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Factors Mediating Remote Preconditioning of Trauma (RPCT) in the Rat Heart: Central Role of the Cytochrome P450 Epoxygenase Pathway in Mediating Infarct Size Reduction

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Abstract

The present study further identified factors involved in the cardioprotective phenomenon of remote preconditioning of trauma (RPCT) with special emphasis on the role of the epoxyeicosatrienoic acids (EETs) in mediating this phenomenon. RPCT was produced by an abdominal incision only through the skin. Subsequently, all rats were subjected to 30 min of left coronary artery occlusion followed by 2 h of reperfusion and infarct size was determined. RPCT produced a reduction in infarct size expressed as a percent of the area-at-risk from 63.0±1.1 to 44.7±1.4%, $P<0.01$ vs. control. To test the 3 major triggers of classical preconditioning in mediating RPCT, blockers of the bradykinin B2 receptor (B2BK), WIN 64338 (1 mg/kg, iv), or HOE 140 (50 µg/kg, iv), the nonselective opioid receptor blocker, naloxone (3mg/kg, iv), or the adenosine A1 receptor blocker, DPCPX (1mg/kg, iv) were administered 10 min prior to RPCT. Only the two B2BK selective antagonists blocked RPCT (60.2±1.1%, WIN 64338; 62.3±2.0%, HOE 140). To test EETs in RPCT, we administered the EET receptor antagonist 14,15-EEZE (2.5 mg/kg, iv) or the EET synthesis inhibitor, MSPPOH (3.0 mg/kg, iv) 10 min prior to RPCT. In both groups, EET antagonists completely blocked RPCT (62.0±0.8%, 14,15-EEZE; 61.8±1.0%, MSPPOH). EET antagonists also blocked the effect of B2BK activation. We also determined if the sarcolemmal K_{ATP} or the mitochondrial K_{ATP} channel mediate RPCT by pretreating rats with HMR 1098, or 5-HD, respectively. Interestingly, 5-HD blocked RPCT (64.7±1.3%), whereas, HMR 1098 did not (50.3±1.3%). The two EET antagonists completely blocked capsaicin-induced cardioprotection. These results clearly suggest that EETs mediate RPCT-, bradykinin- and capsaicin-induced cardioprotection in rat hearts.

Introduction

Previous work from several laboratories, including ours, demonstrated that remote preconditioning of trauma (RPCT) produced by a nonischemic abdominal wall surgical incision markedly reduced infarct size in mice and dog hearts.^{1, 2} Evidence suggests a sensory neural pathway is involved with spinal nerves and sympathetic nerves being activated. Bradykinin, PKC ϵ , the mitochondrial K_{ATP} channel and the epoxyeicosatrienoic acids (EETs, the cytochrome P450 epoxygenase metabolites of arachidonic acid) were also triggers or mediators of RPCT in mice (BK, PKC, mito K_{ATP}) and dogs (EETs) and topical capsaicin mimics RPCT. These results strongly suggest that RPCT is neuronally-mediated, however, one cannot rule out circulating humoral factors such as adenosine and opioids

being involved³ as shown in other models of remote ischemic preconditioning (RIPC) produced by activation of mesenteric nerves and skeletal muscle.^{4, 5} Furthermore, the role of the EETs in RPCT and species other than the dog is not clear. This RPCT phenomenon has not been previously characterized in the rat. Therefore, the present study was designed to determine in a rat model of infarction the major players mediating RPCT and more clearly defined the role of EETs and K_{ATP} channel subtypes in mediating cardioprotection in this model.

Materials and Methods

Materials

14,15-EEZE and MSPPOH were synthesized in the laboratory of Dr. J.R. Falck. 5-Hydroxydecanoic acid (5-HD), naloxone, HOE 140, glibenclamide, bradykinin and DPCPX were obtained from Sigma Chemical Company. WIN 64338 were obtained from Tocris Biosciences. HMR 1098 was kindly provided by Dr. Hans Gogelein, Aventis Pharmaceuticals, Germany. Capsaicin cream was obtained over the counter. 14,15-EEZE and MSPPOH were dissolved in a vehicle composed of 95% ethanol, polyethylene glycol 200 and 1 N sodium hydroxide (5:5:1). Naloxone, HOE 140, 5-HD, HMR 1098 and bradykinin were dissolved in distilled water. WIN 64338, DPCPX, and glibenclamide, were dissolved in DMSO (0.01%).

General preparation of rats

All experiments were conducted in accordance with the “Position of the American Heart Association on Research and Animal Use” adopted by the American Heart Association and the guidelines of the Biomedical Resource Center and the IACUC Committee of the Medical College of Wisconsin. The Medical College of Wisconsin is accredited by the American Association of Laboratory Animal Care (AALAC).

The protocol for the rat preparation and experiments has been previously described in detail.⁶ Male Sprague-Dawley rats (250–300 g) were anesthetized with 100 mg/kg of Inactin, placed on a heating pad and a tracheotomy performed and the rat ventilated with room air supplemented with 100% oxygen. Catheters were placed in the carotid artery and jugular vein for measurement of systemic blood pressure, heart rate, blood gases and for administration of drugs or vehicle. The heart was exposed by a left thoracotomy and the left coronary artery isolated and a suture placed around it to produce an occlusion followed by reperfusion. The hearts were subjected to 30 min of total coronary artery occlusion followed by 2 h of reperfusion at which time the artery was reoccluded and Evans Blue Dye injected into the jugular vein to stain the nonischemic area of the heart blue. Subsequently, the heart was arrested with a bolus of 10% KCl and removed for the measurement of infarct size. After heart removal, it was washed in buffer and cut in a bread loaf fashion into 5–6 pieces and the blue stained normal region and the ischemic region separated and placed in separate vials containing triphenyltetrazolium chloride staining solution and incubated at 37°C in phosphate buffer at pH 7.40 for approximately 15 min. Subsequently, the pieces of tissue were placed into vials containing formaldehyde and stored overnight at room temperature and infarct size determined by gravimetry the following day. Infarct size (IS) was expressed as a percent (%) of the area at risk (IS/AAR, %) as determined by our dual staining technique. In the AAR, the dark red color represents noninfarcted tissue, whereas, the infarcted tissue remains a pale grey color.

Experimental design

Rats were randomly assigned to 30 groups for the different treatments and 8 rats were usually assigned to each group of experiments. In all studies, rats were subjected to 30 min

of left coronary artery occlusion (LCAO) and 2 h of reperfusion. In all slit experiments the abdominal slit was performed 15 min prior to the 30 min occlusion period. A 4-cm transverse skin incision was made *via* the abdominal midline of the rats with a scalpel similar to the previously described protocols.^{1, 2} The pharmacological antagonists or appropriate vehicle were administered alone 15 min preocclusion in control experiments and in the slit experiments (RPCT) the various antagonists were administered iv 15 min prior to RPCT (Figure 1). In some experiments, bradykinin was administered iv 15 min prior to the 30 min occlusion period and the respective receptor antagonist, WIN 64338 or in some experiments the EET antagonists were administered 15 min prior to bradykinin. Finally, the same amount of external capsaicin cream (0.1%) was administered topically on the same surface area at the site of the slit. In 3 groups, the EET antagonists, 14,15-EEZE and MSPPOH were given iv 15 min prior to capsaicin cream application.

Statistical analysis

All values are expressed as the mean $\pm$ SEM. Differences between groups in hemodynamics and blood gases were compared by use of a two-way analysis of variance (ANOVA) followed by Tukey's post hoc test. Differences between groups in AAR, IS and IS/AAR were compared by a one-way ANOVA. Differences between groups were considered significant if $P < 0.05$.

Results

Hemodynamics and blood gases

Heart rate, systolic and diastolic blood pressure and blood gases (data not shown for blood gases) at baseline, at 30 min of occlusion or at 2 h of reperfusion were not significantly different between groups (Table 1 and 2). There were also no differences in the area at risk as a percent of the left ventricle (AAR/LV, Table 1). These data suggest that differences in infarct size were not different as the result of changes in hemodynamics or area at risk.

Mediators of classical ischemic preconditioning (IPC)

To determine the role of the 3 G protein-coupled receptors which have been demonstrated to trigger classical ischemic preconditioning (IPC), adenosine,⁷ opioids or bradykinin B2 receptors,⁸ specific antagonists of these 3 receptors were administered alone 15 min prior to the 30 min occlusion period (no slit control) or 15 min prior to RPCT and IS/AAR determined (Figure 2). Initially, we showed that the transverse abdominal slit resulted in a significant reduction in IS/AAR from 63.0 ± 1.1 to $44.7 \pm 1.4\%$ ($P < 0.01$). The reduction of IS/AAR produced by RPCT is much less than the reduction we have previously published for IPC (IS/AAR = $11.0 \pm 3.0\%$).⁶ Administration of DPCPX, the adenosine A1 receptor antagonist, naloxone, the nonselective opioid receptor antagonist or WIN 64338 or HOE 140, 2 bradykinin B2 receptor antagonists alone in the absence of the abdominal slit had no effect on IS/AAR. However, the two B2 receptor antagonists, WIN 64338 or HOE 140 blocked the protective effect of RPCT (63.2 ± 1.5 , $61.7 \pm 1.3\%$), whereas, DPCPX ($45.4 \pm 2.2\%$) and naloxone ($44.5 \pm 1.2\%$) had no effect on RPCT-induced protection (Figure 2). These results indicate that bradykinin/B2 receptor pathway is a major signaling in the RPCT-induced cardioprotection. The results also suggest the signaling pathway for RPCT is different from classical or remote preconditioning produced by a brief ischemic insult.

We previously showed that bradykinin was a mediator of RPCT in the dog heart since RPCT was blocked by HOE 140² and the epoxyeicosatrienoic acids (EETs) were also important players in RPCT since the EET receptor antagonist, 14,15-EEZE, and the EET synthesis inhibitor, MSPPOH both blocked RPCT in the canine heart. Therefore, we tested the hypothesis that the EETs were downstream mediators of the B2 receptor. Administration of

bradykinin (60 µg/kg, iv) 15 min prior to the 30 min ischemic period produced a significant reduction in IS/AAR (46.9 ± 1.2 , $P < 0.01$) similar in magnitude to that seen with RPCT (Figure 2). This protective effect of bradykinin was completely abolished by 14,15-EEZE (62.91.1%) and MSPPOH ($61.6 \pm 1.8\%$) which suggests that bradykinin released by RPCT is exerting its cardioprotective effect *via* the EETs in rat hearts (Figure 3) similar to that previously shown in the dog heart.²

Role of EETs in capsaicin-induced cardioprotection and RPCT

To further determine a role for sensory C-fibers in mediating RPCT in rat hearts, capsaicin cream (0.1%) was applied at the same position as the abdominal slit 15 min prior to the 30 min ischemic period. In agreement with previous results obtained in the mouse heart,¹ capsaicin produced a significant reduction in IS/AAR ($47.5 \pm 0.3\%$, $P < 0.01$). The effect of capsaicin cream was completely abolished by pretreatment with either of the two EET antagonists, 14,15-EEZE ($60.9 \pm 1.2\%$) or MSPPOH ($61.5 \pm 1.5\%$).

Role of sarcolemmal (sarc) or mitochondrial (mito) K_{ATP} channel in RPCT

Previous data published by Jones et al.¹ showed that the cardiac K_{ATP} channel is a distal mediator of RPCT in the mouse heart, however, the subtype involved, sarc K_{ATP} channel or the mito K_{ATP} channel or both was not addressed. Therefore, we determined if the sarc K_{ATP} or the mito K_{ATP} channel were involved in RPCT by pretreating rats with either glibenclamide, a nonselective K_{ATP} channel antagonist, HMR 1098, a selective sarc K_{ATP} antagonist or 5-HD, a selective mito K_{ATP} blocker. Interestingly, glibenclamide and 5-HD completely blocked the protective effect of RPCT ($61.7 \pm 1.3\%$, $64.7 \pm 1.3\%$), whereas, HMR 1098 did not ($50.3 \pm 1.3\%$, Figure 5). None of the 3 K_{ATP} channel antagonists had any effect on IS/AAR when administered to rats without the abdominal slit (Figure 5).

Discussion

The results of the present study are the first to characterize the signaling pathways responsible for remote preconditioning of trauma (RPCT) in the rat heart. The data suggest that, similar to results obtained in the mouse¹ and dog² heart, the sensory nerve activation produced by a surgically-induced abdominal incision or by topically applied capsaicin results in a marked reduction in myocardial infarct size. In all 3 models, bradykinin appears to be the common mediator of cardioprotection *via* activation of the B2 bradykinin receptor. In all 3 animal species, bradykinin receptor antagonists, HOE 140 or WIN 64338, blocked the protective effect of RPCT and also blocked the cardioprotective effect of exogenously administered bradykinin.

Since bradykinin, adenosine and endogenous opioids are the 3 major G protein-coupled receptors^{7, 8} involved in mediating classical ischemic preconditioning (IPC) and several models of ischemically-induced remote preconditioning (RPC),^{4, 5, 9} we examined a role for these 3 classical mediators in mediating RPCT by using selective receptor antagonists. Interestingly, bradykinin was the only mediator involved in RPCT in the rat heart, making this pathway different from classical IPC and RPC. To further confirm a role for bradykinin, exogenously administered bradykinin produced a reduction in infarct size similar in magnitude to that produced by RPCT (Figure 3).

Previous work from our laboratory^{10, 11} in the canine heart demonstrated an important role for the epoxyeicosatrienoic acids (EETs) as mediators of both ischemic preconditioning and postconditioning. Furthermore, the cardioprotective effect of bradykinin² in dog hearts was abolished by two EET antagonists, 14,15-EEZE,¹² a putative EET receptor antagonist, and MSPPOH, an EET synthesis inhibitor.¹³ Similar experiments were performed in the rat heart

with exogenous bradykinin and the two EET antagonists with confirming results. Bradykinin mimicked RPCT-induced infarct size reduction, blocked by 14,15-EEZE and MSPPOH (see Figure 3). These findings suggest an important role for EETs in mediating RPCT as part of the bradykinin signaling pathway.

Another stimulus mimicking the effect of RPCT in the mouse heart is the sensory nerve activator, capsaicin, which was shown by Jones et al.¹ to mimic RPCT when applied topically to the same area as used for the abdominal slit stimulation. On topical application of 0.1% capsaicin cream to the abdomen, we obtained a reduction in infarct size similar to that produced by RPCT. The cardioprotective effect of capsaicin cream was completely abolished by 14,15-EEZE or MSPPOH which further supports a key role for the EETs in mediating RPCT. More experiments are needed to determine the source of the EETs in mediating RPCT and where these compounds are spatially located in the signaling pathway.

In a final series of experiments, we determined the subtype of K_{ATP} channel which mediates RPCT. In the initial study published by Jones et al.,¹ RPCT in mice was partially blocked by the nonselective K_{ATP} channel antagonist, glibenclamide, and was completely abolished by the putative mitochondrial K_{ATP} channel antagonist 5-HD. In the rat heart, glibenclamide and 5-HD both completely abolished the cardioprotective effect of RPCT, whereas, the selective sarcolemmal K_{ATP} antagonist did not block RPCT (Figure 5). Although it is surprising that glibenclamide did not abolish RPCT in the mouse, whereas, 5-HD did,¹ our results clearly support the mitochondrial K_{ATP} channel as the major mediator of RPCT in rats. In spite of these results obtained with HMR 1098 and 5-HD, caution should be used when interpreting data obtained using these two K_{ATP} channel antagonists. There is considerable controversy surrounding the use of HMR 1098 and 5-HD as selective blockers of the sarc and mito K_{ATP} channels and several published papers suggest the K_{ATP} blockers possess alternative modes of action.^{14, 15} Nevertheless, the data obtained with glibenclamide, a well-known sulfonylurea K_{ATP} blocker¹⁶ strongly suggest a role for a cardiac K_{ATP} channel in mediating RPCT in the rat and mouse heart, most likely, the mito K_{ATP} channel.

We were surprised that naloxone did not block RPCT in the current study since we previously showed that opioids mediate the cardioprotective effect of exogenously administered EETs in rat hearts. In both studies, naloxone itself had no effects on infarct size of the rat heart. However, naloxone abolished the reduction of infarct size mediated by exogenous 11,12- or 14,15-EET in the intact rat heart¹⁷ but did not block the reduction of infarct size produced by RPCT (present study). At the moment we have no easy answer for these puzzling observations. We speculate that the RPCT may release multiple classes of mediators, particularly bradykinin as supported by our current results (Figure 2). Bradykinin stimulates the production of EETs in a variety of systems,^{18,19} and this hypothesis is supported by our results that the effects of both RPCT and bradykinin in cardioprotection are blocked by 14,15-EEZE and MSPPOH (Figure 2&3). EETs can activate numerous downstream signaling pathways including activation of endothelial nitric oxide synthase to release nitric oxide²⁰⁻²¹ and vascular and cardiac K_{ATP} channels.^{22,23} Our current data suggest that at least the mito- K_{ATP} channel is involved in the RPCT-mediated cardioprotection (Figure 5). The RPCT model possesses several questions of where the release of EETs occurs and whether the RPCT influences the downstream signaling of the EETs in which opioids may not be a major participant. Thus, the current results from RPCT may be different from those obtained following the direct administration of the EETs. Measurements of EETs, opioids and bradykinin in hearts or plasma may help furnish the answer.

Unanswered questions and future directions

There are several questions which remain in characterizing the phenomenon of RPCT. Obviously, this is a real endogenous cardioprotective mechanism which has been confirmed to exist in 3 animal species thus far, the mouse, rat and dog with many similarities in the signaling components investigated. One area of controversy that exists concerns the magnitude of the infarct size reduction. The greatest reduction in IS/AAR occurs in the mouse (approximately 80%), followed by the dog (approximately 60%) and then the rat (approximately 30%). It is not clear as to why there is such a large variability between these 3 species. Perhaps a better and more complete understanding of the neural and humoral⁹ signaling pathways involved in these species will lead to a novel compound that will give a reduction in infarct size equivalent to that observed in the mouse heart. Another interesting finding in the rat and dog studies is the important role played by the CYP epoxygenase pathway in mediating RPCT. Thus far, the evidence for an important role for the EETs in mediating RPCT is based on the use of two antagonists, 14,15-EEZE and MSPPOH. To confirm the importance of this pathway in mediating RPCT, it will be necessary to measure EET concentrations in plasma or cardiac tissue prior to and following RPCT, bradykinin or capsaicin administration to demonstrate that these stimuli actually increase EET concentrations and that these EET blockers antagonize these effects. As always in research, there are more questions than answers. At present, the RPCT phenomenon may be most important since its signaling pathway provides valuable new insight into cardioprotective signaling that is well worth studying with the hope of an eventual lead to a clinical application.³

Acknowledgments

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References

1. Jones WK, Fan GC, Liao S, et al. Peripheral nociception associated with surgical incision elicits remote nonischemic cardioprotection via neurogenic activation of protein kinase C signaling. *Circulation*. 2009; 120:S1–S9. [PubMed: 19752352]
2. Gross GJ, Baker JE, Moore J, Falck JR, Nithipatikom K. Abdominal Surgical Incision Induces Remote Preconditioning of Trauma (RPCT) via Activation of Bradykinin Receptors (BK2R) and the Cytochrome P450 Epoxygenase Pathway in Canine Hearts. *Cardiovasc Drugs Ther*. 2011; 25:517–522. [PubMed: 21786213]
3. Przyklenk K, Whittaker P. Remote ischemic preconditioning: current knowledge, unresolved questions, and future priorities. *J Cardiovasc Pharmacol Ther*. 2011; 16:255–259. [PubMed: 21821525]
4. Patel HH, Moore J, Hsu AK, Gross GJ. Cardioprotection at a distance: mesenteric artery occlusion protects the myocardium via an opioid sensitive mechanism. *J Mol Cell Cardiol*. 2002; 34:1317–1323. [PubMed: 12392992]
5. Moses MA, Addison PD, Neligan PC, et al. Mitochondrial KATP channels in hindlimb remote ischemic preconditioning of skeletal muscle against infarction. *Am J Physiol Heart Circ Physiol*. 2005; 288:H559–H567. [PubMed: 15458954]
6. Schultz JE, Hsu AK, Gross GJ. Morphine mimics the cardioprotective effect of ischemic preconditioning via a glibenclamide-sensitive mechanism in the rat heart. *Circ Res*. 1996; 78:1100–1104. [PubMed: 8635241]
7. Cohen MV, Downey JM. Adenosine: trigger and mediator of cardioprotection. *Basic Res Cardiol*. 2008; 103:203–215. [PubMed: 17999026]
8. Cohen MV, Philipp S, Krieg T, et al. Preconditioning-mimetics bradykinin and DADLE activate PI3-kinase through divergent pathways. *J Mol Cell Cardiol*. 2007; 42:842–851. [PubMed: 17292392]

9. Lim SY, Yellon DM, Hausenloy DJ. The neural and humoral pathways in remote limb ischemic preconditioning. *Basic Res Cardiol*. 2010; 105:651–655. [PubMed: 20449597]
10. Gross GJ, Falck JR, Gross ER, Isbell M, Moore J, Nithipatikom K. Cytochrome P450 and arachidonic acid metabolites: role in myocardial ischemia/reperfusion injury revisited. *Cardiovasc Res*. 2005; 68:18–25. [PubMed: 15993870]
11. Gross GJ, Gauthier KM, Moore J, Campbell WB, Falck JR, Nithipatikom K. Evidence for role of epoxyeicosatrienoic acids in mediating ischemic preconditioning and postconditioning in dog. *Am J Physiol Heart Circ Physiol*. 2009; 297:H47–H52. [PubMed: 19448143]
12. Gauthier KM, Deeter C, Krishna UM, et al. 14,15-Epoxyeicosa-5(Z)-enoic acid: a selective epoxyeicosatrienoic acid antagonist that inhibits endothelium-dependent hyperpolarization and relaxation in coronary arteries. *Circ Res*. 2002; 90:1028–1036. [PubMed: 12016270]
13. Gauthier KM, Yang W, Gross GJ, Campbell WB. Roles of epoxyeicosatrienoic acids in vascular regulation and cardiac preconditioning. *J Cardiovasc Pharmacol*. 2007; 50:601–608. [PubMed: 18091575]
14. Hanley PJ, Mickel M, Löffler M, Brandt U, Daut J. K(ATP) channel-independent targets of diazoxide and 5-hydroxydecanoate in the heart. *J Physiol*. 2002; 542:735–741. [PubMed: 12154175]
15. Rainbow RD, Norman RI, Hudman D, Davies NW, Standen NB. Reduced effectiveness of HMR 1098 in blocking cardiac sarcolemmal K(ATP) channels during metabolic stress. *J Mol Cell Cardiol*. 2005; 39:637–646. [PubMed: 16099467]
16. Cleveland JC Jr, Meldrum DR, Cain BS, Banerjee A, Harken AH. Oral sulfonylurea hypoglycemic agents prevent ischemic preconditioning in human myocardium. Two paradoxes revisited. *Circulation*. 1997; 96:29–32. [PubMed: 9236412]
17. Gross GJ, Baker JE, Hsu A, Wu HE, Falck JR, Nithipatikom K. Evidence for a Role of Opioids in Epoxyeicosatrienoic Acid (EET)-induced Cardioprotection in Rat Hearts. *Am J Physiol Heart Circ Physiol*. 2010; 298(6):2201–2207.
18. Weston AH, Félétou M, Vanhoutte PM, Falck JR, Campbell WB, Edwards G. Bradykinin-induced, endothelium-dependent responses in porcine coronary arteries: involvement of potassium channel activation and epoxyeicosatrienoic acids. *Br J Pharmacol*. 2005; 145:775–784. [PubMed: 15895105]
19. Campbell WB, Fleming I. Epoxyeicosatrienoic acids and endothelium-dependent responses. *Pflugers Arch*. 2010; 459:881–895. [PubMed: 20224870]
20. Wang H, Lin L, Jiang J, Wang Y, Lu ZY, Bradbury JA, Lih FB, Wang DW, Zeldin DC. Up-regulation of endothelial nitric-oxide synthase by endothelium-derived hyperpolarizing factor involves mitogen-activated protein kinase and protein kinase C signaling pathways. *J Pharmacol Exp Ther*. 2003; 307:753–764. [PubMed: 12975498]
21. Jiang JG, Chen RJ, Xiao B, Yang S, Wang JN, Wang Y, Cowart LA, Xiao X, Wang DW, Xia Y. Regulation of endothelial nitric-oxide synthase activity through phosphorylation in response to epoxyeicosatrienoic acids. *Prostaglandins Other Lipid Mediat*. 2007; 82:162–174. [PubMed: 17164144]
22. Lu T, Hoshi T, Weintraub NL, Spector AA, Lee HC. Activation of ATP-sensitive K(+) channels by epoxyeicosatrienoic acids in rat cardiac ventricular myocytes. *J Physiol*. 2001; 537:811–827. [PubMed: 11744757]
23. Lu T, Ye D, Wang X, Seubert JM, Graves JP, Bradbury JA, Zeldin DC, Lee HC. Cardiac and vascular K_{ATP} channels in rats are activated by endogenous epoxyeicosatrienoic acids through different mechanisms. *J Physiol*. 2006; 575:627–644. [PubMed: 16793897]

Figure 1.
Experimental protocol for remote preconditioning with trauma (RPCT) in a rat *in vivo* infarct model.

Figure 2.

Remote preconditioning with trauma (RPCT) results in a significant reduction in myocardial infarct size expressed as a percent of the area-at-risk (IS/AAR) in the intact rat heart. This effect was abolished by pretreatment with the selective bradykinin 2 receptor (BK2R) antagonists, WIN 64338 (1.0 mg/kg, iv) and HOE 140 (50 µg/kg, iv) but not by the adenosine A1 receptor blocker, DPCPX (1.0 mg/kg, iv) or by the nonselective opioid receptor antagonist naloxone (3.0 mg/kg, iv) All values are the mean $\pm$ SEM of 8 animals/group. *P < 0.01 vs the Control group.

Figure 3.

Bradykinin (60 $\mu\text{g/kg}$, iv) produced a significant reduction in IS/AAR in the intact rat heart. This cardioprotective effect was abolished by pretreatment with the EET receptor blocker, 14,15-EEZE (2.5 mg/kg, iv), and the EET synthesis inhibitor, MSPPOH (3.0 mg/kg, iv). These effects of bradykinin and 14,15-EEZE and MSPPOH in cardioprotection are identical to the RPCT-mediated cardioprotection. All values are the mean $\pm$ SEM of 8 animals/group. * $P < 0.01$ vs the Control Group.

Figure 4.

Topical application of capsaicin cream (0.1%) produced a significant reduction in IS/AAR similar in magnitude to that observed with RPCT (Figure 2). The cardioprotective effect of capsaicin cream was completely abolished by pretreatment with the two EET antagonists, 14,15-EEZE (2.5 mg/kg, iv) or MSPPOH (3.0 mg/kg, iv). All values are the mean $\pm$ SEM of 8 rats/group. *P < 0.01 vs the Control group.

Figure 5.

Pretreatment with the nonselective K_{ATP} channel antagonist, glibenclamide (1.0 mg/kg, iv), and the selective mito K_{ATP} channel antagonist 5-HD (10 mg/kg, iv) completely abolished the cardioprotective effect of RPCT, whereas, the selective sarcolemmal K_{ATP} channel blocker, HMR 1098 (6.0 mg/kg, iv), did not block the beneficial effect of RPCT. All values are the mean $\pm$ SEM of 8 rats/group. * $P < 0.01$ vs the Control group. + $P < 0.05$ vs the Control group.

Table 1

Hemodynamic Values (Heart Rate and AAR/LV)

Protocol	Heart Rate (beats/min)			AAR/LV
	Con	15-min Occ	2-h Rep	(%)
Control	378 ± 7	388 ± 6	366 ± 5	41.0 ± 1.1
RPCT	378 ± 6	383 ± 6	355 ± 5	45.0 ± 1.5
RPCT + Naloxone	368 ± 7	373 ± 7	366 ± 10	39.3 ± 3.3
Naloxone	398 ± 18	402 ± 16	360 ± 12	40.0 ± 4.1
RPCT + DPCPX	364 ± 12	361 ± 17	370 ± 14	44.7 ± 2.2
DPCPX	376 ± 9	388 ± 9	382 ± 15	42.3 ± 0.9
RPCT + WIN 64338	363 ± 7	361 ± 10	359 ± 7	40.4 ± 1.2
WIN 64338	382 ± 11	398 ± 12	384 ± 13	38.4 ± 1.0
RPCT + HOE 140	365 ± 8	378 ± 10	365 ± 13	38.5 ± 1.4
HOE 140	386 ± 10	386 ± 8	362 ± 5	34.6 ± 2.7
Bradykinin	398 ± 4	392 ± 9	373 ± 6	40.8 ± 2.9
Bradykinin + 14,15 -EEZE	415 ± 8	402 ± 7	383 ± 7	43.1 ± 1.6
14,15-EEZE	368 ± 9	361 ± 10	348 ± 3	40.3 ± 2.7
Bradykinin + MS-PPOH	404 ± 6	395 ± 9	365 ± 7	40.0 ± 1.9
MS-PPOH	402 ± 8	403 ± 6	373 ± 9	41.8 ± 2.4
RPCT + 14,15-EEZE	363 ± 6	368 ± 7	348 ± 8	40.5 ± 1.0
RPCT + MS-PPOH	370 ± 4	375 ± 8	333 ± 8	42.0 ± 1.1
Capsaicin	378 ± 6	385 ± 17	363 ± 17	44.1 ± 1.5
Capsaicin + 14,15-EEZE	383 ± 10	375 ± 8	373 ± 10	40.6 ± 2.1
Capsaicin + MS-PPOH	397 ± 6	390 ± 9	365 ± 8	43.7 ± 1.0
RPCT + Glibenclamide	378 ± 11	380 ± 12	363 ± 18	36.7 ± 2.8
Glibenclamide	390 ± 9	393 ± 16	365 ± 13	38.2 ± 2.0
RPCT + 5-HD	382 ± 12	388 ± 12	354 ± 12	41.7 ± 1.6
5-HD	404 ± 4	414 ± 4	476 ± 2	40.4 ± 2.2
RPCT + HMR 1098	381 ± 9	392 ± 9	354 ± 8	42.4 ± 1.8
HMR 1098	385 ± 10	392 ± 9	378 ± 8	41.7 ± 2.2

All values are the mean ± SEM (N = 8–15 rats).

Abbreviations: AAR = Area-at-risk; Con = Control; LV = Left Ventricle; Occ = Occlusion; Rep = Reperfusion

Table 2

Hemodynamic Values (Blood Pressure, mmHg)

Treatment Protocol	SBP			DBP		
	Con	15-min Occ	2-h Rep	Con	15-min Occ	2-h Rep
Control	156 ± 4	136 ± 4	112 ± 3	124 ± 4	113 ± 4	82 ± 4
RPCT	148 ± 3	123 ± 5	104 ± 3	114 ± 2	110 ± 5	76 ± 3
RPCT + Naloxone	160 ± 6	137 ± 6	114 ± 3	129 ± 2	113 ± 6	87 ± 5
Naloxone	141 ± 6	129 ± 3	105 ± 3	112 ± 7	107 ± 3	73 ± 2
RPCT + DPCPX	167 ± 7	105 ± 11	114 ± 4	133 ± 4	81 ± 12	81 ± 5
DPCPX	161 ± 11	118 ± 5	111 ± 6	129 ± 9	100 ± 6	74 ± 7
RPCT + WIN 64338	175 ± 3	129 ± 7	107 ± 3	139 ± 2	106 ± 8	77 ± 3
WIN 64338	184 ± 7	144 ± 7	113 ± 4	139 ± 4	122 ± 7	77 ± 8
RPCT + HOE 140	137 ± 5	126 ± 8	109 ± 6	107 ± 5	102 ± 7	77 ± 6
HOE 140	143 ± 12	113 ± 8	104 ± 5	115 ± 12	90 ± 9	66 ± 5
Bradykinin	151 ± 5	120 ± 6	105 ± 4	117 ± 3	94 ± 6	69 ± 3
Bradykinin + 14,15-EEZE	145 ± 7	117 ± 7	104 ± 5	113 ± 5	95 ± 6	76 ± 3
14,15-EEZE	142 ± 6	124 ± 8	106 ± 8	116 ± 5	101 ± 8	76 ± 9
Bradykinin + MS-PPOH	144 ± 4	111 ± 9	98 ± 6	112 ± 3	87 ± 9	72 ± 5
MS-PPOH	140 ± 6	125 ± 8	101 ± 5	111 ± 6	104 ± 8	67 ± 5
RPCT + 14,15-EEZE	134 ± 3	119 ± 6	99 ± 8	103 ± 4	96 ± 5	68 ± 8
RPCT + MS-PPOH	122 ± 7	113 ± 5	94 ± 6	88 ± 7	87 ± 6	64 ± 6
Capsaicin	136 ± 7	121 ± 5	105 ± 8	106 ± 8	98 ± 5	77 ± 7
Capsaicin + 14,15-EEZE	131 ± 4	112 ± 7	104 ± 5	95 ± 6	83 ± 9	66 ± 5
Capsaicin + MS-PPOH	130 ± 6	123 ± 7	97 ± 3	106 ± 5	103 ± 6	65 ± 4
RPCT + Glibenclamide	147 ± 4	128 ± 5	114 ± 4	117 ± 3	104 ± 5	80 ± 4
Glibenclamide	145 ± 4	122 ± 6	112 ± 3	116 ± 4	103 ± 8	77 ± 4
RPCT + 5-HD	159 ± 7	137 ± 8	118 ± 5	126 ± 7	114 ± 8	88 ± 6
5-HD	145 ± 3	128 ± 7	106 ± 5	115 ± 4	106 ± 6	68 ± 4
RPCT + HMR 1098	125 ± 6	112 ± 5	96 ± 2	97 ± 4	91 ± 5	66 ± 2
HMR1098	143 ± 26	115 ± 9	109 ± 8	107 ± 23	92 ± 11	78 ± 11

All values are the mean $\pm$ SEM (N = 8–15 rats).

Abbreviations: Con = Control; Oce = Occlusion; Rep = Reperfusion; DBP = Diastolic Blood Pressure; SBP = Systolic Blood Pressure.