N-oleoyldopamine, a novel endogenous capsaicin-like lipid, protects the heart against ischemia-reperfusion injury via activation of TRPV1

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Zhong B, Wang DH. N-oleoyldopamine, a novel endogenous capsaicin-like lipid, protects the heart against ischemia-reperfusion injury via activation of TRPV1. Am J Physiol Heart Circ Physiol 295: H728–H735, 2008. First published June 20, 2008; doi:10.1152/ajpheart.00022.2008.—N-oleoyldopamine (OLDA), a bioactive lipid originally found in the mammalian brain, is an endo-
vanniloid that selectively activates the transient receptor potential vanilloid type 1 (TRPV1) channel. This study tests the hypothesis that OLDA protects the heart against ischemia and reperfusion (I/R) injury via activation of the TRPV1 in wild-type (WT) but not in gene-
targeted TRPV1-null mutant (TRPV1−/−) mice. Hearts of WT or TRPV1−/− mice were Langendorff perfused with OLDA (2 × 10−9 M) in the presence or absence of CGRP8–37 (1 × 10−6 M), a selective calcitonin gene-related peptide (CGRP) receptor antagonist; RP-67580 (1 × 10−6 M), a selective neurokinin-1 receptor antagonist; chelerythrine (5 × 10−6 M), a selective protein kinase C (PKC) antagonist; or tetrabutylammonium (TBA, 5 × 10−4 M), a nonselective K+ channel antagonist, followed by 35 min of global ischemia and 40 min of reperfusion (I/R). Left ventricular end-diastolic pressure (LVEDP), left ventricular developed pressure (LVDP), coronary flow (CF), and left ventricular peak positive dP/dt (+dP/dt) were evaluated after I/R. OLDA improved recovery of cardiac function after I/R in WT but not TRPV1−/− hearts by increasing LVDP; CF, and +dP/dt and by decreasing LVEDP. CGRP8–37, RP-67580, chelerythrine, or TBA abolished the protective effect of OLDA in WT hearts. Radioimmunoassay showed that the release of substance P (SP) and CGRP after OLDA treatment was higher in WT than in TRPV1−/− hearts, which was blocked by chelerythrine or TBA. Thus OLDA exerts a cardiac protective effect during I/R injury in WT hearts via CGRP and SP release, which is abolished by PKC or K+ channel antagonists. The protective effect of OLDA is void in TRPV1−/− hearts, supporting the notion that TRPV1 mediates OLDA-induced protection against cardiac I/R injury.

N-oleoyldopamine; transient receptor potential vanilloid 1; ischemia-reperfusion; substance P; calcitonin gene-related peptide; protein kinase C antagonist; potassium ion channel antagonist; gene knockout

THE TRANSIENT RECEPTOR POTENTIAL VANILLOID 1 (TRPV1) CHANNEL

is a nonselective cation channel mainly expressed in primary sensory neurons and sensory C- and A- fibers (17), although TRPV1 mRNA and proteins have recently been found in vascular smooth muscle and endothelial cells (15, 35, 52). TRPV1 can be activated by physical and chemical stimuli, including noxious heat, protons, vanilloid compounds (6, 10), or endogenous arachidonic acid derivatives (21, 44), which include at least three kinds of lipids: unsaturated N-acylpati-
mines [e.g., N-arachidonoyldopamine (NADA), N-oleoyldopam-
ine (OLDA)] (7, 20); lipoxygenase products [e.g., 12-(S)- and 15-(S)-hydroperoxyicosatetraenoic, leukotriene B4] (21, 39); and the N-acyl ethanolamines (e.g., anandamide, N-oleoyleth-
anolamine, N-linoleoyl ethanolamine) (29, 38, 58). These com-
pounds may activate TRPV1 and other receptors with different affinities and are increasingly recognized as an important class of signaling molecules affecting pain, inflammation, and tissue injury (47).

For example, anandamide may activate TRPV1 as well as cannabinoid receptors, CB1 and CB2, but its potency for TRPV1 is much less than capsaicin (3, 58). Anandamide may protect against ischemia-reperfusion (I/R) injury through activation of CB1 or CB2 in the heart, brain, and liver (1, 42, 45) and may cause release of nitric oxide through TRPV1 activation in arterial mesenteric bed (35). However, the role of TRPV1 in anandamide-induced protective effects is contro-
versial and unclear given the facts that higher concentrations of anandamide are required to stimulate TRPV1 (44) and that the efficacy of anandamide as a TRPV1 agonist is influenced by CB1 receptor activation (39). Although 12(S)- and 15(S)-hydroperoxyicosatetraenoic acid (HpETE) has also been shown to protect against myocardial I/R injury through TRPV1 activation (39), 12(S)-HpETE possesses lower potency when compared with capsaicin (21). Although NADA possesses nanomolar potency for TRPV1 and causes substance P (SP) and calcitonin gene-related peptide (CGRP) release (20), it also has the similar potency for CB1 receptors (3). In contrast to these endogenous lipids that have either low or indistinct affinity or potency, or are rapidly taken up or degraded after binding to TRPV1, OLDA is the most potent and selective endogenous agonist of TRPV1 that has very low affinity for cannabinoid receptors. For example, OLDA has been shown to be 50 times more potent on TRPV1 than on CB1 receptors and at least 30 times more potent than capsaicin (7, 40). Moreover, OLDA is a stable compound that stays for hours in biomem-
branes, which may result from a slower rate of metabolism that provides for a longer period of receptor activation (55). Regard-
less, the role of OLDA in protecting the heart against I/R injury is unknown.

During myocardial ischemia, TRPV1-positive sensory nerves integrate and respond to multiple ischemic metabolites and cause chest pain (31). The responses of TRPV1-positive sensory nerves include transmitting signals to the central ner-
vous system as well as releasing sensory neurotransmitters such as SP and CGRP, which coexpress in TRPV1-positive sensory neurons (17, 18) and may play an important role in protecting the heart from ischemic injury and damage. Indeed,

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we have previously shown that TRPV1 gene knockout impairs preconditioning and postischemic protection against myocardial injury in mice (49, 56). The present study was designed to use the most potent endogenous TRPV1 agonist known to date, namely OLDA, as a model compound to define whether OLDA may protect the heart from I/R injury by increasing SP and CGRP release following TRPV1 activation. With the use of wild-type (WT) and TRPV1−/− mice, this study examines whether OLDA protects the heart from I/R injury by activating TRPV1, leading to SP and CGRP release in WT mice, and whether the protective effect of OLDA is absent in TRPV1−/− mice. We also used the K+ channel antagonist and the protein kinase C (PKC) antagonist to examine whether blockade of these pathways may modulate OLDA-induced cardiac protective effects.

MATERIALS AND METHODS

Langendorff heart preparation and measurements of cardiac function. Male TRPV1 gene knockout (TRPV1−/−) strain B6.129S4-TRPV1tm1lkd and matching control WT strain C57BL/6J mice (25–30 g) were used (Jackson Laboratory, Bar Harbor, ME). 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 (in mmol/l: 118 NaCl, 4.7 KCl, 1.2 MgSO4, 1.2 KH2PO4, 2.5 CaCl2, 25 NaHCO3, 0.5 Na-EDTA, and 11 glucose, saturated with 95% O2-5% CO2, pH 7.4) through the aorta in a noncircularizing Langendorff apparatus as described previously (49). A water-filled balloon was inserted in the left ventricle and adjusted to a left ventricular 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. 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 reintitated 3 min after reperfusion. Left ventricular developed pressure (LVPD, peak systolic pressure – LVEDP) and left ventricular (LV) peak positive dP/dt (+dP/dt) during ischemic contractions were used as indexes of LV systolic function; LVEDP was used as an index 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 perfused at 1% of the CF rate with 1) normal controls (nonischemic) as vehicle; 2) OLDA (2 × 10−6M; Cayman Chemical); 3) OLDA plus CGRP8−37 (10−6 M; Sigma), a selective CGRP receptor antagonist; 4) OLDA plus RP-67580 (10−6 M; Tocris Bioscience), a selective neurokinin 1 (NK1) receptor antagonist; 5) OLDA plus chelerythrine (5 × 10−6 M; Calbiochem), a general PKC antagonist; or 6) OLDA plus tetrabutylammonium (TBA, 5 × 10−4 M; Sigma), a nonselective K+ channel antagonist. Antagonists were added to the perfusate 5 min before adding OLDA and continued for an additional 5 min with OLDA perfusion. Hearts were subsequently subjected to 35 min of no-flow normothermic global ischemia followed by 40-min of reperfusion. Additional antagonists without OLDA groups were performed in which WT hearts were perfused with the same concentrations of antagonists as above before subjected to I/R.

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

Evaluation of myocardial infarct size. The risk area and infarct size were measured after 40 min of postischemic 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. Hearts were then removed 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. Both sides of each slice were photographed and delineated, and photos were quantified with ImageJ version 1.37v (National Institutes of Health). Because hearts were subjected to global ischemia, the total cross-sectional areas were defined as the total risk areas. The infarcted area to total risk area ratio (%infarct size) of the two 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 and saturated with 95% O2-5% CO2 at 37°C continuously for 60 min (the stabilization period). In OLDA-treated groups, OLDA (2 × 10−9 M) was added and incubated for 60 min in WT and TRPV1−/− hearts. To examine the effects of the PKC antagonist and the K+ channel antagonist on OLDA-induced SP and CGRP release, chelerythrine (5 × 10−6 M) and TBA (5 × 10−4 M) were added 5 min before adding OLDA. The samples were purified and analyzed by radioimmunoassay as recommended by the supplier. Commercially available rat CGRP and SP radioimmunoassay kits (Peninsula Laboratories) were used to determine SP and CGRP release that was normalized by the wet heart weight.

Immunofluorescence assay of TRPV1 receptors. The LV tissue blocks were cut to a thickness of 10 µm. The tissues sections were incubated with the primary antibody (goat anti-TRPV1, dilution 1:2,500; Santa Cruz Biotechnology) and then with horseradish peroxidase-conjugated donkey anti-goat IgG secondary antibody (dilution 1:200; Jackson ImmunolResearch). The sections were subsequently incubated with fluorescein isothiocyanate conjugated to tyramide (TSA kit; PerkinElmer Life Sciences) following the protocol recommended by the manufacturer.

Statistical analysis. All values are expressed as means ± SE. Differences among groups measured at the end of the I/R experiments, the release of SP, CGRP, and LDH, and the infarct size were analyzed by Student’s t test. The effects of the PKC antagonist and the K+ channel antagonist on OLDA-induced cardiac protection, the selective CGRP receptor antagonist on OLDA-induced SP and CGRP release, chelerythrine (5 × 10−6 M) and TBA (5 × 10−4 M) were added 5 min before adding OLDA. The samples were purified and analyzed by radioimmunoassay as recommended by the supplier. Commercially available rat CGRP and SP radioimmunoassay kits (Peninsula Laboratories) were used to determine SP and CGRP release that was normalized by the wet heart weight.

RESULTS

OLDA protection against I/R injury was impaired in TRPV1−/− hearts. There were no statistically significant differences in hemodynamics between groups at the baseline (data not shown). After I/R, OLDA pretreatment inhibited the increase in LVEDP and improved recovery by increasing LVDP, CF, and +dP/dt in WT but not in TRV1−/− hearts (Figs. 1–4). Thus OLDA protected WT hearts against ischemic injury, whereas it had no protective effect on TRPV1−/− hearts.

Blockade of the CGRP receptor impaired OLDA protection. To determine whether endogenous CGRP plays a role in OLDA-induced cardiac protection, the selective CGRP receptor antagonist CGRP8−37 (10−6M) was given. CGRP8−37 blocked OLDA-induced cardioprotective effects in WT mice by increasing LVEDP and decreasing LVDP, CF, and +dP/dt in WT but not TRV1−/− hearts (Figs. 1–4). CGRP8−37 (10−6 M) had no effect on cardiac function in WT hearts without I/R (data not shown).
Blockade of the SP receptor impaired OLDA protection. The effect of endogenous SP on OLDA-induced cardiac protection was assessed by pretreatment with the NK1 receptor antagonist RP-67580 (10^{-6} M). The protective effects of OLDA were suppressed in the presence of RP-67580 by increasing LVEDP and decreasing LVDP, +dP/dt, and CF in WT but not TRVR1^{-/-} hearts (Figs. 1–4). Chelerythrine (5 × 10^{-6} M) had no effect on cardiac function in WT hearts without I/R (data not shown).

Blockade of PKC impaired OLDA protection. The effect of PKC activation on OLDA-induced cardiac protection was assessed by pretreatment with the PKC inhibitor chelerythrine (5 × 10^{-6} M). The protective effects of OLDA were suppressed in the presence of chelerythrine by increasing LVEDP and decreasing LVDP, +dP/dt, and CF in WT hearts but not TRVR1^{-/-} hearts (Figs. 1–4). Chelerythrine (5 × 10^{-6} M) had no effect on cardiac function in WT hearts without ischemia (data not shown).

Blockade of K^+ channel impaired OLDA protection. The effect of a nonselective K^+ channel antagonist TBA on OLDA-induced cardiac protection was assessed. The protective effects of OLDA were suppressed in the presence of TBA (5 × 10^{-4} M) by increasing LVEDP and decreasing LVDP, +dP/dt, and CF in WT hearts but not TRVR1^{-/-} hearts (Figs. 1–4). TBA (5 × 10^{-4} M) had no effect on cardiac function in WT hearts without ischemia (data not shown).

Measurements of LDH and infarct area. LDH levels and the infarct area after I/R were significantly lower in WT hearts treated with OLDA than WT hearts treated with vehicle or TRPV1^{-/-} hearts with or without OLDA treatment (Figs. 5 and 6), indicating that OLDA protects hearts in WT, but it’s protection is impaired in TRPV1^{-/-}.

Fig. 1. The changes of left ventricular end-diastolic pressure (LVEDP) at the end of ischemia-reperfusion (I/R). Wild-type (WT) and transient receptor potential vanilloid 1-null mutant (TRPV1^{-/-}) hearts were retrogradely perfused in a Langendorff apparatus and subjected to N-oleoyldopamine (OLDA, 2 × 10^{-9} M, at 1% of coronary flow rate) for 10 min in the presence or absence of the antagonists calcitonin gene-related protein (CGRP)-8–37, RP-67580, chelerythrine, or tetrabutylammonium (TBA), and then I/R. Antagonists were added to the perfusate 5 min before OLDA and continued for 5 min after OLDA. 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. In the ischemia control groups, WT and TRPV1^{-/-} hearts were equilibrated for 40 min, followed by I/R (WT-I/R and TRPV1^{-/-} I/R). Values are means ± SE; n = 6–11. *P < 0.05 vs. OLDA-WT.

Fig. 2. The changes of LVEDP at the end of I/R. WT and TRPV1^{-/-} hearts were treated as described in Fig. 1. Values are means ± SE; n = 6–11 mice. *P < 0.05 vs. OLDA-WT.
Release of SP and CGRP. The release of SP and CGRP at baseline (normal control) was not different between WT and TRPV1−/− hearts. SP and CGRP release in WT but not TRPV1−/− hearts subjected to OLDA treatment increased remarkably compared with the baseline (Fig. 7). The PKC antagonist chelerythrine (5 x 10⁻⁶ M) and the nonselective K⁺ channel antagonist TBA (5 x 10⁻⁴ M) blocked OLDA-induced SP and CGRP release in WT hearts (Fig. 8).

TRPV1 expression in the heart. TRPV1 immunofluorescence labeling was detected mainly in the myocardium and vascular tissues in the WT but not TRPV1−/− hearts, as indicated in Fig. 8. Negative staining was also found in WT control hearts in which the primary antibody was omitted from the immunofluorescence assay (data not shown).

DISCUSSION

TRPV1 expressed in cardiac sensory nerves may function as a molecular sensor to detect tissue ischemia and to modulate cardiac function (33). It has been shown that capsaicin-sensitive sensory nerves play a role in attenuating inflammatory responses and protecting the heart from injury (13, 23, 25). Studies using TRPV1−/− mice showed that the TRPV1 contributes to cardiac protection (39, 49, 56) and protects against the onset of sepsis after endotoxin (10). OLDA is a member of the family of fatty acid amides that includes anandamide and NADA and is increasingly recognized as an important class of lipid signaling molecules (47). OLDA has been found in striatal, dorsal spinal cord, and dorsal root ganglia (47) and identified as the most selective and potent endogenous TRPV1 activator so far (6). Regardless its potentially important role, its pathophysiological function is unknown. The present study showed that OLDA significantly improved recovery of the cardiac function after I/R injury as well as decreased LDH release and the infarct size in WT but not in TRPV1−/− hearts. Basal contractile function and baseline SP and CGRP levels were not different between WT and TRPV1−/− hearts. These results indicate that OLDA protects the heart against I/R injury via activation of the TRPV1.

As immunofluorescence assay showed, TRPV1 is expressed in the myocardium and vascular tissues in WT hearts. Previous
studies in rats showed that TRPV1 protein expression cannot be detected by immunohistochemistry although TRPV1 gene expression is detectable (57). In contrast, by the use of a signal amplification technology, Tyramide Signal Amplification (TSA) Systems that increases in sensitivity significantly, others have showed that TRPV1-positive afferent nerves distribute on the epicardial surface of the ventricle and around coronary vessels in rats (54). Our data obtained from mice are consistent with that of the latter study. TRPV1 can be activated in the setting of myocardial ischemia and mediates the sensation of angina (19). Indeed, TRPV1 may integrate and respond to multiple ischemic metabolites, serving as a polymodal detector of pain-producing chemical and physical stimuli. However, TRPV1 does not merely act in sensory capacity. Its activation also causes the release of CGRP, SP, and other neurokinins from sensory nerve terminals (14, 28, 48). CGRP is one of the most potent vasodilators identified to date in many species. In addition to vasodilation, CGRP has been suggested to play a protective role after myocardial infarction and vascular damage (5).

Fig. 5. 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 OLDA followed by I/R (OLDA-WT and OLDA-TRPV1^{-/-}), or subjected only to I/R as injury control (WT-I/R and TRPV1^{-/-}I/R). Coronary outflow was collected during the first period of 10–20 min of I/R and sampled for the LDH content. Values are means ± SE; n = 5. P < 0.05 vs. OLDA-WT (*) and vs. WT-I/R (†).

Fig. 6. Cardiac injury was assessed and expressed as the percent of infarct size. WT and TRPV1^{-/-} hearts were retrogradely perfused in a Langendorff apparatus and treated with OLDA followed by I/R (OLDA-WT and OLDA-TRPV1^{-/-}), or subjected only to I/R as injury control (WT-I/R and TRPV1^{-/-}I/R). Risk area and infarct size were measured 30 min after I/R. 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 and then removed and incubated for another 10 min at 37°C in 1% TTC. Values are means ± SE; n = 5. P < 0.05 vs. OLDA-WT (*) and P < 0.05 vs. WT-I/R (†).

Fig. 7. Release of CGRP and substance P (SP) from isolated hearts subjected to OLDA (2 × 10^{-8} M) in the presence or absence of the protein kinase C (PKC) inhibitor chelerythrine (5 × 10^{-6} M) or the nonselective K* channel antagonist TBA (5 × 10^{-4} M). WT and TRPV1^{-/-} are the normal control groups. Values are means ± SE; n = 4. P < 0.05 vs. WT (*) and vs. OLDA-WT (†).
TRPV1−/− hearts (53). These results indicate that substitution of SP and CGRP before I/R is capable of inducing preconditioning-like protection. In the present study, OLDA increased SP and CGRP release in WT but not in TRPV1−/− hearts. Blockade of the NK1 receptor with RP-67580 and the CGRP receptor with CGRP8–37 impaired OLDA-induced protective effects in WT but not TRPV1−/− hearts. These data suggest that endogenously released CGRP and SP following TRPV1 activation by OLDA contribute to OLDA-induced cardiac protection.

The PKC signaling cascade has been implicated to play an important role in I/R injury, and PKC antagonists abrogated ischemic preconditioning protection in the heart (4, 53). To assess the role of PKC in mediating the protective effect of OLDA against I/R injury, chelerythrine, a selective PKC antagonist, was used. The primary sequence predicts that TRPV1 contains many putative phosphorylation sites, and PKC- and protein kinase A-mediated phosphorylation of TRPV1 is critical for its functions (2, 26, 31, 36, 46). It has been shown that PKC-mediated phosphorylation of TRPV1 may significantly increase TRPV1-mediated effects, e.g., PKC potentiates and sensitizes heat-, protons-, or agonist-induced TRPV1 currents and TRPV1-induced increases in SP and CGRP release (31, 37, 46). In the present study, OLDA-induced SP and CGRP release can be blocked by chelerythrine, indicating that PKC modulates TRPV1 function by modulating TRPV1-induced neuropeptide release. Although SP and CGRP release induced by OLDA may also activate PKC to protect the heart from I/R injury (24, 50), our data indicate chelerythrine-induced blockade of OLDA effects is probably mediated by inhibiting SP and CGRP release rather than affecting SP and CGRP effects.

It is well known that K+ channel antagonists can block the infarct-limiting effects of ischemic preconditioning, and agonists of the channel mimic the protective effect (16). TBA, a nonspecific calcium-activated K+ channel antagonist, was used to examine whether the K+ channel blocker may inhibit OLDA-mediated cardioprotective effects. We found that TBA impaired OLDA-induced I/R protection and SP and CGRP release. Although TRPV1 contains four subunits and each contains six transmembrane domains with similar topological features and consistent permeation characteristics similarly as the voltage-gated K+ channel (22, 31), no evidence shows that the K+ channel blocker TBA may block TRPV1. Thus TBA may affect OLDA action indirectly via inhibiting OLDA-induced SP and CGRP release. Indeed, it has been shown that CGRP opens K+ channels in patches on smooth muscle cells and hyperpolarizes arterial smooth muscle and dilates arteries through ATP-sensitive potassium channel (30).

Studies using rat basophilic leukemia-1 cells showed that OLDA is a potent inhibitor of 5-lipoxygenase (5-LOX) (41). It has been shown that myocardial I/R promotes the release and metabolism of leukotrienes (LTs), which are metabolites of arachidonic acid formed from the 5-LOX pathway and exert potent vasoactive and proinflammatory effects (12). LTs also play roles in I/R of skin, brain, and kidney (8, 11, 34), and 5-LOX inhibitors have been suggested to be useful in the treatment of conditions associated with I/R of the kidney (34). However, the effects of 5-LOX inhibitors on myocardial I/R are rather controversial. Studies of the 5-LOX inhibitor LY-233569 showed that it failed to reduce the myocardial infarct size after I/R in the dog (18), indicating that 5-LOX may not mediate acute reperfusion injury of ischemic myocardium and that 5-LOX-mediated cardioprotective effects observed in the present study may be 5-LOX pathway independent.

In summary, the present study provides direct evidence that OLDA, an endovanilloid, exerts a cardiac protective effect during I/R injury via activating TRPV1, leading to CGRP and SP release in WT mice. The PKC antagonist and the nonspecific K+ channel antagonist block OLDA-induced SP and CGRP release and impair the protective effect of OLDA. The protective effect of OLDA is void in TRPV1−/− hearts. Our data may have important clinical implications, suggesting that impairment of OLDA production or TRPV1 expression/function may render a rather more vulnerable I/R injury.
GRANTS

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