Cardiac Norepinephrine Release: Modulation by Ovariectomy and Estrogen[1]

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Previously, we have demonstrated that in contrast to male rats, female rats do not show an age-related reduction of depolarization-elicited norepinephrine (NE) release from cardiac synaptosomes (resealed nerve terminals). These results suggest that sex hormones such as estrogen may modulate NE release from cardiac synaptosomes prepared from female rats. The present study was designed to test the hypotheses that long-term estrogen depletion, resulting from ovariectomy, and estrogen replacement alters depolarization-elicited NE release from cardiac synaptosomes. Female F344 rats were divided into two groups, one of which underwent bilateral ovariectomy, whereas the other underwent a sham operation. Three ovariectomized subgroups received daily injections of conjugated equine estrogens, Δ8,9-dehydroestrone or 17α-dihydroequilenin. Another ovariectomized control subgroup and the sham-operated animals received daily injections of vehicle. After 90 or 270 days of treatment, the animals were sacrificed. Cardiac synaptosomes were prepared from each heart, incubated with [^{3}H]-NE, and used to evaluate NE release capacity by exposure to 50 m*M* K^+. The effectiveness of the ovariectomy and the estrogenic actions of the test compounds was confirmed by evaluating vaginal smears, determining uterine weights, and measuring serum luteinizing hormone (LH) concentrations. Ovariectomy (after both 90 and 270 days) significantly increased depolarization-induced NE release compared with sham-operated rats. Treatment with all three estrogenic preparations reduced NE release in ovariectomized rats to values similar to those observed in sham-operated animals. Interestingly, NE release rates from rats treated with conjugated estrogens for 270 but not 90 days were significantly below that observed in age-matched sham animals. These results demonstrate that estrogen modulates depolarization-elicited NE release from cardiac nerve terminals. Such modulation may represent a protective action by estrogen at the cardiac synapse. Exp Biol Med 228:194–199, 2003

Key words: cardiac norepinephrine release; long-term estrogen replacement; cardiac synaptosomes; cardiovascular disease; ovariectomy

Over the past decade, clinical research has suggested that there are age-related gender differences in the incidences of cardiovascular disease (CD) (1, 2). Young women experience a lower incidence of CD compared with age-matched men. Whereas the incidence of CD increases gradually with age in men, it increases suddenly and rapidly during the climacteric in women such that in postmenopausal women, the incidence of CD equals that observed in age-matched men. This observation has led to the suggestion of a cardioprotective role for estrogen. Indeed, young women who undergo bilateral ovariectomy have a higher incidence of heart disease than their menstruating counterparts (2). Observational and clinical trials with estrogen replacement in menopausal women have shown cardioprotection (2–8). However, recent long-range clinical trials such as Heart and Estrogen Replacement Study (HERS) (9, 10), Estrogen Replacement and Arteriosclerosis (ERA) (11), and the ongoing Woman's Health Initiative (WHI) (12) have produced conflicting results concerning the long-term cardioprotective effects of combined hormone therapy (13). Nevertheless, the clinical study of estrogen use alone continues in the WHI long-term evaluation because provisional data is promising (13).

Both α- and β-estrogen receptors are expressed in male and female rodent hearts (14–16). These transcription regulators may either increase or decrease the amounts of specific proteins found in the heart. For example, decreased estrogen levels may increase the number of cardiac L-type calcium channels (17). In contrast, 17β-estradiol enhances the expression of estrogen receptors and other cardiac proteins (15, 18). In addition to these receptor-mediated effects, considerable evidence suggests that the cardiovascular protective effects of estrogen may also be mediated by receptor-independent mechanisms (19).

This work was supported by a basic research grant from Wyeth Ayerst Pharmaceuticals (to B.A.E.).

[1] This paper was presented, in part, at the American College of Cardiology, 49th Annual Scientific Session, March 12–15, 2000, Anaheim, CA.

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Received April 23, 2002.
Accepted October 16, 2002.

1535-3702/03/2282-0194$15.00

Normally, norepinephrine (NE) modulates heart rate and contractility. However, laboratory studies have shown that excess NE release can be responsible for hyperstimulation of the heart, which clinically leads to arrhythmias and coronary occlusion (20). Brain synaptosomes (resealed presynaptic nerve terminals) are a widely used model for the investigation of neurotransmitter release from brain nerve terminals (21). Similarly, we have developed the cardiac synaptosome and have demonstrated that as for brain synaptosomes, depolarization-elicited NE release from cardiac synaptosomes is a calcium-dependent process (22, 23). A major advantage in investigating neurotransmitter release from superfused synaptosomes compared with tissue slices is that reuptake is eliminated (21, 24). Without the confounding variable of reuptake, changes in NE overflow from brain and cardiac synaptosomes are solely due to changes in existing NE release. Thus, cardiac synaptosomes are well suited for the investigation of the physiological regulation of NE release from nerves within the heart (24).

Previously, we have demonstrated that in contrast to male rats, female rats do not show an age-related reduction in depolarization-elicited NE release from cardiac synaptosomes (25). Indeed, female rats exhibit a (nonsignificant) tendency for an age-dependent increase in NE release (25). These results suggest that sex hormones such as estrogen modulate NE release from female cardiac synaptosomes. The purpose of the present study was to determine the long-term effects of estrogen depletion (produced by ovariectomy) and estrogen replacement on depolarization-elicited NE release from cardiac synaptosomes.

Materials and Methods

Design. Sixty 3-month-old female F344 rats were divided into two groups, one of which underwent bilateral ovariectomy, whereas the other underwent a sham operation. Three ovariectomized subgroups received daily injections of conjugated equine estrogens (0.6 mg/kg; Premarin; Wyeth Ayerst Pharmaceuticals, Radnor, PA), Δ8,9-dehydroestrone (0.2 mg/kg), or 17α-dihydroequilenin (3 mg/kg) dissolved in vehicle (NaCl 0.9% with 1% Tween 80 at 0.1 ml/kg). The conjugated estrogen (CEE) used is the estrogen formulation most prescribed clinically for menopausal symptoms. The other estrogens used are components of the conjugated estrogen. The doses of estrogens were calculated from in-house rat data at the Wyeth Research Laboratories (M. Dey, personal communication). The choice was based on dose levels in rats that provide maximum bone protection, are capable of maintaining uterine weight, and retain expected body weights over the 90 and 270 days of the experimental treatment period. Another ovariectomized control subgroup and the sham-operated animals received daily injections of vehicle. After 90 or 270 days of treatment, the animals were sacrificed. The overall design and the number of rats in each subgroup are shown in Table I. All studies were approved by the MCP/Hahnemann College of Medicine (Drexel University) Institutional Animal Care and Use Committee.

Estrogen Effects. Estrogenic responses of the test compounds were monitored by evaluating vaginal smears, determining uterine weights, and measuring serum luteinizing hormone (LH) concentrations. Vaginal smears were taken twice weekly and before termination of the study. A moistened wisp of cotton was used and vaginal cells were obtained placed on slides. After being stained with a modified Papanicolau stain, they were read microscopically by a single investigator (B.A.E.) according to the methods for rodents described by Grollman (26). Particularly important was the cyclicity that continued in the sham-operated rats as opposed to the persistent estrus that occurred when estrogen treatment was given to ovariectomized rats. Uterine weights and blood were obtained at the time of sacrifice. Serum LH was measured using a rat single antibody radioimmunoassay test at the endocrine research laboratory of the Women's Health Research Institute (St. David's, PA).

Superfusion and [^{3}H]-NE Release from Cardiac Synaptosomes. After 90 (Group I) and 270 days (Group II) of treatment, the rats were sacrificed and cardiac synaptosomes (resealed nerve terminals) were prepared (as described below) from each heart to measure depolarization-elicited NE release.

Depolarization-induced release of [^{3}H]-NE from cardiac synaptosomes was performed as previously described (22–25). Briefly, synaptosomes were prepared by digesting minced heart tissue with collagenase (class II; Worthington Biochemical, Lakewood, NJ) followed by homogenization in 0.32 *M* sucrose. The homogenate was centrifuged for 10 min at 650*g* at 4°C, and the resulting supernatant was centrifuged for 20 min at 21,000*g* at 4°C to obtain the P2 frac-

Table I. Study Design

	Group I (90 days) $n = 28$	Group II (270 days) $n = 32$
Sham-operated + vehicle	$n = 5$	$n = 7$
Ovariectomized + vehicle (control)	$n = 6$	$n = 7$
Ovariectomized + conjugated estrogen	$n = 6$	$n = 6$
Ovariectomized + A[a]	$n = 5$	$n = 6$
Ovariectomized + D[b]	$n = 6$	$n = 6$

[a] A = 17α-Dihydroequilenin.
[b] D = Δ8,9-Dehydroestrone.

tion containing the functional cardiac synaptosomes (22). To load internal NE stores, the P_2 fraction was incubated in oxygenated HEPES buffer saline solution (50 m*M* HEPES, 144 m*M* NaCl, 5 m*M* KCl, 1.2 m*M* $CaCl_2$, 1.2 m*M* $MgCl_2$, and 10 m*M* glucose, pH 7.4) plus 300 n*M* [^{3}H]-NE (42 Ci/m*M*; NEN, Boston, MA) for 1 hr at 37°C (24). Aliquots of the [^{3}H]-NE-containing synaptosomes were placed into 300-μl chambers of a Brandel 12-chamber superfusion system (Biomedical Research and Development Laboratories, Gaithersburg, MD) and were perfused with oxygenated HEPES buffer saline at a rate of 250 μl/min. After a 45-min washout period during which unincorporated [^{3}H]-NE was removed, 5-min fractions were collected. Synaptosomes were depolarized with a 2-min pulse of HEPES buffer saline containing 50 m*M* KCl (the final concentration in the chamber). To terminate the experiment and to release the remaining [^{3}H]-NE, the synaptosomes were lysed by perfusion with water. The amount of [^{3}H]-NE in each 5-min fraction was determined by scintillation counting. As previously described (25), the fraction of the total [^{3}H]-NE released into each fraction was calculated. Furthermore, net fractional release of [^{3}H]-NE due to depolarization was calculated as the fractional release induced by the high K^+ buffer minus the basal fractional release.

Statistical analyses were done primarily using two-way analysis of variance (ANOVA) and by Student *t* tests. *Post hoc* calculations used Fisher's PLSD and Bonferroni/Dunn tests.

Results

Estrogen Effects. The results of three different measures (uterine weights, vaginal smears, and LH levels) were used to confirm the loss of estrogen after ovariectomy and to evaluate the effectiveness of the three estrogen preparations for restoring estrogen activity. These are tabulated in Table II. The vaginal smears of sham-operated rats demonstrated complete estrous cycles. The persistent diestrous condition observed in the ovariectomized controls indicated a lack of effective estrogenic activity. In contrast, persistent estrus was observed in rats treated with each of the three estrogenic preparations that showed that an estrogen effect on the vaginal mucosa (estrus) was present while the rat received the hormones.

As anticipated, uterine weights in vehicle-treated ovariectomized rats were significantly less ($P < 0.01$) than those from vehicle-treated sham operated controls at both 90 and 270 days of treatment (Table II). Treatment with conjugated equine estrogens or either of the two components of this preparation (Δ8,9-dehydroestrone or 17α-dihydroequilenin) significantly increased uterine weights ($P < 0.01$) compared with vehicle-treated ovariectomized rats at both 90 ($P < 0.01$) and 270 days ($P < 0.01$). There was no significant difference in uterine weights among the three groups receiving the different estrogenic formulations after either 90 or 270 days of treatment. However, the uterine weights of rats in each of the three subgroups receiving estrogenic formulations were significantly less than those observed in the sham rats ($P < 0.01$).

Elevated serum LH values indicated that in the ovariectomized control group, a condition of hypoestrinism existed; LH values at 90 and 270 days were 19.67 and 23.40 ng/ml, respectively. These LH levels were significantly greater ($P < 0.01$) than the values observed in all other groups. Treatment with all three estrogenic preparations reduced LH levels to values below those observed in sham-operated control rats. The large standard error observed in the sham-operated animals is most likely due to the animals being sacrificed at different stages of the estrous cycle. Accurate techniques for measurement of equine estrogen levels in rats are not available; however, the use of LH levels allows for relative assessment of estrogen level.

Synaptosomes. The results of the depolarization-induced release of [^{3}H]-NE from cardiac synaptosomes obtained from the rats in the various treatment groups are shown in Table III. After 90 days of treatment, depolarization-induced NE release was significantly ($P < 0.05$; 20%) higher in ovariectomized rats treated with vehicle compared with sham-operated rats. There was no significant differ-

Table II. Estrogen Action

	Uterine weights in grams ± SEM		Vaginal smears		Luteinizing hormone in ng/mL ± SEM	
	Group I (90 days)	Group II (270 days)	Group I (90 days)	Group II (270 days)	Group I (90 days)	Group II (270 days)
Sham-operated + vehicle	0.78 ± 0.14 a	0.73 ± 0.13 a′	C	C	4.61 ± 3.39 f	7.87 ± 4.76 f′
Ovariectomized vehicle (controls)	0.12 ± 0.01 b	0.13 ± 0.13 b′	D	D	19.67 ± 3.26 g	23.40 ± 4.09 g′
Ovariectomized conjugated estrogen	0.44 ± 0.02 c	0.58 ± 0.04 c′	E	E	1.00 ± 0.11 h	2.32 ± 0.35 h′
Ovariectomized + A[a]	0.47 ± 0.04 d	0.50 ± 0.01 d′	E	E	1.15 ± 0.26 i	4.18 ± 0.85 i′
Ovariectomized + D[b]	0.47 ± 0.07 e	0.58 ± 0.07 e′	E	E	1.02 ± 0.17 j	2.63 ± 0.25 j′

C, cyclic; D, diestrus persistently; E, estrus persistently; NS, not significant.
[a] A, 17α-Dihydroequilenin.
[b] D, Δ8,9-Dehydroestrone.
P values: a-a′, NS; b-b′, NS; c-c′, <0.01; d-d′, NS; e-e′, NS; a–b,c,d,e, < 0.01; b–a,c,d,e, < 0.01; a-b, < 0.01; a′,b′,c′,d′,e′, < 0.01; b′-a′,c′,d′,e′, < 0.01; a′-b′, < 0.01; f-f′, NS; g-g′, NS; h-h′, < 0.02; i-i′, < 0.01; j-j′, < 0.01; g-f,h,i,j, < 0.01; g′-f′,h′,i′,j′, < 0.01.

Table III. Norepinephrine Release[a]

	Group I (90 days) % ± SEM	Group II (270 days) % ± SEM
Sham-operated + vehicle	5.9 ± 0.6 a	8.17 ± 0.54 a'
Ovariectomized + vehicle	7.1 ± 0.3 b	10.24 ± 0.91 b'
Ovariectomized + conjugated estrogen	6.3 ± 0.3 c	7.04 ± 0.42 c'
Ovariectomized + A[b]	6.3 ± 0.3 d	8.53 ± 0.27 d'
Ovariectomized + D[c]	6.0 ± 0.3 e	8.87 ± 0.70 e'

P values: a-a' < 0.01; b-b' < 0.01; c-c' < .10 NS; d-d' < 0.01; e-e' < 0.01; a-b < 0.05; a-c,d NS; b-c,d,e NS; b-c,d,e NS; b-e < 0.05; a'-b' < 0.01; a'-c' < 0.01; a'-d',e' NS; c'-d' < 0.05; c'-e' < 0.05; b'-c' < 0.01; b'-d' < 0.05; b'-e' NS.
[a] Data presented are a percentage of net fractional release of NE due to K^+-stimulated depolarization.
[b] A, 17α-Dihydroequilenin.
[c] D, Δ8,9-Dehydroestrone.
NS, Nonsignificant.

ence between the sham-operated group and the three ovariectomized groups treated with estrogen, indicating that the 90 days of estrogen treatment prevented significant ovariectomized-induced elevation in NE release.

After 270 days, K^+-induced NE release in ovariectomized rats treated with vehicle was significantly ($P < 0.01$; 25%) higher than in sham-operated controls. Treatment with 17α-dihydroequilenin and Δ8,9 dehydroestrone in ovariectomized rats showed a reduced NE release at 270 days similar to that of sham-operated rats, and NE release from rats treated with conjugated estrogen was significantly ($P < 0.01$) below the sham level. Thus, in the ovariectomized rats given estrogen treatment, NE release was either maintained or reduced as compared with the sham level. At 90 days, there was no significant difference in release among the estrogen-treated rats, but at 270 days, a significant difference was seen between the single estrogens and the conjugated form. The changes seen appear to be either related to long-term treatment or aging.

Discussion

The major finding of this study is that estrogen deficiency by ovariectomy results in an increased depolarization-induced NE release from cardiac synaptosomes in young female rats. Additionally, when compared with the intact rat (sham), estrogen given to ovariectomized rats may provide maintenance or reduction of NE release. Treatment for 90 days (short-term) results in maintaining the NE release level, whereas 270 days (long-term) results in either maintaining or significantly reducing the NE release when compared with the sham control.

Estrogen deficiency, produced by ovariectomy, was verified using three different modalities. The presence of significantly decreased uterine weights, increased LH, and persistent diestrous in our vehicle-treated ovariectomized rats at both 90 and 270 days indicated a severely reduced estrogen level. These were compared with the sham-operated rats that showed normal estrogen activities and were having regular estrous cycles.

Decreased estrogen levels significantly contributed to an enhanced depolarization-induced NE release from cardiac synaptosomes after ovariectomy, at both 90 and 270 days. To test our hypothesis, three separate estrogenic preparations were given and the ability of these hormones to normalize NE release was observed. These compounds were conjugated equine estrogens (Premarin) and two active components of this preparation, Δ8,9-dehydroestrone and 17α-dihydroequilinen. Treatment with these estrogens reversed the effects of ovariectomy on uterine weights and LH levels and resulted in persistent estrus as seen by vaginal smears. More importantly, after 270 days of treatment, conjugated equine estrogens and 17α-dihydroequilenin significantly reduced depolarization-evoked NE release in ovariectomized animals. Conjugated equine estrogens treatment significantly reduced NE release below that observed in sham-operated control rats, whereas NE release from 17α-dihydroequilenin-treated animals was not significantly different from sham rats. A trend toward reduced NE release compared with sham animals was observed after 270 days of treatment with Δ8,9-dehydroestrone and after 90 days of treatment in all three preparations; however, these values did not reach the levels of significance. These results suggest that long-term treatment with conjugated equine estrogens or Δ8,9-dehydroestrone is required to significantly reverse the effects of ovariectomy. Additionally, treatment with conjugated estrogen was more effective in reversing the effects of ovariectomy on NE release than individual estrogen components that were used. Additional experiments using several doses of each compound are needed to establish this factor.

Interestingly, there was a significantly greater release of NE from the cardiac synaptosomes in the sham 270-day group compared with sham animals in the 90-day group. This increased NE release may be due to an age-related reduction in estrogen levels in the 270-day group (12 months of age) compared with the 90-day group (6 months of age). Such a reduction in estrogen levels is suggested by the trend toward higher LH concentrations in 270-day sham rats relative to the 90-day group sham animals.

The current experiments do not address the mechanism(s) by which estrogen reverses the effects of ovariectomy on NE release. A cogent factor may be through spe-

cific receptor activity. Both α- and β-estrogen receptors are well-known transcription factors that up- or downregulate the levels of the proteins involved in depolarization-mediated NE release. Additional dedicated experiments will be required to test this hypothesis.

Our findings are relevant to the results of a number of studies that have linked NE to cardiomyopathies (25–27). They are also consistent with studies that have linked excessive NE stimulation in the heart to arrhythmias and other potentially damaging effects due to increased oxygen demand and free radical formation (18, 28–33). In our studies, ovariectomy resulted in a 20%–25% increase in depolarization-induced NE release when compared with the sham groups ($P < 0.05$ at 90 days; $P < 0.01$ at 270 days), suggesting that *in vivo* the lack of estrogen results in hypersecretion of NE. This result of ovariectomy is corrected by estrogen replacement. Persistent and increasing hypersecretion of NE in the heart could account for effects seen in those clinical studies that link coronary heart disease and estrogen decline (2–4). Suppression of excess NE release would appear to furnish a cardioprotective effect in women who take estrogen replacement therapy during the menopause (34, 35). Given that coronary heart disease occurs later, women already on estrogen may continue therapy because even the HERS and ERA studies support this use (9, 10). The period of effectiveness may be limited; however, further clinical double-blind, placebo-controlled studies are in progress to evaluate long-term use of estrogen in cardiac diseases (13).

The results of this study in the rat shows that estrogen deficiency caused by ovariectomy produced a significant increase in NE release in the heart. Dependent on the specific estrogen compound that was given to the ovariectomized rats, an apparent response was the maintenance or significant reduction of NE release over time. This suppression by estrogens of increased NE in the heart may prevent NE-mediated pathophysiology or cardiotoxicity. Relatively long-term endogenous estrogen was needed to effect this moderation of NE release. Additional studies to provide a time line and the dose-response factors involved could support this hypothesis.

1. Chow M. Benefit/risk of estrogen therapy in cardiovascular disease: current knowledge and future challenges. J Clin Pharmacol **35:** 11S–17S, 1995.
2. Barrett-Connor E. Epidemiology and the menopause: a global overview. Int J Fertil Menopausal Study **38**(Suppl 1):6–14, 1993.
3. Barrett-Connor E. Postmenopausal estrogen and heart disease. Atherosclerosis **118:**S7–S10, 1995.
4. Grodstein F, Stampfer MJ, Colditz GA, Willett WC, Manson JE, Joffe M. Postmenopausal hormone therapy and mortality. N Engl J Med **336:**1769–1775, 1997.
5. Col NF, Eckman MH, Karas RH, Paukder SG, Goldberg RJ, Ross EM, Orr RK, Wong JB. Patient-specific decisions about hormone replacement therapy in post-menopausal women. J Am Med Assoc **277:**1140–1147, 1997.
6. Grodstein F, Stampfer M. The epidemiology of coronary heart disease and estrogen replacement in postmenopausal women. Prog Cardiovasc Dis **38:**199–210, 1995.
7. Martino M, Eskin BA. Estrogen and the postmenopausal heart. Female Patient **24:**19–28, 1999.
8. Subbiah MTR. Mechanisms of cardioprotection by estrogens. Proc Soc Exp Biol Med **217:**23–29, 1998.
9. Hulley S, Grady D, Bush T, Fineberg C, Herrington D, Riggs B, Vittinghoff E. Randomized trial of estrogen plus progestin for secondary prevention of coronary heart disease in postmenopausal women. J Am Med Assoc **280:**608–613, 1998.
10. Grady D, Herrington D, Bittner V, Blumenthal R, Davidson M, Hlatky M, Hsia J, Hulley S, Herd A, Khan S, Newby LK, Waters D, Vittinghoff E, Wenger N. HERS research group. Cardiovascular disease outcomes during 6.8 years of hormone therapy: heart and estrogen/progestin replacement study follow-up (HERS II). J Am Med Assoc **288:**49–57, 2002.
11. Herrington DM, Reboussin DM, Brosnihan KB, Sharp PC, Shumaker SA, Snyder TE, Furberg CD, Kowalchuk GJ, Stuckey TD, Rogers WJ, Givens DH, Waters D. Effects of estrogen replacement on the progression of coronary-artery atherosclerosis. N Engl J Med **343:**522–529, 2000.
12. The Women's Health Initiative Study Group. Design of the Women's Health Initiative clinical trial and observational study. Control Clin Trials **19:**61–109, 1998.
13. Writing Group for the Women's Health Initiative (WHI) Investigators. Risks and benefits of estrogen plus progestin in healthy postmenopausal women: principle results from WHI randomized controlled trial. J Am Med Assoc **288:**321–333, 2002.
14. Griffin M, Lee HW, Zhao L, Eghbali-Webb M. Gender-related differences in proliferative response of cardiac fibroblasts to hypoxia: effects of estrogen. Mol Cell Biochem **215:**21–30, 2000.
15. Grohe C, Kahlert S, Lobbert K, Stimpel M, Karas RH, Vetter H, Neyses L. Cardiac myocytes and fibroblasts contain functional estrogen receptors. FEBS Lett **416:**107–112, 1997.
16. Jankowski M, Rachelska G, Donghao W, McCann SM, Gutkowska J. Estrogen receptors activate atrial natriuretic peptide in the rat heart. Proc Natl Acad Sci U S A **98:**11765–11770, 2001.
17. Johnson BD, Zheng W, Korach KS, Scheuer T, Catterall WA, Rubanyi GM. Increased expression of the cardiac L-type calcium channel in estrogen receptor-deficient mice. J Gen Physiol **110:**135–140, 1997.
18. Nuedling S, Karas RH, Mendelsohn ME, Katzenellenbogen JA, Katzenellenbogen BS, Meyer R, Vetter H, Grohe C. Activation of estrogen receptor-β is a prerequisite for estrogen-dependent up-regulation of nitric oxide synthases in neonatal rat cardiac myocytes. FEBS Lett **502:**103–108, 2001.
19. Dubey RK, Jackson EK. Cardiovascular protective effects of 17β-estradiol metabolites. J Applied Physiol **91:**1868–1883, 2001.
20. Boddi M, Coppo M, Padeletti L, Michelucci A, Gensini GF, Poggesi L, Neri Serneri GG. Enhanced cardiac norepinephrine release in patients with idiopathic ventricular tachycardia related to the occurrence of arrhythmias. Am Heart J **127:**686–689, 1994.
21. Raiteri L, Raiteri M. Synaptosomes still viable after 25 years of superfusion. Neurochem Res **25:**1265–1274, 2000.
22. Aloyo VJ, McIlvain HB, Bhavsar VH, Roberts J. Characterization of norepinephrine accumulation by a crude synaptosomal-mitochondrial fraction isolated from rat heart. Life Sci **48:**1317–1324, 1991.
23. Snyder DL, Johnson MD, Aloyo V, Eskin B, Roberts J. Age-related changes in cardiac norepinephrine release: role of calcium movement. J Gerontol Biol Sci **50A:**B358–B367, 1995.
24. Snyder DL, Aloyo VJ, McIlvain HB, Johnson MD, Roberts J. Effect of age on potassium- and tyramine-induced release of norepinephrine from cardiac synaptosomes in male F344 rats. J Gerontol Biol Sci **47:**B190–B197, 1992.
25. Snyder DL, Johnson MD, Eskin BA, Wang W, Roberts J. Effect of age on cardiac norepinephrine release in the female rat. Aging Clin Exp Res **7:**210–217, 1995.
26. Grollman A. Essentials of Endocrinology (2nd ed). Philadelphia: JP Lippincott Company, pp507–509, 1947.
27. Opie LH, Walpoth B, Barsacchi R. Calcium and catecholamines: rel-

evance to cardiomyopathies and significance in therapeutic strategies. J Mol Cell Cardiol **17**(Suppl 2):21–34, 1985.
28. Downing SE, Lee JC. Contribution of α-adrenoceptor activation to the pathogenesis of norepinephrine cardiomyopathy. Circ Res **52**:471–478, 1983.
29. Rona G. Catecholamine cardiotoxicity. J Mol Cell Cardiol **17**:291–306, 1985.
30. Schomig A, Dart A, Dietz R, Mayer E, Kubler W. Release of endogenous catecholamines in the ischemic myocardium of the rat. Part A: locally mediated release. Circ Res **55**:689–701, 1984.
31. Schomig A, Haass M, Richardt G. Catecholamine release and arrhythmias in acute myocardial ischaemia. Eur Heart J **12**(Suppl F):38–47, 1991.
32. Richardt G, Waas W, Kranzhofer R, Cheng B, Lohse MJ, Schomig A. Interaction between the release of adenosine and noradrenaline during sympathetic stimulation: a feedback mechanism in the rat heart. J Mol Cell Cardiol **21**:269–277, 1989.
33. Singal PK, Kapur N, Dhillon KS, Beamish RE, Dhalla NS. Role of free radicals in catecholamine-induced cardiomyopathy. Can J Physiol Pharmacol **60**:1390–1397, 1982.
34. Esler M, Kaye D, Thompson J, Jennings G, Cox H, Turner A, Lambert G, Seals D. Effects of aging on epinephrine secretion and regional release of epinephrine from the human heart. J Clin Endocrin Metab **80**:435–442, 1995.
35. Eisenhoffer G, Esler M, Meredith I, Dart A, Cannon RO, Quyyumi A, Lambert G, Chin J, Jennings G, Goldstein D. Sympathetic nervous function in human heart as assessed by cardiac spillover of dihydroxyphenylglycol and norepinephrine. Circulation **85**:1775–1785, 1992.