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Rapid communication

Cardioprotection by remote ischemic preconditioning of the rat heart is mediated by extracellular vesicles ☆

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ABSTRACT

Remote ischemic preconditioning (RIPC) of the heart is exerted by brief ischemic insults affected on a remote organ or a remote area of the heart before a sustained cardiac ischemia. To date, little is known about the inter-organ transfer mechanisms of cardioprotection by RIPC. Exosomes and microvesicles/microparticles are vesicles of 30–100 nm and 100–1000 nm in diameter, respectively (collectively termed extracellular vesicles [EVs]). Their content of proteins, mRNAs and microRNAs, renders EV ideal conveyors of inter-organ communication. However, whether EVs are involved in RIPC, is unknown. Therefore, here we investigated whether (1) IPC induces release of EVs from the heart, and (2) EVs are necessary for cardioprotection by RIPC. Hearts of male Wistar rats were isolated and perfused in Langendorff mode. A group of donor hearts was exposed to 3 × 5-5 min global ischemia and reperfusion (IPC) or 30 min aerobic perfusion, while coronary perfusates were collected. Coronary perfusates of these hearts were given to another set of recipient isolated hearts. A group of recipient hearts received IPC effluent depleted of EVs by differential ultracentrifugation. Infarct size was determined after 30 min global ischemia and 120 min reperfusion. The presence or absence of EVs in perfusates was confirmed by dynamic light scattering, the EV marker HSP60 Western blot, and electron microscopy. IPC markedly increased EV release from the heart as assessed by HSP60. Administration of coronary perfusate from IPC donor hearts attenuated infarct size in non-preconditioned recipient hearts (12.9 ± 1.6% vs. 25.0 ± 2.7%), similarly to cardioprotection afforded by IPC (7.3 ± 2.7% vs. 22.1 ± 2.9%) on the donor hearts. Perfusates of IPC hearts depleted of EVs failed to exert cardioprotection in recipient hearts (22.0 ± 2.3%). This is the first demonstration that EVs released from the heart after IPC are necessary for cardioprotection by RIPC, evidencing the importance of vesicular transfer mechanisms in remote cardioprotection.

1. Introduction

Remote ischemic conditioning (RIPC), where a remote area of the heart or another organ is submitted to brief cycles of ischemia–reperfusion, protects the heart against a lethal ischemic insult with efficiency comparable to that of classic in-situ ischemic protocols [1,2]. Although effector pathways of RIPC have been well described, it is currently unclear how cardioprotective signals are propagated between organs [3]. Humoral and neuronal aspects have been hypothesized, but vesicular transfer mechanisms have not been evidenced in inter- or intra-organ communication of RIPC signals.

Exosomes and microvesicles/microparticles (collectively termed extracellular vesicles, EVs) are membrane-bound structures secreted by a wide range of mammalian cell types via distinct mechanisms [4,5]. Since EVs contain a high concentration of RNAs and proteins, and since EVs can be secreted and specifically taken up by other cells, they are prime medium for intercellular signal transfer mechanisms [5]. Thus, it is not surprising that EVs have been shown to modulate several essential cellular functions, including cell survival mechanisms [6,7]. However, to date, it is not known whether EVs are involved in the transmission of cardioprotective signals in ischemic conditioning maneuvers, particularly, their role in the propagation of RIPC has never been studied.

Therefore, here we aimed to investigate whether the release of EVs from the heart is induced by preconditioning stimuli; and to test if EVs are necessary for RIPC-induced cardioprotection by assessing that RIPC can be exerted in the presence and absence of EVs.

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2. Materials and methods

This investigation conforms to the Guide for the Care and Use of Laboratory Animals published by the US National Institutes of Health (NIH publication No. 85–23, revised 1996) and was approved by the animal ethics committee of the Semmelweis University, Budapest, Hungary.

2.1. Experimental setup, heart perfusion protocol, and assessment of infarct size

Male Wistar rats (250–350 g) were anesthetized by 85 mg/kg ketamine and 10 mg/kg xylazine and heparinized. Hearts were isolated and perfused in Langendorff mode with 37 °C Krebs–Henseleit solution for 20 min for stabilization; then hearts were randomized to the following groups. Perfusate donor hearts either received aerobic perfusion for an additional 30 min (CON) or were exposed to 3 × 5-5 min ischemia and reperfusion (PRE). Perfusate recipient hearts were perfused with collected perfusate from either CON or PRE hearts (CON PERF and PRE PERF, respectively). Another group of hearts received perfusate of PRE hearts which had been previously depleted of EVs (DEPL PERF). All hearts were then exposed to a 30 min global ischemia and 2 h reperfusion (Fig. 1).

Hearts then were cut into 6–8 slices, slices were weighed, and infarct size was assessed by TTC-staining. Infarct size was expressed as a percentage of the total heart weight.

2.2. Isolation of EVs and EV depletion

EVs were isolated from collected coronary perfusates by filtration and differential centrifugation. Briefly, perfusates were dialyzed against 0.45% saline containing 5 mM EDTA for 4 h at room temperature then vacuum-distilled to 40 mL. Concentrated perfusates were filtered through 800 nm filter (Merck, Darmstadt, Germany) and centrifuged at 12,200 ×*g* for 20 min at 4 °C. Pellets were saved as microvesicle/microparticle fraction. Then supernatants were filtered through 200 nm filter (Merck, Darmstadt, Germany) and centrifuged at 100,000 ×*g* for 90 min at 4 °C. Pellets were saved as exosome-rich pellet and the supernatant was saved as EV-depleted perfusate. EV-depleted perfusates were then reconstituted to their original volume with Krebs–Henseleit solution and used in heart perfusion experiments.

2.3. Characterization and assessment of quantity and size distribution of EVs

Isolated vesicles were visualized by transmission electron microscopy. Vesicle pellets were fixed with 4% formaldehyde, postfixed in 1% OsO_4. EVs were dehydrated in graded ethanol, block-stained with 1% uranyl acetate in 50% ethanol, and embedded in Taab 812 (Taab Laboratories, Aldermaston, UK). Ultrathin sections were cut and then analyzed with a Hitachi 7100 electron microscope.

Hydrodynamical average particle size of EVs in perfusates was measured by Dynamic Light Scattering (DLS) apparatus Zetasizer Nano ZS (Malvern Instruments, Malvern Hills, UK) (n = 3–4).

The presence and amount of EVs were assessed by HSP60 immunoblots from vesicular pellets and EV-depleted perfusates.

For a detailed Methods section please see Supplementary data online.

2.4. Statistical analysis

Values are expressed as mean ± SEM. One way analysis of variance (ANOVA) followed by Fisher LSD post-hoc test was used to determine differences in infarct size.

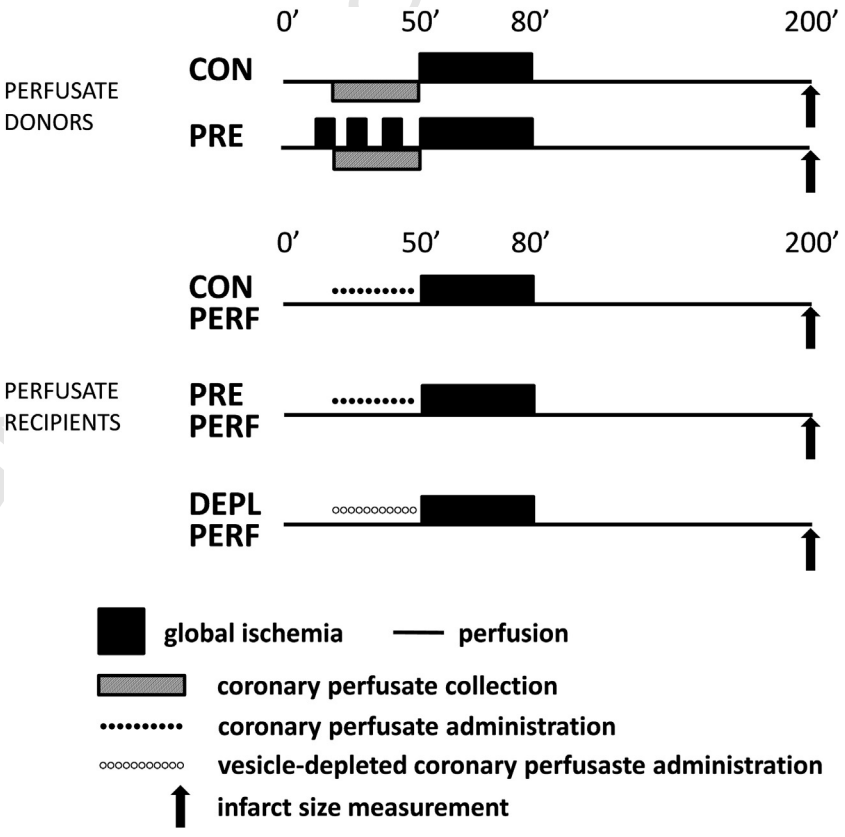

Fig. 1. Experimental protocol of Langendorff-perfused rat hearts. CON: control; PRE: preconditioned; PERF: perfused; DEPL: depleted.

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