Myogenic tone and small artery remodelling: insight into diabetic nephropathy

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Introduction

Diabetic nephropathy is the single most frequent cause of end-stage renal disease (ESRD) in the western world, with an estimated cost in excess of $15.6 billion per annum in the United States alone [1]. In anticipation of an obesity-related diabetes epidemic, coupled with progressively growing rates of hypertension, these figures are forecast to rise exponentially. As both diabetes and renal disease are increasingly recognized as generalized vasculopathic states, there has been renewed interest in identifying potential vascular mechanisms influencing renal damage.

The relationship between diabetes, hypertension and kidney disease is complex, progressive and reciprocal. In type 1 diabetes, the prevalence of microalbuminuria increases from the onset of disease, reaching 50% after 20 years [2], whilst in type 2 diabetes, it is stable at 20–25% [3]. Microalbuminuria is a powerful marker for progression to overt nephropathy [4–6] and renal function continues to decline in 30% of microalbuminuric patients with either type 1 [7] or type 2 diabetes [6]. Whilst in patients with type 1 diabetes, good glycaemic control [8] or relative youth [7] is associated with the remission of microalbuminuria, in patients with type 2 diabetes, institution of antihypertensive therapy is also important [6]. Once nephropathy is established however, ESRD occurs in 75% of individuals within 20 years [1]; at which point the cardiac mortality risk is 20-fold higher than that of the general population [9]. Thus, the stage at which diabetic renal disease is most likely to be arrested and even reversed is at the point at which microalbuminuria is detected by the clinician. The reversibility of microalbuminuria suggests an associated vascular dysfunction rather than a more permanent structural defect, regression of which may be harder to achieve. Therefore, preventative measures and early intervention are essential in reducing the impact of hypertensive renal damage in diabetic individuals.

Prevention strategies are difficult to implement because of the high susceptibility of diabetic individuals to develop microvascular disease, which is frequently encountered with even minor elevations in blood pressure [10]. Furthermore, declining renal function has been associated consistently with elevated blood pressure in pre-diabetic [11] and diabetic populations [12]. In consequence, whilst it is likely hyperglycaemia and metabolic derangements are the initial vascular insults that lead to kidney damage [13], hypertension then advances disease progression [14]. Correspondingly, antihypertensive treatment replaces hypoglycaemic agents as the most important therapeutic intervention after the diagnosis of nephropathy has been made [15].

Against this background, glomerular haemodynamic derangements are perceived as a key to the development and progression of diabetic renal disease [16]. To understand the interactions between central arterial blood pressure and the local autoregulation of blood flow, it is necessary to examine the mechanisms of renal glomerular haemodynamics both in health and in disease states such as hypertension and diabetes.

Renal haemodynamics: clinical correlates

The glomerular filtration rate (GFR) and passage of albumin into the urine are both dependent upon renal blood flow (RBF) and the structural integrity of the glomerular membrane barrier. Large fluctuations in RBF occur in parallel with variations in arterial blood pressure during the cardiac cycle; in normotensive adults, this ranges from ~80 mmHg during diastole to 125 mmHg during systole. In hypertensive individuals and in particular those with high pulse pressures due to arterial stiffening, these fluctuations are more pronounced. Under normal circumstances, the kidney possesses the ability to achieve a stable renal blood flow,
despite these variations in the mean arterial blood pressure, so-called renal autoregulation [17] (illustrated in Figure 1).

Very early on in the natural history of diabetic nephropathy, there is a subclinical stage of renal hyperfiltration, characterized by an increased GFR [18]. Hyperfiltration has been reported in as many as 66% [19] and 50% [20] of type 1 and type 2 diabetic patients, respectively, and can be reversed by normalizing hyperglycaemia [21]. As hyperfiltration precedes the development of glomerular damage, albuminuria and declining renal function [18], there have been suggestions that dysfunctional autoregulation of GFR has a causal role in diabetic nephropathy [16]. Thirty years ago, Parving et al. [22] demonstrated that following acute reduction in blood pressure by administration of clonidine, diabetic individuals experienced significant reductions in both blood pressure, renal blood flow, systolic and diastolic blood pressure [23]. This occurred despite similar reductions in arterial blood pressure in both groups, so providing direct evidence for impaired regulation of GFR in type 1 diabetes. This was later reproduced in a cohort of type 2 diabetic patients [23].

The principle determinant of GFR is the glomerular capillary pressure (\(P_{GC}\)), which in turn is dependent on autoregulation of RBF. RBF is controlled by two mechanisms: a rapid myogenic response of the renal afferent arteriole (0.1–0.3 Hz) and a slower tubuloglomerular feedback (0.02–0.05 Hz). A substantial degree of RBF autoregulation therefore occurs at the level of the renal afferent arteriole: in consequence, changes in GFR partly reflect changes in renal arteriolar resistance. However, there are several caveats in drawing conclusions about the haemodynamics of the renal microcirculation from clinical values of GFR alone. For example, a normal GFR may be the result of compensatory hyperfunction in a decreased number of nephrons [24] or a damaged glomerular membrane barrier and should not therefore be assumed to reflect normal vascular function.

Although the diagnosis of diabetic nephropathy is defined by damage specifically to the kidneys, this complication rarely occurs in isolation and patients are more frequently seen to suffer from widespread vascular disease [25]. Correspondingly, the progressive change in urinary protein excretion from micro- to macroalbuminuria also strongly correlates with heart disease, stroke and general cardiovascular mortality [26]. Although the mechanism by which proteinuria confers an increased cardiovascular risk is uncertain, its predictive value for both global vascular and renal disease implies a common aetiology for subsequent target organ damage. To this end, an understanding of the significance of small artery structure and function throughout the vasculature, in addition to those factors that may influence them, is essential to fully appreciate those mechanisms involved in renal autoregulation of blood pressure.

**Small artery structure and function: fundamental principles**

Functional studies of blood vessels have shown that small arteries, that is, those arteries with a relaxed internal diameter <300 \(\mu\)m, possess the ability to respond to a range of physiological pressures with a level of contraction independent of neurohormonal influences. This process is known as the myogenic response and is only observed in small resistance arteries that determine peripheral vascular resistance. This function can be assessed in arteries in vitro using pressure myography, and a typical pressure/diameter relationship is shown in Figure 2. As intraluminal pressure is increased within the artery, the vascular smooth muscle cells in the wall constrict, so reducing the diameter of the lumen. This occurs independent of neural control and in the absence of endothelium and so is considered an intrinsic function of the vessel wall [27].

There are considered to be three phases of myogenic behaviour [28]. The first consists of the development of myogenic or basal tone. This is associated with a large increase in intra-cellular calcium and changes to the membrane potential via influx through L-type voltage-gated calcium channels. The second phase, known as myogenic
reactivity, is a further constriction in response to an increase in intraluminal pressure. Intra-cellular concentrations of calcium do not change during this phase, but calcium sensitization of mechanical apparatus inside the cell is thought to occur. The third phase occurs when the wall of the artery is unable to maintain a constriction against mounting pressures and is known as forced dilation. The signalling pathways involved in the initiation, maintenance and control of myogenic tone and autoregulation are myriad and include changes to intra-cellular calcium, protein kinases, diacylglycerol and modulation of ion and TRP-like channels (for a review, see Hill et al. [29]).

The myogenic response of resistance arteries is intricately linked to changes in wall structure. This is best demonstrated by observing both functional and structural changes to the small artery wall in hypertension. The hallmark of essential hypertension is an increase in peripheral vascular resistance while cardiac output is normal. In most forms of uncomplicated hypertension, such as the onset of high blood pressure or milder hypertensive states, these changes involve a decrease in internal lumen diameter with an increase in the medial wall thickness, termed eutrophic inward remodelling (Figure 3). Contrary to traditional opinion, this occurs by rearrangement of the existing wall constituents in the absence of any true hypertrophy or hyperplasia, and therefore the medial cross-sectional area remains unchanged. The development of hypertension will clearly imply that there will be prolonged periods of myogenic constriction and this is considered key to the development of inward eutrophic remodelling and/or reduced arterial distensibility. Larger arteries do not undergo prolonged constriction and do not autoregulate, and in consequence, the increased wall stress that pressure exerts upon them is offset by smooth muscle hypertrophy. Human structural and functional studies have predominantly been based on the analysis of small blood vessels harvested from subcutaneous fat [30], although observed changes are reflective of vascular remodelling and functional deficits in the mesenteric [31], coronary [32] and cerebral [33] arteries from patients with hypertension and diabetes. In animals, eutrophic inward remodelling has been reported from the mesenteric vessels of the spontaneously hypertensive rat (SHR) [34] and intramyocardial arteries from a porcine reno-vascular model of hypertension [35].

In some pathological states, there is hypertrophy of the resistance arteries, which may supervene completely and is an adverse prognostic sign. Currently, we believe that these structural changes are determined by the integrity of the myogenic response; when this reflex is intact, eutrophic inward remodelling is the physiological response to raised blood pressure. However, if this reflex breaks down or accelerated pressure overwhelms the ability to autoregulate, then a blood vessel is unable to withstand the increased wall stress and replaces eutrophic inward remodelling with hypertrophy (Figure 3). The result of dysfunctional autoregulation and/or hypertrophy of resistance arteries would be a failure to provide a reduced luminal diameter in response to high blood pressures, in the acute or chronic setting. The laws of Laplace and Poiseuille imply that both flow and generated wall tension are primarily influenced by radius of the vessel. Therefore, in an individual with an appropriate myogenic response and eutrophic remodelling, a reduced lumen diameter and thus flow rate will have a protective effect on the organ that lies downstream. However, in patients where myogenic autoregulation fails and whose arterial remodelling does not result in a lumen of reduced diameter, central pressures elevated by hypertension will be transferred directly to the microcirculation. The resultant passive pressure microcirculatory bed [36] will lead to pressure-related damage in susceptible organs, such as the retina or glomeruli. Therefore, the development of hypertension signals a functional disorder and imminent target organ damage. The relationship between luminal diameter and target organ damage has been addressed in large-scale studies, in which retinal vessel calibres were measured in 5979 subjects (821 with diabetes). It was found that patients with diabetes have larger arteriolar and venular calibres [37] compared with vessels from healthy subjects and in these individuals, larger retinal vessel calibre predicts progression of diabetic retinopathy [38] and nephropathy [39].

Recently, a number of studies have supported this idea, by demonstrating the prognostic importance of the myogenic response and relating this to alterations in small artery structure. We have demonstrated that the myogenic response breaks down in the stroke-prone spontaneously hypertensive rat (SHRSP) and before the development of cerebral injury. Consequently, there is associated vascular wall hypertrophy and alterations in the distribution of collagen throughout the vascular wall [40]. Studies of small arteries from patients with type 2 diabetes with and without hypertension have demonstrated that in both groups of individuals, myogenic tone is severely impaired and in
Is myogenic tone responsible for autoregulation of RBF?

An intact myogenic response in the renal afferent arteriole is integral to stabilizing pressure and so preventing glomerular capillary hypertension and consequent proximal nephropathy. Several studies support this using a variety of different techniques to quantify autoregulation. In addition to pressure myography [47], investigators have used direct visu-

Fig. 4. Active pressure diameter in patients with hypertension and diabetes [41]. Pressure-lumen diameter relations in subcutaneous resistance arteries from (1) control subjects, (2) patients with essential hypertension and (3) patients with type II DM and hypertension. There is a significant differ-
ence in the active pressure diameters between patients with type 2 diabetes and hypertension patients with hypertension alone (P < 0.05). Control subjects and patients with essential hypertension demonstrate a reduction in lumen diameter in response to increasing intraluminal pressures (myo-
genic response). In patients with type 2 diabetes, this reduction in lumen diameter does not occur, reproduced with kind permission from Schofield et al. [41].

Fig. 5. Afferent arteriole constrictions in response to varying re-
al arteriole pressures in kidneys from control (empty circles, N = 35), diabetic (filled squares, N = 60) and insulin-treated diabetic (filled triangles, N = 23) rats. Diameters are expressed in both absolute terms (microns, left) and percentage change from basal (i.e. 80 mmHg) diameter (right). Pressure-induced vasoconstriction was markedly blunted in DM kidneys compared with that in controls. Treatment with insulin restored pressure responsiveness substantively. Results are mean ± SE. Reproduced with kind permission from Hayashi et al. [50].

The fawn-hooded rat (FHR) also displays reduced myo-
genic tone within the renal vasculature [47] and con-
sequently develops proteinuria and glomerular sclerosis despite only having moderate hypertension, resulting in renal failure and, ultimately, death at a young age [59,60].
This is in contrast to the spontaneously hypertensive rat (SHR), which has much higher systemic blood pressures but exhibits a significant increase in preglomerular arteriolar resistance due to an intact myogenic response. This provides protection against the transmission of high systemic pressures to the glomeruli by maintaining normal glomerular capillary pressure, single nephron GFR [51], and therefore preventing against the development of proteinuria [61,62]. At a similar level of systemic hypertension to the SHR, the hypertensive Dahl salt-sensitive (DS) rat experiences glomerular capillary hypertension and increase in single nephron GFR, which corresponds with a decreased preglomerular arteriolar resistance [51]. Furthermore, when mesangial immune injury is inflicted upon these two strains, the DS rat develops hypertensive glomerular injury, whilst the SHR appears to be protected. However, when preglomerular arteriolar vasoconstriction is experimentally reduced following uninephrectomy in the SHR [52], glomerular injury ensues. It is of interest that the only animal model reported to have an impaired myogenic response in the absence of target organ damage is the Brown Norway rat (BNR) [49]. This is most likely explained by its normal blood pressure, so that although susceptible to damage, the glomerular capillary network is not required to autoregulate at high arterial pressures. Indeed, when the kidney from this animal is subjected to high systemic pressures, for example following transplantation into the SHR, glomerular injury follows [48].

Also contributing to local renal autoregulation is tubuloglomerular feedback (TGF), which involves constrictions of preglomerular vessels in response to increased flow within distal renal tubules; executed through purinergic mediated changes in vascular tone [63]. Current consensus suggests that both phenomena are involved in renal autoregulation but hold the myogenic mechanism responsible for responding to elevations in systolic blood pressure found in hypertension and therefore more important in haemodynamic protection. This has been supported by mathematical models closely representing physiological responses (as reviewed by Loutzenhiser et al. [64]). Indeed, studies in the hydronephrotic kidney have observed intact autoregulation in the absence of a TGF [53] and conversely, the FHR develops target organ damage and proteinuria despite an intact TGF response [65].

Mechanisms responsible for wall remodelling and myogenic tone

The mechanisms responsible for eutrophic inward remodelling, in contrast to those of myogenic tone, are less well understood. The vascular extracellular matrix is subject to tensile force produced by blood pressure that must be transferred through integrins across the cell membrane and linked by signalling complexes to the cytoskeleton. Specific integrin subtypes are initially used to transduce pressure and it has been shown by the use of peptides and antibodies that integrins a3b1 and a5b1 indirectly regulate the myogenic response by the control of Ca^{2+} flow through ion channels [27] (see Figure 6). a3b1 is responsible for the initial calcium influx required to establish basal vessel tone and a5b1 to mediate force maintenance via Ca^{2+} sensitization of contractile components during myogenic reactivity [27] (see Figure 2). These integrins can form complexes that regulate cytoskeletal dynamics that maintain a vascular myogenic force at a given pressure. This is reduced by cytoskeletal disruption. Eutrophic inward remodelling must involve a migratory process after prolonged constriction whereby existing vascular smooth muscle cells re-position themselves in the vascular wall. A characteristic of migrating cells is the presence of lamellipodial and filopodial protrusions containing focal adhesion kinases (FAKs) that produce a substrate for other cytosolic proteins [66].

Integrin a3b1 is necessary for the pressure-induced inward remodelling process: it is over-expressed during development of hypertension in SHRs [67] and a b3 antibody enhances arterial remodelling induced by endothelin-1 [68]. The integrin a3b1 binds fibronectin, another component of the extracellular matrix, and also mediates activation of the L-type calcium current resulting in calcium influx [69]. Both a3b1 and fibronectin are also over-expressed in the SHR [70] and inhibition of the fibronectin matrix assembly disrupts the growth of vascular smooth muscle cells [71]. The Dahl salt-sensitive rat, which has a propensity to hypertensive renal injury, also shows upregulation of the profibrotic growth factor TGF-b1 that is associated with deposition of fibronectin and production of collagen [72].

The TGR(mRen2)27 rat develops severe hypertension between 4 and 8 weeks of age [73]. Concurrently, small arteries from these animals undergo eutrophic inward remodelling between 5 and 8 weeks [73,74]. As this process occurs, there is upregulation of the a3 integrin subunit in the media of the arterial wall that dimerizes with subunits b3 and b1 to form a fibronectin receptor [73]. If a3b1 is inhibited during the development of hypertension by intraperitoneal administration of a blocking peptide, eutrophic remodelling is abolished and although growth of the artery occurs, it is in an outward rather than inward fashion, in

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**Fig. 6.** Representative tracing showing the effect of changes in intraluminal pressure on arteriolar diameter occurring in the absence or presence of an anti-αβ3-integrin function blocking antibody. Reproduced with kind permission from Martinez-Lemus et al. [27].
other words hypertrophy takes place [73] (see Figure 7). Although the relationship between myogenic tone and arterial remodelling in small arteries has not yet been clarified, it is interesting to see that the same integrins, $\alpha_5\beta_1$ and $\alpha_v\beta_3$, are necessary for both functions. Furthermore, the propensity to develop renal damage in patients with type 1 diabetes is associated with polymorphisms in the integrin-binding domain [75].

In addition to the integrins, the epithelial sodium channel (ENaC) and related proteins such as the acid-sensing ion channel (ASIC) proteins have also received attention for their mechanosensing and chemosensing properties [76]. Traditionally investigated for their role in the regulation of blood pressure through control of renal salt and water reabsorption, they are now felt to have a role in myogenic autoregulation also. Through gene-specific silencing, Jernigan et al. have demonstrated that this protein family is required for myogenic constriction of isolated renal interlobular arteries [77]. Further, myogenic constriction is absent in cerebral arteries of ASIC2 knockout mice [78].

**Therapeutic and clinical implications**

It is well documented that diabetic individuals experience a higher incidence of cardiovascular complications than non-diabetic individuals with equivalent blood pressures. Based on large clinical studies, such as HOT [79] and UKPDS [10], hypertension guidelines recommend that individuals with diabetes should aim to maintain blood pressures below 130/80 mmHg [80]. Furthermore, nephroprotection from antihypertensive medication only becomes apparent in mild hypertension if there is concomitant diabetes [81]. In consequence, individuals with diabetes and microalbuminuria [82] or high cardiovascular risk [83] are advised to commence antihypertensive management regardless of their blood pressure. The mechanisms underlying these clinical observations and management rationale are, however, unknown.

We believe that the explanation lies in the small arteries, where diabetes causes loss of the myogenic response, therefore resulting in dysfunctional autoregulation of blood flow and subsequent damage to respective organs. This occurs following even minor elevations in blood pressure and explains the cluster of retinopathy, nephropathy and global vascular disease frequently observed in diabetic individuals. Developing microvascular complications in one vascular bed is predictive of further disease in others [25]. Against this background, the established associations between microalbuminuria and surrogate renal [4] and cardiovascular [26] endpoints makes microalbuminuria a likely marker of defective small artery autoregulation in the kidneys and therefore throughout the vasculature. Furthermore, cross-sectional studies have confirmed that diabetic patients with microalbuminuria have higher GFR values than those without [84]. It is therefore possible that diabetics with normalalbuminuria represent those individuals with preserved myogenic autoregulation or normal blood pressure, who are thereby protected against target organ damage. This would provide an explanation for the strong associations between microalbuminuria, renal and vascular disease, currently unexplained by established risk factors [85].

Epidemiological studies, such as the AusDiab project, have shown clearly that target organ damage precedes the diagnosis of type 2 diabetes, with renal [86] and retinal [87] damage seen in patients with impaired glucose tolerance. If myogenic tone and small artery remodelling are affected, and indeed responsible for target organ damage at this early stage of disease progression, one may speculate that early therapeutic intervention to correct the vascular defect would be more likely to succeed than later attempts, when the patient has established type 2 diabetes. Studies of human subcutaneous fat arteries [88] and rat middle cerebral arteries [34] have shown potential for the reversal of remodelling and improvement in myogenic autoregulation [44] using anti-hypertensive therapy, with therapies targeting the renin-aldosterone axis having greater effects than other classes of drugs. Furthermore, a recent...
study demonstrated improvement in the myogenic response of mesenteric arteries in subtotally nephrectomized Wistar rats, following administration of an ACE inhibitor; with consequent reduction in levels of proteinuria [46]. This correlates with various large-scale interventional studies, which have confirmed the clinical efficacy of angiotensin blockade in reducing proteinuria more effectively than traditional anti-hypertensive medications and with nephroprotective benefits independent of blood pressure lowering [89–91]. The influence of early intervention on GFR and small artery function in diabetic individuals with hyperfiltration will further elucidate the associations between arterial function, glomerular filtration and microalbuminuria.

Changes in small artery autoregulation precede damage to the glomerular filtration barrier in diabetic individuals. Concurrent elevations in systolic blood pressure lead to a rise in glomerular capillary pressure, thereby increasing permeability to proteins and initiating hyperfiltration. Glomerular hypertension damages capillaries and endothelium, and initiates a rise in protein glomerular filtration, which is itself nephrotoxic. Over time, changes to mesangial and proximal tubular cells supervene, resulting in fibrotic changes to the connective tissue, characteristic of advanced diabetic nephropathy [92].

Future directions for research

Animal studies have indicated that both genetic [47] and environmental factors [40] can be responsible for deficits in myogenic autoregulation. It is unlikely that diabetes is the only clinical condition that has a detrimental effect on myogenic autoregulation. Recognizing those patients with evidence of microvascular disease not explained by diabetes will further establish the extent to which damage to small artery myogenic autoregulation is responsible for generating chronic disease states. This, when coupled with the identification of therapies capable of reversing structural changes and improving small artery function, will ultimately enable effective management of this increasing cohort of patients.

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