Effects of norepinephrine on expression of IGF-1/IGF-1R and SERCA2 in rat heart

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Abstract

Objectives: The effects of norepinephrine on expression of cardiac genes during pathological cardiac growth and heart failure are not fully understood. Tissue insulin-like growth factor 1 IGF-1 and its receptor IGF-1R play an important role in the regulation of the hyperplastic capacity of cardiac myocytes. Sarcoplasmic reticulum Ca$^{2+}$-ATPase (SERCA2), on the other hand, is important in regulating cardiac contractile function. The present study examined the effects of elevated levels of NE on expression of IGF-1/IGF-1R and SERCA2 mRNAs.

Methods: Rats were infused with NE using osmotic minipumps for 3 and 6 days at a rate of 50 µg/kg/h and also at a higher dose (130 µg/kg/h) for 6 and 14 days. Levels of expression of IGF-1/IGF-1R and SERCA2 mRNAs were determined by ribonuclease protection assay and by Northern blotting, respectively. Results: NE treatment significantly increased IGF-1 mRNA levels in both left- and right-ventricle; however, levels of IGF-1R increased in the left- but not the right-ventricle. By contrast, NE infusion at both the lower dose and the higher dose failed to alter expression of SERCA2 mRNA. Conclusion: Our results suggest that NE treatment differentially regulates expression of IGF-1 and IGF-1R in the ventricles of rat heart and that NE appears not to affect expression of SERCA2 mRNA. © 1998 Elsevier Science B.V.

Keywords: Rat, ventricle; Insulin-like growth factor; Catecholamines; Ca$^{2+}$-ATPase

1. Introduction

The activity of the sympathetic nervous system increases in heart failure and in certain forms of hypertension resulting in increased levels of circulating catecholamines and in increased release of NE from the sympathetic nerve endings within the myocardium [1–3]. The effects of elevated NE level on expression of cardiac genes during pathological cardiac growth and heart failure are not fully understood. The facts that NE is an α-as well as β-adrenergic agonist and that β-adrenergic signaling pathway is downregulated in failing heart [4–6] would seem to suggest that increased catecholamines in heart failure may modulate α-adrenergic effects more than β-adrenergic effects.

Insulin-like growth factor 1 (IGF-1) plays an important role in development and growth [7,8]. Recent data have provided evidence for potential roles of tissue IGF-1 and its receptor (IGF-1R) in the hyperplastic responses of the myocardium [9–13]. In spontaneously hypertensive rats, DOCA-salt hypertensive rats, and aortic constricted rats, levels of IGF-1 mRNA and protein are greatly increased; however, levels of IGF-1R are not altered [10]. In contrast, in cardiac hypertrophy induced by two-kidney, one clip hypertension [9] and in myocardial infarction [14] mRNA levels of both IGF-1 and IGF-1R are elevated. It is important to note that IGF-induced cardiac hypertrophy may be the result of increased cardiac cell size as well as increased proliferation of cardiac cells [15,16]. The study by Reiss et al. [13] demonstrated that overexpression of IGF-1 in the heart of transgenic mice is correlated with myocyte proliferation and not myocyte hypertrophy.

Sarcoplasmic reticulum Ca$^{2+}$-ATPase (SERCA2) is critical in regulating cardiac contractile function. Expression of SERCA2 is downregulated and SR function is
abnormal in hypertrophied and failing heart [17–22]. In a pressure-overload rat model, a significant decrease has been reported in Ca2+/ATPase mRNA levels in young rats 1 month after coarctation of the abdominal aorta [20]. Furthermore, Feldman et al. [17] showed that Ca2+/ATPase levels diminished only in decompensated failure, but not in compensated hypertrophy. Likewise, Zarain-Herzberg et al. [22] demonstrated decreased steady state levels of SERCA2 mRNA and protein in congestive heart failure due to myocardial infarction. Clinical studies also reveal that SERCA2 gene expression is greatly reduced in failing human heart [23–27]. Cellular signals that trigger down-regulation of SERCA2 are not completely understood.

The aim of the present study was to examine the effects of elevated levels of NE on expression of IGF-1, IGF-1R, and SERCA2. It is postulated that under conditions when endogenous catecholamines levels are sufficiently elevated, NE may play a role in modulating cardiac growth and contractile function by, respectively, altering expression of IGF-1/IGF-1R and SERCA2 mRNAs. Although a previous study has examined the effects of isoproterenol on SERCA2 expression [28], we chose to examine the effects of NE, an endogenous catecholamine, on expression of these genes. Our results demonstrate that infusion of NE differentially regulates expression of IGF-1 and IGF-1R mRNAs in the rat ventricles. In contrast, NE treatment failed to alter expression of SERCA2 mRNA.

2. Methods

The investigation conforms with the Guide for the Care and Use of Laboratory Animals published by the US National Institutes of Health (NIH Publication No. 85-23, revised 1985)

2.1. Animal models

Male Sprague Dawley rats (350–450 g) received infusion of NE using Alzet Osmotic minipumps (model 2002, Palo Alto, CA) implanted subcutaneously on the frontoor back. Minipumps delivered L-norepinephrine bitartrate (NE) in ascorbic acid-saline (0.1 mg/ml) at a flow rate of 0.5 μL/h to deliver NE at a rate of 50 μg/kg/h for 3 and 6 days. In a second series of experiments, NE was infused at a rate of 130 μg/kg/h for 6 and 14 days. Sham animals received ascorbic acid-saline at the same flow rate for the same time course as the NE-treated rats. At the specified time, rats were deeply anesthetized with ether and the heart was rapidly excised and rid of connective tissue. The left- and right-ventricular free wall and septum were separated, weighed, and stored at −70°C until used.

2.2. Blood pressure measurement

Systolic blood pressure (SBP) was measured in unanesthetized rats by tail cuff plethysmography using a MOD 59 pulse amplifier (IITC Inc., Woodland Hills, CA). Animals were warmed for 20 min under a lamp which produced an ambient temperature of about 38°C and then gently placed in a restrainer. Tail pulses were displayed on a chart recorder.

2.3. Ribonuclease protection assay (RPA)

Total RNA was extracted from frozen tissue by the method of Chomczynski and Sacchi [29,30] using the TRI reagents (Molecular Research Center, Inc., Cincinnati, OH). RPA was performed using the Ambion RPA II Kit (RPA II®-Ribonuclease Protection Assay Kit, Ambion Inc. Austin, Texas) according to manufacturer’s instructions. Plasmids containing a 690 bp rat IGF-1 insert and a 265 bp rat IGF-1R insert were kindly provided by D. LeRoith (Diabetes Branch, National Institutes of Health, Bethesda, MD) [31,32]. They were linearized with EcoRI and the cRNA antisense probes were transcribed in vitro using the Ambion Maxiscript Kit (MAXIscript™, Ambion Inc., Austin, Texas) with either T7 or SP6 polymerase and labeled with α-32P-dUTP. Linearized pTRI-GAPDH (glyceraldehyde-3-phosphate dehydrogenase) plasmid was purchased from Ambion Inc. and a Sty I digested fragment (134 bp) was used as template to transcribe the cRNA probe. The probes were purified on 5% polyacrylamide/8 M urea denaturing gel. RNA samples (10 μg) were hybridized overnight with 32P-labeled cRNA antisense probe (1 × 105 cpm at 42–45°C). Single stranded RNA was digested by the addition of 200 ul of digestion buffer containing 2.5 U/ml RNase A and 100 U/ml RNase T1 in a 30-minute incubation at 37°C. The protected fragments were separated by electrophoresis on 5% polyacrylamide/8 M urea denaturing gels. Band signal intensities were quantitated by scanning the gels with phosphoimager (Betascope 603 Blot Analyzer, Betagen Corporation, Waltham, MA). The gels were subsequently subjected to autoradiography for the purpose of displaying the images. The signal from each sample was normalized to the corresponding GAPDH signal. In one set of experiment (Fig. 3) because properly exposed images of GAPDH are not available the actual cumulated counts from Phosphoimaging are presented (legend to Fig. 3).

2.4. Northern blot analysis

RNA samples (10 μg) were size fractionated on 1.2% formaldehyde-agarose gels, and transferred to Magnagraph nylon membranes (MSI, Westborough, MA) by downward capillary transfer. The blots were hybridized to cDNA probes labeled with [α-32P]-dCTP by a random primer DNA labeling method (Amersham Corporation, Arlington Heights, IL). The cDNA probe for SERCA2 was a 2.3 kb EcoRI/Pvu I fragment of a clone kindly provided by Dr. W.H. Dillmann (Dept. Medicine, University of California at San Francisco). The probe for GAPDH was a 1.2 kb EcoRI/EcoRI fragment of the HHCMC32 clone pur-
Table 1
Effects of NE infusion on blood pressure and heart weight

<table>
<thead>
<tr>
<th></th>
<th>3 days</th>
<th></th>
<th>6 days</th>
<th></th>
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<tr>
<td></td>
<td>C</td>
<td>NE</td>
<td>C</td>
<td>NE</td>
</tr>
<tr>
<td>BP (mmHg)</td>
<td>110.0 ± 4.6</td>
<td>156.0 ± 8.7 *</td>
<td>123.0 ± 6.5</td>
<td>149.0 ± 1.8 *</td>
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<td>HR (beats/min)</td>
<td>258.8 ± 11.5</td>
<td>350.4 ± 11.0 *</td>
<td>255.2 ± 26.4</td>
<td>325.2 ± 12.9 *</td>
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<tr>
<td>BW (g)</td>
<td>440.4 ± 8.4</td>
<td>420.2 ± 12.7</td>
<td>414.0 ± 20.5</td>
<td>445.0 ± 9.9</td>
</tr>
<tr>
<td>LVW (mg)</td>
<td>570.0 ± 22.4</td>
<td>650.0 ± 35.8</td>
<td>550.0 ± 31.3</td>
<td>700.0 ± 35.8 *</td>
</tr>
<tr>
<td>LVW/BW (mg/100 g)</td>
<td>130.1 ± 5.7</td>
<td>155.1 ± 8.7 *</td>
<td>132.0 ± 5.1</td>
<td>156.7 ± 8.3 *</td>
</tr>
<tr>
<td>RVW (mg)</td>
<td>190.0 ± 13.4</td>
<td>200.0 ± 17.9</td>
<td>210.0 ± 13.4</td>
<td>210.0 ± 4.5</td>
</tr>
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<td>RVW/BW (mg/100 g)</td>
<td>43.6 ± 3.1</td>
<td>46.6 ± 3.4</td>
<td>50.2 ± 3.9</td>
<td>48.2 ± 1.3</td>
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BP - Blood pressure, HR - Heart rate, BW - body weight, RVW - Right ventricle weight, LVW - left ventricle weight.
* p < 0.05, ** p < 0.01 compare with age matched control group (n = 5).

Fig. 1. Linearity of ribonuclease protection assay. RPA was performed as described in Methods. Increasing amounts of total RNA was hybridized with specific cRNA probes: (A) IGF-1, (B) IGF-1R. cRNA was hybridized to yeast RNA to serve as negative control. The right most lane shows the size of undigested probe. (C) Intensity of the specific bands was quantitated by phosphor-imaging. The line is generated by linear regression.
Fig. 2. RPA analysis of expression of IGF-1 and IGF-1R in left ventricular tissue. Total RNA (10 μg/sample) was hybridized with antisense cRNA probe specific for IGF-1 and IGF-1R as described in Methods. Protected fragments were separated by electrophoresis. (A) Autoradiograms showing specific protected fragments (n = 5). Top panels: 3 days; Bottom panels: 6 days. (B) Signal intensity of the bands was quantitated by phosphor-imaging and normalized to GAPDH signals (* p < 0.05; ** p < 0.01). C denotes control, T indicates NE treated animals.

Fig. 3. RPA analysis of expression of IGF-1 and IGF-1R in right ventricular tissue. Protocol is the same as described in Fig. 2. (A) Autoradiograms showing specific protected fragments (n = 5). Top panels: 3 days; Bottom panels: 6 days. (B) Signal intensity of the bands were quantitated by phosphor-imaging and normalized to GAPDH signals (3-day IGF-1: C = 5756 ± 208; T = 5572 ± 380; 3-day IGF-1R: C = 76147 ± 6464; T = 74390 ± 5049; 6-day IGF-1: C = 78581 ± 10470; T = 77113 ± 1231) (* p < 0.05).
chased from ATCC (Rockvill, MD). Hybridization of blot to labeled cDNA probe was performed according to the filter/sandwich method [33] using 50% formamide, 5 × sodium saline citrate (SSC), 1 × Denhard’s solution, 20 mM sodium phosphate pH 6.8, 1% sodium dodecyl sulfate (SDS), and 5% dextran sulfate hybridization buffer at a temperature of 42°C for 16–24 hours. After hybridization, blots were washed sequentially with 5 × SSPE/0.5% SDS at room temperature and 0.1 × SSPE/1.0% SDS at 68°C. Band signal intensities were quantitated by scanning of the blots with Phosphor-imager. The signal from each sample was normalized to the corresponding GAPDH signal. The blots were subsequently subjected to autoradiography for the purpose of displaying the images.

2.5. Statistical analysis

Results are expressed as mean ± SE. Statistical differences between groups were analyzed by Student’s t-test. A p < 0.05 was considered statistically significant.

3. Results

3.1. Blood pressure and heart weight

Rats were infused with NE (50 μg/kg/h) or vehicle for 3 and 6 days. Data in Table 1 show that NE treatment significantly increases systolic blood pressure and heart rate compared to that of controls. Left ventricular weight (LVW) significantly increased after 6 days of NE treatment, and LVW/BW increased after 3 and 6 days of treatment. By contrast, NE treatment had no significant effect on either right ventricular weight (RVW) or RVW/BW in any of the treatment groups when compared to controls.

3.2. Effect of NE infusion on IGF-1 and IGF-1R expression

In order to demonstrate the linearity of the RPA, IGF-1 and IGF-1R cRNA antisense probe was hybridized to increasing amounts of total RNA from rat heart. Fig. 1 shows that signals of the protected fragments increased linearly with the amount of RNA loaded.

In left ventricle, both IGF-1 and IGF-1R mRNA levels, normalized to GAPDH levels, increased significantly by day 6 of NE treatment, with IGF-1 increasing to a greater extent than IGF-1R (Fig. 2). In right ventricle, IGF-1

![Fig. 4. Northern blot analysis of SERCA2 mRNA in left ventricular tissue. Total RNA (10 μg/lane) was size fractionated and transferred to nylon membrane as described in Methods. The blots were hybridized simultaneously with cDNA probes specific for SERCA2 and GAPDH. (A) Autoradiograms showing the specific SERCA2 and GAPDH signals (n = 5). Top panels: 3 days; Bottom panels: 6 days. (B) The blots were quantitated by phosphor-imaging and SERCA2 signals were normalized by corresponding GAPDH signals.](https://academic.oup.com/cardiovascres/article-abstract/37/1/202/540720)
mRNA levels also increased after 6 days of NE treatment (Fig. 3). By contrast, IGF-1R mRNA levels in right ventricle were unchanged during the treatment period.

3.3. Expression of Ca\(^{2+}\)-ATPase mRNA after NE infusion

The level of expression of SERCA2 mRNA in LV was examined by Northern blot analysis. Although SERCA2 signals appear to be decreased in NE treated rats (Fig. 4A), especially at day 3 after treatment, levels of SERCA2 mRNA levels in the left ventricle remained unchanged after 3 and 6 days of treatment when the signals were normalized to that of GAPDH (Fig. 4B).

In order to determine whether the lack of response in SERCA2 levels is dose dependent, in another series of experiments rats were infused with a higher dose of NE (130 \(\mu\)g/kg/h) for 6 and 14 days. Similar significant changes in BP, LVW, LVW/BW, but not RVW, were observed at this dose of NE as were at the lower dose (Table 2). Results in Fig. 5 show that infusion of NE at the higher dose for a longer period of time still failed to elicit significant changes in SERCA2 mRNA levels in both the left- and the right-ventricles.

4. Discussion

The major findings in the present study are that NE infusion in rats differentially regulates the expression of IGF-1 and IGF-1R mRNAs in the left- and right-ventricle and that NE treatment failed to alter expression of SERCA2 mRNA at the doses examined.

The concentrations of NE used in the present study were chosen based on previous reports [34–36] and on our pilot study showing that these concentrations of NE produce significant increases in blood pressure and heart rate without grossly affecting the health of the animals, as indicated by the relatively small changes in body weight (Tables 1 and 2).

In the present study, NE treatment significantly increased IGF-1 mRNA levels in both left- and right-ventricle; however, levels of IGF-1R increased in the left ventricle but not in the right ventricle. These data suggest that expression of IGF-1 and IGF-1R can be modulated by NE. Although it is difficult to separate the pressor effects of NE from direct effects of NE, the lack of hyperplastic response in right ventricle suggests that NE treatment, at the dose examined, appears not to cause overload of the right ventricle. Thus, the increase in expression of IGF-1
mRNA in the right ventricle suggests that NE may affect IGF-1 expression independent of pressure overload. On the other hand, failure of NE to increase IGF-1 mRNA in RV suggests that NE, at least at the dose examined, has little, if any, pressure overload-independent effect on expression of IGF-1R. However, direct measurement of right ventricular pressure will be needed to verify these conclusions.

Previous studies demonstrate that expression of SERCA2 is altered during cardiac hypertrophy and failure [17–22]. Our study shows that NE infusion at relatively high doses did not alter expression of SERCA2 mRNA. Thus, our results do not support a significant direct role of NE on SERCA2 gene expression in the above mentioned disease states. We cannot, however, exclude the possibility that NE plays a permissive role in regulating expression of SERCA2 during cardiac hypertrophy and failure when other hormonal factors may be involved. Whether NE alters expression of SERCA2 protein by translational and/or post-translational mechanisms remains to be determined.

In conclusion, our results demonstrate that NE treatment differentially regulates expression of IGF-1 and IGF-1R in the rat ventricles. The data suggest a possible role of NE in modifying expression of these genes under conditions when NE levels are sufficiently elevated. On the other hand, our results failed to support the hypothesis that NE plays a significant direct role in regulating expression of SERCA2 mRNA. Cellular and molecular mechanisms underlying NE-induced upregulation of IGF-1 and IGF-1R are currently under investigation.

Acknowledgements

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References


