Disruption of TRPV1-mediated coupling of coronary blood flow to cardiac metabolism in diabetic mice: role of nitric oxide and BK channels

Giacinta Guarini,1,2* Vahagn A. Ohanyan,1 John G. Kmetz,1 Daniel J. DelloStritto,1 Roslin J. Thoppil,1
Charles K. Thodeti,1 J. Gary Meszaros,1 Derek S. Damron,2 and Ian N. Bratz1

1Department of Integrative Medical Sciences, Northeast Ohio Medical University, Rootstown, Ohio; and 2Department of Biological Sciences, Kent State University, Kent, Ohio

Submitted 6 January 2012; accepted in final form 14 May 2012

Disruption of TRPV1-mediated coupling of coronary blood flow to cardiac metabolism in diabetic mice: role of nitric oxide and BK channels. Am J Physiol Heart Circ Physiol 303: H216–H223, 2012.—We have previously shown transient receptor potential vanilloid subtype 1 (TRPV1) channel-dependent coronary function is compromised in pigs with metabolic syndrome (MetS). However, the mechanisms through which TRPV1 channels couple coronary blood flow to metabolism are not fully understood. We employed mice lacking TRPV1 (TRPV1−/−), db/db diabetic, and control C57BKS/J mice to determine the extent to which TRPV1 channels modulate coronary function and contribute to vascular dysfunction in diabetic cardiomyopathy. Animals were subjected to in vivo infusion of the TRPV1 agonist capsaicin to examine the hemodynamic actions of TRPV1 activation. Capsaicin (1–100 µg·kg−1·min−1) dose dependently increased coronary blood flow in control mice, which was inhibited by the TRPV1 antagonist capsazepine or the nitric oxide synthase (NOS) inhibitor N-nitro-l-arginine methyl ester (l-NAME). In addition, the capsazepin-mediated increase in blood flow was attenuated in db/db mice. TRPV1−/− mice exhibited no changes in coronary blood flow in response to capsaicin. Vasoreactivity studies in isolated pressurized coronary microvessels revealed a capsazepin-dependent relaxation that was inhibited by the TRPV1 inhibitor SB366791 l-NAME and to the large conductance calcium-sensitive potassium channel (BK) inhibitors iberiotoxin and Penetrem A. Similar to in vivo responses, capsazepin-mediated relaxation was impaired in db/db mice compared with controls. Changes in pH (pH 7.4–6.0) relaxed coronary vessels contracted to the thromboxane mimetic U46619 in all three groups of mice; however, pH-mediated relaxation was blunted in vessels obtained from TRPV1−/− and db/db mice compared with controls. Western blot analysis revealed decreased myocardial TRPV1 protein expression in db/db mice compared with controls. Our data reveal TRPV1 channels mediate coupling of myocardial blood flow to cardiac metabolism via a nitric oxide-dependent, BK channel-dependent pathway that is corrupted in diabetes.

Type II diabetes increases the morbidity and mortality to many cardiovascular-related diseases, such as coronary artery disease (CAD) by as much as fourfold vs. nondiabetic patients (17, 30). This is attributed in part, to a greater development of both micro- and macrovascular disease. Impaired coronary microvascular blood flow contributes to the increased cardiovascular events associated with diabetes through numerous mechanisms (27, 35). The proposed mechanism for the pathogenesis of diabetic cardiomyopathy likely reflects the multifactorial and highly complex nature of diabetes, but arterial dysfunction clearly appears to be a contributing factor. The transient receptor potential vanilloid 1 (TRPV1) channels are characteristically gated by chemical and physical stimuli including changes in pH, heat, ethanol, and endovanilloid compounds such as capsaicin (5). We and others (1, 3, 7, 10, 31, 41, 43) have revealed TRP channel expression in vascular smooth muscle cells (VSMC) and endothelial cells (EC) suggesting these channels regulate vascular functions. Specifically, we found that TRPV1 channels play an important role in the vascular reactivity, largely through Ca2+ entry via endothelial TRPV1 channels, triggering nitric oxide (NO)-dependent vasodilation in endothelium of conduit coronary arteries from Ossabaw swine (3). Importantly, TRPV1 signaling was virtually abolished in pigs exhibiting metabolic syndrome (MetS) and could be a potential mechanism contributing to the endothelial dysfunction and the development of vascular complications, CAD, and diabetic cardiomyopathy often observed in the setting of diabetes. Despite these compelling observations suggesting an important role of TRPV1 in cardiovascular homeostasis, the contribution of TRPV1 channels to myocardial blood flow (MBF) regulation is largely unknown.

The purpose of this investigation was to test the hypothesis that TRPV1 channels regulate MBF via an NO-/large conductance calcium-sensitive potassium channel (BK) channel-dependent pathway, which is disrupted in diabetic cardiomyopathy. This hypothesis was examined using vertically integrated experiments in isolated, pressurized coronary arterioles in vitro and anesthetized mice in vivo. Expression of TRPV1 channels was also assessed by Western blotting. In the present study, we demonstrate that TRPV1 channels directly regulate MBF and thus are an ionic mechanism involved in the coupling of MBF to cardiac metabolism and this coupling is corrupted in db/db mice in part due to altered pH-mediated regulation of TRPV1 in diabetes.

METHODS AND MATERIALS

Mice. All procedures were conducted with the approval of the Institutional Animal Care and Use Committee of the Northeast Ohio Medical University (NEOMED) and in accordance with National Institutes of Health Guidelines for the Care and Use of Laboratory Animals (NIH publication no. 85–23, revised 1996). Mice breeding pairs were originally purchased from Jackson Laboratories (Bar Harbor, ME) after which mice were bred in our animal facility at NEOMED. BKS.Cg-m+/+ LepRb/J (db/db) mice have a mutation on chromosome 4 that inhibits the expression of the leptin receptor,
whereas heterozygous mice cannot be distinguished from normal mice. The type II diabetes mellitus in db/db mice is similar to type II diabetes mellitus in adult humans, characterized by obesity, insulin resistance/hyperinsulinemia, and hyperglycemia that the mice develop by 4–8 wk of age. Experiments were performed in 10- to 12-wk-old male TRPV1⁻/⁻, db/db or aged-matched, C57BKS/J mice as controls. Mice were housed in a temperature-controlled room with a 12:12-h light-dark cycle and maintained with access to food and water ad libitum.

Jugular and femoral artery catheterization and measurement of mouse blood pressure. Mice received a surgical plane of inhaled anesthesia with 1.5–2.5% sevoflurane gas with supplemental oxygen, using a veterinary anesthesia and monitoring device. Animals were placed on controlled heating table, maintained at 37°C with core temperature measured via a rectal probe. Mice were secured in the supine position and placed under a dissecting microscope. The right jugular vein was cannulated with PE-50 polyethylene tubing (Becton Dickinson, Oakville, ON) containing 50 U/ml heparin (50 U/ml in Dulbecco’s PBS) in saline for intravenous drug infusions. Next, a midline incision was made on the ventral right thigh region and the femoral nerve was isolated and pulled aside. The distal and proximal ends of the femoral artery were held with surgical sutures for temporary control of bleeding, and the distal end of the femoral artery was tied off. The femoral artery was isolated and cannulated with a 1.4-Fr (SPR-1.000; Millar Instruments) high-fidelity microtip transducer catheter connected to a data acquisition system (PowerLab ML820; ADInstrument, Colorado Springs, CO) through a pressure interface unit (Millar Instrument, Transducer Balance, TCB 600) designed to measure invasively systolic, diastolic, pulse pressure, mean arterial blood pressure (MAP), and heart rate (HR). The microtip catheter was advanced into the femoral artery, and aortic blood pressure was recorded. All measured variables were continuously recorded and stored on an iMac computer that used the PowerLab system (ADInstruments; Castle Hill, Australia). The blood pressure data were collected and analyzed using ADInstruments Chart v5.1.2 software. To correct HR measurement by the ML820 for movement artifact, an ECG monitor was connected throughout the experiments. All mice were euthanized following the experimental protocol utilizing a lethal dose of pentobarbital sodium (50 μg/kg).

Contrast echocardiography MBF measurements. For myocardial contrast echocardiography (MCE), animals were prepared as above for transthoracic echocardiography. Similar to above, mice were simultaneously infused with capsaicin (1–100 μg·kg⁻¹·min⁻¹) and nontargeted contrast (Vevo MicroMarker NonTargeted Contrast Agent Kit; Visual Sonics, Toronto, ON, Canada; diluted 1/10 in sterile saline). In all mice, the contrast agent was infused intravenously (jugular vein) at 20 μl/min (1 x 10⁸ bubbles/ml). MCE was performed using ACUSON SEQUIOA 512 Ultrasound System (Siemens) with low-MI (0.3) imaging, and high-MI (1.9) destruction was used to detect changes in MBF. All settings for processing were adapted and optimized for each animal: penetration depth was 2–2.5 cm, near field was focused on the middle of the left ventricle (short axis view), and gains were adjusted to obtain images with no signal from the myocardium and then held constant. Measurements were

Fig. 1. Capsaicin increases myocardial blood flow (MBF) in control mice. A: region of interest and analysis of flow measurements. B: contrast echocardiography flow measurements of MBF in control (n = 13) mice during baseline and in response to capsaicin in the absence or presence of the transient receptor potential vanilloid 1 (TRPV1) antagonist, capsaizepine (5 mg/kg; n = 7). TRPV1⁻/⁻ mice displayed no changes in flow at any dose of capsaicin (n = 5). C: MBF in control mice (n = 6) during baseline and in response to capsaicin in the presence of N-nitro-L-arginine methyl ester (L-NAME; 100 mg/kg; n = 6). KO, knockout. *P < 0.05 vs. baseline; #P < 0.05 vs. control at that concentration.
performed offline using iMCE software (Siemens). Regions of interest were positioned within the anterolateral and posterolateral walls in the short axis view. A curve of signal intensity over time was obtained in each region of interest and fitted to an exponential function: $y = A(1 - e^{-\beta t})$, where $y$ is signal intensity at any given time, $A$ is signal intensity, which corresponds to the microvascular cross sectional area, and $\beta$ is initial slope of the curve, which corresponds to blood volume exchange frequency (42) (Fig. 1A). Relative blood volume was calculated as ratio of myocardial and cavity signal intensity (RBV = A/ALV). A/LV corresponds to signal intensity for LV cavity. MBF was estimated by the product RBV $\times$ $\beta$. MAP and HR were recorded using a Millar probe located in abdominal aorta, for double product (MAP $\times$ HR) measurement as a surrogate of cardiac work.

Experimental protocol. Following each surgery, mice were given a bolus injection of the ganglionic blocker hexamethonium (5 mg/kg; Sigma, St. Louis, MO) to eliminate reflex adjustments and focus on the primary actions of capsaicin. Initial studies were performed to determine the effects of continuous infusion of capsaicin (1–100 $\mu$g·kg$^{-1}$·min$^{-1}$) administered in an escalating fashion at the rate of 20 $\mu$l/min for 4 min. The vehicle was 10% ethanol in saline, which had no effect on MAP or MBF. Hemodynamic response curves were performed in control, db/db, and TRPV1$^{-/-}$ mice, before and after inhibition of TRPV1 channels (capsazepine: 10 mg/kg iv; Sigma, via bolus injection) and endothelial nitric oxide synthase (eNOS) [N-nitro-L-arginine methyl ester (L-NAME); 100 mg/kg iv, via bolus injection]. Five to ten minutes elapsed following each inhibitor before capsaicin infusion began to allow for MAP to stabilize. Pressures and/or HR were continuously recorded throughout the experiment.

Isolated coronary microvessel reactivity studies. Mice were anesthetized, and their hearts were excised and placed in ice-cold physiologic salt solution. Mouse coronary microvessel arterioles were dissected free from ventricular wall tissue in buffer containing the following (in mM): 145 NaCl, 5.0 KCl, 2.5 CaCl$_2$, 1.17 MgSO$_4$, 25.0 NaHCO$_3$, and 10 glucose, pH 7.4. Microvessels were cannulated with

### Table 1. Parameters

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>db/db</th>
<th>TRPV1 KO</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body weight, g</td>
<td>28.1 ± 0.8</td>
<td>29.5 ± 1.2</td>
<td>28.4 ± 0.8</td>
</tr>
<tr>
<td>Fasting glucose, mg/dl</td>
<td>85.3 ± 3.8</td>
<td>95.2 ± 17.4</td>
<td>88.8 ± 6.2</td>
</tr>
<tr>
<td>Basal HR, beats/min</td>
<td>418 ± 22</td>
<td>458 ± 20*</td>
<td>460 ± 14*</td>
</tr>
<tr>
<td>MAP, mmHg</td>
<td>72.5 ± 2.9</td>
<td>89.0 ± 2.7*</td>
<td>78.1 ± 1.9</td>
</tr>
<tr>
<td>Hex MAP, mmHg</td>
<td>66.0 ± 3.8</td>
<td>85.0 ± 3.6</td>
<td>77 ± 1.3</td>
</tr>
<tr>
<td>Cap 1 MAP, mmHg</td>
<td>72.8 ± 4.1</td>
<td>85.7 ± 2.7</td>
<td>77 ± 0.9</td>
</tr>
<tr>
<td>Cap 10 MAP, mmHg</td>
<td>74.0 ± 4.7</td>
<td>83.5 ± 2.0</td>
<td>76 ± 1.1</td>
</tr>
<tr>
<td>Cap 20 MAP, mmHg</td>
<td>78.8 ± 5.1</td>
<td>85.8 ± 4.1</td>
<td>77 ± 0.7</td>
</tr>
<tr>
<td>Cap 100 MAP, mmHg</td>
<td>97.5 ± 3.5</td>
<td>93 ± 5.0</td>
<td>76 ± 1.3</td>
</tr>
<tr>
<td>Myocardial pH</td>
<td>7.38 ± 0.08</td>
<td>7.14 ± 0.11*</td>
<td>7.8 ± 0.2</td>
</tr>
<tr>
<td>Baseline diameter, $\mu$m</td>
<td>93.9 ± 2.3</td>
<td>98.8 ± 5.5</td>
<td>68.8 ± 10.6</td>
</tr>
<tr>
<td>Diameter after U46619, $\mu$m</td>
<td>40.1 ± 4.7</td>
<td>37.1 ± 3.6</td>
<td>26.4 ± 5.0</td>
</tr>
<tr>
<td>Cap 1 HR, beats/min</td>
<td>422 ± 15</td>
<td>447 ± 21</td>
<td>448 ± 30</td>
</tr>
<tr>
<td>Cap 10 HR, beats/min</td>
<td>397 ± 16</td>
<td>445 ± 20</td>
<td>458 ± 12</td>
</tr>
<tr>
<td>Cap 20 HR, beats/min</td>
<td>402 ± 18</td>
<td>433 ± 18</td>
<td>465 ± 10</td>
</tr>
<tr>
<td>Cap 100 HR, beats/min</td>
<td>440 ± 18</td>
<td>441 ± 12</td>
<td>455 ± 21</td>
</tr>
<tr>
<td>Ethanol vehicle change MAP, mmHg</td>
<td>-2.2 ± 1.6</td>
<td>-2.9 ± 1.2</td>
<td>-1.8 ± 2.1</td>
</tr>
<tr>
<td>Ethanol vehicle change HR, beats/min</td>
<td>7.8 ± 5.7</td>
<td>8.2 ± 4.9</td>
<td>8.7 ± 6.2</td>
</tr>
</tbody>
</table>

Values are means ± SE. TRPV1, transient receptor potential vanilloid 1; KO, knockout; MAP, mean arterial pressure; HR heart rate; Hex, hexamethonium; Cap, capsaicin. *Significance from control.
glass pipettes and secured with silk suture in a temperature-controlled chamber (Danish Myotech, DMT, Atlanta, GA). The chamber was transferred to the stage of an inverted microscope outfitted with a video camera, and edge detection analyzer. Arterioles were pressurized to 60 mm Hg and warmed to 37°C. Vessel viability was tested using 60 mM KCl. Vessels were contracted with the thromboxane mimetic U46619 (1 μM), and subsequent capsaicin-mediated relaxation was determined in the presence and absence of TRPV1, eNOS, and BK channel inhibition. Vascular reactivity studies were also examined in response to changes in pH by addition of calculated concentrations of 1 N HCl to the bath volume to achieve the desired pH. Similar to above, vessels were contracted with U46619 and relaxation was determined in the presence and absence of TRPV1 inhibition.

Endothelium disruption. The endothelium was disabled in all coronary arteriole experiments by passing ~1 ml of air through the lumen. Disruption of the endothelium was assessed by exposing U46619 (1 μM)-contracted arterioles to acetylcholine (ACh; 1 μM). Only arterioles where ACh-mediated vasodilation was eradicated to 60 mmHg and warmed to 37°C. Vessel viability was tested using 60 mM KCl. Vessels were contracted with the thromboxane mimetic U46619 (1 μM), and subsequent capsaicin-mediated relaxation was determined in the presence and absence of TRPV1, eNOS, and BK channel inhibition. Vascular reactivity studies were also examined in response to changes in pH by addition of calculated concentrations of 1 N HCl to the bath volume to achieve the desired pH. Similar to above, vessels were contracted with U46619 and relaxation was determined in the presence and absence of TRPV1 inhibition.

Immunoblot analysis of myocardial TRPV1. TRPV1 Western blotting was performed as described previously (31). GAPDH staining was utilized as a loading control.

Myocardial blood pH measurements. Left ventricles of the heart were carefully punctured and ~400 μl of blood were collected into a heparinized syringe. Then, ~200 μl of blood were transferred to an i-STAT cartridge (Abbott Labs), which was placed into an i-STAT handheld clinical analyzer (Abbott Labs) to allow for measurement of myocardial blood pH.

Drugs. All drugs were purchased from Sigma Chemicals unless otherwise stated. Initial in vivo studies were generated using capsazepine. However, recent cellular studies in our laboratory were performed using both capsazepine and SB366791. SB366791 proved better at inhibiting pH-induced TRPV1 events in our cellular experiments. Further examination revealed that SB366791 has an IC50 of ~10 nM as determined in electrophysiological assays. Thus SB366791 is ~10-fold more potent than the competitive antagonist capsazepine, which has an IC50 of 0.2–5 μM against capsaicin (18).

In addition, at high concentrations, capsaizepine is thought to interact with other ion channels (8, 16). Thus to potentially avoid any conflicting results, we choose to use the more potent and specific TRPV1 antagonist SB366791. Capsaicin, capsazepine, SB366791, iberiotoxin, and Penitrem A were dissolved in stock solutions of ethanol. Hexamethonium (5 mg/ml) stock solution was made up in saline.

Statistics. The mean of HR and MAP at 30 s before the infusion of drugs were taken as baseline values. The largest value after drug infusion was taken as the maximum (HR or MAP). The percentage of maximal change of HR and MAP was calculated using the formula: percent change (% change) = (maximum − baseline)/baseline × 100. Data are expressed as means ± SE. Statistical comparisons were made with paired t-tests or two-way repeated-measures ANOVA (with Student-Newman-Keuls multiple comparison) as appropriate. For statistical analyses, GraphPad Prism 4.0 software for Windows XP (GraphPad Software, San Diego, CA) was utilized, and calculation of percentage change was performed using Excel 2.007 (Microsoft, Redmond, WA). In all tests, P < 0.05 was considered statistically significant.

RESULTS

TRPV1 channels directly regulate MBF. Body weight was significantly higher in db/db mice and as expected, and db/db mice displayed a significantly higher fasting blood glucose concentration (see Table 1). Capsaicin (1–100 μg·kg−1·min−1) increased MBF in control mice. Pretreatment with capsaizepine (5 mg/kg), a TRPV1 channel antagonist, significantly attenuated capsaizepine-induced increases in MBF in control mice (Fig. 1B), confirming that the response to capsaizepine is specifically mediated through TRPV1 channels. To directly examine the role of TRPV1 channels in the regulation of MBF, we administered capsaizepine to TRPV1−/− mice. TRPV1−/− mice ex-
hibited no changes in MBF in response to capsaicin (Fig. 1B). Importantly, neither ethanol nor capsaicin had an influence on HR, suggesting that TRPV1 channels exclusively act on blood vessels rather than heart itself (Table 1). Further examination of the capsaicin-mediated response revealed eNOS plays a significant role as inhibition of eNOS with L-NAME (100 μM/kg bolus dose, a global inhibitor of all NOS), inhibited the capsaicin increase in MBF (Fig. 1C).

Capsaicin-induced increases in MBF are attenuated in db/db mice. We next evaluated if TRPV1 channel-mediated hemodynamic effects are altered in db/db mice. Capsaicin-mediated increase in MBF was attenuated in db/db mice compared with controls, with an increase in MBF only to 10 μg/kg capsaicin (Fig. 2A). The TRPV1 antagonist capsazepine had no effect in db/db mice, except at the 10 μg/kg dose (Fig. 2B). No changes in HR were observed in response to capsaicin administration. Finally, when plotting MBF against the double product (HR × MAP) for both control and db/db mice, we observed that coupling of MBF to double product is rightward- and downward-shifted in the db/db mice compared with control mice. (Fig. 2C).

Capsaicin mediated relaxation in coronary microvessels involves eNOS and BK channels. To further evaluate the capsaicin-mediated effect, coronary microvessels were dissected from hearts of control mice and vasoreactivity studies were performed. Capsaicin dose dependently relaxed vessels contracted to U46619 (1 μM; Fig. 3B). This relaxation was abolished by preincubation with the TRPV1 inhibitor SB366791 (10 μM; Fig. 3B). The capsaicin-mediated relaxation is largely due to endothelial-dependent NO production as endothelium removal or incubation with L-NAME (100 μM) inhibited capsaicin-induced relaxation in vessels obtained from control mice (Fig. 3C). Interestingly, the combination of eNOS and cyclooxygenase inhibition together did not significantly further attenuate the response to capsaicin (data not shown). Furthermore, incubation with the BK channel antagonist Penitrem A (10 μM) or iberiotoxin (100 nM) also largely blocked the capsaicin mediated relaxation (Fig. 3D).

Attenuated capsaicin-mediated relaxation in coronary microvessels from db/db mice. Capsaicin dose dependently relaxed vessels contracted to U46619 (1 μM) in db/db mice; yet similar to in vivo responses, capsaicin-mediated relaxation was blunted compared with controls (Fig. 4A). Inhibition of TRPV1 (SB366791), eNOS (L-NAME), and BK channels (Penitrem A) had little or no effect on capsaicin-mediated relaxation of coronary microvessels from db/db mice (Fig. 4, B-D, respectively).

Acidic environment and altered pH-mediated relaxation in coronary microvessels from db/db mice. TRPV1 channels are known to be activated by a change in pH. To determine if an altered pH environment partially explains the attenuated capsaicin-mediated responses in db/db mice, vasoreactivity responses to changes in pH were measured in vessels precontracted with U46619. Changes in pH (pH 7.4—6.0) relaxed coronary vessels contracted to U46619 (1 μM) in all three lines of mice, however, pH-mediated relaxation was blunted compared to controls. Inhibition of TRPV1 (SB366791), eNOS (L-NAME), and BK channels (Penitrem A) had little or no effect on capsaicin-mediated relaxation of coronary microvessels from db/db mice compared with controls.
TRPV1 expression is altered in db/db mice. To determine if altered functional expression of the TRPV1 channels correlates with the attenuated hemodynamic responses exhibited in db/db mice, cardiac TRPV1 protein expression was examined. Western blot analysis of whole heart tissue homogenates of mice revealed a ∼35% decrease in TRPV1 protein expression in db/db mice compared with controls (Fig. 6, A and B).

DISCUSSION

This study was designed to delineate the mechanisms by which TRPV1 channels regulate hemodynamic responses while coupling metabolism to coronary blood flow in control and diabetic mice. Our major findings include the following: 1) capsaicin-induced increases in myocardial blood flow are inhibited by NOS inhibition in control mice and are markedly attenuated in db/db mice; 2) coupling of metabolism to MBF is disrupted in db/db mice; 3) robust TRPV1-mediated relaxation in control mice occurs through eNOS and BK channels; 4) TRPV1-mediated relaxations are blunted in db/db mice; 5) pH-mediated relaxation in coronary microvessels is attenuated in TRPV1<sup>(−/−)</sup> and db/db mice; and 6) blunted TRPV1-induced responses correlate with diminished TRPV1 protein expression. This study provides direct evidence that TRPV1 functions in the coronary circulation to regulate blood flow responses. Additionally, impaired TRPV1-induced vasodilation in type II diabetes is associated with decreased myocardial expression of TRPV1 protein and perhaps involves pH-mediated alterations in TRPV1 channel activity.

The heart is highly dependent on a continuous supply of oxygen from the coronary circulation to meet its metabolic requirements (39), which if not met diminish cardiac function (20, 21). Thus tight control of coronary blood flow is essential to maintain myocardial performance. Our data clearly demonstrate an altered coupling of blood flow to metabolism in hearts from db/db mice that may be due, in part, to lack/loss of functional myocardial TRPV1 channels.

Abnormalities of the coronary microcirculation distinct from large-vessel atherosclerosis have been reported in clinical and experimental diabetes mellitus (11). Morphological (37, 44) and functional abnormalities (9, 28, 29) in the coronary circulation of diabetic patients and animals have also been reported. However, coronary microvascular structural abnormalities in diabetes have not been universally observed (36). The current study examined whether coronary dysfunction in diabetes is coupled to impaired contribution of coronary TRPV1 channels. TRPV1-mediated changes in blood flow in various vascular beds have previously been demonstrated (24). In contrast, capsaicin had no effect on blood flow in TRPV1<sup>(−/−)</sup> mice (34). In our study, capsaicin infusion induced a significant concentration-dependent hemodynamic effect in control mice that was attenuated by capsazepine, confirming a role for TRPV1 channels in the regulation of coronary blood flow. A similar effect was seen in db/db mice, albeit depressed compared with controls.

The mechanisms underlying this dysfunction have not been clearly defined but could be related to a variety of pathophysiological conditions associated with type II diabetes including microvascular changes (12, 44). TRPV1 channel involvement in vascular tone regulation has previously been documented; however, limited knowledge exists of the mechanisms involved in the regulation of vascular tone observed following TRPV1 stimulation. TRPV1 involvement in endothelial-dependent vasodilation to capsaicin has been demonstrated (1, 3, 10, 19). Activation of TRPV1 channels induces vasodilation in a variety of vascular beds, via release of known vasoactive factors: calcitonin gene-related peptide, substance P (13, 40),...
and NO (32). Our data indicate TRPV1-induced vasodilation may be a mechanism involved in hemodynamic regulation under physiological/pathophysiological conditions. We found that capsaicin-induced coronary vasodilation is NO dependent (Figs. 1 and 3). In the present study, both endothelial denuding and eNOS inhibition attenuated TRPV1-mediated changes in MBF and microvessel relaxation in control mice, with no effect on either parameter in db/db mice.

The reduced metabolic coronary vasodilation in diabetes may be related to coronary endothelial dysfunction, as impaired endothelium-dependent vasodilation has been described in several animal models of diabetes (14, 38) and in humans (23). Our data suggest an impaired contribution of endothelial-dependent vasodilation and dysfunction of TRPV1 channels in db/db mice as L-NAME had no effect on TRPV1-mediated changes in MBF and little effect on capsaicin-mediated relaxation in isolated vessels. Although our studies did not assess the role of cyclooxygenase products, they may contribute to the present findings. Importantly, cyclooxygenase inhibition may lead to increased arachidonic acid metabolism via lipoxigenase and cytochrome P-450 (CYP) pathways as their products have been shown to directly activate TRPV1 channels (5, 33).

Further studies are needed to investigate a role for both pathways in our TRPV1-induced relaxation. Contribution of other vasoactive factors such as endothelium-derived hyperpolarizing factor, PGI₂, or even calcitonin gene-related peptide and substance P cannot be ruled out and need to be examined further.

Previous studies have demonstrated a link between several K⁺ and TRPV channels in vascular tone regulation (4). Similarly, Fujimoto et al. (15) reported capsaicin-mediated relaxation of guinea pig ileum via voltage-dependent K⁺ channels. The current study examines the role of BK channels in the vasorelaxant response to capsaicin. In our study, we found that both iberiotoxin and Penetrim A administration attenuated capsaicin-induced vasorelaxation responses in control and db/db mice.

It is well documented that TRPV1 acts as a molecular integrator of several noxious stimuli and thus plays a key role in sensing tissue injury, inflammation and vascular functions (6). Cardiac metabolism is altered in diabetes such that these changes may alter intracellular pH levels seen in diabetic hearts (25, 26). Local changes in pH in diabetic cardiomyopathy may contribute to dysfunctional TRPV1 signaling, perhaps via a pH-induced TRPV1 desensitization. Support for pH-dependent TRPV1 regulation of blood flow has been previously reported (2, 22). Our data demonstrate a portion of the pH-mediated relaxation in isolated coronary microvessels is due, in part, to activation of TRPV1 channels. As such, altered activation of TRPV1 to local pH changes during increased metabolic demand could account for the blunted responses seen in diabetic animals. In support of this, myocardial pH was more acidic in db/db mice compared with controls. Although not directly tested, the prolonged acidic environment may lead to TRPV1 desensitization and the resulting perfusion impairments. Further studies are needed to investigate the potential role a prolonged acidic environment has on TRPV1-mediated desensitization.

Decreased TRPV1 expression in diabetic animals has been previously reported (7). However, the impact of decreased TRPV1 channel expression on the diabetic heart has not been thoroughly investigated. Confirming previous studies from our laboratory (31), we found TRPV1 expression in diabetic mouse hearts is markedly reduced compared with nondiabetic hearts, thus providing a potential mechanism to explain at least in part, the attenuated responses in db/db mice.

In summary, we report TRPV1 activation leads to vascular regulation of hemodynamic responses. We propose that TRPV1 has a potential physiological/pharmacological role that represents a promising new therapeutic strategy to control tissue-specific blood distribution. Our findings support the hypothesis that vascular dysfunction in diabetes is related to decreased functional myocardial TRPV1 channel expression. Diminished TRPV1 channel function is accompanied by decreased capsaicin-mediated vasoreactivity and altered hemodynamic regulation. We assert that this impairment of TRPV1 channels could contribute to vascular dysfunction typically observed in diabetes. We conclude that TRPV1 diminished functional expression and/or dysfunction may underlie, at least in part, the diabetic coronary microvascular complications often seen in patients and in animal models of diabetic cardiomyopathy.

ACKNOWLEDGMENTS

We thank Cheryl Hodnichak and the Echocardiography Core Facility for technical contributions for the in vivo studies.

DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author(s).

AUTHOR CONTRIBUTIONS


REFERENCES


