Age-related Effects of Norepinephrine on Rat Ventricular Hypertrophy

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SUMMARY

Norepinephrine (NE) (2.5 $\mu\text{g/kg/min}$) was administered to 5-week-old male Sprague Dawley rats by subcutaneous osmotic mini pumps for 14 days to generate an *in vivo* cardiac hypertrophy model and the responses with respect to aging examined. In the model, ventricles were significantly hypertrophied without myocardial necrosis and without significant increases in heart rate or blood pressure; the beta adrenergic system was down-regulated. In 37-week-old rats receiving 1.0 $\mu\text{g/kg/min}$ NE, there was a tendency towards heart failure, and myocardial necrosis and interstitial fibrosis were revealed by histological examinations. The density of beta adrenergic receptors and adenylyl cyclase activity was lower in the older rats. The excess stimulation of adrenergic receptors caused severe cardiac injury in old rats regardless of down regulation of beta adrenergic receptors. (Jpn Heart J 1997; 38: 433-443)

Key words: Sympathetic nervous system, Norepinephrine, Ventricular hypertrophy, Osmotic mini pump, Aging

CULTURED neonatal cardiomyocytes have been used to determine the role of α adrenergic stimulation in cardiac hypertrophy.^{1,2)} Because many factors interact in *in vivo* experimental systems, it is often difficult to decide which factor(s) most profoundly affect the results. There are limitations in cultured cardiomyocyte systems, so both *in vivo* and *in vitro* experimental systems are needed to uncover the relevant factors. Among *in vivo* experimental systems, reports differ with respect to the changes of catecholamine concentrations of myocardium^{3,4)} and the response of myocardium to α and β adrenergic blockers.^{5,6)} The tonus of sympathetic nervous systems is believed to be different in the different models. Cooper et al.⁷⁾ suggested that mechanical stimulation may be the most important factor for cardiac hypertrophy in adults. It is reasonable to assume that animal species and ages may also be affecting factors. In the present

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study, we created a rat ventricular hypertrophy model using subcutaneously implanted osmotic mini-pumps that delivered norepinephrine (NE), and examined the relationship between the sympathetic nervous system and aging in the development of ventricular hypertrophy.

METHODS

Osmotic mini-pumps (Alzet model 2002) were implanted subcutaneously on the dorsum of 5- and 37- week-old male Sprague Dawley (SD) rats (Charles River Laboratories, Inc., Boston, MA, USA) under ether anesthesia. The mode of action of these pumps *in vivo* has been described by Patel et al.⁸⁾ and King et al.⁹⁾ The pumps deliver solution at rate of 0.5 μ l/hour into surrounding tissues. The concentrations of delivered drug can be adjusted by changing concentrations of solution in the pump. Blood pressures (BP) and heart rates (HR) were measured by the tail cuff method prior to euthanasia.

At appropriate times following the experimental protocol, the rats were decapitated while under deep ether anesthesia and the hearts removed. Body weight (BW) and ventricle weight (VW) were recorded and both right and left ventricle-to-body weight (V/B) ratios calculated.

Membrane preparation: The technique described by Liemas and Liemas¹⁰⁾ was used. The excised heart was minced with scissors and homogenized with a polytron: PT 10 (Kinematica, Lucerne, Switzerland) (Set 5, 20 sec) in Buffer A (250 mM sucrose, 1 mM NaHCO₃, 1 mM MgCl₂, pH 7.4) at 4°C. The homogenate was centrifuged at 500 $\times$ g for 10 min at 4°C, the supernatant filtered through double gauze and then centrifuged again at 40,000 $\times$ g for 20 min at 4°C. After centrifugation, the pellet was resuspended in Buffer A, homogenized by the potter technique, and centrifuged again at 40,000 $\times$ g for 20 min at 4°C. The pellet was resuspended in Buffer B (50 mM Tris, 10 mM MgCl₂, 2 mM EGTA, pH 7.4), frozen, and stored at -70°C until measurement.

Binding assay: Following the technique described by Vatner et al.,¹¹⁾ 8 graded concentrations (240 to 3,000 pM) of ¹²⁵I-iodocyanopindolol (ICYP) (NEN, specific activity 2200 Ci/mmol) and crude membranes were incubated at 37°C for 60 min and filtered through Whatman GF/C glass fiber filters (Whatman Inc., Clifton, NJ). The amount of ¹²⁵I-ICYP on the filter was measured by a gamma counter. Non-specific binding was assessed under 6 μ M propranolol. The results were analyzed by Scatchard analysis and the density of β -adrenergic receptors (Bmax) and dissociation constant (Kd) calculated. Sixteen graded concentrations (6 $\times$ 10⁻⁴ M to 1.5 $\times$ 10⁻⁸ M) of (-)-ISO-HCl were prepared. With these solutions, 300 pM ¹²⁵I-ICYP and crude membranes were incubated at 37°C for 30 min. After filtration through a Whatman GF/C glass fiber filter, the amount of ¹²⁵I-

ICYP on the filter was measured using a gamma counter. The study was repeated under Gpp(NH)p and the affinity for ISO in the presence and absence of Gpp(NH)p was calculated with a computer-assisted program, 'Ligand', developed by Munson and Rodbard,¹²⁾ which uses the F-test for the best fit.

Adenylyl cyclase activity was assayed according to the method of Homma et al.¹³⁾ 0.4 mM IBMX, 0.5 mM App(NH)p, stimulants (0.1 mM ISO + 0.1 mM GTP or 0.1 mM Forskolin) and crude membrane were incubated at 37°C for 10 min, and cAMP was measured with a radioimmunoassay using a cAMP kit (Yamasa Shoyu K.K., Chiba, Japan). Changing Buffer A to Buffer 1 (250 mM sucrose, 1 mM NaHCO₃, 1 mM EGTA, pH 7.4) and Buffer B to Buffer 2 (100 mM Hepes, 2 mM EGTA, pH 7.6), crude membranes were prepared according to the same process, and the activity of adenylyl cyclase stimulated by Mn²⁺ was also measured.

The protein concentrations for each membrane assay were determined by the method of Lowry et al.,¹⁴⁾ but assays of Mn²⁺ stimulation of adenylate cyclase were determined with Biorad protein assay kits (Biorad, Hercules, CA).

NE concentrations in ventricular myocardium were measured by the HPLC method, and the 5'-nucleotidase activity was also determined as a measure of membrane volume, following the method of Hippel and Hilmo.¹⁵⁾

Student's t-test and Fisher's PLSD method were used to evaluate the significance of differences between groups. Differences were considered significant if $p \leq 0.05$.

Protocol, Study 1-1: To examine the changes in β -adrenergic receptors with aging, NE (1.0 and 2.5 $\mu\text{g/kg/min}$) was administered to 5 and 37-week-old male SD rats for 14 days; $n = 8$ in each group. The rats were sacrificed at the end of the 14-day treatment period and BW, HR, BP, VW, β -adrenergic receptor density and adenylyl cyclase activity were measured; ventricles were examined histologically. The NE used was in the form of an HCl salt (Sigma A7381, MW 205.6).

Protocol, Study 1-2: The ventricular hypertrophy model in the present study was generated by an osmotic mini-pump. To this model, we added a second dorsal subcutaneous pump that administered an α -1 blocker (bunazosin, kindly provided by Eisai Co., Ltd, Tokyo, Japan) or a β -1 blocker (bisoprolol, kindly provided by Tanabe Seiyaku Co., Osaka, Japan) with NE simultaneously to examine suppressive effects on ventricular hypertrophy. Because 5-week-old Sprague Dawley rats weigh 150–160 g and the implantation of two pumps would be too invasive, we used 6-week-old male SD rats in this part of the study. The drugs and dosages administered by the 2 pumps in the rats of each group were as follows:

Group 1, $n = 4$: pump 1, NE (2.5 $\mu\text{g/kg/min}$); pump 2, 0.9% saline;

Group 2, $n = 4$: pump 1, NE ($2.5 \mu\text{g/kg/min}$); pump 2, bunazosin ($1.71 \times 10^{-7} \text{ mol/kg/min}$);

Group 3, $n = 5$: pump 1, NE ($2.5 \mu\text{g/kg/min}$); pump 2, bunazosin ($8.53 \times 10^{-8} \text{ mol/kg/min}$);

Group 4, $n = 4$: pump 1, NE ($2.5 \mu\text{g/kg/min}$); pump 2, bisoprolol ($4.40 \times 10^{-7} \text{ mol/kg/min}$);

Group 5, $n = 3$: pump 1, NE ($2.5 \mu\text{g/kg/min}$); pump 2, bisoprolol ($2.20 \times 10^{-7} \text{ mol/kg/day}$).

Protocol, Study 2: The manufacturer stopped production of the HCl salt of NE, forcing us to change to a bitartate salt of NE (Sigma A7825, MW 319.3). We made preliminary studies again to determine a suitable concentration and delivery time of bitartate salts of NE for 5-week-old SD rats. Ascorbic acid (0.1 mg/ml) was added to serve as an antioxidative agent. Throughout the preliminary studies, the bitartate salt of NE ($3.0 \mu\text{g/kg/min}$) for 7 days was thought to be sufficient to induce ventricular hypertrophy.

NE ($3.0 \mu\text{g/kg/min}$) or 0.9% saline was administered to 5-week-old male SD rats for 7 continuous days continuously, and pre- and post BW, HR, BP and post VW were measured. Crude membrane preparations were made to measure β -adrenergic receptor density, affinity and adenylyl cyclase activity. 5'-nucleotidase activity was measured to use as an inner standard for crude membrane measurements. NE concentration in ventricles was measured by HPLC.

RESULTS

Study 1-1: The density of β -adrenergic receptors, dissociation constant, and activity of adenylyl cyclase were significantly reduced ($p < 0.05$) in 37 week old rats compared to 5 week old rats (Table I). NE ($1.0 \mu\text{g/kg/min}$) administration to 5 week old rats for 14 days decreased V/B (Table II-1) but a higher dose level of

Table I. Changes of Beta-adrenergic Receptor and Adenylyl Cyclase Activity with Advancing Age in SD rats.

	Age of rats	
	5 weeks	37 weeks
	($n = 8$)	($n = 8$)
β -AR: Bmax (fmol/mg)	108.2 ± 10.8	$47.4 \pm 7.1^*$
Kd (nM)	0.146 ± 0.036	$0.073 \pm 0.020^*$
AC-assay: basal (pmol/mg/min)	6.4 ± 1.2	5.8 ± 0.9
AC-assay: ISO + GTP (pmol/mg/min)	24.2 ± 5.8	$9.5 \pm 1.2^*$

Values are mean $\pm$ SD; * $p < 0.05$; β -AR: Bmax, β -adrenergic receptor density; Kd = dissociation constant; AC-assay: basal = non-stimulated cAMP quantity; AC-assay = ISO + GTP, isoproterenol + GTP-stimulated cAMP quantity.

Table II. The Administration of NE-HCl (1.0 $\mu\text{g/kg/min}$ and 2.5 $\mu\text{g/kg/min}$) for 14 Days in 5-week-old SD rats

1. NE (1.0 $\mu\text{g/kg/min}$)		
	Control ($n = 8$)	NE ($n = 8$)
BW (g)	280.8 $\pm$ 8.1	275.3 $\pm$ 13.6
HR (bpm)	391.0 $\pm$ 14.5	397.0 $\pm$ 25.0
BP (mmHg)	118.4 $\pm$ 13.0	116.5 $\pm$ 11.1
VW (mg)	897.3 $\pm$ 47.2	933.7 $\pm$ 54.3
V/B (mg/g)	3.20 $\pm$ 0.18	3.40 $\pm$ 0.21*
2. NE (2.5 $\mu\text{g/kg/min}$)		
	Control ($n = 8$)	NE ($n = 8$)
BW (g)	289.1 $\pm$ 8.1	265.4 $\pm$ 14.8*
HR (bpm)	398.8 $\pm$ 8.3	407.5 $\pm$ 14.9
BP (mmHg)	117.5 $\pm$ 4.6	118.0 $\pm$ 4.8
VW (mg)	1026.4 $\pm$ 76.8	1312.9 $\pm$ 126.2*
V/B (mg/g)	3.55 $\pm$ 0.29	4.95 $\pm$ 0.48*

Values are mean $\pm$ SD; BW = body weight; HR = heart rate; BP = blood pressure; VW = ventricular weight; V/B = ventricular to body weight ratio; * $p < 0.05$

Figure 1. The left ventricles of 5 week old SD rats. (Left) control rat, infused with 0.9% saline for 14 days. (Right) experimental rat, infused with NE (2.5 $\mu\text{g/kg/min}$) for 14 days.

NE (2.5 $\mu\text{g/kg/min}$) for 14 days led to significant ($p < 0.05$) ventricular hypertrophy and increased V/B without changes in HR and BP (Table II-2) and there was no necrosis of myocardium and interstitial fibrosis (Figure 1); BW was significantly ($p < 0.05$) reduced. In 37 week old rats, NE at the higher dose level (2.5 $\mu\text{g/kg/min}$) was lethal, and even at the lower dose level (1.0 $\mu\text{g/kg/min}$) NE caused increased HR ($p < 0.05$) and decreased BW ($p < 0.05$) (a tendency to heart failure) but no significant ventricular hypertrophy (Table III). Histological examination revealed myocardial necrosis with an invasion of small round mononuclear cells and interstitial fibrosis, which were not found in the ventricles of control rats (Figure 2).

Table III. The Administration of NE-HCl (1.0 $\mu\text{g/kg/min}$) for 14 Days in 37-week-old SD Rats

	Control (n = 8)	NE (1.0) (n = 7)
BW (g)	623.5 $\pm$ 43.8	582.2 $\pm$ 24.5*
HR (bpm)	325.0 $\pm$ 10.7	357.1 $\pm$ 39.9*
BP (mmHg)	110.1 $\pm$ 6.1	115.1 $\pm$ 6.1
VW (mg)	1635.0 $\pm$ 160.4	1681.0 $\pm$ 140.6
V/B (mg/g)	2.64 $\pm$ 0.35	2.90 $\pm$ 0.34

Values are mean $\pm$ SD; BW = body weight; HR = heart rate; BP = blood pressure; VW = ventricular weight; V/B = ventricular to body weight ratio; * $p < 0.05$

Figure 2. The left ventricles of 37 week old SD rats infused with NE (1.0 $\mu\text{g/kg/min}$) for 14 days.**Table IV.** The Simultaneous Administration of NE-HCl (2.5 $\mu\text{g/kg/min}$) + α -1 (Bunazosin) or β -1 Blocker (Bisoprolol) for 14 Days in 6-week-old SD Rats

	Group 1 (n = 4)	Group 2 (n = 5)	Group 3 (n = 5)	Group 4 (n = 4)	Group 5 (n = 3)
At the start of the study:					
BW (g)	179.7 $\pm$ 4.0	176.1 $\pm$ 7.9	177.7 $\pm$ 3.5	176.9 $\pm$ 4.6	181.9 $\pm$ 2.5
HR (bpm)	415.0 $\pm$ 17.3	398.0 $\pm$ 20.5	420.0 $\pm$ 18.7	427.5 $\pm$ 15.0	430.0 $\pm$ 20.0
BP (mmHg)	113.0 $\pm$ 10.9	103.0 $\pm$ 5.2	111.2 $\pm$ 11.5	124.3 $\pm$ 5.4	113.7 $\pm$ 4.0
After 14 days of drug administration:					
BW (g)	214.6 $\pm$ 15.8	282.3 $\pm$ 13.6*	260.2 $\pm$ 33.8*	240.3 $\pm$ 14.7*	251.1 $\pm$ 8.3*
HR (bpm)	385.0 $\pm$ 61.9	370.0 $\pm$ 23.5	372.5 $\pm$ 35.0	365.0 $\pm$ 33.2	353.3 $\pm$ 25.2
BP (mmHg)	121.5 $\pm$ 19.1	105.4 $\pm$ 46.6	122.8 $\pm$ 13.8	131.8 $\pm$ 4.5	135.7 $\pm$ 1.2
VW (mg)	1215 $\pm$ 117	1185 $\pm$ 66	1124 $\pm$ 58	1146 $\pm$ 112	1165 $\pm$ 92
V/B (mg/g)	5.67 $\pm$ 0.71	4.20 $\pm$ 0.29*	4.38 $\pm$ 0.60*	4.76 $\pm$ 0.18*	4.63 $\pm$ 0.21*
Group 1, controls: NE + 0.9% saline					
Group 2: NE + Bunazosin (1.71×10^{-7} mol/kg/day)					
Group 3: NE + Bunazosin (8.53×10^{-8} mol/kg/day)					
Group 4: NE + Bisoprolol (4.40×10^{-7} mol/kg/day)					
Group 5: NE + Bisoprolol (2.20×10^{-7} mol/kg/day)					

Values are mean $\pm$ SD, * $p < 0.05$ between treatment and controls (Treatment 1). BW = body weight; HR = heart rate; BP = blood pressure; VW = ventricular weight; V/B = ventricular to body weight ratio.

Table V. The Administration of NE-Bitartrate (3.0 $\mu\text{g/kg/min}$) for 7 Days in 5-week-old SD Rats

	Control (<i>n</i> = 8)	NE (3.0) (<i>n</i> = 8)
7 days-BW (g)	195.5 $\pm$ 7.3	190.1 $\pm$ 8.6
BP (mmHg)	113.4 $\pm$ 11.3	129.5 $\pm$ 14.9*
HR (bpm)	413.0 $\pm$ 16.2	388.5 $\pm$ 22.7*
VW (mg)	729.6 $\pm$ 53.7	870.9 $\pm$ 88.5*
V/B (mg/g)	3.74 $\pm$ 0.28	4.66 $\pm$ 0.31*
β -AR:		
:Bmax (fmol/mg)	118.8 $\pm$ 16.4	80.5 $\pm$ 20.9*
:Kd (nM)	0.119 $\pm$ 0.051	0.130 $\pm$ 0.062
AC-assay		
:basal (pmol/mg/min)	6.4 $\pm$ 1.2	5.3 $\pm$ 1.0
:ISO + GTP (pmol/mg/min)	24.2 $\pm$ 5.8	19.7 $\pm$ 8.7
:Forskolin (pmol/mg/min)	144.3 $\pm$ 22.8	131.3 $\pm$ 40.0
:Mn ²⁺ (pmol/mg/min)	155.6 $\pm$ 10.8	130.9 $\pm$ 18.8

Values are mean $\pm$ SD; * $p < 0.05$; HR = heart rate; BP = blood pressure; VW = ventricular weight; V/B = ventricular to body weight ratio; β -AR: Bmax, b-adrenergic receptor density; β -AR: Kd = dissociation constant; AC-assay: basal = non-stimulated cAMP quantity; AC-assay: ISO + GTP = isoproterenol + GTP-stimulated cAMP quantity; AC-assay: forskolin = forskolin-stimulated cAMP quantity; AC-assay: Mn²⁺ = Mn²⁺-stimulated cAMP quantity.

Table VI. The Changes of NE Content and 5'-Nucleotidase Activity in the NE-Bitartrate (3.0 $\mu\text{g/kg/min}$) Stimulated 7 Days Hypertrophied Ventricles in 5-week-old SD Rats

	NE content (ng/g)	5'-nucleotidase activity ($\mu\text{mmol/mg/min}$)
Control	771.6 $\pm$ 429.2 (<i>n</i> = 9)	1.65 $\pm$ 0.74 (<i>n</i> = 8)
NE (3.0)	467.9 $\pm$ 119.1* (<i>n</i> = 7)	1.70 $\pm$ 0.57 (<i>n</i> = 7)

Values are mean $\pm$ SD; * $p < 0.05$.

Study 1-2: HR, BP and VW were not significantly affected by 14 days of treatment with NE (2.5 $\mu\text{g/kg/min}$) plus either an α -1 blocker (bunazosin) or a β -1 blocker (bisoprolol), but BW and therefore V/B ratios were significantly ($p < 0.05$) reduced compared to rats receiving NE plus 0.9% saline (Table IV).

Study 2: The administration of NE (3.0 $\mu\text{g/kg/min}$) for 7 days induced significant ventricular hypertrophy with decreased HR and increased BP (Table V). Histologically, there was no myocardial necrosis or interstitial fibrosis in the hypertrophied ventricles. The density and affinity of β adrenergic receptors was significantly ($p < 0.05$) decreased in the hypertrophied ventricles. There were no significant differences in the activity of adenylyl cyclase. NE content was significantly ($p < 0.05$) lower in the hypertrophied ventricles, but the differences between groups with respect to 5'-nucleotidase activities were not significant (Table VI).

DISCUSSION

When we discuss changes in the sympathetic nervous system with ventricular hypertrophy, we must also consider the dilution of receptors on membranes with hypertrophy. We measured the 5'-nucleotidase activity of crude membranes as membrane units. Because there were no significant differences between controls and hypertrophied ventricles, we ignored the effect of dilution in these studies. Fibroblasts contain more β -2 adrenergic receptors than do cardiomyocytes.¹⁶⁾ If the ventricles have necrotic areas, β -1 adrenergic receptors are underestimated. We examined the ventricles histologically and found no necrotic areas or interstitial fibrosis in hypertrophied ventricles.

The doses of bunazosin and bisoprolol used in the present experiment were determined from standard doses for humans in proportion to BW (Groups 2 and 4) and double that dose (Groups 3 and 5). Bunazosin and bisoprolol suppressed the body weight loss. The reason for this suppression seems to be through the extra-cardiac α - and β -adrenergic receptors. VW did not change significantly so an anti-hypertrophic effect of these drugs was not detected in the present experiments.

By the continuous i.v. infusion of NE for 3 days, the rapid development of cardiac hypertrophy seemed to be directly stimulated by myocardial α - and β -adrenoceptors rather than by hemodynamic changes.⁶⁾ In a conscious dog model, the administration of NE (0.5 μ g/kg/min for 28 days) caused a decreased HR, cardiac output and no BP change.⁸⁾ In our rat model, the NE HCl salt (2.5 μ g/kg/min, 12.2 nM/kg/min) administered for 14 days caused ventricular hypertrophy without HR and BP changes, and the NE tartate salt (3.0 μ g/kg/min, 9.4 nM/kg/min) for 7 days caused ventricular hypertrophy with decreased HR and increased BP. The difference between the two NE salts is thought to be due to their stability at room temperature; NE tartate salt is more stable at room temperature. BP and HR are thought to be concentration-dependent on NE, and increased BP is not considered to be indispensable for ventricular hypertrophy in this model.

NE has $\alpha > \beta$ -adrenergic stimulation and in the NE stimulated hypertrophied ventricle there was downregulation to at least the β -adrenergic receptor levels. NE concentrations in myocardium did not reflect the activity of the sympathetic nervous system in an aortic banding model.¹⁷⁾ In a pacing-induced dog heart failure model, the NE uptake of the myocardium decreased and interstitial NE concentration increased with increasing serum NE concentrations in the heart failure group.¹⁸⁾ Although NE concentrations in myocardium differ in the experimental models, in our model the decrease of NE content of myocardium seemed to reflect the disorder of ventricular sympathetic nervous system with

down regulation of the β adrenergic receptor systems. Kondo et al.¹⁹⁾ report that noradrenergic hyper-innervation in the subepicardium and myocardium of the ventricles of stroke-prone spontaneously hypertensive rats (SHRSP) may be a primary change of the heart before the onset of hypertension. This hyper-innervation of noradrenergic nerves is possibly thought to be a transient phenomenon between compensative and non-compensative cardiac hypertrophy.

β -adrenergic-mediated myocardial inotropic and chronotropic responsiveness reportedly decreases with aging.²⁰⁾ Others reported that the overall cardiovascular response to NE was unaffected by aging in SD rats.²¹⁾ In Fischer 344 rats, the decrease in myocardial beta-adrenergic responsiveness with aging was believed to be unrelated to delayed or incomplete recovery of β -adrenoceptor density after agonist-induced down-regulation.²²⁾ Tobise et al.²³⁾ reported that age-related changes in the activity of the myocardial β -adrenergic receptor-Gs-adenylyl cyclase pathway was not primarily regulated by alteration in the level of Gs or Gi but by the density of β -adrenergic receptors and by the activity of the catalyst adenylyl cyclase, and in particular the decrease in the steady-state mRNA content of the type VI isoform of adenylyl cyclase correlated with catalytic activity with age.

In our model, β -adrenergic receptor systems were attenuated with aging. The loading of NE caused cardiac hypertrophy in younger rats, but caused cardiac injury at lower doses in aged rats. Cardiac injury caused by NE was through activation of the α -adrenergic receptor system in a rabbit model.²⁴⁾ However, rat ventricular α -adrenoceptors, measured by specific binding of [³H]prazosin, was reported to decrease by 33% in rats aged from 3 to 24 months.²⁵⁾ According to the above results, the cardiotoxicity of NE to the aged rat was thought to be dependent on the changes of α and β adrenoceptors with aging. In cultured adult cardiomyocytes, NE caused an increase of intracellular cAMP and Ca²⁺ and decreased viability.²⁶⁾ The association between catecholamines and cardiotoxicity has previously been studied.²⁷⁻³¹⁾ The mechanisms are believed to be attributed to impaired intracellular Ca²⁺ handling, reduced coronary blood flow, and formation of highly cytotoxic free radicals derived from catecholamine auto-oxidation. In the aged rat, the cardiotoxicity of NE might be due to decreased activity of superoxide dismutase³²⁾ that scavenges cytotoxic free radicals. It seems worthwhile to investigate the cardioprotective effect of superoxide dismutase in these aged rats. The results of these studies indicate that NE caused myocardial injury more severely in older rats with decreasing β adrenergic function. The function of β adrenergic receptors is thought to play important roles in the protection of the myocardium against excessive NE.

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