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Adverse vascular remodelling is more sensitive than endothelial dysfunction to hyperglycaemia in diabetic rat mesenteric arteries

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ABSTRACT

Increased vascular stiffness and reduced endothelial nitric oxide (NO•) bioavailability are characteristic of diabetes. Whether these are evident at a more moderate levels of hyperglycaemia has not been investigated. The objectives of this study were to examine the association between the level of glycaemia and resistance vasculature phenotype, incorporating both arterial stiffness and endothelial function. Diabetes was induced in male Sprague Dawley rats with streptozotocin (STZ; 55 mg/kg i.v.) and followed for 8 weeks. One week post STZ, diabetic rats were allocated to either moderate (~20 mM blood glucose, 6–7 U/insulin s.c. daily) or severe hyperglycaemia (~30 mM blood glucose, 1–2 U/insulin s.c. daily as required). At study end, rats were anesthetized, and the mesenteric arcade was collected. Passive mechanical wall properties were assessed by pressure myography. Responses to the endothelium-dependent vasodilator acetylcholine (ACh) were assessed using wire myography. Our results demonstrated for the first time that mesenteric arteries from both moderate and severely hyperglycaemic diabetic rats exhibited outward hypertrophic remodelling and increased axial stiffness compared to arteries from non-diabetic rats. Secondly, mesenteric arteries from severely (~30 mM blood glucose), but not moderately hyperglycaemic (~20 mM blood glucose) rats exhibit a significant reduction to ACh sensitivity compared to their non-diabetic counterparts. This endothelial dysfunction was associated with significant reduction in endothelium-derived hyperpolarisation and endothelium-dependent NO•-mediated relaxation. Interestingly, endothelium-derived nitroxyl (HNO•)-mediated relaxation was intact. Therefore, moderate hyperglycaemia is sufficient to induce adverse structural changes in the mesenteric vasculature, but more severe hyperglycaemia is essential to cause endothelial dysfunction.

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Abbreviations: AGE, advanced-glycation endproducts; BH_4, tetrahydrobiopterin; BK_{Ca}, large conductance calcium-activated potassium channel; COX, cyclooxygenase; EDH, endothelium-derived hyperpolarisation; eNOS, endothelial nitric oxide synthase; GHB, glycated haemoglobin; HNO, nitroxyl; HXC, hydroxocobalamin; IK_{Ca}, intermediate conductance calcium-activated potassium channel; K_V, voltage-gated potassium channel; KcaB, a cocktail of TRAM-34, apamin and iberiotoxin; KPSS, potassium physiological saline solution; L-Cys, L-cysteine; MHG, moderate hyperglycaemia; NG, normal glycaemia; NO, •nitric oxide; NOS, nitric oxide synthase; Nox2, NADPH oxidase 2; ODQ, 1H-[1,2,4]oxadiazolo[4,3-*a*]quinoxaline-1-one; pEC_{50}, negative log of half-maximal effective concentration; PGI_2, prostacyclin; R_{max}, maximum relaxation; SHG, severe hyperglycaemia; sGC, soluble guanylate cyclase; SK_{Ca}, small conductance calcium-activated potassium channel; SNP, sodium nitroprusside; STZ, streptozotocin; WT, wall thickness.

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1. Introduction

Cardiovascular complications (both micro and macrovascular) are the leading cause of diabetes-related morbidity and mortality [1]. These vascular complications, including arterial stiffness (caused by adverse vascular remodelling) and endothelial dysfunction are regarded as critical initiating factors in the development of diabetic vascular complications [2,3], and may represent surrogate markers of prognosis.

Streptozotocin (STZ)-induced diabetes is a well-established animal model of experimental diabetes [4,5]. STZ causes pancreatic beta cell death due to DNA alkylation [6,7], resulting in marked hyperglycaemia in rodents [4]. Hyperglycaemia-induced oxidative stress causes arterial stiffness and endothelial dysfunction [8]. Although these vascular complications are well characterised in STZ-induced hyperglycaemic rats, the majority of studies only address severely hyperglycaemic rats (blood glucose $\geq$30 mM) [9–12]; the potential effects of more moderate hyperglycaemia on the vasculature are largely not considered. Therefore, understanding of the relationship between the level of glycaemia and vascular structure and function is incomplete.

Arterial wall stiffness is a common hallmark of diabetes and can increase the risk of further cardiovascular events such as myocardial infarction or stroke [13]. This increased stiffness results from adverse arterial remodelling, secondary to changes in the composition and structure of the arterial wall and resultant altered passive mechanical wall properties [14]. Diabetes increases the formation of advanced-glycation endproducts (AGEs) [15–17] which, by forming cross-links with the extracellular matrix protein collagen, renders the artery wall more rigid and less able to stretch [18]. Additionally, increased vascular collagen content [19] further exacerbates vascular stiffness [20,21].

The key role of the endothelium in regulating normal vascular tone is well-known; the release of the vasoactive factors such as prostacyclin (PGI_2), nitric oxide ($NO^\bullet$) and endothelium-derived hyperpolarisation (EDH) all contribute to vascular regulation. Reduced production of these endothelial-derived relaxing factors leads to endothelial dysfunction [2,22]. Emerging evidence suggests that the one-electron-reduced and protonated form of $NO^\bullet$, nitroxyl (HNO), is likely produced endogenously in the vasculature [23–25] and contributes to endothelium-dependent relaxation [26,27]. HNO stimulates soluble guanylate cyclase (sGC) in a similar manner to $NO^\bullet$ to elicit vasodilation [26,28,29]. In addition, it has been demonstrated that HNO activates K_v channels in vascular smooth muscle to stimulate hyperpolarisation and subsequent vasorelaxation [26,29].

In diabetic mesenteric arteries, it is well-established that EDH-type relaxation is impaired [9–12]. The residual component of relaxation, after the inhibition of both cyclooxygenase (COX) and calcium-activated potassium channels (K_{Ca}), is likely to be attributed to endothelial nitric oxide synthase (eNOS)-derived $NO^\bullet$, which is also reported to be impaired by diabetes [9,10,12]. Although both $NO^\bullet$ and HNO can be produced by eNOS, previous studies have failed to identify which nitrogen oxide is impacted by diabetes [9,10,12]. Thus it remains unclear whether $NO^\bullet$-mediated, HNO-mediated relaxation, or both, are impaired in diabetes-induced endothelial dysfunction in resistance vessels.

The aims of this study were to characterise the differential changes in arterial wall stiffness and endothelial function in the mesenteric artery induced by moderate versus severe hyperglycaemia in a rat model of experimental diabetes. Furthermore, as HNO offers greater resistance than $NO^\bullet$ to the detrimental consequences of oxidative stress [30,31], which is evident in diabetes [30,32,33]. Thus, we also sought to investigate if endothelium-derived HNO-mediated relaxation remains intact under hyperglycaemic conditions.

2. Methods

2.1. Animals

This investigation complied with the National Health and Medical Research Council (NHMRC) of Australia code of practice for the care and use of animals for scientific purposes. All procedures involved in this project were approved by the Alfred Medical Research Educational Precinct (AMREP) Animal Ethics Committee (approved E/1519/2014/B). Type 1 diabetes was induced in rats as previously described [34]. Briefly, adult male outbred Sprague Dawley rats (n = 50) obtained from AMREP animal services (bodyweight ~230 g; approximately 6 weeks of age) were randomly assigned to receive STZ to induce diabetes (55 mg/kg i.v., n = 35) or citrate buffer (normal glucose (NG), n = 15) via the tail vein following an overnight fast. One week following STZ, diabetic rats were further assigned to two groups: severe hyperglycaemia (SHG, n = 16) and moderate hyperglycaemia (MHG, n = 19) using a single daily insulin (Eli Lilly) injection (long-lasting Humulin NPH) to titrate blood glucose levels to >28 mM (1–2 units, s.c. per day) and ~20 mM (6–7 units, s.c. per day) as required, respectively. Blood glucose and body weight were monitored weekly. Following 8 weeks of diabetes or non-diabetic control, rats were euthanised with an intraperitoneal injection of ketamine-xylazine (100 and 12 mg/kg, respectively) and the mesenteric arcade was collected. Blood glucose and glycated haemoglobin (GHB) levels were measured using a one touch glucometer (Roche, Sydney, NSW, Australia) and Cobas HbA1c analyser (Roche, Sydney, NSW, Australia), respectively.

2.2. Passive mechanical wall properties ex vivo

Following euthanasia, a section of the mesenteric arcade was immediately placed in an ice-cold Ca^{2+}-free physiological saline solution (14.9 mM NaCl, 4.7 mM KCl, 1.7 mM $NaHCO_3$, 1.2 mM KH_2PO_4, 1.7 mM $MgSO_4$, 5 mM glucose, 10 mM HEPES and 2 mM EGTA) and cleared of fat and connective tissue. Third order mesenteric arteries were cleared of fat and connective tissue and leak-free segments were mounted on the cannula of a pressure myograph (Living Systems Instrumentation, Burlington, VT, USA). The lumen was gently flushed to remove any remaining blood, and the distal end occluded. Arteries were incubated at 37 °C for 20 min before measures of vessel wall parameters (vessel length, outer diameter [OD] and wall thickness [WT] were obtained, in 10 mmHg increments from 5 mmHg to 120 mmHg). Inner diameter (ID), wall stress and wall strain were calculated as described previously [35]. Volume distensibility was calculated as Δ volume/([Δ cross-sectional area $\times$ length] $\times$ Δ Pressure), where cross-sectional area was calculated as $(\pi \times ID^2)/4$ [35,36]. The% change in length with pressure was calculated using the following equation: %length = [(value at pressure)/(value at baseline)] $\times$ 100 [37].

2.3. Vascular reactivity ex vivo

The remaining mesenteric arcade was immediately placed in an ice-cold Kreb's bicarbonate solution containing (in mmol/l) NaCl 120, KCl 5, $MgSO_4$ 1.2, KH_2PO_4 1.2, $NaHCO_3$ 25, D-glucose 11.1, $CaCl_2$ 2.5, EDTA 0.0026. Indomethacin (10 μmol/l), a non-selective cyclooxygenase (COX) inhibitor to inhibit the synthesis of prostanoids, was present in the Kreb's solution at all times. Third order mesenteric arteries were cleared of connective fat and tissue, cut into 2 mm rings and mounted on a Mulvany-Halpern wire myograph (model 610 M; Danish Myo Technology, Aarhus, Denmark).

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