Mice lacking functional TRPV1 are protected from pressure overload cardiac hypertrophy

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Abbreviations: TRPV1, transient receptor potential vanilloid type 1

TRPV1 (transient receptor potential cation channel, subfamily V, member 1) is best studied in peripheral sensory neurons as a pain receptor; however TRPV1 is expressed in numerous tissues and cell types including those of the cardiovascular system. TRPV1 expression is upregulated in the hypertrophic heart, and the channel is positioned to receive stimulatory signals in the hypertrophic heart. We hypothesized that TRPV1 has a role in regulating cardiac hypertrophy. Using transverse aortic constriction to model pressure overload cardiac hypertrophy we show that mice lacking functional TRPV1, compared to wild type, have improved heart function, and reduced hypertrophic, fibrotic and apoptotic markers. This suggests that TRPV1 plays a role in the progression of cardiac hypertrophy, and presents a possible therapeutic target for the treatment of cardiac hypertrophy and heart failure.

Introduction

Although TRPV1 inhibition has not been studied in the context of cardiac hypertrophy, TRPV1 activation has been implicated in protection from myocardial ischemia reperfusion injury. In addition, the channel's endogenous ligand, anandamide, has been implicated in multiple cardiac diseases such as cardiotoxicity and hypertension. Therefore, TRPV1 could be a target for the treatment of cardiac disease, and clarifying the roles of TRPV1 would enable the use of the existing, well-characterized pharmacopeia of TRPV1 agonists and antagonists for cardiac and other diseases.

Cardiac hypertrophy is classically considered to be an adaptive and compensatory response that increases the work output of cardiomyocytes and thus maintains cardiac function despite increased load. In mice, cardiac hypertrophy is typically modeled using transverse aortic constriction (TAC) to induce acute pressure overload. The increased resistance created by aortic constriction initially compromises left ventricular (LV) function; the subsequent development of LV hypertrophy begins to restore systolic function in the two weeks following TAC. Concentric LV hypertrophy continues during weeks two to eleven post TAC, potentially doubling the LV mass compared to controls. A decline in LV function accompanies LV chamber dilation and myocardial fibrosis, and around half of TAC treated mice develop pulmonary congestion by week eleven. Thus, TAC is an effective stimulus for rapidly producing cardiac hypertrophy in an experimental setting. Although there are differences between the TAC model and clinical cardiac hypertrophy, such as the acute onset of hypertension in this model rather than the gradual onset in clinical...
cases, the TAC model provides tremendous utility for identifying important therapeutic targets in heart disease and exploring the effects of molecular or pharmacological inhibitors.35,36

We show here that loss of TRPV1 function in mice alters the responses of the heart to TAC-induced pressure overload. Our data suggest that TRPV1 contributes to cardiac hypertrophy, fibrosis, apoptosis and loss of contractile function in response to pressure overload. These results raise the possibility that TRPV1 antagonists currently used as anti-hyperalgesics could have anti-hypertrophic side effects, or could be repurposed as anti-hypertrophic agents.

**Results and Discussion**

To ascertain whether TRPV1 is involved in the progression of cardiac hypertrophy, mice lacking functional TRPV1 and control mice with wild-type TRPV1 were modeled for pressure overload cardiac hypertrophy. Heart dimensions and function were measured and compared over time using unanesthetized trans-thoracic echocardiography and hearts were harvested eight weeks later for molecular, biochemical and histological analysis. Heart dimensions and function were better preserved in mice lacking functional TRPV1. Cellular hypertrophy, markers for hypertrophy, fibrosis and apoptosis were also significantly reduced in these mice, indicating that TRPV1 may be involved in the progression of cardiac hypertrophy.

**Pressure overload model.** To investigate the involvement of TRPV1 in the remodeling associated with cardiac hypertrophy and heart failure, we subjected 10-week-old male B6.129X1-Trpv1tm1Jul mice (TRPV1 KO),35 and age/sex matched C57BL/6J (WT) control mice, to acute pressure overload by transverse aortic constriction (TAC). Sham operated control mice from both strains underwent an identical surgical procedure except for actual aortic constriction. TRPV1 KO TAC mice and WT TAC mice showed no difference in baseline pressures, assessed immediately distal to the TAC banding site by Doppler echocardiography (Table 1).

**Gravimetric analysis of the heart, after pressure overload cardiac hypertrophy.** Reveals that TAC treated hearts were 28% heavier in WT TAC mice than TRPV1 KO TAC mice (Table 1). When normalized to body weight and tibia length, the heart weight/body weight ratio and the heart weight/tibia length ratio were also significantly greater in WT TAC mice than TRPV1 KO TAC mice (Fig. 1A and B).

Heart structure and function are maintained during pressure overload cardiac hypertrophy in mice lacking functional TRPV1. End-diastolic left ventricular internal diameter (LVIDd) was analyzed for eight weeks following TAC by transthoracic echocardiographic analysis. In WT TAC mice, LVIDd began to increase at two weeks and plateaued at approximately six weeks, whereas TRPV1 KO TAC mice showed no change in LVIDd until six weeks (Fig. 1C). The rate of increase in LVIDd is significantly greater in WT TAC mice than in TRPV1 KO TAC mice (Fig. 1D) between weeks two and six post TAC.

Heart function was analyzed by left ventricular ejection fraction (%EF). Heart function declined in WT mice from approximately two to six weeks post TAC treatment, but was preserved in TRPV1 KO TAC mice over the same period of time (Fig. 1E). The change in ejection fraction at six weeks is significantly different between WT TAC mice and TRPV1 KO TAC mice (Fig. 1F).

Mice lacking functional TRPV1 are protected from hypertrophy and apoptosis after modeled pressure overload cardiac hypertrophy. The degree of cellular hypertrophy was examined by staining of the plasma membranes with fluorescently-labeled wheat germ agglutinin (WGA). Cell sizes were compared by imaging and computer aided measurement of the cross-sectional area of cardiomyocytes. This comparison reflects the degree of cellular hypertrophy between samples.38 The data show a significant increase in the cardiomycyte cross sectional area of WT TAC compared to TRPV1 KO TAC mice (Fig. 2A and B). This suggests that, at the cellular level, TRPV1 KO mice develop less cardiac hypertrophy than WT mice, in response to modeled pressure overload cardiac hypertrophy.

To further evaluate and compare the degree of hypertrophy between TRPV1 and WT mice, additional markers of hypertrophy, apoptosis and heart failure were assessed. Plasma concentrations of the circulating hormone atrial natriuretic peptide (ANP) and the growth factor TGFbeta increase during heart failure and are considered late markers of cardiac hypertrophy.39,40 Therefore, expression of ANP and TGFbeta was analyzed by RT-PCR of mRNA isolated from heart tissue. We observed significantly greater increases in ANP and TGFbeta transcript levels in WT TAC mice than in TRPV1 KO TAC mice (Fig. 2C). Western blot analysis confirmed that there was a significant increase in ANP protein expression in TAC WT mice compared to TAC TRPV1 KO mice (Fig. 2D). These data suggest that protection from the stress and or signaling systems associated with the hypertrophic transcriptional responses is observed in the TRPV1 KO mice.

Apoptosis plays a critical role in the progression of cardiac hypertrophy to heart failure. Cardiomyocytes are non-dividing cells, so apoptosis and death of theses cells leads to decreased

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**Table 1. Doppler echocardiography and gravimetric analysis**

|          | n   | Peak Pressure (mmHg) | SD  | Body weight (g) | SD   | Tibia length (mm) | SD   | Heart weight (mg) | SD  |
|----------|-----|----------------------|-----|----------------|------|-------------------|------|------------------|-----|
| WT-mice Sham surgery | 4   | 3.5                  | 0.11| 27.3           | 2.31 | 19.5              | 0.58 | 144.7            | 7.27|
| WT-mice TAC surgery  | 6   | 59.3                 | 7.86| 26.2           | 0.54 | 18.9              | 0.20 | 241.6*           | 76.1|
| TRPV1 KO sham surgery | 5   | 2.72                 | 0.32| 26.8           | 3.31 | 19.6              | 0.57 | 142.0            | 30.4|
| TRPV1 KO TAC surgery | 8   | 53.7                 | 13.5| 27.4           | 2.8  | 19.7              | 0.61 | 174.7            | 32.8|

Summary of Doppler echocardiography and gravimetric analysis of sham and TAC treated WT and TRPV1 KO mice. Heart weights of TAC treated WT mice are significantly larger than TAC treated TRPV1 KO mice, after eight weeks of treatment. (n = 8,6, p = 0.044).
numbers of cardiomyocytes, which are replaced by fibrous tissues. This causes the workload on the remaining cardiomyocytes to increase and consequently induces compensatory cardiac hypertrophy.\(^1\) Here we assessed the degree of cellular apoptosis by measurement of cleaved caspase-3 protein in heart tissue lysates from TAC and sham treated WT and TRPV1 KO mice. Analysis of western blot densitometry of heart tissue lysates showed significantly less caspase-3 cleavage in TRPV1 KO TAC mice than in WT TAC mice (Fig. 2E, bottom). As expected, WT sham and TRPV1 KO sham mice showed no apparent caspase-3 cleavage (Fig. 2E, top). These results indicate that TAC-induced cardiac apoptosis is reduced in TRPV1 KO mice. Again, indicating that
Figure 2. Mice lacking functional TRPV1 present with less hypertrophy and apoptosis than WT control mice, eight weeks post TAC treatment. (A) WT and TRPV1 KO mice have smaller cardiac myocytes and less hypertrophy than TAC WT control mice. (A) Staining of plasma membranes (Wheat germ agglutinin-Alexa488) in heart tissue sections, TAC treated WT (left) and TAC treated TRPV1 KO (right) mice. Scale bar = 20 μm. (B) Measurement of cardiomyocyte cross sectional area was significantly different between TAC WT mice and TAC TRPV1 KO mice (p = 0.025, n = 100), eight weeks post TAC treatment. (C) Expression levels of atrial natriuretic peptide (ANP) and TGFbeta transcripts were significantly greater in TAC WT mice than in TRPV1 KO mice (p = 0.037, p = 0.007) relative to control mice. (D) Western Blot analysis of Prepro-ANP and pro-ANP indicates significantly more Prepro-ANP and pro-ANP protein in heart tissue protein lysates from TAC WT mice (p = 0.016, n = 3) than TAC TRPV1 KO mice (n = 3). Graph indicates integrated density normalized to GAPDH loading control. (E) Western blot analysis of cleaved Caspase-3 indicates significantly more cleaved Caspase-3 protein in heart tissue protein lysates from TAC WT mice (p = 0.001, n = 3) than TAC TRPV1 KO mice (n = 3). Graph indicates integrated density normalized to GAPDH loading control.
there exists some protection from the stress and or signaling associated with cardiac hypertrophy in the TRPV1 KO mice.

Mice lacking functional TRPV1 have reduced fibrosis, tissue remodeling and inflammatory markers after modeled pressure overload cardiac hypertrophy. During the development of ventricular hypertrophy, the composition of cardiac tissue changes, leading to structural remodeling of the myocardium. For example, the disruption of the equilibrium between the synthesis and degradation of collagen results in an excessive accumulation of collagen type I and III fibers within the myocardium. As collagen and other extracellular matrix components accumulate in the interstitial space, myocardial stiffness increases, and diastolic and systolic dysfunction occurs. We analyzed Collagen III levels in hearts from WT TAC mice compared to hearts from TRPV1 KO TAC mice. Our data show that the functional knockout of TRPV1 in mice allows for the preservation of heart structure and heart function under modeled pressure overload. Concomitant with this protection is the downregulation of multiple protein and transcriptional markers associated with initiation and the progression of hypertrophy, apoptosis, fibrosis and heart failure. This data suggest that TRPV1 may play a role as either an initiating stressor, or an upstream signaling transducer of the hypertrophic transcriptional response in the heart.

Multiple factors regulate the progression of hypertrophy and heart failure, including modulation of the second messenger calcium. The involvement of calcium signaling in heart failure is critical; in addition to a stimulatory role in myocyte contraction, calcium also plays a role in the regulation of hypertrophic gene expression and apoptosis. Any changes in the rate or amplitude of calcium influx, release or cycling disrupt the balance of phospho-signaling events, and vice versa. Activation and influx of calcium through TRPV1 could initiate or potentiate, the degradation of the extracellular matrix and in the generation of vasoactive peptides. In the heart and blood vessels, it is CMA1, rather than angiotensin converting enzyme (ACE), that is largely responsible for converting angiotensin I to the vasoactive peptide angiotensin II. Our data show that CMA1 transcripts and protein (Fig. 3E and F) are expressed at significantly higher levels in hearts from WT TAC mice than TRPV1 KO TAC mice.

Our data show that the functional knockout of TRPV1 in mice allows for the preservation of heart structure and heart function under modeled pressure overload. Concomitant with this protection is the downregulation of multiple protein and transcriptional markers associated with initiation and the progression of hypertrophy, apoptosis, fibrosis and heart failure. This data suggest that TRPV1 may play a role as either an initiating stressor, or an upstream signaling transducer of the hypertrophic transcriptional response in the heart.

Mast cell chymase, CMA1, is a chymotryptic serine proteinase that belongs to the peptidase family S1. It functions in the regulation of the enzymes responsible for collagen degradation, the matrix metalloproteinases (MMPs). There was a significant increase in MMP2 transcripts in hearts from WT TAC mice compared to hearts from TRPV1 TAC mice (Fig. 3D).
downstream signaling cascades and subsequent transcriptional programs associated with the progression of cardiac hypertrophy, fibrosis, apoptosis and heart failure. Hypertrophic transcriptional programs are known to be induced downstream of multiple interdependent signaling pathways, engaged after receptor stimulation or by stretch induction. Signaling components such as PKC, 46-48 PI3K, 46,49 PKA, 50 and calcium, 46 through calcium/calmodulin-dependent kinase II alpha (CaMKIIalpha), 54,46,51-55 are known to initiate, potentiate or regulate TRPV1 activation.

There are multiple candidates for cell types in which TRPV1 could play a role in cardiac homeostasis and systemic blood pressure regulation. The major TRPV1 containing effector cell types may include innervating neurons, cardiomyocytes and mast cells. Knockout of TRPV1 in any of these could perhaps be independently responsible for the results we observe. Full analysis of expression patterns for TRPV1 in the heart is lacking, and a necessary next step towards attribution of the effect that we observe. The last of these, mast cells, have been subject to phenotypic analysis for a role in the heart. Mast cells express TRPV1 under certain conditions, and mast cell deficiency shows an intriguingly necessary next step towards attribution of the effect that we observe. The major TRPV1 containing effector cell types may include innervating neurons, cardiomyocytes and mast cells. Knockout of TRPV1 in any of these could perhaps be independently responsible for the results we observe. Full analysis of expression patterns for TRPV1 in the heart is lacking, and a necessary next step towards attribution of the effect that we observe. The last of these, mast cells, have been subject to phenotypic analysis for a role in the heart. Mast cells express TRPV1 under certain conditions, and mast cell deficiency shows an intriguingly necessary next step towards attribution of the effect that we observe. The major TRPV1 containing effector cell types may include innervating neurons, cardiomyocytes and mast cells.

**Methods**

**Animal care.** All animal procedures were approved by the Institutional Animal Care and Use Committee at University of Hawaii.

**Animals.** Mice lacking functional TRPV1, B6.129X1-Trpv1tm1Jul/J (Jackson Labs, ME) were genotyped by PCR. 37 In this strain the Trpv1 gene is disrupted by deletion of the exon encoding part of the fifth and all of the sixth putative transmembrane domains of the channel, together with the intervening pore-loop region and leads to a loss of TRPV1 function. 37 C57BL/6J were used as WT control animals. Mice were housed under a 12-h light/dark cycle and fed with standard diet and water ad libitum. Ten-week-old male mice were used for all experiments. Eight weeks after surgery mice were euthanized by CO2 asphyxiation for histological and molecular analysis.

**Transverse aortic constriction (TAC).** Transverse aortic constriction was performed as described by Rockman, producing left ventricular hypertrophy by constriction of the aorta. 46 The left side of the chest was deplated with Nair and a baseline 2-D echocardiogram was obtained as described below. Mice were then deeply anesthetized with a mixture of ketamine and xylazine. The transverse aorta between the brachiocephalic and left carotid artery was banded using 6-0 silk ligature around the vessel and a 26G blunt needle, after which the needle was withdrawn. Sham surgeries were identical apart from the constriction of the aorta.

**Doppler echocardiography.** Doppler echocardiography was performed one week post TAC to measure the level of constriction. Mice were anesthetized lightly with isoﬂuorane gas and shaved. Doppler was performed using the Visualsonics Vevo 770 system. In the parasternal short-axis view, the pulsed wave Doppler sample volume was placed in the transverse aorta just proximal and distal to the site of banding. Peak velocity was traced using Vevo.770 software, and the pressure gradient was calculated using the simplified Bernoulli equation.

**Transthoracic echocardiography.** Baseline and post TAC transthoracic echocardiography were used to assess changes in mouse heart dimensions and function. Briefly, after 2 d of acclimatization and depilation, unanesthetized transthoracic echocardiography was performed using a 30-Mhz transducer (Vevo 770, VisualSonics). High quality two-dimensional images and M-mode images of the left ventricle were recorded. Measurements of left ventricular end-diastolic (LVIDd) and end-systolic (LVIDs) internal dimensions were performed by the leading_edge-to-leading_edge convention adopted by the American Society of Echocardiography. The left ventricular ejection fraction (%EF) was calculated as: (LV Vol; d-LV Vol; s/LV Vol; d x 100) (Visualsonics Inc.).

**Tissue preparation for histology.** Eight weeks post TAC, mice were euthanized by CO2 asphyxiation and hearts were collected for histological and molecular analysis. For histology, hearts were perfused with phosphate-buffered saline and 10% formalin in situ, collected immediately and fixed overnight in 10% formalin at 4°C. Tissues were then cut in a sagittal orientation, embedded in paraffin, mounted on glass slides and stored until use. Paraffin-embedded sections were stained for the following:

- **Collagen.** Collagen volume fraction was determined by analysis of picrosirius stained sections. Sections cut to 5 μm thickness were deparaffinized, stained with Weigert’s haematoxylin, and then stained with picrosirius red (0.1% Sirius Red in picric acid). Sections were subsequently washed and dehydrated before image analysis.

- **Cardiomyocyte cross sectional area.** Heart sections were deparaffinized and permeabilized, then stained with wheat germ-agglutinin conjugated to Alexa488 (WGA-Alexa488, Invitrogen, W11261) at a concentration of 50 μg/mL to identify sarcolemmal.
membranes and measure cardiomyocyte cross sectional area (described below).

**Image collection and analysis.** Fluorescent and bright field images were collected on an epifluorescence-microscope (Axioscope, Zeiss). Fibrosis and cross-sectional cardiomyocyte area were quantified using ImageJ software (NIH). To quantify fibrosis, collagen fibers were highlighted, and the red-stained pixels were counted to determine the percentage of pixels in each field that represented collagen fibers. Perivascular tissue was excluded from this calculation. Three heart sections from each animal were imaged at five images per heart. Images were averaged for each animal and graphed in Prism GraphPad. Cardiomyocytes from WGA stained sections were randomly selected in a blinded fashion then traced to determine the cross sectional area of individual myocytes (n = 100).

All images were captured and analyzed in a single-blind manner, except for WGA staining, which was analyzed in a double-blind manner.

**RT-PCR.** For RNA extraction, hearts were collected from mice and total RNA was isolated from homogenized hearts in a blind manner. Total RNA was isolated from individual myocytes (n = 100).

For RNA extraction, hearts were collected from WGA stained sections were randomly selected in a blinded manner, except for WGA staining, which was analyzed in a double-blind manner.

Western blot analysis. Western blot extracts were prepared from homogenized heart extracts were prepared from homogenized heart tissue using Western blot analysis. Western blot extracts were prepared from homogenized heart tissue using IGEPAL. Total protein concentrations were determined by the bicinchoninic acid (BCA) colorimetric assay. Absorbance was measured at 562 nm by spectrophotometer (Spectra Max 340), and concentrations determined using a standard curve based on bovine serum albumin (BSA) protein standards. Concentrations were normalized to 30 μg, and samples were separated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE). Protein samples were transferred to polyvinylidene fluoride (PVDF, Millipore, IPFL00010) membrane at 1.4 amps for 3.5 h. Membranes were probed overnight at 4°C with antibodies to cleaved Caspase-3 (Cell Signaling, 9661S), CMA1 (Gene Tex, GTX72388), “ANP (Santa Cruz) and GAPDH (Calbiochem, CB1001). The membranes were visualized with ECL substrate (GE Healthcare, RPN2132) and film. Western blot band intensity was quantified as integrated density by densitometry and normalized to the density of loading control.

**Statistics.** Statistical significance of echocardiography data was evaluated using two-way ANOVA, with a Bonferroni post hoc test and linear regression. Histology and molecular data were evaluated using the two-tailed Student’s t-test. Evaluations were performed using PRISM software (La Jolla, CA) with p < 0.05 regarded as significant. All data are shown as mean ± SD.

**Disclosure of Potential Conflicts of Interest**

No potential conflicts of interest were disclosed.

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**References**

1. Caterina MJ, Schumacher MA, Tominaga M, Rosen TA, Levine JD, Julius D. The capsaicin receptor: a heat-activated ion channel in the pain pathway. Nature 1997; 389:816-24.
2. Sun H, Li DP, Chen SR, Hirtelman WN, Pan HL. Sensing of blood pressure increase by transient receptor potential vanilloid 1 receptors on baroreceptors. J Pharmacol Exp Ther 2009; 331:851-9.
3. Cui M, Honore P, Zhong C, Guavin D, Mikusa J, Hernandez G, et al. TRPV1 receptors in the CNS play a key role in broad-spectrum analgesia of TRPV1 antagonists. J Neurosci 2006; 26:9383-5.
4. Huang SM, Bisogno T, Trevisani M, Al-Hayani A, De Petrocelli L, Femia F, et al. An endogenous capsaicin-like substance with high potency at recombinant and native vanilloid VR1 receptors. Proc Natl Acad Sci USA 2002; 99:8400-5.
5. Olah Z, Karai I, Iadarola MJ. Anandamide Activates Vanilloid receptor 1 (VR1) at acidic pH in dorsal root ganglia neurons and cells ectopically expressing VR1. J Biol Chem 2001; 276:31163-70.
6. Dhaka U, Uzzell V, Dubin AE, Mathur J, Perus M, Bandell M, Patapoutian A. TRPV1 is activated by both acidic and basic pH. J Neurosci 2009; 29:153-8.
7. Bhave G, Hu HJ, Glauer KS, Zhu W, Wang H, Brasier DJ, et al. Protein kinase C phosphorylation sensitizes but does not activate the capsaicin receptor transient receptor potential vanilloid 1 (TRPV1). Proc Natl Acad Sci USA 2003; 100:12480-5.
8. Smuder M, McNaughton PA. Modulation of single-channel properties of TRPV1 by phosphorylation. J Physiol 2011; 588:7343-56.
9. Santhi P, Jenes A, Somogyi C, Nagy I. The endogenous cannabinoid anandamide inhibits transient receptor potential vanilloid type 1 receptor-mediated currents in rat cultured primary sensory neurons. Acta Physiol Hung 2010; 97:149-58.
10. Baron R. Neuropathic pain: a clinical perspective. Handb Exp Pharmacol 2009; 3-30.
11. Cortright DN, Szallasi A. TRP channels and pain. Curr Pharm Des 2009; 15:1736-49.
12. Chizh BA, O’Donnell MB, Napolitano A, Wang J, Brooke AC, Aylott MC, et al. The effects of the TRPV1 antagonist SB-705498 on TRPV1 receptor-mediated activity and inflammatory hyperalgesia in humans. Pain 2007; 132:132-41.
13. Gunthorpe MJ, Chizh BA. Clinical development of TRPV1 antagonists: targeting a pivotal point in the pain pathway. Drug Discov Today 2009; 14:56-67.
14. Szallasi A, Cruz F, Cepeppetti P. TRPV1: a therapeutic target for novel analgesic drugs? Trends Mol Med 2006; 12:545-54.
15. Thilo F, Liu Y, Schulz N, Gergs U, Neumann J, Lodenkemper C, et al. Increased transient receptor potential vanilloid type 1 (TRPV1) channel expression in hypertrophic heart. Biochem Biophys Res Commun 2010; 401:98-103.
16. Basu S, Srivastava P. Immunological role of neuronal receptor vanilloid receptor 1 expressed on dendritic cells. Proc Natl Acad Sci USA 2005; 102:5120-5.
17. Guntheroe MJ, Scalass A. Peripheral TRPV1 receptors as targets for drug development: new molecules and mechanisms. Curr Pharm Des 2008; 14:32-41.
18. Earley S. Vanilloid and melastatin transient receptor potential channels in vascular smooth muscle. Microcirculation 2010; 17:237-49.
19. Pang G, Lu W, Li X, Chen Y, Zhong N, Ran P, Wang J. Expression of Store-operated Ca2+ entry and transient receptor potential canonical and vanilloid-related proteins in rat distal pulmonary venous smooth muscle. Am J Physiol Lung Cell Mol Physiol 2009; 299:L623-30.
20. Wang YX, Wang J, Wang C, Liu J, Shi LP, Xu M. Functional expression of transient receptor potential vanilloid-related channels in chronically hypoxic human pulmonary arterial smooth muscle cells. J Membr Biol 2008; 233:151-9.
21. Zhong B, Wang DH. Protease-activated receptor 2-mediated protection of myocardial ischemia-reperfusion injury: role of transient receptor protein vanilloid receptors. Am J Physiol Regul Integr Comp Physiol 2009; 297:H1681-90.
22. Beatz IN, Dick GM, Tüne JD, Edwards JM, Neeh ZP, Dincer UD, Snarek M. Impaired capsaicin-induced relaxation of coronary arteries in a porcine model of the metabolic syndrome. Am J Physiol Heart Circ Physiol 2008; 294:H496-9.
23. Mohapart SR. Role of natriuretic peptide signaling in modulating asthma and inflammation. Can J Physiol Pharmacol 2007; 85:754-9.
24. Smith SA, Lea AK, Williams MA, Murphy MN, Mitchell JH, Garry MG. The TRPV1 receptor is a mediator of the exercise pressor reflex in rats. J Physiol 2010; 588:1179-89.
25. Yamazaki T, Yazaki Y. Molecular basis of cardiac hypertrophy. Z Kardiol 2008; 97:1-6.
26. Zhong B, Wang DH. TRPV1 gene knockout impairs preconditioning protection against myocardial injury in isolated perfused hearts in mice. Am J Physiol Heart Circ Physiol 2007; 293:H791-8.
27. Sexton A, McDonald M, Cayla C, Thiemermann C, Ablalwila A. 12-Lipoxygenase-derived eicosanoids protect against myocardial ischemia/reperfusion injury via activation of neuronal TRPV1. FASEB J 2007; 21:2605-703.
28. Ho WS, Gardiner SM. Acute hypertension reveals depressor and vasodilator effects of cannabinoids in conscious rats. Br J Pharmacol 2009; 156:104-10.
29. Zakreszka A, Schlicker E, Baranowska M, Kozlowska H, Kwolek O, Malinowska B. A cannabionoid receptor, sensitive to O-1918, is involved in the delayed hypotension induced by anandamide in anaesthetized rats. Br J Pharmacol 2010; 160:574-84.
30. Hydock DS, Lien CY, Hayward R. Anandamide preserves cardiac function and geometry in an acute doxorubicin cardiotoxicity rat model. J Cardiovasc Pharmacol Ther 2009; 14:59-67.
31. Mulhophady P, Rajesh M, Bhatk S, Patel V, Kashiwaya Y, Luidet L, et al. CB1 cannabinoid receptors promote oxidative stress and cell death in murine models of doxorubicin-induced cardiomyopathy and in human cardiomyocytes. Cardiovasc Res 2010; 85:773-84.
32. Hiley CR. Endocannabinoids and the heart. J Cardiovasc Pharmacol 2009; 53:267-76.
33. Bishnoi M, Premkumar LS. Possible consequences of blocking transient receptor potential vanilloid. Curr Pharm Biotechnol 2011; 12:102-14.
34. Holter P. The pharmacological challenge to tame the transient receptor potential vanilloid-1 (TRPV1) nociceptor. Br J Pharmacol 2008; 155:1145-62.
35. Patten RD, Hall-Porter MR. Small animal models of heart failure: development of novel therapies, past and present. Circ Heart Fail 2009; 2:138-44.
36. Lypacevic M. Surgical models of hypertrophy and heart failure: Myocardial infection and transverse aortic constriction. Drug Discovery Today: Disease Models 2006; 3:283-90.
37. Caterina MJ, Leffler A, Malmberg AB, Martin WJ, Tranfont J, Petersen-Zeer KR, et al. Impaired nociception and pain sensation in mice lacking the capsaicin receptor. Science 2000; 288:306-13.
38. Shojiama I, Sato K, Izumiya Y, Schiekofer S, Ito M, Liao R, et al. Disruption of coordinated cardiac hypertrophy and angiogenesis contributes to the transition to heart failure. J Clin Invest 2005; 115:2108-18.
39. Lijnen P, Petrov V. Renin-angiotensin system, hypotrophy and gene expression in cardiac myocytes. Methods Find Exp Clin Pharmacol 1999; 21:363-74.
40. Lijnen P, Petrov V. Antagonism of the renin-angiotensin system, hypertrophy and gene expression in cardiac myocytes. Methods Find Exp Clin Pharmacol 1999; 21:363-74.
41. Deiz J, Fortuno MA, Ravassa A. Apoptosis in hypertensive heart disease.Curr Opin Cardiol 2008; 13:317-25.
42. Deiz J. Mechanisms of cardiac fibrosis in hypertension. J Clin Hypertens (Greenwch) 2007; 9:546-50.
43. Hutchinson KR, Stewart JA Jr, Luzêche J, Luzêche JA. Extracellular matrix remodeling during the progression of volume overloaded heart failure. J Mol Cell Cardiol 2010; 48:564-9.
44. Lompre AM, Flajart RJ, Harding SE, Kranias EG, Lohia MJ, Marks AR. Ca2+-cycling and new therapeutic approaches for heart failure. Circulation 2010; 121:822-30.
45. Berridge MJ, Bootman MD, Lipp P. Calcium—a life and death signal. Nature 1998; 395:645-8.
46. Roderick HL, Fligaz DR, Smyrnias I, Fearnley CM. TRP channel antagonists: 2. Structure-activity relationships of 4-oxopyrimidine-3-carboxamide-4-triazole. Bioorg Med Chem 2007; 50:3515-27.
47. Dominguez C, et al. Novel vanilloid receptor-1 antagonists: 2. Structure-activity relationships of 4-oxopyrimidine-3-carboxamide-4-triazole. Bioorg Med Chem 2007; 50:3515-27.
48. Zhu W, Xu P, Ciausac FX, Hall AK, Oxford GS. Activin acutely sensitizes dorsal root ganglion neurons and induces hyperalgesia via PKC-mediated potentiation of transient receptor potential vanilliod-1. J Neurosci 2007; 27:13770-80.
49. Zhang ZY, Xu H, Clapham DE, Ji RR. Phosphatidylinositol-3-kinase activators ERK in primary sensory neurons and mediates inflammatory heat hyperalgesia through TRPV1 sensitization. J Neurosci 2004; 24:8300-9.
50. Jeske NA, Diogenes A, Ruparel NR, Fehrenbacher JC, Henry M, Akopian AN, Hargreaves KM. A-kinase anchoring protein mediates TRPV1 thermal hyperalgesia through PKA phosphorylation of TRPV1. Pain 2008; 138:604-16.
51. Zhu WZ, Wang SQ, Chakir K, Yang D, Zhang T, Brown JH, et al. Linkage of beta-adrenergic stimulation to apoptotic heart cell death through protein kinase A-independent activation of Ca2+/calmodulin kinase II. J Clin Invest 2003; 111:617-25.
52. Price TJ, Jeske NA, Flores CM, Hargreaves KM. Pharmacological interactions between calcium/calmodulin-dependent kinase II alpha and TRPV1 receptors in rat trigeminal sensory neurons. Neurosci Lett 2005; 389:94-8.
53. Huke S, Desanti S, Kaertel CA, Mesza S, Brown JH, Dedman JR, Bers DM. SR-targeted CaMKII inhibition improves SR Ca2+ handling, but accelerates cardiac remodeling in mice overexpressing CaMKII-B. J Mol Cell Cardiol 2011; 50:230-8.
54. Molkentin JD. Dichotomy of Ca2+ in the heart: contraction versus intracellular signaling. J Clin Invest 2006; 116:623-6.
55. Freeman R, McKinsey TA, Olson EN. Decoding calcium signals involved in cardiac growth and function. Nat Med 2000; 6:1221-7.
56. Camini M, Fazel S, Zhao S, Xaymardan M, Fuji H, Wessel RD, Li BK. C-kinase dysfunction impairs myocardial healing after infarction. Circulation 2007; 116:77-82.
57. Hara M, Ono K, Hwang MW, Iwasaki A, Okada M, Nakatani K, et al. Evidence for a role of mast cells in the evolution to congestive heart failure. J Exp Med 2002; 195:375-81.
58. Beijer S, Andersson DA. TRP channel antagonists for pain—opportunities beyond TRPV1. Curr Opin Investig Drugs 2009; 10:655-63.
59. Doherty EM, Forsch C, Bannon AW, Bo Y, Chen N, Dominguez C, et al. Novel vanilloid receptor-1 antagonists: 2. Structure-activity relationships of 4-oxopyrimidines leading to the selection of a clinical candidate. J Med Chem 2007; 50:3515-27.
60. Roberts LA, Connors M. TRPV1 antagonists as a potential treatment for hyperalgesia. Recent Pat CNS Drug Discov 2006; 1:65-76.
61. Rockman HA, Ono S, Ross RS, Jones LR, Karimi M, Bhargava V, et al. Molecular and physiological alterations in murine ventricular dysfunction. Proc Natl Acad Sci USA 1994; 91:2604-8.