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Negative inotropic effects of epinephrine in the presence of increased β-adrenoceptor sensitivity during hypothermia in a rat model ☆

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ABSTRACT

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

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

Results: In vitro measurements of IC_{50} for propranolol showed a hypothermia-induced increase in β-adrenoceptor sensitivity at 15 °C. Corresponding in vivo experiments at 15 °C showed decreased cardiac output and stroke volume, whereas total peripheral resistance (TPR) increased during Epi infusion, simultaneous with a 4-fold cAMP increase.

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

Introduction

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

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

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

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

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

Materials and methods

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

In vitro beta-adrenoceptor assay

Isolation of cardiomyocytes

Rats ($n = 8$) were sacrificed by an intraperitoneal injection of pentobarbital sodium (220 mg/kg) and fentanyl (50 μg/kg). Hearts were excised and put in ice-cooled, oxygenated Krebs solution before tied to a Langendorff perfusion system. Perfusion rate was 7 ml/min with initial 5 min pure Krebs solution perfusion, followed by addition of 0.6 mg/ml collagenase for 20 min. All solutions were kept at 37 °C. Hearts were removed from the Langendorff system and cut into smaller pieces, before suspended in Krebs solution containing 10 mg/ml albumin and 0.6 mg/ml calcium (calcium solution), kept on a shaking plate for 10 min and minced in collagenase solution. Preparations were continually gassed with medical air (with added 5% CO_2). Following centrifugation, collagenase solution was gradually replaced with calcium solution. In the final cell suspension, rod-shaped cells were counted as fraction of total cell number. This cell suspension was divided in 2 groups, 1 hypothermic group (15 °C) and 1 normothermic group (37 °C) (Table 1).

β-Adrenoceptor labeling

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

Measurement of β-adrenoceptor sensitivity

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

In vivo hemodynamic monitoring and cardiac tissue cAMP measurements

Experimental protocol

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