Adrenal Beta-Arrestin 1 Inhibition In Vivo Attenuates Post-Myocardial Infarction Progression to Heart Failure and Adverse Remodeling Via Reduction of Circulating Aldosterone Levels

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Objectives: We investigated whether adrenal beta-arrestin 1 (βarr1)-mediated aldosterone production plays any role in post-myocardial infarction (MI) heart failure (HF) progression.

Background: Heart failure represents 1 of the most significant health problems worldwide, and new and innovative treatments are needed. Aldosterone contributes significantly to HF progression after MI by accelerating adverse cardiac remodeling and ventricular dysfunction. It is produced by the adrenal cortex after angiotensin II activation of angiotensin II type 1 receptors (AT1Rs), G protein-coupled receptors that also signal independently of G proteins. The G protein-independent signaling is mediated by βarr1 and βarr2. We recently reported that adrenal βarr1 promotes AT1R-dependent aldosterone production leading to elevated circulating aldosterone levels in vivo.

Methods: Adrenal-targeted, adenoviral-mediated gene delivery in vivo in 2-week post-MI rats, a time point around which circulating aldosterone significantly increases to accelerate HF progression, was performed to either increase the expression of adrenal βarr1 or inhibit its function via expression of a βarr1 C-terminal-derived peptide fragment.

Results: We found that adrenal βarr1 overexpression promotes aldosterone elevation after MI, resulting in accelerated cardiac adverse remodeling and deterioration of ventricular function. Importantly, these detrimental effects of aldosterone are prevented when adrenal βarr1 is inhibited in vivo, which markedly decreases circulating aldosterone after MI. Finally, the prototypic AT1R antagonist losartan seems unable to lower this adrenal βarr1-driven aldosterone elevation.

Conclusions: Adrenal βarr1 inhibition, either directly or with AT1R “biased” antagonists that prevent receptor-βarr1 coupling, might be of therapeutic value for curbing HF-exacerbating hyperaldosteronism. (J Am Coll Cardiol 2011;57: 356–65) © 2011 by the American College of Cardiology Foundation

Death due to chronic heart failure (HF) continues to rise, despite recent advances in prevention and management of heart disease, and new treatments are needed (1,2). Aldosterone is 1 of a number of hormones with detrimental effects to the myocardium, where circulating levels are elevated in chronic HF (3). It can contribute significantly to HF progression after myocardial infarction (MI) and to the morbidity and mortality of the disease (3–5). Its main actions on the post-MI heart include but are not limited to cardiac hypertrophy, fibrosis, and increased inflammation and oxidative stress, all of which result in adverse cardiac remodeling and progressive loss of cardiac function and performance (5,6). Accordingly, plasma aldosterone levels are a marker of HF severity (7,8), and aldosterone antagonists—such as spironolactone and eplerenone—have well-documented beneficial effects in HF, constituting a significant segment of the chronic HF pharmacotherapeutic regimen (9,10).

Aldosterone is a mineralocorticoid produced and secreted by the cells of the zona glomerulosa of the adrenal cortex in response to either elevated serum potassium levels or to angiotensin II (AngII) acting through its type 1 receptors.
Plasma aldosterone measurements. Rat plasma aldosterone levels were determined by EIA (Aldosterone EIA kit, ALPCO Diagnostics, Salem, New Hampshire), as described (15,20).

Echocardiographic and hemodynamic measurements. Two-dimensional guided M-mode and Doppler echocardiography with a 14-MHz transducer (Vevo 770 Echograph, VisualSonic, Inc., Toronto, Canada) and closed chest cardiac catheterization were performed in rats, as described previously (16,21). Three independent echocardiographic measurements were taken in both modes.

Western blotting. Western blots to assess protein levels of steroidogenic acute regulatory (StAR) protein (sc-25806), cardiac levels of plasminogen activator inhibitor (PAI)-1 (sc-8979), transforming growth factor (TGF)-β (sc-1460), β-arrestin1 transgenes (A1CT antibody, sc-1460), and glyceraldehyde-3-phosphate dehydrogenase (MAB374, Chemicon, Temecula, California) were done with protein extracts from rat adrenal glands or hearts, as described previously (15,16). Visualiza tion of Western blot signals was performed with Alexa Fluor 680–conjugated donkey antirabbit IgG, IRDye 800CW–coupled streptavidin (Li-COR, Lincoln, Nebraska), and closed chest cardiac catheterization were performed in rats, as described previously (16,21). Three independent echocardiographic measurements were taken in both modes.

Methods

In vivo adrenal gene delivery in post-MI rats. All animal procedures and experiments were performed in accordance with the guidelines of the Institutional Animal Care and Use Committees of Thomas Jefferson and Nova Southeastern Universities. Myocardial infarction was performed with a cryo-infarct method we have previously described (16). Adrenal-specific in vivo gene delivery was done essentially as described (17), via direct delivery of adenovirus in the adrenal gland. Drug treatments were performed with 50 mg/kg/day of losartan potassium (in drinking water) and 100 or 50 mg/kg/day eplerenone (both drugs from Sigma-Aldrich, St. Louis, Missouri).

Construction and purification of adenoviruses. Recombinant adenoviruses that encode full-length wild-type β-arrestin1 (Adβarr1) or a rat β-arrestin1 C-terminal fragment (aa. 369-418, Adβarr1ct) (Online Fig. 1A) were constructed as described previously (15,16). Briefly, transgenes were cloned into shuttle vector pAdTrack-cytomegalovirus, which harbors a cytomegalovirus-driven green fluorescent protein (GFP), to form the viral constructs with standard cloning protocols. As control adenovirus, empty vector that expressed only GFP (AdGFP) was used. The resultant adenoviruses were purified, as described previously, with 2 sequential rounds of cesium chloride density ultracentrifugation (15,16).
for atrial natriuretic peptide (ANP) (NPR-A); and finally, 5′-TCAAGAAGCTGGAGGAGC-3′ and 5′-GGACATCAAGGGGATCAC-3′ for 18S ribosomal RNA. Real time RT-PCR was performed with SYBR Green Supermix (Bio–Rad, Hercules, California). Normalization was done with 18S ribosomal RNA levels. No bands were seen in the absence of RT.

**Masson-trichrome staining.** Masson-trichrome staining was performed as described (22).

**Statistical analyses.** Data are generally expressed as mean ± SEM. Unpaired 2-tailed Student t test and 1- or 2-way analysis of variance with Bonferroni test were generally performed for statistical comparisons, unless otherwise indicated. For most 3-group statistical comparisons, Dunnett’s test with SAS version 8.2 software (SAS, Cary, North Carolina) was used as well. For all tests, a p value of <0.05 was generally considered to be significant.

**Results**

**Adrenal βarr1 and post-MI aldosterone levels.** In the present study, we set out to investigate the potential role played by adrenal βarr1 in modulation of in vivo post-MI HF aldosterone levels. To this end, we overexpressed—specifically in the adrenal glands of 2-week post-MI rats—wild-type βarr1 or a βarr1 C-terminal fragment (βarr1ct), which is unable to bind receptor substrates, thus acting as an inhibitor of βarr1 scaffolding/signaling activity (Online Fig. 1A). To confirm the inhibitory effects of βarr1ct on βarr1 activity in vitro, we performed an extensive molecular characterization of its effects on AngII-induced signaling to aldosterone production in the human AZG cell line H295R (Online Fig. 1B). The βarr1ct was indeed found to abrogate βarr1- and G protein-mediated signaling from AT1R to extracellular signal-regulated kinase activation and StAR protein up-regulation; both signaling events are absolutely necessary for AngII-driven aldosterone production and secretion from these adrenocortical cells (15) (Online Fig. 1B). Thus, after confirming that βarr1ct acts as an inhibitor of adrenal βarr1-mediated aldosterone production in vitro, we overexpressed either the full-length βarr1 (to increase adrenal βarr1 levels/activity) or the βarr1ct (to inhibit adrenal βarr1 activity in vivo) specifically in the adrenals of the post-MI rats. Experimental animals were randomized to 3 different groups: 1 group receiving adrenal gene transfer of AdGFP (control group), 1 receiving full-length wild-type βarr1 (Adβarr1), and 1 receiving the βarr1ct (Adβarr1ct). One day before adrenal gene transfer, all groups were analyzed by echocardiography to confirm presence of similar levels of left ventricular dysfunction and HF before gene delivery. All groups were then studied over the course of the following 7 days (i.e., up to 3 weeks after MI).

In vivo expression of the respective transgenes in the adrenal glands of the animals at 7 days after gene delivery was confirmed by Western blotting (Online Fig. 2). Of note, the adrenal-targeted gene transfer methodology employed results in no ectopic transgene expression (17) (data not shown). As expected, plasma circulating aldosterone levels at 7 days after gene delivery were found markedly elevated in control AdGFP-treated post-MI rats (470 ± 20 pg/ml, approximately 2-fold of the aldosterone levels of normal AdGFP-treated rats) (15), compared with normal (i.e., sham-operated) AdGFP-treated rats, indicating marked MI-induced aldosterone elevation. Importantly, adrenal βarr1 overexpression resulted in an even more pronounced aldosterone elevation after MI, on top of that normally present due to the occurrence of MI (845 ± 150 pg/ml in Adβarr1-treated versus 470 ± 20 pg/ml in control AdGFP-treated post-MI rats, n = 6, p < 0.05) (Fig. 1). In contrast, levels in Adβarr1ct-treated rats (350 ± 30 pg/ml, n = 6, p < 0.05 vs. AdGFP) were significantly lower than in control AdGFP-treated post-MI rats (Fig. 1). Aldosterone levels in post-MI AdGFP rats were similar to saline-treated post-MI rats (data not shown), indicating no nonspecific effects of the adenoviruses used on plasma aldosterone values.

Consistent with the aforementioned findings, βarr1 overexpression led to significant up-regulation of adrenal StAR protein, the most critical enzyme in adrenocortical biosynthesis of aldosterone (as well as of the other adrenal steroids)
(15), compared with control AdGFP-treated post-MI rats, indicating enhanced aldosterone synthesis in vivo, whereas overexpression of βarr1ct reduced adrenal StAR levels below the levels of the control rats (Online Fig. 2). Taken together, these results indicate that adrenal βarr1 promotes post-MI-associated hyperaldosteronism, and inhibition of its activity reduces aldosterone production and plasma circulating aldosterone levels after MI in vivo.

In vivo cardiac function and dimensions at 7 days after gene delivery. Next, we examined the impact of this adrenal βarr1-mediated hyperaldosteronism on the post-MI myocardium. Indeed, we found that ejection fraction (EF) was markedly reduced in Adβarr1-treated post-MI rats at 7 days after gene delivery, compared with control AdGFP-treated post-MI rats (41.4 ± 1.2% vs. 48.7 ± 1.1%, respectively, n = 7, p < 0.05) (Fig. 2A). The EF in both

![Image](https://via.placeholder.com/150)

**Figure 2** Effect of Adrenal βarr1-Mediated Hyperaldosteronism on Cardiac Function, Dimensions, and Contractility

(A) Ejection fraction (EF%) of Adβarr1- and control AdGFP-treated post-myocardial infarction (MI) rats before and after gene delivery (see also Table 1). *p < 0.05 versus AdGFP after gene delivery or Adβarr1 before gene delivery; **p < 0.05 versus AdGFP-pre-gene delivery, n = 7 rats/group. (B) Left ventricular end diastolic diameter (LVEDD) of these rats. *p < 0.05 versus AdGFP after gene delivery or Adβarr1 before gene delivery, n = 7 rats/group. (C) The EF% and (D) LVEDD of Adβarr1-treated post-MI rats administered either with saline (vehicle) or with eplerenone (Adβarr1-Eplerenone) for 7 days, at 1 week after gene delivery (3 weeks after MI). AdGFP post-MI rats (treated with vehicle) are also shown at 1 week after gene delivery (3 weeks after MI) for comparisons. *p < 0.05 versus either AdGFP or Adβarr1-Eplerenone; no significant difference between AdGFP and Adβarr1-Eplerenone was observed at p < 0.05, n = 5 rats/group. (E, F) Basal and maximal dose of isoproterenol (Max. Iso)-stimulated +dP/dt max (E) and −dP/dt max (F) responses of Adβarr1- and control AdGFP-treated post-MI rats at 7 days after adrenal gene delivery (see also Table 1). *p < 0.05 versus AdGFP, n = 7 rats/group. Abbreviations as in Figure 1.
groups was similar before gene delivery, and EF of AdGFP-treated rats at 7 days after gene delivery was slightly but significantly reduced compared with pre-gene delivery, as expected, given that cardiac function deteriorates over time after MI, although at 3 weeks after MI (when post-gene delivery measurements were taken) there is limited dysfunction with this model (Fig. 2A). Indeed, previous studies by us have shown that this model in the rat does not lead to significant cardiac dysfunction before approximately 10 weeks after MI (21). Furthermore, left ventricular end diastolic diameter (LVEDD), a marker of cardiac dimensions, was significantly increased in Adβarr1-treated rats at 3 weeks after MI compared with control AdGFP post-MI rats, in which heart enlargement was less pronounced at 3 weeks after MI (Fig. 2B). This indicates that adrenal βarr1 overexpression significantly accelerates the progression of cardiac hypertrophy by promoting aldosterone elevation after MI. Of note, EF and LVEDD of saline-treated, 3-week, post-MI rats were similar to those of control sham-operated; †p<0.05 versus AdGFP; ‡p<0.05 versus Adβarr1ct (n=7).

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<tr>
<th>Echocardiographic parameters after gene delivery</th>
<th>Sham-Operated (n = 6)</th>
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<th>Adβarr1 (n = 7)</th>
<th>Adβarr1ct (n = 7)</th>
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<td>LVdDd (mm)</td>
<td>3.76 ± 0.08</td>
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<td>LVEDD (mm)</td>
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<td>FS (%)</td>
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<td>EF (%)</td>
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<td>PWTd (mm)</td>
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<td>LV + dp/dtmax (mm Hg/s)</td>
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<td>7,549 ± 512*</td>
<td>5,820 ± 85*†</td>
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<td>HW/BW ratio (mg/g)</td>
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Adrenal gene delivery of adenosin green fluorescent protein (AdGFP), adenoviruses that encode full-length wild-type beta-arrestin 1 (Adβarr1), or adenoviruses that encode rat beta-arrestin 1 C-terminal fragment (Adβarr1ct) was performed at 2 weeks after myocardial infarction, and the parameters in the table were measured 7 days after gene delivery (direct adrenal gland injection). Values of age-matched, sham-operated animals are shown for comparisons. Analysis of variance with Bonferroni test was performed among all groups. Data are presented as mean ± SEM. *p < 0.05 versus sham-operated; †p < 0.05 versus AdGFP; ‡p < 0.05 versus Adβarr1ct.
Cardiac remodeling and functional biomarkers at 7 days after gene delivery. We also performed molecular and structural evaluation of the post-MI rat hearts at 7 days after gene delivery. Real time PCR in total messenger RNA isolated from these hearts showed—consistent with the in vivo functional data—a marked up-regulation of collagen types 1 and 3, markers of cardiac fibrosis, and of ANP and B-type natriuretic peptide, markers of cardiac hypertrophy, in the post-MI hearts of Ad/arr1-treated rats, compared with control AdGFP-treated animals (Figs. 3A to 3D). Conversely, up-regulation of all these markers was prevented in Ad/arr1ct-treated rats (Figs. 3A to 3D), even though this group did not show significant improvement in cardiac function, which is not surprising given the early post-MI time point at which these measurements were taken. Thus, lowering of circulating aldosterone levels by adrenal /arr1 inhibition in vivo causes a marked reduction in the expression of adverse remodeling-related genes, which might help halt the post-MI cardiac decline at later time points. Additionally, heart-weight/body-weight ratio measurements also confirmed the accelerated cardiac hypertrophy (i.e., enhanced at 1 week after adrenal gene delivery, compared with control AdGFP-treated) displayed by Ad/arr1-treated post-MI rats (Table 1, see also preceding text, Fig. 2B).

Cardiac fibrosis at 7 days after gene delivery. Masson-trichrome staining for cardiac fibrosis at 3 weeks after MI (7 days after gene delivery) showed markedly increased fibrosis in Ad/arr1-adrenal-treated rat hearts compared with control AdGFP-treated rat hearts, whereas fibrosis was almost completely absent in Ad/arr1ct-adrenal-treated rat hearts (Figs. 4A and 4B). As expected, no fibrosis was detectable in sham-operated rat hearts (Fig. 4A). In addition, eplerenone treatment markedly reduced fibrosis in Ad/arr1-adrenal-treated rat hearts (Online Fig. 4), thus providing another indication that the cardiac effects of /arr1 are aldosterone-dependent.

Cardiac mediators of aldosterone at 7 days after gene delivery. Immunoblotting in cardiac protein extracts revealed a marked up-regulation of cardiac PAI-1 and TGF-β—2 of the most important molecular mediators of the cardiac fibrotic and adverse remodeling actions of aldosterone (5)—in the post-MI hearts of Ad/arr1-treated rats compared with control AdGFP-treated rats (Figs. 4C and 4D). In contrast, in the hearts of Ad/arr1ct-treated rats, not only was up-regulation of PAI-1 and TGF-β prevented but the levels of these proteins were actually lowered below the levels of control AdGFP-treated rats (Figs. 4C and 4D). Taken together, these results indicate that adrenal /arr1-mediated hyperaldosteronism accelerates cardiac adverse...
remodeling and progression to HF after MI and that these effects can be reciprocally mitigated by adrenal \( \beta \text{arr1} \) inhibition, which significantly reduces circulating aldosterone levels.

**Angiotensin antagonism and \( \beta \text{arr1} \)-mediated aldosterone levels after MI.** Finally, we examined whether adrenal \( \beta \text{arr1} \) can affect the efficacy of AT\(_1\)R antagonism at curbing AngII-induced aldosterone production. For this purpose, we treated post-MI rats with the prototypic AT\(_1\)R antagonist losartan (23,24) for the entire 7-day post-gene delivery period at a dose of 50 mg/kg/day. As expected, in control AdGFP-treated post-MI rats, losartan produced a small but significant plasma aldosterone reduction (from 470 ± 20 pg/ml in saline-treated to 402 ± 10 pg/ml in losartan-treated rats, p < 0.05, n = 6) (Fig. 5). In Ad\( \beta \text{arr1} \)-treated post-MI rats however, losartan is virtually unable to lower aldosterone levels (845 ± 150 pg/ml in saline-treated vs. 880 ± 88 pg/ml in losartan-treated rats, not significant at p < 0.05, n = 6) (Fig. 5). In the Ad\( \beta \text{arr1ct} \)-treated group, no significant aldosterone reduction by losartan was observed, probably because plasma aldosterone levels were already reduced below the levels of AdGFP-treated rats by Ad\( \beta \text{arr1ct} \) alone. Consistent with this, losartan seems also incapable of reducing the cardiac fibrosis induced by adrenal \( \beta \text{arr1} \)-mediated hyperaldosteronism (Online Fig. 4). However, levels in both the saline- and losartan-treated Ad\( \beta \text{arr1ct} \) rats were significantly lower than in vehicle-administered control AdGFP post-MI rats (Fig. 5). These results strongly suggest that the post-MI aldosterone-lowering effects of losartan are antagonized by adrenal \( \beta \text{arr1} \); therefore adrenal \( \beta \text{arr1} \) inhibition can potentiate the hypoaldosteronic actions of this drug in post-MI HF.

**Figure 4** Impact of Aldosterone Levels on Cardiac Fibrosis and Adverse Remodeling Mediators

(A) Trichrome-Masson’s staining in myocardial cross sections from AdGFP-, Ad\( \beta \text{arr1} \)-, or Ad\( \beta \text{arr1ct} \)-treated post-MI rats at 7 days after adrenal gene delivery. Blue denotes collagen fibers, red denotes muscle fibers, and black represents cell nuclei. Representative images are shown from 5 to 6 rat hearts stained/group, along with staining in sham rat hearts, in which no blue staining was detectable. (B) Quantification of the % fibrotic area visualized upon Trichrome-Masson’s staining. *p < 0.05 versus AdGFP; **p < 0.05 versus Ad\( \beta \text{arr1} \), n = 5 to 6 rat hearts/group. (C) Western blotting for cardiac plasminogen activator inhibitor (PAI)-1 and transforming growth factor-\( \beta \) (TGF-\( \beta \)) in AdGFP-, Ad\( \beta \text{arr1} \), or Ad\( \beta \text{arr1ct} \)-treated post-MI rats at 7 days after gene delivery, including glyceraldehyde-3-phosphate dehydrogenase (GAPDH) as loading control. (D) Densitometric analysis of 5 heart samples tested/group. *p < 0.05 versus AdGFP; **p < 0.05 versus Ad\( \beta \text{arr1} \), n = 5 rat hearts/group. Abbreviations as in Figures 1, 2, and 3.

**Figure 5** Adrenal \( \beta \text{arr1} \)-Dependent Aldosterone Levels and Losartan

Plasma aldosterone levels 7 days after adrenal gene delivery of post-myocardial infarction rats after concomitant vehicle (−Los) or losartan (+Los) treatment. *p < 0.05 versus AdGFP/−Los or Ad\( \beta \text{arr1} \)/−Los, n = 5 rats/group/treatment. Abbreviations as in Figures 1, 2, and 3.
Effects of losartan in AdGFP-treated and saline-treated post-MI rats were similar (data not shown).

Discussion

We recently reported that adrenal βarr1 promotes AngII-dependent aldosterone production in vitro in human AZG cells, independently of G-proteins (15). Additionally, adrenal-specific βarr1 overexpression in vivo resulted in marked elevation of circulating aldosterone levels in otherwise normal animals (15). In the present study, we sought to investigate whether adrenal βarr1 plays any role in regulation of circulating aldosterone levels in post-MI HF progression. We found that adrenal βarr1 is indeed a crucial regulator of circulating aldosterone levels in vivo during post-MI HF progression, in that increased adrenal βarr1 levels/activity promote aldosterone elevation after MI, resulting in accelerated cardiac adverse remodeling and deterioration of function, whereas blockade of its activity in vivo lowers post-MI aldosterone levels, attenuating or even preventing these detrimental effects of aldosterone on the failing heart.

These findings strongly suggest that blockade of adrenal βarr1 action on AT1Rs might serve as a novel therapeutic strategy for lowering aldosterone levels after MI and in HF. This is particularly important, because aldosterone has been shown to exert some of its actions (its so-called “non-genomic” actions) independently of the mineralocorticoid receptor (MR), its molecular target that normally mediates its cellular actions (4,5). These MR-independent actions are unaffected by the currently available MR antagonists, such as eplerenone and spironolactone, used in the treatment of HF (9,10). Therefore, curbing aldosterone production at its major source (i.e., the adrenal cortex) by inhibiting βarr1 actions could presumably be more effective therapeutically than inhibiting the actions of aldosterone at its receptor level.

In addition, because adrenal βarr1 seems necessary for up-regulation of StAR, the enzyme that regulates synthesis of all adrenal steroids, its inhibition presumably leads to suppression of the production of the other adrenal steroids as well (i.e., glucocorticoids and corticosterone) (15). Of note, glucocorticoids have been reported to actually occupy the cardiac MRs under normal conditions instead of aldosterone (25). Therefore, adrenal βarr1 inhibition, by suppressing production of glucocorticoids and mineralocorticoids alike, has the unique potential of keeping cardiac MRs completely at bay. For this very same reason, adrenal βarr1 emerges as a much superior target for post-MI cardiac remodeling and HF treatment than MR inhibition (e.g., with eplerenone) or aldosterone synthase inhibition, given that the latter strategies cannot counter all the adverse effects of all adrenal steroids after MI, as suppression of all adrenal steroid production via adrenal βarr1 inhibition is projected to do.

Another important ramification of the present study is that pathological situations that cause elevation of adrenal βarr1 activity toward receptors can lead to abnormally high AngII-induced aldosterone production and hyperaldosteronism. Indeed, we recently reported that in chronic HF, adrenal GRK2—a protein kinase that induces receptor-βarr coupling—is dramatically up-regulated, resulting in chronically elevated catecholamine production by the adrenal medulla (16). Thus, it is entirely plausible that, driven by the enhanced GRK2 activity, adrenal βarr1 activity toward receptors—including the AT1Rs—is also increased in chronic HF or during progression from MI to HF, which could mediate (at least in part) the chronically elevated circulating levels of aldosterone that precipitate this disease. Importantly, we have previously shown that GRK2 can desensitize AngII receptors in the heart in vivo (26) and that overexpression of GRK2 in rat adrenal glands also causes elevation of plasma aldosterone (15). Both of these findings argue in favor of the aforementioned scenario.

Furthermore, it is now well-established that, in addition to the circulatory renin-angiotensin-aldosterone system (RAAS), there are also several other local RAASs in peripheral tissues, including the heart (intracardiac RAAS) and the kidneys (intrarenal RAAS), and these systems also hyperfunction in HF contributing to the HF-associated hyperaldosteronism (27,28). Therefore, it would be worth investigating whether βarr1 is involved in aldosterone production by these local RAASs and whether it contributes to their increased aldosterone output during HF as well. In fact, specifically for the intracardiac RAAS, this possibility is very likely, given the elevated cardiac GRK2 levels in HF (29).

One of the major physiological effects of aldosterone is an increase in blood pressure via salt and water retention (4,5). Thus, alterations in mean arterial pressure by the elevated aldosterone levels caused by adrenal βarr1 overactivity might very well have contributed to the observed cardiac phenotype of adrenal βarr1-overexpressing post-MI rats. It should be noted here, however, that βarr1 knockout mice do not show any changes in blood pressure compared with wild-type age-matched control mice (30). Additionally, the direct effects of aldosterone on cardiac tissue are bound to have played the most important role in the observed cardiac phenotype of the post-MI animals, given the relatively small time period (only 7 days) between genetic manipulation of adrenal βarr1 levels that raises aldosterone levels (i.e., gene delivery) and the day of cardiac measurements/examination, which is rather insufficient for blood pressure to dramatically affect cardiac function and remodeling. Besides, whether changes in blood pressure play any role in the cardiac effects of aldosterone is still an open question in its own right, because there are several reports in the published literature showing aldosterone to affect cardiac function and fibrosis in post-MI rats independently of changes in mean blood pressure (31,32). Indeed, no differences in systemic mean arterial pressure among the 3 post-MI treatment groups of
the present study (i.e., AdGFP, Adβarr1, Adβarr1ct) were observed at 1 week after gene delivery (data not shown), further supporting the notion that blood pressure did not play any major role in the observed cardiac effects of βarr1-dependent aldosterone at this early post-MI time point (3 weeks).

The last finding of the present study is that the aldosterone-lowering actions of losartan, the prototypic drug of the class of AT\(_1\)R antagonists (sartans) (23,24), are countered by adrenal βarr1. Although at normal Barr1 levels (control AdGFP-treated post-MI rats) it is capable of producing a small but significant plasma aldosterone lowering as expected, when adrenal βarr1 is overactive (Adβarr1-treated post-MI rats), losartan does not decrease plasma aldosterone at all. This finding implies that inhibition of adrenal βarr1 in vivo can facilitate the inhibitory effects of losartan (and possibly also of the other sartans) on AngII-induced aldosterone production. Of note, limited efficacy of losartan and other sartans at lowering aldosterone levels in HF patients and in experimental animals, the so-called “aldosterone escape,” has been reported (20,33,34). Therefore, the finding that the effects of losartan on aldosterone production can be antagonized by adrenal βarr1-AT\(_1\)R coupling might explain (at least in part) this reported limited efficacy of losartan and related drugs at curbing aldosterone levels. By contrast, increased activity of the Barr1 co-factor GRK2 on cardiac AT\(_1\)Rs also attenuates the pro-contractile signaling of these receptors (26). Therefore, the development of novel, functionally selective (or “biased”) AT\(_1\)R ligands (35,36)—which would inhibit AT\(_1\)R-induced GRK2/βarr1 activation, at least as effectively as AT\(_1\)R-induced G-protein activation—might prove extremely beneficial in the treatment of HF-related hyperaldosteronism and decreased cardiac function.

**Clinical implications.** We have found that circulating aldosterone levels are reciprocally regulated by adrenal βarr1 activity in vivo, in that they are directly proportional to βarr1 activity toward AngII receptors in the adrenal glands. Therefore, inhibiting adrenal βarr1 action markedly decreases circulating aldosterone and attenuates its detrimental effects on the post-MI heart—such as fibrosis, hypertrophy, and dilation—thereby preventing or even reversing adverse remodeling after MI and maintaining cardiac function in the face of post-MI-driven cardiac decline. Additionally, losartan, a classical AngII receptor antagonist drug used in the treatment of hypertension, seems unable to counter this adrenal βarr1-promoted hyperaldosteronism after MI. Taken together, the present findings suggest adrenal βarr1 as a major driving force behind post-MI aldosterone elevation, whose inhibition in vivo—either via gene therapy or pharmacologically—could potentially be of enormous therapeutic value in the management of post-MI HF patients. Finally, from the pharmacotherapeutic standpoint, an evaluation of the whole class of AT\(_1\)R antagonists (sartans) in terms of their efficacy at antagonizing βarr1-driven hyperaldosteronism is highly warranted, because it could help explain some well-known existing differences in therapeutic efficacy and also identify the most efficacious agents at lowering post-MI aldosterone, within this very important cardiovascular drug class.

**Conclusions**

The present study reports that adrenal βarr1 promotes the well-documented post-MI-associated elevation of circulating aldosterone, and thus direct inhibition of its activity via adrenal-targeted gene therapy or via development of novel AT\(_1\)R “biased” or “functionally selective” ligands that can prevent/reduce GRK2/βarr1 activation by the AT\(_1\)R might be of therapeutic value in post-MI ensuing HF as well as in already established chronic HF—both of which are precipitated by the cardiotoxic actions of elevated aldosterone.

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**REFERENCES**


Key Words: adrenal beta-arrestin 1 • aldosterone • angiotensin II • heart failure • myocardial infarction.

APPENDIX

For supplementary figures, please see the online version of this article.