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Adiponectin Inhibits Oxidative/Nitrative Stress during Myocardial Ischemia and Reperfusion
via PKA Signaling

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Running Head: PKA in APN’s cardioprotective signaling

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Abstract

The cardioprotective effects of adiponectin (APN) against myocardial ischemia/reperfusion (MI/R) injury are well known. However, comprehension of the mechanisms mediating intracellular APN signaling remains incomplete. We recently demonstrate the antioxidant/antinitrative effects of APN are not dependent upon AMPK. Protein Kinase A (PKA) has been previously shown to be activated by APN, with uncertain relevance to APN cardiac protection. The current study determined whether the anti-oxidative/anti-nitrative effect of APN is mediated by PKA. Administration of APN (2 µg/g) 10 minutes before reperfusion significantly enhanced cardiac PKA activity, reduced oxidative stress and decreased infarct size. Knockdown of cardiac PKA expression (PKA-KD) by intramyocardial injection of PKA-siRNAs (>70% suppression) significantly inhibited APN cardioprotection determined by cardiac apoptosis, infarct size, and cardiac function. Moreover, PKA-KD virtually abolished the suppressive effect of APN on MI/R induced NADPH oxidase overexpression and superoxide overproduction, and partially inhibited the effect of APN on nitrative protein modification in MI/R heart. Mechanistically, APN significantly inhibited MI/R-induced IKK/IκB phosphorylation and NFκB activation, which were blocked in PKA-KD heart. Finally, the PKA-mediated antioxidant/antinitrative and cardioprotective effects of APN are intact in AMPK deficiency mice, suggesting that there is no cross-talk between AMPK and PKA signaling in APN cardioprotection. Collectively, we demonstrate for the first time that APN inhibits oxidative/nitrative stress during MI/R via PKA-dependent NFκB inhibition.

Keywords: Protein Kinase A; Adiponectin; Reperfusion Injury; Oxidative Stress;
The adipokine adiponectin (APN), of primarily adipose origin, regulates glucose and lipid metabolism(23). Prospective clinical observations and animal studies have demonstrated that reduced adiponectin function may be a major contributor to increased ischemic heart disease morbidity in diabetic patients(21). Administration of the adiponectin attenuates myocardial ischemia and reperfusion (MI/R) injury in mice(20; 24). However, the detailed molecular mechanisms responsible for the cardioprotective effect of adiponectin remain elusive.

It is well accepted that excess superoxide-mediated oxidative stress plays an important role in MI/R injury. Superoxide reacts with nitric oxide (NO) to form the highly cytotoxic molecule peroxynitrite (ONOO’), which induces nitrative stress and exacerbates MI/R injury. We demonstrated that APN deficient mice exhibited much more severe I/R injury from overproduction of superoxide, peroxynitrite, and resultant nitrative stress, reversible by gAPN administration (22). Therefore, APN is cardioprotective against ischemia/reperfusion injury by inhibiting oxidative/nitrative stress. We have recently demonstrated that the anti-oxidative/anti-nitrative property of APN is not mediated by AMP activated protein kinase (AMPK), the most recognized APN intracellular signaling molecule (24). A comprehensive understanding of intracellular APN anti-oxidative signaling mechanisms remains lacking.

Protein kinase A (PKA) is an important mediator of signal transduction downstream of G-protein-coupled receptors, and plays a key role in the regulation of metabolism and triglyceride storage. In endothelial cells, APN inhibits palmitate-induced apoptosis by suppressing reactive oxygen species (ROS) generation via PKA pathways (27). However, whether PKA signaling mediates APN’s anti-oxidative/anti-nitrative effect during MI/R injury has never been previously investigated.

Therefore, the aims of the present study were to investigate whether PKA mediates APN’s anti-oxidative/anti-nitrative and cardioprotective effects against MI/R injury; and if so
to identify the possible responsible intracellular signaling mechanisms.
Materials and Methods

Adult male WT mice, adult male AMPK-DN mice or their male littermate controls were utilized in this study. Generation, breeding, phenotype characteristics, and genotyping of AMPK-DN mice (>80% inhibition of cardiac AMPK activity) have been previously described in detail (28). The experiments were performed in adherence with the National Institutes of Health Guidelines on the Use of Laboratory Animals and were approved by the Thomas Jefferson University Committee on Animal Care.

Myocardial ischemia/reperfusion: Mice were anesthetized with 2% isoflurane. MI/R was induced by temporarily exteriorizing the heart via a left thoracic incision, and placing a 6-0 silk suture slipknot around the left anterior descending coronary artery. Twenty minutes after MI, animals were randomized to receive either vehicle or gAPN (2 μg/g, ip) (24). After 30 minutes of MI, the slipknot was released, and the myocardium was reperfused for 3 hours (all assays except cardiac function and infarct size) or 24 hours (cardiac function and infarct size only). All assays were performed utilizing tissue from the ischemic/reperfused area, the area at risk (AAR), identified by Evans blue negative staining. Sham-operated control mice (Sham MI/R) underwent the same surgical procedure, except the suture placed under the left coronary artery was not tied.

Inhibition of PKA with in vivo siRNAs-mediated knock-down (PKA-KD): To specifically confirm the role of PKA in gAPN’ protective effect on MI/R, siRNA gene silencing technique was utilized to knock-down mouse cardiac PKA expression (PKA-KD). In brief, two mouse-specific PKAα subunit-selective siRNA targeting the nucleotide sequences 5’-CGTCCTGACCTTTGAGTATCT-3’ and 5’-CAGTGTGCTGTTGTAACATA-3’ were employed. siRNA oligos of the same size possessing scrambled nucleotide sequences served as control. All siRNAs were obtained from Integrated DNA Technologies. The siRNAs were diluted in 5% glucose and mixed with in vivo-jet PEI (Genesee Scientific). Adult mice were
anesthetized with 2% isoflurane. The heart was exposed via left thoracotomy at the fifth intercostal space. 20 μl PKA-specific siRNAs (dose 0.8 μg/μl) or scrambled control was delivered via three separate intramyocardial injections (by 32G needle), temporarily blanching the left ventricular free wall. 48 hours after siRNAs injection, the mice were subjected to MI/R.

**Determination of myocardial apoptosis and myocardial infarct size:** Myocardial apoptosis was determined by terminal deoxynucleotidyl transferase-mediated dUTP nick-end labeling (TUNEL) staining and caspase-3 activity as described in our previous study (24). Myocardial infarct size was assessed by Evans blue-2,3,5-triphenyl tetrazolium chloride double staining methods (24).

**Determination of cardiac function:** Cardiac function was determined by echocardiography and left ventricular catheterization methods 24 hours after reperfusion before thoracotomy, as described in our previous study (24).

**Measurement of PKA activity:** PKA activity was measured utilizing non-radioactive PepTag assays (Promega, Southampton, Hampshire, UK) reliant upon a change in charge of the PepTag A1 peptide from +1 to -1 following phosphorylation (14). Sample reaction mixtures were incubated at room temperature for 15 minutes. After incubation, samples were separated on a 0.5% (w/v) agarose gel at 100V for 15–20 minutes. Purified PKA catalytic subunit served as a positive control, while the negative control contained only buffer. Bands were visualized under ultraviolet light.

**Quantification of Superoxide Production:** Superoxide production in I/R heart tissue was measured by lucigenin-enhanced chemiluminescence as previously described (24). Superoxide production was expressed as relative light units (RLU) per second per mg heart weight (RLU/s/mg wet tissue).

**Determination of Total NOx Content in Cardiac Tissue:** Cardiac tissue samples from AAR
were rinsed, homogenized in deionized water (1:10, weight/volume), and centrifuged at 14,000 g for 10 minutes. Tissue NO and its in vivo metabolic products (NO₂ and NO₃, collectively known as NOₓ) in the supernatant were determined via chemiluminescence NO detector (SIEVER 280i NO Analyzer) as described in our previous study(24).

**Quantitation of Tissue Nitrotyrosine Content:** Ischemic/reperfused cardiac tissue nitrotyrosine content (pmol of nitrotyrosine/mg protein), an accepted footprint of in vivo peroxynitrite formation and a reliable index of nitrative stress, was determined via ELISA, described in our previous publication(22; 26).

**Measurement of nuclear translocation of NF-κB p65:** The relative increase of NF-κB p65 translocation into the nuclei of the ischemic/reperfused cardiomyoctyes was determined via ELISA per manufacturer’s protocol (IMGENEX, San Diego, CA). In brief, cardiac tissue was cut into small pieces and homogenized. The mixture was centrifuged for 10 minutes at 10,000 rpm at 4°C. The supernatant represents the cytoplasmic fraction. The pellet was resuspended in nuclear lysis buffer, and subsequently centrifuged for 10 minutes at 14,000 rpm at 4°C. This supernatant represents the nuclear fraction. A plate coated with anti-p65 antibody captured free p65 (both nuclear or cytoplasmic). Bound p65 was detected by adding a secondary antibody followed by alkaline phosphatase-conjugated secondary antibody. Each well’s absorbance value was determined at 405 nm by a microplate reader (Molecular Devices). The relative ratio of nuclear to cytoplasmic p65 was calculated by: (absorbance value of nucleus)/(absorbance value of cytoplasm) (16).

**Western blot analysis:** Protein from tissue homogenates was separated on SDS-PAGE gels, transferred to nitrocellulose membranes, and incubated with primary antibodies (against PKA C-α Antibody, IKKβ, Phospho-IKKβ, IκBα, Phospho-IκBα [Cell Signaling Technology, Danvers, MA], iNOS [Upstate, Chicago, IL], gp91phox [Transduction Laboratories, San Jose, CA]) and horseradish peroxidase-conjugated secondary antibody. The blot was developed
with a Supersignal Chemiluminescence detection kit (Pierce, Rockford, IL), and observed
with a Kodak Image Station 400 (Rochester, NY).

Statistical analysis: All values in the text and figures are presented as mean±standard error of
the mean of n independent experiments. All data (except Western blot density) were
subjected to one-way ANOVA followed by Bonferoni correction for post-hoc t test. Western
blot densities were analyzed with the Kruskal-Wallis test followed by Dunn’s post-hoc test.
Probabilities of 0.05 or less were considered to be statistically significant.
Results

PKA deficiency blocked gAPN inhibition of MI/R-induced myocardial infarct, apoptosis, and cardiac dysfunction. MI/R did not alter PKA expression and slightly reduced PKA activity. Administration of APN 10 min before reperfusion had no effect upon PKA expression but significantly increased PKA activity (Figure 1) and reduced myocardial infarct size (Figure 2). To determine whether APN activation of PKA plays a causative role in APN cardioprotection, PKA expression was downregulated via intramyocardial siRNA injection. Pretreatment with the PKA-selective siRNAs reduced PKA expression by 70% ($P<0.01$ relative to scramble group, n=6, Figure 2A). Cardiac specific PKA knockdown blocked APN-induced PKA activation (Figure 2B) and attenuated APN’s infarct reduction effects (Figure 2C).

MI/R resulted in significant cardiac injury including cardiac systolic and diastolic dysfunction (Figure 3) and myocardial apoptotic cell death (Figure 4). Consistent with our previous observations (22), gAPN administration significantly improved cardiac function after reperfusion (Figure 3, Scramble) and reduced apoptosis (Figure 4, Scramble). The knockdown of cardiac PKA expression (PKA siRNA) slightly worsened MI/R-induced cardiac dysfunction measured by LVEF and LVEDP (Figure 3A, B), but significantly increased infarct size (Figure 2C) and apoptosis (Figure 4A). More importantly, cardioprotective effect of APN was either completely abolished (anti-apoptotic effect on Figure 4) or significantly attenuated (Figure 2C, Figure 3) in the PKA-KD mice. Taken together, these results demonstrated that PKA activation plays a critical role in APN cardioprotection after MI/R.

PKA deficiency abolished the anti-oxidative and inhibited anti-nitrative effects of gAPN post I/R. Having demonstrated that gAPN protects against reperfusion injury in a partially PKA-dependent manner, we investigate the underlying mechanisms potentially responsible. We report previously gAPN cardioprotection involves reduction of oxidative/nitrative stress...
We performed two series of experiments assessing the relationship between PKA signaling and oxidative/nitrative stress in MI/R hearts. In the first experiment series, MI/R-induced oxidative stress was detected. Under basal conditions, no significant difference existed between superoxide production in scramble and PKA-KD mice. MI/R induced greater increases in superoxide production in PKA-KD animals (Figure 5A). APN administration significantly inhibited MI/R-induced superoxide production in scramble animals (Figure 5A). However, APN treatment did not reduce superoxide production in PKA-KD animals (Figure 5A). We next identified potentially responsible molecular sources for the impaired antioxidant actions of gAPN in PKA-KD mice. Expression of gp91phox, the primary isoform of NADPH oxidase expressed in heart (1; 2; 29) was significantly increased after MI/R in both scramble and PKA-KD heart (Figure 5B). gAPN treatment of scramble animals subjected to MI/R significantly inhibited gp91phox expression. In contrast, the inhibitory effect of APN upon MI/R-induced gp91phox overexpression was abolished in PKA-KD mice, in consistent fashion with superoxide generation trends in PKA-KD mice (Figure 5B). Together, these results demonstrate that MI/R-induced oxidative stress was modestly increased in PKA-KD mice, and the anti-oxidant effect of gAPN was completely abolished by PKA signaling deficiency.

In the second experiment series, we assessed MI/R-induced myocardial nitrative stress. There was no significant total nitric oxide (NOx) content difference between PKA-KD and scramble animals during basal conditions or after MI/R (Figure 6A). Administration gAPN significantly inhibited MI/R-induced NOx overproduction in both scramble and PKA-KD mice (Figure 6A). Inducible nitric oxide synthase (iNOS) expression was significantly increased after MI/R-induced in scramble and PKA-KD animals (Figure 6B). Treatment with gAPN significantly inhibited iNOS expression, an effect not inhibited by PKA-KD (Figure 6B). These results demonstrate that although the anti-oxidant effect of gAPN is mediated by
PKA, iNOS-inhibitory effect of gAPN is not mediated by PKA.

Superoxide rapidly reacts with NO to form the cytotoxic molecule peroxynitrite. Nitrotyrosine (NT) content, the accepted footprint of peroxynitrite production, was increased by MI/R injury both in scramble and PKA-KD mice (Figure 6C). Administration of gAPN significantly inhibited MI/R-induced NT generation in scramble animals, an effect that was partially blocked in PKA-KD animals (Figure 6C).

gAPN decreased IKK/IκB/NF-κB signaling activation in I/R myocardium, which was blocked by PKA deficiency. Decreased IκB phosphorylation preventing nuclear NF-κB translocation attenuates myocardial reperfusion injury (17) and preserves cardiac function post MI. PKA signaling is involved in gAPN-suppressed IKK-IκB-NFκB activation in endothelial cells subjected to tumor necrosis factor-α or high glucose treatment (27). We investigated the possible mechanism underlying the PKA-dependent cardioprotective effects of gAPN, and determined activation of IKK-IκB-NFκB by MI/R. Consistent with others’ previous results (15; 30), MI/R induced significant IKK phosphorylation (Figure 7A), resulting in IκBa phosphorylation (Figure 7B) and NF-κB activation (Figure 7C) in both scramble and PKA-KD mice. MI/R-induced NFκB activation by IKK/IκB phosphorylation was significantly inhibited by gAPN in scramble animals (Figures 7). However, gAPN did not inhibit NFκB activity during PKA-knockdown (Figure 7). These data suggest the cardioprotective effect of gAPN was partially due to inhibition of IKK/IκB/NF-κB activation, and such effect was dependent upon PKA signaling.

PKA-mediated cardioprotective effect of APN against MI/R injury is AMPK-independent:

Despite considerable evidence supporting the essential role of AMPK in adiponectin intracellular signaling (4; 5; 7; 8; 11), we recently provided direct evidence of AMPK-independent gAPN-mediated cardioprotection in the intact animal (24; 25). The current study suggests gAPN is cardioprotective in mice subjected to MI/R, with involvement
of PKA signaling. We next investigated whether there is crosstalk between PKA and AMPK pathways. Adult male mice overexpressing a dominant negative α2 subunit (D157A) of AMPK (AMPK-DN) mice were pretreated with PKA siRNAs, and the effects of gAPN administration upon MI/R injury were determined. Consistent with our previous data (24), gAPN inhibited MI/R-induced infarction (Figure 8A) and improved cardiac function (Figure 8B) in AMPK-DN animals. Importantly, PKA knockdown significantly attenuated this protective effect, consistent with wild type (WT) mice results presented in Figures 1-3. Moreover, PKA deficiency blocked the anti-oxidative/antinitrative effects of gAPN, evidenced by augmented superoxide (Figure 8C) and peroxynitrite (Figure 8D) generation. Together, these results support PKA-mediated gAPN cardioprotective effect against MI/R injury is independent of AMPK signaling.
**Discussion**

Several important observations have been made in the present study. Firstly, we demonstrate gAPN could not inhibit post-ischemic myocardial apoptosis, infarction, or cardiac dysfunction in cardiac specific PKA knockdown animals. This is the first evidence that PKA signaling mediates the cardioprotective effects of APN in ischemic heart disease in vivo. Secondly, we provide the first direct evidence that the anti-oxidative and antinitrative effects of gAPN in ischemic/reperfused hearts are mediated by PKA. Thirdly, we demonstrate PKA signaling is involved with adiponectin-mediated inhibition of IKK-IκB-NFκB activation after MI/R. Finally, we provide evidence that PKA-mediated gAPN’s cardioprotective effect against MI/R injury is independent of AMPK signaling.

APN is an abundant circulating adipocytokine secreted primarily from adipose tissue, exerting at least three major functions, including an insulin sensitization/metabolic-regulatory function (in the liver and muscle), an anti-inflammatory/vasculoprotective function, and an anti-ischemic/cardioprotective function. Numerous epidemiological studies underline the correlation between hypoadiponectinemic states and increased morbidity/mortality of cardiovascular ischemic diseases. Consistent with our and others’ previous results, the present study confirms exogenous APN supplementation can significantly mitigate myocardial apoptosis, infarct size, and cardiac dysfunction.

The signal transduction pathway mediating the antioxidant effect of adiponectin remains intensely investigated. PKA is involved in adiponectin-mediated suppression of ROS and apoptosis in endothelial cells. Therefore, the current study sought to determine the role of PKA in gAPN’s cardioprotective effect against MI/R injury, utilizing in vivo gene silencing techniques. In wild type mice, we demonstrate gAPN reversed MI/R-induced myocardial infarction, cardiomyocytes apoptosis, and cardiac dysfunction. However, in mice subjected to intramyocardial PKA knockdown, such protective effects of gAPN were either
completely abolished or significantly attenuated. This is direct evidence that a significant portion of gAPN-mediated cardioprotection is PKA-dependent in intact animals.

Simultaneous overproduction of superoxide and NO not only causes inactivation of the cytoprotective NO, but generates the highly cytotoxic molecule peroxynitrite. Therefore, those therapeutic interventions preventing concomitant NO/superoxide overproduction effectively block peroxynitrite formation, and offer great tissue protection. The current study’s results demonstrate that gAPN administration reduced synchronized NO/superoxide overproduction from NADPH oxidase and iNOS, and effectively block peroxynitrite formation in ischemic/reperfused hearts. Here, we demonstrate for the first time that gAPN inhibits I/R-induced oxidative injury in a PKA-dependent fashion, which may be the mechanism responsible for reduced cardioprotection by gAPN observed in PKA-deficient animals.

Nuclear factor kappa B (NF-κB) is a crucial transcription factor in the induction of genes involved in various physiological processes, including response to injury and inflammation. Activation of NF-κB requires phosphorylation of the inhibitor of NF-κB (IκB) by IκB kinase (IKK), resulting in proteasome-mediated degradation of IκB. This allows the translocation of NFκB from the cytosol to the nucleus, where the heterodimer binds the promotor region of specific target genes. Activation of NF-κB leads to the transcription of factors promoting inflammation (i.e. adhesion molecules, cytokines, and chemokines), but may also contribute to tissue remodeling, inflammation resolution, and the transcription of genes resulting in anti-inflammatory effects. Notably, NF-κB activation is implicated in the pathophysiology underlying acute myocardial infarction, heart failure, endothelial dysfunction, and unstable angina pectoris(3; 10; 30). Furthermore, IKK inhibition attenuates myocardial injury and dysfunction following acute I/R (30), and inhibition of IκB phosphorylation and resultant NF-κB activation attenuates MI/R injury in cardiomyocytes. We currently demonstrate gAPN inhibits IKK/IκB/NFκB activation in the scramble heart.
However, gAPN could not block MI/R-induced IKK and IkB phosphorylation and resultant NFkB activation in PKA-deficient mice. Together, these results indicate gAPN confers cardioprotection via inhibition of IKK/IkB/NFkB-mediated inflammation, and intact PKA signaling is necessary for such gAPN-mediated anti-inflammatory protection against I/R injury.

Substantial evidence supports the essential role AMPK plays in adiponectin intracellular signaling. Pharmacological or genetic inhibition of AMPK activity virtually abolishes the metabolic, anti-inflammatory and vasculoprotective effects of adiponectin. However, we previously demonstrate APN-cardioprotection against MI/R injury is retained in AMPK-DN mice. APN administration to AMPK-DN mice reduces MI/R-induced oxidative and nitratrive stress, indicating that AMPK-independent mechanisms mediate gAPN’s cardioprotective effect (24). Our present study data is consistent with our previous findings. In AMPK-DN mice, the antioxidative/antinitrative effects (and consequent cardioprotective effects) of gAPN were dependent upon whether PKA was silenced or not. Our data supports the responsibility of PKA signaling in part for the protective actions of adiponectin against MI/R injury.

In summary, we demonstrate for the first time that APN inhibits oxidative/nitrative stress during MI/R via PKA signaling, in an AMPK independent fashion. This represents new understanding in the intracellular signaling mechanisms of APN, as we continue to identify novel therapeutic targets in the treatment of diabetic ischemic/reperfusion injury.
Acknowledgments

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Author Disclosure Statement

No conflicts of interest, financial or otherwise, are declared by the authors.
**Figure Legend**

**Figure 1. The influence of APN upon PKA expression and activation.** (A) Cardiac PKA expression in control (Sham MI/R) or MI/R treated with vehicle or gAPN. (B) Cardiac PKA activity in control or MI/R treated with vehicle or gAPN. n=6-9 hearts/group. *P<0.05 vs. vehicle.

**Figure 2. The influence of PKA signaling blockade by PKA-selective siRNAs upon myocardial PKA expression and activity and the resultant effect on myocardial infarct size.** (A) Cardiac specific PKA knockdown decreased PKA expression. (B) PKA siRNAs significantly inhibited PKA expression. (C) PKA-specific siRNA significantly blocked gAPN inhibition of MI/R-induced myocardial infarction. n=9-12 hearts/group. *P<0.05, **P<0.01 vs. vehicle; #P<0.05, ##P<0.01 vs. scramble with the same treatment.

**Figure 3. PKA deficiency blocked gAPN inhibition of MI/R-induced myocardial dysfunction.** Cardiac function determined by echocardiography (A, LVEF) and left ventricular catheterization hemodynamic assay (B, LVEDP; C, +dp/dtmax). n=12-14 animals/group. *P<0.05, **P<0.01 vs. vehicle; #P<0.05, ##P<0.01 vs. scramble with the same treatment.

**Figure 4. PKA deficiency blocked gAPN inhibition of MI/R-induced cardiomyocyte apoptosis.** (A) TUNEL staining; (B) caspase-3 activity. n=5-6 hearts/group. **P<0.01 vs. vehicle; #P<0.05, ##P<0.01 vs. scramble with the same treatment.

**Figure 5. PKA deficiency blocked the anti-oxidative effects of gAPN post MI/R.** (A) Production of superoxide (n=6-7 hearts/group); (B) gp91phox expression, representative
Western blots shown (n=5–6 hearts/group). **P<0.01 vs. vehicle; *P<0.05, ***P<0.01 vs. scramble with the same treatment.

**Figure 6.** PKA deficiency partially blocked the anti-nitrative effects of gAPN post MI/R
(A) NOx content (n=5-6 hearts/group); (B) Inducible nitric oxide synthase (iNOS) expression, representative Western blots shown (n=5-6 hearts/group); (C) Nitrotyrosine content, determined by ELISA (n=6-8 hearts/group). *P<0.05, **P<0.01 vs. vehicle; *P<0.05, **P<0.01 vs. scramble with the same treatment.

**Figure 7.** gAPN decreased IKK/IκB/NF-κB signaling activation in MI/R myocardium, which was blocked by PKA deficiency. (A) Phosphorylated IKK expression, representative Western blots shown (n=6 hearts/group); (B) Phosphorylated IκB expression, representative Western blots shown (n=5-6 hearts/group); (C) Activated NFκB, determined by ELISA (n=6-8 hearts/group). *P<0.05, **P<0.01 vs. vehicle; *P<0.05, **P<0.01 vs. scramble with the same treatment.

**Figure 8.** PKA-mediated cardioprotective effect of APN against MI/R injury is AMPK-independent (A) Myocardial infarction, determined by Evans blue/TTC double staining (n=9-12 hearts/group); (B) Cardiac function, determined by echocardiography assay (n=12-15 animals/group); (C) Superoxide production (n=6-8 hearts/group); (D) Nitrotyrosine content, determined by ELISA (n=6-8 hearts/group). *P<0.05, **P<0.01 vs. vehicle; *P<0.05, **P<0.01 vs. scramble with the same treatment.


15. **Li C, Kao RL, Ha T, Kelley J, Browder IW and Williams DL.** Early activation of IKK{beta} during in


22. Tao L, Gao E, Jiao X, Yuan Y, Li S, Christopher TA, Lopez BL, Koch W, Chan L, Goldstein BJ and Ma XL. Adiponectin cardioprotection after myocardial ischemia/reperfusion involves the reduction of


Fig 1
Fig 2

A

PKA
GAPDH

PKA/GAPDH (Density, AU)

Scramble PKA siRNA

B

Phosph-A1
NonPhosph-A1

Neg. Pos. - + - + - + MI gAPN

pA1/non-pA1

Scramble PKA siRNA

C

Infarct Size (% of AAR)

Sham MI/R MI/R +Vehicle MI/R +gAD MI/R +Vehicle MI/R +gAD

Scramble PKA siRNA

** * # #

Fig 2
Fig 3
**A**

Scramble PKA siRNA

**B**

Scramble PKA siRNA

Fig 4
Fig 5

A

Superoxide Production (RLU/sec/mg wet tissue)

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<th>PKA siRNA</th>
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B

![Western Blot](gp91phox.png)

![Western Blot](gadph.png)

gp91phox/GAPDH

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Fig 6
A

p-IKK
IKK

B

p-IκBα
IkBα

C

NF-κBp65 Activity (ng/ml/mg protein)

Fig 7
Fig 8