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Nociceptive-induced Myocardial Remote Conditioning Is Mediated By Neuronal Gamma Protein Kinase C

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Abstract

Deciphering the remote conditioning molecular mechanism may provide targets to develop therapeutics that can broaden the clinical application. To further investigate this, we tested whether two protein kinase C isozymes, the ubiquitously expressed epsilon PKC (εPKC) and the neuronal specific gamma PKC (γPKC), mediate nociceptive-induced remote myocardial conditioning.

Male Sprague-Dawley rats were used for both *in vivo* and *ex vivo* myocardial ischemia-reperfusion protocols. For the *in vivo* studies, using a surgical abdominal incision for comparison, applying only to the abdomen either bradykinin or the εPKC activator (ψεRACK) reduced myocardial infarct size (45±1%, 44±2%, respectively, versus incision: 43±2%, and control: 63±2%, $P < 0.001$). Western blot showed only εPKC, and not γPKC, is highly expressed in the myocardium. However, applying a selective γPKC inhibitor (γV_{5-3}) to the abdominal skin blocked remote protection by any of these strategies.

Using an *ex vivo* isolated heart model without an intact nervous system, only selective εPKC activation, unlike a selective classical PKC isozyme activator (activating α, β, β_{II} and γ), reduced myocardial injury. Importantly, the classical PKC isozyme activator given to the abdomen *in vivo* (with an intact nervous system including γPKC) during myocardial ischemia reduced infarct size as effectively as an abdominal incision or ψεRACK (45±1% versus 45±2% and 47±1%, respectively). The classical PKC activator-induced protection was also blocked by spinal cord surgical transection.

These findings identified potential remote conditioning mimetics, with these strategies effective even during myocardial ischemia. A novel mechanism of nociceptive-induced remote conditioning, involving γPKC, was also identified.

Keywords

infarct size; remote; incision; protein kinase C; gamma; epsilon

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Introduction

Remote conditioning either via a blood pressure cuff, by surgical abdominal incision or by femoral nerve stimulation, robustly decreases myocardial injury in rodents and canines [16, 17, 25, 28, 38]. Remote conditioning is a highly translatable technique for use in humans with planned ischemia-reperfusion events, such as coronary artery bypass grafting (CABG), and early clinical trials are encouraging [21, 46].

There is little known whether pharmacological agents given remotely can induce cardioprotection. Presently few pharmacological mimetics of remote conditioning exist [17, 25]. Identifying additional pharmacological mimetics to induce remote conditioning may provide a wider utility for use, including during percutaneous coronary intervention, myocardial infarction or out of hospital arrest. Further, the efficacy of remote conditioning during or after myocardial ischemia is largely unknown, and if effective even during ischemia, could provide an extended therapeutic window to administer remote conditioning agents.

Here we set out to identify further the molecular mechanism leading to remote conditioning to provide therapeutic alternatives. The trigger for remote conditioning is largely unknown. However, initial studies suggest both neuronal and humoral signaling components are involved [25, 28, 38]. We focused on the neuronal-mediated component of remote conditioning and surmised perhaps the protective signal occurs *via* ubiquitously and neuronal specific proteins modulating nociception.

Therefore, we investigated whether components of nociceptive signaling, including bradykinin and two protein kinase C (PKC) isozymes, are pivotal in mediating nociception-induced myocardial infarct size reduction. We focused on the ubiquitously expressed epsilon PKC (εPKC) and a PKC isozyme that is only highly expressed in neuronal cells, gamma PKC (γPKC) [4, 31, 45]. We describe here potential pharmacological mimetics, their timing to induce myocardial remote conditioning, and how this pathway is mediated via the neuronal specific PKC isozyme, γPKC.

Methods

All animal studies conformed to the National Institute of Health *Guide for the Care and Use of Laboratory Animals.* The procedures and protocols used in this study were approved by the Animal Care and Use Committee at both the Medical College of Wisconsin and Stanford University. Eight to ten-week-old male Sprague Dawley rats (Harlan Laboratories) were used for the studies outlined.

Pharmacological Agents and Peptide Synthesis

All agents were dissolved in water. Peptides were designed based upon uniquely evolutionary conserved sequences for specific PKC isozymes or a specific isozyme class (such as classical or novel), as previously described [2, 44]. All peptides used in this manuscript were previously validated and published, including the εPKC activator (ψεRACK) [11, 22, 23, 42], the εPKC inhibitor ($\varepsilon V_{1\text{-}2}$) [14, 30], the classical PKC activator (ψβRACK) [7, 39, 40], which selectively targets only the classical PKC isozymes (α, β_I, β_{II}, and γ), including γPKC, and the γPKC inhibitor ($\gamma V_{5\text{-}3}$) [26, 45].

Activator peptides stabilize the PKC transition state *via* intra-molecular interactions (Figure 1a). The peptide sequence allows for release of a PKC isozyme allowing for cellular translocation, or as termed in this manuscript, activation. Additional peptide sequences unique to specific εPKC and γPKC isozymes were previously designed which in turn limit

isozyme binding to PKC protein partners. This is termed in this manuscript as inhibition and occurs by blocking anchoring of PKC to a protein by the peptide blocking the interaction site (Figure 1b). To allow for intracellular delivery, the peptide sequences were conjugated to a partial TAT sequence that penetrates cellular membranes (Figure 1c and d).

Agents administered included bradykinin (60ng/kg, Tocris), TAT_{47-57} carrier peptide (1mg/kg) and TAT_{47-57}-conjugated εPKC- and γPKC activator or inhibitor specific peptides (1mg/kg). Drug doses were determined based upon previous studies [17, 37].

In Vivo Myocardial Infarction Model

The model performed was summarized in the supplemental material and previously described in a number of publications (Supplemental Methods) [15, 17]. The experimental protocol is an *in vivo* myocardial infarction model consisting of 30 minutes ischemia and 2 hours of reperfusion followed by infarct size assessment. Hemodynamics, including heart rate, blood pressure and rate pressure product, arterial blood gasses and body temperature were measured throughout the experiment. The performer of the experimental protocol, AKH, was blinded to the drugs given with each drug identity described as a random number. AKH could not be blinded to the surgical incision.

Briefly, rats were divided into groups to first test the ability for pharmacological agents to cause remote conditioning when compared to a surgical incision. Drugs were given in equal volume per weight (0.25-0.3mL) over a surface area approximately 4 cm by 1–2 mm using a 25 gauge needle to the abdominal dermal layer. A second group of rats were used to test the involvement of εPKC and γPKC in triggering remote conditioning by surgical incision or mimetics. A surgical transection of the spinal cord at T10 was further used to test whether the effects seen by the mimetic occurs through neuronal signaling *via* the spinal cord. An additional series of rats were used to test whether the mimetics are effective even when given during ischemia.

Isolated Heart Myocardial Ischemia-Reperfusion Model

The protocol used has been extensively previously described [3, 23]. The performer of the experiments (TJU) was blinded to the drugs administered. Briefly, rats were anesthetized with beuthanasia and heparin given intra-peritoneal to the abdomen. After adequate anesthesia was maintained, hearts were quickly excised and the aorta was secured to the aortic cannula of a Langendorff apparatus. Hearts were perfused with Krebs buffer (120mM NaCl, 5.8mM KCl, 1.0mM CaCl, 1.2mM $MgSO_4$, 25mM $NaHCO_3$, 1.2mM NaH_2PO_4, 10mM Dextrose). Left ventricular pressure balloons were made from plastic wrap as described (Cast Away Cling Wrap, gift from Dr. Jason Peart) [35]. The balloons were connected to a pressure transducer (MLT-0699, ADI Instruments) to measure left ventricular hemodynamics including, end diastolic pressure, left ventricular developed pressure, heart rate, time to contracture, +dP/dt, and –dP/dt.

Biochemical Analysis

Western Blot—To determine the protein expression for γPKC and εPKC, Western blotting was performed on left ventricular myocardium and spinal cord tissue homogenate. Brain homogenate was used as a positive control, in addition to recombinant protein to quantify PKC isozyme amounts in tissues. Recombinant protein was used instead of a loading control since housekeeping genes, such as GAPDH, substantially differ between tissues [12]. Tissue was excised, finely minced with scissors and homogenized with mannitol-sucrose lysis buffer (210mM mannitol, 70mM sucrose, 5mM MOPS, 1mM EDTA with pH 7.4), protease/phosphatase inhibitors and 1% Triton-X (Sigma, St. Louis, MO). Homogenates were centrifuged at 800g to remove cellular debris and the supernatant kept as the total fraction.

Protein content was determined by Bradford assay, prepared in sample buffer at a concentration of 1μg/μl, boiled and 30μg of each homogenate was run on 10% SDS page gels. Membrane proteins were transferred to PVDF membrane and probed overnight for specific antibodies to εPKC and γPKC isozymes (1:500 dilution in 5% milk, Santa Cruz). The next day, membranes were washed and incubated in secondary anti-rabbit antibody for 90 minutes (1:3000 dilution in 5% milk, ThermoScientific). Membranes were reacted with ECL.

Statistical Analysis

All values were expressed as the mean ± SEM. A one-way ANOVA comparing each group to the vehicle treated group was performed to determine statistical significance for *in vivo* and isolated heart infarct size. A two-way ANOVA was performed to assess all hemodynamic measurements (Prism Software, La Jolla, CA).

Results

For *in vivo* remote conditioning infarct size studies, 118 rats were used in 114 successful experiments. Four rats were excluded secondary to intractable ventricular fibrillation during myocardial occlusion or reperfusion (2 from $\varepsilon V_{1\text{-}2}$ + incision group, 1 from incision during ischemia group, 1 from $TAT_{47\text{-}57}$ group). No significant differences were noted in heart rate, mean arterial pressure and rate pressure product compared to control (Supplemental Table 1). No significant hemodynamic differences were noted immediately after surgical incision or drug administration. No statistical differences in myocardial area at risk compared to left ventricle were noted when compared to the untreated control group (Supplemental Figure 1 and 2).

As previously reported [17], we found that an abdominal surgical incision prior to ischemia reduced myocardial infarct size by 30%. Either bradykinin or the εPKC activator, ψεRACK, given to the same abdominal area, produced a comparable myocardial infarct size reduction (Figure 2b). Administration of the εPKC selective inhibitor, $\varepsilon V_{1\text{-}2}$, to the abdomen prior to abdominal incision or bradykinin, blocked cardiac protection from either of these two treatments (Figure 3b).

We next confirmed the tissue distribution of the PKC isozymes, εPKC and γPKC, and tested whether the γPKC isozyme triggers remote nociceptive conditioning by bradykinin or surgical incision. Western blot of normoxia tissues revealed εPKC is present ubiquitously for all tissue homogenates tested. Unlike εPKC, γPKC was not expressed in left ventricular myocardial tissue homogenate (Figure 4a). Selective inhibition of γPKC given to the abdomen blocked cardiac protection afforded by εPKC activation, in addition to either bradykinin or surgical incision (Figure 4c).

To determine whether γPKC activation requires an intact nervous system, we further continued our studies using an isolated heart protocol (Figure 5a). For isolated heart studies, 31 rats were used for 25 successful experiments. Six rats were excluded secondary to 1 rat heart taking greater than 3 minutes to be hung on the aortic cannula, 2 rats with LVEDP greater than 10mmHg at the start of baseline, 2 rats for failure to establish a LVDP greater than 70mmHg and 1 rat for intractable ventricular fibrillation upon reperfusion in the control group. Myocardial infarct size was similar when comparing TAT and the classical PKC activator, ψβRACK, which selectively targets all classical PKC isozymes (α, β, β_{II}, γ), including γPKC, compared to control [39]. Infarct size was significantly reduced when the εPKC activator was given compared to control (Figure 5b and c). Neither agent significantly affected the time to contracture (Figure 5d). The infarct size findings were consistent with +dP/dt, −dP/dt and the percentage recovery of LVDP at reperfusion when compared to

baseline LVDP values (Figure 5e-g). No baseline differences in heart rate, LVDP or EDP were noted (Supplemental Table 2).

We next found although the γPKC activator was ineffective *ex vivo*, the γPKC activator delivered to the abdomen *in vivo* during ischemia reduced myocardial infarct size (Figure 6b). To confirm γPKC mediated activation is mediated by a neuronal signal, we severed the spinal cord at T10, above the site of where the γPKC activator was administered. Although displaying no noticeable hemodynamic effects after the cord was severed at T10 (Supplemental Table 3), the γPKC activator when given to the abdomen failed to reduce myocardial infarct size (Figure 6b).

Further, the γPKC activator-induced myocardial salvage mimicked the protection of an abdominal incision or the εPKC activator when given during ischemia (Figure 7b). In contrast, the protection induced by an abdominal surgical incision did not reduce infarct size when performed immediately after reperfusion (Figure 7b). Both the εPKC and γPKC activators had no effects on hemodynamics when compared to the abdominal incision groups (Supplemental Table 3).

Discussion

Although remote preconditioning of the myocardium is well described, besides capsaicin, pharmacological strategies to mimic the effect have not been identified [25, 28]. Here we describe cardioprotective mimetic agents given remotely that target a γPKC-dependent neuronal pathway and reduce myocardial injury even when given during ischemia. The mimetics used had no negative hemodynamic effects, avoiding the potential for further ischemic injury by increasing myocardial demand when triggering a nociceptive-inducing pathway. We also demonstrate a specific role for γPKC, which is expressed only in neurons, in mediating remote nociceptive-induced cardioprotection.

The contribution of γPKC in tissue protection is not unprecedented since γPKC is important to reduce injury from ischemia in the brain [1, 19] and oxidative stress in the lens [29]. How γPKC mediates the signal caused by the abdominal intervention to the heart is likely through the highly innervated abdominal skin, since γPKC was present in homogenized mouse skin transmitting a signal to the spinal cord [27]. This is supported by findings that a neuronal specific fluorescent dye, dil, locally injected to the abdominal skin at the same site of our abdominal incision travels from the abdominal neurons, to the spinal cord, and up to nerve endings innervating the heart at T1-T4 [25]. Moreover, the pathway inducing cardioprotection does not require input from the brain since transection above C7 does not alter the incision-induced cardioprotective effect [25].

In the spinal cord, γPKC is selectively localized to the posterior dorsal horn mainly in Rexed lamina II (substantia gelatinosa) [34], with perhaps a portion of γPKC localized in lamina I and lamina III [36]. Interestingly, lamina II is highly populated with opioid receptors [49], and where unmyelinated C fibers and a portion of the myelinated nerve fibers terminate. Positive stained γPKC neurons in lamina II were those of the myelinated nerve fibers, rather than unmyelinated fibers [34]. In a γPKC knockout mouse, deletion of γPKC alters neurotransmitter release of substance P, neurokinin-1 and neuropeptide Y during sciatic nerve injury [31]. Thus, γPKC, unlike other PKC isozymes, is selectively expressed only in the brain, peripheral nerves and select regions of the spinal cord that in turn regulate neurotransmitter release.

Although we did not provide biochemical evidence displaying γPKC translocation in the spinal cord, two independent pain studies using a paw surgical incision showed an incision-

induced translocation of γPKC from the cytosolic to particulate fraction in the spinal cord by Western blot that persisted for hours [9, 48]. Specifically, of all classical γPKC isozymes, only γPKC translocation after incision was noted in the spinal cord, with α, β_I and β_{II} isozymes unchanged [48]. After nerve injury, γPKC knockout mice display less neuropathic pain behavior and further, γV_{5-3} reduces pain response in strychnine injected rats [31, 33]. In addition, formalin-induced translocation of γPKC is blocked by the administration of the εPKC inhibitor, εV_{1-2} [45]. These data, in combination with our present findings, support the role for γPKC in mediating the cardioprotective signal of an abdominal incision and the incision mimetics *via* nociceptive pathways.

As a classical PKC isozyme, translocation of γPKC from the cytosol to membrane is dependent upon calcium, unlike the εPKC and δPKC isozymes belonging to the novel PKC isozyme class. A number of protein partners for γPKC were identified through proteomic analysis [51] and recent evidence suggests a γPKC-synapsin pathway may be important for hypoxia-induced neuroprotection [51]. In turn, γPKC subsequently regulates receptors including opioids, NMDA, AMPA, GLUR1 and TRPV1 [24, 32, 48], which all may be potential candidates to further initiate the cardioprotective signal. In addition, whether the humoral component of remote cardioprotection, previously shown by others [28, 38], is connected to the nociceptive-mediated pathway, will require further examination.

Remote cardioprotection is dependent on the sympathetic nervous system, without parasympathetic nerve involvement [25]. The classical PKC peptide, activating α, β_I, β_{II}, and γPKC, without an intact nervous system in isolated hearts, was ineffective at reducing myocardial infarct size, even though both α and β PKC are present in the adult rat heart [41]. Additionally, when directly applied to myocytes, the classical PKC activator did not reduce ischemic injury [7], indicating γPKC reduces myocardial infarct size only remotely. Findings in homogenized human hearts suggest that γPKC is expressed in the myocardium only at a very low abundance (<1ng/μg tissue) [18]. Since γPKC is present in only heart homogenates and not expressed in the rat ventricle derived H9C2 cell line, nerve endings expressing γPKC are likely present in the homogenized tissue [18, 50].

Moreover, since εPKC is ubiquitously expressed, εPKC provides a dual role as a specific trigger, initiating the cardioprotective signal in remote tissue and also acting as an end effector at the myocardium after the signal is transmitted through the neuronal system [23, 25]. This is supported by our findings that 1) abdominal administration of the εPKC activator is blocked by γPKC inhibition, and 2) when the εPKC activator is given directly to the heart in an isolated heart model, the εPKC activator is also cardioprotective. Where PKC signaling contributes to other components as triggers of this pathway, including nitric oxide [38] and end effectors such as STAT5 [20], will need further elucidation. The contribution of εPKC in remote cardioprotection is also supported since aldehyde dehydrogenase 2 (ALDH2), downstream of εPKC, is required for remote cardioprotection in humans [6, 8].

The ability for remote conditioning to protect the myocardium during ischemia by a surgical incision, εPKC, or γPKC activation also suggests that an extended window exists into the ischemic period that produces remote conditioning. This finding also supports that a surgical incision is just as protective during as compared to before myocardial ischemia [25]. We extend these findings to show a surgical incision is ineffective after reperfusion.

Our study should be interpreted with considerations of the following limitations. The reperfusion period selected for these studies was short, however, our studies here were aimed to identify additional mimetics and the molecular mechanism of remote conditioning. We were able to compare the effects of these mimetics with prior studies, showing the degree of myocardial infarct size reduction is comparable to bradykinin, opioids, glycogen

synthase kinase 3β inhibitors and K_{ATP} channel openers given intravenously [15, 17]. To determine the therapeutic potential for these newly discovered remote conditioning mimetics, investigating longer reperfusion periods and cardiac functional status weeks after a myocardial infarction will be a focus of future studies.

The γPKC isozyme, with the slight ataxia reported for the γPKC knockout mouse [5], parallels the finding human ataxia occurs by point mutations in the γPKC C1 domain region [10]. Even though in general PKC isozyme differences may occur based on the species used [47], γPKC isozyme function appears similar across mammalian species. Additionally, an abdominal incision is effective in reducing myocardial damage in a canine large animal model [16], however, since the signaling cascades of cardioprotection differ between species and in particular larger animals [43], validation of this molecular pathway in large animals will be needed. Further, since the classical PKC activator in this study is not specific for the C2 domain of γPKC, and sustained activation of αPKC and βPKC are linked to the pathophysiology of heart failure [13], a peptide that selectively activates only γPKC, instead of all classical isozymes will also be an area of further investigation.

We also cannot completely exclude the agents administered subdermal reach the circulation. However, our findings support this does not occur since 1) surgical transection of the spinal cord blocks the protective effect of the classical PKC activator and 2) the εPKC activator, if reaching the circulation, should reduce infarct size in the presence of the γPKC inhibitor, $\gamma V_{5\text{-}3}$.

Here we identified γPKC as a neuronal trigger for remote cardioprotection. A summary for the proposed signaling process leading to remote cardioprotection is shown (Figure 8). The cardioprotective mimetics described here are effective when applied remotely. Importantly, this treatment is beneficial even during ischemia. Although requiring further study, these findings may have broad applications for organ preservation from ischemic damage during transplantation, percutaneous coronary interventions, myocardial infarction and out of hospital arrest.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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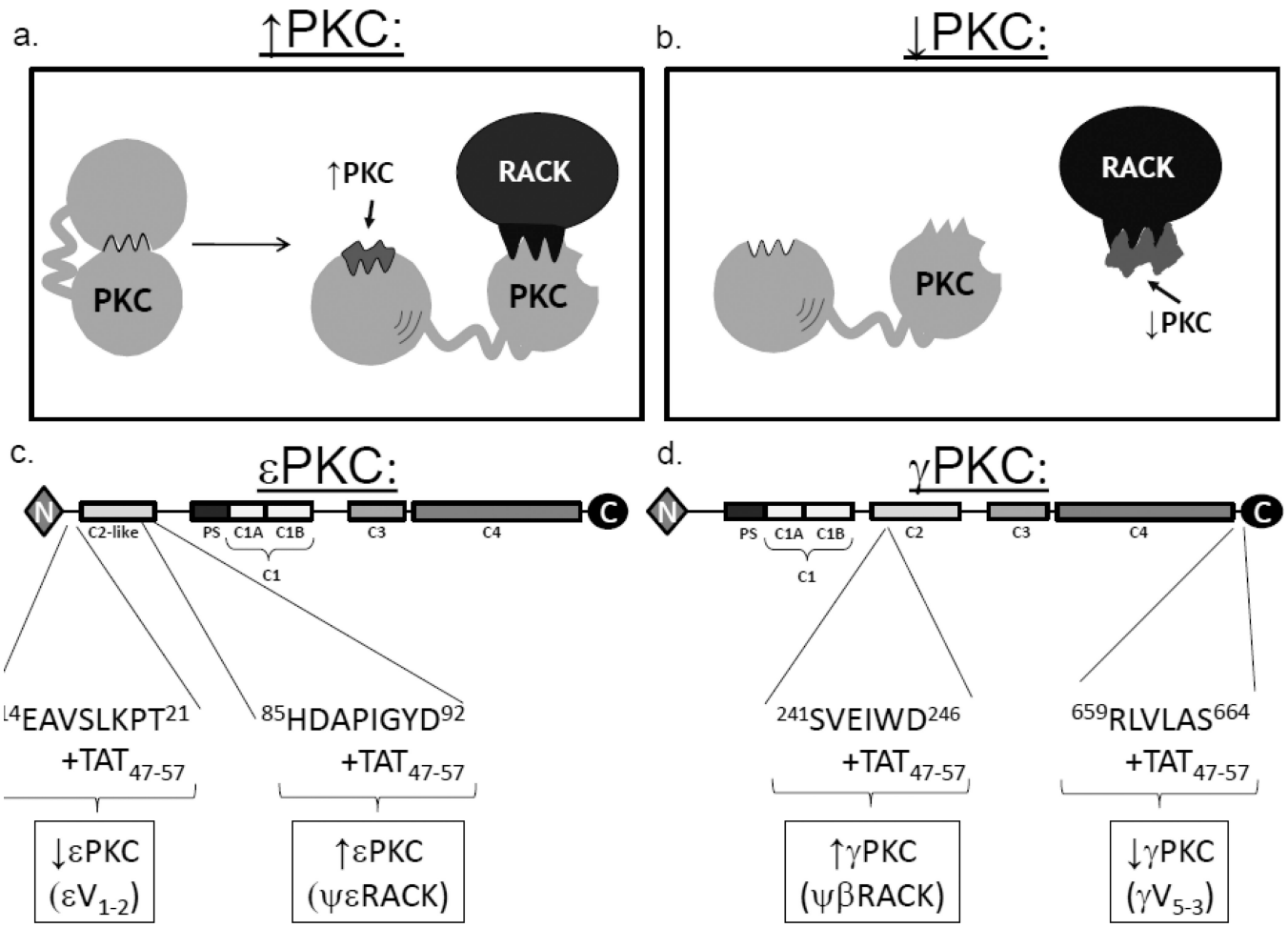

Fig. 1.

Cartoon and schematic describing PKC activator and inhibitor peptides. **a**. PKC activator (↑PKC) cartoon. PKC is present in a state allowing for intra-molecular interactions, limiting translocation and PKC-protein interaction. An activator peptide, constructed by a short peptide sequence homologous to the intra-molecular interaction site, disrupts the intra-molecular interactions, allowing a PKC isozyme to interact with a protein partner, traditionally described as a receptor for activated C kinase (RACK). **b**. PKC inhibitor (↓PKC) cartoon. Short peptide sequences unique to a PKC isozyme or PKC class limit the inter-molecular interactions between a PKC isozyme and protein partner. Inhibition is based upon limiting the ability for the PKC isozyme to interact with a RACK. **c** and **d**. Amino acid location and sequences used for the peptide design of activators and inhibitors for both εPKC and γPKC. Sequences were conjugated to a partial TAT sequence consisting of amino acids 47–57 for intracellular entry [44].

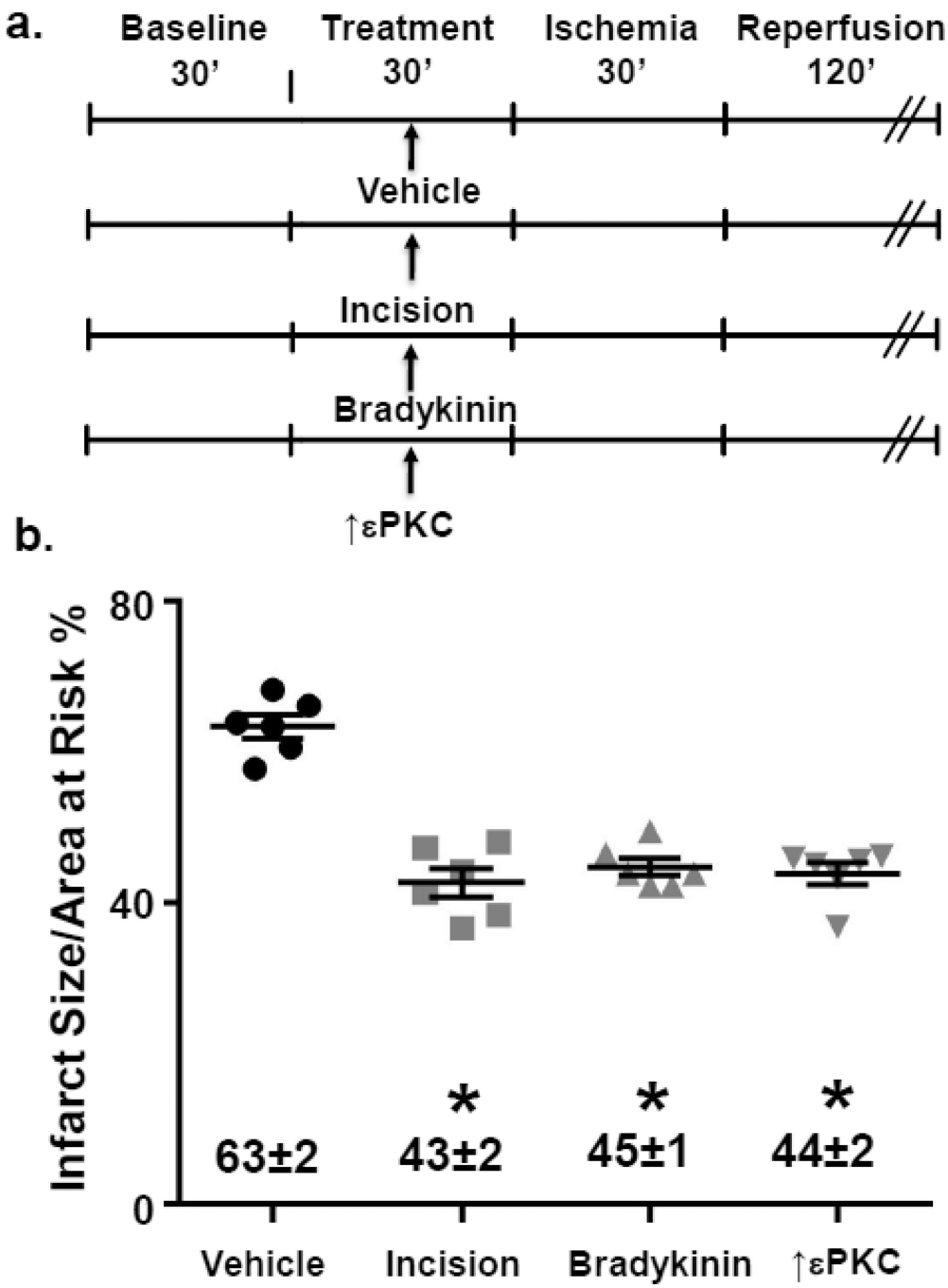

Fig. 2.

Pharmacological mimetics of nociceptive-induced remote conditioning. Experimental protocol and myocardial infarct size expressed as area at risk percentage. **a**. Experimental protocol, with incision or drugs given 15 minutes prior to ischemia to the abdominal dermal layer. **b**. Bradykinin or PKC activator (↑εPKC) reduces infarct size equally compared to an abdominal incision. *P <0.001 versus control. The individual infarct sizes for each animal are plotted in addition to the mean ± SEM numerical value listed below each individual group.

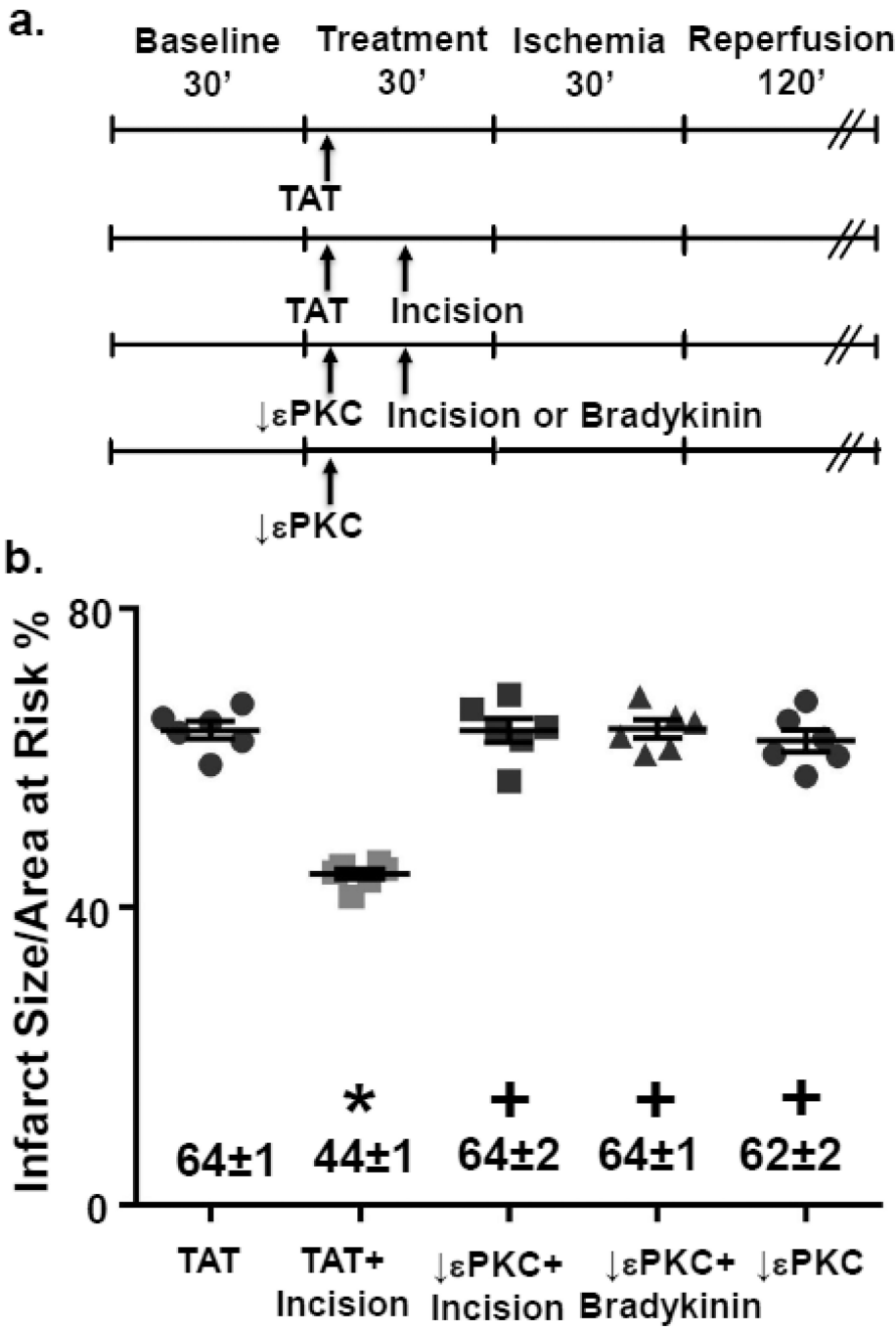

Fig. 3.

εPKC inhibition blocks nociceptive-induced remote conditioning. **a**. Experimental protocol with either TAT or the PKC inhibitor (↓εPKC) given 25 minutes before ischemia. Some groups either received an abdominal incision or bradykinin 15 minutes before ischemia. **b**. The εPKC inhibitor (↓εPKC) blocks the protective effect of the abdominal incision or bradykinin. *P <0.001 versus TAT control, $^{+}$P<0.001 versus TAT + incision group.

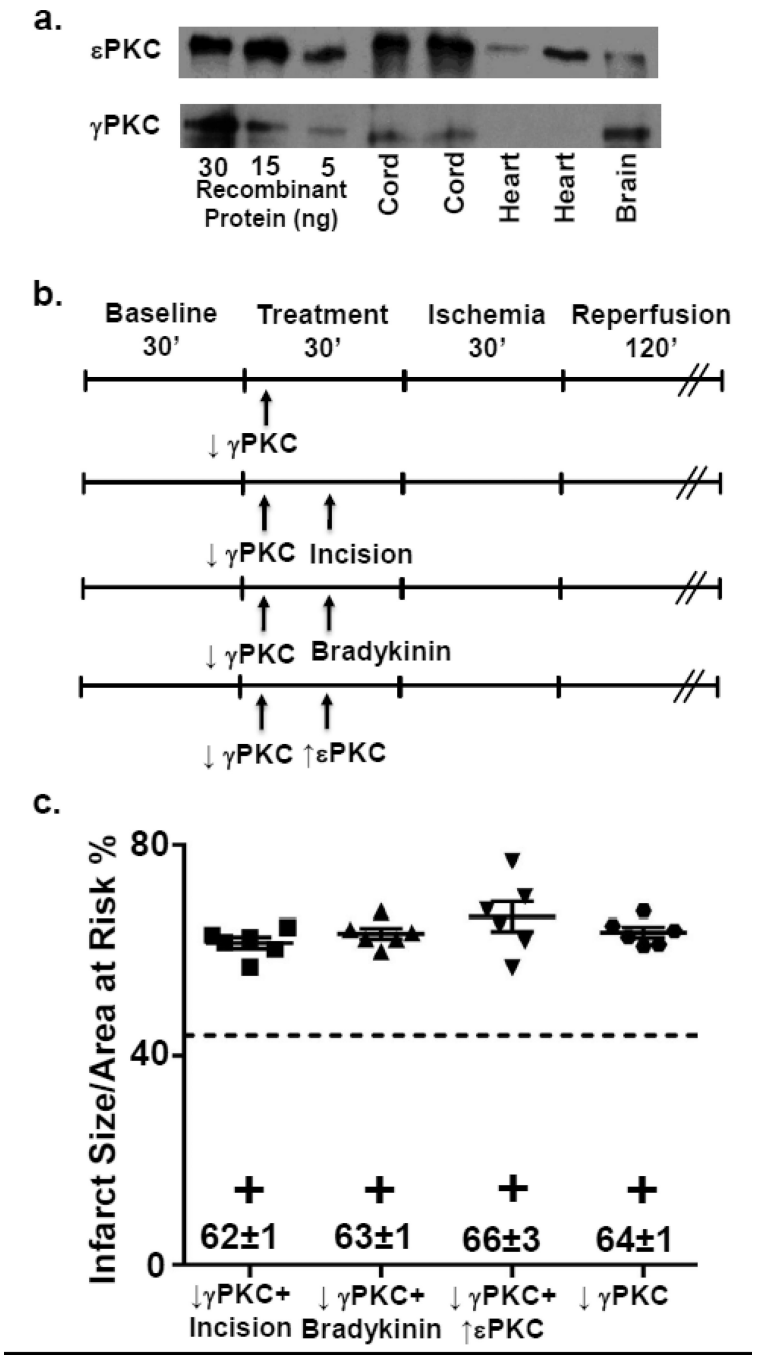

Fig. 4.

Neuronal specific γPKC inhibition blocks nociceptive-induced remote conditioning **a**. Western blot of rat heart left ventricle and spinal cord for εPKC and γPKC. Both brain tissue and recombinant protein for εPKC and γPKC were used as a positive control. Although εPKC is ubiquitously expressed, γPKC is only expressed in the nervous system. **b**. Experimental protocol with either TAT or the γPKC inhibitor (↓γPKC) given 25 minutes before ischemia. Some groups received either bradykinin, the εPKC activator (↑γPKC) or an abdominal incision, 15 minutes before ischemia. **c**. The γPKC inhibitor (↓γPKC) blocks protection of bradykinin, ↑εPKC, or abdominal incision. $^{+}P<0.001$ versus TAT + incision

group. The individual infarct sizes for each animal are plotted in addition to the mean ± SEM numerical values listed below each individual group. A dashed line represents the average infarct size seen by the TAT + incision group (44±1%) presented in Figure 3B for comparison.

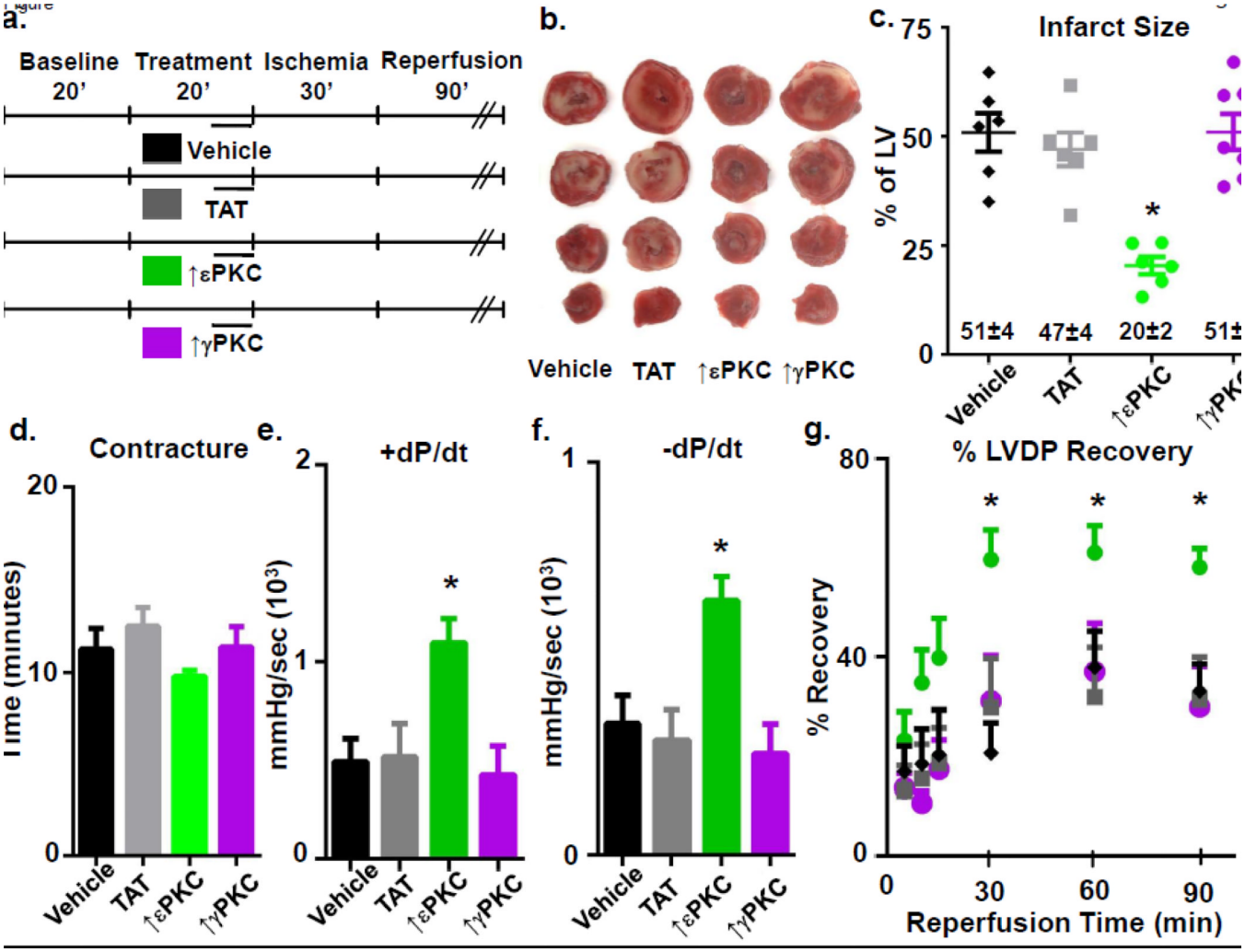

Fig. 5.

Isolated heart studies. **a**. Experimental protocol. Rats were administered 1μM of either vehicle (water), TAT, the εPKC activator (↑εPKC) or the classical PKC activator (↑γPKC) as an infusion for 10 minutes prior to ischemia and reperfusion. **b**. Representative left ventricle images of infracted myocardial tissue as assessed by TTC staining at the completion of reperfusion with viable tissue stained red and non-viable infarcted tissue white. **c**. Quantitative assessment of myocardial infarct size, assessed as infarct size per percentage of left ventricle. Individual data points are plotted for each experiment. **d**. Time to contracture for each heart during ischemia. **e**. and **f**. +dP/dt and –dP/dt assessed at 90 minutes of reperfusion. **g**. Percentage of LVDP recovery from baseline for each group assessed at 5, 10, 15, 30, 60 and 90 minutes after reperfusion. *P<0.05 versus vehicle, TAT or ↑γPKC, n=6–7/group, data presented as mean ± SEM.

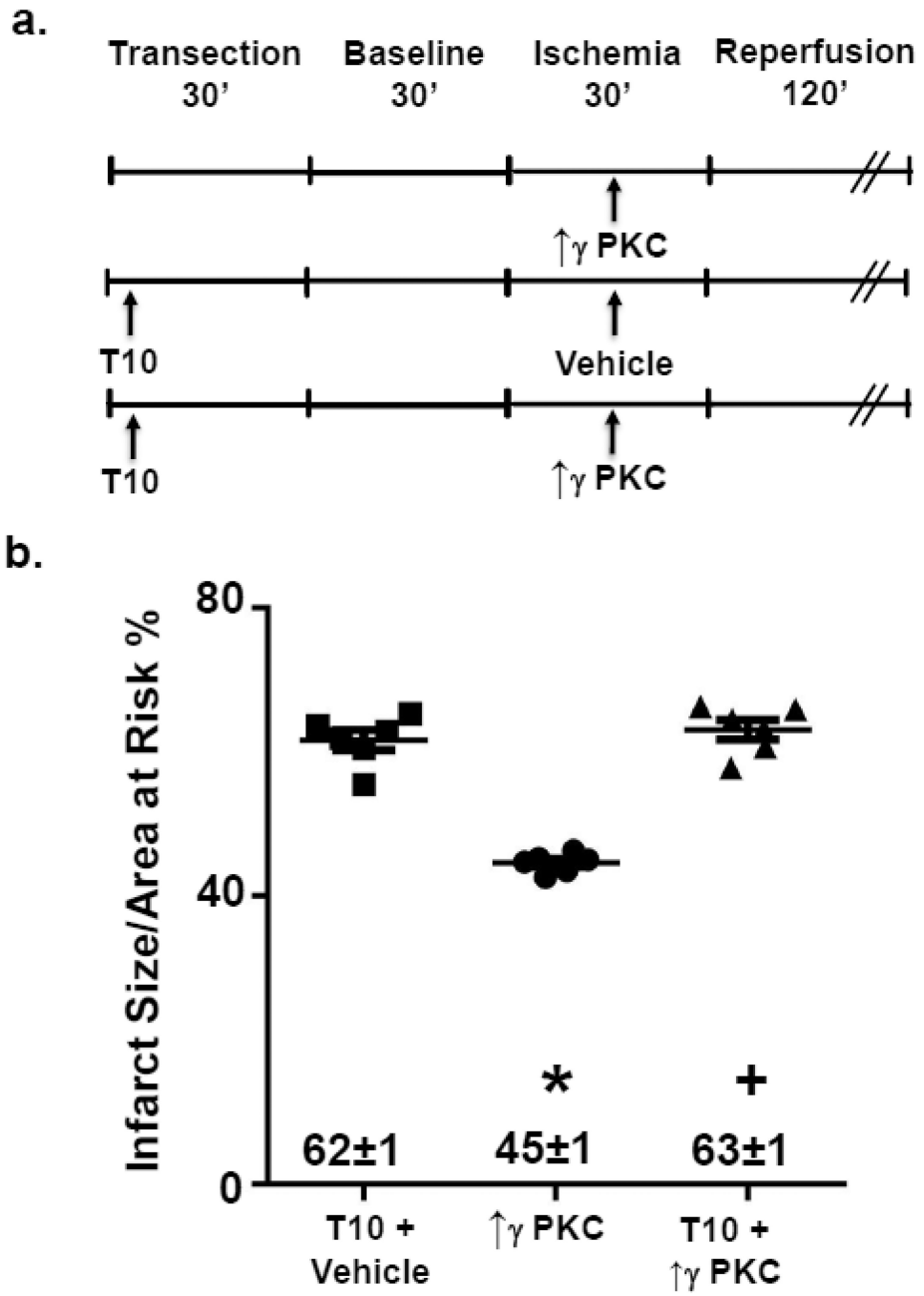

Fig. 6.

Classical PKC activation (↑γPKC) reduces infarct size during ischemia *in vivo* and is blocked by T10 spinal cord transection. **a**. Experimental protocol. Some rats during surgical instrumentation underwent a T10 spinal cord transection prior to ischemia-reperfusion. Either the classical PKC activator (↑γPKC) or vehicle were given 15 minutes prior to reperfusion. **b**. Myocardial infarct size expressed as a percentage of area at risk. ↑γPKC was effective during ischemia and blocked by T10 spinal cord transection. The individual infarct sizes for each animal are plotted in addition to the mean ± SEM numerical values listed

below each individual group. *P <0.001 versus groups: T10+ Vehicle, and T10+ ↑γPKC, in addition to control (Figure 2: 63±2), TAT (Figure 3: 64±1), +P<0.001 versus ↑γPKC.

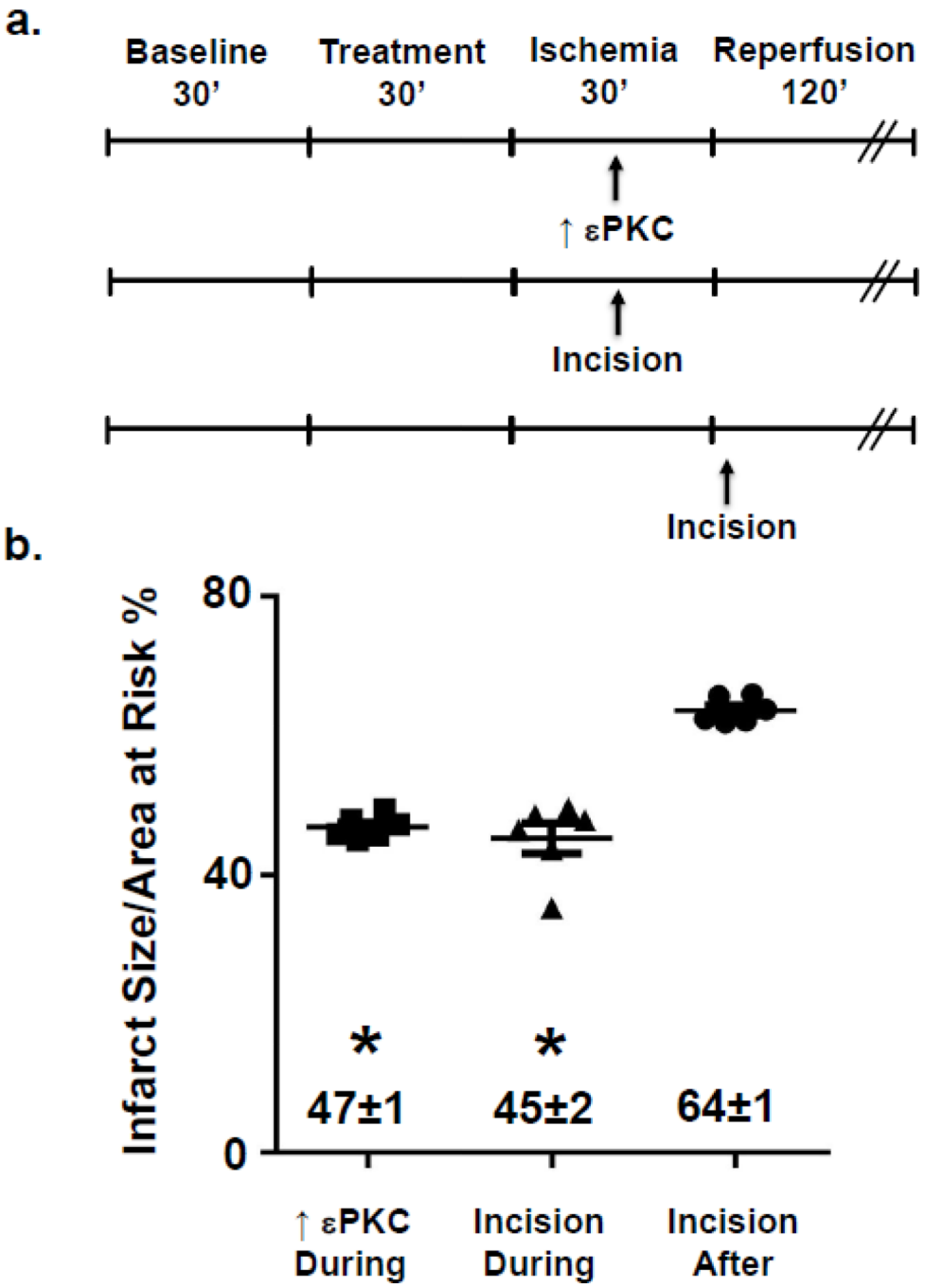

Fig. 7.

Abdominal incision or εPKC activation given during ischemia reduced infarct size. **a**. Experimental protocol with either an abdominal incision or ↑εPKC given 15 minutes prior to reperfusion. A surgical incision was also performed in an additional group 5 seconds after reperfusion. **b**. Myocardial infarct size expressed as a percentage of area at risk. Surgical incision was ineffective after reperfusion. The individual infarct sizes for each animal are plotted in addition to the mean ± SEM numerical values listed below each individual group. *P <0.001 versus groups: incision after, control (Figure 2: 63±2) and TAT (Figure 3: 64±1).

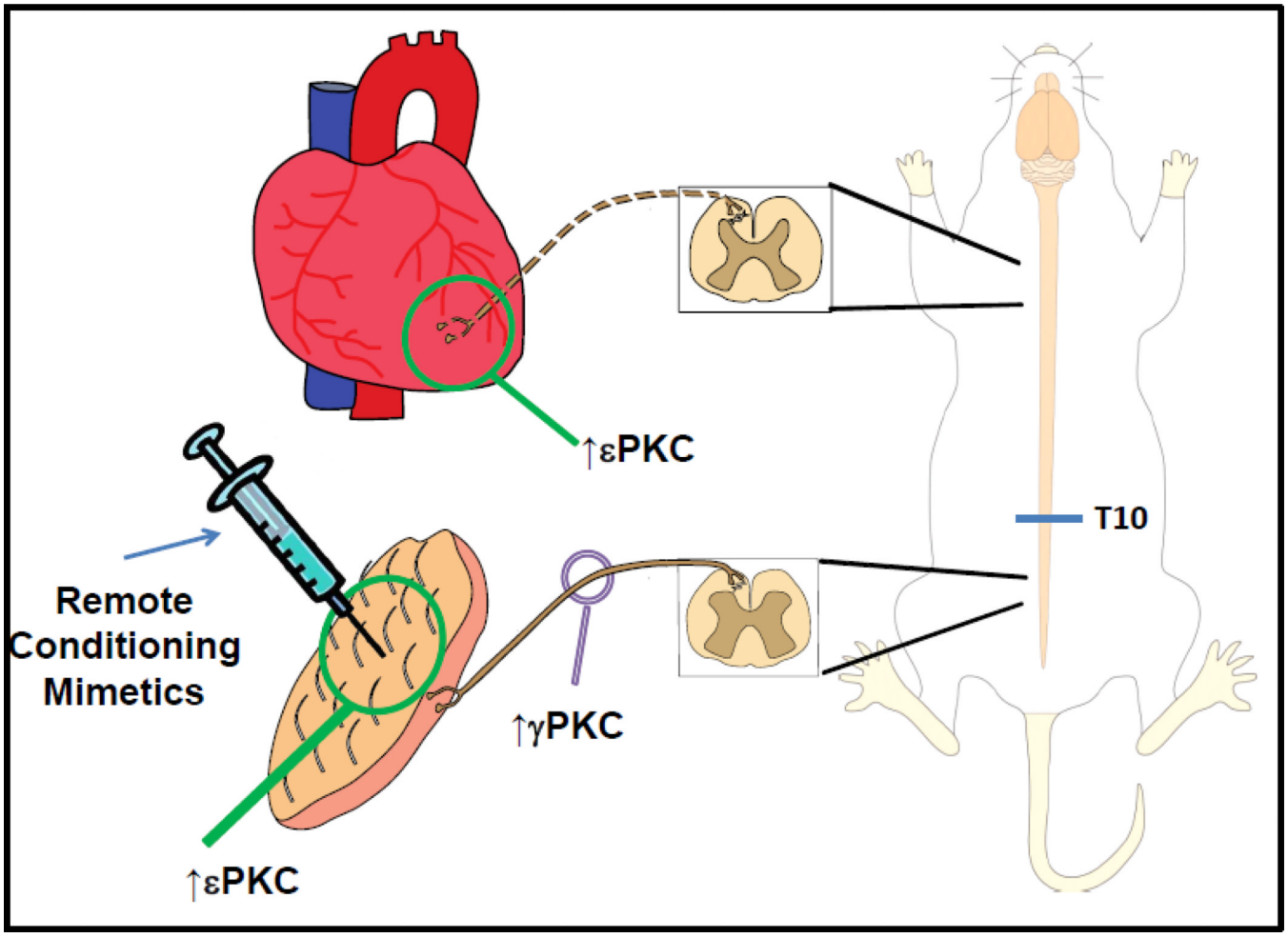

Fig. 8.

Schematic for the remote conditioning mechanism. Activation of εPKC occurs in local tissue, followed by transmission of the cardioprotective signal via γPKC in the nervous system. This signal is then relayed to the myocardium to promote protection from ischemic injury through local myocardial activation of εPKC. A T10 transection of the spinal cord blocks the remote conditioning signal.