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Vasopressin type 1A receptor deletion enhances cardiac contractility, β -adrenergic receptor sensitivity and acute cardiac injury-induced dysfunction

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Abstract

Vasopressin type 1A receptor (V1AR) expression is elevated in chronic human heart failure (HF) and contributes to cardiac dysfunction in animal models, in part via reduced β -adrenergic receptor (β AR) responsiveness. Although cardiac V1AR overexpression and V1AR stimulation are each sufficient to decrease β AR activity, it is unknown whether V1AR inhibition conversely augments β AR responsiveness. Further, although V1AR has been shown to contribute to chronic progression of HF, its impact on cardiac function following acute ischaemic injury has not been reported. Using V1AR knockout (V1AR KO) mice we assessed the impact of V1AR deletion on cardiac contractility at baseline and following ischaemic injury, β AR sensitivity and cardiomyocyte responsiveness to β AR stimulation. Strikingly, baseline cardiac contractility was enhanced in V1AR KO mice and they experienced a greater loss in contractile function than control mice following acute ischaemic injury, although the absolute levels of cardiac dysfunction and survival rates did not differ. Enhanced cardiac contractility in V1AR KO mice was associated with augmented β -blocker sensitivity, suggesting increased basal β AR activity, and indeed levels of left ventricular cAMP, as well as phospholamban (PLB) and cardiac troponin I (cTnI) phosphorylation were elevated compared with control mice. At the cellular level, myocytes isolated from V1AR KO mice demonstrated increased responsiveness to β AR stimulation consistent with the finding that acute pharmacological V1AR inhibition enhanced β AR-mediated contractility in control myocytes. Therefore, although V1AR deletion does not protect the heart from the rapid

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AUTHOR CONTRIBUTION

Melissa Wasilewski, Laurel Grisanti, Jianliang Song, Rhonda Carter, Ashley Repas, Valerie Myers and Erhe Gao participated in either the conception and design of the work and/or acquisition, analysis and interpretation of the data. Walter Koch, Joseph Cheung, Arthur Feldman and Douglas Tilley participated in the conception of the work, provided material, scientific expertise and writing of the manuscript.

DECLARATIONS OF INTEREST

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development of cardiac dysfunction following acute ischaemic injury, its effects on β AR activity suggest that acute V1AR inhibition could be utilized to promote myocyte contractile performance.

Keywords

β AR; cardiac contractility; cardiomyocyte; myocardial infarction; V1AR

INTRODUCTION

Heart failure (HF) secondary to reduced ejection fraction (HFrEF) affects over 6 million individuals in the U.S. and costs the healthcare economy over \$60 billion per year [1]. Approximately 70 % of HFrEF in the U.S. is due to ischaemic heart disease [2]. The development of ventricular dysfunction in response to either acute ischaemic injury of chronic ongoing ischaemia is mediated in part by the release of neurohormones and their action at specific G protein-coupled receptor (GPCR) targets. Arginine vasopressin (AVP) is one such neurohormone whose level is elevated early following a myocardial infarction (MI) in humans [3], though its impact on cardiac function following acute injury is unclear.

In the heart, AVP acts at its type 1A receptor (V1AR), which has recently garnered attention due to the finding that V1AR levels are increased in small (mouse) and large (dog) animal models of HF, as well as in human HF [4–6]. Our previously published studies in mice showed that: (1) cardiac-specific transgenic overexpression of V1AR was sufficient on its own to induce cardiomyopathy over a period of months [7] and (2) mice that underwent transaortic constriction-induced pressure overload were prevented from developing cardiac dysfunction and β -adrenergic receptor (β AR) down-regulation by infusion of SR 49059, a V1AR-selective blocker [8]. Further, we previously reported that although activation of endogenous V1AR in isolated mouse heart preparations did not affect cardiac contractility on its own, it did desensitize β AR responsiveness to catecholamine stimulation [8], therefore inhibition of V1AR signalling could act to enhance β AR-mediated contractility. However, *in vivo* studies investigating the impact of V1AR following acute cardiac injury are scarce and conflicting. For instance, two independent studies using a rat model of MI reported opposing effects of chronic administration of a V1AR antagonist beginning one day post-MI [9,10]. Therefore, a definitive role for V1AR in the regulation of cardiac contractility either normally or following cardiac injury is lacking. Herein, using V1AR knockout (V1AR KO) mice, we sought to determine whether complete V1AR deletion results in altered cardiac contractile parameters and sensitivity to β AR activation under resting conditions or following acute cardiac injury.

MATERIALS AND METHODS

Animals

All experiments were performed following the National Institutes of Health (NIH) Guide for the Care and Use of Laboratory Animals, and were approved by the Temple University IACUC. 8–12-week-old male and female wild-type (WT) and V1AR KO mice, attained

from and previously described by [11] and back-crossed on to a 25% C57BL/6, 75% FVB background, were used as indicated.

Echocardiography

Left ventricular (LV) function was evaluated using the VisualSonics Vevo 2100 imaging system and a 30 MHz (MS400) transducer. After mice were sedated with isoflurane (1.5 %) with heart rates (HRs) stabilized at approximately 500 beats/min, a parasternal short-axis view was obtained for LV M-mode imaging at the papillary muscle level. M-mode images were used for measurements of LV internal dimension (LVID), LV volume (LV Vol) and LV posterior wall thickness (LVPW) at diastole (d) and systole (s) in two separate images for two to three consecutive beats. Ejection fraction (EF) was calculated as $\%EF = [(LV\ Vol\ d - LV\ Vol\ s)/LV\ Vol\ d] \times 100\ %$ and fractional shortening (FS) was calculated as $\%FS = [(LVEDD - LVESD)/LVEDD] \times 100\ %$. β AR responsiveness was examined by echocardiography at baseline and in response to metoprolol (MET, 50 ng IP), where measurements were analysed at the time of peak change in HR in response to MET.

Myocardial infarction (MI) surgery

MI was produced as previously described [12]. Briefly, isoflurane (2.5 %) was used as general anaesthesia during surgery. Hearts of WT and V1AR KO mice were exposed through thoracotomy, a suture ligation was placed in the distal half of the left descending coronary artery, and hearts were immediately placed back into the chest. Sham operations were performed in the same manner but without ligation to provide controls. After surgery, animals were monitored until recovery from anaesthesia, and given 0.1 mg/kg buprenorphine for analgesia every 12 h as needed. Mice were monitored at least every 24 h after surgery for signs of pain or distress such as inability to move or eat, difficulty breathing, and matted or dull hair.

Assessment of left ventricular infarct size

Hearts were excised 24 h following surgery and sliced into five 1.0 mm thick sections. The sections were incubated with 1 % triphenyltetrazolium chloride (TTC; Sigma) in phosphate buffered saline (PBS; pH 7.4) for 5 min at 37 °C. Slices were immediately imaged for infarct size using NIS Elements software for recording images and image analysis. Myocardial infarct size was quantified by measuring TTC-stained area (red) and TTC-negative stained area (white; infarcted myocardium) and was expressed as a percentage of the total LV area.

Radioligand binding

Membrane preparations from heart were prepared by homogenization in ice-cold lysis buffer (25 mM Tris pH 7.4, 5 mM EDTA, 1 μ g/ml Aprotinin, 1 μ g/ml Leupeptin) and centrifuged at $1000 \times g$ for 5 min at 4 °C. The supernatant was centrifuged at $30000 \times g$ and the crude membrane pellet was resuspended in lysis buffer containing 10 % glycerol and stored at -80 °C until use. It was determined that the density of β AR adds V1AR on membranes by saturation binding experiments. Membrane preparations (25 μ g of protein for β AR, 40 μ g of protein for V1AR) were incubated with 125 I-cyanopindolol (CYP, 200 pM; PerkinElmer) or 125 I-p-AVP (150 pM; PerkinElmer) in binding buffer (75 mM Tris pH 7.4, 2 mM EDTA,

12.5 mM MgCl₂, 1 µg/ml Aprotinin, 1 µg/ml Leupeptin). Incubations were performed in the presence or absence of propranolol (non-specific β AR antagonist, 10 µM) or SR 49059 (V1AR-selective antagonist, 5 µM) to determine non-specific binding in 250 µl volume and allowed to equilibrate at 37 °C for 1 h prior to filtering the membranes through a Whatman glass fibre filter (Whatman GF/C, Brandel). Each filter was washed five times with 5 ml of ice-cold wash buffer (10 mM Tris pH 7.4, 10 mM EDTA) to remove unbound ligand. The amount of total and non-specific radiolabel bound to cell membranes was determined in a gamma counter. All assays were performed in triplicate and receptor density was normalized to mg of membrane protein.

Measurement of cAMP

Left ventricular samples were collected, weighed and flash frozen in liquid nitrogen. These were subsequently homogenized in a 10× volume of 0.1 M HCl for a final concentration of 0.1 g/ml. cAMP levels in the samples were measured and compared with known standards using the cAMP Complete ELISA kit (Enzo Life Sciences) following manufacturer's protocol. A Tecan M1000 multimode plate reader was used to read the absorbance of the samples at 405 nm and Prism 5.0 software (GraphPad Software) was used to calculate the concentration of cAMP in the samples via interpolation from the linear regression of the standard curve.

Immunoblotting

Left ventricular samples were homogenized in lysis buffer containing 20 mM Tris (pH 7.4), 137 mM NaCl, 10 % glycerol, 1 mM EDTA, 1 % NP-40, 10 mM NaF (Fisher Scientific), 1× HALT protease inhibitor cocktail (Thermo Scientific) and phosphatase inhibitor cocktail set IV (Calbiochem). Lysates were run on SDS/15 % PAGE gels and transferred to Immobilon-PSQ polyvinylidene fluoride 0.2 mm pore size membranes (Millipore), which were then incubated with anti-Phospho-Ser-16 phospholamban (PLB) rabbit pAb (1:5000, Badrilla), anti-PLB mouse mAb (1:1000, Badrilla), anti-Phospho-Ser-23/24 cTnI rabbit pAb (1:1000, Cell Signaling Technology) or anti-cTnI rabbit pAb (1:1000, Cell Signaling Technology). Membranes were subsequently incubated with appropriate anti-rabbit or anti-mouse IRDye (680 or 800)-labelled antibodies and detected using the LiCOR Biosciences Odyssey system. Phosphorylation data are calculated as phospho-protein/total protein and normalized to WT.

Isolation of adult murine cardiac myocytes and contractility measurements

Adult murine cardiac myocytes were isolated from the septum and LV free wall of 8–12 week old mice. Briefly, mice were heparinized (1500 U/kg IP) and anesthetized (pentobarbital sodium, 50 mg/kg IP). Excised hearts were mounted on a steel cannula and retrograde perfused (100 cm H₂O, 37 °C) with Ca²⁺-free bicarbonate buffer followed by enzymatic digestion (collagenases B and D, protease XIV). Isolated myocytes were plated on laminin-coated glass coverslips, and the Ca²⁺ concentration of the buffer was incrementally increased (0.05, 0.125, 0.25, 0.5 mM) with 10 min of exposure at each concentration. The final Ca²⁺ buffer was then aspirated and replaced with MEM (Sigma–Aldrich) containing 1.2 mM Ca²⁺, 2.5 % FBS and 1 % penicillin/streptomycin. The pH was adjusted to 7.0 in 4 % CO₂ by the addition of NaHCO₃ (0.57 g/l). After 1 h (4 % CO₂,

37 °C), medium was replaced with FBS-free MEM containing 0.1 mg/ml BSA and antibiotics. Myocytes adherent to coverslips were bathed in 0.7 ml of air- and temperature-equilibrated (37 °C), HEPES-buffered (20 mM, pH 7.4) medium 199 containing 1.8 mM [Ca²⁺] and used within 2–8 h of isolation. Measurements of myocyte contraction at a pacing frequency of 2 Hz were performed continuously at baseline and up to 3 min after the addition of isoproterenol (ISO, 0.1 μ M). For V1AR inhibitor experiments, cardiomyocytes were pretreated with either vehicle (0.1 % DMSO) or SR 49059 (1 μ M) for 2 min prior to ISO stimulation. Responses were attained from three cardiomyocytes per genotype/drug treatment per isolation, with three independent mouse heart isolations per condition performed.

Statistical analysis

Data are presented as mean $\pm$ standard error of the mean (S.E.M.). Statistical analyses were performed using either a two-tailed unpaired *t* test, a one-way analysis of variance (ANOVA) with Tukey's multiple comparison post-test, a two-way ANOVA followed by a Bonferroni's repeated measures comparisons test or a log-rank (Mantel–Cox) test using Prism 5.0 software (GraphPad Software), as indicated.

RESULTS

V1AR KO mice have enhanced cardiac contractility and experience a greater loss of cardiac function after acute MI

To determine the impact of V1AR deletion on cardiac function, we performed echocardiography in WT and V1AR KO mice (Figure 1A). Compared with WT mice, the V1AR KO mice had significantly different contractile parameters at baseline (Supplementary Table S1). Both EF (Figure 1B) and FS (Figure 1C) were increased in V1AR KO mice compared with WT mice. Additionally, both diastolic (Figure 1D) and systolic (Table 1) LVID were significantly reduced in V1AR KO mice, with corresponding decreases in diastolic (Figure 1E) and systolic (Supplementary Table S1) LV Vol. Neither LV wall thickness (Table 1) nor gross morphology (Supplementary Figure S1) were different between WT and V1AR KO mouse hearts (Supplementary Table S1), suggesting that enhanced contractility in V1AR KO mice is not secondary to cardiac hypertrophy.

In order to investigate the impact of V1AR deletion in the response to acute myocardial injury, WT and V1AR KO mice underwent proximal left anterior descending coronary artery ligation (MI) or sham surgery. Cardiac function was evaluated by echocardiography both before and two days after surgery to evaluate early progress toward cardiac dysfunction (Figure 2A). As expected, V1AR KO mice displayed enhanced contractility at baseline compared with their WT counterparts, but following MI both WT and V1AR KO mice displayed similar cardiac function (Table 1). Although post-MI function was similar in both lines, since the V1AR KO mice had enhanced contractility to begin with, they experienced more dramatic changes in function in response to injury (Table 1), including EF (Figure 2B) and LVID at diastole (Figure 2C). The difference in loss of contractile function between WT and V1AR KO mice was not due to differences in surgery as both cohorts experienced the same degree of initial infarct size (Figure 2D and Supplementary Figure S1). To determine if

these acute changes in cardiac function are maintained over time, we monitored cardiac function at both 1 and 2 weeks post-MI. As was observed 2 days post-MI, V1AR KO mice experienced greater overall changes in cardiac function as measured via echocardiography at 1 and 2 weeks post-MI, but both WT and V1AR KO experienced reductions in cardiac function to final levels that were not different (Figure 2E, Table 2). The lack of functional differences after cardiac injury were reflected in survival rates of the mice, where 70–80 % of both mouse lines died within 1 week of MI (Figure 2F), with no significant difference between them.

V1AR KO mice have increased β AR activity and sensitivity to β -blockade

Since we previously showed V1AR can repress β AR signalling [8], we hypothesized that the increased cardiac contractility observed in V1AR KO mice may be due to enhanced β AR activity. Thus, we next performed ^{125}I -p-AVP and ^{125}I -cyanopindolol binding on WT and V1AR KO cardiac membranes to determine whether increased β AR density in the absence of V1AR accounts for the enhanced contractility. Although V1AR expression was confirmed to be ablated in the KO mice (Figure 3A), β AR expression was detected at similar levels in both lines (Figure 3B). Although β AR expression was unaltered in V1AR KO hearts, downstream β AR signalling could be enhanced in the absence of V1AR-mediated repression, therefore we measured levels of cAMP from LV homogenates and found that levels of the second messenger were indeed elevated in V1AR KO hearts (Figure 3C). Since elevated cAMP levels could increase PKA-mediated phosphorylation of proteins involved in the cardiac contractile cycle, we compared levels of PLB phosphorylation at the PKA-sensitive Ser-16 site and cardiac troponin I (cTnI) phosphorylation at the PKA-sensitive Ser-23/24 sites in WT and KO cardiac lysates. As observed with cAMP, basal P-PLB and P-cTnI levels were each found to be significantly elevated in V1AR KO hearts compared with WT hearts (Figure 3D). Thus, although β AR expression does not differ between WT and V1AR KO mice, β AR activity appears to be enhanced in V1AR KO hearts, thereby increasing basal cardiac contractility.

To functionally assess whether the absence of V1AR expression increases β AR sensitivity, cardiac function of WT and V1AR KO mice was examined by echocardiography at baseline and in response to acute administration of the β -blocker MET (50 ng IP). Echocardiography measurements were analysed at the time of peak HR responses to MET (Table 3). Indeed, MET effectively decreased cardiac contractility in both WT and V1AR KO mice (Figure 3E), with the MET-induced change in LVID d (Figure 3F) and LV Vol d (Figure 3G) being significantly different between the two groups, indicating that V1AR KO mice have increased sensitivity to β -blockers.

V1AR deletion or inhibition enhances β AR responsiveness in cardiomyocytes

Although our data suggest that cardiac β AR responsiveness is increased in the absence of V1AR, the use of global KO mice limits our ability to dissect this effect *in vivo* as V1AR is normally expressed in numerous tissues such as the CNS and vasculature that could affect cardiovascular function [13–16]. Thus, to determine whether enhanced cardiac β AR responsiveness in the absence of V1AR expression indeed occurs at the level of individual cardiomyocytes, we next evaluated contractility in isolated cardiomyocytes in response to

ISO (0.1 μM). Cardiomyocytes from both WT and V1AR KO mice displayed similar contractility at baseline (Figure 4A), however upon ISO stimulation, V1AR KO cardiomyocytes displayed significantly enhanced shortening compared with WT cardiomyocytes. The enhanced βAR -mediated contractility in V1AR KO cardiomyocytes was not further sensitive to pretreatment with the V1AR-selective antagonist SR 49059 (1 μM) (Figure 4B). Finally, to determine if acute V1AR blockade in WT cardiomyocytes could recapitulate these findings, we pretreated them with vehicle (0.1 % DMSO) or SR 49059 (1 μM) prior to ISO stimulation. Similar to responses observed in cardiomyocytes isolated from V1AR KO hearts, WT cardiomyocytes pretreated with SR 49059 displayed an enhanced response to ISO stimulation (Figure 4C), confirming that deficiency of V1AR itself in V1AR KO cardiomyocytes is directly responsible for enhanced βAR responsiveness.

DISCUSSION

V1AR studies to date have provided conflicting information regarding its impact on cardiac function normally and following injury. Physiologically, AVP depressed cardiac function both in conscious dogs [17] and conscious rabbits, with increased AVP sensitivity in response to experimental HF in the rabbits [18]. Although these responses may be secondary to increased after-load, AVP was shown to elicit a biphasic contractile response in *ex vivo* rat hearts [19] and a slight reduction in contractility in *ex vivo* mouse hearts [8]. Previously, V1AR KO mice were reported to have reduced blood pressure [11] and reduced hypertrophy 1 week after transaortic constriction (TAC) [20], whereas V1AR mRNA expression was shown to increase in a hypertensive rat model of HF [21]. Consistent with these studies, cardiomyocyte-restricted ($\alpha\text{MHC-tTA}$ driven) V1AR transgenic (V1ARTg) mice, with five times the expression of endogenous V1AR, developed LV hypertrophy, dilatation, reduced contractile performance and fetal gene reprogramming with progressive aging [7]. In addition, we reported that cardiac V1AR expression is increased 2-fold in human HF [6], as well as in mouse [8] and dog [5] models of HF, and that chronic V1AR inhibition during transaortic constriction (TAC) restored V1AR expression and cardiac function in mice [8].

Despite the growing evidence for beneficial effects of V1AR inhibition in different models of HF, there is a paucity of information about the role of V1AR in the response to acute cardiac injury, and although we showed V1AR stimulation *in vitro* engaged protective signalling events against hypoxic injury [22], *in vivo* reports are lacking. A study in sheep showed that V1AR antagonism just after MI had no effect on cardiac haemodynamics [23], whereas two independent studies using a rat model of MI reported opposing effects of chronic administration of a V1AR antagonist beginning one day post-MI on HF development [9,10]. Here, using V1AR KO mice we have shown that V1AR deletion is unable to reduce infarct size, absolute contractile dysfunction or long-term mortality in response to acute cardiac injury. Thus, although V1AR inhibition has been shown to protect the heart against the development of hypertrophic HF [8], its role in the acute response to cardiac ischaemia may be limited. Of note, we observed a higher level of mortality (70–80 %) following MI in our mice on a 25 % C57BL/6, 75 % FVB background than that of pure C57BL/6 mice, in which we routinely observe between 5 and 20 % [12]. Since genetic background is known to relay differing degrees of sensitivity to cardiac injury [24,25] our

results may reflect strain-dependent susceptibility, however since there was no difference in mortality rates between V1AR KO and WT mice, V1AR deletion clearly did not offer a survival advantage at least within 2 weeks post-MI.

The most striking finding from our study is the significant enhancement of cardiac contractility in V1AR KO mice mediated by increased β AR responsiveness. Although a previous study comparing WT and V1AR KO mice reported no significant changes in baseline cardiac contractile parameters via echocardiography [11], the WT contractile parameters in their study were much higher than ours, which could possibly be due to strain differences (129Sv/C57BL/6 compared with C57BL/6/FVB) or technique. However, the echocardiography data in their study did show trends toward decreased left ventricular dimensions in V1AR KO mice as we observed in our study. We also demonstrated that the alterations in contractile parameters we observed in V1AR KO mice, including LV dimension, were sensitive to β -blockade, supporting our previous observations that cardiomyocyte-specific V1AR overexpression reduces cardiac contractility [7], and V1AR stimulation of whole hearts or isolated cardiomyocytes reduces β AR responsiveness [8].

Although a limitation of our current study is the use of a global V1AR KO mouse in the absence of a cell type-specific V1AR KO mouse, cardiomyocytes isolated from V1AR KO hearts exhibited greater contractile response to β AR stimulation, confirming that the enhanced contractile phenotype indeed occurs via changes within the cardiomyocytes themselves. Furthermore, when V1AR was inhibited with SR 49 059 in WT cardiomyocytes, β AR responsiveness was significantly accentuated suggesting a direct relationship between V1AR deletion and enhanced responsiveness to β AR stimulation in V1AR KO cardiomyocytes. Of note, although V1AR KO hearts have enhanced basal contractility, the baseline contractility of cardiomyocytes isolated from V1AR KO compared with WT hearts was not different. We surmise that this may be due to a lack of direct cardiac innervation in the *in vitro* system, resulting in an absence of basal catecholamine stimulation. Indeed, the enhanced levels of cAMP, PLB and cTnI phosphorylation and contractility observed at baseline in V1AR KO mouse hearts would suggest that V1AR normally represses β AR responsiveness to intrinsic catecholamine stimulation. Further, since deletion or pharmacologic blockade of V1AR enhances isolated cardiomyocyte contractility when treated with ISO, our results suggest that V1AR may itself have intrinsic activity that can be antagonized to improve β AR responsiveness to endogenous catecholamine.

In summary, we have shown that genetic deletion of V1AR is sufficient to enhance β AR responsiveness and baseline cardiac contractility. This builds upon our previous work showing that cardiac-specific overexpression of V1AR leads to decreased β AR responsiveness, whereas V1AR inhibition during HF development prevented cardiac β AR down-regulation [7,8]. Although V1AR deletion did not confer protection against acute cardiac dysfunction or survival following cardiac ischaemia, our data illustrate the ability of V1AR inhibition to enhance β AR responsiveness. Such a strategy could be helpful to provide indirect inotropic support in established HF when β AR activity and cardiac contractility are severely reduced. Since we previously reported that the expression ratios of cardiac-expressed V1AR to β AR changed from 1:3 in normal hearts to 1:1 after HF

development [8], the relative impact of V1AR on β AR responsiveness may be more pronounced after HF is manifested. Overall, since both HF and V1AR signalling contribute to β AR desensitization, reduced or absent V1AR signalling could provide some degree of relief of β AR desensitization to enhance acute inotropic therapy.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Abbreviations

AVP	arginine vasopressin
βAR	β -adrenergic receptor
CO	cardiac output
cTnI	cardiac troponin I
EF	ejection fraction
FS	fractional shortening
HF	heart failure
HFrEF	heart failure secondary to reduced ejection fraction
HR	heart rate
ISO	isoproterenol
KO	knockout
LV Vol	left ventricular volume
LVID	left ventricular internal dimension
LVPW	left ventricular posterior wall thickness
MET	metoprolol
MI	myocardial infarction
PLB	phospholamban
SV	stroke volume
TTC	triphenyltetrazolium chloride

V1AR	vasopressin type 1A receptor
WT	wild-type

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CLINICAL PERSPECTIVES

- Cardiac V1AR expression is increased in small and large animal models of left ventricular dysfunction, as well as in patients with HFrEF, contributing to the progression of cardiac dysfunction and β AR desensitization. Further, acute V1AR stimulation negatively regulates β AR activity, resulting in reduced catecholamine-mediated contractility.
- We show that ablation of V1AR expression results in increased cardiac contractility and a greater loss in contractile function following acute ischaemic injury. Enhanced cardiac contractility in V1AR KO mice was associated with augmented β -blocker sensitivity and β AR activity. Myocytes isolated from V1AR KO mice, or control myocytes pretreated acutely with a V1AR antagonist, demonstrated increased responsiveness to β AR stimulation.
- Our results suggest that acute V1AR inhibition may offer a novel approach to enhancing cardiac contractile performance.

Figure 1. V1AR KO mice have enhanced cardiac contractility

(A) Representative short-axis M-mode echocardiography images of WT and V1AR KO mouse hearts at baseline. (B–E) Quantification of contractile parameters of WT ($n = 15$) and V1AR KO ($n = 18$) mice at baseline, including %EF (B), %FS (C), diastolic left ventricular internal diameter (LVID, D) and diastolic LV Vol (E); *** $P < 0.001$, two-tailed unpaired t test.

Figure 2. V1AR KO mice experience a greater loss of cardiac function after acute MI
 (A) Representative short-axis M-mode echocardiography images of WT and V1AR KO mouse hearts before and 2 days post-MI. Comparison of MI-induced changes in %EF (B) and LVID (C) at diastole in WT ($n = 6$) and V1AR KO ($n = 12$) mouse hearts; * $P < 0.05$, two-tailed unpaired t test. (D) Infarct size measured 24 h post-MI was not significantly different between WT and V1AR KO mouse hearts ($n = 4$ each). (E) Comparison of MI-induced changes in %EF in WT ($n = 6$) and V1AR KO ($n = 10$) mouse hearts after 1 and 2 weeks; ** $P < 0.01$, one-way ANOVA with Tukey's multiple comparison test. (F) Kaplan-Meier survival curves of WT and V1AR KO mice after MI surgery; ns = not significant, log-rank (Mantel-Cox) test. WT and V1AR KO sham-operated mice had 100 % survival and are not shown.

Figure 3. V1AR KO mice have increased β AR activity and sensitivity to β -blockade
 125 I-p-AVP (A, $n = 9$ WT and $n = 13$ V1AR KO) and 125 I-cyanopindolol (B, $n = 7$ each) binding on cardiac membranes from WT and V1AR KO mice; ns = not significant, *** $P < 0.001$, two-tailed unpaired t test. (C) cAMP levels measured in LV samples from WT ($n = 5$) and V1AR KO ($n = 6$) mice; ** $P < 0.001$, two-tailed unpaired t test. (D) Immunoblots for phosphorylated (P-Ser-16) and total PLB, as well as phosphorylated (P-Ser-23/24) and total cTnI, in LV lysates of WT ($n = 7$) and V1AR KO ($n = 8$) mouse hearts, with data summarized in the bar graphs to the right; * $P < 0.05$, *** $P < 0.001$ two-tailed unpaired t test. (E) Representative short-axis M-mode echocardiography images of WT and V1AR KO mouse hearts at baseline and following IP injection with MET (50 ng). Comparison of MET-induced changes in LVID (F) and LV Vol (G) at diastole in WT and V1AR KO mouse hearts ($n = 9$ each); * $P < 0.05$, two-tailed unpaired t test.

Figure 4. V1AR deletion or inhibition enhances β AR responsiveness in cardiomyocytes
(A) Adult mouse LV cardiomyocytes were isolated from WT and V1AR KO mice and representative tracings of cardiomyocyte length attained at baseline (dashed line) and after stimulation with ISO (0.1 μ M, solid line) with quantification of cardiomyocyte shortening (%) shown in bar graph to the right; *** P < 0.001, two-way repeated measures ANOVA with Bonferroni post-test. Similarly, adult mouse LV cardiomyocytes were isolated from either V1AR KO **(B)** or WT **(C)** mice and pretreated with DMSO (0.1 %) or SR 49059 (1 μ M) 2 min prior to stimulation with ISO (0.1 μ M). Representative tracings are shown with quantification of cardiomyocyte shortening (%) shown in bar graphs to the right; ** P < 0.01, *** P < 0.001, two-way repeated measures ANOVA with Bonferroni post-test. ns, not significant.

Table 1
Echocardiographic measurements of WT and V1AR KO mice at baseline and 2 days post-MI

Measurement	WT sham (<i>n</i> = 10)			WT MI (<i>n</i> = 6)		
	Baseline	Day 2	Baseline	Day 2	(Day 2 - Base)	
LVID d (mm)	3.66 ± 0.13	3.41 ± 0.12	3.53 ± 0.09	3.83 ± 0.14	0.30 ± 0.22	
LVID s (mm)	2.50 ± 0.13	2.35 ± 0.15	2.30 ± 0.13	3.50 ± 0.15 ^{***}	1.19 ± 0.24	
LVPW d (mm)	0.85 ± 0.06	1.02 ± 0.08	0.83 ± 0.06	0.69 ± 0.04 ^{**}	-0.15 ± 0.09	
LVPW s (mm)	1.25 ± 0.04	1.31 ± 0.07	1.25 ± 0.07	0.75 ± 0.04 ^{***}	-0.50 ± 0.08	
CO (ml/min)	19.59 ± 3.34	14.25 ± 2.34	15.75 ± 0.84	6.03 ± 1.39	-9.72 ± 1.47	
EF (%)	60.01 ± 3.09	59.83 ± 3.41	64.79 ± 3.34	19.69 ± 2.06 ^{***}	-45.09 ± 3.95	
FS (%)	31.73 ± 2.22	31.45 ± 2.28	34.90 ± 2.35	8.76 ± 0.95 ^{***}	-26.14 ± 2.56	
LV Vol d (μl)	57.45 ± 4.72	48.47 ± 4.08	52.00 ± 3.21	63.57 ± 4.98	11.57 ± 7.38	
LV Vol s (μl)	23.34 ± 2.67	20.41 ± 3.16	18.64 ± 2.63	51.43 ± 4.71 ^{**}	32.78 ± 6.50	
SV (μl)	34.16 ± 2.76	27.99 ± 1.81	33.32 ± 1.75	9.58 ± 1.40 ^{***}	-23.75 ± 2.51	
Measurement	V1AR KO sham (<i>n</i> = 6)			V1AR KO MI (<i>n</i> = 12)		
	Baseline	Day 2	Baseline	Day 2	(Day 2 - base)	
LVID d (mm)	3.13 ± 0.14	3.06 ± 0.13	3.02 ± 0.11	4.34 ± 0.21 ^{***}	1.32 ± 0.24 ^a	
LVID s (mm)	1.91 ± 0.19 [†]	1.84 ± 0.15	1.72 ± 0.11 [†]	3.97 ± 0.20 ^{***}	2.25 ± 0.74 ^{da}	
LVPW d (mm)	0.84 ± 0.70	1.09 ± 0.12	0.80 ± 0.05	0.56 ± 0.03 ^{***}	-0.24 ± 0.17	
LVPW s (mm)	1.18 ± 0.09	1.46 ± 0.08	1.26 ± 0.05	0.63 ± 0.03 ^{***}	-0.63 ± 0.20	
CO (ml/min)	25.61 ± 2.31	18.61 ± 1.76	22.31 ± 2.36	10.44 ± 1.56	-11.87 ± 1.98	
EF (%)	70.48 ± 4.64 [†]	72.36 ± 3.21 [†]	75.25 ± 2.43 [†]	18.68 ± 2.02 ^{***}	-56.56 ± 2.47 ^a	
FS (%)	39.52 ± 3.74 [†]	40.56 ± 2.50 [†]	43.41 ± 2.31 [†]	8.43 ± 0.97 ^{***}	-34.98 ± 2.11 ^a	
LV Vol d (μl)	39.45 ± 4.42	37.41 ± 3.77	36.26 ± 3.22	88.18 ± 11.91 ^{***, †}	51.92 ± 12.35 ^a	
LV Vol s (μl)	12.24 ± 3.03	10.85 ± 2.18	9.43 ± 1.45	71.90 ± 10.40 ^{***, †}	62.47 ± 10.22	
SV (μl)	26.39 ± 2.47	26.68 ± 2.14	27.94 ± 1.93	13.72 ± 2.45 ^{***}	-14.22 ± 3.14	

^{**} *P* < 0.01,

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*** $P < 0.001$ compared with own MI baseline,

[†] $P < 0.05$ compared with corresponding WT sham timepoint,

[‡] $P < 0.05$ compared with corresponding WT MI timepoint, two-way repeated measures ANOVA with Bonferroni post-test.

^a $P < 0.05$,

^{aa} $P < 0.01$ compared with WT[†], two-tailed unpaired *t* test.

Table 2

Echocardiographic measurements of WT and VIAR KO mice at baseline, 1 and 2 weeks post-MI

Measurement	WT sham (<i>n</i> = 4)			WT MI (<i>n</i> = 6)		
	Baseline	Week 1	Week 2	Baseline	Week 1	Week 2
LVID d (mm)	3.58 ± 0.22	3.53 ± 0.19	3.59 ± 0.10	3.50 ± 0.09	4.68 ± 0.14 ^{***}	4.95 ± 0.17 ^{***}
LVID s (mm)	2.45 ± 0.29	2.33 ± 0.09	2.31 ± 0.13	2.59 ± 0.09	4.40 ± 0.14 ^{***}	4.68 ± 0.19 ^{***}
LVPW d (mm)	0.88 ± 0.03	0.91 ± 0.08	0.92 ± 0.06	0.80 ± 0.10	0.86 ± 0.06	0.77 ± 0.05
LVPW s (mm)	1.17 ± 0.05	1.17 ± 0.07	1.25 ± 0.10	1.08 ± 0.09	0.92 ± 0.04	0.83 ± 0.05 ^{**}
CO (ml/min)	17.55 ± 2.98	14.84 ± 2.63	15.82 ± 1.80	13.05 ± 0.90	7.51 ± 0.40 ^{**}	5.87 ± 0.69 ^{***}
EF (%)	59.53 ± 7.23	63.64 ± 2.33	65.57 ± 4.06	51.96 ± 1.91	13.59 ± 0.56 ^{***}	12.38 ± 1.65 ^{***}
FS (%)	31.82 ± 5.57	33.87 ± 1.84	35.55 ± 3.10	25.98 ± 1.20	6.03 ± 0.25 ^{***}	5.52 ± 0.75 ^{***}
LV Vol d (μl)	54.78 ± 8.59	52.64 ± 6.24	54.10 ± 3.80	51.19 ± 3.31	102.19 ± 7.42 ^{***}	116.39 ± 9.58 ^{***}
LV Vol s (μl)	22.77 ± 6.72	18.82 ± 1.78	18.64 ± 2.56	24.67 ± 2.13	88.42 ± 6.82 ^{***}	102.53 ± 10.14 ^{***}
SV (μl)	35.39 ± 4.35	31.39 ± 4.87	32.71 ± 4.02	26.96 ± 1.40	14.02 ± 1.29 ^{***}	12.12 ± 1.21 ^{***}

Measurement	VIAR KO sham (<i>n</i> = 7)			VIAR KO MI (<i>n</i> = 10)		
	Baseline	Week 1	Week 2	Baseline	Week 1	Week 2
LVID d (mm)	3.12 ± 0.10	3.22 ± 0.07	3.30 ± 0.13	2.87 ± 0.10 [‡]	4.50 ± 0.13 ^{***}	4.85 ± 0.13 ^{***}
LVID s (mm)	1.83 ± 0.12	1.86 ± 0.12	1.93 ± 0.17	1.63 ± 0.13 ^{†††}	4.16 ± 0.14 ^{***}	4.46 ± 0.15 ^{***}
LVPW d (mm)	0.99 ± 0.06	0.92 ± 0.07	0.92 ± 0.04	0.91 ± 0.03	0.64 ± 0.03 ^{*,‡}	0.69 ± 0.06 [*]
LVPW s (mm)	1.38 ± 0.10	1.32 ± 0.10	1.30 ± 0.08	1.26 ± 0.03	0.69 ± 0.04 ^{***}	0.75 ± 0.07 ^{***}
CO (ml/min)	13.07 ± 0.64	16.04 ± 0.97	17.20 ± 1.97	12.78 ± 0.93	7.48 ± 0.82 ^{***}	9.27 ± 0.70 ^{***}
EF (%)	73.55 ± 2.65	73.88 ± 3.14	73.02 ± 4.20	76.07 ± 2.88 ^{†††}	17.21 ± 1.52 ^{***}	17.92 ± 1.87 ^{***}
FS (%)	41.64 ± 2.26	42.20 ± 2.76	41.96 ± 3.67	44.01 ± 2.50 ^{†††}	7.72 ± 0.70 ^{***}	8.12 ± 0.88 ^{***}
LV Vol d (μl)	39.10 ± 3.08	41.65 ± 2.28	44.75 ± 4.23	32.23 ± 2.84	93.34 ± 6.08 ^{***}	111.20 ± 7.20 ^{***}
LV Vol s (μl)	10.67 ± 1.65	11.09 ± 1.82	12.59 ± 2.54	8.37 ± 1.73	77.92 ± 6.11 ^{***}	92.10 ± 7.43 ^{***}
SV (μl)	27.44 ± 1.54	31.22 ± 1.71	31.36 ± 2.38	24.93 ± 1.35	14.40 ± 0.86 ^{***}	18.25 ± 1.46 ^{***}

* $P < 0.05$,

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$P < 0.01$,

 $P < 0.001$ compared with corresponding sham timepoint,
*
 $P < 0.05$,
†
 $P < 0.001$ compared with corresponding WT MI timepoint, two-way repeated measures ANOVA with Bonferroni post-test,
†††

Table 3
Echocardiographic measurements of WT and V1AR KO mice before and after IP injection with MET

Measurement	WT (<i>n</i> = 9)			V1AR KO (<i>n</i> = 9)		
	Baseline	MET	(MET - Base)	Baseline	MET	(MET - Base)
LVID d (mm)	3.55 ± 0.10	3.90 ± 0.11	0.35 ± 0.05	3.14 ± 0.11 [*]	3.73 ± 0.09	0.59 ± 0.07 ^a
LVID s (mm)	2.53 ± 0.12	3.26 ± 0.12	0.73 ± 0.12	1.90 ± 0.12 ^{**}	2.84 ± 0.11 [†]	0.95 ± 0.11
LVPW d (mm)	0.72 ± 0.04	0.65 ± 0.04	-0.07 ± 0.02	0.89 ± 0.05 [*]	0.78 ± 0.04	-0.11 ± 0.05
LVPW s (mm)	1.04 ± 0.07	0.76 ± 0.05	-0.28 ± 0.06	1.28 ± 0.06 [*]	0.99 ± 0.05 [†]	-0.29 ± 0.05
CO (ml/min)	14.67 ± 1.01	8.40 ± 0.63	-6.27 ± 1.15	13.85 ± 0.83	9.40 ± 0.82	-4.46 ± 0.60
EF (%)	56.45 ± 2.98	34.93 ± 3.56	-21.52 ± 4.56	71.35 ± 2.66 ^{**}	48.45 ± 2.41 ^{††}	-22.91 ± 3.02
FS (%)	29.15 ± 2.07	16.62 ± 1.96	-12.53 ± 2.81	39.87 ± 2.22 ^{***}	24.05 ± 1.43 [†]	-15.83 ± 2.28
LV Vol d (μl)	53.25 ± 3.39	66.64 ± 4.29	13.39 ± 2.16	39.56 ± 3.56 [*]	59.82 ± 3.67	20.26 ± 2.32 ^a
LV Vol s (μl)	23.55 ± 2.50	43.52 ± 3.83	19.96 ± 3.35	11.76 ± 1.90 [*]	31.21 ± 2.82 ^{††}	19.45 ± 2.23
SV (μl)	29.81 ± 1.63	23.23 ± 1.67	-6.58 ± 1.65	27.85 ± 1.82	27.80 ± 1.48	-0.05 ± 1.60 ^a
HR (bpm)	497 ± 17	362 ± 9	-130 ± 19	499 ± 8	334 ± 14	-165 ± 14

^{*} *P* < 0.05,

^{**} *P* < 0.01,

^{***} *P* < 0.001 compared with WT baseline,

[†] *P* < 0.05,

^{††} *P* < 0.01 compared with WT MET, two-way repeated measures ANOVA with Bonferroni post-test.

^a *P* < 0.05 compared with WT, two-tailed unpaired *t*-test.