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Gender Differences in the Expression of Endothelin Receptors in Human Saphenous Veins *In Vitro*[1]

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ABSTRACT

The contractile response to endothelin-1 (ET-1) appears to be modulated by the relative density of ET_A and ET_B receptors. To determine the effects of gender on the distribution of ET receptors, we analyzed the endothelin receptor subtypes on membrane fractions prepared from saphenous vein samples obtained from patients of different genders undergoing coronary artery bypass graft surgery. The contractile response to ET-1 in the presence and absence of 1 μM of the ET_A receptor antagonist BQ-123 was also investigated. Similar studies were repeated with endothelium-denuded samples to study the role of endothelium- and smooth muscle-derived ET_B receptors. Competitive binding experiments were performed on membrane fractions using [^{125}I]ET-1 and unlabeled ligands ET-1, ET-3, sarafatoxin 6c and BQ-123. Analysis of the binding data with endothelium-intact samples yielded two classes of binding sites in both women and men. In women, the maximum binding capacities were 83 $\pm$ 6 and 97 $\pm$ 10 fmol/mg protein for ET_A and ET_B receptors, respectively; the corresponding values in men were 618 $\pm$ 121 and 201 $\pm$ 10 fmol/mg protein. In addition, ET-1-induced contractions were 2-fold greater in men than in women at high ET-1 concentrations. Competitive binding studies with endothelium-denuded saphenous veins demonstrated the presence of only ET_A receptors in both female and male tissue. These results indicate that the ratio and the density of ET receptors are different in men and women, which might be an important factor in the regulation of the contractile response.

ETs constitute a family of peptides with extremely potent vasoconstrictor actions (Yanagisawa *et al*., 1988; Inoue *et al*., 1989). ET-1, the major isoform in the vascular endothelium, is generated in two steps from a 203-amino acid residue precursor, preproendothelin-1. In addition to its vasocontractile action, ET-1 has been shown to enhance mitogenesis in various cell lines such as vascular (Komura *et al*., 1988) and airway (Glassberg *et al*., 1994) smooth muscle cells and fibroblasts (Takuwa *et al*., 1989) and to stimulate release of endothelium-derived relaxing factors and prostaglandin (DeNucci *et al*., 1988).

The mature peptide mediates its effects *via* two distinct G protein-coupled receptor subtypes. The ET_A receptor subtype binds ET-1 and ET-2 with higher affinity than ET-3 and S6c (Arai *et al*., 1990; Takasuka *et al*., 1993). The ET_B receptor subtype displays similar affinities for all ET isoforms and S6c (Sakurai *et al*., 1990; Williams *et al*., 1991). Both receptors are distributed in various tissues and cells in different proportions. ET_A receptors, localized mainly on smooth muscle cells of blood vessels, are believed to be involved in the vasocontractile response to ET-1 (Masaki, 1995). The ability of vascular smooth muscle cells to produce bioactive ET-1 suggested that ET-1 might be involved in the contraction and growth of these cells in a paracrine and autocrine fashion (Hahn *et al*., 1990; Kanse *et al*., 1991). The role of ET_B receptors in smooth muscle contraction is more complex. For instance, ET_B receptors located on endothelial cells mediate vasodilation *via* the release of relaxing factors. This receptor subtype can also exert vasoconstriction when located on the smooth muscle cells (Masaki, 1995). However, it is believed that ET_A receptors are the major receptor subtype involved in the vasoconstriction induced by ET-1 (Davenport and Maguire, 1994; Rubanyi and Polokoff, 1994; Masaki, 1995; Goto *et al*., 1996; Webb and Meek, 1997). Thus, the net contractile effect of ET-1 depends mainly on the relative density of ET_A receptors on smooth muscle cells and of ET_B receptors on endothelial cells. Because receptor subtype-specific and nonspecific antagonists are available, characterization of receptor subtypes and their contribution to vascular reactivity becomes quite important in determining the potential use of an ET receptor antagonist as a therapeutic agent.

Gender differences in the development of cardiovascular diseases have been recognized in numerous epidemiological studies. For instance, men are more susceptible to coronary

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ABBREVIATIONS: ET, endothelin; S6c, sarafatoxin 6c.

artery disease than women, whereas the incidence of vascular disorders such as primary pulmonary hypertension and migraine headache is higher in women (White *et al*., 1995). However, the mechanisms of these gender differences have not yet been elucidated. Recently, we reported that plasma ET-1 levels were elevated in black hypertensive women and men compared with white hypertensive patients (Ergul *et al*., 1996), but there is no information on the effects of gender on ET receptors and on the receptor subtype distribution. In this study, we investigated the presence and function of ET_A and ET_B receptors on both endothelium-intact and endothelium-denuded human saphenous veins obtained from patients undergoing coronary artery bypass graft surgery.

Methods

Materials. Synthetic ET-1 and ET-3 were generous gifts of Dr. Marc E. Freeman (Department of Biological Science, Florida State University, Tallahassee, FL). BQ-123 and S6c were obtained from Peninsula Laboratories (Belmont, CA). [^{125}I]ET-1 (2200 Ci/mmol) was from New England Nuclear Research Products (Boston, MA).

Subjects. Saphenous veins were obtained from female (ages 55–69 years) and male (ages 51–83 years) patients undergoing bypass surgery. Only postmenopausal women who were not receiving estrogen replacement therapy were included in this study. Fresh tissues were used in the vascular reactivity experiments. Binding experiments were conducted with membrane fractions prepared from both fresh and frozen samples (stored at −125°C), and no difference was observed. Receptor binding and vascular reactivity assays were performed with both endothelium-intact and endothelium-denuded saphenous veins. For studies with endothelium-denuded samples, the endothelium was removed gently by rubbing the internal surface of the veins with forceps, and the removal of endothelium was confirmed by testing the rings with acetylcholine because it induces an endothelium-dependent vasorelaxation.

Preparation of membranes for receptor binding experiments. The tissue was weighed and then frozen in liquid nitrogen. Next, it was pulverized and homogenized on ice in 50 mM Tris-HCl/0.25 M sucrose buffer, pH 7.5, containing 1 mM EDTA, 1–2 μg/ml aprotinin and 100 μg/ml phenylmethylsulfonyl fluoride. For every 100 mg of tissue, 2 ml of buffer was used. The homogenate was centrifuged at 1200 $\times$ *g* for 20 min at 4°C, and the supernatant was transferred to 15-ml tubes and centrifuged at 30,000 $\times$ *g* for 30 min. The pellet was resuspended in 0.5 ml of 50 mM Tris-HCl, pH 7.5. The protein content in the membrane preparation was measured using the BCA Protein Assay Kit from Pierce (Rockford, IL).

Receptor binding experiments. Two types of binding experiments were performed with the membrane fractions: (1) saturation binding and (2) competitive binding assays as described by Ergul *et al*. (1995). Briefly, for the saturation binding experiments, 30 μg of membrane protein was incubated with 25 to 500 pM [^{125}I]ET-1 in 0.5 ml of binding buffer [Hanks' balanced salt solution supplemented with 0.1% (w/v) bovine serum albumin] for 2 hr at 37°C in a shaking water bath. The nonspecific binding was determined in the presence of excess unlabeled ET-1 (1 μM) at each concentration of the radiolabeled ligand. Due to the high nonspecific binding, radiolabeled ligand concentrations of >500 pM could not be used in the saturation binding experiments. For the competitive binding experiments, 30 μg of membrane protein was incubated with 100 pM [^{125}I]ET-1 in the presence of various concentrations (1 pM to 1 μM) of the unlabeled ligands, ET-1, ET-3, BQ-123 and S6c, for 2 hr at 37°C. The nonspecific binding was determined in the presence of excess (2 μM) unlabeled ligand ET-1, ET-3, BQ-123 or S6c, and similar counts were obtained with each ligand. At the end of the incubation period, the membrane fraction was centrifuged, and the pellet was rinsed twice with ice-cold Hanks' balanced salt solution/bovine serum albumin. The pellet was then solubilized with 1 N NaOH, and the membrane-bound radioactivity measured using a Wallac 1470 Wizard gamma counter. Both the saturation and competitive binding data were analyzed with Prism and InPlot programs (GraphPAD Software, San Diego, CA). The IC_{50} value of the specific binding and B_{max} values obtained from competitive binding experiments with membranes from endothelium-intact and endothelium-denuded tissue are given as mean ± S.E.M. of five and three independent experiments, respectively. The data were analyzed for the presence of one or two classes of binding sites, and the best fit was chosen with statistical significance of $P < .05$. The total number of ET_A and ET_B receptors was calculated as the mean ± S.E.M. of B_{max} values determined with each unlabeled ligand.

Vascular reactivity. The tissues were kept in chilled oxygenated Kreb's buffer (4.6 mM KCl, 2.5 mM $CaCl_2$, 1.2 mM KH_2PO_4, 118 mM NaCl, 2.5 mM $NaCHO_3$ and 11 mM dextrose). After the fat tissue was carefully removed, the saphenous veins (with or without endothelium) were cut into 2- to 4-mm rings and mounted at the optimal diastolic tension (2 $\times$ *g*) in 5-ml tissue baths containing oxygenated Kreb's buffer maintained at pH 7.4 and 37°C. Isometric contractions were recorded with a Grass model 7B polygraph (Quincy, MA). The rings were allowed to equilibrate for 1 hr and were sequentially treated with 70 mM KCl to determine the viability of the rings. Then, KCl-contracted rings were challenged with 1 μM acetylcholine, and an endothelium-mediated vasorelaxation (44%) was observed. There was no difference between the relaxation observed in female and male subjects. After the rings were washed with Kreb's buffer, the dose-response curve was generated using various concentrations of ET-1 (0.25–750 nM), and the contractile response obtained with each concentration of ET-1 was expressed as the percentage of the maximal KCl response. To identify the receptor subtype on the saphenous veins, the dose-response curves were repeated in the presence of 1 μM BQ-123, an ET_A receptor antagonist. The data are given as a mean ± S.E.M. of at least six or three independent experiments with endothelium-intact and endothelium-denuded tissue, respectively. EC_{50} values for vascular reactivity experiments were calculated as described previously (Fleming *et al*., 1972).

Statistical analysis. All results are expressed as a mean ± S.E.M.. The binding (B_{max} and K_d values) and contractility data obtained from female and male patients were evaluated statistically using the unpaired Student's *t* test. In addition, individual binding and contractility experiments were analyzed by repeated measures analysis of variance. With both methods, a value of $P < .05$ was considered significant.

Results

Receptor subtypes on human saphenous veins. To determine the extent of individual variations of the ET-1 binding to human saphenous veins, saturation binding experiments were performed initially. Membranes prepared from male and female patient samples were individually assayed with little variation noted between samples from different patients of the same gender. Thus, the results from the analysis of the binding data for each patient sample are given as the mean ± S.E.M. (fig. 1 and table 1). The findings indicated that although there was a difference in B_{max} values in samples from women and men (***$P < .001$), the K_d values were independent of gender.

Competition experiments between [^{125}I]ET-1 and ET-1 were conducted to compare the K_d and B_{max} values obtained using the two different binding methods. As shown in table 1, the K_d values were in reasonable agreement with those obtained using saturation binding, and the B_{max} values for men were comparable. Because competitive binding experiments using different unlabeled competing ligands, such as ET-1,

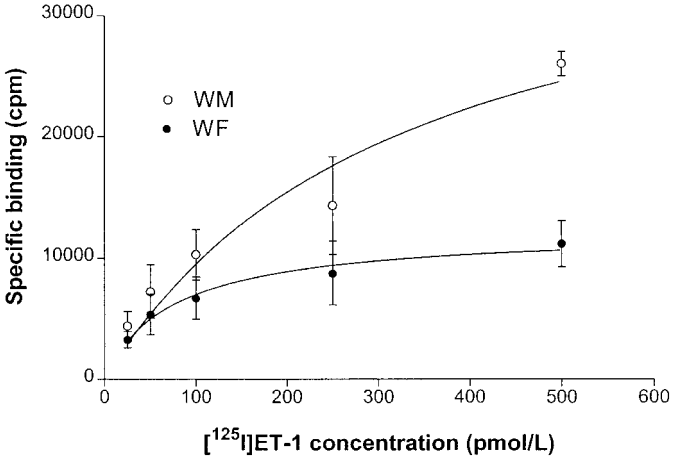

Fig. 1. Saturation (specific) binding of [^{125}I]ET-1 to the membrane fractions prepared from endothelium-intact saphenous veins of female (●) and male (○) patients. Membrane fractions were incubated with various concentrations of [^{125}I]ET-1 (10–500 pM) in the presence and absence of unlabeled ET-1 for 2 hr at 37°C, and membrane-bound radioactivity was measured. Each value represents the mean ± S.E.M. specific binding (cpm) obtained from six individuals.

TABLE 1

The analysis of saturation binding (n = 6) and competitive binding (n = 6) experiments performed with membranes from individual endothelium-intact samples

Saturation binding	K_d	B_{max}
	nM	*fmol/mg protein*
Women	0.1 ± 0.04	122 ± 23
Men	0.3 ± 0.08	402 ± 108
Competitive binding	K_i	
	nM	
Women	0.3 ± 0.1	113 ± 6
Men	0.8 ± 0.2	491 ± 31

ET-3, the ET_A receptor antagonist BQ-123 and the ET_B-selective agonist S6c, are more useful in identifying receptor subtypes, these assays were also performed. However, the amount of membrane obtained from each patient was limited for this type of study. Based on the saturation binding results indicating that there was no significant difference in ET-1 binding characteristics among individual patients within each gender group, membrane fractions were randomly pooled and competitive binding experiments were conducted with each pool (n = 5 and n = 3 for endothelium intact and endothelium-denuded samples, respectively).

The competition curves obtained using samples from men are shown in figure 2A. ET-1 displaced the ligand in a monophasic manner with apparent IC_{50} and B_{max} values of 0.5 ± 0.1 nM and 696 ± 75 fmol/mg protein, respectively. BQ-123 displaced only 70% of the radiolabeled ET-1 at the highest concentration used (1 μM), indicating that the remaining 30% was of the ET_B receptor subtype. Data from displacement with ET-3 and S6c provided further evidence for the presence of high and low affinity binding sites (fig. 2A). The IC_{50} value for the low affinity binding site (80 nM) was several hundred-fold greater than that of ET-1, and the IC_{50} of the higher affinity binding site was equal to that of ET-1 (0.1 nM), as expected for the ET_A and ET_B receptor subtypes, respectively. Displacement with S6c also yielded

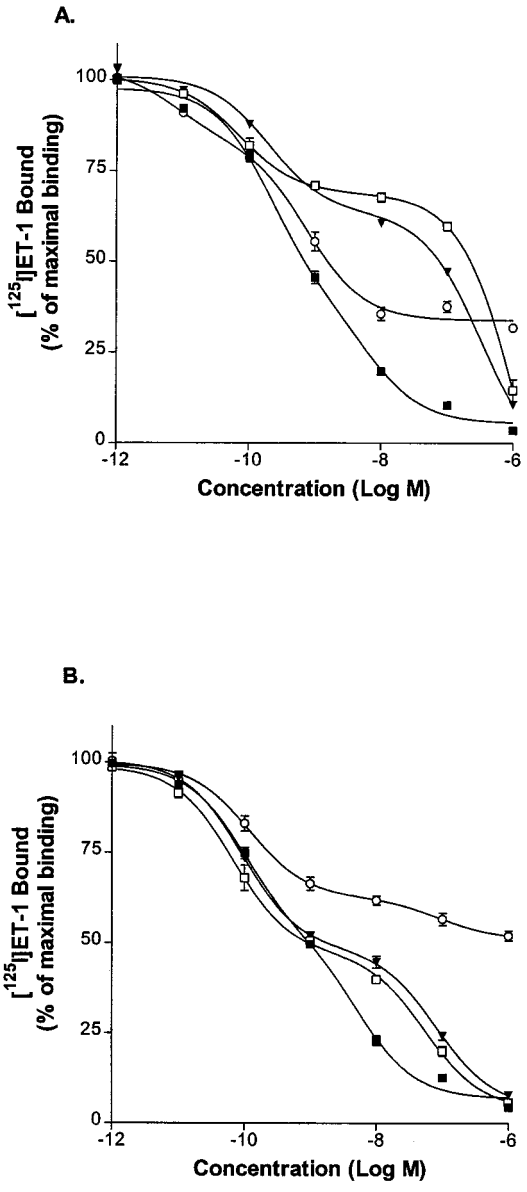

Fig. 2. Competition of the specific binding of [^{125}I]ET-1 with the pooled membrane fractions obtained from endothelium-intact saphenous veins of male (A) and female (B) patients. Membranes were incubated with 100 pM [^{125}I]ET-1 and various concentrations of the unlabeled ligands (1 pM to 1 μM) ET-1 (■), ET-3 (□), S6c (▼) and BQ-123 (○) for 2 hr at 37°C, and membrane-bound radioactivity was measured. Each value represents the mean ± S.E.M. from at least five independent experiments.

one class of high affinity binding sites with an IC_{50} value of 0.1 nM and one class of lower affinity binding sites with an IC_{50} of 82 nM, indicating the presence of ET_A and ET_B receptors, respectively. The B_{max} values suggest that the ET_A receptor subtype constitutes ~70% of the receptor population, with the remaining 30% being of the ET_B subtype. Because the age of the patients ranged between 51 and 83 years, we also analyzed whether age would be a contributing factor to receptor density. B_{max} values did not differ significantly ($P < .09$) in this limited sampling between group 1 (ages 51–66 years, n = 3) and group 2 (ages 68–83 years, n = 3).

As shown in figure 2B and table 2, the competitive binding experiments with endothelium-intact samples from women

TABLE 2
Summary of the competitive binding experiments with pooled samples

	ET-1		ET-3		BQ-123		S6c	
	IC_{50}	B_{max}	IC_{50}	B_{max}	IC_{50}	B_{max}	IC_{50}	B_{max}
	nM	*fmol/mg*	*nM*	*fmol/mg*	*nM*	*fmol/mg*	*nM*	*fmol/mg*
Endothelium-intact tissue								
Women	0.3 ± 0.1	159 ± 8[a]	0.1 ± 0.02	100 ± 5[a]	0.2 ± 0.04	85 ± 6[a]	0.1 ± 0.01	93 ± 5[a]
			51 ± 10	81 ± 5[a]			67 ± 6	84 ± 4[a]
Men	0.5 ± 0.1	696 ± 75[a]	0.1 ± 0.01	200 ± 28[a]	0.2 ± 0.1	542 ± 34[a]	0.1 ± 0.02	202 ± 16[a]
			80 ± 14	739 ± 233[a]			82 ± 46	497 ± 52[a]
Endothelium-denuded tissue								
Women	0.7 ± 0.1	77 ± 4[a]	26 ± 4	75 ± 3[a]	1.2 ± 0.3	81 ± 1.5[a]	37 ± 6	79 ± 7[a]
Men	0.7 ± 0.1	566 ± 10[a]	33 ± 10	706 ± 25[a]	1 ± 0.1	605 ± 19[a]	20 ± 4	525 ± 13[a]

[a] The B_{max} values obtained from female and male saphenous veins with each unlabeled ligand were significantly different ($P < .001$).

yielded similar IC_{50} values; however, the total number of binding sites were different (***$P < .001$). The total number of receptors was less than that found in men, and the ET_A:ET_B ratio was 50:50 in comparison to 75:25 in men (table 2).

Similar experiments were performed using membranes prepared from endothelium-denuded saphenous veins (fig. 3, A and B). Interestingly, in both women and men, only one class of binding sites with the characteristics of ET_A receptor subtype was detected. The total number of binding sites (B_{max}) on male samples was ~6- to 7-fold higher than that of women (table 2).

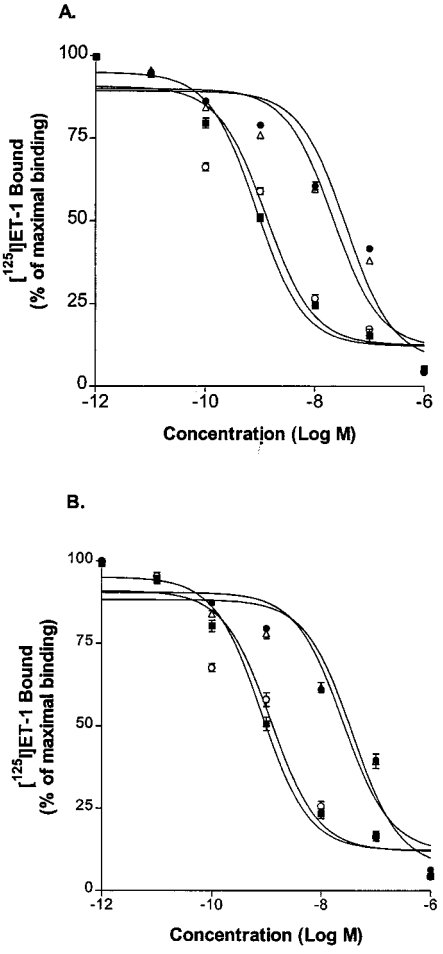

Fig. 3. Competition of the specific binding of [^{125}I]ET-1 to the pooled membrane fractions obtained from endothelium-denuded saphenous veins of male (A) and female (B) patients. Binding experiments were performed as described in the legend to figure 2, and all symbols have the same meaning.

Vascular reactivity experiments. Functional studies of saphenous veins were performed on blood vessels with or without endothelium, obtained from the same patients in which receptor binding studies were performed. Initially, the veins were exposed to 70 mM KCl to assess their ability to contract. In all vessels tested, KCl produced a consistent contraction with no gender differences observed. Dose-response curves to ET were then constructed with the responses normalized as a percentage of the KCl response. Saphenous veins from both male and female patients exhibited prominent, dose-dependent constrictions (0.9 ± 0.3 and 1.2 ± 0.5 × *g* tension in women and men, respectively). The EC_{50} values for female and male tissues were 1.4 ± 4.3 and 3.5 ± 2.2 nM, respectively. However, as seen in figure 4A, the maximal ET-1-induced constrictions at 1 μM in saphenous veins from men were approximately twice that observed in women (*$P < .05$).

Because our receptor binding studies demonstrated that both ET_A and ET_B receptors were present in endothelium-intact saphenous veins, the above experiments with men were repeated in the presence of BQ-123. As shown in fig ure 4A, BQ-123 (1 μM) completely blocked the response of ET-1, indicating that the observed effect was mediated by the ET_A receptor (***$P < .001$). In addition, when BQ-123 was added to a blood vessel that was contracted with ET-1, it displaced the bound ligand, and relaxation was induced (data not shown). Preliminary studies with samples from female patients using BQ-123 yielded similar results.

To determine whether ET_B receptors on smooth muscle cells contribute to vascular reactivity, ET-1 dose-response curves in the presence and absence of 1 μM BQ-123 were repeated with endothelium-denuded saphenous vein rings (fig. 4B). The results obtained with both female and male samples were quite similar to the contractile response observed in endothelium-intact tissue. The EC_{50} values for female and male tissues were 1.4 ± 6.3 and 28 ± 12 nM, respectively. These findings support the competitive binding data and suggest that vasoconstriction is mediated primarily by ET_A receptors.

Discussion

This study represents the first report of gender differences in ET receptor density, as well as the ratio of receptor subtypes in human vascular tissue. Our findings demonstrate

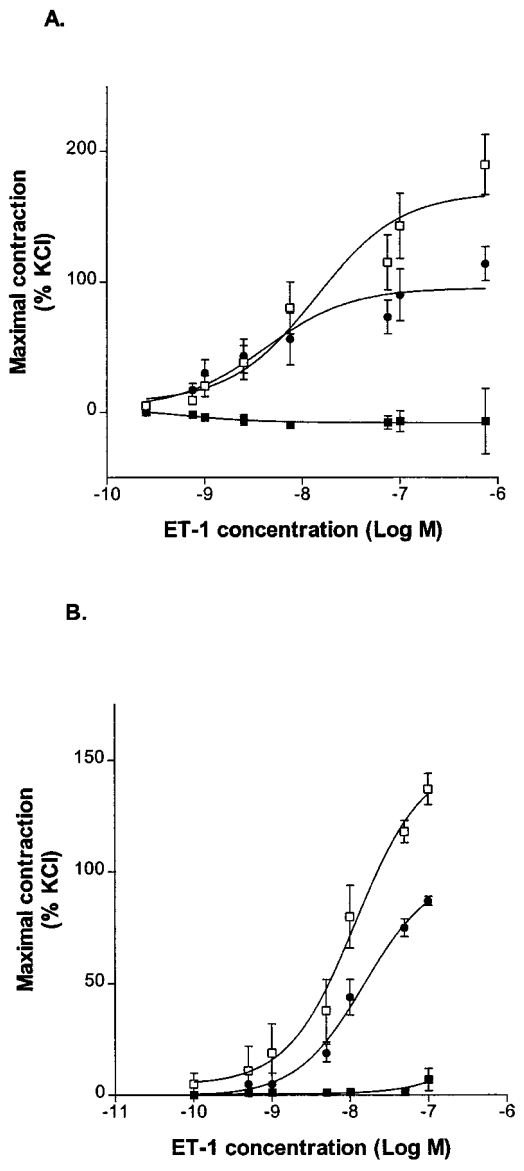

Fig. 4. Dose-response curves of endothelium-intact (A) endothelium-denuded (B) human saphenous veins to ET-1 in the absence of 1 μM BQ-123 in men (□) and postmenopausal women (●) and in the presence of 1 μM BQ-123 in men (■). Rings were treated with various concentrations of ET-1 (0.25–750 nM), and the response obtained was expressed as the percentage of the isometric tension produced by 70 mM KCl. Data are given as mean ± S.E.M. (n = 6 and 5 in the absence and presence of BQ-123, respectively).

that ET_A and ET_B receptors in human saphenous veins are localized to smooth muscle and endothelium, respectively. Saphenous veins from men contain a greater number of ET_A receptors than women, and the ratio of ET_A to ET_B receptors on endothelium-intact tissue is 3:1 and 1:1 in men and women, respectively. Vascular reactivity studies performed with saphenous vein rings *in vitro* demonstrated that these differences in receptor density and subtype distribution are reflected by the contractile responses observed with ET-1. At lower doses of ET-1, only a small fraction of the receptors are occupied and the difference in the ET-1-induced contractility between female and male tissues is not that prominent ($P < .09$). At saturating levels of ET-1 (1 μM), the contractile response in men is 2-fold higher than that observed in women (**$P < .01$). It seems reasonable to attribute this difference to the greater number of ET_A receptors in men, but the possibility of spare receptor recruitment cannot be totally discounted.

We used the human saphenous vein to examine ET receptors and ET-1-induced responses for a number of reasons. First, the saphenous vein has been shown to be more responsive to ET than arterial tissue in a number of studies (Cocks *et al*., 1989; Miller *et al*., 1989; Haynes *et al*., 1991). Second, by using veins, we avoided the potentially confounding factor of vascular hypertrophy, which can occur in resistance vessels. Third, the venous system has been reported to contribute to the pathophysiology of hypertension and congestive heart failure (Ellis and Julius, 1973; Goto *et al*., 1996). Last, the incidence of reoccurence of occlusion in the grafted saphenous veins after bypass surgery is higher in men than in women (Tyras *et al*., 1978; Douglas *et al*., 1981).

Several studies have characterized the distribution of ET receptors in vascular tissue, and it is apparent from these reports that the ET receptor population and response varies depending on the species and vascular bed (Davenport and Maguire, 1994). In our study, both ET_A and ET_B receptors were identified in human endothelium-intact saphenous veins, which is consistent with that of a recent study of Nishiyama *et al*. (1995). In their study, however, they did not evaluate gender differences with regard to receptor distribution or function. In addition, our study demonstrated that the vasoconstriction induced by ET-1 was mediated by ET_A receptors as evidenced by the complete blockade of the ET response by BQ-123, which is consistent with the results of Davenport and Maquire (1994). In addition, when the endothelium was removed from the saphenous veins, competitive binding experiments demonstrated the presence of only the ET_A receptor subtype on remaining smooth muscle, and contractility studies yielded similar results with the studies performed using endothelium-intact tissue. Thus, our data do not support a role for a direct vasoconstrictor action of ET_B receptors in the human saphenous vein in either men or women.

Since the initial discovery of ET-1, researchers have focused on the potential role of ET-1 in the maintenance of blood pressure and the pathogenesis of essential hypertension or vasospasm due to its potent contractile effects. Yet, the influence of ET-1 in the regulation of vascular tonus and in hypertension remains unclear. However, plasma ET-1 levels have been demonstrated to be elevated in several disease states such as chronic heart failure (Goto *et al*., 1996; Love *et al*., 1996a), hypertension (Ergul *et al*., 1996), coronary vasospasm (Matsuyama *et al*., 1991; Rubanyi and Polokoff, 1994), myocardial ischemia (Yasuda *et al*., 1990), atherosclerosis (Lerman *et al*., 1991) and pulmonary hypertension (Stewart *et al*., 1991). The use of an orally active nonselective ET receptor antagonist, bosentan, in animals proved to be useful in treating congestive heart failure, cerebral vasospasm and pulmonary hypertension, thus providing additional evidence for the involvement of ET-1 in the pathogenesis of these disorders (DiCarlo *et al*., 1994). In addition, administration of the ET_A receptor antagonist BQ-123 and an anti-ET-1 antibody before an ischemic insult to dogs and rats, respectively, has been shown to substantially reduce the myocardial infarct size (Watanabe *et al*., 1991; Grover *et al*., 1993). Recently, it has

been reported that systemic ET receptor blockade decreases peripheral vascular resistance and blood pressure in healthy humans (Haynes *et al*., 1996), and ET_A receptor antagonists may be useful as vasodilator agents in chronic heart failure (Love *et al*., 1996a, 1996b). These observations emphasize the potential role of endogenous ET-1 in the pathogenesis of cardiovascular disorders and the importance of the characterization of ET receptor subtypes.

The gender differences observed in this study, noted in both the receptor binding and vascular reactivity studies, raise interesting questions with regard to our understanding of the gender-related differences in cardiovascular disease reported in epidemiological studies. The enhanced vasoconstrictor response associated with the higher percentage of ET_A receptors is consistent with a higher rate of vascular disease observed in men. Interestingly, our study demonstrated a lower maximal response to ET-1 and a different receptor subtype distribution in women compared with men. Because postmenopausal women are known to be at a higher risk for cardiovascular disease than premenopausal women, it is of great interest to determine whether the gender difference we detected in saphenous veins is present in the arterial tissue as well. To the best of our knowledge, the only study investigating the gender effects on ET receptors has been reported in pigs (Miller *et al*., 1996). It was demonstrated that the total number of binding sites in coronary arteries were similar in female and male pigs and that ET-1-induced greater contractions in artery rings of female pigs in a fashion independent of their estrogen levels. It was also suggested that these gender differences might be due to the differential regulation of intracellular calcium rather than a regulation at the transcriptional level (Miller *et al*., 1996). Our findings in studies with saphenous veins that men possess a greater number of binding sites than women and that ET-1 causes greater contractions in men indicate that the regulation of ET receptor distribution is different in humans, and as shown in the literature, there might be differences in the tissues examined (Davenport and Maguire, 1994).

Previous studies in our laboratory have demonstrated reduced or impaired vasodilation in response to acetylcholine in phenylephrine-constricted vessels obtained from postmenopausal women without an alteration in response to vasoconstrictor effects (Tackett *et al*., 1995a, 1995b). In this study, we found that the ratio of ET_A to ET_B receptors is in favor of the ET_B subtype in women, which is believed to be involved mainly in the vasodilator effects of ET-1. These results suggest that the function of the ET_B receptor subtype might be under hormonal regulation and that ET-1 may have a different role in the regulation of vascular tone as well as the development and maintenance of cardiovascular pathophysiological states in women. Although our studies were performed with veins, our findings strongly suggest that the gender factor has to be taken into consideration during the evaluation of ET receptor subtype distribution in various tissues, as well as the use of antagonists as potential therapeutic agents.

Acknowledgments

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