Postinfarct sympathetic hyperactivity differentially stimulates expression of tyrosine hydroxylase and norepinephrine transporter

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Departments of 1Physiology and Pharmacology, 2Anesthesiology and Perioperative Medicine, and 3Neurology, Oregon Health and Science University School of Medicine, Portland; 4Portland Veterans Affairs Medical Center, Portland, Oregon; and 5Max-Delbrück-Center for Molecular Medicine, Berlin-Buch, Germany

Submitted 4 May 2007; accepted in final form 17 October 2007

Parrish DC, Gritman K, Van Winkle DM, Woodward WR, Bader M, Habecker BA. Postinfarct sympathetic hyperactivity differentially stimulates expression of tyrosine hydroxylase and norepinephrine transporter. Am J Physiol Heart Circ Physiol 294: H99–H106, 2008. First published October 19, 2007; doi:10.1152/ajpheart.00533.2007.—The balance between norepinephrine (NE) synthesis, release, and reuptake is disrupted after acute myocardial infarction, resulting in elevated extracellular NE. Stimulation of sympathetic neurons in vitro increases NE synthesis and the synthetic enzyme tyrosine hydroxylase (TH) to a greater extent than it increases NE reuptake and the NE transporter (NET), which removes NE from the extracellular space. We used TGR(ASrAOGEN) transgenic rats, which lack postinfarct sympathetic hyperactivity, to test the hypothesis that increased cardiac sympathetic nerve activity accounts for the imbalance in TH and NET expression in these neurons after myocardial infarction. TH and NET mRNA levels were identical in the stellate ganglia of unoperated TGR(ASrAOGEN) rats compared with Sprague Dawley (SD) controls, but the threefold increase in TH and twofold increase in NET mRNA seen in the stellate ganglia of SD rats 1 wk after ischemia-reperfusion was absent in TGR(ASrAOGEN) rats. Similarly, the increase in TH and NET protein observed in the base of the SD ventricle was absent in the base of the TGR (ASrAOGEN) ventricle. Neuronal TH content was depleted in the left ventricle of both genotypes, whereas NET was unchanged. Basal heart rate and cardiac function were similar in both genotypes, but postinfarct relaxation was enhanced in TGR(ASrAOGEN) hearts. These data support the hypothesis that postinfarct sympathetic hyperactivity is the major stimulus increasing TH and NET expression in cardiac neurons.

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Animals and experimental groups. Adult Sprague Dawley (SD) rats (225–250 g) were obtained from Charles River SASCO and were kept on a 12:12-h light-dark cycle with ad libitum access to food and water for 7 days before death and tissue harvest. Buprenex (0.1 mg/kg) was administered as needed to ensure the animals were comfortable following surgery. All surgical procedures were performed under aseptic conditions. All procedures were approved by the Institutional Animal Care and Use Committee and comply with the Guide for the Care and Use of Laboratory Animals published by the United States National Institutes of Health (NIH publication No. 85–23, revised 1996). Control animals did not undergo any surgical procedures, whereas sham animals underwent the procedure described above except for the LAD ligation.

Immunoblot analysis. TH and NET levels were quantified by Western blot as described previously (21). Hearts were excised and cut in 3- to 5-mm transverse cross sections, excluding the area spanning the LAD ligation, as shown in Fig. 1. The base was processed as a single sample that included the top 3 mm of both ventricles. Below the site of LAD occlusion, the left (LV) and right (RV) ventricles were separated and processed individually (Fig. 1) for Western blot analysis. LV samples included both infarct and peri-infarct tissue. Tissue was pulverized to a powder on dry ice, homogenized in lysis buffer, sized fractionated on SDS-PAGE, and transferred to membranes for blotting. Multiple samples of a single heart region from control, sham, and MI rats were run together, and aliquots of each sample were fractionated simultaneously on two gels. Each blot was first incubated with mouse monoclonal anti-PGP (diluted in the 4th intercostal space, and the pericardium was opened. The left anterior descending coronary artery (LAD) was reversibly ligated with a 7–0 suture for 30 min and then reperfused by release of the ligature (21). Occlusion was confirmed by sustained S-T wave elevation, regional cyanosis, and wall motion abnormalities. When sustained (20+ beats) arrhythmia or ventricular fibrillation occurred during the occlusion, two to five drops of 2% lidocaine were applied topically to the myocardium to stabilize heart rhythm. Reperfusion was confirmed by the return of color to the ventricle distal to the ligation. Core body temperature was monitored by a rectal probe and maintained at 37°C, and a three-lead electrocardiogram was monitored throughout the surgery using a PowerLab data acquisition system. The suture remained within the wound for identification of the ligature site, and the chest and skin were closed in layers. After surgery, animals were returned to individual cages and given regular food and water for 7 days before death and tissue harvest. Buprenex (0.1 mg/kg) was administered as needed to ensure the animals were comfortable following surgery. All surgical procedures were performed under aseptic conditions. All procedures were approved by the Institutional Animal Care and Use Committee and comply with the Guide for the Care and Use of Laboratory Animals published by the United States National Institutes of Health (NIH publication No. 85–23, revised 1996). Control animals did not undergo any surgical procedures, whereas sham animals underwent the procedure described above except for the LAD ligation.

Myocardial ischemia-reperfusion. Infarction was generated using ischemia-reperfusion because reperfusion of occluded coronary arteries is standard treatment for humans. Anesthesia was induced with 4% isoflurane and maintained with 2% isoflurane. Rats were then intubated and mechanically ventilated. A left thoracotomy was performed in the 4th intercostal space, and the pericardium was opened. The left anterior descending coronary artery (LAD) was reversibly ligated with a 7–0 suture for 30 min and then reperfused by release of the ligature (21). Occlusion was confirmed by sustained S-T wave elevation, regional cyanosis, and wall motion abnormalities. When sustained (20+ beats) arrhythmia or ventricular fibrillation occurred during the occlusion, two to five drops of 2% lidocaine were applied topically to the myocardium to stabilize heart rhythm. Reperfusion was confirmed by the return of color to the ventricle distal to the ligation. Core body temperature was monitored by a rectal probe and maintained at 37°C, and a three-lead electrocardiogram was monitored throughout the surgery using a PowerLab data acquisition system. The suture remained within the wound for identification of the ligature site, and the chest and skin were closed in layers. After surgery, animals were returned to individual cages and given regular food and water for 7 days before death and tissue harvest. Buprenex (0.1 mg/kg) was administered as needed to ensure the animals were comfortable following surgery. All surgical procedures were performed under aseptic conditions. All procedures were approved by the Institutional Animal Care and Use Committee and comply with the Guide for the Care and Use of Laboratory Animals published by the United States National Institutes of Health (NIH publication No. 85–23, revised 1996). Control animals did not undergo any surgical procedures, whereas sham animals underwent the procedure described above except for the LAD ligation.

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1:2,000), immunoreactive bands were visualized by chemiluminescence using SuperSignal Dura, and band intensity was recorded by a −40°C charge-coupled device camera. Blots were stripped and then rebotted for either polyclonal anti-TH (diluted 1:1,000) or rabbit anti-NET (diluted 1:1,000). After visualization with goat anti-mouse (diluted 1:10,000) or anti-rabbit (diluted 1:5,000) horseradish peroxidase, the blots were stripped again and then rebotted for the opposite antibody. Data were analyzed using LabWorks software (UVP, Upland, CA). The ratio of TH/PGP or NET/PGP of unoperated control animals was set as 100%, and values from operated animals were calculated as a percentage of control. Each region of the heart (base, LV, RV; Fig. 1) was compared with the corresponding region in control hearts of the same genotype.

Area at risk and infarct size. After the onset of reperfusion (24 h), the rats were placed in an induction chamber and anesthetized with −4°C isoflurane. Rats were intubated and maintained on 2% isoflurane, and the chest cavity was reopened at the site of the initial incision. The coronary artery was then reocluded, and fluorescent particles (4 ng/ml in deionized water with 0.01% Tween 20; Duke Scientific no. 34–1, 2- to 8-μm size) were infused through a polyethylene catheter (PE-60) with a 22-gauge needle tip in the left ventricle (LV) of the heart. Microspheres were infused at a rate of 2 μl/min for 5 min to delineate the area at risk (Fig. 2). The heart was then excised for infarct size analysis and cut into transverse slices 2 mm thick using a cutting block. Both sides of all slices were photographed under ultraviolet light, and the captured images were saved in Photoshop for measurement of area at risk. The slices were then placed in 2,3,5-triphenyltetrazolium chloride solution (TTC, 1% wt/vol in sodium phosphate buffer at 37°C, pH 7.4) for 20 min. The staining procedure was carried out in the dark to prevent breakdown of the TTC by light. The slices were then placed in 10% neutral buffered formalin overnight to increase the contrast between stained and unstained tissue. Myocardium that did not stain red was presumed to be infarcted (Fig. 2). Both sides of each 2-mm section were photographed under white light, and images were saved in Photoshop. LV, risk, and infarct areas for each slice were traced and digitized. The volume of myocardium at risk and infarcted myocardium was calculated from the measured areas and slice thickness. Infarct size was normalized as a fraction of the area at risk or as percent of the entire LV. All analyses were performed in a blinded fashion by two people. The data presented are the average of the two independent determinations of infarct/risk.

Immunohistochemistry. Hearts from unoperated rats of each genotype were fixed for 1 h in 4% paraformaldehyde, rinsed in PBS, cryoprotected in 30% sucrose overnight, and frozen in mounting media for sectioning. Transverse sections (10 μm) through both ventricles were thaw-mounted on charged slides. Sections were incubated overnight in rabbit anti-TH (1:300 Chemicon), washed, incubated with anti-rabbit Alexa 488 (Molecular Probes), diluted 1:300, and visualized by fluorescence microscopy.

HPLC analysis of NE. NE levels in heart tissue were measured by HPLC with electrochemical detection, as described in detail previously (21). Heart tissue was homogenized in perchloric acid (0.1 M) containing 1.0 μM of the internal standard dihydroxybenzylamine to correct for sample recoveries. Catecholamines were purified by aluminia extraction before analysis by HPLC. Detection limits were ~0.05 pmol with recoveries from the aluminia extraction >60%.

Real-time PCR. Stellate ganglia were harvested 7 days after ischemia-reperfusion and stored immediately in RNAlater. RNA was isolated from individual stellate ganglia using the Ambion RNeasy micro kit. Total RNA was treated with DNase, quantified by determining optical density at 260 nm, and then 25 ng of total RNA were reverse transcribed. An RNA alone control was included for each sample to test for genomic DNA contamination. Real-time PCR was performed with either ABI TaqMan Universal PCR master mix or ABI Syber green PCR master mix in the ABI 7500. The pan-neuronal marker PGP was used as a normalization control because its expression is unaltered in the stellate ganglia following ischemia-reperfusion (21). Samples were assayed using previously described primer sets for TH, NET, and PGP (15, 21) or using ABI prevalidated TaqMan gene expression assays for rat TH, NET, and PGP. For the PCR amplification, 2 μl of reverse transcription reactions were used in a total volume of 20 μl, and each sample was assayed in duplicate. Standard curves for TH, NET, and PGP were generated with known amounts of RNA from control sympathetic ganglia, ranging from 0.39 to 100 ng. Values for TH and NET were normalized to PGP from the same sample. Postinfarct mRNA ratios (TH/PGP, NET/PGP) were compared with unoperated controls of the same genotype to determine the percent change following ischemia-reperfusion.

Heart rate, left ventricular peak systolic pressure, dp/dt, and drug treatments. After ischemia-reperfusion or sham surgery (1 wk), anesthesia was induced with 4% isoflurane and maintained with 2–3% isoflurane. Rats were intubated and placed on a rodent ventilator. A micropipette pressure transducer (2.0 French; Millar) was inserted in the right carotid artery and advanced in the LV for measurement of left ventricular pressure using a PowerLab data acquisition system. A small polyvinyl catheter was placed in the left femoral vein for drug administration. When the animal was stable, it was given hexametho-

Table 1. Animal characteristics

<table>
<thead>
<tr>
<th>Region</th>
<th>Heart Rate, beats/min</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Unoperated (n = 5)</td>
</tr>
<tr>
<td>Basal</td>
<td>318±16</td>
</tr>
<tr>
<td>Δ Hex</td>
<td>−16±11</td>
</tr>
<tr>
<td>Δ Tyr</td>
<td>62±11</td>
</tr>
<tr>
<td></td>
<td>333±13</td>
</tr>
<tr>
<td></td>
<td>18±3*</td>
</tr>
<tr>
<td></td>
<td>55±4</td>
</tr>
</tbody>
</table>

Table 2. Cardiac NE

<table>
<thead>
<tr>
<th>Region</th>
<th>Unoperated (n = 5)</th>
<th>Sham (n = 5)</th>
<th>MI (n = 4)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Base</td>
<td>10.7±2.4</td>
<td>8.8±0.5</td>
<td>6.8±1.4</td>
</tr>
<tr>
<td>RV</td>
<td>12.1±1.0</td>
<td>7.3±0.9</td>
<td>8.0±1.5</td>
</tr>
<tr>
<td>LV</td>
<td>7.3±0.5</td>
<td>5.2±0.4</td>
<td>3.6±1.4*</td>
</tr>
<tr>
<td></td>
<td>8.0±1.1</td>
<td>6.6±1.3</td>
<td>6.1±0.4</td>
</tr>
<tr>
<td></td>
<td>8.5±0.3*</td>
<td>6.9±0.4</td>
<td>6.0±1.4</td>
</tr>
<tr>
<td></td>
<td>5.5±0.5</td>
<td>5.6±1.1</td>
<td>4.2±0.2</td>
</tr>
</tbody>
</table>

Values are means ± SE; n, no. of rats. Units are pmol/mg. NE, norepinephrine; RV, right ventricle; LV, left ventricle. *P < 0.05 vs. SD unoperated.
nium chloride (5 mg/kg) to abolish ganglionic transmission (1). After a new baseline was established, a single dose of tyramine hydrochloride was infused (200 μg/kg) to assess the cardiac response to release of endogenous NE. After parameters returned to baseline, animals received a single dose of the β-agonist dobutamine (32 μg/kg) to assess β-receptor sensitivity. Left ventricular peak systolic pressure (LVPSP), cardiac contractility (dP/dtMAX), cardiac relaxation (dP/dtMIN), and heart rate were analyzed using ChartPro software.

Statistics. Student’s t-test was used for comparisons of just two samples. In experiments where sham and MI groups were normalized to unoperated controls of the same genotype, the differences between all groups within the genotype were analyzed by one-way ANOVA using the Newman-Keul’s post hoc test. Experiments that did not require normalization to an intragenotype control were analyzed by two-way ANOVA using the Bonferroni post hoc test. All statistical analyses were carried out using Prism 4.02.

RESULTS

Basal heart rate (Table 1) was similar in AOGEn rats and in age-matched SD rats, although atrial NE content in was higher in SD rats [15.7 ± 1.3 (SE) pmol/mg SD (n = 5) vs. 10.3 ± 0.7 pmol/mg AOGEn (n = 3); P < 0.03]. Blockade of ganglionic transmission with hexamethonium decreased heart rate in SD rats but increased heart rate in AOGEn transgenic rats of all surgical groups. Following ganglionic blockade, infusion of tyramine to stimulate release of NE triggered similar increases in SD and AOGEn heart rate across all surgical groups (Table 1).

Immunohistochemical detection of TH revealed a similar pattern and density of sympathetic innervation in the LV and RV of unoperated SD and AOGEn rats (Fig. 1, B–E). NE content was somewhat lower in the ventricles of AOGEn rats compared with SD rats, but this was statistically significant only in the right ventricle (RV; Table 2). TH and NET mRNA levels in the stellate ganglia, normalized to PGP as an internal control, were indistinguishable between control animals of each genotype [TH/PGP: SD 1.5 ± (SE) 0.2, AOGEn 1.4 ± 0.2; NET/PGP: SD 1.3 ± 0.2, AOGEn 1.4 ± 0.1; n = 5].

Infarct size normalized to area at risk (Fig. 2) or as percent of the LV was identical in SD and AOGEn rats [infarct as %LV: wild type 12.9 ± (SE) 1.9, AOGEn 12.4 ± 1.3; n = 5], but TH and NET mRNA levels were altered differentially by ischemia-reperfusion surgery. TH and NET mRNA were increased significantly in the stellate ganglia of SD rats 1 wk after ischemia-reperfusion surgery compared with shams and unoperated controls (Fig. 3). However, TH and NET mRNA levels in cardiac sympathetic neurons of AOGEn rats were not elevated after MI compared with sham or unoperated control animals.

The effect of ischemia-reperfusion on TH and NET protein levels was dependent on the region of the heart examined (see Fig. 1A for schematic). In the undamaged base of the ventricles above the infarct, TH content normalized to PGP was increased more than threefold in SD rats compared with unoperated controls and shams but was not increased in AOGEn rats (Fig. 4A). Likewise, NET protein normalized to PGP was increased twofold in the base of the ventricles in SD but not AOGEn rats (Fig. 4B). The LV below the site of LAD ligation...
exhibited a very different set of changes. TH protein content was decreased significantly in the LV of both genotypes compared with unoperated controls and shams, even after normalizing to the pan-neuronal marker PGP to control for denervation of the infarct and peri-infarct tissue (Fig. 5A). In contrast, left ventricular NET levels were not changed significantly in either genotype following ischemia-reperfusion surgery (Fig. 5B). Neither TH nor NET was decreased in the LV following sham surgery, consistent with the retention of both proteins in the base of the heart following sham surgery. Likewise, TH and NET levels in the RV were not changed significantly after sham or ischemia-reperfusion surgery in either genotype (data not shown).

Although TH content was elevated in the base of SD hearts after MI, neuronal NE content in the base of the ventricles was not altered significantly by sham or ischemia-reperfusion surgery in either genotype (Table 2). NE content in the LV was decreased after MI in SD rats, but NE content in AOGEN rats was not altered significantly by sham or MI surgery in any of the regions of the heart examined (data not shown).

Basal $dP/dt_{\text{MAX}}$ and $dP/dt_{\text{MIN}}$ were similar across all groups 1 wk after sham or ischemia-reperfusion surgery (Fig. 6). In contrast, basal LVPSP was elevated significantly in the AOGEN rats regardless of surgical group and changed to a greater extent following ganglionic blockade (Fig. 6). The β-agonist dobutamine stimulated LVPSP, $dP/dt_{\text{MAX}}$, and $dP/dt_{\text{MIN}}$ in both genotypes, and the responses in AOGEN rats were generally enhanced compared with SD rats regardless of surgical group (Fig. 7). Tyramine-induced release of endogenous NE stimulated LVPSP and $dP/dt_{\text{MAX}}$ to a similar extent in both genotypes regardless of surgical group (Fig. 8). NE-stimulated $dP/dt_{\text{MAX}}$ was significantly lower after MI in both genotypes, consistent with impaired $dP/dt_{\text{MAX}}$. In contrast, the NE-stimulated increase in $dP/dt_{\text{MIN}}$ was blunted after MI in SD rats but unimpaired in AOGEN rats (Fig. 8).

**DISCUSSION**

The major of findings of our studies with SD and AOGEN rats were that basal noradrenergic parameters were indistinguishable between the genotypes, and there were no differences in infarct size normalized to the area at risk following ischemia-reperfusion. The increase in TH and NET mRNA seen in SD rats after ischemia-reperfusion was absent in AOGEN rats, and this corresponded with a significant increase in TH protein in the base of SD ventricles that was absent in the base of AOGEN ventricles. TH content was depleted significantly in the LV in both genotypes, and LVPSP differed between the genotypes in both the sham and MI groups. AOGEN rats exhibited elevated responses to dobutamine infusion but not to release of endogenous NE with tyramine.

Normal noradrenergic transmission results from a balance between NE synthesis, release, and reuptake. The balance between synthesis, release, and reuptake of NE is disrupted...
after AMI, resulting in elevated extracellular NE (2, 24, 26). The reasons for this are not known, but AMI leads to sympathetic hyperactivity (11, 13, 14). Stimulation of sympathetic nerve activity in vivo or depolarization of sympathetic neurons in vitro increases NE production by stimulating both the expression and activity of TH (9, 17, 29, 33). Chronic depolarization of sympathetic neurons in vitro also increases NE uptake and NET expression (22), but the effect on NE uptake is smaller than the stimulation of TH expression and NE synthesis. We hypothesized that this differential effect of nerve activity (a greater increase in NE synthesis than in NE reuptake) could account for the imbalance in TH and NET found in the stellate ganglia and base of the ventricles after MI. We used AOGEN transgenic rats, which do not exhibit the normal degree of cardiac sympathetic hyperactivity after chronic ischemia (34), to test this hypothesis.

Expression of TH and NET mRNA in the stellate ganglia of unoperated AOGEN rats was similar to that seen in SD control rats, but regulation of TH and NET gene expression differed following ischemia-reperfusion surgery. Expression of both genes increased significantly in the stellate ganglia of SD rats after MI, but neither increased in AOGEN rats. Similarly, TH and NET protein levels were elevated in the base of the RV and LV after infarct in SD but not AOGEN rats. That increase was specific for the base of the ventricles however, since below the LAD ligation, TH content in both genotypes was decreased significantly in the LV and unchanged in the RV. It is not clear why there are such distinct regional differences in cardiac TH content, but local changes in neurotrophins and inflammatory cytokines may play a role (3, 19). The greater increase in TH protein compared with NET in the base of the ventricles is

Fig. 7. Cardiac responses after dobutamine infusion in SD (filled bars, n = 5, mean ± SE) and AOGEN transgenic (hatched bars, n = 5, mean ± SE) rats. A: change in left ventricular peak systolic pressure after dobutamine infusion (*P < 0.05). B: change in +dP/dtMAX after dobutamine infusion. C: change in −dP/dtMIN after dobutamine infusion (*P < 0.05).

Fig. 8. Cardiac responses after tyramine infusion in SD (filled bars, n = 5, mean ± SE) and AOGEN transgenic (hatched bars, n = 6, mean ± SE) rats. A: change in left ventricular peak systolic pressure after tyramine infusion (P = 0.06 AOGEN vs. SD). B: change in +dP/dt after tyramine infusion (*P < 0.05). C: change in −dP/dt after tyramine infusion (*P < 0.05 and **P < 0.01).
consistent with the buildup of extracellular NE and depletion of neuronal NE seen after MI. Similar changes in TH and NET expression are observed in cultured sympathetic neurons depolarized with high KCl (22). These data suggest that sympathetic hyperactivity after ischemia-reperfusion is the primary stimulus driving the increased expression of TH and NET in cardiac sympathetic neurons.

Angiotensin II signaling in the brain alters reflex control of heart rate (31), and indirect evidence suggests that basal sympathetic outflow to the heart is decreased in AOGEN rats (8) in addition to the blunted sympathetic hyperactivity after cardiac ischemia (34). Our study provides further evidence for decreased basal sympathetic outflow in AOGEN rats, since activation of β-receptors with dobutamine generated enhanced responses in AOGEN hearts regardless of surgical group. Although the pattern and density of the cardiac sympathetic innervation appeared normal in AOGEN transgenic rats, NE content trended lower throughout the AOGEN heart compared with age-matched SD control rats, consistent with decreased basal sympathetic outflow. The decrease in NE content was statistically significant only in the atria and RV, but decreased central stimulation of sympathetic neurons would impact release and synthesis of NE.

In contrast to the dobutamine experiments, administration of tyramine to trigger release of endogenous NE from sympathetic nerve terminals did not generate significantly larger responses in AOGEN hearts compared with SD controls. Ventricular pressure trended higher in AOGEN rats, but the difference was not significant. The only parameter that was elevated in AOGEN rats compared with SD rats after tyramine infusion was dP/dt\text{MIN}, and that was only elevated in the postinfarct animals. Given the increased sensitivity to β-receptor agonists in AOGEN hearts recently described by Campos et al. (8) and confirmed by our experiments, the normal responses seen in AOGEN hearts following tyramine administration suggest that less NE is released from sympathetic terminals in the AOGEN heart. Thus the lower NE content in AOGEN hearts may be physiologically significant even if it is not statistically significant.

Although basal heart rate did not differ between the genotypes or surgical groups, ganglionic block decreased mean heart rate in SD rats and increased heart rate in AOGEN rats. This surprising result suggests that parasympathetic outflow to the heart may be increased in AOGEN rats relative to the level of sympathetic tone. This is consistent with a telemetry study in conscious rats that proposed vagal tone was higher in AOGEN animals than SD controls (4) but is not supported by the observation that atropine stimulated similar increases in heart rate in anesthetized AOGEN and SD rats (8). The consistent finding among our study and the others is that blockade of sympathetic transmission does not generate a significant drop in heart rate in AOGEN rats.

In general, we observed fewer differences in cardiac physiology between AOGEN and SD rats 1 wk after ischemia-reperfusion than were reported 6–8 wk after chronic myocardial ischemia (34). Tyramine-induced release of endogenous NE revealed impaired dP/dt\text{MAX} in both genotypes. In contrast to the change in dP/dt\text{MAX} that was consistent in both genotypes after MI, the change in dP/dt\text{MIN} following tyramine infusion was significantly greater in AOGEN rats than in SD rats. The reason for this differential response in RV following infarction is not understood, but sympathetic transmission stimulates dP/dt\text{MIN} to a greater extent than dP/dt\text{MAX} (18), although both effects are mediated through activation of cardiac β-adrenergic receptors. NE content is spared in the peri-infarct LV of AOGEN rats relative to SD rats, and release of this endogenous NE may stimulate a greater change in dP/dt\text{MIN} than dP/dt\text{MAX} in the AOGEN rats.

In summary, we used AOGEN transgenic rats that have attenuated postinfarct sympathetic hyperactivity to determine if increased sympathetic nerve activity was responsible for changes in TH and the NET following ischemia-reperfusion. We found that the MI-induced increase in TH and NET mRNA and protein observed in SD rats was absent in AOGEN rats. This suggests that increased nerve activity stimulates expression of these proteins and the genes that encode them. The high levels of extracellular NE in the heart after AMI contribute to increased risk of cardiac arrhythmia and mortality (6, 23, 27). Thus identifying the factors that regulate NE synthesis and reuptake after MI is an important step in preventing pathological changes in neural transmission.

ACKNOWLEDGMENTS

We thank T. Jarred Ewert, Andrew Roland, and Eric Alston for technical assistance.

GRANTS

This work was supported by National Heart, Lung, and Blood Institute Grant R01 HL-68231 (B. A. Habecker).

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