Gene Transfer of Heterologous G Protein–Coupled Receptors to Cardiomyocytes
Differential Effects on Contractility

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Abstract—In heart failure, reduced cardiac contractility is accompanied by blunted cAMP responses to β-adrenergic stimulation. Parathyroid hormone (PTH)–related peptide and arginine vasopressin are released from the myocardium in response to increased wall stress but do not stimulate contractility or adenylyl cyclase at physiological concentrations. To bypass the defective β-adrenergic signaling cascade, recombinant P1 PTH/PTH-related peptide receptors (rPTH1-Rs) and V2 vasopressin receptors (rV2-Rs), which are normally not expressed in the myocardium and which are both strongly coupled to adenylyl cyclase, and recombinant β2-adrenergic receptors (rβ2-ARs) were overexpressed in cardiomyocytes by viral gene transfer. The capacity of endogenous hormones to increase contractility via the heterologous, recombinant receptors was compared. Whereas V2-Rs are uniquely coupled to Gs, PTH1-Rs and β2-ARs are also coupled to other G proteins. Gene transfer of rPTH1-Rs or rβ2-ARs to adult cardiomyocytes resulted in maximally increased basal contractility, which could not be further stimulated by adding receptor agonists. Agonists at rPTH1-Rs induced increased cAMP formation and phospholipase C activity. In contrast, healthy or failing rV2-R–expressing cardiomyocytes showed unaltered basal contractility. Their contractility and cAMP formation increased only at agonist exposure, which did not activate phospholipase C. In summary, we found that gene transfer of PTH1-Rs to cardiomyocytes results in constitutive activity of the transgene, as does that of β2-ARs. In the absence of receptor agonists, rPTH1-Rs and rβ2-ARs increase basal contractility, coupling to 2 G proteins simultaneously. In contrast, rV2-Rs are uniquely coupled to Gs and are not constitutively active, retaining their property to be activated exclusively on agonist stimulation. Therefore, gene transfer of V2-Rs might be more suited to test the effects of cAMP-stimulating receptors in heart failure than that of PTH1-Rs or β2-ARs. (Circ Res. 2001;88:688-695.)

Key Words: gene transfer ■ receptor ■ heart failure

Congestive heart failure is characterized by an increased sympathetic drive; a stimulation of the renin-angiotensin-aldosterone system; and an augmented release of several peptide hormones, among them atrial natriuretic peptide, arginine vasopressin (AVP), and parathyroid hormone (PTH)–related peptide (PTHrp).1–3 AVP is released both systemically from the pituitary gland and locally from the myocardium.2,4 In addition, PTHrp is released from the heart in response to increased wall stress or hypoxia,5 and PTHrp mRNA has been detected in isolated cardiomyocytes.6,7 PTH and PTHrp are devoid of direct effects on ventricular contraction at physiological concentrations in vivo.7 In the cardiomyocyte, PTH and PTHrp induce hypertrophy acting via an endogenously occurring, yet uncloned, myocardial PTH receptor subtype8 and therefore contribute to further worsening of heart failure. At physiological concentrations, neither PTH nor PTHrp nor AVP stimulates cAMP generation in cardiomyocytes.9,10 Whereas PTH and PTHrp exert strong vasodilatory effects on the coronary arteries,8 AVP is one of the strongest coronary vasoconstrictors.

Our laboratory has examined several approaches to bypass the defective β-adrenergic signaling cascade of the failing heart9,10 via viral gene transfer of heterologous receptors that respond to intracardially released hormones. Recently, we reported the use of recombinant V2 vasopressin receptors (rV2-Rs) to increase contractility in healthy cardiomyocytes.10 Similar to V2-Rs, P1 PTH/PTHrp receptors (PTH1-Rs) are strongly coupled to Gs and induce a considerable adenylyl cyclase stimulation in the presence of subnanomolar concentrations of receptor agonists.13,14 Neither receptor is expressed in normal myocardium. In contrast to V2-Rs, however, PTH1-Rs are strongly coupled to both Gs and Gq, hence being able to equally stimulate phospholipase C (PLC) activity.15–17

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As both AVP and PTHrp are released from the heart in response to increased wall stress, recombinant receptors to these hormones might be used to create a positive inotropic paracrine loop. Inducing a receptor subtype shift by gene transfer of the recombinant cAMP–stimulating receptors into the myocardium should therefore allow the use of the endogenously released receptor agonists for a positively inotropic effect in myocardial distress. Overexpression of recombinant PTH1-Rs (rPTH1-Rs) seemed especially attractive, because the specific receptor agonists induce marked coronary dilation.

Recombinant cAMP–stimulating receptors have been investigated previously by overexpressing β-adrenergic receptors (β-ARs) in the myocardium. Quite surprisingly, cardiac-specific β2-AR–transgenic mice displayed increased contractility independently of the presence of receptor agonists that could not be antagonized by standard receptor blockers. In addition, after somatic gene transfer, animals or cardiomyocytes expressing β2-ARs showed increased basal contractility. Therefore, it has been generally concluded that gene transfer of β2-ARs results in the expression of constitutively active receptors. Similar to PTH1-Rs, β2-ARs are coupled to G proteins, Gs and Gi. As we sought to learn more about gene transfer of recombinant cAMP–stimulating receptors to normal and failing adult cardiomyocytes, the present study compares the effects of gene transfer of PTH1-Rs and of V1a-Rs with the effects of gene transfer of β2-ARs.

For this purpose, we chose infection conditions that resulted in moderate overexpression of recombinant receptors at levels that are generally reached after gene transfer in vivo. This approach was aimed at complementing studies in transgenic mice that have often used extremely high expression levels of recombinant receptors.

Materials and Methods

Construction and Purification of Recombinant Adenovirus

Generation of a recombinant (E1/E3-deficient) adenovirus (serotype 5) expressing the human V1a-R13 under control of a cytomegalovirus promotor (Ad-V1a-R-GFP) has been described recently. Similarly, recombinant adenoviruses for the PTH1-R14 (Ad-PTH1-R-GFP) and for the human β2-AR (Ad-β2-AR-GFP) were generated. All receptors were expressed in a baculotropic system together with green fluorescent protein (GFP). As a control, Ad-GFP without further transgenes was used. Large virus stocks were prepared as described previously. Adenoviral titers were determined using plaque titration in non–E1-expressing cells.

Preparation and Culture of Adult Ventricular Cardiomyocytes

Single calcium-tolerant ventricular myocytes were isolated from New Zealand White rabbit hearts. The hearts were excised, suspended in a Langendorff apparatus, and perfused retrogradely. Collagenase type II (Cell Systems; 1.6 mg/mL) was infused in the presence of 0.04 mmol/L CaCl2. After 15 to 20 minutes, the softened tissue was cut with scissors, resuspended in carbogen-gassed Powell medium (composition in mmol/L, NaCl 110, KCl 2.5, KH2PO4 1.2, MgSO4 1.2, NaCO3 25, and glucose 11 [pH 7.4]) and filtered. Increasing concentrations of CaCl2 were added slowly. Finally, the cells were layered on top of Powell medium containing BSA (40 mg/mL) and 1 mmol/L CaCl2, and were allowed to sediment.

Isolated cardiomyocytes were cultured in M199 (supplemented with MEM vitamins; MEM nonessential amino acids; 25 mmol/L HEPES; 100 IU/mL penicillin; and [in μg/mL] insulin 10, streptomyces 100, and gentamicin 100) on laminin- precoated dishes (5 to 10 μg/cm2) at a density of 104 cells/cm2 (at 5% CO2 and 37°C). The cells were infected with adenovirus (multiplicity of infection for Ad-V1a-R-GFP and Ad-PTH1-R-GFP, 50 plaque-forming units/cell; for Ad-β2-R-GFP, 20 plaque-forming units/cell) 5 hours after plating. Extensive titration had shown that 90% to 95% of cardiomyocytes express the transgene at this titer.

Preparation of Failing Cardiomyocytes

Pacemakers were implanted into New Zealand White rabbits, and animals were paced at 380/min for 2 weeks. After that period, echocardiographic assessment showed a clear decrease in left ventricular (LV) fractional shortening, and tip catheter measurement documented a marked decrease in LV dP/dtmax, and an increase in LV end-diastolic pressure (Table). Cardiomyocytes were isolated from these hearts as described above. The altered morphology (cell length >150 μm) and the depressed contractility of failing cardiomyocytes were stable for several days after isolation. Institutional guidelines for care and use of laboratory animals were followed.

Contraction Experiments

Contractility of infected cardiomyocytes was measured by an electro-optical monitoring system connected to online digitalized assessment of amplitude and velocity of shortening and of relaxation. The experiments were performed on ventricular cardiomyocytes in a single-cell investigation system (Scientific Instruments), in a temperature-controlled cuvette (37°C), at a constant medium flow of 0.5 mL/min, and at a constant electrical field. A 1.8 mmol/L Ca2+-Tyrode solution was used. Transgene expression in individual cells was monitored on determining green fluorescence by switching to monochromatic light at 470 nm. The cardiomyocytes were paced by an external stimulation of 50 V and an 800-ms pulse duration to achieve a contraction frequency of 70/min. After the contraction amplitude reached stability, the experiments were started by applying increasing concentrations of PTH(1–34), PTHrp(1–34), AVP, desmopressin (DDAVP), or isoproterenol. All cells were finally superfused with isoproterenol (1 μmol/L) to control their viability.

Radioligand Binding

Cells were harvested 48 hours after adenoviral infection. Membranes were prepared as described previously. The protein content of each sample was determined by the method of Bradford. Radioligand binding of [125I]-labeled (Nle8,18)[Tyrr34]-PTH(1–34) (Amersham) in 7 concentrations ranging from 0.01 to 5 nmol/L with or without unlabeled PTH(1–34) (5×10−9 mol/L) was carried out for 6 hours at 4°C in the presence of (in mmol/L) Tris-HCl (pH 7.4) 50, NaCl 100, and KCl 5 in triplicate. Binding of [3H]AVP was performed as described. Membrane preparations were incubated with increasing concentrations of [3H]AVP in 7 concentrations ranging from 0.02 to 8 nmol/L with or without unlabeled AVP (10−6 mol/L) in the presence of 50 nmol/L Tris-HCl (pH 7.4) at 37°C for 1 hour. For determination of β-AR density, membrane preparations were incubated with increasing concentrations of [3H]CGP 12177 in 7 concentrations ranging from...

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*P<0.05.

Hemodynamic data of rabbits after 2 weeks of rapid ventricular pacing or after sham operation. Table shows heart rate, echocardiographic fractional shortening (transsternal M mode), LV end-diastolic pressure (LVEDP), and the first derivative of LV pressure (dp/dtmax).
Determination of Intracellular cAMP Concentrations

Cardiomyocytes were investigated 48 hours after adenoviral infection. The cells were stimulated with the respective agonists for 10 minutes. The reaction was stopped by adding 100 μL of a 20 mmol/L phosphate-EDTA buffer (pH 7.0) in the presence of 3-isobutyl-L-methylxanthine (1 mmol/L), followed by cooking at 100°C for 7 minutes. This suspension was centrifuged, and the supernatant was used for ELISA assays with cAMP-specific antibodies (Stratagene, catalog No. 200020), using the manufacturer’s instructions.

Determination of PLC Activity

For inositol triphosphate (IP₃) assays, adenovirus-infected cardiomyocytes were stimulated with the respective agonists for 1 minute, and the reaction was stopped by adding perchloric acid (4%) and scratching the cells off. They were centrifuged at 2000 g, and then 10 μL of KOH (10 mol/L) was added. The solution was resuspended and centrifuged again, and the protein content of each sample was determined by the method of Bradford. The supernatant was used for ELISA assays with cAMP-specific antibodies (Stratagene, catalog No. TRK 1000) to measure IP₃ formation, according to the manufacturer’s instructions.

Determination of PTH and PTHrp Levels in Culture Medium

Medium (2 mL) was harvested from cardiomyocytes in culture for 1 and 2 days. Levels of PTHrp were determined by a routine ELISA using 2 specific antibodies.

Data Analysis

Data are mean±SEM. For statistical analysis, we used ANOVA for repeated measurements followed by Scheffé testing or, where appropriate, Student t test with 2-tailed distribution.

Results

Infection With Recombinant Adenoviruses

Adenoviral constructs for PTH1-R (Ad-PTH1-R-GFP), V₂-R (Ad-V₂-R-GFP), β₂-AR (Ad-β₂-AR-GFP), and GFP (Ad-GFP) were used in all experiments. Robust expression of all transgenes was demonstrated after adenoviral infection of isolated adult cardiomyocytes by comparing images under normal light and at 470 nm (green fluorescence), as shown before."

Radioligand binding with [³H]labeled PTH(1–34), with [³H]AVP, or with [³H]CGP 12177 showed overexpression of rPTH1-Rs, rV₂-Rs, and rβ₂-ARs in clear excess over the respective natively occurring receptors as measured in control cells (Figure 1; β₂-AR density was 210±20 fmol/mg protein after Ad-β₂-AR-GFP infection versus 15±2 fmol/mg protein in controls). The V₂-selective ligand, SR 1214163A, specifically displaced the increase in [³H]AVP binding in membranes from Ad-V₂-R-GFP–infected cells (not shown), showing that it was due to the appearance of a rV₂-R population. The Kᵢ value of [³H]AVP binding (in nmol/L) to rV₂-Rs was 0.16 (0.09 to 0.28), that of [³H]labeled PTH(1–34) binding to rPTH1-Rs 0.2 (0.08 to 0.4), and that of [³H]CGP 12177 to β-ARs 0.5 (0.3 to 0.8), which are similar to the values reported elsewhere."

Intracellular cAMP Formation After Infection With Recombinant Viruses

In Ad-GFP–infected or noninfected cardiomyocytes, intracellular cAMP formation did not change after addition of increasing concentrations of PTH(1–34) (Figure 2) or PTHrp in a range of physiological concentrations lower than 1 μmol/L. With 1 μmol/L PTHrp, a small, 1.5-fold increase in cAMP formation was observed, compatible with what has been reported earlier."

In cells infected with Ad-PTH1-R-GFP, agonist-induced intracellular cAMP formation was maximally increased to 3.4-fold (PTH, Figure 2) and to 4-fold (PTHrp, not shown) with EC₅₀ values of ≈1 nmol/L (PTH) and of 0.5 nmol/L...
(PTHr). In Ad-V2-R-GFP–infected cells, cAMP formation was 3.6-fold increased in the presence of agonists AVP (EC50, 0.5 nmol/L) or DDAVP (V2-R-selective; EC50, 0.2 nmol/L), similar to the results of our previous studies.10,23 Basal cAMP formation was 1.7±0.25 (Ad-GFP) and 1.6±0.3 (Ad-V2-R-GFP) pmol/min per 10^5 cells. Infection with Ad-β2-AR-GFP or Ad-PTH1-R-GFP resulted in trends toward increased basal cAMP formation (2.1±0.5 and 2.1±0.25 pmol/min per 10^5 cells, respectively), which, however, did not reach statistical significance. Ad-β2-AR-GFP–infected cells showed a markedly increased maximum cAMP formation in response to 1 μmol/L isoproterenol (6-fold increase versus 3.4-fold increase in controls).

**Effect of rPTH1-Rs and rV2-Rs on PLC Activity**

To study the effects of heterologous expression of rPTH1-Rs or rV2-Rs on PLC activity, intracellular IP3 formation was determined. Figure 3 shows the effect of increasing concentrations of DDAVP and of PTH(1–34) on IP3 formation in cardiomyocytes. Whereas overexpression of rV2-Rs had no influence on intracellular IP3 formation, rPTH1-R–expressing cells showed a markedly increased maximum cAMP formation in response to 1 μmol/L isoproterenol (6-fold increase versus 3.4-fold increase in controls).

**Effects of rPTH1-Rs, rV2-Rs and rβ2-ARs on Contractility of Ventricular Cardiomyocytes**

To study the effect of heterologous expression of rPTH1-Rs, rV2-Rs, and rβ2-ARs on contractile responsiveness of ventricular cardiomyocytes, myocyte shortening was measured after infection with recombinant viruses. Baseline contraction amplitude (3.4 to 3.8 μm) was not significantly altered by infection with Ad-GFP or Ad-V2-R-GFP. Baseline and isoproterenol-dependent contractility did not differ significantly between freshly isolated cardiomyocytes and isolated cardiomyocytes after 48 to 72 hours of culture. Control cells infected with Ad-GFP did not show any contractile response to stimulation with AVP, DDAVP, PTH, or PTHrp at the physiological, submillimolar concentrations used (Figure 4).

After infection with Ad-PTH1-R-GFP, baseline contractility in the absence of agonists was 2.5-fold increased (Figure 4A). In parallel, shortening fraction was increased to 11.5±0.6% (4.7±0.7% in controls). This increased baseline contractility was not significantly altered by adding PTH1-R antagonists Asn-Leu-Trp-PTHrp(7–34) or His-Tyr-PTH(3–34) in concentrations of up to 0.1 μmol/L (Figure 4B).

Addition of specific PTH1-R agonists PTH(1–34) or PTHrp(1–34) did not result in any further increase in contractility (Figure 4A). Also, the addition of increasing concentrations of isoproterenol did not result in any further increase in contractility (Figure 5).

In contrast, superfusion of Ad-V2-R-GFP–infected cardiomyocytes with AVP or DDAVP led to a significant increase in contractility (Figure 4C) and shortening fraction to the same extent as could be reached by β-adrenergic stimulation (at 10^{-7} mol/L isoproterenol: 299±25 μm/second; 10.7±1.2%). The observed EC50 values for AVP and DDAVP were in the picomolar range, far below the concentrations needed for stimulation of natively present V1-Rs. The V2-selective antagonist, SR 1214163A, specifically inhibited the effects of AVP. These results confirm those of earlier studies.10,23

Additionally, in rβ2-AR–expressing cardiomyocytes, basal contractility was 2-fold increased compared with control cells (to 260±23 μm/second) and did not change significantly after the addition of isoproterenol (up to 1 μmol/L).

**Effect of Pertussis Toxin (PTX) on Contractility in rPTH1-R-Expressing Cardiomyocytes**

To exclude additional coupling of rPTH1-Rs to Gi proteins, rPTH1-R–expressing cardiomyocytes were incubated for 12 hours with PTX (1 μg/mL). As would be expected with diminished inhibitory Gi tonus, basal contractility in PTX-
incubated cells was slightly increased (from 280 to 320 μm/second). However, there was no change in contractile response to increasing concentrations of PTH or PTHrp, as would be expected if the effect of PTH1-R–dependent cAMP activation on contractility had been counteracted by simultaneous Gi coupling.19

Effect of rPTH1-Rs and rV2-Rs on Contractility in Failing Cardiomyocytes

Also, in failing cardiomyocytes isolated from rabbits after rapid pacing, rPTH1-Rs and rV2-Rs induced effects similar to those in healthy cells. Figure 6 shows that basal contractility was clearly reduced in failing as compared with healthy cardiomyocytes. Overexpression of PTH1-Rs in these cells led to a clear increase in basal contractility that could not be further stimulated by addition of receptor agonists. In contrast, V2-R–expressing failing cardiomyocytes showed decreased basal contractility similar to control virus–infected cells. Addition of DDAVP clearly increased contractility. This effect was in addition to mere stimulation of native β-ARs (Figure 6).

Levels of PTHrp and PTH in Medium From Cultured Cardiomyocytes

PTH and PTHrp levels were determined in the supernatant from cardiomyocytes after 1 and 2 days of culture. In neither the supernatant from normal nor from failing cardiomyocytes was any PTH or PTHrp detected.

Discussion

In the present study, we demonstrate the feasibility of using gene transfer of rPTH1-Rs or rV2-Rs to stimulate contractility in healthy and failing cardiomyocytes. Both receptors are strongly coupled to adenyl cyclase and are normally not expressed in the myocardium. rPTH1-Rs, however, couple to intracellular signal transduction more promiscuously than do V2-Rs, inducing PLC activation in addition. rV2-R–expressing cardiomyocytes display normal basal contractility and can be maximally stimulated by addition of receptor agonists, whereas cells expressing rPTH1-R and rβ2-AR show markedly increased basal activity that cannot be further stimulated. Consequently, rV2-Rs are only activated on external addition of agonists, whereas rPTH1-Rs and rβ2-ARs appear to be constitutively active.

Overexpression of rPTH1-Rs, rV2-Rs, and rβ2-ARs in Cardiomyocytes

All recombinant receptors were expressed in adenoviral bicistronic constructs together with GFP, so that their expression could be detected in individual cells. Additionally, receptor expression was shown by specific radioligand binding that documented genetic receptor subtype shifts, resulting in a clear overexpression of rPTH1-Rs over natively occurring PTH receptors, of rV2-Rs versus native V2-Rs, and of β-ARs over native β2-ARs at similar expression levels. In contrast to previous studies, however, recombinant receptor density was kept at physiologically occurring levels to min-
imize nonspecific effects observed at higher overexpression levels. Agonists at all receptors strongly stimulated adenylyl cyclase, which was determined by the accumulation of intracellular cAMP in intact cardiomyocytes.

Effects of rPTH1-Rs, rV2-Rs, and rβ2-ARs on Contractility
Gene transfer of rPTH1-Rs and of rβ2-ARs to cardiomyocytes led to clearly increased basal contractility while measured at the same, fixed frequency as control cells. This phenomenon occurred in the obvious absence of receptor agonists as determined in the supernatant of cardiomyocytes after up to 48 hours of incubation. As contractility could not be further stimulated by adding receptor agonists, nor could it be significantly antagonized by specific antagonists, it must have been due to a continuously activated state of the receptor. These findings corroborate previous studies on rβ2-AR expression in cardiomyocytes and in the myocardium. Also, the addition of β-adrenergic agonists did not further stimulate contractility in rPTH1-R–expressing cells.

In contrast, gene transfer of GFP or of V2-Rs resulted in basal contraction amplitude and contractility similar to those in noninfected controls. On external addition of receptor agonists to rV2-R–expressing cells, however, their contractility increased to the same extent as on addition of β-adrenergic agonists. This was true for both normal and failing cardiomyocytes. Although the proportional loss of positive inotropic potency of rV2-Rs in failing cardiomyocytes was somewhat disappointing, their stimulation did provide additive contractile power compared with mere stimulation of native β-ARs (Figure 6).

Stimulation of PLC
As previous reports have documented coupling of cloned PTH1-Rs to Gq and to PLC by measuring [α-32P]GTP-γ-azidoanilide photoaffinity labeling and IP3 response, we also investigated the effects of gene transfer of both heterologous receptors on PLC activity. After gene transfer of V2-Rs, V2-R agonists did not induce PLC activation, whereas gene transfer of PTH1-Rs led to strong agonist-dependent PLC stimulation at nanomolar concentrations. One might therefore be led to believe that simultaneous stimulation of adenylyl cyclase and PLC does not result in a net increase in contractility; this hypothesis should be investigated in further studies.

Findings Parallel Those With Other Recombinant Receptors in Cardiomyocytes
The present study shows that overexpression of both PTH1-Rs and β2-ARs in cardiomyocytes at low, physiologically occurring levels lead to enhanced basal contractility as much as did gene transfer of β2-ARs in vivo. Overexpression of rPTH1-R resembled that of rβ2-ARs inasmuch as rβ2-AR activity could not be antagonized by standard receptor blockers (with the exception of inverse agonists). Because of the lack of inverse agonists for PTH receptors, we

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**Figure 5.** Measurement of velocity of shortening (contractility) in healthy ventricular cardiomyocytes. Ad-PTH1-R-GFP–infected or Ad-GFP–infected cardiomyocytes were electrically paced as described in Materials and Methods. Cells were stimulated with increasing concentrations of isoproterenol. Data are mean±SEM of 22 independently measured cells taken from at least 5 different animals in each group.

**Figure 6.** Effects of rPTH1-Rs and rV2-Rs on velocity of shortening (contractility) in failing cardiomyocytes (n=25 in each group±SEM). Contractility was measured in control cells (A) and in rPTH1-R–expressing or rV2-R–expressing failing cells (B). Agonists, in mol/ L, isoproterenol (iso; 10^{-11}), DDAVP (10^{-9}), and PTH(1–34) (10^{-6}) were added as indicated. As in healthy cardiomyocytes, basal contractility was clearly increased in PTH1-R–expressing cells and could not be further stimulated by addition of receptor agonists. In contrast, V2-R–expressing failing cardiomyocytes showed decreased contractility similar to that of control virus-infected cells. Addition of DDAVP (10^{-9} mol/L) induced contractility to levels seen after stimulation with isoproterenol and was additive to mere stimulation with isoproterenol. Insert shows representative original tracings of a healthy cell and a failing cell. *P<0.05, **P<0.01 vs isoproterenol-stimulated, Ad-GFP–infected failing cardiomyocytes.
Effects of cAMP-Stimulating Transgenes on Models of Heart Failure

Importantly, β2-AR overexpression worsens heart failure when tested in mice made hypertrophic by aortic banding27 or by cross-breeding with muscle LIM protein–negative (MLP−/−) mice.28 In contrast, transgenic mice with increased cAMP levels due to cardiac-specific overexpressions of the β-AR kinase (ARK) inhibitor, βARKct,28 or of adenylyl cyclase VI29 fare better (have increased survival) when tested in the same or in similar settings. These latter mice also display markedly better cardiac function compared with Gsα or β2-AR–transgenic mice30,31 even at an advanced age.

In summary, the question whether cAMP stimulation per se should always be deleterious in heart failure has raised considerable interest. We have identified gene transfer of V2-Rs as cAMP-stimulating receptors to result in transgenes that allow potent positive inotropic effects only in the presence of receptor agonists and that do not alter basal contractility. In contrast, gene transfer of PTH1-R leads to constantly increased basal contractility, as did overexpression of other cAMP-stimulating transgenes, such as β2-ARs, β-ARs (at young age31), βARKct,28 or adenylyl cyclase VI.29 The choice of rV2-Rs as promising transgenes is also supported by the fact that, unlike the native V2-Rs, they do not seem to induce vasoconstriction in the endothelium (H.J. Weig, C. Städele, unpublished observations, 2000).

Possible Consequences for Treatment of Heart Failure

As wall stress varies with hemodynamic conditions of patients with heart failure, AVP secretion from the myocardium will differ depending on loading conditions in those patients and will therefore lead to intermittent cAMP stimulation via heterologous rV2-Rs. Gene transfer of V2-Rs into the myocardium of these patients might allow for the use of this secretion of AVP,24 which is involved in reducing ventricular contractility via V2-Rs, for an intermittent stimulation of contractility mediated by rV2-Rs.

Moreover, rV2-Rs in cardiomyocytes are not subject to downregulation10 even after prolonged agonist exposure. They can be used to increase LV function after somatic gene transfer to the myocardium in vivo.23 Whether this is also true for the long-term overexpression of V2-Rs in vivo remains to be determined in an ongoing study.

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