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Enhancement of crystalloid cardioplegic protection by structural analogs of apelin-12

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ABSTRACT

Background: C-terminal fragments of adipokine apelin are able to attenuate myocardial ischemia–reperfusion (I/R) injury, but whether their effects are manifested during cardioplegic arrest remain obscure. This study was designed to evaluate the efficacy of natural apelin-12 (H-Arg-Pro-Arg-Leu-Ser-His-Lys-Gly-Pro-Met-Pro-Phe-OH, A12) and its novel structural analogs (H-(N^{α}Me)Arg-Pro-Arg-Leu-Ser-His-Lys-Gly-Pro-Nle-Pro-Phe-OH, AI, and N^G-Arg(N^GNO_2)-Pro-Arg-Leu-Ser-His-Lys-Gly-Pro-Nle-Pro-Phe-NH_2, AII) as additives to crystalloid cardioplegia and explore benefits of early reperfusion with these peptides.
Methods: Isolated working rat hearts subjected to normothermic global ischemia and further reperfusion were used. St. Thomas' Hospital cardioplegic solution No.2 (STH2) containing 140 μM A12, AI, or AII was infused for 5 min at 25°C before ischemia. In separate series, peptide administration was used for 5 min after ischemia. Metabolic state of the hearts was evaluated by myocardial content of high energy phosphates and lactate. Lactate dehydrogenase (LDH) leakage was assessed in myocardial effluent on early reperfusion.
Results: Addition of the peptides to STH2 enhanced functional and metabolic recovery of reperfused hearts compared with those of control (STH2 without additives). Cardioplegia with analog AII was the most effective and accompanied by a reduction of postischemic LDH leakage. Infusion of A12, AI, or AII after ischemia improved the majority indices of cardiac function and metabolic state of the heart by the end of reperfusion. However, the overall protective effect of the peptides was less than when they were added to STH2.
Conclusions: Enhancement of apelin bioavailability may minimize myocardial I/R damage during cardiac surgery. Structural analogs of A12 are promising components of clinical cardioplegic solutions.

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1. Introduction

Ischemia–reperfusion (I/R) injury is the major problem in open-heart operations, including heart transplantation. Various pharmacologic interventions have been tested as adjunctive therapies to further improve cardioplegic protection and reduce myocardial injury during reperfusion. Strategies for additives to cardioplegic and reperfusion solutions include calcium antagonists, NO donors, antioxidants, metabolic protectors, osmotic agents, and Na^+/H^+ exchange inhibitors [1]. Inspite of progress in myocardial protection achieved over the past years, the search of appropriate new additives and formulations for cardiac surgery remains an important task. Targeting the molecular mechanisms of

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endogenous cardioprotection by preconditioning and post-conditioning mimetics is a promising approach to this issue. Certain pharmacologic agents engage the cytoprotective signaling pathways via activation of G-protein-coupled receptors and kinase cascades that converge on inhibition of the mitochondrial permeability transition pore [2]. One of them is the adipocytokine apelin, the endogenous ligand for the G-protein-coupled APJ receptor [3]. Apelin is produced as a 77 amino acid prepropeptide, which is cleaved to shorter biologically active C-terminal fragments. To date, apelin and APJ receptors have been shown to play an important role in the maintenance of cardiovascular homeostasis and protection against I/R injury [4].

Apelin-12 (A12) and apelin-13 are the most potent C-terminal fragments of the polypeptide and possess a high affinity to APJ receptor and bioactivity *in vivo* [5,6]. However, apelin peptides rapidly cleared from the circulation with a half-life of no longer than several minutes [7]. It is believed that this is due to their rapid hydrolysis by various peptidases including angiotensin-converting enzyme 2 [8]. Based on these observations, many researchers have attempted to create apelin analogs, which possess increased binding with the receptor and higher stability than the parent peptides [9–11]. To increase stability of A12 during storage and its resistance to peptidase cleavage, we synthesized several structural analogs of A12 [12]. Treatment with some of these original peptides is able to attenuate myocardial I/R injury in *ex vivo* and *in vivo* rat heart. It is essential that the overall protective effect exerted by these compounds is comparable with that of A12 [13,14]. These data suggest that addition of drug-like apelin analogs to cardioplegic solutions may enhance cardioprotection in human clinical and surgical settings. This study was designed to explore the efficacy of novel structural analogs of A12 added to St. Thomas' Hospital cardioplegic solution No 2 (STH2) in isolated working rat hearts subjected to global ischemia. The other aim of the study was to evaluate benefits of early reperfusion with A12 synthetic derivatives using the same model. We evaluated effects of the peptides on functional and metabolic recovery of reperfused hearts by comparing them with the action of the natural peptide A12.

2. Materials and methods

2.1. Synthesis of A12 and its analogs

Peptides A12, AI, and AII were synthesized by the automatic solid phase method using an Applied BioSystems 431A peptide synthesizer (Germany, Weiterstadt) and Fmoc technology as previously described [12]. We replaced easily oxidized methionine by norleucine and included N^{α}-methylarginine moiety in N-terminus of A12 to increase its stability (analog AI). To protect A12 from carboxypeptidase action and to endow the peptide properties of NO donor, phenylalanine residue in C-terminus was amidated and the nitro group was placed into the guanidinum group of arginine moiety (analog AII). The synthesized peptides were purified by preparative high performance liquid chromatography and identified by ^{1}H-NMR spectroscopy and mass spectrometry (Table 1).

Table 1 – Structure of A12 and its analogs.

Peptide	Structure	Mw, g/mol
A12	H-Arg-Pro-Arg-Leu-Ser-His-Lys-Gly-Pro-Met-Pro-Phe-OH	1422, 7
Analog AI	H-(**N^{α}Me**)Arg-Pro-Arg-Leu-Ser-His-Lys-Gly-Pro-**Nle**-Pro-Phe-OH	1418, 7
Analog AII	N^G-Arg(**$N^G NO_2$**)-Pro-Arg-Leu-Ser-His-Lys-Gly-Pro-**Nle**-Pro-Phe-**NH_2**	1448, 7

The substitutions are shown in bold.

2.2. Animals

Male Wistar rats weighing 290–340 g were used in this study. All animals were housed in cages in groups of three, maintained at 20–30°C with a natural light–dark cycle, and had free access to standard pelleted diet (Aller Petfood, St. Petersburg, Russia) and tap water. The care and use of the animals were conducted in accordance with the European Convention for the Protection of Vertebrate Animals used for Experimental and other Scientific Purposes (No 123 of 18 March 1986).

2.3. Heart perfusion

Rats were heparinized by intraperitoneal injection (1600 IU/kg body weight) and anesthetized with urethane (1.3 g/kg body weight). Hearts were excised and immediately placed into ice-cold Krebs–Henseleit bicarbonate buffer (KHB) until the contraction stopped. The aorta was then cannulated, and Langendorff perfusion was performed at a constant pressure equivalent to 75 cm H_2O for 15 min. Working perfusion was performed according to a modified method of Neely *et al.* [15] under constant left atrium pressure and aortic pressure of 20 and 100 cm H_2O, respectively. KHB containing (in millimolar): NaCl 118; KCl 4.7; $CaCl_2$ 3.0; Na_2EDTA 0.5; KH_2PO_4 1.2; $MgSO_4$ 1.2; $NaHCO_3$ 25.0; glucose 11.0 was oxygenated with a mixture of 95% O_2 and 5% CO_2; pH was 7.4 $\pm$ 0.1 at 37°C; it was passed through a 5-μm Millipore filter (Millipore Corp, Bedford, MA) before use. A needle was inserted into the left ventricular (LV) cavity to register LV pressure via a Gould Statham P50 transducer, SP 1405 monitor, and a Gould Brush SP 2010 recorder (Gould, Oxnard, CA). The contractile function intensity index was calculated as the LV developed pressure-heart rate product (LVDP $\times$ HR), where LVDP is the difference between LV systolic and LV end-diastolic pressure. Cardiac pump function was assessed by cardiac output, the sum of aortic output, and coronary flow. Coronary resistance was calculated as aortic pressure/coronary flow.

2.4. Experimental design

The steady state values of cardiac function were recorded after preliminary 20-min perfusion in the working mode. In control, after preliminary working perfusion, a 5-min infusion of STH2 at 25°C was applied in Langendorff mode at a flow rate of 4 mL/min for 5 min. The hearts were subjected to 40-min normothermic global ischemia followed by 5-min Langendorff reperfusion at a rate of 4 mL/min with subsequent 25-

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