Endothelin A Receptor Blockade Improves Endothelium-Dependent Relaxation in Obese Woman

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Summary

Hypertension in obesity is associated with increased insulin resistance, vascular mass and body mass index (BMI). The purpose of the study was to visualize endothelin-1 (ET-1) mediated constriction in arteries isolated from subcutaneous adipose tissue from obese hypertensive women previously operated by gastric bypass. Functional studies were conducted in a microvascular myograph. Expressed as percentage of contraction elicited by 124 mM KCl concentration-response curves for ET-1 were shifted leftward in arteries from obese hypertensive patients compared to healthy normotensive subjects. The vasodilator response to the ET-1 antagonist BQ123 (1 µM) was significantly higher in arteries from obese hypertensive patients ($p<0.001$). BQ123 induced relaxation was inhibited by NO synthase inhibitor L-NAME (0.1 nM). Preincubation with BQ123 enhanced the relaxation induced by acetylcholine (ACh; 0.1 nM – 0.1 mM) ($p<0.001$), but not that induced by NO donor sodium nitroprusside (SNP; 0.1 nM – 0.1 mM), in arteries from obese hypertensive patients. The present study show that hypertension yet prevail after gastric bypass surgery and the ET_A receptor antagonist BQ123 may be a useful tool in reducing blood pressure in obese hypertensive patients.

Key words

Human small arteries • Endothelin • Endothelium • BQ123 • Nitric oxide • Women • Obesity

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Introduction

Patients with essential hypertension have impaired endothelium-dependent vasodilatation mainly due to decreased bioavailability of endothelial nitric oxid (NO) (Gradin *et al.* 2003, Panza *et al.* 1990). Endothelial dysfunction has been associated with the development of atherosclerosis and other vascular complications observed in hypertensive patients. The mechanisms leading to impaired endothelial function in obese hypertensive vessels have not been fully elucidated. A disruption of the critical equilibrium between opposing forces may result in thickening of the vascular wall and may predispose the vascular smooth muscle to increased tone and decreased vasomotion (Panza *et al.* 1993). Among substances produced by vascular endothelial cells is endothelin (ET-1) a powerful vasoconstrictor peptide (Yanagisawa *et al.* 1988). ET-1 exerts its vasoactive effects through interaction with 2 receptor subtypes, ET_A and ET_B (Luscher and Noll 1995, Sakurai *et al.* 1990). The ET_A receptor is a classic G-protein-coupled receptor, involving a pertussis toxin-insensitive G-protein at the receptor site, localized to the vascular smooth muscle cells (VSMC) (Böhm and Pernow 2007, Lin *et al.* 1991). The ET_A receptor appears to be the dominant receptor mediating vasoconstriction to endogenous and exogenous ET-1 and accounts for the role of ET-1 in basal vascular tone in humans under physiological conditions (Seo *et al.* 1994, Warner 1999, Haynes and Webb 1994). Moreover, in isolated vessel studies and *in vivo* ET_A receptors play an integral role in vasoconstriction to endogenous or exogenous ET-1. Vascular ET_B receptors are localized on

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VSMC and endothelial cells (Maguire *et al*. 2001). Previous studies have described that nonselective antagonism of $ET_{A/B}$ receptors resulted in a significant vasodilator response in hypertensive patients, but not in healthy controls (D´Orleans-Just *et al*. 2002). This indicates that increased activity of the ET-1 system indeed contributes to the augmented vascular tone of patients with hypertension.

Vascular health declines earlier in aging male subjects compared to female subjects (Derbyshire and Marietta 2012). Thus, estrogen regulates a number of signaling pathways that are protective to the vessel structure and function, both during short term and in the long term (Tang and Vanhoutte 2012). Major hormonal changes associated with cessation of function or menopause, such as circulating estrogen and progesterone levels contribute to vascular dysfunction in women *via* loss of the beneficial mechanisms (Celemajor *et al.* 1994). Recent results describe a more profound association between total or abdominal adiposity and arterial properties in women than in men (Quio *et al*. 2008).

The purpose of this study was to investigate the contribution of the ET_A receptor to vascular reactivity and obese hypertension after gastric bypass surgery. Since the hypertension is increased the response to the ET_A receptor antagonist BQ123 was characterized in subcutaneous arteries isolated from obese hypertensive woman compared to arteries from healthy woman. Endothelium cell dysfunction seems to contribute to the vasoconstriction.

Materials and Methods

Subjects

Obese women aged 54.8±2.4 years who had undergone gastric bypass surgery within the last 2 years and with a documented history of hypertension (blood pressure >140/90 mm Hg), at the outpatient clinic of the Department of Plastic surgery, Sahlgrenska University Hospital for removal of excess abdominal adipose tissue were recruited in the study. None of the patients had a history of diabetes.

A group of healthy women (control group) matched for age was recruited from the same department to serve as a control group. The subjects were scheduled for esthetic surgery.

Each subject was screened by clinical history body mass index, blood pressure and routine chemistry. The control subjects had no history of hypertension, and none were taking medications at the time of the study. The study protocol was approved by the Swedish ethical board in Gothenburg and all participants gave their written informed consent.

Baseline measurements

All measurements were collected in the morning. Blood samples were collected and a physician measured systolic and diastolic blood pressure twice in the left arm of the subject in the sitting position using a standardized protocol. The average of 2 such readings constituted the examination blood pressure and body mass index.

Tissue preparation

During abdominal surgery of obese and control subjects, 5 g of subcutaneous adipose tissue was removed, put in prechilled salt solution and immediately transported to the laboratory for functional studies. Arteries in the adipose tissue were carefully freed from surrounding tissue. Segments of 2 mm length were mounted onto stainless wires and suspended in 5 ml microvascular myograph baths (Danish Myotechnology, Aarhus, Denmark). The tissue baths were temperature controlled (37 °C) and contained physiological salt solution (PSS) of the following composition (mM): NaCl 119, $NaHCO_3$ 25, glucose 5.5, KCl 4.7, $CaCl_2$ 2.5, KH_2PO_4 1.18, $MgSO_4$ 1.17 and ethylenediaminetetra-acetic acid (EDTA) 0.026. KPSS was similar to PSS except that NaCl was exchanged with KCl on an equimolar basis. Solutions were equilibrated with 5 % CO_2 in O_2 to maintain a pH of 7.4. The vessels were allowed to stabilize for 60 min. The relation between resting wall tension and internal circumference L_{100} corresponding to a transmural pressure of 100 mm Hg for a relaxed vessel *in situ* was calculated. The vessels were set to the internal circumference L_1, given by L_1=0.9.

Recording of mechanical activity

The contractile ability of the vessels was tested by stimulating with 124 mM KPSS until reproducible responses were obtained; i.e. when the arteries developed contraction was within 10 % of the previous contraction to KPSS. In most experiments this was reached with a third stimulation to KPSS.

Cumulative concentration response curves were obtained by addition of endothelin-1 (ET-1: 1 pM – 0.1 mM) in arteries under baseline conditions or following incubation with the ET_A receptor antagonist BQ123 (0.3 μM, 1 μM and 3.0 μM) for 30 min was investigated, since limited amount of artery was available the effect of 1 µM BQ123 was determined in these preparations.

Concentration response curves to the endothelium-dependent dilator acetylcholine (ACh: 0.1 nM – 0.1 mM) and the endothelium-independent dilator sodium nitroprusside (SNP; 0.1 nM – 0.1 mM), in arterial segments precontracted with phenylephrine (Phe) (300 nM) for 15 min. The relaxations were determined in the absence and presence of the selective ET_A receptor antagonist BQ123 (1 µM). The relaxations induced by ACh were in addition determined following blockade of NOS by N-mono-methyl-L-arginine, L-NAME (0.1 nM). All chemicals were purchased from Sigma. Stock solutions were prepared and stored at -20 °C and fresh dilutions were prepared daily.

Analysis of data and statistics

Mechanical responses of vessels were measured as force and expressed as active wall tension (T) which is the increase in force above baseline (F) divided by twice the segment length. The contractile response to ET-1 is expressed as a percentage of that induced by KPSS. Relaxations are expressed as percentages of the precontractile tone. Sensitivity to the agonists is given as pD_2 = -log EC50 (M).

The results from the vessel studies are expressed as means ± SEM and n represents the number of arteries (one/subject). Clinical characteristics of participating woman are presented as means ± SD. Statistical differences between arteries in each group were tested with the use of Student`s paired or unpaired t test when appropriate. Statistical differences yielding p values less than 0.05 were considered significant.

Results

Study subject characteristics

Clinical characteristics of control subjects and obese hypertensive patients are presented in Table 1. Systolic and diastolic blood pressure was significantly higher in obese hypertensive patients compared to healthy control subjects. Blood pressure taken prior to the gastric bypass surgery was 165±3.2/91.9±1.9 mm Hg. Thus, blood pressure remained significantly elevated (AFTER) the gastric bypass surgery. The obese patients had significantly higher BMI and lower high density lipoprotein cholesterol than the control group at follow-up (Table 1). Fasting blood glucose, HbA1c and low density lipoprotein cholesterol did not differ between the groups.

Table 1. Clinical characteristics of the study participants.

	Control subjects	**Obese hypertensive subjects**
Age, years	55.9±1.3	54.80±2.43
Weight, kg	70.9±2.7	106.4±3.1***
Height, cm	163.3±2.4	165.0±1.3
Body mass index, kg/m²	20.50±0.32	30.70±0.94*
Biochemical features		
Brain natriuretic peptide, pg/ml	27.13±5.65	41.63±18.52**
Fasting glucose, mmol/l	5.11±0.14	5.26±0.25
HbA1c, mmol/mol	3.75±0.23	4.09±0.17
HDL cholesterol, mmol/l	1.74±0.65	1.55±0.17*
LDL cholesterol, mmol/l	3.56±0.32	3.28±0.33
Triglycerides, mmol/l	1.34±1.51	1.51±0.34
Morphological measurements		
Systolic blood pressure, mm Hg	112.9±1.6	154.4±3.5***
Diastolic blood pressure, mm Hg	65.6±1.8	95.1±2.1***

Values are means ± SD. * p<0.05, ** p<0.001, *** p<0.0001 significant differences vs. healthy control subjects (n=8). ET-1, endothelin-1; LDL, low-density lipoprotein; HDL, high-density lipoprotein.

Table 2. Effect of BQ123 (1 μM) on ACh and SNP relaxation of subcutanouse small arteries.

Drug	**Control subjects**		**Obese hypertensive subjects**	
	Rmax %	**pD_2**	**Rmax %**	**pD_2**
ACh	92.6±1	7.93±0.1	51.5±3*	7.97±0.1*
ACh + BQ123	98.1±1	7.58±0.1	77.0±2*	8.0±1*
SNP	99.0±0.3	7.77±0.1	57.5±2*	7.85±0.1*
SNP + BQ123	85.7±0.3	7.10±0.04	51.5±3*	8.15±0.12*

Rmax, maximal relaxation; pD_2, -log (EC50). Preactivation with Phe (300 nM). Values are means ± SEM. * $p<0.05$ vs. controls. Each group consisted of 8 arteries from control subjects and obese hypertensive patients, respectively.

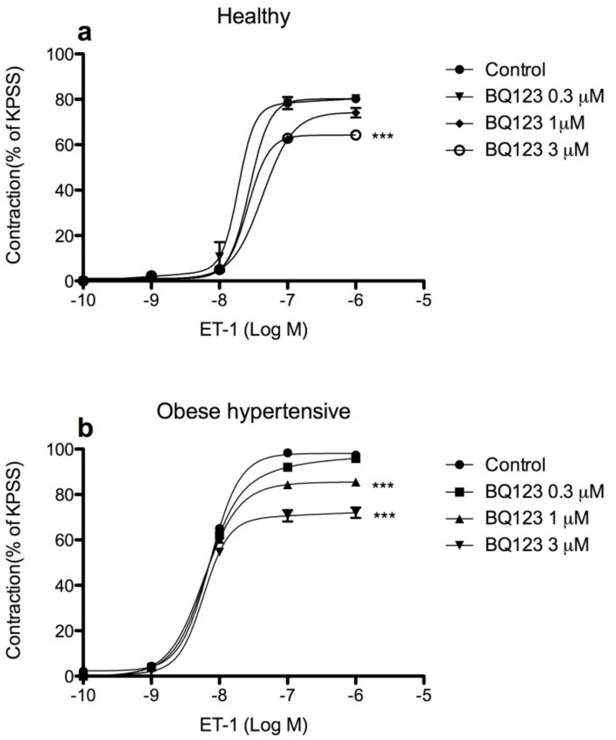

Fig. 1. Endothelin vasoconstriction of human small arteries in the absence and presence of three different concentrations of the ET_A receptor antagonist BQ123: 0.3 μM, 1 μM and 3 μM. The arteries are precontracted with 124 mM KPSS and relaxations are expressed as percentage of the initial constriction level. Values are means ± SEM of arteries from 8 healthy subjects (**a**) and 8 obese hypertensive patients (**b**), respectively. Significantly different responses were evaluated by t test: *** $p<0.0001$.

Response to ET-1

ET-1 evoked more pronounced contraction in arteries from obese hypertensive patients than in arteries from healthy controls. The maximal contraction induced by ET-1 was 98.2±0.3 % of KPSS in arteries from hypertensive patients vs. 85.8±0.5 % in control arteries ($p<0.001$; n=8). Furthermore, the concentration-response curve to ET-1 was shifted leftward in arteries from obese hypertensive compared to healthy control subjects (Figs 1a and 1b). The ET_A receptor antagonist BQ123 (1 μM), inhibited these contractions, significantly in arteries from obese hypertensive patients whereas it did not significantly influence the contractions induced by ET-1 in arteries from healthy controls. The highest dose of 3.0 μM inhibited the contractile responses in arteries from control subjects (Figs 1a and 1b).

Vasodilator response to ACh and SNP

The vasorelaxing effects were determined on arteries precontracted with Phe (300 nM). The relaxation induced by ACh (0.1 nM – 0.1 mM) was significantly impaired in arteries from obese hypertensive patients in comparison with those from healthy controls. The NOS inhibitor L-NAME (0.1 nM) significantly attenuated the relaxing effect of ACh in arteries from both healthy and obese hypertensive patients. Preincubation with the ET_A receptor antagonist BQ123 (1 μM) significantly enhanced the ACh induced relaxation in arteries from obese hypertensive patients with max value of (51±2 % to 77±2 %; n=8). In contrast, the relaxing effect of ACh in arteries from control subjects were unaffected by BQ123 (1 μM). Furthermore, the ACh induced relaxation showed pD_2 values for control and obese hypertensive arteries, respectively, and after BQ123 (1 μM) antagonism (Table 2). The response to L-NAME (0.1 nM) were of similar magnitude in arteries from healthy and obese hypertensive patients (Figs 2a and 2b).

The NO donor SNP (0.1 nM – 0.1 mM) induced relaxation of Phe (300 nM) precontracted arteries and the response to the ET_A receptor antagonist BQ123 (1 μM) did not further modify the relaxation induced by SNP in arteries from obese hypertensive patients. The control response described a difference of the maximal response. Further, the SNP induced relaxation and vascular sensitivity, pD_2, was more pronounced in arteries from healthy subjects (Table 2) (Figs 3a and 3b).

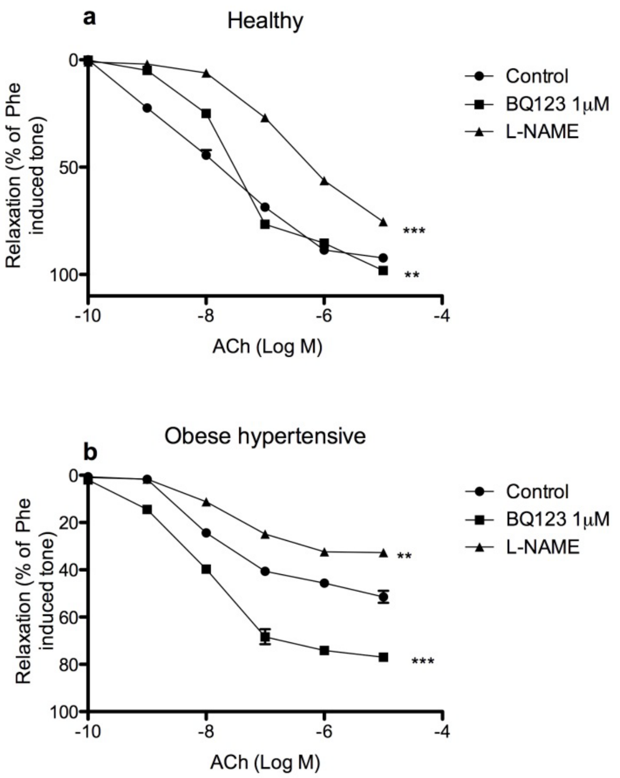

Fig. 2. ACh relaxation of human small arteries in the absence and presence of the ET_A receptor antagonist BQ123 (1 µM) or the NO synthase inhibitor L-NAME (0.1 nM). The arteries were constricted with Phe (300 nM) and relaxation are expressed as percentage of the initial constriction level. Data are means ± SEM of arteries from 8 healthy subjects (**a**) and 8 obese hypertensive patients (**b**), respectively. Significantly different responses were evaluated by t test. ** p<0.001, *** p<0.0001.

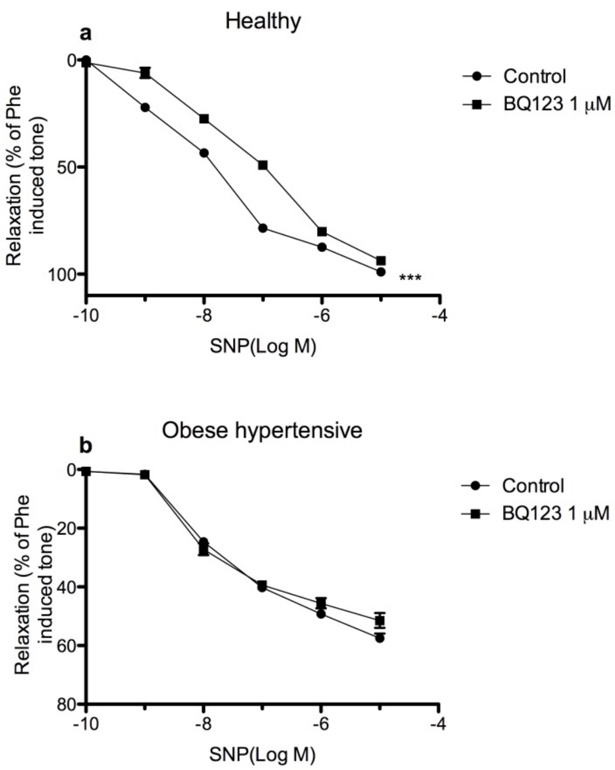

Fig. 3. SNP relaxation of human small arteries in the absence and presence of the ET_A receptor antagonist BQ123 (1 µM). The arteries were constricted with Phe (300 nM) and relaxations are expressed as percentage of the initial constriction level. Data are means ± SEM of arteries from 8 healthy subjects (**a**) and 8 obese hypertensive patients (**b**), respectively. Significantly different responses were evaluated by t test. ** p<0.001.

Discussion

The main finding is increased blood pressure, altered vascular response to ET-1 and endothelial dysfunction contribute to enhanced vasoconstriction in arteries from obese hypertensive woman, a response after post gastric bypass surgery. With menopause abdominal adiposity increase neurohormonal drive and nitric oxide contribute to the increased release of ET-1 and hypertension (Stein *et al.* 1998, Teeds *et al*. 2010). Previous studies show increased ET-1 levels after bypass surgery and increased blood pressure in obese hypertensive patients found in this study supports the prevalence of hypertension among obese subjects (Stauffer *et al.* 2008, Scheen and Luyckx 1999).

Obesity is one component of a risk factor constellation that consists of impaired glucose uptake, low density lipoprotein glyceride, cholesterol and triglyceride levels. Furthermore, decreased levels of HDL were found in samples from obese patients; however, the levels of LDL cholesterol were similar between the groups. Interestingly similar levels of LDL are found in elder male patients with atherosclerosis and their controls (Böhm *et al*. 2002a). Also NO may contribute since it inhibits smooth muscle cell proliferation, thus protecting the wall against atherosclerosis (Jessup 1996, Pernow *et al*. 2012). Therefore both the duration of the increased blood pressure and morphological changes may contribute to the increased vasoconstrictor stimulus and endothelial dysfunction (Gibbons and Dzau 1994).

Our results show that after administration of the selective ET_A receptor antagonist BQ123 the vasodilator response to ACh was significantly enhanced in arteries from obese hypertensive patients compared to arteries from healthy patients. Further, after administration of L-NAME the vasodilator response was blocked. In order to support these findings administration of the NO donor, SNP, did not significantly modify the ET_A blockade in arteries from hypertensive patients. Thus supporting that endothelium-independent vasodilation was not influenced and that the VSMCs to relax did not differ between the 2 study groups (Böhm *et al.* 2002a). Previous results describe an improvement in endothelial vasodilator function after either non selective $ET_{A/B}$, selective ET_A or selective ET_B blockade in segments of the internal mammary artery obtained from patients with various combinations of risk factors undergoing coronary artery bypass grafting in whom the ET-1 system is similarly attended (Hoogerwerf *et al*. 2001). Further, results report showing that ET-1 receptor antagonism is able to prevent upregulation of vascular ET-1 and correct endothelial dysfunction in an experimental model of hypertension (Verma *et al.* 2001). Since in endothelium denuded arteries ET-1 contraction is enhanced compared to arteries from normal patients (Ruscitzka *et al.* 2001, Verhaar *et al*. 1998). Therefore, in addition to endothelial cell dysfunction an increased smooth muscle activation appears to contribute to ET-1 contraction in arteries from obese hypertensive patients. NO antagonizes ET-release from the endothelium and ET_B relaxant receptors may be involved in the contractile responses (Riezebos *et al*. 1994, Porteri *et al*. 2002, Stefano *et al.* 1999, Bigaud and Pelton 1992). Moreover, contractile responses to phenylephrine and serotonin are highly increased by NO synthase inhibition which through the endothelium regulates smooth muscle sensitivity to NO (Meininger and Davis 1992, Thorin *et al*. 1998). Accordingly, the improvement of ACh induced NO-dependent relaxation by blockade of ET_A receptors was due to improvement of endothelial function. Therefore it seems reasonable to postulate that augmentation of NO activity is a likely mechanism for the improved endothelium-mediated vasorelaxation supported by results from Pussard *et al.* (1995).

In the present study, concentration-response curves for ET-1 were shifted leftward in arteries from obese hypertensive patients. The ET_A receptor antagonist BQ123 antagonized the ET-1 contraction significantly further to the right in arteries from obese hypertensive patients compared to arteries from healthy subjects. Similar results have been found in forearm blood flow responses from hypertensive patients (Taddei *et al.* 2001, Cardillo *et al.* 2002, Rafnsson *et al*. 2012, Böhm *et al.* 2002b). Interestingly others have found that also blood pressure has been lowered substantially after administration of $ET_{A/B}$ nonselective antagonists (Weil *et al.* 2011). Further, ET-1 may largely cause vasoconstriction and hypertension as shown by others (Zhu *et al.* 1999, Böhm *et al*. 2007). In the present study the nonparallel shifts in concentration response curves by BQ123 in arteries from obese hypertensive patients cannot be ascribed to the presence of endothelial receptors which oppose ET-1 contraction (Haynes and Webb 1994), since ET-1-induced contraction mediated by ET_A receptors is considered to be endothelium independent (Spieker *et al.* 2006, Cardillo *et al.* 2002). Hereby, these results provide both indirect and direct evidence for endothelial cell dysfunction.

The main finding in the present study is increased blood pressure. Blockade of ET-1 receptors improves the endothelium dependent relaxation as well as reduces the alteration of the smooth muscle response to ET-1. The capacity of the ET-1 receptor antagonist to reduce vascular tone may represent an alternative treatment in cardiovascular disease and obese hypertension.

Conflict of Interest

There is no conflict of interest.

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