attachment 5:

Fig. 1B

Attachment 6:

Fig. 1C

Attachment 7:

Fig. 1D

Attachment 8:

Fig. 1E

Attachment 9:

Fig. 1F