Dietary Capsaicin Ameliorates Pressure Overload–Induced Cardiac Hypertrophy and Fibrosis Through the Transient Receptor Potential Vanilloid Type 1

Qiang Wang,1,* Shuangtao Ma,1,* De Li,1 Yan Zhang,1 Bing Tang,1 Chenming Qiu,1 Yongjian Yang,1 and Dachun Yang1

BACKGROUND
Dietary capsaicin plays a protective role in hypertension, atherosclerosis, obesity, and hyperlipidemia through activating the transient receptor potential vanilloid type 1 (TRPV1), a nonselective cation channel. This study was designed to investigate the role of capsaicin in cardiac hypertrophy and fibrosis in a pressure overload model.

METHODS
TRPV1 knockout (KO) mice and their wild-type (WT) littermates, aged 8 weeks, were randomly divided into sham and aortic banding surgery groups and were fed with chow or chow plus capsaicin for 10 weeks.

RESULTS
Dietary capsaicin significantly attenuates pressure overload–induced increase in heart weight index, enlargement of ventricular volume, decrease in cardiac function, and increase in cardiac fibrosis in WT mice. However, these effects of capsaicin were absent in TRPV1 KO mice. Additionally, capsaicin blunted pressure overload–induced upregulation of transforming growth factor β, connective tissue growth factor, and the phosphorylation of Smad2/3 in WT mice but not in TRPV1 KO mice. Moreover, capsaicin attenuated pressure overload–induced overexpression of metalloproteinase (MMP)-2, MMP-9 and MMP-13 in WT mice but not in TRPV1 KO mice. Capsaicin also attenuated angiotensin II–induced proliferation of cardiac fibroblasts from mice with the TRPV1 channel.

CONCLUSIONS
Our results suggest that dietary capsaicin protects against cardiac hypertrophy and fibrosis in pressure overload mice through TRPV1.

Keywords: blood pressure; capsaicin; cardiac fibrosis; cardiac hypertrophy; hypertension; transient receptor potential vanilloid type 1.

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*These authors contributed equally.

1Department of Cardiology, Chengdu Military General Hospital, Chengdu, Sichuan, PR China.

Correspondence: Dachun Yang (y.dachun@gmail.com).

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Heart failure is a growing public health problem with cumulating cardiovascular risk factors and increasing incidence with aging of the population.1 Cardiac fibrosis, a marker of cardiac hypertrophy, includes the processes of reactive perivascular and interstitial fibrosis, which lead to diastolic stiffness, ventricular enlargement, and systolic dysfunction.2–4 Despite the improvement in treatment strategies in past decades, an ideal antifibrotic treatment has not yet been established. There is a body of evidence indicating that inflammation plays a crucial role in the development of cardiac fibrosis.5,6 It has been shown that inflammatory cells first infiltrate the perivascular domain, where fibroblasts synthesize collagen I and develop perivascular fibrosis, and subsequently infiltrate also the interstitial space, and the fibrosis extends to between the cardiomyocytes, the so-called interstitial fibrosis.7 Capsaicin is the active component of chili peppers and is considered to exhibit an anti-inflammatory effect.8 Capsaicin is used to help relieve a certain type of pain known as neuralgia and rheumatoid arthritis associated with inflammation.9 Dietary capsaicin has been proved to ameliorate several cardiovascular diseases, including obesity, dyslipidemia, glycemia, hypertension, and atherosclerosis.10,11

The action of capsaicin is mostly dependent on its receptor transient receptor potential vanilloid type 1 (TRPV1).12 TRPV1 is known as a nonselective cationic channel expressed in primary sensory C-fibers and is also found in other excitable and nonexcitable tissues, including cardiomyocytes and fibroblasts.13 Capsaicin activates a TRPV1 channel and promotes calcium entry that is necessary to activate several cellular events and promotes some gene expression. According to the previous studies, TRPV1 was upregulated in hypertrophied myocardium,14 suggesting that TRPV1 might play a potent role in cardiac remodeling. Thus, we hypothesized that dietary capsaicin could regulate pressure overload–induced cardiac hypertrophy and fibrosis though the TRPV1 channel.

In this study, we investigated the role of dietary capsaicin in pressure overload–induced cardiac hypertrophy and fibrosis in TRPV1 knockout (KO) mice and wild-type (WT)
littermates and the underlying molecular mechanisms. Because angiotensin II plays an important role in pressure overload–induced cardiac fibrosis, we also examined the effect of capsaicin in angiotensin II–induced proliferation of cardiac fibroblasts.

METHODS

Animals

All animal procedures were performed in accordance with the Guide for the Care and Use of Laboratory Animals published by the US National Institutes of Health (NIH Publication No. 85–23, revised 1996) and approved by the Institutional Animal Care and Use Committee. The TRPV1 KO mice on a C57BL/6J genetic background and their WT littermates were purchased from the Model Animal Research Center of Nanjing University (Nanjing, Jiangsu, China). Suprarenal aortic banding (AB) was carried out as described previously with some modifications. Briefly, male mice aged 8 weeks were anesthetized by intraperitoneal injection of pentobarbital (50 mg/kg of body weight). Suprarenal AB was performed by placing a suture around the abdominal aorta and a 30-gauge blunted needle, which was subsequently removed. An identical procedure was used for sham surgery, with the exception of band placement. Three days after AB surgery, mice were given the normal standard chow (control group) or normal chow plus 0.01% capsaicin (capsaicin group). Ten weeks after surgery, the hearts of the mice were harvested and weighed to calculate the heart weight/body weight ratio (HW/BW; mg/g) and the left ventricular weight/body weight ratio (LVW/BW; mg/g).

Echocardiography and hemodynamics

Mice were anesthetized with 1.5% isoflurane, and echocardiographic images were obtained with a Visualsonics Vevo 2100 (Visualsonics Inc., Toronto, Ontario, Canada) ultrasound system with a 40-MHz transducer. For evaluation of left ventricular hemodynamics, a 1.4-F microconductance pressure catheter (ARIA SPR-853; Millar Instruments, Houston, TX) was introduced through the right common carotid artery into the ascending aorta and then advanced into the left ventricle as described previously. Data were collected on Chart via PowerLab (ADInstruments, Castle Hill, Australia).

Histological analyses

Hearts were excised and retrograde-perfused with phosphate-buffered saline. They were fixed with 10% (v/v) formalin and embedded in paraffin. Paraffin sections (5-mm thickness) were then stained with either hematoxylin and eosin or Masson Trichrome (for collagen). The positively stained (green) fibrotic area was expressed as a percentage of total area.

Cardiac fibroblasts culture

Mouse cardiac fibroblasts were isolated and cultured according to the method described previously. Isolated cells were cultured in Dulbecco modified Eagle medium nutrient mixture F-12 (Invitrogen, Carlsbad, CA) containing 10% fetal bovine serum. Adherent cells were characterized at passage 1 using immunohistochemistry microscopy and found to be positive for vimentin but negative for smooth muscle α-actin and von Willebrand factor. Fibroblasts up to passage 3 were used in the following studies.

Angiotensin II measurement

Angiotensin II concentrations in plasma and left ventricular tissue were measured by radioimmunoassay using a commercial RIA kit (Phoenix Pharmaceuticals, Mountain View, CA) according to the manufacturer’s instruction.

Cell proliferation assay

The cell proliferation was measured using a CCK-8 cell proliferation kit (Dojindo Laboratories, Kumamoto, Japan) according to the manufacturers’ instructions. Cells were seeded into a 96-well plate with 100 µl complete medium and cultured at 37 °C. Cells were divided into 3 groups: control, angiotensin II (1 µM), and angiotensin II plus capsaicin (1 µM). Ten microliters of CCK-8 solution was added to each well after 1, 2, 3, 4, 5, 6, and 7 days, respectively. Plates were incubated at 37 °C for 2 hours, and then the absorbance at 450 nm was measured with a microplate reader (Multiskan MK3; Thermo Labsystem, Waltham, MA).

Flow cytometry analysis

Cell cycle analysis was performed by flow cytometry. Briefly, cultured cells were trypsinized into single cell suspensions and fixed with 70% ethanol at −20 °C overnight. Cells were incubated with propidium iodide (Sigma Aldrich, St. Louis, MO) and RNase (Sigma-Aldrich) for 30 minutes at 4 °C. DNA content was assessed by flow cytometry (BD FASAria Cell Sorter; BD Biosciences, San Jose, CA).

Western blot analysis

Cell lysates and heart extracts were electrophoresed under reducing conditions through 8%–12% sodium dodecyl sulfate–polyacrylamide gels and electroblotted on an immobilon-PVDF membrane (Millipore, Billerica, MA). The membranes were blocked with 5% BSA in 0.1% Tween-20 Tris-buffered saline for 60 minutes before overnight incubation with either anti-TRPV1 (1:500 dilution; ab111973; Abcam, Cambridge, UK), anti–collagen I (1:250 dilution; sc-8784; Santa Cruz Biotechnology, Santa Cruz, CA), anti–collagen III (1:300 dilution; sc-8781; Santa Cruz Biotechnology), antifibronectin (1:300 dilution; sc-9068; Santa Cruz Biotechnology), anti–transforming growth factor β1 (TGF-β1) (1:300 dilution; sc-146; Santa Cruz Biotechnology), anti–collagen I (1:250 dilution; sc-8784; Santa Cruz Biotechnology), anti–collagen III (1:300 dilution; sc-8781; Santa Cruz Biotechnology), antifibronectin (1:300 dilution; sc-9068; Santa Cruz Biotechnology), anti–transforming growth factor β1 (TGF-β1) (1:300 dilution; sc-146; Santa Cruz Biotechnology), anti–phospho-Smad2 (1:800 dilution; sc-7681; Cell Signaling Technology), anti–phospho-Smad3 (1:800 dilution; sc-7683; Cell Signaling Technology), anti–phospho-Smad4 (1:700 dilution; sc-7682; Cell Signaling Technology), anti–phospho-Smad2 (1:900 dilution; sc-3539; Cell Signaling Technology), anti–phospho-Smad3 (1:700 dilution; sc-5339; Cell Signaling Technology), anti–phospho-Smad4 (1:700 dilution; sc-5339; Cell Signaling Technology), anti–phospho-Smad3 (1:700 dilution; sc-5339; Cell Signaling Technology), anti–phospho-Smad4 (1:700 dilution; sc-5339; Cell Signaling Technology), anti–connective tissue growth factor (CTGF)
Capsaicin attenuates cardiac hypertrophy and fibrosis

Capsaicin attains overload-induced cardiac hypertrophy and fibrosis

Statistical analysis

Data are means ± SEs. The differences among groups were analyzed using 1-way analysis of variance followed by a Bonferroni test. *P < 0.05 was considered significant.

RESULTS

Capsaicin attenuates overload-induced cardiac hypertrophy through TRPV1

To evaluate the effect of capsaicin on cardiac hypertrophy, TRPV1 KO and WT mice subjected to AB surgery were fed with capsaicin diet and standard chow diet. Ten weeks after surgery, both KO and WT mice showed significant increased HW/BW ratio, LVW/BW ratio, interventricular septal thickness at end diastole, left ventricular posterior wall thickness at end diastole, left ventricular end-diastolic diameter, and ejection fraction and fractional shortening (Figure 1a–c; Table 1). The cardiac hypertrophy was confirmed by the morphology of the gross hearts and hematoxylin-and-eosin staining (Figure 1d,e). Interestingly, dietary capsaicin significantly attenuates the effects induced by pressure overload in WT mice but did not affect these parameters in TRPV1 KO mice (Figure 1a–e; Table 1). Hemodynamic analysis revealed that AB significantly increased the left ventricular end-diastolic pressure and left ventricular end-systolic pressure and decreased maximal rate of pressure development and maximal rate of pressure decay in both TRPV1 KO mice and
Table 1. Echocardiographic and hemodynamic parameters in transient receptor potential vanilloid type 1 knockout and wild-type mice 10 weeks after surgery

<table>
<thead>
<tr>
<th></th>
<th>WT-Sham Cont</th>
<th>WT-AB Cont</th>
<th>KO-Sham Cont</th>
<th>KO-AB Cont</th>
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</thead>
<tbody>
<tr>
<td>IVSd, mm</td>
<td>0.660 ± 0.033</td>
<td>0.656 ± 0.017</td>
<td>0.791 ± 0.034*</td>
<td>0.717 ± 0.023**</td>
</tr>
<tr>
<td>LVPWd, mm</td>
<td>0.676 ± 0.022</td>
<td>0.680 ± 0.014</td>
<td>0.853 ± 0.030*</td>
<td>0.735 ± 0.022***</td>
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<td>LVEDD, mm</td>
<td>3.786 ± 0.053</td>
<td>3.747 ± 0.068</td>
<td>5.003 ± 0.285*</td>
<td>4.309 ± 0.146***</td>
</tr>
<tr>
<td>LVSSD, mm</td>
<td>2.849 ± 0.080</td>
<td>2.917 ± 0.083</td>
<td>4.536 ± 0.329*</td>
<td>3.609 ± 0.128***</td>
</tr>
<tr>
<td>EF, %</td>
<td>49.73 ± 2.32</td>
<td>52.42 ± 2.87</td>
<td>22.71 ± 3.25*</td>
<td>38.84 ± 1.76***</td>
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<tr>
<td>FS, %</td>
<td>24.84 ± 1.39</td>
<td>26.17 ± 1.54</td>
<td>9.90 ± 1.52*</td>
<td>16.29 ± 0.59**</td>
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<tr>
<td>LVEDP, mm Hg</td>
<td>2.50 ± 0.36</td>
<td>2.63 ± 0.44</td>
<td>12.75 ± 0.96*</td>
<td>7.86 ± 1.036***</td>
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<tr>
<td>dP/dtmax, mm Hg/s</td>
<td>12,416 ± 694</td>
<td>12,494 ± 618</td>
<td>7,038 ± 549*</td>
<td>9,209 ± 741**</td>
</tr>
<tr>
<td>dP/dtmin, mm Hg/s</td>
<td>12,049 ± 660</td>
<td>12,379 ± 613</td>
<td>6,607 ± 657*</td>
<td>8,849 ± 653**</td>
</tr>
</tbody>
</table>

Values are means ± SEM (n = 8).
Abbreviations: AB, aortic banding; CAP, capsaicin diet–fed mice; Cont, normal diet–fed mice; dP/dtmax, maximal rate of pressure development; dP/dtmin, maximal rate of pressure decay; EF, ejection fraction; FS, fractional shortening; IVSd, interventricular septum diameter at end diastole; KO, knockout transient receptor potential vanilloid type 1 mice; LVEDD, left ventricular end-diastolic diameter; LVEDP, left ventricular end-diastolic pressure; LVESP, left ventricular end-systolic pressure; LVPWd, left ventricular posterior wall diameter at end diastole; LVSSD, left ventricular end-systolic diameter; WT, wild-type mice.

*P < 0.05 vs. Sham + Cont; **P < 0.05 vs. AB + Cont; ***P < 0.01 vs. AB + Cont.
Capsaicin attenuates cardiac hypertrophy and fibrosis

WT littermates (Table 1). Dietary capsaicin ameliorated the worsened hemodynamic dysfunction in response to AB in WT mice but not in TRPV1 KO mice (Table 1).

Capsaicin attenuates overload-induced cardiac fibrosis through TRPV1

Cardiac fibrosis was evaluated by Masson’s Trichrome staining performed on paraffin-embedded sections. The perivascular and interstitial fibrosis were observed in both TRPV1 KO and WT mice in response to pressure overload (Figure 2a,b). The cardiac fibrosis in WT mice was remarkably rescued by dietary capsaicin (Figure 2a). However, the fibrosis in TRPV1 KO mice was not attenuated after treatment with capsaicin (Figure 2b). AB induced a significant increase in collagen synthesis, as measured by the protein expression of collagen I and collagen III and fibronectin expression in both TRPV1 KO and WT mice (Figure 2c–f). As expected, dietary capsaicin significantly blunted the upregulation of collagen and fibronectin in WT mice (Figure 2c–e). However, the collagen synthesis in loaded TRPV1 KO mice was not affected by the treatment of capsaicin (Figure 2d–f).

Capsaicin regulates signals responsible for collagen turnover

The expressions of TGF-β1 and CTGF and the phosphorylation of Smad2/3, which are responsible for cardiac fibrosis, were dramatically increased in pressure-loaded TRPV1 KO and WT littermates (Figure 3a,b,e). Additionally, the expressions of MMP-2, MMP-9, MMP-13, which are responsible for the degradation of collagen, were also upregulated by AB (Figure 3c,d). Interestingly, these effects were blunted by treatment of capsaicin in WT mice but not in TRPV1 KO mice (Figure 3a–e). Moreover, the induction of MT1-MMP, TIMP-1, and TIMP-2 were not affected by capsaicin (Figure 3c,d).

Capsaicin inhibits angiotensin II–induced proliferation of cardiac fibroblasts

We found that mice in the AB group had significant increases in plasma (261.63 ± 29.64 vs. 135.75 ± 14.83 pg/ml; P < 0.01) and left ventricular (1.941 ± 0.245 vs. 0.749 ± 0.104 pg/mg protein; P < 0.05) levels of angiotensin II compared with mice in the sham group. Then, we investigated the role of capsaicin in angiotensin II–induced proliferation of cardiac fibroblasts. Fibroblasts are the principal determinants of cardiac fibrosis. Cardiac fibroblasts were isolated from TRPV1 KO mice and WT littermates, and cell proliferation was measured using a CCK-8 kit. The results showed that the cardiac fibroblast proliferation was significantly increased by angiotensin II (Figure 4a,b). These effects of angiotensin II were abrogated by TRPV1

![Figure 2. Effect of capsaicin on cardiac fibrosis. (a,b) Representative Masson stained sections of gross heart (upper panel), interstitial fibrosis (middle panel), and perivascular fibrosis (lower panel) from the normal diet (Cont)–fed or capsaicin diet (CAP)–fed wild-type (WT) and transient receptor potential vanilloid type 1 (TRPV1) knockout (KO) mice subjected to sham surgery and aortic banding (AB). (c,d) Representative Western blots of collagen I, collagen III, and fibronectin of the heart tissue from WT and TRPV1 KO mice, respectively. (e,f) Representative immunohistochemical images of collagen I (upper panel), collagen III (middle panel), and fibronectin (lower panel) of the heart tissue from WT and TRPV1 KO mice, respectively.](http://ajh.oxfordjournals.org/)

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agonist capsaicin in fibroblast isolated from WT mice but not in that from TRPV1 KO mice (Figure 4a,b). The cell cycle analyses were performed using a flow cytometer with propidium iodide staining. The angiotensin II significantly increased the percentage of cells in the S plus G2/M phases, whereas these effects were attenuated by capsaicin in fibroblasts isolated from WT mice but not in that from TRPV1 KO mice (Figure 4c–e).

DISCUSSION

In this study, we investigated the role of capsaicin in pressure overload–induced cardiac hypertrophy and fibrosis and used TRPV1 KO mice to examine the role of TRPV1 in the antihypertrophic and antifibrotic actions of capsaicin. Dietary capsaicin significantly attenuated cardiac hypertrophy, fibrosis, and dysfunction under conditions of pressure overload in WT mice, whereas these effects were abolished in TRPV1 KO mice. Moreover, capsaicin significantly attenuated angiotensin II–induced proliferation of cardiac fibroblasts. These results suggest that capsaicin plays a protective role in the cardiac response to pressure overload though TRPV1 channel.

A significant finding of this study is that dietary capsaicin ameliorates pressure overload–induced cardiac hypertrophy. Moreover, this study showed that the antihypertrophic action of capsaicin is dependent on the presence of TRPV1 channels. These findings indicated that, in addition to the pressure effect, there is another important mechanism operating for the hypertrophy, which is mediated by the TRPV1 channels. According to a previous study, capsaicin activated the TRPV1 channel, resulting in an increase of intracellular calcium concentration that subsequently triggered several cellular processes.25 However, the function of TRPV1 in cardiac hypertrophy has not been fully understood. Buckley et al.26 reported that the pressure-overloaded mice lacking functional TRPV1, compared with WT, have improved heart function and reduced hypertrophic, fibrotic, and apoptotic markers. However, our study demonstrated that TRPV1 KO mice and WT littermates have similar hypertrophic response to pressure overload.

Accompanying hypertrophy, cardiac fibrosis is characterized by a disproportionate accumulation of collagen and other extracellular matrix. The type I and III collagen are the most abundant types in the heart and are the main contributors to pressure overload–induced fibrosis.27 The most important finding of this study is that dietary capsaicin ameliorates pressure overload–induced cardiac fibrosis. As shown in this study, capsaicin significantly decreased the deposition of collagen I, collagen III, and fibronectin after pressure overload though a TRPV1-dependent manner. A similar action of TRPV1 has been identified in renal fibrosis in hypertensive mice.

TGF-β1/Smad2/3 signaling and CTGF play a pivotal role in the activation of cardiac fibroblast and the subsequent formation of extracellular matrix.28 A previous study demonstrated that, during the formation of renal fibrosis, the increases of TGF-β1 expression and Smad2/3 phosphorylation were significantly enhanced in TRPV1 KO mice.
Capsaicin Attenuates Cardiac Hypertrophy and Fibrosis

Figure 4. Abbreviations: G0, Gap 0; G1, Gap 1; G2, Gap 2; S, synthesis; M, mitosis. Capsaicin inhibits angiotensin (ANG) II–induced proliferation of cardiac fibroblasts. (a, b) Cell proliferation was detected using a CCK-8 kit. Cardiac fibroblasts from wild-type (WT) and transient receptor potential vanilloid type 1 (TRPV1) knockout (KO) mice were treated with saline (Cont), Ang II, or Ang II plus capsaicin (Ang II + CAP). (c–e) The cell cycle analyses were performed using a flow cytometer. *P < 0.01 vs. Cont; **P < 0.05 vs. Ang II.
compared with WT littermates. These data indicate that the protective effects of TRPV1 against renal fibrosis may be mediated by inhibiting TGF-β1/Smad2/3 signaling. Our study showed that capsaicin, a TRPV1 agonist, significantly attenuated pressure overload–induced upregulation of TGF-β1 and phosphorylation of Smad2/3. Taken together, the antifibrotic action of capsaicin might be mediated by TRPV1-dependent inhibition of TGF-β1/Smad2/3 signaling.

The proliferation of cardiac fibroblasts serves as a key cellular event in the development of cardiac fibrosis. Here we demonstrated that dietary capsaicin, under similar experimental conditions, inhibits cardiac fibroblast proliferation, and subsequently attenuates pressure overload–induced cardiac hypertrophy and fibrosis.

**REFERENCES**
