Lipid peroxidation-derived aldehydes and oxidative stress in the failing heart: role of aldose reductase

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Srivastava, Sanjay, Bysani Chandrasekar, Aruni Bhatnagar, and Sumanth D. Prabhu. Lipid peroxidation-derived aldehydes and oxidative stress in the failing heart: role of aldose reductase. Am J Physiol Heart Circ Physiol 283: H2612–H2619, 2002. First published August 22, 2002; 10.1152/ajpheart.00592.2002.—Lipid peroxidation-derived aldehydes (LP-DA) can propagate oxidative injury and are detoxified by the aldose reductase (AR) enzyme pathway in myocardium. Whether there are alterations in the AR axis in heart failure (HF) is unknown. Sixteen instrumented dogs were studied before and after either 24 h or 4 wk of rapid left ventricular (LV) pacing (early and late HF, respectively). Six unpaced dogs served as controls. In early HF, there was subtle depression of LV performance (maximum rate of LV pressure rise, P = 0.05 vs. baseline) but no chamber enlargement, whereas in late HF there was significant (P < 0.05) contractile depression and LV dilatation. Oxidative stress was increased at both time points, indexed by tissue malondialdehyde, total glutathione, and free C6–C9 LP-DA (P < 0.025 vs. control). AR protein levels and activity decreased progressively during HF (P < 0.025 early/late HF vs. control); however, AR mRNA expression decreased only in late HF (P < 0.005 vs. early HF and control). DNA binding of toxic-kin-responsive enhancer binding protein (TonE, a transcriptional regulator of AR) paralleled AR mRNA, declining >50% in late HF (P < 0.025 vs. control). We conclude that AR levels and attendant myocardial capacity to detoxify LP-DA decline during the development of HF. In early HF, decreased AR occurs due to a translational or posttranslational mechanism, whereas in late HF reduced TonEBP transcriptional activation and AR downregulation contribute significantly. Reduced AR-mediated LP-DA metabolism contributes importantly to LP-DA accumulation in the failing heart and thus may augment chronic oxidative injury.

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in failing myocardium, whether there are pathological alterations in the activity, abundance, and transcriptional regulation of AR in HF is unknown. Accordingly, the purpose of this investigation was to evaluate the magnitude and time course of alterations in LP-DA, AR expression and activity, and TonE activation during tachycardia HF in dogs.

METHODS

**HF groups and hemodynamic measurements.** All studies were performed in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals (Revised 1996). Twenty-two mongrel dogs of either sex, 25–30 kg, were instrumented for physiological monitoring (19, 21). Chronic instrumentation included a LV apex microcatheter (Konigsberg Instruments), endocardial piezoelectric crystals along the anterior-posterior, septal-lateral, and long-axis diameters, LV epicardial pacing leads, and inferior vena cava pneumatic occluders. With the dogs conscious and lying in a sling, pressure and diameter signals were recorded and digitized at 500 Hz during steady state and caval occlusion before and after autonomic blockade with intravenous atropine (2 mg) and hexamethonium (25 mg/kg). Autonomic blockade was used to minimize reflex effects during caval occlusion and thereby isolate myocardial mechanical properties during this maneuver. LV volume was derived from the three diameters using the equation for an ellipse. Systolic function was indexed by the maximum rate of LV pressure rise (dP/dt max), mean velocity of circumferential fiber shortening (V c), and end-systolic pressure-volume relation (ESPVR; derived after autonomic blockade). Diastolic function was indexed by LV end-diastolic pressure (EDP) and the time constant of relaxation (τ). Instantaneous end-diastolic circumferential force (EDF) was used as an index of diastolic wall stress. The mathematical derivation of these parameters has been previously described (19, 21).

After baseline recordings, rapid LV pacing was instituted at a heart rate of 210 beats/min using customized pulse generators (model 5985, Medtronic). Hemodynamic measurements were repeated after 24 h in eight dogs (early HF) and after 4 wk in eight dogs (late HF). The pacemaker was turned off at least 30 min before the protocol was repeated. After this, the animals were euthanized by lethal KCl injection at least 30 min before the protocol was repeated. After this, the animals were euthanized by lethal KCl injection at least 30 min before the protocol was repeated.

**Measurement of tissue MDA.** Myocardial MDA content was measured in LV homogenates by either thiobarbituric acid-reactive substances (TBARS) or the lipid peroxidation 586 colorimetric assay (Bioxytech). For TBARS (17), LV tissue was homogenized in 4 volumes of 0.1 M potassium phosphate buffer (pH 7.0) containing 1 mM EDTA, 500 μM butylated hydroxytoluene, 20 μM desferal, and 50 μl protease inhibitor cocktail (Sigma). Trichloroacetic acid (10%) was then added, and the precipitated proteins were removed by centrifugation at 3,000 g for 10 min at room temperature. The supernatant was aspirated, mixed with an equal volume of 0.67% thiobarbituric acid, and incubated at 100°C for 15 min. The samples were cooled to room temperature, and optical density was measured at 532 nm. MDA generated by acid hydrolysis of tetra-ethoxy propane was used as a standard. For the lipid peroxidation 586 kit MDA measurement (35), the LV homogenate was incubated with N-methyl-2-phenylindole (NMP)-1 for 1 h at 45°C as per the manufacturer’s instructions. The resultant MDA-NMP adduct was measured at 586 nm.

**Gas chromatography-chemical ionization mass spectrometry.** LP-DA in LV homogenates was derivatized as described by Luo et al. (12). The derivatized extracts (1-μl aliquots) were separated on a bonded-phase capillary column (DB-5MS, 15 m, 0.25 mm inner diameter, 0.25 mm film thickness, J & W Scientific) and analyzed with a HHP5890/HF5973 gas chromatography (GC)-mass spectrometry (MS) system (Hewlett-Packard). The GC injection port and the interface temperature were set to 280°C with the helium carrier gas maintained at flow rate of 1.2 ml/min. Splitless mode injections were performed with the inlet port purged after 1 min and increased at a rate of 8°C/min to 200°C, which was held for 10 min, then increased at a rate of 25°C/min to 280°C, and held at this temperature for 5 min. Components eluting from GC were detected in negative chemical ionization (CI) mode with ammonia as the reagent gas and quantified using D₃-benzaldehyde as an internal standard.

**Myocardial glutathione.** Total glutathione (GSH + GSSG) content was measured with a GSH/GSSG-412 kit from Bioxytech (5,5’-dithio-bis-[2-nitrobenzoic acid (DTNB)]-dependent reductase recycling assay (35). Samples were homogenized in 10 volumes of 5% metaphosphoric acid at 4°C. The homogenates were centrifuged at 10,000 g for 10 min, and the supernatant was used for the recycling assay.

**AR protein activity and expression.** AR activity was determined as described previously (28). LV samples were homogenized in 5 volumes of 0.01 M potassium phosphate buffer (pH 7.0) containing 1 mM EDTA and 5.0 mM dithiothreitol (DTT). The homogenate was centrifuged at 20,000 g for 20 min at 4°C. The supernatant was aspirated and incubated with 0.1 M DTT for 1 h at 37°C. Excess DTT was removed by gel filtration through a Sephadex G-25 column (PD-10) and precalibrated with nitrogen-saturated 0.1 M potassium phosphate (pH 7.0) containing 1 mM EDTA. All operations were performed at 4°C to prevent enzyme oxidation. Reduced homogenates were stored under nitrogen and used within 1 h. AR activity was determined at 25°C in a 1-ml system containing 0.1 M potassium phosphate (pH 7.0), 10 mM D,L-glyceraldehyde, and 0.1 mM NADPH. The reaction was monitored by measuring the rate of NADPH disappearance at 340 nm. One activity unit was defined as the amount required to oxidize 1 μmol NADPH/min. The control cuvette contained all components of the reaction mixture except glyceraldehyde. AR protein expression was assessed by standard SDS-PAGE Western immunoblotting techniques using a polyclonal anti-AR antibody as previously described (23). AR signals were quantitated by an image scanning densitometer and expressed as a percentage of control.

**AR mRNA expression.** Total RNA extraction and Northern blotting, autoradiography, and densitometry were performed as previously described (5, 21). For Northern blots, human AR cDNA (1 kb, pET vector) was used as a probe. Human 28S rRNA (40-bp single-stranded oligonucleotide, Oncogene Science) was used as an internal control, with results expressed as a ratio of the AR mRNA to the corresponding 28S rRNA.

**TonEBP DNA binding.** Isolation of nuclear protein extract from frozen myocardium and electrophoretic mobility shift assay (EMSA) were performed as previously described (5). 32P-labeled consensus double-stranded oligonucleotides (5’-TGGAAAAAGTCCTCCAGCT-3’) corresponding to the binding site for TonE (5’-TGGAAAAAGTCCTCCAGCT-3’) were used as probes (13). In competition experiments, a 100-fold molar excess of unlabeled consensus or mutant TonE oligonucleotides (mutant, 5’-TGGTGTCCCAGCTCCAGCT-3’) was added to the reaction mixture for 20 min, followed by the addition of labeled con-
Hemodynamic parameters

Table 1. Hemodynamic parameters

<table>
<thead>
<tr>
<th></th>
<th>Early HF Group</th>
<th>Late HF Group</th>
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<tr>
<td></td>
<td>Baseline</td>
<td>24-h RVP</td>
</tr>
<tr>
<td>HR, beats/min</td>
<td>80 ± 7</td>
<td>98 ± 8</td>
</tr>
<tr>
<td>LVESP, mmHg</td>
<td>103 ± 6</td>
<td>95 ± 5</td>
</tr>
<tr>
<td>LVEDP, mmHg</td>
<td>7 ± 2</td>
<td>9 ± 2</td>
</tr>
<tr>
<td>LVEDV, ml</td>
<td>57 ± 6</td>
<td>45 ± 4*</td>
</tr>
<tr>
<td>dP/dt_{max}, mmHg/s</td>
<td>2,728 ± 161</td>
<td>2,010 ± 179*</td>
</tr>
<tr>
<td>EDF, g</td>
<td>178 ± 48</td>
<td>177 ± 30</td>
</tr>
<tr>
<td>V_{cf}, circumferences/s</td>
<td>1.06 ± 0.06</td>
<td>0.84 ± 0.08*</td>
</tr>
<tr>
<td>τ, ms</td>
<td>37.2 ± 2.3</td>
<td>37.7 ± 4.0</td>
</tr>
<tr>
<td>E_{es}, mmHg/ml</td>
<td>7.1 ± 0.8</td>
<td>9.0 ± 1.0</td>
</tr>
<tr>
<td>V_{100}, ml</td>
<td>32.2 ± 3.3</td>
<td>35.2 ± 4.5</td>
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</table>

Values are means ± SE. HF, heart failure; RVP, rapid ventricular pacing; HR, heart rate; LVESP, left ventricular (LV) end-systolic pressure; LVEDP, LV end-diastolic pressure; LVEDV, LV end-diastolic volume; dP/dt_{max}, maximum rate of LV pressure rise; EDF, end-diastolic force; V_{cf}, mean velocity of circumferential fiber shortening; τ, time constant of LV relaxation; E_{es}, end-systolic elastance; V_{100}, volume intercept of the end-systolic pressure-volume relation at 100 mmHg. E_{es} and V_{100} were measured after autonomic blockade. *P < 0.05 vs. respective baselines.

RESULTS

Hemodynamic effects of rapid pacing. Figure 1 displays representative steady-state pressure-volume loops and corresponding ESPVRs determined during caval occlusion at baseline and after either 24 h or 4 wk of pacing (early and late HF, respectively). As described in our previous study (21), short-term tachypacing resulted in mild reductions in LV end-diastolic volume, but no change in LVEDP, and no change in either the slope (end-systolic elastance (E_{es})) or relative position of the ESPVR, suggesting maintenance of LV contractile performance (Fig. 1A). In contrast, long-term tachypacing resulted in marked LV dilatation, increased LV filling pressure, reduced E_{es}, and prominent rightward shift of the ESPVR, indicating severely depressed systolic function (Fig. 1B). Table 1 displays group data. Early HF animals displayed mild but significant (P < 0.05) reductions in dP/dt_{max} and V_{cf}, but no change in LVEDP, τ, E_{es}, or end-systolic volume at an end-systolic pressure of 100 mmHg (V_{100}; an index of ESPVR position), indicating subtle depression of LV performance without circulatory congestion or HF. Additionally, there was no change in EDF, an index of total LV diastolic load. In contrast, late HF animals demonstrated significant LV dilatation, increased LVEDP, and marked depression of contractility (E_{es}, dP/dt_{max}, and V_{cf}) and relaxation (τ), consistent with dilated cardiomyopathy and overt HF. Furthermore, EDF was markedly increased indicating increased diastolic wall stress.

Myocardial oxidative stress and LP-DA during the development of HF. Table 2 displays group data for biochemical parameters of oxidative stress. Oxidative stress, as indicated by increased myocardial MDA levels (P < 0.025) and decreased myocardial glutathione correction for multiple comparisons. Given three experimental groups, a P value of <0.025 was considered significant. Comparisons of hemodynamic parameters before and after pacing was made using the paired t-test. A P value of <0.05 was considered significant. Group data are expressed as means ± SE.
**Table 2. Oxidative stress parameters**

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Early HF</th>
<th>Late HF</th>
</tr>
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<tbody>
<tr>
<td>Total glutathione, nmol/mg protein</td>
<td>2.21 ± 0.11</td>
<td>1.90 ± 0.08*</td>
<td>1.80 ± 0.12*</td>
</tr>
<tr>
<td>MDA, nmol/g tissue</td>
<td>7.68 ± 0.43</td>
<td>10.08 ± 0.55*</td>
<td>14.52 ± 0.55†</td>
</tr>
<tr>
<td>Lipid peroxidation kit</td>
<td>16.95 ± 0.65</td>
<td>21.82 ± 1.03*</td>
<td>29.32 ± 3.60†</td>
</tr>
<tr>
<td>GC-CI/MS data, nmol/g tissue</td>
<td>0.88 ± 0.07</td>
<td>1.26 ± 0.12*</td>
<td>1.49 ± 0.22†</td>
</tr>
<tr>
<td>Hexanal</td>
<td>0.43 ± 0.03</td>
<td>0.66 ± 0.06*</td>
<td>0.75 ± 0.07†</td>
</tr>
<tr>
<td>Heptanal</td>
<td>0.53 ± 0.06</td>
<td>0.80 ± 0.05*</td>
<td>1.08 ± 0.06†</td>
</tr>
<tr>
<td>Nonanal</td>
<td>0.21 ± 0.01</td>
<td>0.31 ± 0.04*</td>
<td>0.49 ± 0.03†</td>
</tr>
<tr>
<td>Nonenal</td>
<td>0.19 ± 0.01</td>
<td>0.26 ± 0.03*</td>
<td>0.31 ± 0.02†</td>
</tr>
<tr>
<td>4-Hydroxy-trans-2-nonenal</td>
<td>0.44 ± 0.03</td>
<td>0.60 ± 0.04*</td>
<td>0.83 ± 0.05†</td>
</tr>
</tbody>
</table>

Values are means ± SE. MDA, malondialdehyde; TBARS, thiobarbituric acid-reactive substances; GC-CI/MS, gas chromatography-chemical ionization mass spectroscopy. *P < 0.025 vs. control; †P < 0.025 vs. early HF.

PEG < 0.001), increased even in the early stages of HF, before LV chamber dilatation. These changes were accompanied by significant increases in medium- and long-chain (C6–C9) free aldehydes (GC-CI/MS data), which are the preferred substrates of AR (28, 32). The free LP-DA levels increased progressively in late HF, reflecting a cumulative effect of prolonged oxidative stress and attendant lipid peroxidation.

**AR protein expression and activity.** Figure 2 displays Western blots for AR from control, early HF, and late HF (A) along with group densitometry data (B). Protein levels of AR consistently declined during the development of HF, with significant reductions in both early and late HF (23% and 33% reductions, \( P < 0.005 \) vs. control). Figure 2C shows group data for myocardial AR enzyme activity in the three groups. AR activity progressively declined in HF, with significantly reduced activity in both early and late HF (\( P < 0.025 \) vs. control). Furthermore, the specific AR inhibitor sorbinil (2 \( \mu \)M) inhibited AR activity by >80% in all three experimental groups (data not shown), consistent with our prior work that AR is the major aldehyde reducing enzyme in myocardium (27). Additionally, AR enzyme activity directly correlated with both the magnitude of LV diastolic wall stress (vs. EDF, \( r = 0.656 \)) and the degree of contractile dysfunction (vs. \( E_{\text{es}} \), \( r = 0.771 \)). Thus the results indicate that increased LP-DA in failing myocardium occurs in the setting of reduced AR-mediated aldehyde metabolism.

**AR mRNA expression and TonEBP DNA binding.** Figure 3 shows Northern blots and densitometry for AR mRNA expression in the three groups. In early HF, AR mRNA was unchanged (actually tending to increase overall), whereas in late HF there was a significant (~40%) reduction in AR gene expression. Figure 4 displays myocardial DNA binding activity (EMSA and corresponding densitometry data) for the transcription factor TonEBP, a bidirectional regulator of AR expression (13, 33). TonEBP DNA binding, although maintained in early HF, declined by >50% in late HF (\( P < 0.025 \) vs. control), indicating that at this stage reduced transcriptional activation of the AR gene contributes to reduced AR mRNA and protein expression.

**DISCUSSION**

This study demonstrates several novel findings regarding lipid peroxidation and oxidative stress in HF. We demonstrate, for the first time, that decreased AR abundance and function and consequent depression of myocardial aldehyde metabolism contribute to the ac-
cumulation of medium and long chain (C6–C9) LP-DA in the failing heart. This indicates that, in addition to increased levels of ROS per se, reduced AR-mediated LP-DA metabolism also plays an important role in the buildup of cytotoxic aldehydes and comprises a potential, and previously unrecognized, mechanism for myocardial injury secondary to chronic oxidative stress in HF. Second, although AR abundance and activity progressively decrease during the development of HF, the mechanisms by which this occurs are multifactorial. In early HF, AR gene expression tended to increase despite reduced protein levels and function suggesting either a translational defect, posttranslational modification of AR, or increased AR degradation. In more advanced HF, AR gene downregulation resulting in part from reduced TonEBP transcriptional activation also plays an important role. Third, increased oxidative stress occurs early in the development of HF, before chamber dilatation or increased diastolic wall stress.

Experimental studies have provided direct evidence of increased ROS in failing myocardium in direct proportion to degree of LV dysfunction (4, 9), whereas treatment with ROS scavengers in murine postinfarction HF (11) and the antioxidant probucol in canine tachycardia HF (15) improves LV remodeling. Several factors considered to be involved in the pathogenesis of HF increase myocardial ROS in vitro, including angiotensin II (14), inflammatory cytokines (14), adrenergic stimulation (1), and mechanical stretch (18). However, the relative contribution of these stimuli to increased oxidative stress in vivo has not been assessed. In this study, oxidant stress, as manifested by an increase in LP-DA and a decrease in total glutathione (Table 2), occurred very early in of HF, after 24 h of rapid pacing. This time point, although characterized by subtle reductions in contractility, was not associated with chamber dilatation, increased LV filling pressures, or increased diastolic wall stress (Fig. 1 and Table 1). Thus mechanical stretch, although clearly a hallmark of HF, is not required for the genesis of myocardial oxidative stress in this pacing-dependent model of ventricular dysfunction. In this study, we found that HF is accompanied by increased LP-DA, which probably reflects a combination of decreased AR-mediated metabolism as well as increased ROS generation in myocardium.

Peroxidation of membrane-derived phospholipids is a well-known biochemical consequence of ROS (7). LP-DA formed via this process are chemically more stable (7) and, as a consequence, are spatially and temporally more potent as secondary oxidants. The oxidative effects associated with LP-DA are cumulative, such that these toxicants can alter cellular function over the long term. Via the formation of aldehyde-protein adducts, the α,β-unsaturated aldehyde HNE can impact several cellular processes, including inhibition of key enzymes (29) and disruption of ion transporters (3, 25). Furthermore, HNE has also been shown to induce growth factor expression and cell growth in vascular smooth muscle (24), β-adrenergic receptor desensitization and contractile dysfunction in atrial tissue (8), and alter excitability in cardiac myocytes (3), all of which are features reminiscent of the HF phenotype. Taken together with studies demonstrating increased HNE-modified proteins in failing myocardium (10), these data suggest that LP-DA such as HNE are potentially important mediators of long-term cellular toxicity due to ROS in HF. We have previously shown that in myocardium, AR is an important route for the detoxification of LP-DA (27). However, the status of this enzymatic pathway in HF has received little attention, and whether reduced aldehyde metabolism contributes to increased abundance of these secondary oxidants is unknown.

Our results establish, for the first time, that the AR axis is depressed in HF suggesting that reduced myocardial capacity for LP-DA detoxification contributes to an overall reduction in antioxidant defenses and to the long-term cardiotoxicity of increased oxidative stress. This notion is supported by our findings of reductions in AR expression and activity throughout the development of HF (Fig. 2), accompanied by attendant increases in C6–C9 LP-DA (Table 2), the preferred aldehyde substrates for AR (28, 32). Furthermore, AR activity in failing myocardium directly correlated with both the degree of contractile dysfunction ($E_{cw}$) and end-diastolic wall stress (EDF). Although the absolute reduction in AR abundance and activity (~30%) is not markedly pronounced, it is important to consider that LP-DA are much more stable than their precursor ROS with a significantly greater half-life (7). As a result, even moderate reductions in LP-DA metabolism can...
result in significant and persistent cellular alterations over an extended time period such as during the development of chronic HF. Thus decreased AR in the failing heart may represent a heretofore unrecognized mechanism for the amplification of ROS bioactivity and perpetuation of oxidant stress-induced LV dysfunction.

Aside from LP-DA detoxification, AR has a variety of other roles in cellular metabolism. AR has classically been studied as a catalyst for the reduction of glucose to sorbitol (2). As sorbitol is a compatible organic osmolyte that can counter the deleterious effects of hypertonicity, AR gene expression is responsive to changes in cellular ionic strength, and, as such, AR comprises one of the toxicity stress response genes (13, 26, 33). These genes are regulated by TonE, which is bound by the transcription factor TonEBP, a member of the nuclear factor of activated T cell (NFAT) family of transcription factors (NFAT4) (13). TonEBP is ubiquitously expressed and constitutively active under isotonic conditions (13, 33). As TonEBP can be upregulated or downregulated in response to increases or decreases in toxicity (33), TonEBP serves as a bidirectional regulator of AR gene expression.

A key observation of this study is the divergent behavior of TonEBP transcriptional activation and AR gene expression between early and late HF (Figs. 3 and 4) despite progressive reductions in AR protein and activity, indicating that the underlying mechanisms for decreased AR in HF are multifactorial. In early HF, AR mRNA expression was not reduced and TonEBP DNA binding was unchanged, suggesting that reduced AR abundance was related to either increased AR degradation or posttranslational protein modification resulting in loss of AR function. In contrast, in late HF, AR gene downregulation contributed importantly to reduced AR protein levels as evidenced by reduced AR mRNA levels and reduced TonEBP DNA binding activity. Although the mechanisms underlying TonEBP regulation in the heart remain unknown, reduced TonE activation in late HF suggests that advanced HF is a hypotonic state. In fact, HF is a fluid overloaded state often associated with hyponatremia, especially in advanced stages. As sodium concentration is the main determinant of interstitial toxicity and ionic strength, relative hypotonicity in late HF can potentially serve as a negative stimulus for TonEBP activity. Thus, in the failing heart, both transcription-dependent and -independent mechanisms can account for decreased AR activity and reduced AR-mediated LP-DA metabolism.

To our knowledge, this is the first systematic investigation of AR and LP-DA metabolism during the progression of HF. Recently, using high-density oligonucleotide arrays to examine a large panel of genes in two nonfailing and two failing human hearts, Yang et al. (34) reported that AR gene expression was increased in advanced HF, in apparent conflict with our results. Although the reasons for this discrepancy in AR gene expression may be related to differences in species and HF etiology, it is important to consider that AR protein levels and activity were not examined. Also, the results were from only two patients, both of whom were treated with a variety of cardioactive drugs with undefined effects on aldehyde metabolism, AR expression, and toxicity. Given the complex nature of AR regulation at both the mRNA and protein levels, variables such as pharmacological agents, alterations in toxicity
due to diuresis, and the temporal stage of the disease may have significant effects on these pathways. Further studies are needed to more precisely define the pathophysiological role of AR and aldehydeic toxicants in HF.

In summary, we demonstrate, for the first time, that depression of the AR axis and reduced myocardial aldehyde metabolism contribute to the accumulation of LP-DA in the failing heart. Although AR abundance and activity progressively decrease during the development of HF, the mechanisms by which this occurs are heterogeneous. In early HF, detrimental posttranslational modification of AR and/or increased AR degradation may be primarily responsible, whereas in late HF reduced TonEBP transcriptional activation and AR gene downregulation play important roles. Increased oxidative stress occurs early in the development of HF, in the setting of normal end-diastolic wall stress and in the absence of LV dilatation, suggesting that mechanical stretch is not necessary to initiate oxidative stress in HF. These findings indicate that, in addition to increased ambient levels of ROS, alterations in the AR metabolic axis also play an important role in the buildup of cytotoxic aldehydic oxidants and that reductions in AR-mediated LP-DA metabolism may serve to amplify myocardial injury due to oxidative stress in HF.

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