Treatment of Heart Failure by a Methanocarba Derivative of Adenosine Monophosphate: Implication for a Role of Cardiac Purinergic P2X Receptors

Si-Yuan Zhou, Mohammed Mamdani, Khaled Qanud, Jian-Bing Shen, Achilles J. Pappano, T. Santhosh Kumar, Kenneth A. Jacobson, Thomas Hintze, Fabio A. Recchia, and Bruce T. Liang

The Pat and Jim Calhoun Cardiology Center, University of Connecticut Health Center, Farmington, Connecticut (J.-B.S., A.J.P., B.T.L.); National Institute of Diabetes and Digestive and Kidney Diseases, National Institutes of Health, Bethesda, Maryland (T.S.K., K.A.J.); Department of Physiology, New York Medical College, Valhalla, New York (S.-Y.Z., M.M., K.Q., T.H., F.A.R.); and Scuola Superiore Sant'Anna, Pisa, Italy (F.A.R.)

Received November 30, 2009; accepted February 25, 2010

ABSTRACT

Evidence is accumulating to support a potentially important role for purinergic (P2X) receptors in heart failure (HF). We tested the hypothesis that a hydrolysis-resistant nucleotide analog with agonist activity at myocardial P2X receptors (P2XRs) improves the systolic HF phenotype in mouse and dog models. We developed a hydrolysis-resistant adenosine monophosphate derivative, (1'S,2R,3S,4'R,5'S)-4-(6-amino-2-chloro-9H-purin-9-yl)-1-[phosphoryloxymethyl] bicycle[3.1.0]hexane-2,3-diol (MRS2339), with agonist activity at native cardiac P2XRs. Chronic MRS2339 infusion in postinfarct and calsequestrin (CSQ) mice with HF resulted in higher rates of pressure change (+dP/dt), left ventricle (LV)-developed pressure, and cardiac output in an in vitro working heart model. Heart function in vivo, as determined by echocardiography-derived fractional shortening, was also improved in MRS2339-infused mice. The beneficial effect of MRS2339 was

dose-dependent and was identical to that produced by cardiac myocyte-specific overexpression of the P2X₄ receptor. The HF improvement was associated with the preservation of LV wall thickness in both systole and diastole in postinfarct and CSQ mice. In dogs with pacing-induced HF, MRS2339 infusion reduced left ventricular end-diastolic pressure, improved arterial oxygenation, and increased +dP/dt. MRS2339 treatment also decreased LV chamber size in mice and dogs with HF. In murine and canine models of systolic HF, in vivo administration of a P2X nucleotide agonist improved contractile function and cardiac performance. These actions were associated with preserved LV wall thickness and decreased LV remodeling. The data are consistent with a role of cardiac P2XRs in mediating the beneficial effect of this agonist.

Interest has grown in the physiological role of purinergic (P2) receptors for extracellular ATP within the cardiovascular system. There are two subfamilies of P2 receptors: ligand-gated ion channel and G protein-coupled receptors, termed P2X and P2Y, respectively (North, 2002; Abbracchio et al.,

2006). Seven subtypes of P2X (P2X₁₋₇) receptors have been identified by molecular cloning. The various cloned P2Y receptors are coupled to phospholipase C (PLC) (for P2Y₁, P2Y₂, P2Y₄, P2Y₆), PLC and Gs (for P2Y₁₁) (Communi et al., 1997; Hollopeter et al., 2001; Zhang et al., 2001), and G_i (P2Y₁₂, P2Y₁₃, P2Y₁₄). Members of both the P2X and P2Y families are expressed in the heart (Erlinge and Burnstock, 2008). P2X₁ receptor expression is increased in the left atria of patients with dilated cardiomyopathy (Berry et al., 1999), whereas the P2Y₁₁ receptor is present in all four chambers of the heart (Communi et al., 1997).

In the heart, extracellular ATP can elicit an increase in

This work was supported in part by the National Institutes of Health National Heart Lung and Blood Institute [Grant R01-HL48225], a Ray Neag Distinguished Professorship, and the Intramural Research Program of the National Institutes of Health National Institute of Diabetes and Digestive and Kidney Diseases.

Article, publication date, and citation information can be found at <http://jpet.aspetjournals.org>.
doi:10.1124/jpet.109.164376.

ABBREVIATIONS: P2, purinergic; P2XR, P2X receptor; CSQ, calsequestrin; PLC, phospholipase C; LV, left ventricle; LVDevP, left ventricular developed pressure; LVEDP, left ventricular end-diastolic pressure; LAD, left anterior descending; LVPW, left ventricular posterior wall; LVEDD, left ventricular end-diastolic diameter; LVESD, left ventricular end-systolic diameter; FS, fractional shortening; MRS2339, (1'S,2R,3S,4'R,5'S)-4-(6-amino-2-chloro-9H-purin-9-yl)-1-[phosphoryloxymethyl] bicycle[3.1.0]hexane-2,3-diol; WT, wild type; TG, transgenic; 2-meSATP, 2-methylthio-ATP; ANOVA, analysis of variance; bpm, beats per minute; IVS, interventricular septum; NS, normal saline; MRS2500, 2-iodo-N⁶-methyl-(N)-methanocarba-2'-deoxyadenosine-3',5'-bisphosphate.

contractility without an attendant increase in cAMP (Podrasky et al., 1997). The ATP analog 2-methylthioATP (2-meSATP) mimics the stimulatory effect of ATP on cardiac contractile function and induces a membrane current in mouse ventricular myocytes (Shen et al., 2006). 2-meSATP has negligible agonist activity on P2Y receptors and does not stimulate PLC effectively (Mei and Liang, 2001). Nucleotidase degrades 2-meSATP to 2-methylthioadenosine 5'-diphosphate; this metabolite had no effect on membrane current in murine ventricular myocytes (Shen et al., 2007). This is of interest because 2-methylthioadenosine 5'-diphosphate was reported to be a selective agonist at P2Y₁ receptors in amphibian heart (Ju et al., 2003). Thus, the P2Y receptor–PLC pathway is unlikely to be involved in mediating positive contractile response to 2-meSATP.

Recent evidence suggests that P2X receptors (P2XRs) may play an important biological role in the heart. Mice with cardiac-specific overexpression of the P2X₄R [P2X₄R transgenic (Tg) mice], which is an important subunit of the native cardiac myocyte P2XR (Shen et al., 2006), displayed an enhanced basal contractile function (Hu et al., 2001) and protected against the progression of HF (Yang et al., 2004; Sonin et al., 2008; Shen et al., 2009). Mice overexpressing calsequestrin (CSQ) develop dilated cardiomyopathy and die prematurely of HF (Jones et al., 1998). When such animals also overexpress P2X₄R (binary TG), they exhibit better cardiac function and survive longer (Yang et al., 2004). Cardiac overexpression of P2X₄R also increased survival in mice subjected to ligation of the left anterior descending (LAD) coronary artery. These data indicate a beneficial phenotype of the P2X₄R Tg mice in systolic HF.

We hypothesized that the stimulation of the endogenous cardiac P2X receptor by an agonist can exert a similar salutary effect in both CSQ and LAD-ligated animals with HF. To test this, we synthesized (1'S,2R,3S,4'R,5'S)-4-(6-amino-2-chloro-9H-purin-9-yl)-1-[phosphoryloxymethyl] bicycle[3.1.0]hexane-2,3-diol (MRS2339), a hydrolysis-resistant (N)-methanocarba derivative of adenosine monophosphate. MRS2339 has agonist activity in cardiac myocytes of both wild-type (WT) mice and mice with HF (Shen et al., 2007). Thus, MRS2339 (10 μ M) elicited a membrane current having the same magnitude, I-V (current voltage) relation, and reversal potential as the P2X agonist 2-meSATP (3 μ M). Furthermore, another P2Y₁ antagonist, MRS2500 [2-iodo-N⁶-methyl-(N)-methanocarba-2'-deoxyadenosine-3',5'-bisphosphate], had no effect on the membrane current induced by either 2-meSATP or MRS2339 (Shen et al., 2007). Inclusion of GDP β S in the pipette solution to inhibit guanine nucleotide exchange and interrupt G protein signaling did not affect the current induced by 2-meSATP or MRS2339.

We extended the study of MRS2339 to include dogs with pacing-induced HF because of the limits imposed by the examination of the small-animal model. Pacing-induced HF is a well established and highly reproducible animal model of dilated cardiomyopathy, characterized by systolic and diastolic dysfunction and a very predictable time course (Kiuchi et al., 1993; Recchia et al., 1998). Numerous studies have shown that this model recapitulates many of the fundamental characteristics of human HF, including progressive LV chamber dilation, LV wall thinning, decreased contractility, neurohormonal activation, and β -adrenergic receptor desensitization (Kiuchi et al., 1993; Shannon et al., 1997; Recchia et al., 1998; Spinale et al., 1998; O'Rourke et al., 1999). By comparing the effects of MRS2339 in HF models in mouse (CSQ overexpression and ischemia) and dog hearts, we could broadly define the role of endogenous P2X receptors in ameliorating systolic HF.

Materials and Methods

Mouse Models of Systolic Heart Failure: LAD Ligation and CSQ Overexpression Models of Dilated Cardiomyopathy. Age-matched (14–16 weeks old) WT or P2X₄R Tg mice with similar body weights were used. The P2X₄R construct was generated and bred as previously described (Hu et al., 2001). Ligation of the LAD in anesthetized mice was carried out by using a procedure similar to that described previously (Sonin et al., 2008). LAD ligation-mediated ischemic cardiomyopathy was induced in both WT and P2X₄R Tg male and female mice. All animals that recovered from anesthesia and lived for 24 h were analyzed for HF. All animal procedures were performed according to guidelines approved by the University of Connecticut School of Medicine Review Board.

The infarct size was quantified after Masson trichrome to measure and quantify the area of fibrosis as described previously (Tsoporis et al., 2005; Sonin et al., 2008). After infarct, mice developed LV dilatation and impaired contractile function (Tsoporis et al., 2005; Sonin et al., 2008).

Measurements of Intact Heart Function ex Vivo by Working Heart Preparation. Various parameters of intact heart function, such as left ventricular developed pressure (LVDevP), rates of pressure change ($\pm$ dP/dt), forward output (stroke volume in 1 min), and total cardiac output (forward output plus coronary flow) were quantitatively determined by using the working heart model as described previously (Hu et al., 2001; Mei and Liang, 2001). The afterload against which buffered perfusate solution was ejected from left ventricle (LV) was constant and set at 56 mm Hg of hydrostatic pressure from a column of buffer connected to the aorta. Hemodynamic parameters from the various groups of mice were obtained under the same afterload. To determine the acute effect of various doses of MRS2339, the following method was used. After exposure to each dose, the heart was perfused with drug-free buffer to obtain a separate baseline +dP/dt before being perfused with the next higher drug dose. Data were analyzed by computer software (WorkBench for Windows+, Kent Scientific Corp., Torrington, CT). The signals from the transducers were constantly displayed and analyzed.

Mice displaying the CSQ model of severe cardiomyopathy and HF were bred and maintained according to a previously described method (Yang et al., 2004). The CSQ TG mice, originally provided by Dr. Larry Jones (Krannert Institute of Cardiology, Indiana University School of Medicine, Indianapolis, IN), developed hypertrophy and dilated cardiomyopathy followed by a lethal HF phenotype with death near the age of 3 months (Jones et al., 1998; Knollman et al., 2000).

Echocardiography. Transthoracic echocardiography was performed with a linear 30-MHz transducer according to the manufacturer's instructions (Vevo 660 High-Resolution Imaging System, VisualSonics, Toronto, Canada) as described previously (Hu et al., 2001; Sonin et al., 2008; Shen et al., 2009). Two-dimensional-targeted M-mode echocardiographic measurements were carried out at midpapillary muscle level. Mice were anesthetized with 1% isoflurane by using a vaporizer as described previously (Tsoporis et al., 2005). All mice were exposed to the same isoflurane concentration. Conditions for animal handling and anesthesia induction were standardized. LV end-diastolic diameter (LVEDD) and LV end-systolic diameter (LVESD), left ventricular posterior wall (LVPW), interventricular septum (IVS) thickness, and fractional shortening (FS = LVEDD – LVESD/LVEDD) were measured. LVPW and IVS were measured digitally on the M-mode tracings, and all echocardiographic

graphic parameters were averaged from more than three cardiac cycles. There were no significant differences in heart rate between WT mice, P2X₄R Tg mice, and MRS2339-treated WT mice at any time measured after the infarction. The various echocardiographic parameter values in postinfarct WT mice reported here were similar to those obtained in WT mice from other studies (Bridgman et al., 2005; Franz et al., 2006; Miyamoto et al., 2006; Niizeki et al., 2007).

Drug Infusion. MRS2339 was dissolved in phosphate-buffered saline, pH 7.4 at the indicated concentrations (from 3.3 to 30 μ M) in 200 μ l of total volume, filtered for sterility for in vivo administration at 6 μ l per day for 28 days via a mini-osmotic pump (Alzet, Cupertino, CA), and implanted in the postinfarct and CSQ mice with HF. After 28 days of infusion, echocardiography was performed. Then, isolated working heart studies were carried out within 3 days of obtaining the various echocardiographic parameters. Doses were selected based on an initial study that showed protection against HF when MRS2339 was present at 3.3 μ M in the mini-pump (Shen et al., 2007). We estimated that this may be a minimally efficacious dosage and selected the next two higher doses at 3-fold increments (9.9 and 30 μ M) in CSQ mice. MRS2339 concentration was 9.9 μ M in the mini-pump implanted in postinfarct mice.

Heart Failure by Rapid Pacing in Dogs as a Model of Dilated Cardiomyopathy. Nine adult mongrel dogs (weight 25–27 kg) were sedated with acepromazine maleate (1 mg/kg i.m.), anesthetized with sodium pentobarbital (25 mg/kg i.v.), and ventilated with room air. A thoracotomy was performed in the left 5th intercostal space. Tygon catheters were placed in the descending thoracic aorta and left atrial appendage. A solid-state pressure gauge (P6.5, Konigsberg Instruments, Inc., Pasadena, CA) was inserted in the left ventricle through the apex. A Doppler flow probe (provided by Craig Hartley, Baylor College of Medicine, Houston, TX) was placed around the left circumflex coronary artery, and a pair of 3-MHz piezoelectric crystals was implanted on opposite endocardial surfaces at the base of the left ventricle. A screw-type, unipolar myocardial pacing lead was implanted on the LV free wall. Wires and catheters were run subcutaneously to the intrascapular region, the chest was then closed in layers, and the pneumothorax was reduced. Antibiotics were given after surgery, and the dogs were allowed to fully recover. After 10 days, dogs were trained to lie quietly on the laboratory table (Recchia et al., 1998; Osorio et al., 2002; Trochu et al., 2003; Lionetti et al., 2005).

Heart failure was induced by pacing the heart at 210 bpm for 3 weeks. After the 3rd week, the pacing rate was increased to 240 bpm. Dogs were considered in end-stage HF when LV end-diastolic pressure (LVEDP) reached 25 mm Hg. Other profound hemodynamic alterations were also present at earlier time points compared with the baseline prepping state, but were still compatible with acceptable clinical conditions. However, in our experience, a LVEDP of 25 mm Hg is typically associated with a reduction of arterial PO₂, various signs of decompensation, and imminent death. The dogs were divided into two groups. In group 1 (five dogs), at the beginning of the 3rd week of pacing, a vehicle solution of normal saline (NS) was continuously infused at 60 μ l/h via an external pump until the end of 4th week for a total of 2 weeks. In group 2 (four dogs), at the beginning of 3rd week of pacing, MRS2339 was continuously infused at 10 μ g/h via an external pump connected to the left atrial catheter until the end of the 4th week for a total of 2 weeks. MRS2339 was dissolved in a concentration of 0.168 mg/ml NS and infused at a rate of 60 μ l/h to yield 10 μ g/h.

Hemodynamic parameters for both groups were measured after cessation of rapid pacing at the end of the 4th week, at which time, drug infusion had also stopped. For hemodynamic measurements, the aortic catheter was attached to a P23ID strain-gauge transducer for measurement of aortic pressure. LV pressure was measured by using the solid-state pressure gauge. Coronary blood flow was measured with a pulsed Doppler flowmeter (model 100, Triton Technology, San Diego, CA). LV diameter was obtained by connecting the implanted piezoelectric crystals to a sonomicrometer, which generated a voltage linearly propor-

tional to the transit time of ultrasounds traveling between the two crystals (1.55×10 m/s). Hemodynamic signals were recorded by a polygraph and simultaneously digitized at a sampling rate of 250 Hz. Arterial blood O₂ was measured in a blood gas analyzer (model GEM Premier 3000; Instrumentation Laboratory, San Jose, CA), and the PO₂ was multiplied by 0.003 and then added to the O₂ content obtained from a hemoglobin analyzer (IL-682 CO-oximeter, Instruments Laboratory, San Jose, CA) to provide total O₂ content (vol.%). Arterial blood samples were obtained with plastic syringes treated with heparin or EDTA and promptly stored on ice (Recchia et al., 1998).

Data Analysis. Unless otherwise indicated, data are provided as mean $\pm$ S.E.M. Student's *t* test for paired or unpaired samples was used to evaluate the effects of experimental interventions; *P* < 0.05 was taken as statistically significant. For multiple groups, one-way analysis of variance (ANOVA) and post-test comparison were carried out.

Results

MRS2339 Has Cardiac Agonist-Like Activity in Normal Mice and Mice with Heart Failure. MRS2339 was synthesized as a relatively nucleotidase-resistant adenine nucleotide analog (Ravi et al., 2002). Injection of MRS2339 induced a dose-dependent increase in +dP/dt (Fig. 1a) and LVDevP (not shown) in nonfailing adult WT murine hearts in the ex vivo working heart model. Spontaneous heart rate did not change before (334.3 ± 7.1 bpm, *n* = 8) or after (324.5 ± 6.7 bpm, *n* = 8, *p* > 0.05) injection of 1 μ M MRS2339 and was not altered by any other drug concentrations (not shown). In the nonfailing hearts, the actual increase or the percentage increase above basal caused by 30, 100, or 1000 nM MRS2339 was higher than that elicited by 10 nM MRS2339 (one-way ANOVA and post-test comparison, *P* < 0.05). There was no difference among the actual or percentage increases caused by 30, 100, or 1000 nM MRS2339 (*P* > 0.05). Injection of MRS2339 in the coronary circulation of postinfarct ischemic mice with HF also produced an increase in +dP/dt (Fig. 1b) and LVDevP (not shown) at each dose in a working heart model. Heart rate did not change before (298.6 ± 15.2 bpm, *n* = 9) or after (299.6 ± 16.1 bpm, *n* = 9, *p* > 0.05) injection of 1 μ M MRS2339 and was not altered by any other drug concentrations (not shown). There was no difference between the percentage increase above basal in either +dP/dt or LVDevP that was caused by any of the doses of MRS2339.

In the CSQ mice with HF, MRS2339 also caused an increase in +dP/dt (Fig. 1c) and LVDevP (not shown) at each dose. Again, the spontaneous heart rate did not change before (299.8 ± 6.9 bpm, *n* = 17) or after (298.9 ± 8.1 bpm, *n* = 17, *p* > 0.05) injection of 1 μ M MRS2339 and was not altered by any other drug concentrations (not shown). The extent of stimulation of +dP/dt or LVDevP by MRS2339 (at 100 or 1000 nM) was similar in nonfailing versus failing postinfarct or CSQ hearts (one-way ANOVA and post-test comparison, *P* > 0.1). These data demonstrate an agonist-like activity of MRS2339 in both the nonfailing and failing myocardium. The data are consistent with the previous finding showing that MRS2339 has agonist activity capable of causing a P2X-like current in normal nonfailing WT and failing CSQ cardiac myocytes (Shen et al., 2007).

Chronic Infusion of MRS2339 in Postinfarct Ischemic Mice with Heart Failure Caused Enhanced Contractile Function as Determined by ex Vivo and in Vivo Measures. We tested the hypothesis that chronic in

a MRS2339 has agonist-like effect in non-failing heart **b** MRS2339 has agonist-like effect in the post-infarct failing heart **c** MRS2339 has agonist-like effect in the post-CSQ failing heart

Fig. 1. Acute MRS2339 has an agonist-like effect in failing and nonfailing hearts. Isolated working heart preparations of WT and P2X₄R Tg animals were prepared (see *Materials and Methods*). MRS2339 was injected to assess its acute effect on cardiac contractile function. **a**, the percentage increase above basal +dP/dt for nonfailing adult WT murine hearts is shown. The basal +dP/dt (mm Hg/s) for 10, 30, 100, and 1000 nM MRS2339 was 7567 ± 302, 7290 ± 236, 7276 ± 241, and 7239 ± 285, respectively. **b**, the percentage increase in postinfarct hearts is shown. The basal +dP/dt (mm Hg/s) for 20, 100, and 1000 nM MRS2339 was 4965 ± 285, 4863 ± 223, and 4818 ± 178, respectively. **c**, the percentage increase in CSQ hearts is shown. The basal +dP/dt (mm Hg/s) for 20, 100, and 1000 nM MRS2339 was 5091 ± 291, 4836 ± 254, and 4779 ± 313, respectively. $P < 0.05$ for increase by each dose compared with its respective basal value (paired t test) in all animals. Data are mean ± S.E.M.

vivo administration of MRS2339 may have a salutary effect on ischemic HF progression, similar to that caused by the cardiac-specific overexpression of P2X₄ receptors (Sonin et al., 2008). The effect of MRS2339 was tested in both male and female mice because of gender difference in susceptibility to ischemia-induced injury. After 28 days of its infusion, MRS2339 caused an improvement in the cardiac contractile function in the postinfarct mice with HF for both genders.

The MRS2339-treated male mice with HF compared with vehicle-treated male animals ($n = 12$) showed a higher +dP/dt and cardiac output in the ex vivo working heart preparation (Fig. 2, **a** and **b**). In female mice, MRS2339 infusion was also able to cause higher +dP/dt and cardiac output than could vehicle infusion (not shown). The LVDevP was also higher in MRS2339-treated (129.6 ± 2.76 mm Hg, $n = 12$) versus vehicle-treated (111.8 ± 3.57 mm Hg, $n = 5$, $P < 0.05$) male mice. A similar difference in LVDevP also existed for drug-treated (129.6 ± 4.19 mm Hg, $n = 8$) versus vehicle-treated (107.7 ± 3.3 mm Hg, $n = 8$, $P < 0.05$) female mice. Consistent with an improved systolic function, the for-

a Chronic MRS2339 infusion could enhance +dP/dt in post-infarct heart failure male mice **b** Chronic MRS2339 infusion could improve cardiac output in post-infarct heart failure male mice **c** Chronic MRS2339 infusion achieved similar increase in FS as cardiac-specific P2X₄R overexpression in male mice

Fig. 2. Chronic infusion of MRS2339 caused an improved contractile performance in mice with postinfarct ischemic HF. Myocardial function ex vivo was determined in the working heart preparation as described in the legend of Fig. 1. Cardiac function in vivo was assessed by echocardiography as described under *Materials and Methods*. After 28 days of infusion (MRS2339 concentration was 9.9 μ M in the mini-pump), +dP/dt (**a**), cardiac output (**b**), and FS (**c**) were higher in drug-treated mice than in vehicle (NS)-treated mice with HF. The postinfarct FS was also greater in transgenic mice with cardiac-specific overexpression of the P2X₄R compared with the FS of postinfarct WT mice treated with vehicle (one-way ANOVA and post-test comparison, $P < 0.05$). The FS in Tg mice ($n = 10$ male mice) was similar to the FS of MRS2339-treated mice ($P > 0.05$).

ward output, that is, stroke volume in 1 min, was higher in drug-treated (7.3 ± 0.36 ml/min, $n = 12$) versus vehicle-treated (4.26 ± 0.64 ml/min, $n = 5$, $P < 0.05$) male mice. The drug-treated female mice (7.29 ± 0.41 ml/min, $n = 8$) also had a higher forward output than vehicle-treated female mice (4.25 ± 0.41 ml/min, $n = 8$, $P < 0.05$). The spontaneous heart rate in the working heart model was similar in vehicle-treated (318 ± 10.8 bpm, $n = 13$) versus drug-treated (348 ± 10 bpm, $n = 20$, $P > 0.05$) ischemic mice with HF. The similar heart rates indicate that the increased cardiac output was caused by a greater contractility in the working heart model.

The echocardiography-derived FS was also higher in MRS2339-treated animals ($n = 10$) than in vehicle-treated animals ($n = 14$) in vivo. This was the case for both male (Fig. 2c) and female mice (not shown). Compared with MRS2339 treatment, cardiac-specific overexpression of the P2X₄ receptor caused a similar increase in the FS as shown in Fig. 2c and in +dP/dt as shown previously (Sonin et al., 2008). The data show for the first time a rescuing effect of this small-molecule agent in ischemia-induced HF.

Chronic Infusion of MRS2339 in CSQ Mice with Heart Failure Caused Improved Cardiac Contractile Performance. CSQ mice with HF, after chronic infusion with MRS2339, showed a higher +dP/dt (Fig. 3a), a higher LVDevP (Fig. 3b), and a larger cardiac output (Fig. 3c) compared with vehicle-treated CSQ animals. The spontaneous heart rate in the working heart model was similar in vehicle-infused (315.1 ± 8.7 bpm, $n = 20$) and 30 μ M MRS2339-infused (315.9 ± 7.7 bpm, $n = 13$, $P > 0.05$) CSQ mice with HF. Heart rate was also not different in vehicle-infused versus 3.3 μ M ($n = 13$) or 9.9 μ M ($n = 20$) drug-infused CSQ mice (not shown). The similar heart rates suggest that the increased cardiac output arose from a higher +dP/dt in the working heart model. The improvement in these in vitro heart function parameters was correlated with a similar increase in the contractile FS in vivo by echocardiography in MRS2339-treated CSQ mice ($n = 10$ –15) (Fig. 3e). The enhanced cardiac contractile function was associated with a lower heart weight/body weight ratio (Fig. 3d). LV chamber dimension was measured after chronic MRS2339 infusion.

Fig. 3. Chronic infusion of MRS2339 caused an improved contractile performance in CSQ mice with HF. Myocardial function ex vivo was determined in the working heart preparation (see Fig. 1 legend). The in vivo cardiac function was assessed by echocardiography as described under *Materials and Methods*. a–c, after 28 days of infusion of MRS2339 at the indicated concentrations, +dP/dt (a), LVDevP (b), and cardiac output (c) were progressively higher than those in NS-treated mice with HF (one-way ANOVA and post-test comparison, $P < 0.05$ for NS versus any drug concentration). The improvement in these working heart hemodynamic parameters occurred in a dose-dependent manner ($P < 0.05$ in 30 μ M versus 9.9 or 3.3 μ M comparison; 3.3 μ M versus 9.9 μ M, $P > 0.05$). d, the heart weight/body weight ratios were also reduced in a dose-dependent manner ($P < 0.05$ for NS versus any drug concentrations; $P < 0.05$ for 3.3 μ M versus 9.9 or 30 μ M; $P > 0.05$ for 30 μ M versus 9.9 μ M). e, after infusion of the same concentrations of MRS2339, the FS was also larger than in NS-treated CSQ mice with HF in a dose-dependent manner ($P < 0.05$ for NS versus 9.9 or 30 μ M; $P < 0.05$ for 3.3 μ M versus 9.9 or 30 μ M).

The LVESD was reduced by MRS2339 after its infusion at doses of 9.9 μM (3.09 ± 0.11 mm, $n = 15$ mice) and 30 μM (3.23 ± 0.11 mm, $n = 11$ mice). These values of LVESD are smaller than those obtained in vehicle-infused mice (3.72 ± 0.15 mm, $n = 14$ mice, $P < 0.05$). The LVEDD did not differ in drug-infused versus vehicle-infused CSQ mice ($P > 0.05$). The improvement in all of the functional parameters studied, whether they were ex vivo or in vivo endpoints, showed an apparent dose dependence (Fig. 3).

Preservation of LV Wall Thickness as a Beneficial Effect in Treatment of Heart Failure. In experiments with postischemic mice with HF, echocardiographic measurements showed a larger LVPW thickness in the noninfarcted region of MRS2339-infused mice. The preserved wall thickness was evident during systole in male (Fig. 4a) and female (not shown) mice. During diastole, the LVPW thickness was significantly larger in MRS2339-treated male mice (0.497 ± 0.0137 mm, $n = 10$) than in vehicle-treated male mice (0.409 ± 0.0131 mm, $n = 14$, $P < 0.05$). Similar data were obtained in female postinfarct mice treated with MRS2339 and female mice treated with vehicle (data not shown). In the noninfarcted septal area, the wall thickness was also better preserved in drug-treated than in vehicle-treated male mice during both systole (Fig. 4b) and diastole. Again, the ability of MRS2339 infusion to preserve LV septal wall thickness

was found in both male and female (not shown) mice with ischemic HF. In male mice, the diastolic septal thickness was larger after MRS2339 treatment (0.452 ± 0.0164 mm, $n = 10$) than vehicle treatment (0.36 ± 0.010 mm, $n = 14$, $P < 0.05$). Similar preservation of septal thickness was also found in drug-treated female mice with ischemic HF (not shown).

Although the cause of systolic HF is different, chronic infusion of MRS2339 was also able to preserve LV thickness in the CSQ mice with HF ($n = 10$ –15 mice). The IVS during systole was also thicker in CSQ mice infused with MRS2339 (Fig. 5a). A better preserved wall thickness was also evident in the LVPW of drug-infused CSQ mice during systole (Fig. 5b) and diastole (not shown). Thus, a better preserved wall thickness occurred in both mouse models of systolic HF.

MRS2339 Improves Cardiac Function in Dogs with Tachycardia-Induced Heart Failure. After 2 weeks of MRS2339 infusion, hemodynamic measurements were compared between control dogs (group 1) and drug-treated dogs with HF (group 2). The data are summarized in Table 1. There was no difference in the heart rate, the systolic, diastolic, or mean arterial blood pressures, or the mean coronary artery blood flow between MRS2339-infused or vehicle-infused dogs with HF ($P > 0.1$). After a 2-week infusion of MRS2339, the paced dogs showed a lower LVEDP (Fig. 6a), higher arterial O_2 content (Fig. 6b), and a higher $+dP/dt_{\text{max}}$ (Fig. 6c) than dogs infused with NS ($P < 0.05$, t test). The

a Chronic MRS2339 infusion achieved similar increase in systolic LVPW thickening as cardiac-specific P2X₄R overexpression in male mice

b Chronic MRS2339 infusion achieved similar increase in systolic septal thickening as cardiac-specific P2X₄R overexpression in male mice

Fig. 4. Chronic MRS2339 infusion or cardiac-specific overexpression of P2X₄R resulted in preservation of LV wall thickness in postinfarct mice with HF. The in vivo cardiac function was assessed by echocardiography as described under *Materials and Methods*. a, after 28 days of infusion, the LVPW thickness during systole (LVPW@S) was larger in male ($P < 0.05$) postinfarct mice with HF. The LVPW@S in P2X₄ Tg mice ($n = 10$ male mice) was similar to that in MRS2339-treated mice ($P > 0.05$). b, similar data were obtained when noninfarcted septal thickness during systole (IVS@S) was compared between NS-treated, P2X₄ Tg, and MRS2339-treated postinfarct mice, $P < 0.05$ NS-treated WT versus P2X₄ Tg or MRS2339-treated animals; $P > 0.05$ P2X₄ Tg versus MRS2339-treated mice. Data are expressed as mean $\pm$ S.E.M.

a Chronic infusion of MRS2339 produced a dose-dependent increase in septal systolic thickening in the CSQ failing heart

b Chronic infusion of MRS2339 produced a dose-dependent increase in LVPW systolic thickening in the CSQ failing heart

Fig. 5. Chronic infusion of MRS2339 caused a dose-dependent increase in LV systolic wall thickness in CSQ hearts. The in vivo cardiac function was assessed by echocardiography as described under *Materials and Methods*. After 28 days of infusion of MRS2339 at the indicated concentrations, noninfarcted septal thickness during systole (IVS@S) (a) and LVPW thickness during systole (LVPW@S) (b) was progressively larger than in NS-treated CSQ mice (one-way ANOVA and post-test comparison, $P < 0.05$ for NS versus 9.9 or 30 μM ; $P < 0.05$ for 3.3 μM versus 9.9 or 30 μM ; $P > 0.05$ for 9.9 versus 30 μM). Data are expressed as mean $\pm$ S.E.M.

TABLE 1

Hemodynamic characterization in MRS2339-infused dogs with HF

Heart failure was induced by rapid pacing in dogs that were infused with either saline (control) or MRS2339 as described under *Materials and Methods*. Data are means $\pm$ S.E.

Parameter	Group 1, Control (n = 5 Dogs)	Group 2, MRS2339-Treated (n = 4 Dogs)
Heart rate (bpm)	126 $\pm$ 5.1	117.5 $\pm$ 6.6
Systolic blood pressure (mm Hg)	96.4 $\pm$ 4.1	99.5 $\pm$ 4.8
Diastolic blood pressure (mm Hg)	71.6 $\pm$ 3.9	68.7 $\pm$ 3.5
Mean arterial blood pressure (mm Hg)	79.6 $\pm$ 6.1	80 $\pm$ 3.5
Mean coronary flow (ml/min)	26.9 $\pm$ 3.8	22.4 $\pm$ 1.3
LVEDP* (mm Hg)	23.8 $\pm$ 2.3	16.0 $\pm$ 0.7
dP/dt _{max} * (mm Hg/s)	1364 $\pm$ 48	1674 $\pm$ 89.9
-dP/dt _{min} (-mm Hg/s)	-1323 $\pm$ 202	-1444 $\pm$ 121
LVESD (cm)	4.35 $\pm$ 0.18	3.84 $\pm$ 0.08
LVEDD* (cm)	5.21 $\pm$ 0.15	4.59 $\pm$ 0.11
FS (%)	16.48 $\pm$ 1.69	16.35 $\pm$ 0.72
Arterial pO ₂ * (mm Hg)	70.4 $\pm$ 4.5	86.5 $\pm$ 1.5
Tau (Weiss) (ms)	22.97 $\pm$ 1.29	24.94 $\pm$ 1.89
Tau (Suga) (ms)	21.23 $\pm$ 1.70	19.47 $\pm$ 2.61

*, $P < 0.05$ in control vs. MRS2339-treated comparison.

LVEDD was also smaller in drug-infused dogs with HF ($P < 0.05$). There was a trend for a smaller LVESD in MRS2339-infused dogs with HF ($P = 0.05$) (Table 1). The data indicate that chronic infusion of MRS2339 could also confer a salutary effect in the canine model of pacing-induced systolic HF.

Discussion

Recent evidence suggests an important role of P2X receptors in protecting against progression of HF. Much of the evidence was based on a salutary phenotype in mice with HF with transgenic overexpression of the P2X₄R (Yang et al., 2004; Sonin et al., 2008; Shen et al., 2009), an important subunit of the endogenous cardiac P2X receptor (Shen et al., 2006). The role of the endogenous cardiac P2X receptor and characterization of its action in improving HF are poorly understood. We used MRS2339, a hydrolysis-resistant adenosine monophosphate derivative with agonist activity at endogenous cardiac P2X receptors, and transgenic mice with cardiac-specific overexpression of the P2X₄R as tools to study this question. Our study showed that chronic infusion of MRS2339 can improve the HF phenotype in both postinfarct and CSQ mice. The beneficial effect was identical to that produced by cardiac myocyte-specific overexpression of the P2X₄R with similar improvements in contractile performance and preservation of LV wall thickness.

Evidence for a Role of Cardiac P2X Receptors in Mediating the Protective Effect of MRS2339. We previously showed that MRS2339 infusion reproduced some of the beneficial effects that arose from cardiac-specific P2X₄R overexpression in CSQ mice (Shen et al., 2007). In the present study, we compared the phenotype after chronic MRS2339 infusion with that from cardiac-specific P2X₄R overexpression in CSQ mice. We extended the comparative study to include another murine model of systolic HF, the LAD-ligated postinfarct mice. Taken together, the data supported the notion that the beneficial effect of chronic MRS2339 infusion may be caused, at least in part, by activation of endogenous cardiac myocyte P2X receptors. Several lines of evidence support this concept. First, MRS2339 could exert an agonist-like effect in both nonfailing and failing myocardium.

a MRS2339 infusion for 2 weeks lowers LVEDP

b MRS2339 infusion for 2 weeks causes better oxygenation

c MRS2339 infusion for 2 weeks causes enhanced +dP/dt

Fig. 6. MRS2339 infusion in dogs with tachycardia-induced HF. MRS2339 or NS (control) was infused for 2 weeks via an intra-atrial catheter (see *Materials and Methods*). Compared with control dogs ($n = 5$), dogs infused with MRS2339 ($n = 4$) had a significantly lower LVEDP (a), higher arterial O₂ content (b), and higher +dP/dt (c) ($P < 0.05$ for each comparison). Data are expressed as mean $\pm$ S.E.M.

With a working heart model, infusion of MRS2339 could cause an increase in +dP/dt and LVDevP acutely in nonfailing WT hearts and failing postinfarct and CSQ hearts. These data showed for the first time that MRS2339 has agonist-like activity in the intact heart preparation. The ATP analog, 2-meSATP, had the same action (Hu et al., 2001). The data extended a previous study that showed that MRS2339 can activate a P2X-like current in nonfailing WT and failing CSQ cardiac myocytes (Shen et al., 2007). This also replicated the membrane action of 2-meSATP (see Introduction). Second, chronic infusion of MRS2339 in the intact mice with HF resulted in a favorable phenotype in both postinfarct and CSQ animals. In the present study, this favorable phenotype was characterized by an improved contractile function of the failing heart ex vivo and in vivo. We compared and characterized the phenotype from MRS2339-treated animals with that from mice with HF with transgenic cardiac myocyte-

restricted overexpression of P2X₄R. The characteristics of both phenotypes were similar. Like WT postinfarct mice, postinfarct mice with cardiac-specific P2X₄R overexpression also showed an increase in +dP/dt, LVdevP, and total cardiac output ex vivo (shown by current data) and in FS in vivo as reported previously (Sonin et al., 2008). After LAD ligation, the extent of the increases of these variables in P2X₄R Tg mice was similar to that in WT mice treated with MRS2339. Chronic infusion of MRS2339 or cardiac-specific P2X₄R overexpression did not affect the infarct size. The salutary effect of either MRS2339 infusion or transgenic cardiac-specific P2X₄R overexpression was independent of gender in postinfarct mice.

Preservation of LV Wall Thickness as a Feature of Salutary Effect on Heart Failure in Mice. In the CSQ model of HF, cardiac-specific P2X₄R overexpression caused an increase in +dP/dt and LVDevP (Yang et al., 2004) in a working heart preparation similar to that produced by chronic MRS2339 infusion in the present study. Consistent with improved systolic contractile function, the forward output was also greater in MRS2339-infused than in vehicle-infused CSQ mice. By in vivo heart function measure, the FS was also similarly improved in CSQ animals infused with MRS2339 (current data) and those overexpressing the P2X₄R (Shen et al., 2009). The present data showed that the HF rescuing effect by either MRS2339 infusion or cardiac overexpression of P2X₄R was associated with a better preservation of LV wall thickness during systole and diastole in both mouse models. Under pressure overload, cardiac-specific overexpression of sarcoplasmic reticulum calcium ATPase (Ito et al., 2001; Suarez et al., 2004) or neuronal nitric-oxide synthase (Loyer et al., 2008) can improve calcium handling and enhance LV wall thickening or thickness after aortic banding. In the postinfarct model, cardiac monocyte chemoattractant protein-1 overexpression can also preserve non-infarcted LV wall thickness (Morimoto et al., 2006). The larger LV wall thickness, along with an improved LV function, however, was also associated with a smaller infarct size in the monocyte chemoattractant protein-1 transgenic mice. Our findings differ in that LV wall thickness was better preserved despite a similar infarct size in vehicle-infused versus MRS2339-infused mice or in WT versus P2X₄R Tg mice. Although the exact mechanism by which preservation of wall thickness occurs is not known, the prevention of LV wall thinning appears to be another consequence after activation of the endogenous cardiac P2X receptor or receptor overexpression. The preservation of LV wall thickness and FS in the CSQ mice showed a dose-dependent relationship to MRS2339 and argues against a nonspecific effect of the drug. Although there are few data on selectivity of MRS2339 at various P2 receptors, these data are consistent with an increasing activation of the native P2X receptor by the escalating doses of MRS2339. Definitive proof of this concept awaits availability of antagonist selective at the cardiac P2X receptor or animals with cardiac-specific knockout of P2X receptors.

Protective Effect of MRS2339 in Canine Heart Failure. MRS2339, when infused in chronically instrumented dogs with systolic HF, exerted beneficial effects. This model of dilated cardiomyopathy with no ischemic injury is characterized by a very predictable time course (Kiuchi et al., 1993; Recchia et al., 1998). Therefore, any efficacious therapeutic

intervention is necessarily reflected by a delay in the progression toward decompensation (Shannon et al., 1997; Spinale et al., 1998; O'Rourke et al., 1999; Lionetti et al., 2005). The increase in LVEDP was attenuated in MRS2339-treated dogs compared with vehicle-infused dogs, consistent with better arterial oxygenation. Moreover, MRS2339 infusion for 2 weeks was associated with a more preserved contractile performance, as determined by a higher +dP/dt, similar to the +dP/dt we observed in MRS2339-infused mice with HF. Although the FS was unchanged after chronic MRS2339 infusion, the LV chamber dimension was decreased. A sustained improvement in the intrinsic cardiac contractile function in drug-treated dogs may be the primary underlying mechanism that delayed the LV remodeling. An inhibition of neurohormonal activation as a result of enhanced mechanical contractile performance cannot be excluded. Neither $-dP/dt_{min}$ nor tau values were different in MRS2339-treated dogs versus placebo-treated dogs with HF, whereas $-dP/dt$ was enhanced in MRS2339-infused CSQ or postinfarct mice ($P < 0.05$, not shown). The reason for this difference is not clear but it may be related to a shorter duration of MRS2339 treatment in the dogs (2 weeks) versus mice (4 weeks) or the difference in causation of HF in the models, or more simply, because $-dP/dt_{min}$ and tau are less sensitive in detecting moderate diastolic dysfunction compared with other indexes such as the end-diastolic pressure volume (or diameter) relationship. The generation of series of pressure-volume loops would have required invasive procedures that we did not use in these dogs. Nevertheless, common to the beneficial phenotype in both the mouse and canine models is an improved contractile function of the failing heart. In both CSQ mice and dogs with pacing-induced HF, MRS2339 was able to decrease LV remodeling, as manifested by a smaller LV dimension.

In summary, MRS2339 is a nucleotide analog with agonist activity at the cardiac P2X receptor. When infused chronically, MRS2339 can ameliorate systolic HF in murine and canine models. These data are consistent with an important salutary function of the endogenous cardiac P2X receptor during HF progression. The present study demonstrates that key downstream events underlying an improved phenotype in systolic HF are a reduction in LV remodeling and preservation of LV wall thickness.

Acknowledgments

We thank Carol McGuinness for technical assistance.

References

- Abbracchio MP, Burnstock G, Boeynaems J-M, Barnard EA, Boyer JL, Kennedy C, Fumagalli M, Gachet C, Jacobson KA, and Weisman GA (2006) International Union of Pharmacology LVIII. Update on the P2Y G protein-coupled nucleotide receptors: from molecular mechanisms and pathophysiology to therapy. *Pharmacol Rev* 59:281–341.
- Berry DA, Barden JA, Balcar VJ, Keogh A, and dos Remedios CG (1999) Increase in expression of P2X₁ receptors in the atria of patients suffering from dilated cardiomyopathy. *Electrophoresis* 20:2059–2064.
- Bridgman P, Aronovitz MA, Kakkar R, Oliverio MI, Coffman TM, Rand WM, Konstam MA, Mendelsohn ME, and Patten RD (2005) Gender-specific patterns of left ventricular and myocyte remodeling following myocardial infarction in mice deficient in the angiotensin II type 1a receptor. *Am J Physiol Heart Circ Physiol* 289:H586–H592.
- Communi D, Govaerts C, Parmentier M, and Boeynaems JM (1997) Cloning a human purinergic P2Y receptor coupled to phospholipase C and adenylyl cyclase. *J Biol Chem* 272:31969–31973.
- Erlinge D and Burnstock G (2008) P2 receptors in cardiovascular regulation and disease. *Purinergic Signaling* 4:1–20.
- Frantz S, Brandes RP, Hu K, Rammelt K, Wolf J, Scheuermann H, Ertl G, and Bauersachs J (2006) Left ventricular remodeling after myocardial infarction in

- mice with targeted deletion of the NADPH oxidase subunit gp91^{PHOX}. *Basic Res Cardiol* **101**:127–132.
- Hollopeter G, Jantzen HM, Vincent D, Li G, England L, Ramakrishnan V, Yang RB, Nurdan P, Nurdan A, Julius D, et al. (2001) Identification of the platelet ADP receptor targeted by antithrombotic drugs. *Nature* **409**:202–207.
- Hu B, Mei QB, Smith E, Barry WH, and Liang BT (2001) A novel cardiac inotropic phenotype with cardiac transgenic expression of human P2X₄ receptor transgenic mouse. *FASEB J* **15**:2739–2741.
- Ito K, Yan X, Feng X, Manning WJ, Dillmann WH, and Lorell BH (2001) Transgenic expression of sarcoplasmic reticulum Ca²⁺-ATPase modifies the transition from hypertrophy to early heart failure. *Circ Res* **89**:422–429.
- Jones LR, Suzuki YJ, Wang W, Kobayashi YM, Ramesh V, Franzini-Armstrong C, Cleemann L, and Morad M (1998) Regulation of Ca²⁺ signaling in transgenic mouse cardiac myocytes overexpressing calsequestrin. *J Clin Invest* **101**:1385–1393.
- Ju YK, Huang W, Jiang L, Barden JA, and Allen DG (2003) ATP modulates intracellular Ca²⁺ and firing rate through a P2Y₁ purinoceptor in cane toad pacemaker cells. *J Physiol* **552**:777–787.
- Kiuchi K, Shannon RP, Komamura K, Cohen DJ, Bianchi C, Homcy CJ, Vatner SF, and Vatner DE (1993) Myocardial β -adrenergic receptor function during the development of pacing-induced heart failure. *J Clin Invest* **91**:907–914.
- Knollman BC, Knollmann-Ritschel BEC, Weismann NJ, Jones LR, and Morad M (2000) Remodeling of ionic currents in hypertrophied and failing hearts of transgenic mice overexpressing calsequestrin. *J Physiol* **525**:483–498.
- Lionetti V, Linke A, Chandler MP, Young ME, Penn MS, Gupta S, d'Agostino C, Hintze TH, Stanley WC, and Recchia FA (2005) Carnitine palmitoyl transferase-I inhibition prevents ventricular remodeling and delays decompensation in pacing-induced heart failure. *Cardiovasc Res* **66**:454–461.
- Loyer X, Gomez AM, Milliez P, Fernandez-Velasco M, Vangheluwe P, Vinet L, Charue D, Vaudin E, Zhang W, Sainte-Marie Y, et al. (2008) Cardiomyocyte overexpression of neuronal nitric-oxide synthase delays transition toward heart failure in response to pressure overload by preserving calcium cycling. *Circulation* **117**:3187–3198.
- Mei Q and Liang BT (2001) P2 purinergic receptor activation enhances cardiac contractility in isolated rat and mouse hearts. *Am J Physiol Heart Circ Physiol* **281**:H334–H341.
- Miyamoto S, Kawamura T, Morimoto T, Ono K, Wada H, Kawase Y, Matsumori A, Nishio R, Kita T, and Hasegawa K (2006) Histone acetyltransferase activity of p300 is required for the promotion of left ventricular remodeling after myocardial infarction in adult mice in vivo. *Circulation* **113**:679–690.
- Morimoto H, Takahashi M, Izawa A, Ise H, Hongo M, Kolattukudy PE, and Ikeda U (2006) Cardiac overexpression of monocyte chemoattractant protein-1 in transgenic mice prevents cardiac dysfunction and remodeling after myocardial infarction. *Circ Res* **99**:891–899.
- Niizeki T, Takeishi Y, Arimoto T, Takahashi H, Shishido T, Koyama Y, Goto K, Walsh RA, and Kubota I (2007) Cardiac-specific overexpression of diacylglycerol kinase ζ attenuates left ventricular remodeling and improves survival after myocardial infarction. *Am J Physiol Heart Circ Physiol* **292**:H1105–H1112.
- North RA (2002) Molecular physiology of P2X receptors. *Physiol Rev* **82**:1013–1067.
- O'Rourke B, Kass DA, Tomaselli GF, Käb S, Tunin R, and Marbán E (1999) Mechanisms of altered excitation-contraction coupling in canine tachycardia-induced heart failure. I: Experimental studies. *Circ Res* **84**:562–570.
- Osorio JC, Stanley WC, Linke A, Castellari M, Diep QN, Panchal AR, Hintze TH, Lopaschuk GD, and Recchia FA (2002) Impaired myocardial fatty acid oxidation and reduced protein expression of retinoid X receptor- α in pacing-induced heart failure. *Circulation* **106**:606–612.
- Podrasky E, Xu D, and Liang BT (1997) A novel phospholipase C- and cyclic AMP-independent positive inotropic mechanism via the P2 purinergic receptor in cardiac ventricular myocyte. *Am J Physiol Heart Circ Physiol* **273**:H2380–H2387.
- Ravi RG, Kim HS, Servos J, Zimmermann H, Lee K, Maddileti S, Boyer JL, Harden TK, and Jacobson KA (2002) Adenine nucleotides analogues locked in a Northern methanocarpa conformation: enhanced stability and potency as P2Y₁ receptor agonists. *J Med Chem* **45**:2090–2100.
- Recchia FA, McConnell PI, Bernstein RD, Vogel TR, Xu X, and Hintze TH (1998) Reduced nitric-oxide production and altered myocardial metabolism during the decompensation of pacing-induced heart failure in the conscious dog. *Circ Res* **83**:969–979.
- Shannon RP, Friedrich S, Mathier M, and Knight DR (1997) Effects of renin inhibition compared to angiotensin-converting enzyme inhibition in conscious dogs with pacing-induced heart failure. *Cardiovasc Res* **34**:464–472.
- Shen J-B, Pappano AJ, and Liang BT (2006) Extracellular ATP-stimulated current in wild-type and P2X₄ receptor transgenic mouse ventricular myocytes: implications for a cardiac physiologic role of P2X₄ receptors. *FASEB J* **20**:277–284.
- Shen JB, Cronin C, Sonin D, Joshi BV, Nieto M, Harrison D, Jacobson KA, and Liang BT (2007) P2X purinergic receptor-mediated ionic current in cardiac myocytes of calsequestrin model of cardiomyopathy. Implications for the treatment of heart failure. *Am J Physiol Heart Circ Physiol* **292**:H1077–H1084.
- Shen JB, Shutt R, Agosto M, Pappano A, and Liang BT (2009) Reversal of cardiac myocyte dysfunction as a unique mechanism of rescue by P2X₄ receptors in cardiomyopathy. *Am J Physiol Heart Circ Physiol* **296**:H1089–H1095.
- Sonin D, Zhou S-Y, Cronin C, Sonina T, Wu J, Jacobson KA, Pappano A, and Liang BT (2008) Role of P2X purinergic receptors in the rescue of ischemic heart failure. *Am J Physiol Heart Circ Physiol* **295**:H1191–H1197.
- Spinale FG, Coker ML, Thomas CV, Walker JD, Mukherjee R, and Hebbard L (1998) Time-dependent changes in matrix metalloproteinase activity and expression during the progression of congestive heart failure: relation to ventricular and myocyte function. *Circ Res* **82**:482–495.
- Suarez J, Gloss B, Belke DD, Hu Y, Scott B, Dieterle T, Kim YK, Valencik ML, McDonald JA, and Dillmann WH (2004) Doxycycline-inducible expression of SERCA2a improves calcium handling and reverts cardiac dysfunction in pressure overload-induced cardiac hypertrophy. *Am J Physiol Heart Circ Physiol* **287**:H2164–H2172.
- Trochu JN, Mital S, Zhang X, Xu X, Ochoa M, Liao JK, Recchia FA, and Hintze TH (2003) Preservation of NO production by statins in the treatment of heart failure. *Cardiovasc Res* **60**:250–258.
- Tsoporis JN, Marks A, Haddad A, Dawood F, Liu PP, and Parker TG (2005) S100B expression modulates left ventricular remodeling after myocardial infarction in mice. *Circulation* **111**:598–606.
- Yang A, Sonin D, Jones L, and Liang BT (2004) A beneficial role of cardiac P2X₄ receptors in heart failure: rescuing the calsequestrin-overexpression model of cardiomyopathy. *Am J Physiol Heart Circ Physiol* **287**:H1096–H1103.
- Zhang FL, Luo L, Gustafson E, Lachowicz J, Smith M, Qiao X, Liu Y-H, Chen G, Pramanik B, Laz TM, et al. (2001) ADP is the cognate ligand for the orphan G protein-coupled receptor SP1999. *J Biol Chem* **276**:8608–8615.

Address correspondence to: Dr. Bruce T. Liang, The Pat and Jim Calhoun Cardiology Center, MC-3946, University of Connecticut Health Center, 263 Farmington Avenue, Farmington, CT 06030. E-mail: bliang@uchc.edu