Administration of 5-Hydroxydecanoate, a Selective Inhibitor of Mitochondrial ATP-sensitive Potassium Channels, Inhibits Apelin-Induced cardioprotection in Ischemia/Reperfusion Model of Male Rats

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Abstract

Apelin, a recently discovered endogenous peptide has shown to protect myocardium against infarction in the animal model. The aim of this study was to evaluate the effects of mitochondrial ATP-sensitive potassium channels inhibition on myocardial protection afforded by apelin treatment during ischemia period. Thirty-two male Wistar rats were divided into four groups; (1) Ischemia/reperfusion, (2) Apelin + ischemia/reperfusion, (3) 5-hydroxydecanoate + Apelin + ischemia/reperfusion and (4) 5-hydroxydecanoate + ischemia/reperfusion. Hemodynamic parameters and infarct size were measured for all groups. There were no significant differences in hemodynamic functions during ischemia and reperfusion periods between the experimental groups. Further, there was a decrease in infarct size in apelin group when compared to ischemia/reperfusion group. However, selective inhibition of mitochondrial ATP-sensitive potassium channels by administration of 5-hydroxydecanoate, diminished the infarct-sparing effect of apelin. These findings suggest that apelin-induced protection was removed by using 5-hydroxydecanoate as a selective inhibitor of mitochondrial ATP-sensitive potassium channel.

Introduction

Ischemic/Reperfusion injury remains one of the primary causes of the myocardial cell death. Rapid restoration of blood flow, either by thrombolysis or by percutaneous coronary interventions is the most efficient and effective plan in patients with temporary coronary blood flow occlusion. Reperfusion halts the ischemic cell death but at the cost of severe toxic changes in cardiac cells leading to a ‘no reflow’ phenomena (1).

Since the last decade, quite a many advances have been made in order to diminish these additional lethal effects brought about by reperfusion (2, 3). Among
these strategies, Ischemic Post conditioning (IPostC; brief intermittent episodes of blood flow occlusion and reperfusion, following a sustained ischemic event) has shown to induce numerous protective effects including: reduction in infarct size, apoptosis, endothelial dysfunction, arrhythmic effect, neutrophil adhesion and stunning (4-8). Pharmacological PostC with various agents has also shown to promote cardioprotection (8). At the time of reperfusion, administration of adenosine, estradiol, bradykinin, insulin, natriuretic peptides, have shown to induce cardioprotection against myocardial infarction (2, 8, 9). It has been proposed that the ligand-induced G-protein coupled receptor activation is involved in the closure of mitochondrial Permeability Transition Pore (mPTP) and opening of ATP sensitive mitochondrial potassium channels (mK_{ATP}) (8). Emerging evidences suggest that the activation of Protein Kinase C (PKC) leads to the opening of mK_{ATP} channels, which in turn can reduce Ca^{2+} overload and ROS production during ischemic postconditioning (IPost) (10-13). These channels have been seen as terminal effector in both, ischemic preconditioning (IPC) and IPostC, and thus, play a vital role in cardiomyocytes survival.

Apelin, an endogenous vasoactive peptide and an adipokine serves as a ligand for orphan G-protein-coupled receptor APJ (putative receptor protein related to the angiotensin receptor AT1) (12-14). The mRNA encoding apelin and APJ is richly expressed in brain, spinal cord, heart, vessels, lungs, placenta and mammary glands (15). The wide spectrum of apelin’s protective effects covers many aspects of cardiovascular system (16). Apelin has shown to protect myocardium against infarction by facilitating angiogenesis and decreasing the permeability of endothelial cells. Of our particular interest, apelin-13 is the significant regulator of the myocardial response to infarction (17).

Keeping in view the importance of apelin-13 in offering cardioprotection via PKC activation (18), the current study was designed to study the role of mK_{ATP} channels in apelin-induced cardioprotection. To our knowledge, this is the first study revealing relationship between apelin-13 and mKATP channels.

## Materials and Methods

### Animal Care

The experimental protocols followed in this study were conformed to the Guidelines for the Care and Use of Laboratory Animals published by National Institutes of Health (NIH Publication No. 85-23, revised 1996) and further approved by the institutional ethical committee at Tehran University of Medical Sciences (Tehran, Iran). Thirty-two male Wistar rats weighing 200-250 g were housed in an air-conditioned colony room on a 12 hours light-dark cycle at 21–23°C with free access to food and water.

### Experimental Groups

Animals were randomly allotted to one of the four groups (n=8): (1) ischemia/reperfusion group (I/R): animals underwent 30 min ischemia and 60 min reperfusion, (2) Apelin group (AP): I/R was induced along with a bolus dose of apelin-13 (0.01 µg/kg) 15 minutes before reperfusion, (3) 5-hydroxydecanoate – Apelin (5-HD+AP): In addition to IR and Apelin-13, rats also received 5-HD (5 mg/kg) (19), 5 minute before apelin injection and (4) 5-hydroxydecanoate (5-HD): 5-HD was injected (5 mg/kg) during ischemia, 15 minute before reperfusion (Fig. 1).

### Optimal Apelin dose:

Three doses (0.01 µg/kg, 0.1 µg/kg and 1 µg/kg) of apelin were studied in order to select an optimal dose. This was done by comparing mortality rate among the treated groups. Apelin at the dose of 0.01 µg/kg demonstrated lowest mortality rate.

### Rat Model of Myocardial Ischemia/Reperfusion and Hemodynamic Studies:

At the beginning of surgical procedure, all the rats included in the study were anesthetized with sodium thiopental (60 mg/kg, i.p). The body temperature was measured using an anal thermometer and kept constant (approximately at 37°C) using a thermal pad. A cannula was inserted in the exposed jugular vein for drugs and dye administration. After tracheotomy, tracheal intubation was performed to
ventilate animals by using a rodent ventilator (tidal volume 2–3 mL; Harvard rodent ventilator model 683, Holliston, MA, USA), thus maintaining a respiratory rate of about 65–70 breaths per minute. Next, we exposed the right common carotid artery and cannulated it with an angiocatheter which was in turn connected to the PowerLab data acquisition system via pressure transducer (AD Instrument Pty Ltd, Mountain View, CA, USA) to monitor systolic blood pressure (SBP), diastolic blood pressure (DBP) and mean arterial pressure (MAP). A standard limb Lead-II electrocardiogram was used to monitor heart rate (HR) via subcutaneous stainless steel electrodes employing a PowerLab data acquisition system (AD Instrument).

Left intercostal thoracotomy (between the fourth and fifth costal space) was performed, and the heart was exposed by removing the intercostal muscles and pericardium. After this surgical procedure, a stabilization period for 15 minutes was observed, after which, all animals underwent 30 minutes of ischemia and 60 minutes of reperfusion. The coronary artery was ligated using a 6-0 silk suture underneath (approximately 1-2 mm) left anterior descending coronary artery (LAD) to induce regional ischemia. The ST segment elevation followed by the cyanosis of the apex of heart, were indicators of a successful LAD occlusion. Releasing the tension from the snare resulted in reperfusion.

### Assessment of Infarct Size:

At the end of reperfusion, LAD was re-occluded and Evans Blue dye (3 mL of 2% solution) was injected via jugular vein to delineate non-ischemic (healthy) zone from ischemic zone. The excised and frozen (−20°C for 24 h) hearts were cut into transverse slices (2 mm thick) and incubated in 1% 2, 3, 5 triphenyltetrazolium chloride (TTC in 0.1 M phosphate buffer, pH 7.4 Sigma) solution for 15–20 min at 37°C to delineate infarct zone from ischemic zone. TTC reacts with the viable (ischemic/reperfused) tissue, producing a red formazan derivative, while 10% formalin gives a pale color to the infarcted zone. The area at risk and infracted area were calculated using

![Diagram of Experimental Groups](image_url)
Photoshop program (Version 7.0, Adobe System, San Jose, CA, USA). Area at risk was expressed as the percentage of left ventricle volume (AAR/LV) while the infarct size was expressed as the percentage of area at risk (IS/AAR).

Statistical Analysis:

The statistical analysis was performed using GraphPad Software v 3.0 (San Diego, USA) and Microsoft Excel (2010). Data were expressed as Mean±SEM. Hemodynamic functions were analyzed using Two-way analysis of variance (ANOVA). One-Way analysis of variance followed by Tukey post hoc test was used for assessing infarct size and area at risk. P<0.05 was considered statistically significant.

Results

Hemodynamic Functions:

As demonstrated in Table I, no significant differences were observed in hemodynamic parameters (mean blood pressure (MAP), systolic blood pressure (SBP), diastolic blood pressure (DBP) and heart rate (HR)) among the experimental groups in baseline. Moreover, no significant differences were observed in ischemia and reperfusion periods among the experimental groups.

Infarct size and Area at Risk:

There were no significant differences in the ratio of AAR to LV area between the experimental groups (Fig. 2A). However, the ratio of IS to AAR was considerably lower in AP group (28.5±3.36%) as compared to the IR group (28.53.36%) (Fig. 2B). When compared with the AP group, the infarct-sparing effect of apelin was abolished by the administration 5HD before apelin injection (28.5±3.36% vs 51.6. Pretreatment with 5HD alone before

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Data are expressed as Mean±SEM. Heart Rate, SBP = Systolic Blood Pressure, DBP = Diastolic Blood Pressure, MAP = Mean Arterial Pressure, IR = Ischemia Reperfusion, AP = Apelin, 5-HD = 5-hydroxydecanoate

Fig. 2: The ratio of area at risk to total left ventricular area (AAR/LV) expressed as percentage (A). The ratio of infarct size to area at risk (IS/AAR) expressed as percentage (B). I/R = Ischemia/Reperfusion, AP = Apelin, AP+5HD = 5-hydroxydecanoate.

*p<0.05 vs IR group **p<0.01 vs. AP group
Reperfusion increased IS/AAR to 492.5% as compared with the Apelin group (Fig. 2B). To assess the cardioprotective effect of apelin, we used ratio of infarct size to Area At Risk (%IS/AAR). Figure 3 reveals that apelin significantly reduced infarct size when compared to IR group and this effect was eliminated with the administration of 5HD.

**Discussion**

The current study demonstrated that administration of apelin-13 (0.01/kg) during ischemia reduces infarct size in IR rat model. Furthermore, selective inhibition of mKATP channels by administration 5HD (5 mg/kg; before apelin injection) resulted in diminishing the infarct-sparing effect of apelin.

In this study, apelin administration did not affect hemodynamic parameters like HR, systolic blood pressure, diastolic blood pressure and mean arterial pressure. Mitra et al. has reported similar results showing that apelin injection does not cause any changes in blood pressure (20). Tao et al, and Aziziet al, showed altered hemodynamic parameters during 2 hours or even longer reperfusion period (21, 22). However, the current study employed reperfusion for 60 min, which may explain the insignificant changes in hemodynamic parameters during our study. It has been shown previously that repeated post-infarct administration of apelin can protect myocardium against IR injury (21). In this regards, the current study is of particular interest as we observed apelin-induced infarct-sparing effect with a single drug dose.

Moreover, in the current study, administration of the higher doses of apelin-13 (1 and 0.1/kg) during ischemia led to high mortality due to severe reduction in blood pressure (data not shown).

Apelin is an endogenous ligand for orphan G protein coupled receptor. This peptide appears as a propeptide before its cleavage by endopeptidase. Apelin is a strong endothelium dependent vasodilator and positive inotropic agent which plays a crucial role in IR (23, 24). Apelin-13 can induce cardioprotection; however, this effect seems to ward off in case of preconditioning (25, 26). After an ischemic reperfusion event, apelin and APJ expression rises in cardiomyocytes (27). Apoptosis is another major aspect of I/R damage (28). Apelin has been shown to hold back apoptosis in numerous types of cells by phosphorylating PI3K-Akt and ERK1/2 (28, 29). Based on the above studies, it seems that apelin induces cardioprotection by activating APJ. In addition, apelin diminishes oxidative injury by decreasing ROS which is intermediated by the PI3K-PKCs (PKC1)-mKATP channels pathway (30).

In our study, administration of 5HD (selective inhibitor for mKATP) diminished apelin-induced cardioprotective effect, as seen by the increase in infarct size. Thus, being a terminal effector, opening of mKATP channels may have inhibited cell death and defended myocardium against IR injury. In addition, the reperfusion injury survival kinases (RISK), PI3 kinase and MEK-ERK1/2 are ultimate targets of GPCR activation. The PI3 kinase/Akt upstream to eNOS has been shown to offer cardioprotection during...
PostC(31) by targeting mK<sub>ATP</sub> channels (32). Activation of mK<sub>ATP</sub> channels has been reported to elevate the influx of potassium within mitochondria, which in turn leads to matrix extension, mitochondrial depolarization, elevated mitochondrial respiration, and decreased ATP production (33, 34). In normal heart mitochondria, 5HD does not influence the rate of ATP production, but in case of hypoxic heart, 5-HD has shown to reduce the rate of ATP production (34). On the other hand many studies have reported that protective role of NO against ischemia/reperfusion injuries (35). In this regards, Azizi et al observed apelin-induced rise in NO levels in the infarcted myocardium 1, 3 and 5 day after I/R injury (21).

Hence, it can be suggested that apelin administration during ischemia period can offer prominent cardioprotection by diminishing oxidative damage in sarcoplasmic reticulum membrane protein along with the inhibition of ROS generation. Furthermore, by potentiating NO and thereby activating PKG and PKC, apelin can cause opening of mK<sub>ATP</sub> channels. According to the study of Azizi et al, it is possible that the effect of apelin can be mediated by increasing the activity of NO and vasodilation of vessels (especially coronary artery) in long term, however, we did not assess this indicator in the current study.

**Conclusion**

Administration of 5HD abolishes apelin induced cardioprotection.

**Acknowledgements**

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**References**


