Genome and Hormones: Gender Differences in Physiology
Selected Contribution: Gender differences in the endothelin-B receptor contribution to basal cutaneous vascular tone in humans

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Kellogg, D. L., Jr., Y. Liu, and P. E. Pérgola. Selected Contribution: Gender differences in the endothelin-B receptor contribution to basal cutaneous vascular tone in humans. J Appl Physiol 91: 2407–2411, 2001.—To test whether the contribution of endothelin-B (ET-B) receptors to resting vascular tone differs between genders, we administered the ET-B receptor antagonist BQ-788 into the forearm skin of 11 male and 11 female subjects by intradermal microdialysis. Skin blood flow was measured using laser-Doppler flowmetry at the microdialysis site. The probe was perfused with Ringer solution alone, followed by BQ-788 (150 nM) and finally sodium nitroprusside (28 mM) to effect maximal cutaneous vasodilation. Cutaneous vascular conductance (CVC) was calculated (laser-Doppler flowmetry/mean arterial pressure) and normalized to maximal levels (%max). In male subjects, baseline CVC was (mean ± SE) 19 ± 3%max and increased to 26 ± 5%max with BQ-788 (P < 0.05 vs. baseline). In female subjects, baseline CVC was 13 ± 1%max and decreased to 10 ± 1%max in response to BQ-788. CVC responses to BQ-788 differed with gender (P < 0.05); thus the contribution of ET-B receptors to resting cutaneous vascular tone differs between men and women. In men, ET-B receptors mediate tonic vasoconstriction, whereas, in women, ET-B receptors mediate tonic vasodilation.

Circulation through the cutaneous vasculature is controlled by neural, paracrine, and autocrine mechanisms. Endothelial cells produce a variety of vasoactive substances, including endothelin-1 (ET-1), an endothelium-derived contracting factor produced from Big endothelin by the action of endothelin-converting enzyme (19). ET-1 is secreted in a polarized fashion into abluminal interstitial fluid (26) and can bind to endothelin-A (ET-A) and/or endothelin-B (ET-B) receptors on vascular smooth muscle in a paracrine fashion and effect vasoconstriction. In addition, ET-1 can activate ET-B receptors on endothelial cells in an autocrine fashion and cause vasodilation. ET-1 can cause vasoconstriction or vasodilation depending on the receptor subtype activated and the location of those receptors.

In the cutaneous vasculature, ET-1 produces a long-lasting vasoconstriction through activation of ET-A receptors (2, 4, 27). In addition, blockade of ET-A receptors in the human forearm causes vasodilation, suggesting that ET-1 contributes to the maintenance of vascular tone acting on these receptors (10). Recently, Lipa et al. (18) reported from in vitro studies using human skin tissue and selective receptor antagonists that ET-1 mediated cutaneous vasoconstriction primarily through ET-A receptors.

In contrast, ET-B receptors can mediate vasoconstrictor or vasodilator effects of ET-1, depending on their localization. In the human forearm, intra-arterial infusions of selective ET-B agonists cause sustained vasoconstriction, suggesting the presence of vasoconstrictor ET-B receptors on vascular smooth muscle cells (8, 9); however, intra-arterial infusion of the selective ET-B antagonist BQ-788 causes a 20% reduction in forearm blood flow, suggesting a tonic vasodilator effect of ET-B receptors in vivo (25). All of these studies used venous occlusion plethysmography to...
measure vascular responses. This technique measures blood flow from the combination of both skin and skeletal muscle and is thus unable to differentiate between these two vascular beds. Little work has been done on the role of ET-B receptors in the control of the cutaneous circulation; however, Lipa et al. (18) suggested from in vitro studies with human skin flaps that ET-B receptors do not participate in the cutaneous vasoconstriction induced by exogenous ET-1.

Endothelial paracrine and autocrine control mechanisms, including the ET-1 system, are under the control of sex hormones and could differ between the genders (21, 22). For example, circulating ET-1 levels are significantly higher in men than in women. Polderman et al. (21) reported that plasma endothelin levels averaged 5.9 ± 1.2 pg/ml in men and 4.17 ± 0.67 pg/ml in women. During the hormone manipulation phase of transsexual procedures, Polderman et al. (21) also found that plasma ET-1 levels decreased significantly in male-to-female transsexuals, whereas ET-1 levels rose significantly in female-to-male transsexuals. Ylikorkala et al. (28) found that plasma levels of ET-1 declined during hormone replacement therapy in postmenopausal women. These studies illustrate a gender difference in the endothelin system and support a role for sex hormones in controlling this system, at least in terms of the levels of circulating ET-1.

Recent studies by Ergul et al. (5) support the existence of a gender difference in the receptors of the endothelin system. These authors found that, in isolated human saphenous veins, the ratio of ET-A to ET-B receptors was greater in men than in women. Men had a ratio of ET-A to ET-B receptors of 3:1, whereas women had a ratio of ET-A to ET-B receptors of 1:1 (5). They concluded that the ratio of receptors of the endothelin system differed between the genders and suggested that, as a consequence, there could be functional differences between the genders in the regulation of vascular tone by this system.

On the basis of the foregoing suggestions of gender differences reported in the endothelin system, we hypothesized that, in vivo, physiological differences would exist in the tonic actions of ET-1 in men and women. We further hypothesized that the functional balance between the vasoconstrictor and vasodilator effects of endothelin could be different between the genders and that this difference would be mediated by ET-B receptors. To test our hypothesis, we locally administered the selective ET-B receptor antagonist BQ-788 into forearm skin while monitoring skin blood flow (SkBF) in young adult men and women to selectively antagonize the effects of endogenous ET-1.

METHODS

ET-B receptor blockade was achieved by intradermal administration of BQ-788, a potent, selective, and competitive ET-B receptor antagonist (Ref. 11; RBI, Natick, MA). Drug delivery was achieved by intradermal microdialysis, which permitted local administration of drug directly into the interstitial space of a small area of skin. This approach limited the effects of drugs to a limited area of forearm skin without potentially confounding systemic effects (12, 14). Drug delivery by microdialysis was combined with local SkBF measurements by laser-Doppler flowmetry (LDF; MBF3D dual-channel flowmeter, Moor Instruments, Devon, UK) to monitor cutaneous vascular responses to the infused drug (12, 15). LDF measurements are specific to skin, being uninfluenced by blood flow in the underlying skeletal muscle tissue (23); thus our approach permitted a specific evaluation of the cutaneous vascular responses.

Twenty-two healthy subjects (11 men and 11 women) participated in this study. For male subjects, the average age was 30 ± 3 (SE) yr, average weight was 75 ± 3 kg, and average height was 175 ± 2 cm. For female subjects, the average age was 29 ± 3 (SE) yr, average weight was 65 ± 3 kg, and average height was 164 ± 2 cm. All subjects were in good health, as documented by medical history and physical examination, were taking no medications, and were non-smokers. All subjects gave their informed consent to participate in this institutionally approved study, which followed Declaration of Helsinki regulations. There was no caffeine or alcohol intake during the 24 h before arrival to the laboratory for the study.

Microdialysis probes were made in our laboratory from polyimide tubing and a 1-cm length of capillary microdialysis membrane (200 µm diameter, molecular mass cutoff of 20 kDa) reinforced by a 51 µm diameter of coated stainless steel wire placed in the lumen of the membrane and tubing. After subjects arrived at the laboratory, each had a probe placed on the ventral aspect of one forearm as follows. A 25-gauge needle was inserted through the dermis using sterile technique with entry and exit points ~2.5–3 cm apart. The microdialysis probe was then threaded through the internal lumen of the needle, the needle was withdrawn, and the probe left in place. The microdialysis membrane remained entirely within the dermis connected through the skin via the polyimide tubing. Ultrasound measurements showed that probes were placed 0.3–1 mm under the epidermal surface. After probe insertion and before additional instrumentation, subjects waited for at least 2 h to permit resolution of insertion trauma. The injury caused by insertion of an intradermal microdialysis probe resolves after a period of 90–135 min, thus permitting in vivo studies without the confounding effects of trauma (1). In addition, we have found that neural and endothelial control mechanisms are preserved after microdialysis probe placement (12, 15) and that SkBF levels remain at baseline levels during perfusion of the microdialysis fiber with Ringer solution alone for up to 5 h (12, 15). These findings support the use of this combined technique in the present investigation and obviate the need to subject volunteers to placement of a second microdialysis probe that receives only Ringer solution.

Mean arterial pressure was recorded continuously via a finger monitor (Finapres blood pressure monitor, Ohmeda, Madison, WI). LDF probes were held in special probe holders that permitted LDF measurements and control of local skin temperature (16). After LDF probe placement, the local temperature at the LDF measurement sites was held constant at 34°C. Room temperature was kept constant at 22°C.

For the study, subjects were placed in the supine position during instrumentation. Data collection began with a 10- to 15-min baseline control period during which the microdialysis probe was perfused with Ringer solution at a rate of 2 µl/min using a microinfusion pump. After this control period, the microdialysis probe was perfused with BQ-788 dissolved in Ringer solution at a concentration of 150 nM. BQ-788 was perfused for 1 h. The concentrations were chosen based on separate preliminary studies that showed that concentra-
tions below 150 nM caused no change in SkBF. Higher concentrations of BQ-788 were not used because we were not able to verify that those concentrations did not antagonize ET-A receptors as well. Perfusion with BQ-788 was followed by perfusion with 28 mM sodium nitroprusside (SNP; Sigma Chemical) in Ringer solution for 30 min to effect maximal vasodilation for data analysis purposes (13, 14).

Data are presented as means ± SE. For data analysis, cutaneous vascular conductance (CVC) was indexed as LDF (in V) divided by mean arterial pressure (in mmHg). Values of CVC were normalized to maximal levels of CVC as achieved with SNP perfusion to better reflect absolute changes in SkBF and thus facilitate comparisons between the genders (13, 14). Vasomotor responses were analyzed by comparing the average levels of CVC over the last 5 min of the baseline and drug-infusion periods. CVC responses were analyzed by repeated-measures ANOVA (1 within, 1 between). Statistical significance was taken at the 5% confidence level.

RESULTS

No difference was found between men and women in the degree of maximal CVC values effected by SNP (3.9 ± 0.3 mV/mmHg in men and 4.1 ± 0.4 mV/mmHg in women; P > 0.05). These values were used to normalize all other data to maximal levels of SkBF to facilitate comparisons.

During perfusion of the microdialysis fibers with Ringer solution alone, baseline CVC values averaged 19 ± 3% of maximal levels in men and 13 ± 1% of maximal levels in women. These values were significantly different between the genders (P < 0.05).

Perfusion of the microdialysis fibers with BQ-788 caused different SkBF responses in men and in women. In men, CVC averaged 19 ± 3% of maximal levels during perfusion of the microdialysis fibers with Ringer solution in the control period and increased during subsequent perfusion of BQ-788 to 26 ± 5% of maximal levels during perfusion with 150 nM BQ-788 (P < 0.05 vs. baseline). In women, CVC averaged 13 ± 1% of maximal levels during perfusion of the microdialysis fibers with Ringer solution in the control period and decreased during subsequent perfusion with 150 nM BQ-788 to 10 ± 1% of maximal levels (P < 0.05 vs. baseline). These responses are illustrated in Fig. 1.

Analysis of the above data showed a significant difference in overall CVC values between men and women (P < 0.01). In addition, there was a significant effect of BQ-788 on CVC (P < 0.01) in both groups. Finally, there was a significant difference in the CVC responses between genders and drug treatment (P < 0.01).

DISCUSSION

This human study shows for the first time in vivo a gender difference in the physiological vascular actions of the endothelin system. ET-B receptors functionally contribute to the maintenance of basal vascular tone in human skin, but this contribution fundamentally differs between men and women. Blockade of ET-B receptors by the competitive antagonist BQ-788 causes a vasoconstriction in women and a vasodilation in men. In men, BQ-788 caused skin vasodilation consistent with removal of a tonic vasoconstrictor effect of ET-1. In women, BQ-788 caused a vasoconstriction, demonstrating release of tonic vasodilator activity. During our preliminary studies, neither higher nor lower doses of BQ-788 were ever able to elicit a vasoconstriction in the male subjects. Overall, our results show gender differences in the contribution of ET-B receptors to basal vascular tone in the human skin: men have ET-B receptor-mediated vasodilator tone, whereas women have ET-B receptor-mediated vasoconstrictor tone.

The simplest explanation for this difference is as follows: 1) ET-B receptors in the cutaneous vessels of men are located predominantly on vascular smooth muscle cells and mediate a tonic ET-1 induced cutaneous vasoconstriction, and (2) ET-B receptors in the cutaneous vessels of women are located predominantly in the endothelium and mediate a tonic ET-1-induced vasodilation that was abolished by BQ-788. Our results do not exclude the possibility that men possess vasodilator ET-B receptors in the cutaneous circulation; however, the results suggest that any vasodilator ET-B contribution to vascular tone is overshadowed by vasoconstrictor ET-B receptor-mediated tone. Conversely, our results do not exclude the possibility that women possess vasoconstrictor ET-B receptors on blood vessels in the skin; however, any vasoconstrictor ET-B contribution to vascular tone is overshadowed by ET-B receptor vasodilator tone.

The main finding of our study is that BQ-788 caused a vasodilation in men and a vasoconstriction in women. On the basis of this observation, gender differences in endothelial vasodilator production such as nitric oxide or prostanoids may exist. In women, a significant por-
tion of this vasodilator tone appears to be coupled to ET-B; however, this is not the case in men. This again might explain the observed differences in the response to ET-B receptor blockade, even if endothelial ET-B receptors are present in men.

Recent studies by Ergul et al. (5) in isolated human saphenous veins support our findings in the cutaneous circulation. These authors showed that ET-1 produced a greater vasoconstrictor response in men than in women and showed that saphenous veins from both men and women had ET-A receptors localized to smooth muscle cells and ET-B receptors localized to endothelial cells. The ratio of ET-A to ET-B receptors appeared to be in favor of the ET-B subtype in women. Men had an ET-A-to-ET-B receptor ratio of 3:1, whereas women had a ratio of 1:1, with a greater overall receptor density found in men than in women (5). Ergul et al. concluded 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 in women. These authors did not find a role for a direct vasoconstrictor action of ET-B receptors in the saphenous vein in either men or women.

Studies have also shown that the endothelin system can attenuate sympathetic neurotransmission, while enhancing the contractile response to exogenous norepinephrine (7, 17, 20, 24). Garcia-Villalon et al. (7) proposed that an inhibitory action of endogenous endothelin on sympathetic vasoconstriction may be present under basal conditions through activation of prejunctional ET-B receptors, which may inhibit norepinephrine release from peripheral sympathetic nerves. Our findings of a significant gender difference in the ET-B receptor-mediated cutaneous vascular tone could also involve sympathetic noradrenergic vasoconstrictor function, since our results are consistent with an effect of ET-B inhibition of sympathetic noradrenergic neurotransmission in women that is not evident in men and/or an ET-B receptor-mediated augmentation of the contractile response to endogenous norepinephrine in men that is not evident in women.

Our results in the skin of male subjects contrast with those of Verhaar et al. (25) in the whole forearm (skin and muscle vascular beds). They studied 21 male and 1 female subject and used intra-arterial infusion of BQ-788 in combination with forearm blood flow measurements by venous occlusion plethysmography. They showed that blockade of ET-B receptors led to a consistent vasoconstriction; thus ET-B receptors contributed to basal vasodilator tone in the whole forearm. Venous occlusion plethysmography measures blood flow to both cutaneous and skeletal muscle; thus their results represent the combined responses of the two vascular beds. Our results are specific to skin (23). It is thus possible that cutaneous vessels receive tonic vasoconstrictor effects of ET-B receptors in men and that skeletal muscle vessels receive a tonic vasodilator influence. Another difference between the two studies is the route of administration of BQ-788. Verhaar et al. used an intra-arterial infusion of BQ-788 directly into the vascular lumen. The authors speculated that their approach could preferentially affect endothelial ET-B receptors, although the authors expressed skepticism of this based on observations that the ET-1 and the ET-A receptor blocker, BQ-123, easily accesses vascular smooth muscle during intra-arterial infusions. In contrast, Haynes et al. (9) found evidence for vasoconstrictor ET-A and ET-B receptors in human resistance and capacitance vessels in vivo. High doses of the ET-B-selective agonist ET-3 infused in the brachial artery of normal volunteers caused a significant vasodilation followed by vasoconstriction. The transient vasodilation was likely due to activation of ET-B receptors on endothelial cells. They postulated that ET-B receptors may be confined to endothelial cells but cause late-onset vasoconstriction through stimulation of the generation of endothelium-derived vasoconstrictor agents (constrictor prostanoids or even ET-1). They concluded that the most likely explanation is that functionally active ET-A and ET-B receptors exist on vascular smooth muscle cells causing vasoconstriction.

In addition to differences in the ET-B receptor contribution to basal cutaneous vascular tone, we found that basal CVC is lower in women than in men. This is consistent with work by Cooke et al. (3), who also found that basal SkBF in women was lower than that in men. They proposed that this difference was due to gender differences in central sympathetic rather than local, control mechanisms. These differences were proposed to effect increased sympathetic outflow in women, leading to a reduction in SkBF relative to men. Our findings that baseline CVC was lower in women than in men demonstrate that local control mechanisms of the cutaneous vasculature also differ between the genders.

Overall, our results demonstrate a gender difference in the endothelin system. It is thus possible that this system contributes to the gender differences noted in the incidence of cardiovascular disease (6). Specifically, increased endothelial ET-B receptor effects in the cardiovascular system in women could have protective cardiovascular effects that delay the development of cardiovascular diseases in women relative to men. In addition, our finding of ET-B receptor-mediated vasodilator tone in women has implications for future therapeutic applications of endothelin-receptor antagonists. ET-B receptor-mediated vasodilator tone quite likely exerts beneficial cardiovascular effects through increased production of NO and prostacyclin in women. Use of combined ET-A and ET-B receptor antagonists, such as bosentan, could negate the beneficial effects of endothelial ET-B receptors in female patients. Clearly, gender differences should be considered in evaluating the therapeutic potential of ET-1 receptor antagonists.

In summary, we found that blockade of ET-B receptors with BQ-788 altered skin blood flow in humans, demonstrating that this system contributes to basal tone in cutaneous blood vessels. In addition, the vascular response to ET-B receptor blockade differed between men and women. In men, blockade of ET-B receptors caused vasodilation, whereas, in women, blockade of ET-B receptors caused vasoconstriction. This shows that the contribution of ET-B receptors to
basal cutaneous vascular tone differs in a fundamental way between men and women.

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REFERENCES