Protease-activated receptor 2-mediated protection of myocardial ischemia-reperfusion injury: role of transient receptor potential vanilloid receptors

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Zhong B, Wang DH. Protease-activated receptor 2-mediated protection of myocardial ischemia-reperfusion injury: role of transient receptor potential vanilloid receptors. Am J Physiol Regul Integr Comp Physiol 297: R1681–R1690, 2009. First published October 7, 2009; doi:10.1152/ajpregu.90746.2008.—Activation of the protease-activated receptor 2 (PAR2) or the transient receptor potential vanilloid type 1 (TRPV1) channels expressed in cardiac sensory afferents containing calcitonin gene-related peptide (CGRP) and/or substance P (SP) has been proposed to play a protective role in myocardial ischemia-reperfusion (I/R) injury. However, the interaction between PAR2 and TRPV1 is largely unknown. Using gene-targeted TRPV1-null mutant (TRPV1−/−) or wild-type (WT) mice, we test the hypothesis that TRPV1 contributes to PAR2-mediated cardiac protection via increasing the release of CGRP and SP. Immunofluorescence labeling showed that TRPV1 coexisted with PAR2, PKC-ε, or PKAc in cardiomyocytes, cardiac blood vessels, and perivascular nerves in WT but not TRPV1−/− hearts. WT or TRPV1−/− hearts were Langendorff perfused with the selective PAR2 agonist, SLIGRL, in the presence or absence of various antagonists, followed by 35 min of global ischemia and 40 min of reperfusion (I/R). The recovery rate of coronary flow, the maximum rate of left ventricular pressure development, left ventricular end-diastolic pressure, and left ventricular developed pressure were evaluated after I/R. SLIGRL improved the recovery of hemodynamic parameters, decreased lactate dehydrogenase release, and reduced the infarct size in both WT and TRPV1−/− hearts (P < 0.05). The protection of SLIGRL was significantly surpassed for WT compared with TRPV1−/− hearts (P < 0.05). CGRP8−37, a selective CGRP receptor antagonist, RP67580, a selective neurokinin-1 receptor antagonist, PKC-ε V1–2, a selective PKC-ε inhibitor, or H-89, a selective PKA inhibitor, abolished SLIGRL protection by inhibiting the recovery of the rate of coronary flow, maximum rate of left ventricular pressure development, and left ventricular developed pressure, and increasing left ventricular end-diastolic pressure in WT but not TRPV1−/− hearts. Radioimmunoassay showed that SLIGRL increased the release of CGRP and SP in WT but not TRPV1−/− hearts (P < 0.05), which were prevented by PKC-ε V1–2 and H-89. Thus our data show that PAR2 activation improves cardiac recovery after I/R injury in WT and TRPV1−/− hearts, with a greater effect in the former, suggesting that PAR2-mediated protection is TRPV1 dependent and independent, and that dysfunctional TRPV1 impairs PAR2 action. PAR2 activation of the PKC-ε or PKA pathway stimulates or sensitizes TRPV1 in WT hearts, leading to the release of CGRP and SP that contribute, at least in part, to PAR2-induced cardiac protection against I/R injury.

transient receptor potential vanilloid 1; protease-activated receptors; protein kinase C-ε; ischemia-reperfusion; substance P; calcitonin gene-related peptide; gene knockout

PROTEASE-ACTIVATED RECEPTORS (PARs) belong to a family of G protein-coupled receptors, which mediate cellular effects of serine proteases and play important roles in mediating inflammation and tissue repair in response to injury (28, 35). PAR2 is widely expressed in various tissues, including endothelial cells, smooth muscle cells, epithelial cells, fibroblasts, sensory neurons, whole heart homogenates, and inflammatory cells, including mast cells, neutrophils, eosinophils, and macrophage (23, 28, 35). PAR2 may be activated by trypsin, mast cell tryptase, factors VIIa and Xa, and neuronal serine proteases, but not by thrombin, a factor that may activate other PARs (17, 28, 35). During myocardial ischemia-reperfusion (I/R), enhanced generation, and/or release of proteases from different sources, including inflammatory cells, such as mast cells and neutrophils and the coagulation cascade, may activate or up-regulate PAR2 (12). PAR2 agonists have been shown to enhance the efficiency of ischemic preconditioning (24), to improve myocardial functional recovery after I/R (23), and to decrease the incidence of ventricular arrhythmias in I/R injury model (23). PAR2 agonists promote vasodilatation via an endothelium-dependent, but nitric oxide (NO)-independent mechanism (5, 21, 40). In addition, it has been postulated that vasodilatation caused by PAR2 activation is mediated by activation of sensory C-fiber afferents innervating the coronary vasculature (21).

The transient receptor potential vanilloid 1 (TRPV1) is a nonselective cation channel mainly expressed in primary sensory neurons and sensory C- and Aδ-fibers (11). Recently, TRPV1 mRNA and proteins have also been detected in vascular smooth muscle and endothelial cells (9, 31, 42). TRPV1 coexpresses with PAR2 in dorsal root ganglia (DRG) neurons that contain substance P (SP) and/or calcitonin gene-related peptide (CGRP) (36, 38). PAR2 activation sensitizes DRG neurons that mediate pain sensation and stimulates the release of SP and CGRP from primary spinal afferent neurons (6, 10, 36). We and others have recently shown that TRPV1 plays a key role in protecting the heart from I/R injury (34, 39, 43). TRPV1–positive sensory nerves integrate and respond to stimuli generated or released during myocardial ischemia (29) by transmitting signals to the central nervous system, as well as releasing sensory neurotransmitters, including SP and CGRP (13, 14), which have been shown to protect the heart from ischemic injury (18, 37, 41). While TRPV1 and PAR2 have been independently shown to protect the heart, the link and interaction between TRPV1 and PAR2 in cardiac protection remain unknown.

A growing body of evidence indicates that PKC and PKA play a crucial role in cardiac protection signaling transduction pathways (27, 33). PKC-ε and PKA colocalize with PAR2 in DRG neurons, and PAR2 activation promotes translocation of PKC-ε and PKA catalytic subunits from the cytosol to the plasma membrane (1, 2, 4). In addition, PKC and PKA may induce or potentiate TRPV1 activity (4, 19, 32). However, the
The interrelation between PKC/PKA- and PAR2/TRPV1-mediated cardiac protection is unknown. Using gene-targeted TRPV1-null mutant (TRPV1−/−) and wild-type (WT) mice, the aim of the present study was to investigate 1) whether PAR2-mediated cardiac protection is TRPV1 dependent; 2) whether PAR2-mediated TRPV1 activation is via activation of PKC-ε and/or PKA pathways; and 3) whether PAR2-mediated, TRPV1-dependent cardiac protection is the result of SP and/or CGRP release.

MATERIALS AND METHODS

Immunofluorescence assay of TRPV1, PAR2, PKC-ε, and PKAc in the heart. The left ventricular (LV) tissue blocks were cut to a thickness of 10 μm. The tissues sections were incubated with the primary antibody (Santa Cruz): goat anti-TRPV1 (1:2,500); rabbit anti-PAR2 (1:50); goat anti-PKC-ε (1:50); or rabbit anti-PKA-α cat (PKAc) (1:200) for 24 h at 4°C. The sections were then incubated with anti-rabbit or anti-goat conjugated to cyanine 3 (1:200) or with horseradish peroxidase-conjugated donkey anti-goat IgG secondary antibody (1:200; Jackson ImmunoResearch). The sections were subsequently incubated with FITC-conjugated to tyramide (TSA kit, PerkinElmer Life Sciences) based on the protocol recommended by the manufacturer. In controls, primary antibodies were preabsorbed with 10 μmol/l peptides used for immunization for 48 h at 4°C.

Langendorff heart preparation and measurements of cardiac function. Male TRPV1−/− strain B6.129S4-TRPV1tm1Jul and control WT strain C57BL/6J mice, to which TRPV1−/− mice were backcrossed for at least six generation, were used. Mice were heparinized (500
U/kg ip) and anesthetized with pentobarbital sodium (50 mg/kg ip). Hearts from TRPV1−/− and WT mice were cannulated and retrogradely perfused at 37°C and 80 mmHg with Krebs-Henseleit buffer (118 mmol/l NaCl, 4.7 mmol/l KCl, 1.2 mmol/l MgSO4, 1.2 mmol/l KH2PO4, 2.5 mmol/l CaCl2, 25 mmol/l NaHCO3, 0.5 mmol/l Na-EDTA, and 11 mmol/l glucose, saturated with 95% O2-5% CO2, pH 7.4) through the aorta in a noncirculating Langendorff apparatus. A water-filled balloon was inserted into the LV and adjusted to a LV end-diastolic pressure (LVEDP) of 5–8 mmHg. The distal end of the catheter was connected to a Digi-Med Heart Performance Analyzer via a pressure transducer. The recovery rate of coronary flow (%CF) was continuously measured using an ultrasonic flow probe placed in the aortic perfusion line. Hearts were paced at 400 beats/min, except during sustained global ischemia, to avoid inducing excessive ventricular tachyarrhythmia during reperfusion, and pacing was reinitiated 2 min after reperfusion. The maximum rate of LV pressure development (dP/dt) during isovolumic contraction and LV developed pressure (LVDP) were used as indexes of LV systolic function; LVEDP were used as indexes of LV diastolic function. The experiments were approved by the Michigan State University Animal Care and Use Committee.

Experimental protocols. All hearts were allowed to stabilize for 25 min and then were perfused at 1% of the CF rate with 1) LSIGRL (10−7 M, Peptides International), an inactive control peptide; 2) SLIGRL (10−7 M, Peptides International), a selective PAR2 agonist; 3) SLIGRL plus CGRPα3–37 (10−6 M, Sigma), a selective CGRP receptor antagonist; 4) SLIGRL plus RP67580 (10−6 M, Tocris Bioscience), a selective neurokinin 1 (NK1) receptor antagonist; 5) SLIGRL plus chelerythrine (5 × 10−6 M, Calbiochem), a general PKC inhibitor; 6) SLIGRL plus PKC-ε V1–2 (10−4 M, Thomas Scientific), a selective PKC-ε inhibitor; or 7) SLIGRL plus H-89 (5 × 10−4 M, Calbiochem), a selective PKA inhibitor. SLIGRL or LSIGRL control was perfused for 15 min, and antagonists/inhibitors were added to the perfusate 5 min before adding SLIGRL and continuously perfused for an additional 5 min after SLIGRL perfusion. Hearts were subsequently subjected to 35 min of no-flow normothermic global ischemia followed by 40 min of reperfusion.

Measurement of lactate dehydrogenase release. In addition to the measurement of cardiac function, cardiac injury was assessed by measurement of lactate dehydrogenase (LDH) release. Perfusion effluent was collected during the first 5–15 min of I/R and stored at −80°C until analysis. Total LDH levels were determined with the use of a cytotoxicity detection kit (Roche Applied Science). The data were expressed as absorbance units released per milliliter per minute per gram of wet heart tissues.

Evaluation of myocardial infarct size. Risk area and infarct size were measured 40 min after postischemia reperfusion. Hearts were perfused for 10 min at a flow rate of 2 ml/min with a 1% 2,3,5-triphenyltetrazolium chloride (TTC) dissolved in Krebs buffer. TTC stains all living tissue brick red and leaves the infarct area unstained (white). Hearts were then removed from the cannula and sliced perpendicularly along the long axis from apex to base in 2-mm sections. Sections were incubated for another 10 min at 37°C in 1% TTC. Once the color was established, the slices were fixed in 10% formalin for 48 h and weighed. Both sides of each slice were photographed and delineated with photos that were quantified with Image J version 1.37v (National Institutes of Health). Since hearts were subjected to global ischemia, the total cross-sectional areas were defined as the total risk areas. The ratio of the infarct area to the total risk area (%infarct size) of both sides of each slice was calculated and multiplied by the weight of the slice.

Measurement of SP and CGRP. WT and TRPV1−/− hearts were cut into pieces and put into tubes containing Krebs-Henseleit buffer with 1 μM phosphoramidon and 1 μM captopril that were saturated with 95% O2-5% CO2 at 37°C continuously for 60 min (the stabilization period). SLIGRL (10−6 M) or LSIGRL control (10−6 M) was then added and incubated for 60 min. To determine the role of PKC-ε and PKA in SLIGRL-induced SP and CGRP release, PKC-ε V1–2 (10−4 M), a selective PKC-ε inhibitor, or H-89 (5 × 10−6 M), a selective PKA inhibitor, was added 5 min before adding SLIGRL. The

Fig. 2. Effect of SL on cardiac function at the end of ischemia-reperfusion (I/R). WT and TRPV1−/− hearts were retrogradely perfused in a Langendorff apparatus and subjected to SL [10−7 M, at 1% of coronary flow (CF) rate] for 15 min, followed by I/R. Hearts were paced at 400 beats/min during the initial equilibration period. Pacing was terminated during ischemia and reinitiated at 3 min into the reperfusion period. As SL controls, WT and TRPV1−/− hearts were also perfused with LS (inactive control peptide). ∗dP/dt, maximum rate of left ventricular pressure development; LVEDP, left ventricular end-diastolic pressure; LVEDP, left ventricular developed pressure. Values are means ± SE; n = 7. ∗P < 0.05 vs. WT-SL; †P < 0.05 vs. TRPV1−/−-SL hearts; ‡P < 0.05 vs. WT-SL hearts.
samples were purified and analyzed with the rat CGRP and SP radioimmunoassay kits (Peninsula Laboratories) for determination of CGRP and SP release that was normalized by the wet heart weight.

Statistical analysis. All values are expressed as means ± SE. The groups of WT and TRPV1−/− hearts treated with SLIGRL represent the same animals in Figs. 2–6. Differences among groups with multiple measurements over time were determined by two-way ANOVA for repeated measurements, and differences between means were identified by the least significant difference test. Comparisons among groups measured at the end of the I/R experiments in the bar charts of Figs. 2–9 and in SP, CGRP, and LDH release and infarct size experiments were performed by one-way ANOVA analysis followed by the Tukey-Kramer multiple-comparison test. The results were considered statistically significant at P < 0.05.

RESULTS

Colocalization of TRPV1, PAR2, PKC-ε, and PKAc in the heart. Immunofluorescence assay showed that, while it was absent in TRPV1−/− hearts (Fig. 1, A1), positive TRPV1 immunostaining was detected on the epicardial surface, blood vessels, and perivascular nerves in WT hearts (Fig. 1, A2). Similar patterns of PAR2 immunostaining were detected in WT and TRPV1−/− hearts (Fig. 1, B1 and B2). Western blot showed that there was no significant difference in the expression of PAR2 between WT and TRPV1−/− hearts (supplemental data; the online version of this article contains supplemental data), indicating that ablation of TRPV1 may not affect PAR2 expression. TRPV1 and PAR2 coexpressed mainly on the epicardial surface and blood vessels in WT but not TRPV1−/− hearts (Fig. 1, C1 and C2), which is consistent with previous findings (7, 15, 22) and supports the notion that TRPV1 may mediate the effect of PAR2 on its activation. Similarly, expression of potential regulatory kinases, PKC-ε and PKAc, was examined in WT and TRPV1−/− hearts treated with SLIGRL or LSIGRL control, and only the results in WT hearts were shown, because expression of these kinases was not detected differently in TRPV1−/− hearts. PKC-ε and PKAc expressed mainly in the cytosol of myocardium, vascular, and perivascular tissues when treated with LSIGRL control, but SLIGRL treatment caused translocation of PKC-ε and PKAc from the cytosol to the plasma membrane that maximally colocalized with TRPV1 (Fig. 1, A3–A6, B3–B6, and C3–C6). Western blot showed that there was no significant difference in the expression of PKC-ε and PKAc between WT and TRPV1−/− hearts (supplemental data). These results indicate that activation of these kinases may regulate TRPV1 activity/expression. Positive staining for TRPV1, PAR2, PKC-ε, and PKAc was abolished by preabsorption of the primary antibodies with the peptides used for immunization (supplemental data).

SLIGRL protection against I/R injury was impaired in TRPV1−/− hearts. There were no statistically significant differences in hemodynamics between groups under baseline conditions. After I/R, SLIGRL pretreatment groups improved recovery of %CF, dP/dt, and LVDP and inhibited the increase in LVEDP in both WT and TRVR1−/− hearts compared with their respective controls (Fig. 2), indicating that PAR2-mediated cardiac protection is TRPV1 dependent and independent.

Blockade of the CGRP receptor suppressed SLIGRL protection in WT hearts. To determine whether endogenous CGRP plays a role in SLIGRL-induced cardiac protection, CGRP8−37

Fig. 3. Effect of the calcitonin gene-related peptide (CGRP) receptor antagonist, CGRP8−37, on SL-induced cardiac protection at the end of I/R. WT and TRPV1−/− hearts were treated with the SL or 10−6 M CGRP8−37, a selective antagonist of the CGRP receptor, added to the perfusion (at 1% of CF rate) 5 min before and after SL. Values are means ± SE; n = 5–7. *P < 0.05 vs. WT-SL.
(10−6 M), a selective CGRP receptor antagonist, was given and showed to block SLIGRL-induced cardiac protection by inhibiting recovery of %CF, dP/dt, and LVDP and increasing LVEDP in WT but not TRVR1−/− hearts compared with their respective controls (Fig. 3). These results suggest that PAR2-mediated cardiac protection is through, at least in part, CGRP receptor activation by its agonist release on TRPV1 activation. Higher (10−5 M) or lower (10−7 M) concentrations of CGRP8–37 had similar effects on post-I/R recovery as that evoked by 10−6 M CGRP8–37 in WT hearts (data not shown).
Moreover, 10^{-6} M CGRP_{8-37} had no effect on cardiac function in the absence of I/R injury in WT hearts.

Blockade of the SP receptor impaired SLIGRL protection in WT hearts. The effect of endogenous SP on SLIGRL-inducing cardiac protection was assessed by pretreatment of the hearts with the NK1 receptor antagonist, RP67580 (10^{-7} M). The protective effects of SLIGRL were suppressed in the presence of RP67580, evidenced by decreased dP/dt and LVDP in WT but not TRVR1^{-/-} hearts compared with their respective controls (Fig. 4). There were no statistically significant differences in %CF and LVEDP in the presence of RP67580 in WT hearts. These results are consistent with the notion that SP release on cardiac function in the absence of I/R injury in WT hearts.

Blockade of PKC, PKC-\(\varepsilon\), or PKA impaired SLIGRL protection in WT hearts. The effect of PKC-\(\varepsilon\) and PKA activation on SLIGRL-induced cardiac protection was assessed by pretreatment of the hearts with PKC-\(\varepsilon\) V1–2 (10^{-4} M), a selective PKC-\(\varepsilon\) inhibitor, or H-89 (5 \times 10^{-6} M), a selective PKA inhibitor. The protective effects of SLIGRL were suppressed in the presence of PKC-\(\varepsilon\) V1–2 by inhibiting recovery of %CF, dP/dt, and LVDP and increasing LVEDP in WT but not TRVR1^{-/-} hearts compared with their respective controls (Fig. 5). Likewise, chelerythrine (5 \times 10^{-6} M), a PKC general inhibitor, abolished SLIGRL-induced cardiac protection in WT but not TRVR1^{-/-} hearts (data not shown). Moreover, H-89 impaired SLIGRL protection by inhibiting the recovery of %CF, dP/dt, and LVDP and increasing LVEDP in WT but not TRVR1^{-/-} hearts (Fig. 6). These results support the hypothesis that SLIGRL treatment causes activation of the kinases that may alter TRPV1 activity/expression. Higher or lower doses of PKC-\(\varepsilon\) V1–2 (10^{-3} M and 10^{-5} M) and H-89 (5 \times 10^{-5} M and 5 \times 10^{-7} M) presented dose-dependent suppression on SLIGRL-induced protection in WT hearts (data not shown).

Measurements of LDH and infarct area. LDH levels and the infarct area after I/R were significantly lower in WT and TRPV1^{-/-} hearts treated with SLIGRL compared with their respective control groups (Figs. 7 and 8). Moreover, LDH levels and the infarct area after I/R were significantly smaller in WT hearts treated with SLIGRL than in TRPV1^{-/-} hearts treated with SLIGRL (Figs. 7 and 8), indicating that SLIGRL protects the hearts from I/R injury in both WT and TRPV1^{-/-} hearts, but its protection is impaired when TRPV1 gene is deleted.

Measurements of SP and CGRP release. The release of SP and CGRP at the baseline (LSIGRL control) was not different between WT and TRPV1^{-/-} hearts (Fig. 9). SP and CGRP release increased remarkably in WT but not TRPV1^{-/-} hearts treated with SLIGRL (Fig. 9), indicating that SLIGRL-induced SP and CGRP release is TRPV1 dependent. Inhibiting PKC-\(\varepsilon\) or PKA with PKC-\(\varepsilon\) V1–2 (10^{-4} M) or H-89 (5 \times 10^{-6} M), respectively, abolished SLIGRL-induced SP and CGRP release in WT hearts (Fig. 9), indicating that these pathways mediate SLIGRL-induced SP and CGRP release.

DISCUSSION

The aim of the present study was to define whether TRPV1 plays a role in PAR2-induced cardiac protection, and, if so, what mechanisms underlie the TRPV1-dependent pathway of PAR2 protection. Indeed, interaction between TRPV1 and PAR2 has been shown in which PAR2 agonist-induced vaso-
dilation was attenuated by a selective antagonist of TRPV1, capsazepine (21). However, the limitation of the pharmacological approaches is that capsazepine has been shown to induce cellular apoptosis and necrosis via a nonreceptor-mediated mechanism (3). To avoid the drawback, the present study uses the hearts from TRPV1 gene knockout mice or WT mice to study.

Our data show that 1) TRPV1 coexpresses with PAR2, PKC-ε, or PKAc in cardiomyocytes, cardiac blood vessels, and perivascular nerves in WT but not TRPV1−/− hearts, providing an anatomical basis for TRPV1 and PAR2/PKC-ε/PKAc interaction under pathophysiological conditions; 2) PAR2 activation improves cardiac recovery after I/R injury in both WT and TRPV1−/− hearts, suggesting that PAR2-mediated protection is TRPV1 dependent and independent; 3) PAR2-induced improvement in cardiac morphology and enzymatic index after I/R injury is significantly surpassed in WT compared with TRPV1−/− hearts, suggesting that ablation of TRPV1 impairs PAR2 action; 4) blockade of the PKC-ε or PKA pathway abolishes PAR2 agonist-induced CGRP/SP release in WT but not TRPV1−/− hearts, indicating that activation of the PKC-ε or PKA pathway by PAR2 stimulates CGRP/SP release via activating TRPV1; and 5) blockade of the CGRP or NK1 receptors abolishes PAR2 agonist-induced cardiac protection in WT but not TRPV1−/− hearts, indicating that CGRP/SP release on TRPV1 activation mediates, at least in part, PAR2-induced cardiac protection against I/R injury.

The protective effects of the PAR2 agonist against injury induced by I/R have been reported in the heart (15, 23, 24), which have been suggested to be linked to the stimulation of several pathways, including activation of PKC or extracellular signal regulated kinase-1/2, increases in superoxide dismutase activity, or TNF-α release (15, 23, 24). Moreover, recent studies showed that PAR2-mediated protection against acute pancreatitis is via the modulation of mitogen-activated protein

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Fig. 7. The cardiac injury was assessed by the release of lactate dehydrogenase (LDH) during I/R. WT and TRPV1−/− hearts were retrogradely perfused in a Langendorff apparatus and subjected to SL or LS and followed by I/R (WT-SL, TRPV1−/−-SL, WT-LS, TRPV1−/−-LS, respectively). Coronary outflow was collected during the first period of 10–20 min of I/R and sampled for the LDH content (top), and SL protective effects compared with respective baselines are shown (bottom). Absorbance. Values are means ± SE; n = 5. *P < 0.05 vs. WT-SL; †P < 0.05 vs. WT-LS; ‡P < 0.05 vs. TRPV1−/−-SL hearts.

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Fig. 8. The cardiac injury was assessed by the percentage of infarct size. WT and TRPV1−/− hearts were retrogradely perfused in a Langendorff apparatus and treated with SL or LS and followed by I/R (WT-SL, TRPV1−/−-SL, WT-LS, TRPV1−/−-LS, respectively). Risk area and infarct size were measured 40 min after ischemia reperfusion (top), and SL protective effects compared with respective baselines are shown (bottom). Values are means ± SE; n = 5. *P < 0.05 vs. WT-SL; †P < 0.05 vs. WT-LS; ‡P < 0.05 vs. TRPV1−/−-SL hearts.
kinase and mitogen-activated protein kinase phosphatase signaling (25), a pathway that also plays a role in myocardial I/R injury (20). PAR2 agonists may exert protective effects directly or via mechanisms similar to preconditioning via interaction with TRPV1. Our previous data showed that activation of TRPV1 renders the heart preconditioning protection (43). The data in the present study show that, although PAR2 protects the hearts from I/R injury in both WT and TRPV1−/− mice, the protection of PAR2 in terms of reducing myocardial infarct size and decreasing LDH release is significantly enhanced when TRPV1 function is intact. These results suggest that TRPV1 contributes, at least in part, to PAR2-mediated cardiac protection.

It has been shown that PAR2 may protect the hearts from I/R injury via triggering dose-dependent coronary vasodilation in a NO-dependent or -independent manner (5, 21, 23, 24). Likewise, TRPV1 mRNA and proteins have been found in vascular smooth muscle and endothelial cells (9, 31, 42), and anandamide activation of TRPV1 expressed in endothelial cells elicits an acute release of NO that may contribute to TRPV1-dependent vasodilation or modulation of vascular tone (30). Although it is unknown whether a NO-dependent pathway is operant in PAR2-mediated protection in both WT and TRPV1−/− mice, the fact that TRPV1 and PAR2 coexpress in cardiac vessels and cardiomyocytes indicates that TRPV1 and PAR2 may share common signaling pathways or act synergistically to protect the heart from I/R injury.

Indeed, the primary sequence of TRPV1 predicts several putative phosphorylation sites, and PKC-ε- and PKA-mediated phosphorylation and sensitization of TRPV1 by PAR2 activation have been evident in neurons and several cell lines (1, 2, 26). Activation of PAR2 may sensitize TRPV1 by reducing the temperature threshold from 42 to 32°C for TRPV1 activation in HEK-293 cells expressing both PAR2 and TRPV1 (8). Moreover, PAR2 agonists stimulate, via the PKC-ε- or PKA-dependent phosphorylation of TRPV1 (1, 2, 26), the release of CGRP and SP from C-fibers in peripheral tissues and in the spinal cord, where PAR2 and TRPV1 colocalize (6, 36, 38). Our data in the present study show that TRPV1 coexpresses with PAR2, PKC-ε, or PKAc in cardiomyocytes, cardiac blood vessels, and perivascular nerves in WT hearts, providing an anatomical basis for TRPV1 and PAR2/PKC-ε/PKAc interaction under pathophysiological conditions. Moreover, blockade of the PKC-ε or PKA pathway inhibits PAR2 agonist-induced CGRP/SP release and cardiac protection in WT but not TRPV1−/− hearts, indicating that the PKC-ε or PKA pathway mediates PAR2 sensitization of TRPV1, leading to CGRP/SP release and cardiac protection in WT hearts. A cautionary note:

**Fig. 9. Release of CGRP and SP from isolated hearts subjected to SL (10−6 M) or LS in the absence or presence of the PKC-ε inhibitor, PKC-ε V1–2 (10−4 M), or the PKA inhibitor, H-89 (5 × 10−6 M), in WT and TRPV1−/− hearts. Values are means ± SE; n = 4. *P < 0.05 vs. WT-LS; †P < 0.05 vs. WT-SL.**
although it is highly likely that phosphorylation of TRPV1 is mediated by PAR2 activation, it cannot be ruled out that downstream events, e.g., those triggered by CGRP/NK1 receptor activation, may also participate in TRPV1 phosphorylation. CGRP and SP may protect the hearts from I/R injury via causing coronary vasodilation and negative inotropic and chronotropic effects (18, 37, 41). Our laboratory has previously shown that exogenous CGRR and SP improve cardiac recovery after I/R injury in the isolated and perfused mouse hearts (39). In the present study, blockade of the CGRP or NK1 receptors eliminates the beneficial effects of the PAR2 agonist in WT but not TRPV1–/– hearts, indicating that endogenously released CGRP and SP, on TRPV1 activation by PAR2 in WT hearts, are attributed to cardiac protection induced by PAR2. Furthermore, a non-CGRP/SP-dependent pathway(s) of PAR2 is operant in TRPV1–/– hearts, given the fact that PAR2 activation also protects TRPV1–/– hearts, albeit with smaller magnitude.

Perspectives and Significance

Our data using the TRPV1 gene ablation model show that PAR2-induced cardiac protection against I/R injury may depend on, at least in part, PAR2 activation of TRPV1 via stimulation of the PKA or PKC-ε pathway that leads to sensitization of TRPV1 and release of CGRP and SP. The cardioprotective effects afforded by these pathways may have significant clinical and translation implications. For example, impairment of TRPV1 function by hypertension, diabetes, obesity, or aging may jeopardize the effectiveness of well-established cardiac protection caused by PAR2, where it is activated by inflammatory or thrombogenic pathways, especially in the border zone surrounding the infarction. As a result, these pathological conditions may increase the risk of or exacerbate myocardial infarction. It is conceivable that each molecule in these pathways, including PAR2, PKA, PKC-ε, TRPV1, SP, or CGRP, may serve as molecular targets for future development of therapies for cardiac injury and inflammation.

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DISCLOSURES

No conflicts of interest are declared by the author(s).

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