Estrogen Alters Relative Contributions of Nitric Oxide and Cyclooxygenase Products to Endothelium-Dependent Vasodilation

JENNIFER CASE and CATHY A. DAVISON

Department of Pharmacology and Neuroscience, Albany Medical College, Albany, New York

Accepted for publication July 13, 1999 This paper is available online at http://www.jpet.org

ABSTRACT

The purpose of this study was to determine the effects of in vivo estrogen manipulations on mechanisms of endothelium-dependent vasodilation. Ovary-intact, ovariectomized (OVX), or OVX with estrogen replacement (OVX + E2) female Sprague-Dawley rats were studied (n = 8). Mesenteric arteries (~300 μm) were isolated, cannulated, and pressurized to 60 mm Hg in an arteriograph containing bicarbonate buffer and vessel diameter was monitored. Concentration-response curves to the endothelium-dependent histamine H1 agonist 2-thiazolylethylamine (2-TEA; 1 nM–100 μM) and to acetylcholine (1 nM–10 μM) were performed in preconstricted arteries. The effect of Nω-nitro-L-arginine (LNA; 100 μM) or LNA + indomethacin (INDO) (10 μM) on agonist-induced vasodilation was determined. There was no difference between treatment groups in the sensitivity of mesenteric arteries to 2-TEA or acetylcholine. LNA produced a significant decrease in sensitivity to 2-TEA in arteries from ovary-intact and OVX + E2 rats but not in those from OVX rats. The addition of INDO produced a small additional decrease in sensitivity to 2-TEA in arteries from ovary-intact rats, a significant decrease in OVX, and no shift in OVX + E2. LNA + INDO produced a similar degree of inhibition of the 2-TEA response in the three treatment groups. In contrast, when acetylcholine was used, the decrease in sensitivity produced by LNA or LNA + INDO was similar in the three rat groups. We conclude that estrogen increases the nitric oxide component of endothelium-dependent dilation and decreases the cyclooxygenase component. These effects of estrogen appear to be agonist-specific. Our findings suggest that estrogen modulates cross talk between the nitric oxide synthase and cyclooxygenase pathways of vasodilation.

Premenopausal women are known to have a lower incidence of cardiovascular disease compared with age-matched men (Kannel et al., 1976; Lerner and Kannel, 1986; Messerli et al., 1987). The gender difference in the incidence of cardiovascular disease persists until the onset of menopause. Menopause is associated with an increase in the incidence of cardiovascular disease in women and the abolition of the gender difference (Knopp et al., 1994). Postmenopausal women on estrogen replacement therapy experience a decrease in cardiovascular morbidity of ~50% compared with postmenopausal women not taking estrogen (Gruchow et al., 1988; Stampfer and Colditz, 1991). Taken together, this evidence suggests a role for estrogen in the observed gender difference for cardiovascular disease. The mechanism through which estrogen has this effect, however, has yet to be fully elucidated.

Recent evidence has suggested that estrogen may have direct beneficial effects on the vascular endothelium. Endothelial dysfunction in women coincides with the onset of menopause, suggesting a protective effect of estrogen on the vascular endothelium (Celermajer et al., 1994; Taddei et al., 1996; Pinto et al., 1997). Estrogen has been found to both potentiate the response to the endothelium-dependent vasodilator acetylcholine in rabbit femoral arteries (Gisclard et al., 1988) and increase endothelium-dependent flow-mediated dilation in postmenopausal women (Taddei et al., 1996). There is evidence to support the hypothesis that estrogen increases the production and release of nitric oxide (NO) from the endothelium. Estrogen appears to increase basal levels of NO in coronary arteries from female rats (Wellman et al., 1996). In addition, it has been found that circulating levels of the NO metabolites nitrite and nitrate are directly correlated with estrogen levels in women (Rosselli et al., 1995). Despite the evidence supporting the hypotheses that estrogen increases NO production and enhances endothelium-dependent vasodilation, there are few studies that link these two actions of estrogen. In addition, few studies have examined the effect of estrogen on the cyclooxygenase pathway of endothelium-dependent vasodilation. Our study is designed to...
examine the effect of estrogen on the relative contributions of NO and cyclooxygenase products to endothelium-dependent vasodilation.

Much of the experimental evidence demonstrating an effect of estrogen on NO production has been generated in vitro studies and acute estrogen administration. The role of estrogen in vivo, and its effect on the endothelium at physiological concentrations, is less well understood. The purpose of this study was 2-fold: 1) to determine whether 3-week manipulations of estrogen affect endothelium-dependent vasodilation in female Sprague-Dawley rats and 2) to establish the mechanism(s) by which in vivo estrogen influences endothelium-dependent vasodilation.

**Materials and Methods**

**Animals.** Female Sprague-Dawley rats, 13 weeks of age, were used in this study (Taconic Farms, Inc., Germantown, NY). Rats were housed in groups of two or three in temperature and humidity controlled, light-cycled (600–1800 h) quarters with ad libitum access to standard rat chow and water. All procedures involving the use of animals were approved by the Institutional Animal Care and Use Committee and conform to federal, state, and institutional guidelines.

Rats were separated into three treatment groups (n = 8 for each group): ovary-intact, ovariectomized (OVX), and ovariectomized followed by 10^<6> M EDTA, and 1.6 mM CaCl2·2H2O. With a dissecting microscope, two experiment was performed. 1996). The efficacy of ovariectomy and estrogen replacement was OVX rats has been demonstrated to restore plasma estrogen concentrations in female Sprague-Dawley rats and 2) to establish

**Preparation of Vascular Tissue.** Three to 4 weeks after surgery, at 16–17 weeks of age, the rats were weighed and euthanized with an overdose of sodium pentobarbital (120 mg/kg i.p.). The sides of the rats were shaved and cleaned, small incisions were made on each flank, and the ovaries were removed. Incisions were closed with wound clips. The OVX + E2 treatment group received a small incision in the back of the neck at the same time as the ovariectomy surgery into which a s.c. 17β-estradiol pellet (0.25 mg/pellet, 60-day continuous release; Innovative Research of America, Sarasota, FL) was placed. This protocol for estrogen replacement in OVX rats has been demonstrated to restore plasma estrogen concentrations to within the normal physiological range (Wellman et al., 1996). The efficacy of ovariectomy and estrogen replacement was confirmed by measuring uterus and body weights at the time the experiment was performed.

**Vasodilator Studies.** Vessels were preconstricted to 60% of their resting baseline diameter with phenylephrine. In the first vessel, a control cumulative concentration-response curve was performed to acetylcholine (10^<−6>–10^<−5> M) or to the selective histamine H1 agonist 2-thiazoylthylamine (2-TEA; 10^<−7>–10^<−2> M). The endothelium-dependent vasodilators acetylcholine and 2-TEA were chosen due to the fact that they produce graded dose-response curves and the vessels do not develop tachyphylaxis. 2-TEA curves were performed in the presence of the histamine H2 blocker tiotidine (1 μM). Each concentration of agonist was added only after the vessel had reached a plateau from the previous dose. The vessel was then rinsed with fresh PSS and allowed to reequilibrate for 30 min. The control dose-response curve to acetylcholine or 2-TEA was then repeated in the presence of either the NO synthase inhibitor L-N-nitro-arginine (LNA; 100 μM), or LNA plus the cyclooxygenase inhibitor indomethacin (INDO; 10 μM). In the second vessel the remaining intervention was tested, either LNA or LNA + INDO. No more than two curves were performed in each vessel; the order of the experiments was randomized. Preliminary studies confirmed that multiple dose-response curves could be reliably performed with both acetylcholine and 2-TEA.

Cumulative concentration-response curves also were performed to sperrmine NO complex (SPERNO, 10^<−6>–10^<−5> M) in phenylephrine preconstricted vessels. SPERNO curves were performed in the presence of LNA (100 μM) to block endogenous NO production.

**Pharmacological Characterization of 2-TEA Vasodilation.** To verify that the vasodilator response was endothelium-dependent and mediated by the histamine H2 receptor, some 2-TEA concentration-response curves were performed in endothelium-denuded vessels or in the presence of the histamine H1 antagonist pyrilamine (3 × 10^<−6> M). Endothelium denudation was accomplished by first rubbing the vessel lumen with a human hair and then passing air bubbles through the lumen (Osol et al., 1989; White et al., 1996). Denudation was verified by the absence of a vasodilator response to acetylcholine in a vessel preconstricted with phenylephrine.

**Drugs.** Acetylcholine, phenylephrine, pyrilamine, LNA, and INDO were all purchased from Sigma Chemical Company (St. Louis, MO). SPERNO was obtained from Research Biochemicals Inc. (Natick, MA). Tiotidine was purchased from Toecis (St. Louis, MO). Buffer reagents were purchased from Fisher Scientific (Pittsburgh, PA). 2-TEA was a generous gift from SmithKline Beecham (Harlow, Essex, UK). Stock solutions of acetylcholine (100 mM), phenylephrine (10 mM), LNA (10 mM), and SPERNO (10 mM) were all made in distilled water. Pyrilamine (0.3 mM) and 2-TEA (10 mM) stock solutions were prepared in buffer. Tiotidine stock solution (1 mM) was made in 0.1 N HCl and INDO stock (10 mM) was made in ethanol; for both drugs, further dilutions were made in water.

**Statistical Analysis.** All data are expressed as means ± S.E. Body weight, uterus weight, and uterus/body weight ratio were compared with one-way ANOVA (STATISTICA for Windows 4.0; StatSoft, Inc., Tulsa, OK). Individual comparisons were performed with Student-Newman-Keuls test. Relaxations are expressed as a percentage of the phenylephrine-constricted diameter. Data were analyzed with nonlinear regression of sigmoidal dose-response curves (GraphPad Prism 2.01), which was used to calculate the EC50 (concentration of agonist that elicited 50% of the maximum response), maximum response, and slope. The negative log EC50 values (pD2) were compared with repeated measures ANOVA (STATISTICA for Windows 4.0; StatSoft, Inc.). Individual comparisons were then performed with Student-Newman-Keuls test. For all comparisons, p < .05 was considered significant.

**Results**

**Efficacy of Ovariectomy and Estrogen Replacement.** Following ovariectomy, there was a significant increase in body weight relative to ovary-intact rats (p < .001), this
increase was absent when ovariectomy was accompanied by estrogen replacement ($p = .65$) (Table 1). Ovariectomy also resulted in a significant decrease in uterine weight relative to ovary-intact animals ($p = .004$), whereas OVX + E$_2$-treated rats showed a significant increase in uterine weight ($p = .008$). Normalizing uterine weight for body weight did not affect these statistical relationships (Table 1).

**Characterization of 2-TEA Vasodilator Response.** Mechanical denudation significantly attenuated 2-TEA-induced vasodilation (Fig. 1). There was a small amount of residual relaxation at the highest concentration of 2-TEA used. Pretreatment of endothelium-intact arteries with 0.3 μM pyrilamine, a selective histamine H$_1$ receptor antagonist, significantly inhibited 2-TEA-induced relaxation (data not shown). At a concentration of 3 μM, pyrilamine blocked the response to the same extent as endothelium denudation (Fig. 1).

**Effect of In Vivo Estrogen Manipulations on 2-TEA-Induced Vasodilation.** Cumulative concentration-response curves to 2-TEA in ovary-intact, OVX, and OVX + E$_2$ rats are shown in Fig. 2A. The baseline diameters of the arteries at the beginning of the experiment were 299 ± 15, 309 ± 8, and 308 ± 9 μm in ovary-intact, OVX, and OVX + E$_2$ animals, respectively (N.S., $p = .77$). The phenylephrine-constricted diameters were 178 ± 10, 185 ± 49, and 186 ± 8 μm, respectively (N.S., $p = .71$). In response to cumulative additions of 2-TEA, there were no statistically significant differences in the pD$_2$ values upon ovariectomy or ovariectomy with estrogen replacement relative to ovary-intact rats (N.S., $p = .18$) (see Fig. 2 legend for pD$_2$ values).

**Effect of In Vivo Estrogen Manipulations on Acetylcholine-Induced Vasodilation.** Cumulative concentration-response curves to acetylcholine in ovary-intact, OVX, and OVX + E$_2$ rats are shown in Fig. 2B. The baseline diameters of the arteries at the beginning of the experiment were 288 ± 11, 277 ± 7, and 289 ± 15 μm in ovary-intact, OVX, and OVX + E$_2$ animals, respectively (N.S., $p = .69$). The phenylephrine-constricted diameters were 188 ± 14, 171 ± 7, and 181 ± 8 μm, respectively (N.S., $p = .33$). In response to cumulative additions of acetylcholine, there were no statistically significant differences in the pD$_2$ values upon ovariectomy or ovariectomy with estrogen replacement relative to ovary-intact rats (N.S., $p = .67$) (see Fig. 2 legend and Table 2 for pD$_2$ values).

**Effect of In Vivo Estrogen Manipulations on Components of 2-TEA-Induced Vasodilation.** The components of the 2-TEA-induced vasodilation were examined by incubating the arteries with pharmacological inhibitors of endothelium-derived vasodilators. As shown in Fig. 3A, the NO synthase inhibitor NLA (100 μM) produced a statistically significant decrease in the sensitivity to 2-TEA (Fig. 3B, see legend for pD$_2$ values). In contrast to results in ovary-intact rats, incubation of arteries from OVX rats with NLA had no effect on the vasodilation induced by 2-TEA. In the presence of both NLA and indomethacin, however, there was a statistically significant decrease in the sensitivity to 2-TEA (Fig. 3B, see legend for pD$_2$ values). Finally, as shown in Fig. 3C, in the OVX + E$_2$ treatment group LNA resulted in a significant rightward shift in the 2-TEA-induced vasodilation. Incubation with both NLA and indomethacin did not produce any further shift than that caused by LNA alone (Fig. 3C, see legend for pD$_2$ values).

**Effect of In Vivo Estrogen Manipulations on the Components of Acetylcholine-Induced Vasodilation.** The components of the acetylcholine-induced vasodilation were examined by incubating the arteries with pharmacological inhibitors of endothelium-derived vasodilators. As shown in Table 2, the NO synthase inhibitor NLA (100 μM) produced a statistically significant decrease in the sensitivity to acetylcholine in all three treatment groups. The combination of both LNA and the cyclooxygenase inhibitor INDO (10 μM) produced an additional rightward shift in the curve (Fig. 3A, see legend for pD$_2$ values).

**TABLE 1**

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Body weight</th>
<th>Uterus weight</th>
<th>Uterus:Body Weight Ratio $\times 10^3$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ovary-intact (8)</td>
<td>216 ± 7</td>
<td>0.465 ± 0.070</td>
<td>2.17 ± 0.36</td>
</tr>
<tr>
<td>OVX (8)</td>
<td>276 ± 16$^a$</td>
<td>0.150 ± 0.067$^a$</td>
<td>0.581 ± 0.06$^a$</td>
</tr>
<tr>
<td>OVX/E$_2$ (8)</td>
<td>209 ± 7</td>
<td>0.749 ± 0.800$^a$</td>
<td>3.60 ± 0.42$^a$</td>
</tr>
</tbody>
</table>

Values are the means ± S.E. $^a$ Significant difference from ovary-intact group.

**Discussion.**

Women are known to have a lower incidence of cardiovascular disease relative to men, and estrogen has been suggested to play a role in this gender difference (Stamper and Colditz, 1991; Knopp et al., 1994). The Framingham study reported that postmenopausal women are twice as likely to
develop cardiovascular disease than premenopausal women of the same age, suggesting that the loss of estrogen is associated with an increased risk of cardiovascular disease (Kannel et al., 1976). Grodstein et al. (1996) found that the risk of cardiovascular disease in postmenopausal women was decreased by estrogen replacement therapy, thereby supporting a protective role for estrogen.

The mechanism of the cardioprotective effect of estrogen is thought to involve, in part, an increase in endothelium-dependent vasodilation (Gisclard et al., 1988; Taddei et al., 1995). Estrogen has been shown to effect NO production by transcriptional (Kleinert et al., 1998) and nontranscriptional (Hishikawa et al., 1998) mechanisms. Recently, investigators have begun to examine whether or not estrogen affects the enzyme cyclooxygenase and the release of cyclooxygenase-derived vasodilators and constrictors from the endothelium. Estrogen has been found to inhibit cyclooxygenase-2 mRNA expression in cultured bovine chondrocytes (Morisset et al., 1998) and to up-regulate cyclooxygenase-1 mRNA as well as prostacyclin production in ovine fetal pulmonary artery endothelium (Jun et al., 1998). Myers et al. (1999) have reported that in vivo estrogen manipulations with LNA alone and in combination with INDO on sensitivity to acetylcholine.

TABLE 2
Effect of LNA alone and in combination with INDO on sensitivity to acetylcholine

<table>
<thead>
<tr>
<th>Rat Group</th>
<th>Control</th>
<th>LNA, 100 μM</th>
<th>LNA, 100 μM and INDO, 10 μM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ovary-intact (7)</td>
<td>6.87 ± 0.10</td>
<td>6.38 ± 0.10</td>
<td>6.00 ± 0.13a</td>
</tr>
<tr>
<td>OVX (8)</td>
<td>6.76 ± 0.11</td>
<td>6.34 ± 0.06</td>
<td>6.05 ± 0.08b</td>
</tr>
<tr>
<td>OVX/E2 (8)</td>
<td>6.88 ± 0.10</td>
<td>6.37 ± 0.12</td>
<td>6.05 ± 0.19b</td>
</tr>
</tbody>
</table>

Values are the means ± S.E. of pD2 (-log EC50) for acetylcholine in mesenteric arteries from ovary-intact, OVX, and OVX/E2. *p* values for each group are given parenthetically.
a Significant difference compared with control.
b Significant difference compared with LNA.

Age is a risk factor for the development of cardiovascular disease. Advancing age is associated with an impairment of vascular endothelium function (Chauhan et al., 1996; Gerhard et al., 1996). Celermajer et al. (1994) reported a gender difference in endothelium dysfunction, with men developing a decrease in endothelium-dependent vasodilation a decade before women. Women do not exhibit a decrease in endothelial function until 50 years of age, which is the average age of menopause onset. These findings suggest that estrogen may maintain the vascular endothelium.

Estrogen replacement therapy in postmenopausal women has been found to: 1) improve endothelium-dependent vasodilation (Arora et al., 1998; Bush et al., 1998) and 2) produce an increase in serum nitrite and nitrate levels, the metabolites of NO (Rosselli et al., 1995). These clinical findings are supported by experimental studies in rats in which: 1) estrogen suppressed pressure-induced constriction of coronary arteries by stimulating endothelium-derived NO (Wellman et al., 1996), and 2) estrogen increased flow-induced vasodilation in gracilis arteries (Huang et al., 1998). Furthermore, it has been found that incubation of human aortic endothelial cells with 17-estradiol resulted in an increase in both NO synthase protein and NO production (Hishikawa et al., 1995). Estrogen has been shown to effect NO production by transcriptional (Kleinert et al., 1998) and nontranscriptional (Caulin-Glaser et al., 1997) mechanisms.
Histamine is known to cause endothelium-dependent vasodilation by stimulating the histamine H1 receptor (Toda, 1988; Kelm et al., 1993). In addition, histamine can cause vasodilation by acting at the H2 receptors on vascular smooth muscle. We chose 2-TEA because of its high degree of selectivity for H1 receptors. Our experiments verified that 2-TEA causes vasodilation by stimulating a histamine H1 receptor on the vascular endothelium. Results with 2-TEA were compared with the prototypical endothelium-dependent vasodilator acetylcholine.

In vivo estrogen manipulations were accomplished through ovariectomy and ovariectomy + estrogen replacement. As expected, after ovariectomy there was an increase in body weight (Shimizu et al., 1990; Albert et al., 1991) and a decrease in uterine weight (Westerlind et al., 1998; Sato et al., 1998) relative to ovary-intact rats. In the OVX+E2 rats, body weight was decreased back to control levels, whereas uterine weight was significantly higher than control. The increased uterine weight suggests that in the estrogen-replacement treatment group, the plasma concentrations of estrogen were higher than those normally found physiologically.

We found no differences in the sensitivity (pD2 values) to 2-TEA in arteries from ovary-intact, OVX, and OVX+E2 rats. Our findings, however, indicate that although the overall vasodilation is not altered, estrogen increases the NO component and decreases the cyclooxygenase component of 2-TEA-induced vasodilation. These results with 2-TEA are in contrast to our findings with acetylcholine. Like with 2-TEA, there were no differences in the sensitivity to acetylcholine in arteries from ovary-intact, OVX, and OVX+E2 rats. However, although both NO and cyclooxygenase products are involved in acetylcholine-induced vasodilation, in vivo estrogen manipulations do not appear to alter the relative contri-

Fig. 3. Effect of LNA and LNA + INDO on 2-TEA-induced vasodilation in ovary-intact, OVX, and OVX + E2 rats. A, arteries were significantly less sensitive to 2-TEA in the presence of LNA compared with the control response in ovary-intact rats (pD2 = 4.58 ± 0.15 and 4.93 ± 0.08, respectively; p = .023). LNA + INDO produced an additional decrease in the sensitivity to 2-TEA; pD2 = 4.29 ± 0.14 (p = .055 versus LNA alone). In OVX rats (B), LNA had no effect on the pD2 relative to the control (4.78 ± 0.08 and 4.69 ± 0.12, respectively; p = .473). LNA + INDO produced a significant decrease in the sensitivity to 2-TEA; pD2 = 4.12 ± 0.08 (p = .0007 versus LNA alone). In OVX + E2 rats (C), LNA produced a significant decrease in the pD2 relative to the control; pD2 values = 4.99 ± 0.08 for control and 4.42 ± 0.08 in the presence of LNA (p = .0003). The pD2 value in the presence of LNA + INDO (4.12 ± 0.08) was not significantly different from that in the presence of LNA alone (p = .23). Number in parentheses, number of rats in each group. Values represent means ± S.E.
butions of these endothelium-derived vasodilators to acetylcholine-induced vasodilation. Based on these results, the ability of estrogen to modulate the relative contributions of endothelium-derived vasodilators is an agonist-specific effect, characteristic of 2-TEA but not acetylcholine.

Our results support the notion of cross-talk between the NO and cyclooxygenase pathways in 2-TEA-induced vasodilation. In the ovariectomized animals, there was a balance between the NO and cyclooxygenase product contributions to 2-TEA-induced vasodilation. In the ovariectomized animals, the loss of estrogen was associated with a decrease in the NO component of the 2-TEA-induced vasodilation and an up-regulation of the cyclooxygenase component. At the supraphysiological estrogen levels in the ovariectomized + estrogen rats, there is an apparent up-regulation of the NO component of the 2-TEA-induced relaxation and a concomitant depression of the cyclooxygenase-product component relative to controls in all three treatment groups, the overall effect of inhibiting both NO synthase and cyclooxygenase was the same. It is possible that NO exerts a negative feedback on cyclooxygenase; therefore, in the absence of estrogen, NO levels fall and there is a compensatory rise in cyclooxygenase.

Our findings corroborate reports in the literature that suggest an interaction between the NO synthase and cyclooxygenase pathways of vasodilation. In the bovine aorta, it was found that high levels of NO inhibited the release of prostacyclin (Doni et al., 1988). In studies with murine J774.2 macrophages, sodium nitroprusside inhibited prostaglandin release, whereas the NO synthase inhibitor A$N^+$-monomethyl-l-arginine enhanced prostaglandin release (Swierkosz et al., 1995). Fernandez et al. (1996) have reported a compensatory increase in NO levels in male rats with portal hypertension (1995). Fernandez et al. (1996) have reported a compensatory interaction between the NO and cyclooxygenase. In contrast to the above reports of a dose-response curves to the NO donor SPERNO. Because there were no differences in the sensitivity to SPERNO with estrogen manipulations, differences in the effect of LNA on 2-TEA responses appear to be due to estrogen effects on synthesis rather than smooth muscle sensitivity to NO.

We conclude that estrogen does not alter the ability of 2-TEA or acetylcholine to induce endothelium-dependent vasodilation in small mesenteric arteries from the rat. Estrogen does, however, enhance the NO component and decrease the cyclooxygenase component of 2-TEA-induced vasodilation. This effect of estrogen is agonist-specific because the same results were not found with acetylcholine. Our findings suggest that estrogen modulates the cross-talk between NO synthase and cyclooxygenase pathways of 2-TEA-induced vasodilation.

Acknowledgments

We acknowledge the excellent technical assistance of Carlos O. Rivera. 2-TEA was a generous gift from SmithKline Beecham.

References


Morisset S, Patry C, Lora M and de Brun-Fandernes AJ (1998) Regulation of


Send reprint requests to: Cathy A. Davison, Ph.D., Department of Pharmacology and Neuroscience, Albany Medical College, 47 New Scotland Ave., Albany, NY 12208. E-mail: Cathy_Davison@ccgateway.amc.edu