Effects of obesity on endothelium-dependent reactivity during acute nitric oxide synthase inhibition: modulatory role of endothelin

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ABSTRACT

This study investigated vascular reactivity in response to acetylcholine, in the presence of acute inhibition of nitric oxide synthase, in the carotid artery and aorta of obese C57Bl6/J mice fed on a high-fat diet for 30 weeks, and of control mice. A subgroup of obese animals was also treated with the ETA receptor antagonist darusentan (50 mg·kg⁻¹·day⁻¹). In vascular rings from control animals, acetylcholine caused endothelium-dependent contractions in the carotid artery, but not in the aorta. In vascular rings from obese mice, contractility to acetylcholine was also evident in the aorta, and that in the carotid artery was increased compared with control mice. ETA receptor blockade by darusentan treatment of the obese mice prevented enhanced vasoconstriction to acetylcholine, resulting in mild vasodilatation. Thus obesity increases endothelium-dependent vasoconstriction in the absence of endothelial nitric oxide. This effect can be completely prevented by chronic ETA receptor blockade, suggesting that endothelin modulates increased endothelium-dependent vasoconstriction in obesity.

INTRODUCTION

Obesity is an important cardiovascular risk factor, and is frequently associated with disease, such as hypertension, diabetes and dyslipidaemia [1]. These conditions are characterized by early vascular changes that determine the progression of vascular disease (reviewed in [2]); however, the mechanisms underlying this impairment are poorly defined. Endothelin-1 (ET-1), an endogenous vasoactive peptide, has been implicated in obesity-associated hypertension [3], and maintains the obesity-induced activation of renal angiotensin-converting enzyme in the kidney [4]. Moreover, a role for ET-1 in the enhanced contractility in response to angiotensin II of normotensive obese mice has been described [4]. In the present study we studied a model of diet-induced obesity to investigate mechanisms of endothelium-dependent responses to acetylcholine in the aorta and carotid artery during acute nitric oxide synthase (NOS) blockade. We also studied the potential role of ET-1 by chronically treating a subgroup of obese animals with the ETA receptor antagonist darusentan.

METHODS

Animal treatments, tissue preparation and vascular physiology experiments

Study design and protocols were in accordance with the institutional animal care committee and the American Heart Association Guidelines for Research Animal Use.

Key words: aorta, carotid artery, endothelium, high-fat diet, prostanoid, risk factors, thromboxane, vascular, vasoconstriction.

Abbreviations: ET-1, endothelin-1; NOS, nitric oxide synthase; t-NAME, N⁶-nitro-l-arginine methyl ester.

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Obesity was induced in male C57BL/6J mice by feeding them on a high-calorie, Western-type diet [4–6] for 30 weeks, with or without treatment with the ET$_4$ receptor antagonist darusentan (LU135252; 50 mg $\cdot$ kg$^{-1}$ $\cdot$ day$^{-1}$; kindly donated by Knoll AG) [4]. After pentobarbital anaesthesia (50 mg/kg; intraperitoneal), animals ($n = 6–8$ per group) were killed and the thoracic aorta and both carotid arteries were excised and placed into Krebs-Ringer bicarbonate solution (4 °C; mmol/l: NaCl 118.6, KCl 4.7, CaCl$_2$ 2.5, KH$_2$PO$_4$ 1.2, MgSO$_4$ 1.2, NaHCO$_3$ 25.1, EDTA calcium disodium 0.026, glucose 11.1). In vitro experiments were performed as described previously [4,7]. Vascular rings (from the thoracic aorta and both common carotid arteries; $n = 4$ rings each) were suspended in organ chambers containing Krebs-Ringer bicarbonate solution (pH 7.4, 37 °C, 95 % $\text{O}_2$/5 % $\text{CO}_2$) and connected to force transducers. Rings were exposed repeatedly to KCl (100 mmol/l), and responses to acetylcholine (30 $\mu$mol/l) were determined in vascular rings precontracted with noradrenaline in the presence of the NO synthase inhibitor N$\text{O}$-nitro-l-arginine methyl ester (l-NAME; 0.3 mmol/l). Rings were preincubated with l-NAME for at least 30 min before responses to acetylcholine were measured.

**Materials**

Acetylcholine chloride, l-NAME HCl, noradrenaline HCl and KCl were purchased from Sigma Chemical Co. (Buchs, Switzerland). Pentobarbital was from Abbott Laboratories (Chicago, IL, U.S.A.).

**Calculations and statistical analysis**

Data are expressed as means $\pm$ S.E.M., where $n$ is the number of animals used. Responses to acetylcholine were expressed as a percentage of precontraction to the vasoconstricting agonist. Data were analysed by ANOVA followed by Bonferroni’s correction [8]. For simple comparisons between two values, the unpaired Student’s $t$ test or the Mann-Whitney $U$ test was used as appropriate. $P$ values of $< 0.05$ were considered significant.

**RESULTS**

Responses to acetylcholine were dependent on the presence of an intact endothelium (results not shown). For vascular rings from control mice, in the presence of acute NOS inhibition with l-NAME, acetylcholine induced a mild vasorelaxant response in the aorta (Figure 1A, open bar), whereas a vasoconstrictor response was observed in the carotid artery (Figure 1B, open bar). For rings from obese mice (Figure 1, filled bars), contractility to acetylcholine was now evident in the aorta, and was increased in the carotid artery when compared with controls. Concomitant ET receptor blockade caused

**DISCUSSION**

This study demonstrates that obesity is associated with endothelium- and ET-1-dependent vasoconstriction in response to acetylcholine in the mouse vasculature. These responses were unmasked by acute NOS inhibition, and were more pronounced in the carotid artery, which suggests that obesity does not modify endothelium-dependent vasoreactivity uniformly. Our results demonstrate that inhibition of the ET-1 system can prevent endothelium-dependent vasoconstriction, and that these chronic effects are independent of endothelial NO bioactivity. Recent experiments from our laboratory have suggested that the endothelium-derived vasoconstrictor that is released in response to acetylcholine in mouse arteries is cyclo-oxygenase-derived thromboxane $A_2$ or prostaglandin $H_2$ (T. Traupe and M. Barton, unpublished work). Taken together, these data, which were obtained using arteries from animals that were normotensive [4], support the concept of enhanced ET$_4$-receptor-dependent thromboxane $A_2$ and/or prostaglandin $H_2$ release by the vascular endothelium in obesity. These findings are also in accordance with those from previous acute studies, which demonstrated ET-1-mediated throm-
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boxane release from reperfused rat hearts and ET-1-mediated endothelium-dependent vasoconstrictor release from the rat aorta [9,10]. Moreover, chronic studies have demonstrated a role for ET-1-mediated vasoconstrictor prostanoid release in renal arteries of hypertensive salt-sensitive Dahl rats [11].

The findings described here were observed in an animal model of obesity. Increased prostaglandin levels have been described in the plasma of normotensive obese patients [12], suggesting that abnormal regulation of the cyclo-oxygenase pathway may also occur in human obesity. The results of our experiments suggest further that endothelin receptor antagonists may contribute to vasoprotection by inhibiting endothelium-dependent vasoconstrictor activity by pressure-independent mechanisms. These properties might suggest new therapeutic potential for ET antagonists in reducing vascular complications in obesity and related diseases.

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REFERENCES