Cryobiology xxx (2014) xxx–xxx

Contents lists available at ScienceDirect

Cryobiology

journal homepage: www.elsevier.com/locate/ycryo

Cardiovascular effects of levosimendan during rewarming from hypothermia in rat ☆

Erik Sveberg Dietrichs [b,a,*], Brage Håheim [a,c], Timofei Kondratiev [a], Gary C. Sieck [c,a], Torkjel Tveita [a,d]

[a] Anesthesia and Critical Care Research Group, Institute of Clinical Medicine, UiT, The Arctic University of Norway, 9037 Tromsø, Norway
[b] Dept. of Research and Education, Norwegian Air Ambulance Foundation, 1441 Drøbak, Norway
[c] Dept. of Physiology and Biomedical Engineering, Mayo Clinic College of Medicine, Rochester, MN 55905, USA
[d] Division of Surgical Medicine and Intensive Care, University Hospital of North Norway, 9038 Tromsø, Norway

ARTICLE INFO

Article history:
Received 8 August 2014
Accepted 22 September 2014
Available online xxxx

Keywords:
Accidental hypothermia
Hypothermia
Levosimendan
Rewarming
Rewarming shock
PDE3 inhibitor
Cardiovascular support
Troponin I
Troponin phosphorylation
Calcium sensitizer

ABSTRACT

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

Introduction

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

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

☆ *Statement of funding:* The Norwegian Research Council supported this study.

* Corresponding author at: Anesthesia and Critical Care Research Group, Department of Clinical Medicine, Faculty of Health Sciences, University of Tromsø, 9037 Tromsø, Norway.

E-mail addresses: erik.sveberg.dietrichs@norskluftambulanse.no (E.S. Dietrichs), bha035@post.uit.no (B. Håheim), timofey.kondratiev@uit.no (T. Kondratiev), sieck.gary@mayo.edu (G.C. Sieck), torkjel.tveita@uit.no (T. Tveita).

http://dx.doi.org/10.1016/j.cryobiol.2014.09.007
0011-2240/

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

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

Materials and methods

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

Anesthesia

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

Respiratory support

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

Core cooling and rewarming

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

Hemodynamic measurements

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

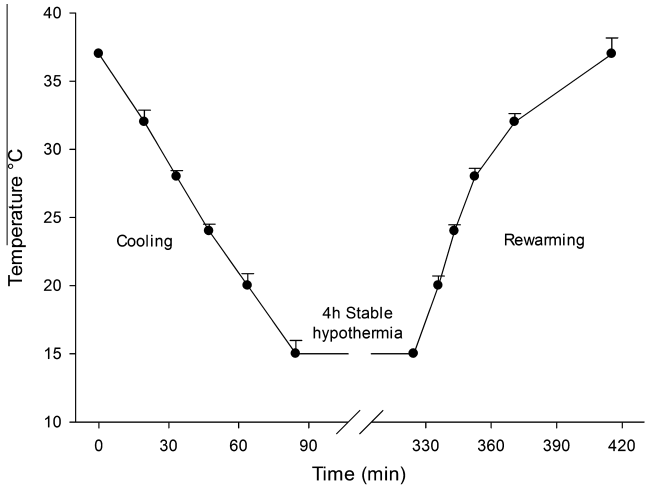

Fig. 1. Temperature profile of the experiments in rats assigned to either of the hypothermic groups, showing the cooling (37–15 °C) and rewarming (15–37 °C) rates and the stable hypothermic period (15 °C): Values are mean ± SEM.

Please cite this article in press as: E.S. Dietrichs et al., Cardiovascular effects of levosimendan during rewarming from hypothermia in rat, Cryobiology (2014), http://dx.doi.org/10.1016/j.cryobiol.2014.09.007