β-Adrenergic Receptor–Mediated Cardiac Contractility Is Inhibited via Vasopressin Type 1A-Receptor–Dependent Signaling

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Background—Enhanced arginine vasopressin levels are associated with increased mortality during end-stage human heart failure, and cardiac arginine vasopressin type 1A receptor (V1AR) expression becomes increased. Additionally, mice with cardiac-restricted V1AR overexpression develop cardiomyopathy and decreased β-adrenergic receptor (βAR) responsiveness. This led us to hypothesize that V1AR signaling regulates βAR responsiveness and in doing so contributes to development of heart failure.

Methods and Results—Transaortic constriction resulted in decreased cardiac function and βAR density and increased cardiac V1AR expression, effects reversed by a V1AR-selective antagonist. Molecularly, V1AR stimulation led to decreased βAR ligand affinity, as well as βAR-induced Ca^{2+} mobilization and cAMP generation in isolated adult cardiomyocytes, effects recapitulated via ex vivo Langendorff analysis. V1AR-mediated regulation of βAR responsiveness was demonstrated to occur in a previously unrecognized Gq protein–independent/G protein receptor kinase–dependent manner.

Conclusions—This newly discovered relationship between cardiac V1AR and βAR may be informative for the treatment of patients with acute decompensated heart failure and elevated arginine vasopressin. **(*Circulation*. 2014;130:1800-1811.)**

Key Words: cardiomyopathies ■ myocardial failure ■ myocardium ■ receptors, adrenergic, beta ■ vasopressin type 1A receptor

The neurohormone arginine vasopressin (AVP) is elevated in patients with heart failure (HF), and there is a direct relationship between plasma levels of AVP and disease severity and mortality.[1–5] AVP is released from the hypothalamus in response to changes in arterial pressure and plasma osmolality. Subsequently, AVP acts at 3 related but distinct G protein–coupled receptors (GPCRs): vasopressin type 1A receptor (V1AR; heart, vascular smooth muscle, myometrium, central nervous system, and liver), vasopressin type 1B receptor (V1BR; anterior pituitary), and V2 vasopressin receptor (V2R; vascular endothelial cells and renal tubule collecting ducts). AVP acts at V1ARs to induce peripheral vasoconstriction and cardiac hypertrophy via Gq protein–mediated signaling. Activation of the V2R leads to release of von Willebrand factor from vascular endothelial cells and insertion of aquaporin 2 into collecting duct cells, thereby resulting in increased transepithelial water permeability, water retention, and urine concentration via Gs protein–mediated signaling.[6] Therefore, either inappropriate or persistent AVP release causes profound hyponatremia, itself a risk factor for increased death and hospitalization in HF patients.[7] Because of the adverse clinical consequences associated with hyponatremia, basic science investigations and drug development have focused on V2R antagonists, which have been approved for the treatment of the hyponatremia associated with HF.

Clinical Perspective on p 1811

The physiological effects of AVP on the heart have been far less clear than its effects on the kidney owing in part to the fact that AVP mediates peripheral vasoconstriction through activation of the V1AR.[8–10] To better understand the role of V1AR in the heart independent of its effects on the vasculature, we

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previously created transgenic mice with inducible and cardiac-restricted overexpression of V1AR (V1AR-TG). These mice developed left ventricular (LV) hypertrophy, dilatation, diminished contractile performance, and reprogramming of the HF gene profile in a Gq protein–dependent manner,[11] findings consistent with effects observed with other Gq protein–coupled receptors (GqPCRs).[12] However, these results did not clarify the potential acute effects of V1AR activation in the heart, because the HF phenotype was only observed in the V1AR-TG mice 20 weeks after transgene activation.

Assessment of the acute effects of V1AR signaling on the heart and identification of its cognate signaling pathways are relevant because of the recent observations that V1AR density is significantly increased in hearts from patients with end-stage HF,[13] and we showed in our V1AR-TG model that enhanced cardiac V1AR expression diminished the hemodynamic response to β-adrenergic receptor (βAR) stimulation in vivo.[11] Thus, if V1AR signaling acts to impair βAR activation, even modest increases in AVP might have substantial effects on myocardial performance. Here, using cultured adult myocytes, genetically engineered nonmyocytes, ex vivo Langendorff-perfused hearts, and adult mice with HF secondary to transaortic constriction (TAC), we report that AVP acutely inhibits βAR-mediated cardiac contractility via a novel G protein receptor kinase (GRK)–dependent and Gq protein–independent mechanism. These results may explain the increased mortality observed in patients with acute HF and elevated AVP levels and provide support for the potential use of a V1AR antagonist in the treatment of these patients.

Methods

Materials

Angiotensin II (A9525), arginine vasopressin (V9879), CGP 20712A (C231), dimethyl sulfoxide (D4540), forskolin (F6886), ICI 119,551 (I127), 3-isobutyl-1-methylxanthine (I5879), isoproterenol (I6504), rolipram (R6520), and SR 49059 (S5701) were purchased from Sigma-Aldrich (St. Louis, MO). X-tremeGENE 9 DNA transfection reagent was purchased from Roche Applied Science (Indianapolis, IN). UBO-QIC was purchased from Professor Evi Kostenis, University of Bonn, Germany.

Animal Protocols

All experiments were performed according to the National Institutes of Health *Guide for the Care and Use of Laboratory Animals* and were approved by the Temple University Institutional Animal Care and Use Committee (ACUP#4031). GqI-TG mice had cardiac-restricted overexpression of a peptide derived from a carboxyl-terminal peptide of the α-subunit of Gq protein,[14] and V1AR-TG mice had controlled and cardiac-restricted overexpression of the V1AR.[11] Wild-type (WT; 75% C57Bl6/J, 25% FVB) littermate controls were used where appropriate.

Transverse Aortic Constriction

Pressure overload was produced by TAC as described previously.[15] After administration of anesthesia with isoflurane (2.5%), the chest was opened and an aortic band was created in 8-week-old WT mice by placement of a ligature (7-0 nylon suture) securely between the origin of the right innominate and left common carotid arteries with a 27-gauge needle as a guide. The sham procedure was identical except that the aorta was not ligated. After the procedure, the chest was closed, and the animal was allowed to recover after anesthesia. Doppler velocity was measured in the right and left carotid arteries, and right carotid artery/left carotid artery velocity ratio was calculated to ensure that TAC produced equal aortic pressure gradients in all experimental groups.

Echocardiography

LV function was evaluated in mice before TAC and at weekly intervals after TAC using a VisualSonics Vevo 770 imaging system and a 707 scan head (Miami, FL) as described previously.[16] After mice were lightly sedated with isoflurane (2.0%), a parasternal short-axis view was obtained for LV M-mode imaging at the papillary muscle level. Three independent M-mode images were used for measurements of LV end-diastolic internal diameter (LVEDD) and LV end-systolic internal diameter (LVESD) in 2 consecutive beats according to the American Society of Echocardiography leading edge method. Fractional shortening (FS) was calculated as FS%=[(LVEDD−LVESD)/LVEDD]×100. Anterior and posterior wall thicknesses were also measured.

Subcutaneous Implantation of ALZET Minipumps

One week after sham or TAC surgery, the mice were anesthetized with isoflurane (1.5%–2.5%) and underwent subcutaneous implantation with minipumps (ALZET, Cupertino CA; model #2006) that contained SR49059 (1 $mg \cdot kg^{-1} \cdot d^{-1}$) or vehicle control (0.1% DMSO in sterile saline) and were subsequently monitored for 6 weeks.

Cardiac Membrane Preparation and Radioligand Binding for V1AR and βAR

Membrane preparation and radioligand binding assays were performed as described previously.[13] Cleaned and minced myocardium was homogenized in ice-cold buffer (in mmol/L: Tris-HCl 10, pH 7.4, EDTA 10) with a Polytron homogenizer (Brinkmann Instruments), filtered through 3 layers of cheesecloth and centrifuged at 1000*g* for 10 minutes at 4°C. The supernatant was then filtered through 2 layers of cheesecloth and centrifuged at 45 000*g* for 30 minutes at 4°C to yield membranes. The plasma membrane pellet was adjusted to a final concentration of 1 mg/mL protein with binding buffer (in mmol/L: Tris 50, EDTA 1, pH 7.4). Protein concentration was determined by the Lowry method.

Membrane preparations (25 μg of protein for βAR binding or 40 μg of protein for V1AR binding) were incubated with ^{125}I-cyanopindolol (PerkinElmer, Waltham, MA; 4–300 pmol/L) or ^{125}I-p-AVP (PerkinElmer, Waltham, MA; 0.67–150 pmol/L) in incubation buffer (in mmol/L: Tris 50, EDTA 5, 0.1% BSA). Incubations were performed either alone or with propranolol (βAR nonspecific antagonist, 10 μmol/L) or SR49059 (V1AR-selective antagonist, 5 μmol/L) to determine nonspecific binding for subtraction from total binding to calculate specific binding. The incubation was performed at room temperature (25°C) for 2 hours in a total volume of 250 μL (βAR) or 100 μL (V1AR) in which steady state kinetics were achieved in specific binding. The reaction was terminated by the addition of 4°C incubation buffer and vacuum filtration through glass fiber filters (Whatman GF/C, Brandel, Inc). Each filter was washed 3 times with 7 mL of ice-cold 10 mmol/L Tris-HCl plus 0.1% BSA. Radioactivity of the wet filters was determined in a gamma counter. All assays were performed in duplicate. Receptor density was normalized to milligrams of membrane protein. K_d and the maximal number of binding sites (Bmax) were determined by Scatchard analysis of saturation binding isotherms with Prism 5.0 (Graph Pad Software Inc).

Isolation of Adult Murine Cardiac Myocytes and Intracellular Ca2+ Transient Measurements

Cardiac myocytes were isolated from the septum and LV free wall of 8- to 12-week-old male WT mice as described previously,[17] and intracellular Ca^{2+} transient measurements were performed as described previously.[18] 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 subjected to retrograde perfusion (100 cm H_2O, 37°C) with Ca^{2+}-free bicarbonate buffer followed by

enzymatic digestion (collagenases B and D, protease XIV). Isolated myocytes were plated on laminin-coated glass coverslips, and the Ca^{2+} concentration of the buffer was increased incrementally (0.05, 0.125, 0.25, 0.5 mmol/L), with 10 minutes of exposure at each concentration. The final Ca^{2+} buffer was then aspirated and replaced with MEM (Sigma-Aldrich) containing 1.2 mmol/L Ca^{2+}, 2.5% FBS, and 1% penicillin/streptomycin. The myocytes were exposed to 0.67 μmol/L Fura 2-AM for 15 minutes at 37°C, then field stimulated to evoke intracellular Ca^{2+} transients (1 Hz, 37°C) in medium 199 containing 1.8 mmol/L extracellular Ca^{2+}.

Adult Feline LV Myocyte Isolation and Infection

Adult feline left ventricular myocytes (AFVMs) were isolated as described previously.[19] Felines were anesthetized with sodium pentobarbital, and hearts were excised rapidly, cannulated, and mounted on a constant-flow Langendorff apparatus. Hearts were rinsed with a physiological Krebs-Henseleit buffer and then subjected to retrograde perfusion with collagenase that contained Krebs-Henseleit buffer. When the tissue softened, the LV was isolated and gently minced, filtered, and equilibrated in Krebs-Henseleit buffer with 0.2 mmol/L $CaCl_2$ and 1% BSA at room temperature. Isolated myocytes were washed with serum-free culture medium (Medium 199, Sigma-Aldrich) supplemented with penicillin-streptomycin-glutamine (Gibco) and seeded on 10-mm glass coverslip-containing 35-mm culture dishes (MatTek Corp, Ashland, MA) coated with laminin (BD Bioscience). A total of 5×10^4 cells per 10-mm coverslip were infected with adenovirus containing the cAMP fluorescence resonance energy transfer (FRET) reporter ICUE3[20] (Ad-ICUE3) at a multiplicity of infection of 40 for 36 hours.

Site-Directed Mutagenesis of Human V1AR to Attain GRK Phosphorylation-Deficient V1AR (GRK⁻V1AR)

The nucleotide sequences encoding the 17 carboxy-terminal serine (S352, S362, S380, S382, S389, S393, S393, S404, S407, S408, S410, S417) and threonine (T378, T386, T395, T398, T418) residues in hemagglutinin-tagged human V1AR underwent mutagenesis to alanine-encoding sequences with a Stratagene QuikChange II XL Site-Directed Mutagenesis Kit as per kit instructions (primers listed in Table I in the online-only Data Supplement).

Cell Culture and Transfection

Human embryonic kidney (HEK) 293 cells stably expressing FLAG-tagged β1AR,[21] hemagglutinin-tagged angiotensin II type 1A receptor (AT1R)[22] or human U2S osteosarcoma cells were grown in 10% FBS and 1% PSF-containing MEM. A total of 5×10^4 cells per coverslip were seeded on 10-mm glass coverslip–containing 35-mm dishes as described above and transfected for 24 hours with 1 μg of WT-V1AR (for β1AR cells or U2S cells), GRK⁻V1AR (for β1AR cells) or WT-β1AR (for AT1R cells) with 1 μg of either ICUE3 or the diacylglycerol FRET reporter (DAGR) using a 3:1 ratio of X-tremeGENE 9 to DNA.

FRET Measurements

AFVMs or HEK 293 cells expressing FRET reporters were rinsed, and media was replaced with imaging buffer (in mmol/L: NaCl 125, KCl 5, $MgCL_2$ 1.5, $CaCl_2$ 1.5, glucose 10, HEPES [pH 7.4] 10, and BSA at 0.2%) before imaging with a Leica DMI4000B inverted microscope with a Leica DFC365 FX 1.4-megapixel monochrome digital camera, and cyan fluorescent protein (CFP) excitation and CFP and yellow fluorescent protein (YFP) emissions were measured every 2 seconds. Cells were pretreated for 5 minutes with buffer or antagonists. After 30 seconds of baseline reads, the cells were stimulated with buffer, AVP, or angiotensin II, followed by isoproterenol at 90 seconds. Single-cell measurements at 20× magnification were used to assess changes in FRET, and each treatment condition was performed in a minimum of 3 independent cell preparations. Quantification of the changes in FRET ratio were calculated as change in CFP emission/YFP emission (ICUE3) or YFP emission/CFP emission (DAGR) over time, normalized to baseline.

Langendorff Perfusion System

The isolated heart perfusion technique was described previously.[23] Briefly, hearts were perfused under constant pressure (80 mm Hg) with a solution containing (in mmol/L): NaCl 13.8, $NaHCO_3$ 22, KCl 4.7, KH_2PO_4 1.2, $MgSO_4$ 1.1, glucose 1.1, $CaCl_2$ 2, Na pyruvate 2. A balloon was placed in the LV, connected to a Millar pressure transducer and an ADInstruments Physiograph (Colorado Springs, CO), and filled with H_2O to set the LV end-diastolic pressure at 10 mm Hg. Hearts were maintained at a temperature of 37°C and were paced at a rate of 480 bpm. After a 15-minute stabilization period, pharmacological agents were added to the perfusion media at 5-minute intervals. LV pressure, LV end-diastolic pressure, and the maximum rate of positive and negative change in LV pressure (±LV dP/dt) were recorded. LV developed pressure was calculated by subtracting the LV end-diastolic pressure from the LV systolic pressure. Data were analyzed with LabChart Pro-6.0 (ADInstruments).

Statistical Analysis

Data are summarized as mean±SEM for continuous variables. All experiments in which the same subject (mouse or heart) was tested over time or using different drug concentrations were analyzed with 2-way repeated-measures ANOVA with Bonferroni multiple-comparisons adjustments. The number of comparisons with Bonferroni post-test adjustments is indicated in figure legends along with *P* values whenever applicable. Pairwise 2-group comparisons with non–repeated-measures data were analyzed with nonparametric Wilcoxon rank sum tests, and exact test *P* values were reported when the combined sample size was <58, as well as whenever feasible for larger sample sizes. Two-sample *t* tests or 1-way ANOVA with Bonferroni post-test adjustments were also performed as sensitivity analyses, but the results were not reported herein. $P<0.05$ was considered statistically significant. Prism 5.0 or SAS version 9.3 (SAS Institute, Inc, Cary, NC) were used for statistical analyses.

Results

The V1AR-Selective Antagonist SR 49059 Preserves Cardiac Function and Restores V1AR and βAR Expression Levels During the Development of Pressure Overload–Induced Hypertrophy In Vivo

We previously reported that V1AR expression increases in end-stage human HF[13] and that mice with cardiac-restricted overexpression of V1AR (V1AR-TG) undergo progressive development of cardiomyopathy and decreased βAR responsiveness with age.[11] To determine whether changes in endogenous V1AR expression are associated with the development of cardiomyopathy and βAR dysfunction, WT mice underwent TAC in conjunction with osmotic minipump-mediated delivery of the V1AR-selective antagonist SR 49059. As expected, TAC decreased cardiac function and increased cardiac hypertrophy; however, coadministration of SR 49059 preserved fractional shortening (Figure 1A) and ejection fraction (Figure 1B) without impacting hypertrophy (Figure IA and IB in the online-only Data Supplement). Cardiac V1AR and βAR expression were also assessed in each of the cohorts via radioligand binding. Interestingly, TAC induced a significant 2-fold increase in V1AR expression, similar to that observed in human hearts,[13] which was inhibited by SR 49059 (Figure 1C). βAR downregulation is associated with the development and progression of HF,[24] and in response to 7

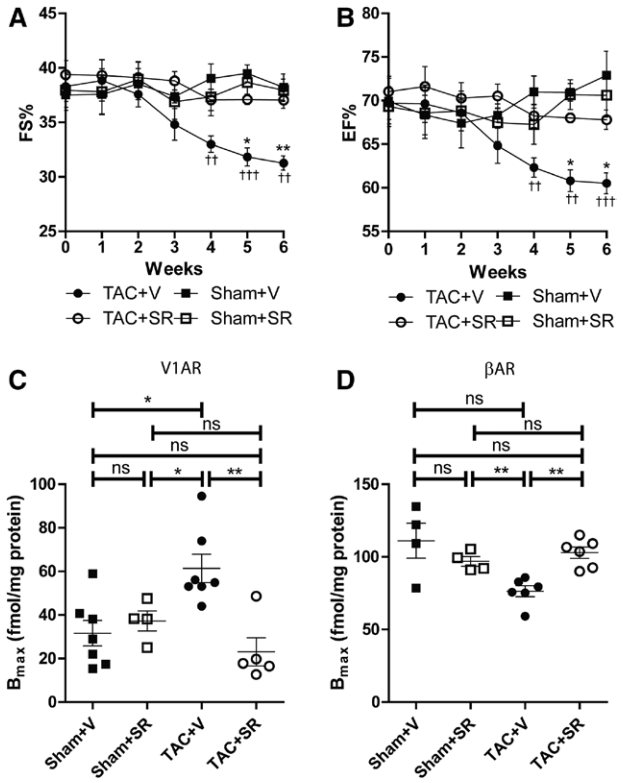

Figure 1. Vasopressin type 1A receptor (V1AR) antagonist SR 49059 (SR) protects against transaortic constriction (TAC)–induced cardiac dysfunction. In response to TAC, fractional shortening (FS; **A**) and ejection fraction (EF; **B**) were significantly decreased in vehicle-treated mice (TAC+V) compared with vehicle-treated sham surgery mice (Sham+V), effects that were prevented with SR 49059 (1 mg·kg^{-1}·d^{-1} starting 1 week after TAC). ††*P*<0.01, †††*P*<0.001 vs Sham+V; **P*<0.05, ***P*<0.01 vs TAC+SR at corresponding weeks; 2-way repeated-measures ANOVA with Bonferroni multiple comparisons test (6 comparisons total). n = 9 (Sham+V), 8 (Sham+SR), 13 (TAC+V), and 14 (TAC+SR). As shown by radioligand binding analysis, TAC increased V1AR membrane density (**C**; n=7 [Sham+V], n=4 [Sham+SR], n=7 [TAC+V], and n=5 [TAC+SR]) and decreased β-adrenergic receptor (βAR) membrane density (**D**; n=4 [Sham+V], n=4 [Sham+SR], n=6 [TAC+V], n=6 [TAC+SR]), effects that were prevented by treatment with SR 49059. **P*<0.05, ***P*<0.01, exact Wilcoxon rank sum test (ns indicates not significant).

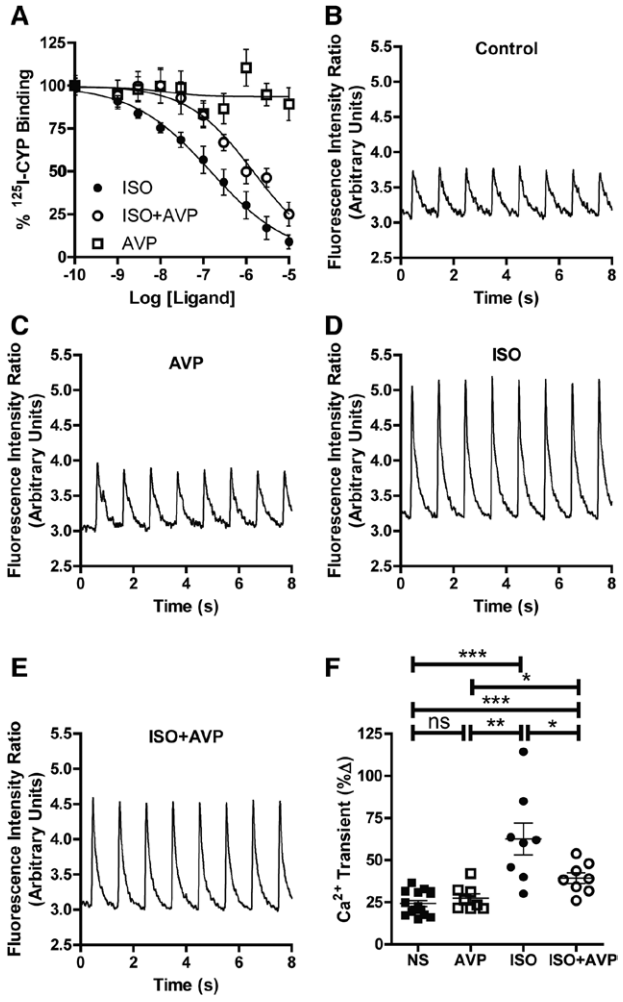

Figure 2. Arginine vasopressin (AVP) negatively impacts mouse cardiac β-adrenergic receptor (βAR) ligand binding and Ca^{2+} transients in adult mouse cardiomyocytes. **A**, Competition radioligand binding on adult mouse left ventricular membranes showed that AVP (0.5 μmol/L) decreased the affinity of isoproterenol (ISO) for βAR (red) compared with ISO alone (blue), whereas increasing concentrations of AVP alone (green) did not alter βAR ligand binding. n=4 per ligand concentration. ^{125}CYP indicates ^{125}I-cyanopindolol. **B** through **E**, Representative tracings from field stimulation–induced Ca^{2+} transients in Fura2-loaded adult mouse left ventricular myocytes in response to nonstimulated control (**B**), AVP (10 nmol/L; **C**), ISO (50 nmol/L; **D**), or AVP+ISO (**E**). **F**, Summary of the changes in Ca^{2+} transients indicates a significant decrease in ISO-mediated transients in the presence of AVP. **P*<0.05, ***P*<0.01, ****P*<0.001, exact Wilcoxon rank sum test. ns indicates not significant. n=16 (NS), n=8 (AVP, ISO, AVP+ISO).

weeks of TAC in this study was reduced by ≈30% (Figure 1D). However, concomitant treatment of TAC mice with SR 49059 completely restored cardiac βAR expression to normal levels. These results confirm the involvement of endogenous V1AR signaling in the progression of HF and indicates that a loss of βAR density in the presence of chronically enhanced V1AR expression may account for the diminished βAR responsiveness we previously observed in V1AR-TG mice.[11]

AVP Negatively Regulates Endogenous Cardiac β1AR Activation

To begin to understand a possible influence of cardiac-expressed V1AR on βAR signaling more acutely, we began to study the molecular relationship between these receptors. Initially, we performed ^{125}I-cyanopindolol competition binding analysis with increasing concentrations of isoproterenol with or without AVP in adult mouse heart membrane preparations. Although AVP alone did not alter ^{125}I-cyanopindolol binding to βAR, AVP induced a significant rightward shift in the ability of isoproterenol to displace ^{125}I-cyanopindolol from βAR (IC_{50} of isoproterenol from 0.14 to 1.4 μmol/L, *P*<0.0001 [2-tailed *t* test]; Figure 2A), indicative of a loss in affinity of βAR for isoproterenol that could be associated with diminished receptor coupling to downstream G protein–dependent signaling.[25,26] Because Gs protein–dependent Ca^{2+} mobilization is enhanced downstream of βAR in cardiomyocytes, we measured field stimulation–induced Ca^{2+} transient responses in Fura2-loaded adult mouse LV myocytes in the presence or

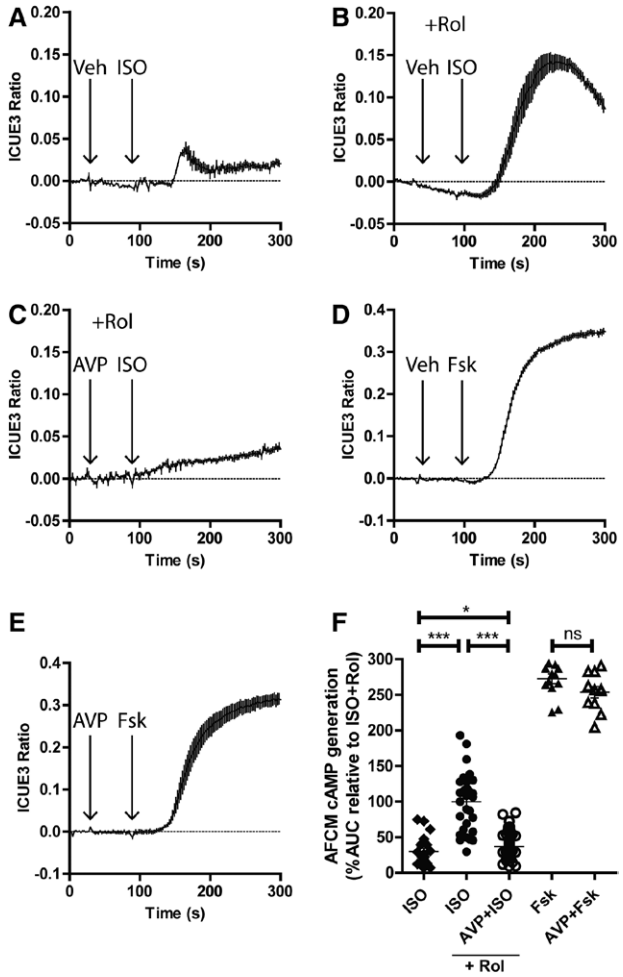

Figure 3. Arginine vasopressin (AVP) reduced isoproterenol (ISO)-mediated cAMP generation in adult feline ventricular myocytes (AFVMs). cAMP generation responses were monitored in AFVMs infected with the fluorescent biosensor ICUE3 in response to vehicle (Veh)+ISO (100 nmol/L; **A**), rolipram (Rol, 1 μmol/L)+Veh+ISO (**B**), Rol+AVP (1 μmol/L)+ISO (**C**), Veh+forskolin (Fsk, 1 μmol/L; **D**), or AVP+Fsk (**E**). **F**, Summarized area under the curve (AUC) data relative to the Rol+Veh+ISO condition show that AVP pretreatment significantly reduced ISO-mediated cAMP generation, while not affecting Fsk-mediated cAMP generation. **P*<0.05, ****P*<0.001, exact Wilcoxon rank sum test. ns=not significant. n=20 (ISO), n=31 (Rol+ISO), n=58 (Rol+AVP+ISO), n=13 (Fsk), and n=11 (AVP+Fsk). AFCM indicates adult feline cardiomyocyte.

absence of AVP and isoproterenol (Figure 2B through 2E). Although AVP did not evoke a change in Ca^{2+} mobilization alone even at micromolar concentrations (Figure IC in the online-only Data Supplement), isoproterenol-induced Ca^{2+} transients were significantly reduced in the presence of AVP (Figure 2F).

βAR-induced Ca^{2+} transients occur in response to Gs protein–dependent generation of cAMP; thus, we tested whether AVP stimulation impacts isoproterenol-mediated cAMP production in AFVMs infected with adenovirus encoding the fluorescent cAMP biosensor ICUE3.[20] Stimulation of AFVMs with isoproterenol produced only a small increase in cAMP production (Figure 3A). Because βAR-dependent cAMP signaling is tightly controlled by phosphodiesterase 4 variants,[27] we pretreated AFVMs with the phosphodiesterase 4–selective antagonist rolipram, which greatly enhanced the isoproterenol-induced cAMP generation (Figure 3B). Treatment of AFVMs with AVP 1 minute before isoproterenol addition blocked the cAMP response regardless of phosphodiesterase 4 inhibition (Figure 3C), which suggests that V1AR stimulation impacts βAR signaling at least at the level of cAMP generation and not cAMP degradation. To test whether cAMP generation is altered by AVP, AFVMs were stimulated with the adenylyl cyclase activator forskolin with or without AVP (Figure 3D and 3E). AVP did not alter cAMP production in response to direct activation of adenylyl cyclase (Figure 3F), which suggests that V1AR stimulation impacts βAR-mediated cAMP generation at the receptor–G protein level.

β1AR and β2AR are the predominant βAR isoforms present in normal adult cardiomyocytes that enhance cAMP generation, although β1AR was predominantly responsible for cAMP generation in AFVMs (Figure IIA and IIB in the online-only Data Supplement). To determine whether AVP stimulation can also modulate β2AR responsiveness, we tested the cAMP generation response to isoproterenol stimulation in the presence or absence of AVP in human U2S osteosarcoma cells, which express βAR at similar levels as myocardium[21] and predominantly signal via β2AR (Figure IIC and IID in the online-only Data Supplement). U2S cells were transfected with V1AR and either ICUE3 or the fluorescent diacylglycerol (DAG) biosensor DAGR.[28] Despite the ability of AVP to evoke V1AR-dependent signaling in these cells (Figure IIE in the online-only Data Supplement), AVP was unable to alter isoproterenol-mediated cAMP generation (Figure IIF and IIG in the online-only Data Supplement), which suggests that V1AR-dependent effects on cardiac βAR signaling are β1AR selective.

V1AR-Mediated Regulation of β1AR Signaling Is Gq Protein Independent

To assess the mechanism by which V1AR stimulation decreases β1AR signaling, we used HEK 293 cells stably expressing β1AR[21] and transiently expressing V1AR and either ICUE3 or DAGR. In these cells, AVP induced a concentration-dependent increase in DAG production (Figure IIIA and IIIB in the online-only Data Supplement) with a maximal effect produced by 1μM, used subsequently to test the effects of V1AR stimulation on β1AR responsiveness. Isoproterenol stimulation rapidly enhanced cAMP production (Figure 4A), whereas AVP alone had no impact (Figure 4B). As observed in AFVMs, AVP pretreatment greatly reduced the isoproterenol-mediated cAMP production (Figure 4C), and similar to the binding data above, AVP induced a competitive rightward shift in isoproterenol-mediated cAMP generation (Figure 4D), altering the EC_{50} of isoproterenol-dependent cAMP generation from 43 to 256 pmol/L. Although SR 49059 pretreatment did not prevent isoproterenol-mediated cAMP production in the absence of AVP (Figure 4F), it completely blocked AVP-dependent DAG production (Figure 4E) and, importantly, restored isoproterenol-dependent cAMP production in the presence of AVP (Figure 4G), which confirms that AVP-mediated regulation of β1AR occurs via V1AR.

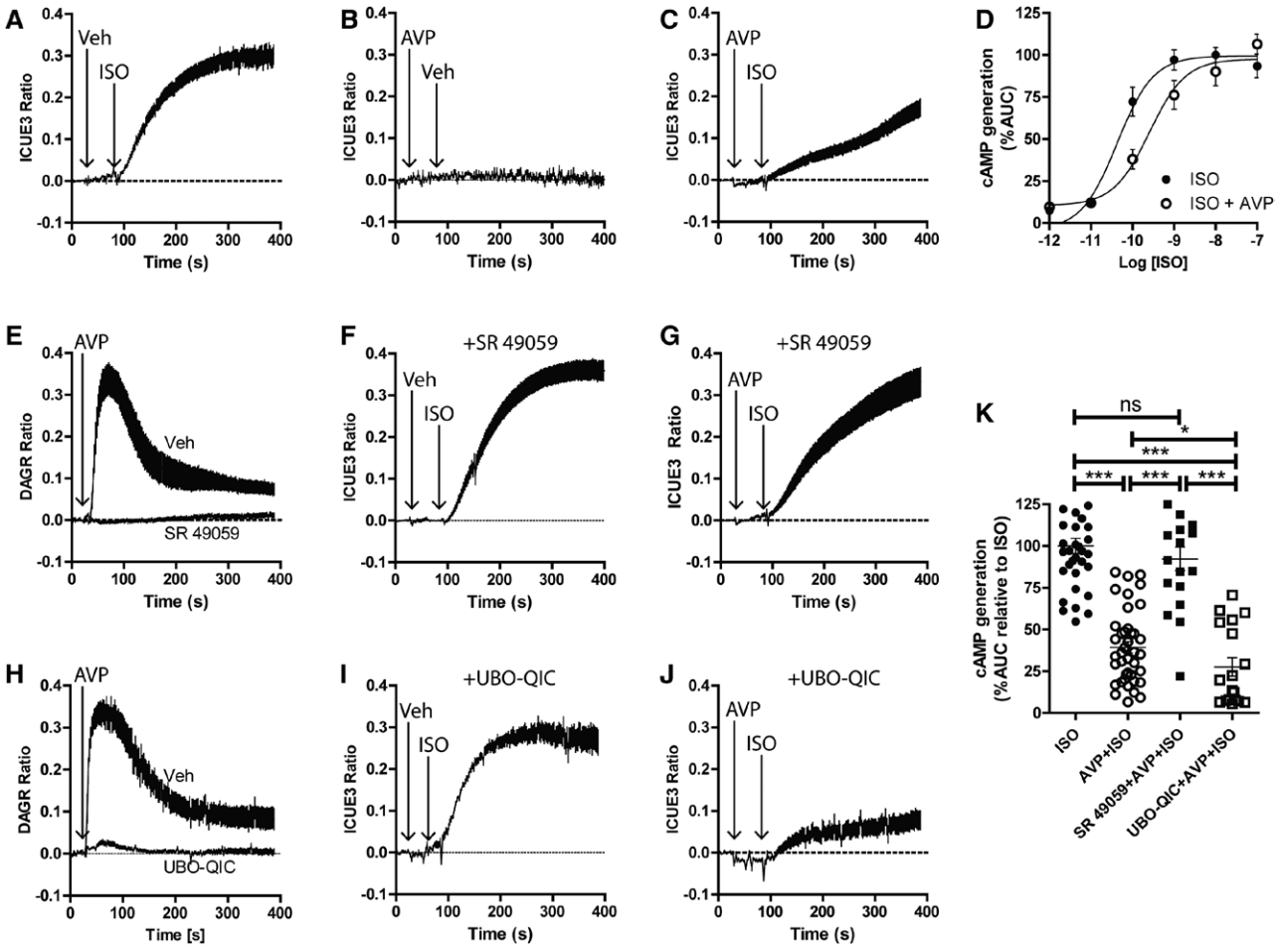

Figure 4. Arginine vasopressin (AVP) effects on β-adrenergic receptor (βAR)–mediated cAMP generation are Gq protein independent. cAMP and diacylglycerol (DAG) generation responses were monitored in human embryonic kidney (HEK) 293 cells stably expressing β1AR and transiently transfected with vasopressin type 1A receptor (V1AR) and either ICUE3 or diacylglycerol reporter (DAGR). Isoproterenol (ISO; 100 pmol/L; **A**), but not AVP (1 μmol/L; **B**), increased cAMP generation. AVP pretreatment reduced ISO-mediated cAMP production (**C**) in a competitive manner (**D**). **E**, AVP (1 μmol/L) induced a rapid DAG generation response that was blocked by SR 49059 (10 μmol/L). Pretreatment of cells with SR 49059 did not block ISO-induced cAMP generation (**F**) but did prevent the AVP-dependent reduction in the ISO response (**G**). **H**, The rapid DAG response to AVP stimulation was blocked by UBO-QIC (10 nmol/L). Pretreatment of the cells with UBO-QIC blocked neither the ISO-induced cAMP generation (**I**) nor the AVP-dependent reduction in the ISO response (**J**). **K**, Summarized area under the curve (AUC) results relative to ISO treatment alone. **P*<0.05, ****P*<0.001, exact Wilcoxon rank sum test. ns=not significant. n=34 (ISO), n=40 (AVP+ISO), n=18 (SR+AVP+ISO), and n=18 (QIC+AVP+ISO).

To test whether the acute effect of AVP on β1AR signaling is a common response to stimulation of GqPCRs, we assessed the ability of the AT1R, known to chronically modulate βAR signaling,[29] to alter β1AR-mediated cAMP generation in HEK 293 cells overexpressing both AT1R and β1AR. Similar to AVP, angiotensin II induced a concentration-dependent increase in DAG production that achieved maximal effect by 1 μmol/L (Figure IIIC and IIID in the online-only Data Supplement). However, pretreatment of the cells with angiotensin II was unable to modulate β1AR-mediated cAMP production (Figure IIIE and IIIF in the online-only Data Supplement), which suggests that the acute effect of V1AR signaling on β1AR responsiveness is selective with regard to other GqPCRs. Because chronic Gq protein–dependent signaling has been shown to mediate βAR desensitization,[29–31] we used the small molecule inhibitor of Gq protein, UBO-QIC,[32] to assess the impact of acute Gq protein–dependent V1AR signaling on βAR responsiveness. As expected, UBO-QIC blocked AVP-Gq protein–dependent DAG production (Figure 4H) with no impact on isoproterenol-mediated cAMP production alone (Figure 4I). Interestingly, UBO-QIC pretreatment was unable to restore isoproterenol-mediated cAMP responsiveness in the presence of AVP (Figure 4J). Thus, although AVP signaling through V1AR negatively regulates β1AR-dependent cAMP formation, it does so in a Gq protein–independent manner (Figure 4K).

V1AR-Mediated Regulation of β1AR Signaling Is GRK Dependent

We next tested whether Gq protein–independent V1AR signaling through GRKs reduces β1AR responsiveness. Multiple GRK isoforms have been shown to play distinct roles in mediating receptor signaling responses in different tissues,[33] but generalized ablation of GRKs would impact βAR signaling regardless of the role of V1AR in

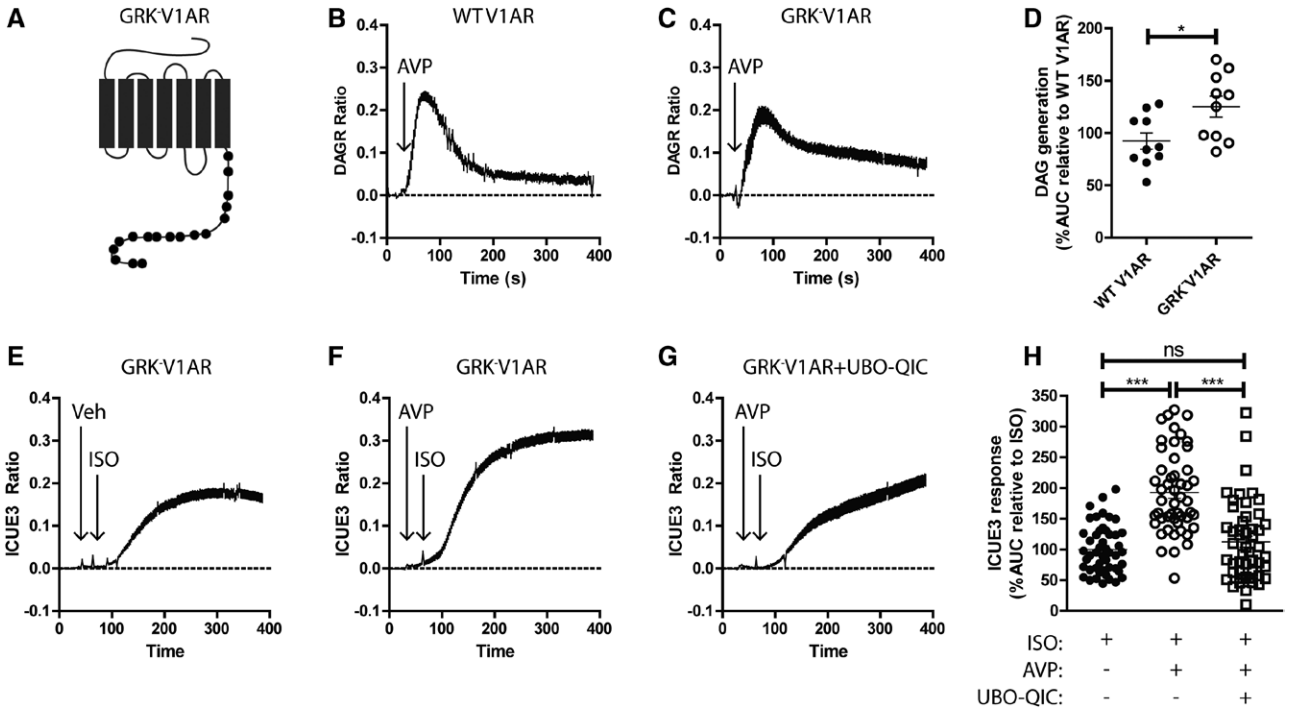

Figure 5. G protein receptor kinase (GRK) phosphorylation–deficient vasopressin type 1A receptor (GRK$^-$V1AR) retained Gq protein coupling and augmented β-adrenergic receptor (βAR) responsiveness. **A**, Schematic of the GRK$^-$V1AR, wherein black circles indicate serine/threonine sites mutated to alanine, corresponding to those in Table I in the online-only Data Supplement. Human embryonic kidney (HEK) 293 cells stably expressing β_1-adrenergic receptor (β1AR) were transiently transfected with diacylglycerol reporter (DAGR) or ICUE3 with either wild-type (WT) V1AR or GRK$^-$V1AR. Both WT V1AR (**B**) and GRK$^-$V1AR (**C**) induced diacylglycerol (DAG) formation responses to AVP (1 μmol/L). **D**, DAG formation area under the curve (AUC) responses relative to WT V1AR indicated stimulation of GRK$^-$V1AR led to more DAG accumulation. *P<0.05, exact Wilcoxon rank sum test. n=10 each. **E**–**G**, Stimulation of GRK$^-$V1AR with AVP (1 μmol/L) led to enhanced isoproterenol (ISO)-mediated cAMP formation, an effect blocked by the Gq protein inhibitor UBO-QIC. **H**, Summarized AUC results relative to ISO treatment of GRK$^-$V1AR. ***P<0.001, exact Wilcoxon rank sum test. n=48 (ISO), n=50 (AVP+ISO), and n=50 (UBO-QIC+AVP+ISO).

regulating βAR desensitization. Thus, to specifically explore the impact of GRK-dependent V1AR signaling, we developed a V1AR construct that lacked all possible C-terminal GRK phosphorylation sites (GRK$^-$V1AR; Figure 5A; Table I in the online-only Data Supplement). Transfection of HEK 293 cells stably expressing β1AR with either WT-V1AR or GRK$^-$V1AR resulted in similar levels of membrane expression and AVP affinity (Figure IVA and IVB in the online-only Data Supplement). Stimulation of WT-V1AR (Figure 5B) and GRK$^-$V1AR (Figure 5C) with AVP each induced rapid DAG formation, with GRK$^-$V1AR producing a small but significantly enhanced accumulation of DAG over WT-V1AR (Figure 5D), consistent with prolonged Gq protein–dependent activity in the absence of GRK phosphorylation. Interestingly, in contrast to WT-V1AR, which reduced isoproterenol-mediated cAMP generation (Figure 4C), AVP stimulation of GRK$^-$V1AR significantly enhanced isoproterenol-mediated cAMP formation, an effect that was blocked by Gq protein inhibition with UBO-QIC (Figure 5E through 5H), which suggests that in the absence of GRK-dependent regulation of V1AR signaling, Gq protein–dependent V1AR signaling promotes β1AR sensitivity to adrenergic stimulation. These results also suggest that when both Gq protein– and GRK-dependent V1AR signaling mechanisms are present, the GRK-dependent branch is dominant with regard to the regulation of β1AR responsiveness.

Endogenous and Overexpressed V1ARs Decrease βAR-Dependent Ex Vivo Cardiac Contractility

Because isoproterenol-induced Ca^{2+} transients were reduced in the presence of AVP, and we previously reported diminished isoproterenol-dependent cardiac contractility in hearts with V1AR overexpression,[11] we next assessed the impact of V1AR signaling on βAR function in the whole heart. Ex vivo Langendorff analysis was performed with WT versus V1AR-TG mouse hearts (which contain ≈5-fold higher V1AR expression but a similar AVP affinity; Figure IVC and IVD in the online-only Data Supplement). Contractility, including LV developed pressure, +dP/dt, and −dP/dt, was compared between WT and V1AR-TG hearts in response to an increasing concentration of the nonselective phosphodiesterase inhibitor IBMX (3-isobutyl-1-methylxanthine). Although IBMX infusion at higher concentrations increased contractility in WT hearts, V1AR-TG hearts were resistant to IBMX even at the highest concentrations tested (Figure 6A through 6C), which suggests that basal cAMP generation in the heart is suppressed by V1AR overexpression. To determine whether adenylyl cyclase activity is negatively regulated by V1AR signaling, WT hearts underwent infusion with forskolin with or without AVP (1 nmol/L) pretreatment. In accordance with the FRET experiments, pretreatment of WT hearts with AVP had little to no significant impact on forskolin-mediated effects on contractility (Figure 6D through 6F), which confirms that in

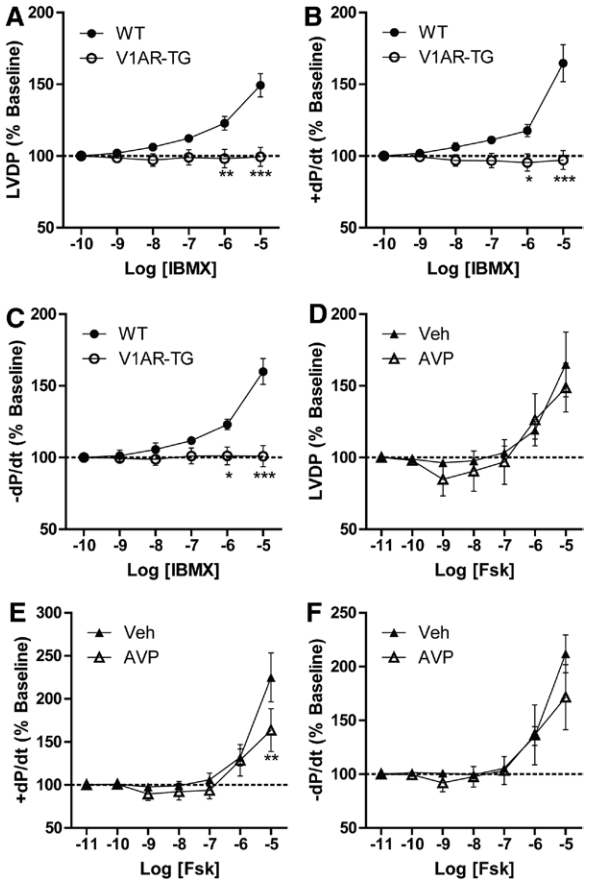

Figure 6. Vasopressin type 1A receptor (V1AR) overexpression blocks basal cardiac contractility ex vivo, but V1AR activation does not impact adenylyl cyclase activity. Cardiac contractile parameters were measured in ex vivo Langendorff preparations from wild-type (WT) mice or mice with cardiac-restricted V1AR expression (V1AR-TG). Infusion of increasing concentrations of the nonselective phosphodiesterase inhibitor IBMX led to an increase in contractility in WT hearts as measured by left ventricular developed pressure (LVDP; **A**), +dP/dt (**B**), and −dP/dt (**C**), expressed as % of baseline, effects that were absent in V1AR-TG hearts. **P*<0.05, ***P*<0.01, ****P*<0.001 vs WT hearts at corresponding concentration, 2-way repeated-measures ANOVA with Bonferroni post-test. n=5 hearts each. WT hearts received increasing concentrations of the adenylyl cyclase activator forskolin (Fsk), which enhanced LVDP (**D**), +dP/dt (**E**), and −dP/dt (**F**) in either the presence or absence of arginine vasopressin (AVP; 1 nmol/L) pretreatment. ***P*<0.01 vs vehicle (Veh)-treated hearts at corresponding concentration, 2-way ANOVA with Bonferroni multiple comparisons test. n=5 hearts each.

the whole heart, V1AR-mediated effects on contractile signaling occur proximal to adenylyl cyclase activation.

To assess βAR responsiveness directly, hearts were perfused with increasing concentrations of isoproterenol alone (WT and V1AR-TG) or after AVP pretreatment (WT+AVP). Compared with the WT hearts alone, both V1AR-TG hearts and WT+AVP hearts produced significantly diminished responses to isoproterenol perfusion, including LV developed pressure, +dP/dt, and −dP/dt (Figure 7A through 7C), which suggests that cardiac overexpression of V1AR mimics the effects of exogenous AVP on βAR responsiveness. Decreased isoproterenol responsiveness in the presence of AVP cannot be explained by noncardiac effects, because AVP produced only a small concentration-dependent decrease in cardiac hemodynamics (≈20% from baseline; Figure IVE through IVG in the online-only Data Supplement), whereas AVP diminished isoproterenol-mediated contractility by ≈70% at maximum concentration of isoproterenol. The modest diminution in ex vivo cardiac contractility in the presence of AVP has been reported to be secondary to reduced coronary blood flow.[34] Additionally, we tested the effect of AVP on isoproterenol responsiveness in isolated hearts of mice overexpressing the Gq protein inhibitory peptide GqI.[14] Similar to the FRET data above, Gq protein inhibition did not prevent AVP from dampening isoproterenol-mediated contractility in the isolated hearts (Figure 7A through 7C). In fact, GqI overexpression resulted in an even more statistically significant AVP-dependent ablation of isoproterenol-mediated contractility. Altogether, the present data highlight a previously unknown role for cardiac V1AR-mediated desensitization of βAR signaling via a novel Gq protein–independent, GRK-dependent mechanism.

Discussion

Neurohormone-binding GPCRs, their cognate G proteins, and downstream signaling effectors, including GRKs, play a critical role in the development of the complex phenotype of the failing heart: cardiac hypertrophy, dilatation, and apoptosis. Similarly, activation of V1AR contributes to the development of HF, because both constitutive and controlled cardiac-restricted overexpression of the V1AR in mice results in the development of cardiac hypertrophy, dilatation, and diminished performance through activation of Gq protein–dependent signaling.[11] In the present study, we demonstrate for the first time that AVP is a potent inhibitor of βAR signaling in cultured adult cardiomyocytes and in a murine ex vivo Langendorff preparation through a GRK-dependent but Gq protein–independent manner. Interestingly, the impact of V1AR stimulation on βAR responsiveness appears to be selective not only with regard to GqPCRs, because AT1R did not reduce isoproterenol-mediated signaling, but also βAR subtype, because only β1AR- but not β2AR-dependent signaling was negatively regulated by AVP treatment. Furthermore, we show that mice with HF secondary to TAC recapitulate the V1AR molecular phenotype found in humans with HF: a 2-fold increase in myocardial V1AR density and decreased βAR expression. Interestingly, although the hypertrophic response to TAC was unchanged in the presence of the V1AR antagonist SR 49059, likely because of enhanced activity of other neurohormone pathways, SR 49059 normalized expression levels of both V1AR and βAR and significantly improved the ejection fraction in TAC mice compared with vehicle controls. These findings provide important information regarding the impact of AVP during the development of HF and provide new rationale for treatment strategies aimed at supporting βAR responsiveness.

An increase in the expression of cardiac Gq protein has been shown to inhibit βAR signaling,[30] and thus, our finding that activation of the V1AR inhibits cardiac β1AR-mediated cAMP signaling through a GRK-dependent but Gq protein–independent pathway was unexpected and contrasts with studies of other cardiac GPCRs. For instance, although both AT1R and V1AR have been shown to enhance V2R-Gs protein–mediated cAMP formation in Cho cells, this effect was Gq protein dependent.[35,36] Similarly, β2AR-mediated cAMP

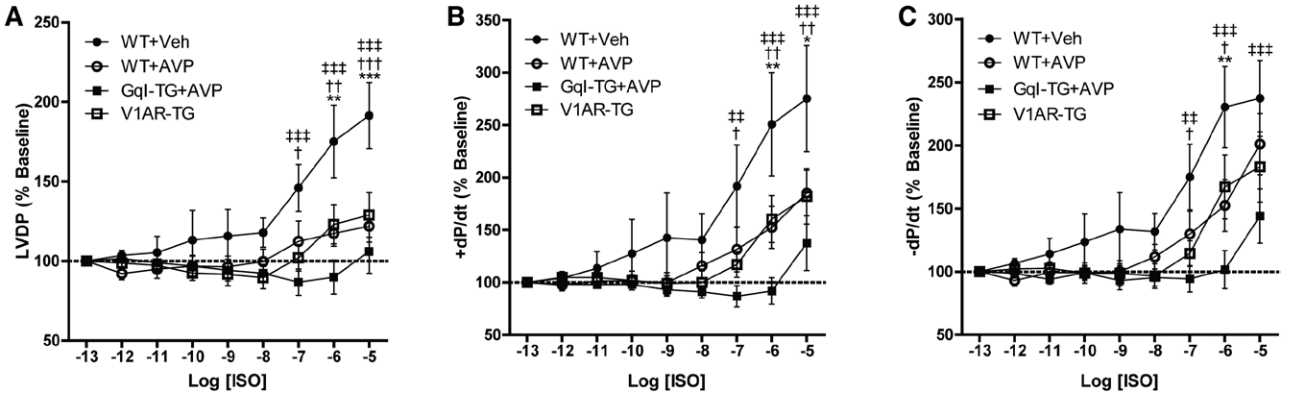

Figure 7. Either vasopressin type 1A receptor (V1AR) overexpression or stimulation can block isoproterenol (ISO)-mediated cardiac contractility ex vivo, even in the presence of genetic Gq protein inhibition. Cardiac contractile parameters were measured in ex vivo Langendorff preparations from wild-type (WT) or transgenic mice with inducible and cardiac-restricted overexpression of V1AR (V1AR-TG). Infusion of increasing concentrations of ISO led to an increase in contractility in WT hearts as measured by left ventricular developed pressure (LVDP; **A**), +dP/dt (**B**), and −dP/dt (**C**), expressed as % of baseline. These effects were blocked in V1AR-TG hearts, in WT hearts pretreated with arginine vasopressin (AVP; 1 nmol/L), and in GqI-expressing hearts pretreated with AVP. **P*<0.05, ***P*<0.01, ****P*<0.001 vs WT+AVP; †*P*<0.05, ††*P*<0.01, †††*P*<0.001 vs V1AR-TG; and ‡‡*P*<0.01, ‡‡‡*P*<0.001 vs GqI-TG+AVP at corresponding concentration; 2-way repeated-measures ANOVA with Bonferroni multiple comparisons test (6 comparisons total). n=5 (WT+Veh), n=5 (WT+AVP), n=8 (V1AR-TG), and n=7 (GqI-TG+AVP) hearts each.

formation was increased by stimulation of several GqPCRs in cardiac fibroblasts,[37] effects that have been attributed to protein kinase C–dependent effects such as enhanced Gs protein–adenylyl cyclase coupling. Although desensitization of one GPCR on activation of another distinct GPCR has been shown (eg, opioid receptor-like 1–mediated desensitization of the μ-opioid receptor and M3 muscarinic receptor–mediated desensitization of β2AR), these effects have also been shown to be protein kinase C dependent, even when they involve GRK signaling.[38,39] Similarly, although mechanical stretch activates the AT1R with subsequent upregulation of GRK2 and reduction in βAR signaling in neonatal rat ventricular myocytes, inhibition of either AT1R-Gq protein coupling or protein kinase C–dependent phosphorylation of GRK2 restored normal βAR signaling.[40]

Here, GRK-mediated regulation of β1AR signaling occurred even in the absence of Gq protein activity, and we only observed a V1AR-mediated increase in β1AR signaling when all possible C-terminal GRK phosphorylation sites were mutated to alanine. These results suggest that GRK-dependent V1AR signaling predominantly reduces β1AR activity and that β1AR-enhancing Gq protein–dependent V1AR effects are either not normally present or unable to significantly impact β1AR activity in the presence of intact V1AR coupling to GRK. Additionally, GqI peptide–mediated inhibition of Gq protein activity did not impact the ability of AVP to inhibit βAR responsiveness in isolated perfused hearts but led to a more significant reduction in βAR-induced cardiac contractility than in WT hearts with intact Gq protein–dependent signaling. These acute results were disparate with our previous finding that GqI peptide overexpression blocked the development of chronic HF 20 weeks after cardiac-restricted and controlled overexpression of the V1AR.[11] Thus, the acute effects of AVP-V1AR signaling on βAR-mediated cardiac contractility occur via GRK-dependent mechanisms, whereas the chronic effects of AVP-V1AR signaling on cardiac morphology occur via Gq protein–dependent signaling.

Although the GRK⁻V1AR construct reveals a required role for GRK-dependent regulation of βAR activity after acute V1AR stimulation, the precise mechanism of this effect remains to be defined. Because GRKs are primarily known for their role in phosphorylation-mediated GPCR desensitization, a likely molecular explanation exists in which V1AR stimulation results in enhanced association of active GRK at the plasma membrane, which enables more rapid GRK-mediated phosphorylation of βAR on subsequent catecholamine stimulation, thereby enhancing the kinetics of βAR-Gs protein uncoupling and desensitization (Figure 8). βAR-Gs protein uncoupling would account for the rightward shift in βAR ligand affinity,[25,26] as well as the diminished cAMP generation, Ca^{2+} mobilization, and contractile responses to isoproterenol stimulation observed in the present study. The fact that this effect on βAR responsiveness occurs selectively in response to stimulation of V1AR and not AT1R suggests that beyond the requirement for GRK activity, close proximity of V1AR

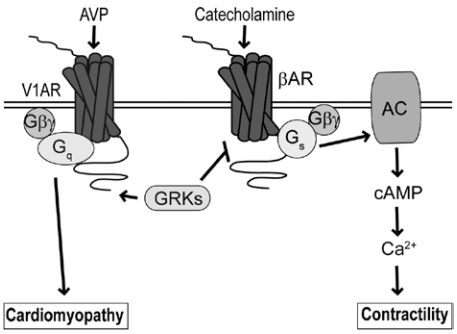

Figure 8. Gq protein–dependent vs G protein receptor kinase (GRK)–dependent cardiac vasopressin type 1A receptor (V1AR) signaling. Increased levels of arginine vasopressin (AVP) led to enhanced stimulation of Gq protein–dependent V1AR signaling to promote cardiomyopathy, whereas GRK-dependent V1AR signaling reduced β-adrenergic receptor (βAR)–mediated signaling and downstream contractility. AC indicates adenylyl cyclase.

and β1AR, possibly via colocalization or heterodimerization, may be an essential component of this desensitizing mechanism. However, follow-up studies will be required to address these possible scenarios.

That GRK-dependent V1AR signaling can mediate acute effects of AVP in cardiomyocytes is consistent with our previous report demonstrating that V1AR stimulation results in rapid and sustained activation of both Gq protein/protein kinase C–dependent and GRK2/βarr1-dependent ERK1/2 signaling in cardiac-like H9c2 cells.[41] However, whereas V1AR/GRK2/βarr1-dependent signaling also participated in cellular protection against hypoxia/reoxygenation, the present results suggest that GRK-dependent V1AR signaling may ultimately be maladaptive in vivo in part via its rapid inhibition of βAR responsiveness. GRKs, in particular GRK2 and GRK5, have been demonstrated to regulate a number of maladaptive effects during the development of HF, including βAR desensitization, and show promise as therapeutic targets for the treatment of HF, alone or in conjunction with conventional β-blockade.[42] Although we have demonstrated that GRK-dependent V1AR signaling impacts βAR responsiveness and cardiac contractility during HF, the potential roles for specific GRK isoforms in these processes remain unknown. GRKs are ubiquitous and interact with multiple GPCRs, and their action at particular GPCRs is ligand and substrate specific.[43,44] Therefore, with more fine-tuned molecular and genetic approaches, it will be desirable to discriminate the impact of specific cardiac GRK isoforms in the regulation of V1AR-mediated effects on βAR responsiveness versus potential prosurvival signaling during the development of HF.

Both basic science investigation and drug development have focused primarily on the renal V2R and not on the cardiac V1AR; however, the clinical effects of V2R antagonists in patients with HF have been disappointing.[3,45,46] The selective V2R antagonist tolvaptan raised serum Na^+ levels in patients with HF but failed to affect the dual primary endpoints of all-cause mortality or cardiovascular deaths or HF hospitalizations in a well-powered trial.[45] The highly selective V2R antagonist lixivaptan was associated with an increase in deaths within the first 10 days of administration of drug when administered to patients with an acute exacerbation of HF, nearly all of the deaths being secondary to worsening HF.[47] Because both tolvaptan and lixivaptan increase plasma AVP levels, it is possible that the adverse results with lixivaptan might be explained by AVP-induced inhibition of βAR signaling in a group of patients with elevated levels of AVP (hyponatremia) and limited inotropic reserve. Others have demonstrated in a mouse model of ischemia-reperfusion injury that AVP infusion significantly increased myocardial dysfunction and mortality compared with both saline and dobutamine.[48] These results are consistent with our observation that cardiac contractility is diminished in hearts of animals with HF and elevated plasma AVP. By contrast, AVP has been shown to improve survival in swine after experimental cardiac arrest, presumably because of enhanced myocardial blood flow secondary to enhanced peripheral vasoconstriction.[49–53] Unfortunately, AVP has not proven consistently beneficial in humans in large clinical trials of patients who have sustained cardiogenic shock.[54–57] Thus, further exploration of the role of AVP in distinct forms of cardiovascular stress is warranted.

In summary, we present data that show for the first time that AVP stimulation (1) acutely inhibits βAR-dependent cAMP generation and Ca^{2+} mobilization in adult cardiomyocytes and ex vivo cardiac contractility, (2) activates a previously unknown GRK-dependent V1AR signaling mechanism to diminish βAR responsiveness, and (3) chronically impacts in vivo contractile function and βAR expression in mice during TAC-induced hypertrophy. These results have led us to hypothesize that V1AR antagonists might be useful in the treatment of patients with acute HF and high circulating levels of AVP.

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Disclosures

Drs Tilley, Koch, Houser, and Feldman have equity in Renovacor, Inc, which has neither funded this study nor has a relevant product related to this study. The remaining authors report no conflicts.

References

1. Bell NH, Schedl HP, Bartter FC. An explanation for abnormal water retention and hypoosmolality in congestive heart failure. *Am J Med*. 1964;36:351–360.
2. Francis GS, Benedict C, Johnstone DE, Kirlin PC, Nicklas J, Liang CS, Kubo SH, Rudin-Toretsky E, Yusuf S. Comparison of neuroendocrine activation in patients with left ventricular dysfunction with and without congestive heart failure: a substudy of the Studies of Left Ventricular Dysfunction (SOLVD). *Circulation*. 1990;82:1724–1729.
3. Gheorghiade M, Pang PS, Ambrosy AP, Lan G, Schmidt P, Filippatos G, Konstam M, Swedberg K, Cook T, Traver B, Maggioni A, Burnett J, Grinfeld L, Udelson J, Zannad F. A comprehensive, longitudinal description of the in-hospital and post-discharge clinical, laboratory, and neurohormonal course of patients with heart failure who die or are rehospitalized within 90 days: analysis from the EVEREST trial. *Heart Fail Rev*. 2012;17:485–509.
4. Szatalowicz VL, Arnold PE, Chaimovitz C, Bichet D, Berl T, Schrier RW. Radioimmunoassay of plasma arginine vasopressin in hyponatremic patients with congestive heart failure. *N Engl J Med*. 1981;305:263–266.
5. Voors AA, von Haehling S, Anker SD, Hillege HL, Struck J, Hartmann O, Bergmann A, Squire I, van Veldhuisen DJ, Dickstein K; OPTIMAAL Investigators. C-terminal provasopressin (copeptin) is a strong prognostic marker in patients with heart failure after an acute myocardial infarction: results from the OPTIMAAL study. *Eur Heart J*. 2009;30:1187–1194.
6. Knepper MA. Molecular physiology of urinary concentrating mechanism: regulation of aquaporin water channels by vasopressin. *Am J Physiol*. 1997;272(pt 2):F3–F12.
7. Klein L, O'Connor CM, Leimberger JD, Gattis-Stough W, Piña IL, Felker GM, Adams KF Jr, Califf RM, Gheorghiade M; OPTIME-CHF Investigators. Lower serum sodium is associated with increased short-term mortality in hospitalized patients with worsening heart failure: results from the Outcomes of a Prospective Trial of Intravenous Milrinone for Exacerbations of Chronic Heart Failure (OPTIME-CHF) study. *Circulation*. 2005;111:2454–2460.
8. Goldsmith SR. Vasopressin as vasopressor. *Am J Med*. 1987;82:1213–1219.
9. Naitoh M, Suzuki H, Murakami M, Matsumoto A, Arakawa K, Ichihara A, Nakamoto H, Oka K, Yamamura Y, Saruta T. Effects of oral AVP receptor antagonists OPC-21268 and OPC-31260 on congestive heart failure in conscious dogs. *Am J Physiol*. 1994;267(pt 2):H2245–H2254.

10. Yatsu T, Kusayama T, Tomura Y, Arai Y, Aoki M, Tahara A, Wada K, Tsukada J. Effect of conivaptan, a combined vasopressin V(1a) and V(2) receptor antagonist, on vasopressin-induced cardiac and haemodynamic changes in anaesthetised dogs. *Pharmacol Res*. 2002;46:375–381.
11. Li X, Chan TO, Myers V, Chowdhury I, Zhang XQ, Song J, Zhang J, Andrel J, Funakoshi H, Robbins J, Koch WJ, Hyslop T, Cheung JY, Feldman AM. Controlled and cardiac-restricted overexpression of the arginine vasopressin V1A receptor causes reversible left ventricular dysfunction through Gαq-mediated cell signaling. *Circulation*. 2011;124:572–581.
12. Tilley DG. G protein-dependent and G protein-independent signaling pathways and their impact on cardiac function. *Circ Res*. 2011;109:217–230.
13. Zhu W, Tilley DG, Myers VD, Tsai EJ, Feldman AM. Increased vasopressin 1A receptor expression in failing human hearts. *J Am Coll Cardiol*. 2014;63:375–376.
14. Akhter SA, Luttrell LM, Rockman HA, Iaccarino G, Lefkowitz RJ, Koch WJ. Targeting the receptor-Gq interface to inhibit *in vivo* pressure overload myocardial hypertrophy. *Science*. 1998;280:574–577.
15. Funakoshi H, Chan TO, Good JC, Libonati JR, Piuhola J, Chen X, MacDonnell SM, Lee LL, Herrmann DE, Zhang J, Martini J, Palmer TM, Sanbe A, Robbins J, Houser SR, Koch WJ, Feldman AM. Regulated overexpression of the A1-adenosine receptor in mice results in adverse but reversible changes in cardiac morphology and function. *Circulation*. 2006;114:2240–2250.
16. Hamad EA, Zhu W, Chan TO, Myers V, Gao E, Li X, Zhang J, Song J, Zhang XQ, Cheung JY, Koch W, Feldman AM. Cardioprotection of controlled and cardiac-specific over-expression of A(2A)-adenosine receptor in the pressure overload. *PLoS One*. 2012;7:e39919.
17. Wang J, Gao E, Rabinowitz J, Song J, Zhang XQ, Koch WJ, Tucker AL, Chan TO, Feldman AM, Cheung JY. Regulation of *in vivo* cardiac contractility by phospholemman: role of Na^+/Ca^{2+} exchange. *Am J Physiol Heart Circ Physiol*. 2011;300:H859–H868.
18. Most P, Seifert H, Gao E, Funakoshi H, Völkers M, Heierhorst J, Remppis A, Pleger ST, DeGeorge BR Jr, Eckhart AD, Feldman AM, Koch WJ. Cardiac S100A1 protein levels determine contractile performance and propensity toward heart failure after myocardial infarction. *Circulation*. 2006;114:1258–1268.
19. Silver LH, Hemwall EL, Marino TA, Houser SR. Isolation and morphology of calcium-tolerant feline ventricular myocytes. *Am J Physiol*. 1983;245(pt 1):H891–H896.
20. Allen MD, DiPilato LM, Rahdar M, Ren YR, Chong C, Liu JO, Zhang J. Reading dynamic kinase activity in living cells for high-throughput screening. *ACS Chem Biol*. 2006;1:371–376.
21. Tilley DG, Kim IM, Patel PA, Violin JD, Rockman HA. β-Arrestin mediates beta1-adrenergic receptor-epidermal growth factor receptor interaction and downstream signaling. *J Biol Chem*. 2009;284:20375–20386.
22. Tilley DG, Nguyen AD, Rockman HA. Troglitazone stimulates beta-arrestin-dependent cardiomyocyte contractility via the angiotensin II type 1A receptor. *Biochem Biophys Res Commun*. 2010;396:921–926.
23. MacDonnell SM, García-Rivas G, Scherman JA, Kubo H, Chen X, Valdivia H, Houser SR. Adrenergic regulation of cardiac contractility does not involve phosphorylation of the cardiac ryanodine receptor at serine 2808. *Circ Res*. 2008;102:e65–e72.
24. Bristow MR. Treatment of chronic heart failure with β-adrenergic receptor antagonists: a convergence of receptor pharmacology and clinical cardiology. *Circ Res*. 2011;109:1176–1194.
25. Pontier SM, Percherancier Y, Galandrin S, Breit A, Galés C, Bouvier M. Cholesterol-dependent separation of the beta2-adrenergic receptor from its partners determines signaling efficacy: insight into nanoscale organization of signal transduction. *J Biol Chem*. 2008;283:24659–24672.
26. De Lean A, Stadel JM, Lefkowitz RJ. A ternary complex model explains the agonist-specific binding properties of the adenylate cyclase-coupled beta-adrenergic receptor. *J Biol Chem*. 1980;255:7108–7117.
27. Fu Q, Chen X, Xiang YK. Compartmentalization of β-adrenergic signals in cardiomyocytes. *Trends Cardiovasc Med*. 2013;23:250–256.
28. Violin JD, Dewire SM, Barnes WG, Lefkowitz RJ. G protein-coupled receptor kinase and beta-arrestin-mediated desensitization of the angiotensin II type 1A receptor elucidated by diacylglycerol dynamics. *J Biol Chem*. 2006;281:36411–36419.
29. Harris DM, Chen X, Pesant S, Cohn HI, MacDonnell SM, Boucher M, Vinge LE, Raake P, Moraca SR, Li D, Most P, Houser SR, Koch WJ, Eckhart AD. Inhibition of angiotensin II Gq signaling augments beta-adrenergic receptor mediated effects in a renal artery stenosis model of high blood pressure. *J Mol Cell Cardiol*. 2009;46:100–107.
30. Dorn GW 2nd, Tepe NM, Wu G, Yatani A, Liggett SB. Mechanisms of impaired beta-adrenergic receptor signaling in G(alphaq)-mediated cardiac hypertrophy and ventricular dysfunction. *Mol Pharmacol*. 2000;57:278–287.
31. Jenie RI, Nishimura M, Fujino M, Nakaya M, Mizuno N, Tago K, Kurose H, Itoh H. Increased ubiquitination and the crosstalk of G protein signaling in cardiac myocytes: involvement of Ric-8B in Gs suppression by Gq signal. *Genes Cells*. 2013;18:1095–1106.
32. Jacobsen SE, Nørskov-Lauritsen L, Thomsen AR, Smajilovic S, Wellendorph P, Larsson NH, Lehmann A, Bhatia VK, Bräuner-Osborne H. Delineation of the GPRC6A receptor signaling pathways using a mammalian cell line stably expressing the receptor. *J Pharmacol Exp Ther*. 2013;347:298–309.
33. Kamal FA, Travers JG, Blaxall BC. G protein-coupled receptor kinases in cardiovascular disease: why "where" matters. *Trends Cardiovasc Med*. 2012;22:213–219.
34. Ryckwaert F, Virsolvy A, Fort A, Murat B, Richard S, Guillon G, Colson PH. Terlipressin, a provasopressin drug exhibits direct vasoconstrictor properties: consequences on heart perfusion and performance. *Crit Care Med*. 2009;37:876–881.
35. Klingler C, Ancellin N, Barrault MB, Morel A, Buhler JM, Elalouf JM, Clauser E, Lugnier C, Corman B. Angiotensin II potentiates vasopressin-dependent cAMP accumulation in CHO transfected cells: mechanisms of cross-talk between AT1A and V2 receptors. *Cell Signal*. 1998;10:65–74.
36. Klingler C, Ancellin N, Barrault MB, Morel A, Corman B. Potentiation of receptor-mediated cAMP production: role in the cross-talk between vasopressin V1a and V2 receptor transduction pathways. *Biochem J*. 1998;330(pt 2):1023–1028.
37. Meszaros JG, Gonzalez AM, Endo-Mochizuki Y, Villegas S, Villarreal F, Brunton LL. Identification of G protein-coupled signaling pathways in cardiac fibroblasts: cross talk between G(q) and G(s). *Am J Physiol Cell Physiol*. 2000;278:C154–C162.
38. Budd DC, Challiss RA, Young KW, Tobin AB. Cross talk between m3-muscarinic and beta(2)-adrenergic receptors at the level of receptor phosphorylation and desensitization. *Mol Pharmacol*. 1999;56:813–823.
39. Mandyam CD, Thakker DR, Christensen JL, Standifer KM. Orphanin FQ/nociceptin-mediated desensitization of opioid receptor-like 1 receptor and mu opioid receptors involves protein kinase C: a molecular mechanism for heterologous cross-talk. *J Pharmacol Exp Ther*. 2002;302:502–509.
40. Malhotra R, D'Souza KM, Staron ML, Birukov KG, Bodi I, Akhter SA. G alpha(q)-mediated activation of GRK2 by mechanical stretch in cardiac myocytes: the role of protein kinase C. *J Biol Chem*. 2010;285:13748–13760.
41. Zhu W, Tilley DG, Myers VD, Coleman RC, Feldman AM. Arginine vasopressin enhances cell survival via a G protein-coupled receptor kinase 2/β-arrestin1/extracellular-regulated kinase ½-dependent pathway in H9c2 cells. *Mol Pharmacol*. 2013;84:227–235.
42. Huang ZM, Gold JI, Koch WJ. G protein-coupled receptor kinases in normal and failing myocardium. *Front Biosci (Landmark Ed)*. 2011;16:3047–3060.
43. Vinge LE, Andressen KW, Attramadal T, Andersen GØ, Ahmed MS, Peppel K, Koch WJ, Freedman NJ, Levy FO, Skomedal T, Osnes JB, Attramadal H. Substrate specificities of g protein-coupled receptor kinase-2 and -3 at cardiac myocyte receptors provide basis for distinct roles in regulation of myocardial function. *Mol Pharmacol*. 2007;72:582–591.
44. Heitzler D, Durand G, Gallay N, Rizk A, Ahn S, Kim J, Violin JD, Dupuy L, Gauthier C, Piketty V, Crépieux P, Poupon A, Clément F, Fages F, Lefkowitz RJ, Reiter E. Competing G protein-coupled receptor kinases balance G protein and β-arrestin signaling. *Mol Syst Biol*. 2012;8:590.
45. Konstam MA, Gheorghiade M, Burnett JC Jr, Grinfeld L, Maggioni AP, Swedberg K, Udelson JE, Zannad F, Cook T, Ouyang J, Zimmer C, Orlandi C; Efficacy of Vasopressin Antagonism in Heart Failure Outcome Study With Tolvaptan (EVEREST) Investigators. Effects of oral tolvaptan in patients hospitalized for worsening heart failure: the EVEREST Outcome Trial. *JAMA*. 2007;297:1319–1331.
46. Lanfear DE, Sabbah HN, Goldsmith SR, Greene SJ, Ambrosy AP, Fought AJ, Kwasny MJ, Swedberg K, Yancy CW, Konstam MA, Maggioni AP, Zannad F, Gheorghiade M; EVEREST Trial Investigators. Association of arginine vasopressin levels with outcomes and the effect of V2 blockade in patients hospitalized for heart failure with reduced ejection fraction: insights from the EVEREST trial. *Circ Heart Fail*. 2013;6:47–52.
47. Feldman AM, Hamad E, Tsai EJ, Zhu W, Tilley DG, Alvarez R, Cheung JY. Vasopressin antagonists for patients with acute heart failure: interpreting new clinical and translational data. *Clin Pharmacol Ther*. 2014;95:373–375.

48. Indrambarya T, Boyd JH, Wang Y, McConechy M, Walley KR. Low-dose vasopressin infusion results in increased mortality and cardiac dysfunction following ischemia-reperfusion injury in mice. *Crit Care*. 2009;13:R98.
49. Coute RA, Mader TJ, Sherman LD. Outcomes by rescue shock number during the metabolic phase of porcine ventricular fibrillation resuscitation. *Am J Emerg Med*. 2014;32:586–591.
50. Krismer AC, Lindner KH, Wenzel V, Mayr VD, Voelckel WG, Lurie KG, Strohmenger HU. The effects of endogenous and exogenous vasopressin during experimental cardiopulmonary resuscitation. *Anesth Analg*. 2001;92:1499–1504.
51. Mayr VD, Wenzel V, Voelckel WG, Krismer AC, Mueller T, Lurie KG, Lindner KH. Developing a vasopressor combination in a pig model of adult asphyxial cardiac arrest. *Circulation*. 2001;104:1651–1656.
52. Voelckel WG, Lurie KG, McKnite S, Zielinski T, Lindstrom P, Peterson C, Krismer AC, Lindner KH, Wenzel V. Comparison of epinephrine and vasopressin in a pediatric porcine model of asphyxial cardiac arrest. *Crit Care Med*. 2000;28:3777–3783.
53. Voelckel WG, Lurie KG, McKnite S, Zielinski T, Lindstrom P, Peterson C, Wenzel V, Lindner KH, Benditt D. Effects of epinephrine and vasopressin in a piglet model of prolonged ventricular fibrillation and cardiopulmonary resuscitation. *Crit Care Med*. 2002;30:957–962.
54. Gueugniaud PY, David JS, Chanzy E, Hubert H, Dubien PY, Mauriaucourt P, Bragança C, Billères X, Clotteau-Lambert MP, Fuster P, Thiercelin D, Debaty G, Ricard-Hibon A, Roux P, Espesson C, Querellou E, Ducros L, Ecollan P, Halbout L, Savary D, Guillaumée F, Maupoint R, Capelle P, Bracq C, Dreyfus P, Nouguier P, Gache A, Meurisse C, Boulanger B, Lae C, Metzger J, Raphael V, Beruben A, Wenzel V, Guinhouya C, Vilhelm C, Marret E. Vasopressin and epinephrine vs. epinephrine alone in cardiopulmonary resuscitation. *N Engl J Med*. 2008;359:21–30.
55. Mentzelopoulos SD, Malachias S, Chamos C, Konstantopoulos D, Ntaidou T, Papastylianou A, Kolliantzaki I, Theodoridi M, Ischaki H, Makris D, Zakynthinos E, Zintzaras E, Sourlas S, Aloizos S, Zakynthinos SG. Vasopressin, steroids, and epinephrine and neurologically favorable survival after in-hospital cardiac arrest: a randomized clinical trial. *JAMA*. 2013;310:270–279.
56. Mentzelopoulos SD, Zakynthinos SG, Siempos I, Malachias S, Ulmer H, Wenzel V. Vasopressin for cardiac arrest: meta-analysis of randomized controlled trials. *Resuscitation*. 2012;83:32–39.
57. Ong ME, Tiah L, Leong BS, Tan EC, Ong VY, Tan EA, Poh BY, Pek PP, Chen Y. A randomised, double-blind, multi-centre trial comparing vasopressin and adrenaline in patients with cardiac arrest presenting to or in the Emergency Department. *Resuscitation*. 2012;83:953–960.

CLINICAL PERSPECTIVE

Elevated levels of arginine vasopressin (AVP) are a characteristic feature of patients with heart failure (HF) and are directly related to worsening symptoms and increased mortality. Although AVP activates both cardiac V1A receptors (V1AR) and renal V2R, the development of pharmacological agents for HF therapy has focused on V2R antagonism to treat hyponatremia. However, V2R antagonism can increase circulating AVP levels, and cardiac V1AR expression has been shown to increase 2-fold in human patients with end-stage HF. We now demonstrate that acute stimulation of V1AR reduces β-adrenergic receptor (βAR) ligand affinity, downstream signaling, and cardiac contractility. Although V1AR is a Gq protein–coupled receptor, inhibition of Gq protein–dependent signaling did not prevent AVP-mediated attenuation of βAR responsiveness. However, mutation of the putative C-terminal GPCR kinase (GRK) phosphorylation sites of V1AR completely blocked the ability of AVP to inhibit βAR-mediated signaling. The ability of AVP to inhibit βAR responsiveness via a GRK-dependent mechanism is unique, because another GqPCR, the angiotensin II type 1A receptor, was unable to impact βAR signaling. Administration of the V1AR-selective antagonist SR 49059 normalized cardiac performance and both V1AR and βAR expression levels in mice with HF secondary to transaortic constriction. This newly discovered relationship between cardiac-expressed V1AR and βAR may explain the increased mortality observed in patients with acute HF and elevated AVP levels, alone or secondary to treatment with V2R-selective antagonists, and may provide support for the potential use of a V1AR antagonist in the treatment of these patients.