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Endothelial dysfunction accompanies a pro-oxidant, pro-diabetic challenge in the insulin resistant, obese Zucker rat in vivo

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Abstract

We have recently made the novel observation that a pro-oxidant challenge with hydroquinone in combination with buthionine sulfoximine (each at 50 mg/kg i.p. daily for 7 days) provokes the onset of type II diabetes mellitus in a model of insulin resistance, the obese Zucker rat. Since endothelial dysfunction in oxidant stress may aggravate in vivo insulin resistance, we have now investigated endothelium-dependent and nitric oxide (NO)-mediated vascular responses in the obese Zucker rat in vivo following this pro-oxidant insult. Pro-oxidant-treated animals exhibited defective vasodepression to the endothelium-dependent agent acetylcholine and to a lesser extent, the NO donor glyceryl trinitrate, together with a reduction in circulating levels of cGMP. Our data therefore suggest that the progression to type II diabetes mellitus in the obese Zucker rat mediated by a pro-oxidant insult is associated with impairments in agonist-stimulated, endothelium-dependent vasodilation and vascular NO signalling.

Keywords: Oxidant stress; Endothelium; Nitric oxide (NO); Insulin resistance; Obese Zucker rat

1. Introduction

The obese Zucker rat is a widely employed, prediabetic model of insulin resistance, which shares many other characteristics of insulin resistance syndrome or Syndrome X, including dyslipidaemia and dysglycaemia (Reaven, 1995; Zucker and Antoniades, 1972). Furthermore, hyperinsulinaemia is associated with oxidant stress in the obese Zucker rat and can be reduced by the lipophilic antioxidant vitamin E in vivo (Laight et al., 1999b). In further support of this relationship, we have been able to demonstrate for the first time that a pro-oxidant challenge with the redox cycling agent hydroquinone in combination with low dose buthionine sulfoximine, a GSH-depleting agent, aggravates insulin resistance and provokes the onset of type II diabetes mellitus in this animal (Laight et al., 1999c).

Oxidant stress can disrupt insulin signalling involved in insulin-stimulated glucose uptake, generating insulin resistance at the tissue level (Jacob et al., 1996). In addition, oxidant stress is commonly associated with endothelial dysfunction, which principally involves the destruction of the endogenous nitrovasodilator nitric oxide (NO) (see Moncada et al. (1991)) by reactive oxygen species such as superoxide anion (Gryglewski et al., 1986; Laight et al., 1998a). This is also of relevance to the pro-oxidant-mediated progression of insulin resistance and diabetes in an established insulin resistant state, since any deterioration in endothelial vasodilator function, particularly in metabolically relevant arterioles, is likely to further impair insulin action in vivo by haemodynamically opposing glucose disposal in insulin-sensitive tissue (Petrie et al., 1996; Steinberg et al., 1996). Indeed, both in vivo insulin resistance and diabetic macroangiopathy have been suggested to be manifestations of endothelial dysfunction at distinct vascular sites (for review, see Laight et al. (1999a)).

While endothelium-dependent vasodilation in the obese Zucker rat has paradoxically been found to be well preserved relative to its lean, insulin-sensitive littermate (Auguet et al., 1989; Cox and Kikta, 1992; Laight et al., 1998b), possibly owing to a chronic, protective adaptation in the endothelium to the endogenous level of oxidant stress in this animal (Andrews et al., 2000; Kaw et al.,

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1999), the vascular effects of increasing the oxidant burden with an exogenous pro-oxidant insult have not previously been reported. We have therefore now examined haemodynamic and biochemical evidence of endothelial injury following hydroquinone + buthionine sulfoximine treatment in the obese Zucker rat in vivo. Our findings suggest that pro-diabetic changes in insulin resistant, obese animals following a pro-oxidant challenge (Laight et al., 1999c), are accompanied by endothelial dysfunction in vivo.

2. Materials and methods

The experiments were conducted in accordance with the Animals (Scientific Procedures) Act 1986 (Home Office, London, UK).

2.1. Pro-oxidant challenge in vivo

Male, 12-week-old lean (fa/+) and obese (fa/fa) Zucker rats (Harlan, Blackthorn, Bicester, UK) were treated daily with hydroquinone + buthionine sulfoximine (each at 50 mg/kg i.p.) while a control group received normal saline (2 ml/kg i.p.) for 7 days, as previously described (Laight et al., 1999c). This pro-oxidant treatment is associated with both evidences of lipid peroxidation and a rise in the intracellular level of oxidised glutathione relative to reduced glutathione (Laight et al., 1999c).

2.2. Vascular reactivity studies in vivo

Thirteen-week old animals were anaesthetised with thiopentone sodium (120 mg/kg i.p.) and instrumented for the recording of mean arterial pressure (MAP) and the administration of reagents. Following 30-min stabilisation, bolus dose–response curves for vasodepression to acetylcholine (0.02–2 μg/kg i.v.) and glyceryl trinitrate (0.1–50 μg/kg i.v.) were constructed (see Laight et al. (1998b)). Subsequently, the vasopressor effect of a bolus dose of N^G-nitro-L-arginine methyl ester (L-NAME) (100 mg/kg i.v.) was assessed (see Rees et al. (1989)).

2.3. Analysis of plasma cGMP

A venous blood sample was collected in EDTA at the end of the 30-min stabilisation period, for the determination of plasma cGMP by radio-immunoassay following sample acetylation, essentially as described by Dundore et al. (1993).

2.4. Statistical analysis

Data represent the mean ± SEM. The difference between two means was assessed by Student's unpaired, two-tailed *t*-test while the comparison of dose–response curves was conducted using two-way analysis of variance. Vasodepression was also assessed by determining area under the dose–response curve (AUC). Statistical significance was accepted at the 5% level.

3. Results

3.1. Animal body weights

The body weight of 13-week-old obese animals in control and hydroquinone + buthionine sulfoximine groups was 439.5 ± 10.4 and 378.7 ± 15.6 g ($P < 0.01$, $n = 4–5$), respectively, and was significantly greater ($P < 0.01$) than the weight of age-matched lean animals (control: 290.0 ± 10.2 g; hydroquinone + buthionine sulfoximine: 270.3 ± 6.2 g; $n = 6$). The modest weight deficit in the hydroquinone + buthionine sulfoximine-treated obese group was related to a relative hypophagia and was not associated with any adverse toxicology according to plasma markers of organ toxicity including urea, creatinine, creatinine ki-

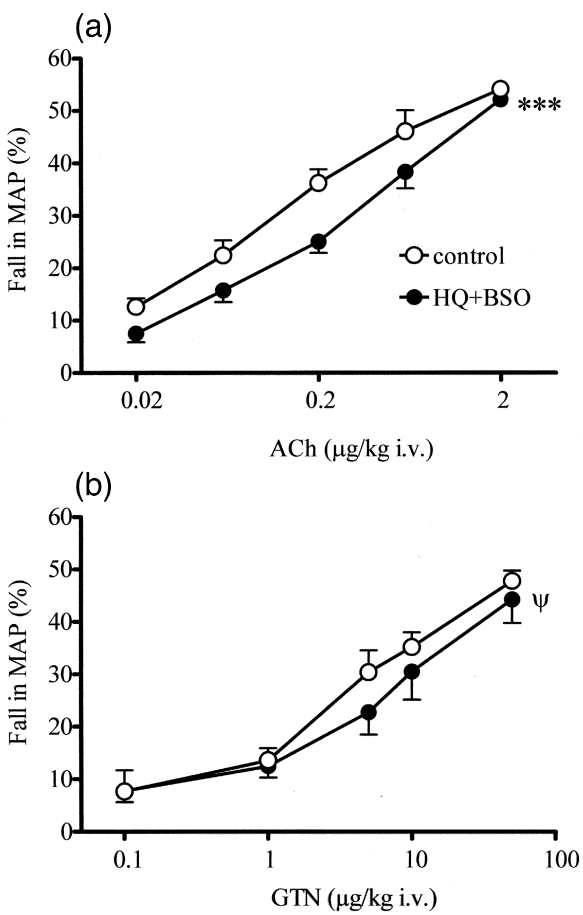

Fig. 1. Effects of pro-oxidant challenge with hydroquinone (HQ) and buthionine sulfoximine (BSO) (each at 50 mg/kg i.p. daily for 7 days) on vasodepressor responses to acetylcholine (ACh) (a) and glyceryl trinitrate (GTN) (b) in the 13-week-old, anaesthetised obese Zucker rat, assessed as the peak fall in MAP. $^{***}P < 0.01$, Ψ $P = 0.06$ (two-way analysis of variance for entire dose–response curve), $n = 4–5$.

nase, alanine aminotransferase and pancreatic lipase (data not shown).

3.2. Effects of hydroquinone + buthionine sulfoximine on basal MAP

Basal MAP in the obese Zucker rat (143.2 ± 5.3 mm Hg), which was not significantly different from that in the lean Zucker rat (132.2 ± 4.3 mm Hg) in the control group, was apparently raised by the pro-oxidant challenge ($P = 0.09$) such that obese basal MAP became significantly higher relative to lean animals after hydroquinone + buthionine sulfoximine (obese: 155.0 ± 2.3 mm Hg; lean: 136.6 ± 2.8 mm Hg) ($P < 0.01$, $n = 4–6$).

3.3. Effects of hydroquinone + buthionine sulfoximine on endothelium-dependent and NO-mediated vascular responses

In obese animals, vasodepression to acetylcholine (0.02–2 μg/kg i.v.) (AUC: 69.4 ± 4.6 units) was significantly reduced by hydroquinone + buthionine sulfoximine (AUC: 54.9 ± 4.0 units) ($P = 0.05$, $n = 4–5$) (Fig. 1a), while an apparent impairment in vasodepression to glyceryl trinitrate (0.1–50 μg/kg i.v.) (AUC: 53.2 ± 4.7 units) did not achieve statistical significance by AUC analysis (AUC: 46.3 ± 7.4 units) ($P > 0.05$, $n = 4–5$) (Fig. 1b). Similarly, hydroquinone + buthionine sulfoximine inhibited vasodepression to acetylcholine in lean animals (control AUC: 71.7 ± 4.2 units; hydroquinone + buthionine sulfoximine AUC: 57.1 ± 3.1 units) ($P < 0.02$, $n = 6$) (Fig. 2a) and tended to impair vasodepression to glyceryl trinitrate, although statistical significance was not achieved by AUC analysis (control AUC: 50.9 ± 3.8 units; hydroquinone + buthionine sulfoximine AUC: 41.9 ± 3.4 units) ($P > 0.05$, $n = 6$) (Fig. 2b). Furthermore, the vasopressor effect of L-NAME in obese (33.8 ± 2.2%) and lean (31.7 ± 4.0%) animals was unaffected by hydroquinone + buthionine sulfoximine (obese: 31.0 ± 3.0%; lean: 35.8 ± 7.3%) ($P > 0.05$, $n = 4–6$). Hydroquinone + buthionine sulfoximine-mediated impairments in vasodepressor responses to acetylcholine and glyceryl trinitrate in both the lean and obese Zucker rat were abolished by pretreatment with the hydrophilic antioxidant tiron (1% (w/v) added to

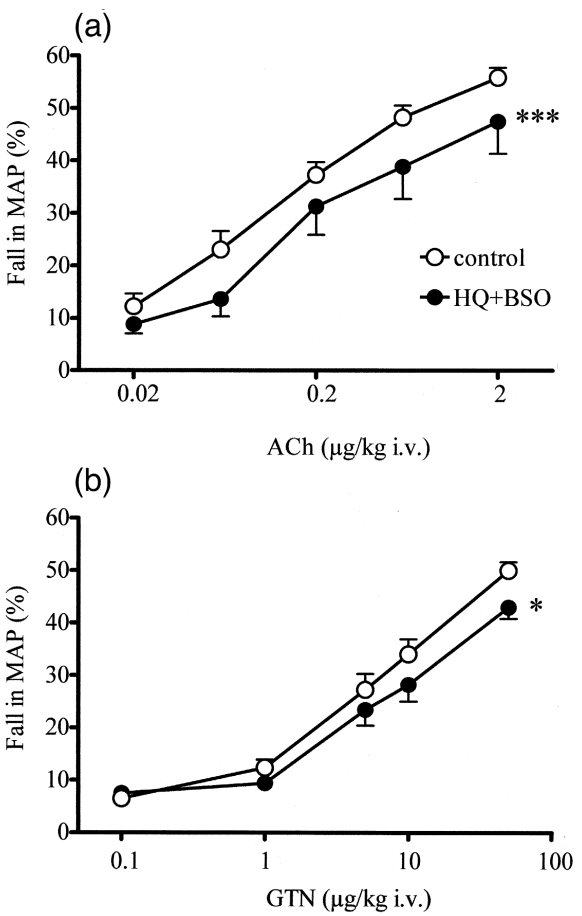

Fig. 2. Effects of pro-oxidant challenge with hydroquinone (HQ) and buthionine sulfoximine (BSO) (each at 50 mg/kg i.p. daily for 7 days) on vasodepressor responses to acetylcholine (ACh) (a) and glyceryl trinitrate (GTN) (b) in the 13-week-old, anaesthetised lean Zucker rat, assessed as the peak fall in MAP. $^{***}P < 0.01$, $^{*}P < 0.05$ (two-way analysis of variance for entire dose–response curve), $n = 6$.

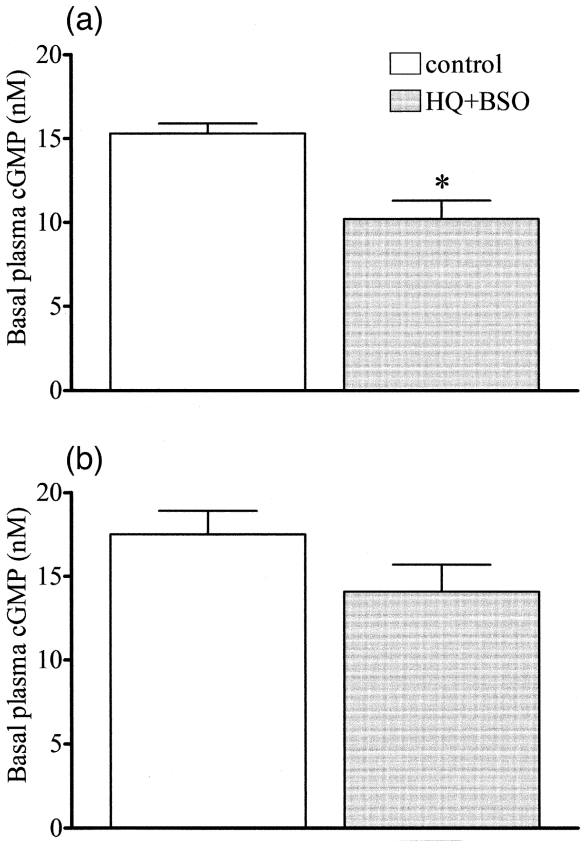

Fig. 3. Effects of pro-oxidant challenge with hydroquinone (HQ) and buthionine sulfoximine (BSO) (each at 50 mg/kg i.p. daily for 7 days) on basal cGMP plasma levels in the 13-week-old, anaesthetised obese (a) and lean (b) Zucker rat. $^{*}P < 0.05$ (Student's unpaired t-test), $n = 4–6$.

the drinking water (see Laight et al., 1998b)) ($n = 5–6$, data not shown).

3.4. Effects of hydroquinone + buthionine sulfoximine on plasma cGMP levels

Basal plasma cGMP was depressed by hydroquinone + buthionine sulfoximine by approximately 19% and 33% in lean and obese animals, respectively, attaining statistical significance in the obese Zucker rat (Fig. 3) (not shown).

4. Discussion

Basal circulating levels of cGMP were diminished following the pro-oxidant challenge, indicating a reduction in vascular intracellular signalling by endogenous NO (see Dundore et al. (1993)). This could arise from a reduced synthesis/release of NO and/or an enhanced degradation of NO by reactive oxygen species during oxidant stress (Gryglewski et al., 1986; Laight et al., 1998a). Indeed, oxidant stress in response to hydroquinone + buthionine sulfoximine in the Zucker rat has been confirmed by an elevation in plasma levels of 8-epi-prostaglandin $F_{2\alpha}$, a sensitive lipid peroxidation marker, together with a reduction in the intracellular ratio of reduced to oxidised GSH (Laight et al., 1999c). This pro-oxidant cocktail has also been shown to reduce plasma total antioxidant status in the rat (Laight et al., 1999d). Hence, while the assay of plasma cGMP does not distinguish between the causes or consequences of reduced NO signalling, NO-mediated vasodilation in vivo could reasonably be expected to be impaired following the pro-oxidant challenge in the present study.

However, our observation that hydroquinone + buthionine sulfoximine clearly elicits a more pronounced inhibition of vasodepression to acetylcholine than that to an NO donor points to a lesion in endothelium-dependent vasodilation, at least in peripheral resistance vessels of the Zucker rat, which is not entirely attributable to a defect in the vasodilator activity of NO. This notion is supported by the inability of hydroquinone + buthionine sulfoximine to modulate the vasopressor effect of the NO synthase inhibitor L-NAME, which indeed implies an intact regulation of peripheral vascular resistance by endogenous vascular NO. This additionally suggests that the hypertensive effect of hydroquinone + buthionine sulfoximine seen in obese animals is unlikely to be related to a deficiency in NO vasodilator signalling.

Furthermore, insofar as MAP may be regulated by endogenous basal NO (Rees et al., 1989), continuously derived from the endothelium during shear stress, the intact L-NAME vasopressor effect after hydroquinone + buthionine sulfoximine suggests that the primary pro-oxidant-mediated endothelial lesion may only concern the agonist — and in this study, muscarinic receptor-mediated stimulation of NO synthesis/release. Of course, a deficiency in the stimulation of other endothelium-derived relaxing factors such as prostacyclin and hyperpolarizing mediators could also account for the observed inhibition in endothelium-dependent vasodepression. This interpretation would be in good agreement with other reports describing a selective impairment in muscarinic receptor signalling in the endothelium in conditions associated with oxidant stress, such as atherosclerosis and diabetes mellitus (Stewart-Lee et al., 1994, 1995; Flavahan, 1992; Gazis et al., 1999). Such studies demonstrate that oxidant stress may evoke specific lesions in endothelial function in the absence of gross impairments in NO-mediated vasodilation.

In summary, a pro-oxidant challenge with hydroquinone + buthionine sulfoximine generates an impairment in agonist-stimulated endothelial function in the Zucker rat. Although there is biochemical evidence to support a reduction in endogenous vascular NO-cGMP signalling, this does not seem to be associated with any major disruption in NO-mediated vasodilation in vivo. While this pro-oxidant-induced pattern of endothelial dysfunction per se is apparently insufficient to generate insulin resistance de novo in the lean Zucker rat (see Laight et al. (1999b)), our data are nevertheless consistent with a role for a haemodynamic defect in the exacerbation of established insulin resistance observed in the obese Zucker rat following a pro-oxidant challenge. In conclusion, our study therefore suggests that endothelial dysfunction is a feature of the pro-oxidant-mediated conversion of insulin resistance syndrome to type II diabetes mellitus, with serious implications for insulin resistance and associated atherogenicity (see Laight et al., 1999a; Haffner et al., 2000).

Acknowledgements

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