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ALTERED ELECTROMECHANICAL COUPLING IN THE RENAL MICROVASCULATURE DURING THE EARLY STAGE OF DIABETES MELLITUS

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SUMMARY

1. The early stage of Type 1 diabetes mellitus (DM) is characterized by renal hyperfiltration, which promotes the eventual development of diabetic nephropathy. The hyperfiltration state is associated with afferent arteriolar dilation and diminished responsiveness of this vascular segment to a variety of vasoconstrictor stimuli, while efferent arteriolar diameter and vasoconstrictor responsiveness are typically unaltered.

2. The contractile status of preglomerular vascular smooth muscle appears to be tightly coupled to membrane potential (E_m) and its influence on Ca^{2+} influx through voltage-gated channels. Efferent arteriolar tone is largely independent of electromechanical events. Hence, defective electromechanical mechanisms in vascular smooth muscle should engender selective changes in preglomerular microvascular function such as those evident during the early stage of DM.

3. Afferent arteriolar contractile responses to K^+ -induced depolarization and BAY K 8644 are diminished 2 weeks after onset of DM in the rat. Similarly, depolarization-induced Ca^{2+} influx and the resulting increase in intracellular $[Ca^{2+}]$ are abated in the preglomerular microvasculature of diabetic rats. The intracellular $[Ca^{2+}]$ response to depolarization is rapidly restored by normalization of extracellular glucose levels. These observations suggest that hyperglycemia in DM impairs regulation of afferent arteriolar voltage-gated Ca^{2+} channels.

4. Dysregulation of E_m may also contribute to afferent arteriolar dilation in DM. Vasodilator responses to pharmacological opening of ATP-sensitive K^+ channels are exaggerated in afferent arterioles from diabetic rats. Moreover, blockade of these channels normalizes afferent arteriolar diameter in kidneys from diabetic rats. These observations suggest that increased functional availability and basal activation of ATP-sensitive K^+ channels promote afferent arteriolar dilation in DM.

5. We propose that dysregulation of E_m (involving ATP-sensitive K^+ channels) and a diminished Ca^{2+} influx response to depolarization (involving voltage-gated Ca^{2+} channels) may act synergistically to promote preglomerular vasodilation during the early stage of DM.

Keywords

afferent arteriole; ATP-sensitive potassium channels; diabetes mellitus; efferent arteriole; rat; renal circulation; voltage-gated calcium channels

INTRODUCTION

Alterations in renal function represent a primary complication of type 1 diabetes mellitus (DM). The renal consequences of DM evolve with disease duration. In humans, renal hypertrophy and glomerular hyperfiltration characterize the initial 5–7 years after onset of DM. Diabetic hyperfiltration is believed to contribute to the appearance of microalbuminuria, the hallmark of impending progression to more severe functional and morphologic glomerular injury. Most investigators concur that hyperfiltration or a consequent maladaptation to this process engenders the decline in glomerular filtration rate and development of diabetic glomerulopathy in 20–40% of individuals with DM. However, the mechanism underlying diabetic hyperfiltration remains poorly understood.

Myriad investigative efforts have failed to provide compelling evidence that any single vasoactive agent or membrane-bound receptor is responsible for the development of diabetic hyperfiltration. Indeed, decreased renal vascular resistance and diminished responsiveness to numerous vasoconstrictor stimuli are evident during the early stage of DM. In rats with streptozotocin-induced DM, the renal vasodilation is localized to the preglomerular vasculature while efferent arteriolar resistance is either normal or minimally decreased,^{1,2} a situation that promotes glomerular hypertension and hyperfiltration. In this review, we summarize evidence suggesting that alterations in electromechanical control of vascular smooth muscle (VSM) tone contribute to the selective preglomerular vasodilation that occurs during the early stage of DM.

VASCULAR SMOOTH MUSCLE SIGNALING EVENTS IN THE NORMAL KIDNEY

Whole kidney studies

In vivo studies employing systemic or intrarenal infusion of pharmacologic agents that influence intracellular Ca^{2+} signaling have uniformly indicated that renal vascular resistance is regulated through both Ca^{2+} -mobilization and Ca^{2+} -influx.³ Navar and coworkers⁴ first suggested that the impact of Ca^{2+} -channel blockade on angiotensin II (AngII)-dependent renal vascular tone is restricted to the preglomerular vasculature and that AngII-dependent influences on efferent arterioles might be insensitive to Ca^{2+} -channel blockade. These considerations led to the postulate that the pre- and postglomerular vasculature might be differentially reliant on Ca^{2+} -mobilization and Ca^{2+} -influx in achieving regulation of contractile tone and in responding to vasoconstrictor agonists.

Preglomerular microvascular smooth muscle (PMVSM) signaling events

Fluorescence imaging studies using fura-2 as an indicator of intracellular Ca^{2+} concentration ($[\text{Ca}^{2+}]_i$) in isolated afferent arterioles first revealed that AngII evokes an initial rise in $[\text{Ca}^{2+}]_i$ that is sensitive to dantrolene (Ca^{2+} release blocker), and a sustained response that was dantrolene-insensitive.⁵ More recently, depletion of the intracellular Ca^{2+} store has been shown to abolish the afferent arteriolar constrictor response to AngII,^{6,7} a process that is also sensitive to phospholipase C inhibition.⁷ These studies suggest that PMVSM responses to AngII involve IP_3 -dependent Ca^{2+} -mobilization.

Ca^{2+} -mobilizing agonists such as AngII rely on Ca^{2+} influx to maintain elevations in $[\text{Ca}^{2+}]_i$ and VSM tone. Organic compounds that block voltage-gated Ca^{2+} channels (VGCCs) are potent preglomerular vasodilators and curtail or abolish every afferent vasoconstrictor response thus far tested.³ These findings underscore the functional importance of VGCCs in the regulation of preglomerular microvascular resistance under normal conditions. Indeed, whole cell voltage-clamp studies have revealed L -type and T -type voltage-sensitive Ca^{2+} currents in PMVSM cells, with current densities that are relatively large compared with VSM cells from

other sources.⁸ Recent evidence indicates that P-type VGCCs are also present in the afferent arteriole.⁹ While voltage-independent pathways are undoubtedly available to allow Ca^{2+} influx in PMVSM cells, the overwhelming mass of data indicates that VGCCs represent the primary Ca^{2+} influx pathway in these cells.

Because Ca^{2+} influx through VGCCs is governed by membrane potential (E_m), regulation of this parameter is crucial for appropriate control of preglomerular microvascular tone. Sarcolemmal K^+ conductance is a primary determinant of E_m in PMVSM cells, which express at least 4 types of K^+ channels. Little investigative attention has focused on elucidating the functional impact of inwardly rectifying K^+ channels and voltage-dependent K^+ channels in PMVSM cells; however, ATP-sensitive K^+ channels (K_{ATP} channels) and Ca^{2+} -activated K^+ channels (K_{Ca} channels) have attracted more interest. K_{ATP} channels in VSM are envisioned to provide a mechanism that links glycolysis-derived ATP to the control of vascular resistance. Pharmacological evidence suggests that K_{ATP} channels are capable of influencing afferent arteriolar resistance,^{10,11} and hyperpolarization induced by opening of K_{ATP} channels has been suggested to impair afferent arteriolar myogenic responses during hypoxia.¹¹ K_{Ca} channels are prominent in PMVSM cells,¹² providing a mechanism whereby P450 metabolites of arachidonic acid exert their influence on E_m and vascular tone.^{13,14} Opening of K_{Ca} channels during agonist-induced constriction is presumed to limit membrane depolarization, although this mechanism does not seem to represent a major determinant of the afferent arteriolar constrictor response to depolarizing agonists.¹⁵ In contrast, gating of Ca^{2+} -activated Cl^- channels (Cl_{Ca} channels) appears to play an important role in eliciting agonist-induced depolarization of PMVSM cells.^{8,16} Cl_{Ca} channel blockade impairs endothelin-stimulated depolarization of PMVSM cells⁸ and afferent arteriolar contractile responses to AngII, endothelin and norepinephrine.^{17,18,19,20} Decreasing extracellular $[\text{Cl}^-]$ exaggerates afferent arteriolar constrictor responses to AngII and norepinephrine,²⁰ while removal of extracellular Cl^- attenuates these responses (apparently by reducing intracellular $[\text{Cl}^-]$).²¹ Thus, opening of Cl_{Ca} channels seems to contribute to the PMVSM depolarization evoked by Ca^{2+} -mobilizing agonists, although few vasoconstrictor agents have been evaluated in this regard.

Efferent arteriolar signaling events

Preglomerular and efferent arteriolar VSM cells appear to differ with regard to the mechanisms exploited to achieve elevations in $[\text{Ca}^{2+}]_i$. Most studies indicate that the efferent arteriole is refractory to the actions of VGCC agonists and antagonists, as well as K^+ -induced depolarization.³ Figure 1 illustrates typical $[\text{Ca}^{2+}]_i$ responses of rabbit afferent and efferent arterioles to membrane depolarization achieved by increasing extracellular $[\text{K}^+]$ from 5 to 100 mM. K^+ -induced depolarization evokes increases in afferent arteriolar $[\text{Ca}^{2+}]_i$ that require extracellular Ca^{2+} and are blocked by 1 μM nifedipine, behavior indicative of Ca^{2+} influx through VGCCs in afferent arteriolar VSM.²² In contrast, efferent arterioles do not exhibit increased $[\text{Ca}^{2+}]_i$ in response to membrane depolarization; rather, a slight decline in $[\text{Ca}^{2+}]_i$ is typically observed.²² This behavior is consistent with a depolarization-induced reduction in the electrochemical gradient driving the continuous Ca^{2+} “leak” into the cytoplasm of cells lacking functional VGCCs. These observations provide a striking distinction between intracellular signaling processes utilized by the VSM populations comprising afferent and efferent arterioles. Although a few studies have reported efferent vasoconstrictor responses to K^+ -induced depolarization,^{23,24} efferent arteriolar contractile responses to AngII, norepinephrine and endothelin are typically unaffected by VGCC blockers.^{23,25,26} Moreover, although AngII evokes depolarization of afferent arteriolar VSM, the peptide does not alter E_m in efferent arteriolar VSM.²⁷ Hence, depolarization-induced Ca^{2+} entry is a minor participant in efferent arteriolar vasoconstriction. Some evidence suggests that AngII provokes Ca^{2+} -influx into efferent arteriolar VSM via a DHP-insensitive pathway,^{5,7} apparently a store-operated Ca^{2+} channel.²⁸ Efferent arteriolar vasoconstrictor responses to AngII and

norepinephrine also appear to involve Ca^{2+} release from intracellular stores,^{5,6,23} although the issue of whether this requirement is absolute or partial remains unsettled.

RENAL VASCULAR Ca^{2+} -SIGNALING IN DIABETES

Because of the apparent segment-specific functional distribution of VGCCs within the renal microvasculature, a defect in expression or regulation of these channels should selectively influence preglomerular function. Indeed, the renal microvascular effects of pharmacological VGCC blockade are reminiscent of the functional changes that engender hyperfiltration during the early stages of DM. Videometric studies in our laboratory have confirmed that juxtamedullary afferent arterioles are dilated and exhibit reduced constrictor responsiveness to norepinephrine during diabetic hyperfiltration, while these parameters of efferent arteriolar function remain unaltered.²⁹ DM also impairs afferent arteriolar myogenic and tubuloglomerular feedback responses,^{30,31} as well as autoregulation of renal blood flow.³² These processes are all normally subject to potent inhibition by VGCC blockers and are almost exclusively localized to the preglomerular microvasculature.³ Based on these considerations, it is intriguing to speculate that a defect in VGCC activity, or in the regulation of E_m in renal PMVSM cells, might contribute to the altered renal hemodynamic function evident during the early stages of DM.

Evidence for altered VGCC function

Few studies have examined by direct methods the impact of DM or hyperglycemia on VGCC activity. Ventricular myocytes from diabetic rats have been reported to exhibit normal³³ or reduced³⁴ I_L -type VGCC current densities. Forty-eight hour glucose infusion reduces I_L -type VGCC mRNA and blunts the response to BAYK8644 in rat pancreatic β -cells, suggesting that hyperglycemia may directly reduce channel expression.³⁵ However, the literature contains conflicting evidence regarding the number of dihydropyridine binding sites (a reflection of I_L -type VGCCs) in diabetic rat heart and skeletal muscle.^{36,37,38}

Bank et al.³⁹ first suggested that insulinopenia in DM might impair Ca^{2+} movement through VGCCs in the renal vasculature. Cultured rat aortic VSM cells exposed to high extracellular glucose concentrations for >12-hr exhibit suppressed voltage-sensitive and agonist-induced $^{45}\text{Ca}^{2+}$ uptake responses,⁴⁰ an observation linking hyperglycemia with depressed VGCC activation. We have employed two complimentary experimental approaches to examine the postulate that impaired afferent arteriolar responsiveness to opening of VGCCs occurs during the hyperfiltration stage of streptozotocin-induced DM in the rat (2 wk after onset).

First, afferent arteriolar contractile responses to increasing bath concentrations of BAYK8644 were monitored by videomicroscopy using the in vitro blood-perfused juxtamedullary nephron technique. In this experimental setting, the renal microvasculature exhibits basal tone that involves voltage-gated Ca^{2+} influx. Hence, the ability of BAYK8644 to prolong opening of VGCCs results in afferent arteriolar constriction. In kidneys harvested from normal rats, BAYK8644 evoked concentration-dependent reductions in afferent arteriolar diameter that achieved statistical significance at 1 nM. In contrast, 100 nM BAYK8644 was required to contract afferent arterioles from rats with DM.⁴¹ Figure 2 illustrates the impact of DM on afferent arteriolar contractile responses to 10 nM BAYK8644, indicating that these vessels are less sensitive to pharmacological activation of VGCCs during the early stage of DM. Because the impact of BAYK8644 relies in part on the activation state of the cells, additional experiments examined the impact of DM on the vasoconstrictor response to K^+ -induced membrane depolarization. In kidneys from both normal and diabetic rats, stepwise increases in bath $[\text{K}^+]$ over the range of 2.7–80 mM K^+ caused progressive decreases in afferent arteriolar diameter, with 80 mM K^+ evoking complete closure of all vessels (a response that was reversed by diltiazem). Analysis of the concentration-response profiles revealed that the EC_{50} for K^+ -

induced reductions in afferent arteriolar lumen diameter was greater in kidneys from rats with streptozotocin-induced DM (40 ± 4 mM) than in kidneys from normal rats (28 ± 4 mM; $P < 0.05$).⁴¹ Figure 2 illustrates the impact of DM on afferent arteriolar diameter responses to 40 mM K^+ , indicating that afferent arteriolar contractile responses to membrane depolarization are attenuated during the early stage of DM.

To further evaluate this phenomenon, afferent arterioles were isolated by microdissection and loaded with fura-2 to allow quantification of the $[Ca^{2+}]_i$ response to membrane depolarization. Figure 3 summarizes the effect of DM on afferent arteriolar $[Ca^{2+}]_i$ responses to increasing bath $[K^+]$ from 5 to 40 mM. Arterioles isolated from Sham rats and studied in a normoglycemic environment (5 mM glucose) responded to K^+ -induced depolarization with a ~ 100 nM increase in $[Ca^{2+}]_i$. In contrast, 40 mM K^+ only increased $[Ca^{2+}]_i$ by ~ 50 nM in arterioles harvested from diabetic rats and studied in a hyperglycemic environment mimicking the diabetic milieu (20 mM glucose).⁴¹ In both groups of vessels, diltiazem completely reversed the response to K^+ -induced depolarization. We recently utilized Mn^{2+} quench of fura-2 fluorescence to confirm that Ca^{2+} influx responses to K^+ -induced membrane depolarization are abated in isolated PMVSM cells from diabetic rats.⁴²

Viewed en masse, these observations indicate that the early stage of DM is associated with diminished responsiveness of preglomerular microvascular VGCCs to membrane depolarization. The response to K^+ -induced depolarization in afferent arterioles from diabetic rats is normalized within 10 min of decreasing extracellular glucose concentration from 20 mM to 5 mM (Figure 3). The beneficial impact of normalizing bath glucose concentration cannot be attributed to an osmotic effect, as it remains evident when bath osmolality is maintained with mannitol. This observation suggests that normalization of bath glucose concentration exerts its effect via the metabolic impact of glucose on cellular function. It is intriguing to note that the impaired $[Ca^{2+}]_i$ response to depolarization is rather slow in onset (hours or days) but is rapidly reversible (minutes). Hence, glycosylation of the VGCC or one of its regulatory proteins is probably not involved in this phenomenon, as protein glycosylation develops over a period of several weeks and is generally irreversible.⁴³ Moreover, it is unlikely that a change in VGCC expression accounts for the rapidly reversible decrease in the afferent arteriolar response to depolarization in DM. It seems more likely that hyperglycemia during the early stage of DM alters regulation of VGCC activity, perhaps by changing the sensitivity of the channel to changes in E_m . Possible mechanisms include glucose-induced activation of protein kinase C to alter the VGCC response to depolarization,⁴⁴ or a hyperglycemia-induced redox imbalance that alters the oxidative state of cysteine residues within the VGCC.^{45,46}

Evidence for altered K_{ATP} channel function

It is also possible that dysregulation of E_m in PMVSM cells contributes to suppressed afferent arteriolar VGCC responses in DM. Dysregulation of E_m in DM involving a functional over-expression of K^+ channels would be expected to promote membrane hyperpolarization, thereby reducing Ca^{2+} influx and through VGCCs and causing vasodilation. We have explored the postulate that K_{ATP} channels may contribute to afferent arteriolar vasodilation during the early stage of DM. K_{ATP} channel agonists (pinacidil and PCO-400) were found to be more potent afferent arteriolar dilators in kidneys from diabetic rats than in normal kidneys. Figure 4 illustrates the exaggerated vasodilator response to pinacidil in kidneys from diabetic rats, which suggests that the functional availability of K_{ATP} channels or their impact on E_m may be increased during the early stage of DM.⁴⁷ Moreover, glibenclamide (a sulfonylurea K_{ATP} channel antagonist) caused a concentration-dependent decrease in afferent arteriolar diameter two weeks after onset of streptozotocin-induced DM, but had virtually no effect on arterioles from normal (Sham) rats. As afferent arteriolar dilation is evident during this early stage of DM, glibenclamide treatment restored afferent arteriolar diameter in diabetic kidneys to a value

not different from Sham afferent arterioles (Figure 4). These observations support earlier reports that K_{ATP} channels do not contribute significantly to basal tone under normal conditions,^{10,11} but implicate open K_{ATP} channels in the afferent arteriolar dilation accompanying the early stage of DM. Decreased GLUT4 expression and an associated reduction in glucose uptake are evident in the afferent arteriole within 1 week after onset of DM in the rat.⁴⁸ Because glucose uptake is rate-limiting for glycolysis in vascular smooth muscle, these events should decrease glycolytic ATP production, thereby increasing K_{ATP} channel activity by relieving the inhibitory impact of normal intracellular ATP levels. The resulting hyperpolarization would favor closing of VGCCs and relaxation of afferent arteriolar VSM. Further studies at the single cell level will be necessary to determine the true nature of these changes and their relevance with regard to regulation of vascular tone and vasoconstrictor responsiveness. It is intriguing to note that the impact of K_{ATP} channels on vascular function may reverse as DM progresses. In non-renal vascular beds, mixed effects on K_{ATP} channel function are reported 4–8 weeks after onset of streptozotocin-induced DM, while K_{ATP} channel function is suppressed 2.5–4 months after onset.⁴⁹ If this phenomenon is also evident in the renal vasculature, it may contribute to reversal of the hyperfiltration and the decline in glomerular filtration rate associated with development diabetic nephropathy.

SUMMARY AND PERSPECTIVE

While DM alters myriad paracrine and endocrine systems, their ultimate impact on the renal microvasculature relies on the signaling processes expressed in arteriolar VSM. Our working hypothesis (Figure 5) states that a disruption of electromechanical coupling engenders afferent vasodilation and reduced contractile responsiveness during the early stages of DM. Increased functional availability and basal activation of K_{ATP} channels should result in dysregulation of E_m during DM, a situation exacerbated by impaired VGCC responsiveness to membrane depolarization. These synergistic effects of DM on K_{ATP} channels and VGCCs would tend to diminish afferent arteriolar responsiveness to myriad constrictor stimuli, thus promoting the characteristic afferent arteriolar dilation that evokes diabetic hyperfiltration.

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REFERENCES

1. Bank N. Mechanisms of diabetic hyperfiltration. *Kidney Int* 1991;40:792–807. [PubMed: 1745032]
2. Anderson S, Brenner BM. Pathogenesis of diabetic glomerulopathy: Hemodynamic considerations. *Diabetes/Metab. Rev* 1988;4:163–77.
3. Navar LG, Inscho EW, Majid DSA, Imig JD, Harrison-Bernard LM, Mitchell KD. Paracrine regulation of the renal microcirculation. *Physiol. Rev* 1996;76:425–536. [PubMed: 8618962]
4. Navar LG, Champion WJ, Thomas CE. Effects of calcium channel blockade on renal vascular resistance responses to changes in perfusion pressure and angiotensin-converting enzyme inhibition in dogs. *Circ. Res* 1986;58:874–81. [PubMed: 3013463]
5. Conger JD, Falk SA, Robinette JB. Angiotensin II-induced changes in smooth muscle calcium in rat renal arterioles. *J. Am. Soc. Nephrol* 1993;3:1792–803. [PubMed: 8329674]
6. Inscho EW, Imig JD, Cook AK. Afferent and efferent arteriolar vasoconstriction to angiotensin II and norepinephrine involves release of Ca^{2+} from intracellular stores. *Hypertension* 1997;29:222–7. [PubMed: 9039106]
7. Takenaka T, Suzuki H, Fujiwara K, Kanno Y, Ohno Y, Hayashi K, Nagahama T, Saruta T. Cellular mechanisms mediating rat renal microvascular constriction by angiotensin II. *J. Clin. Invest* 1997;100:2107–14. [PubMed: 9329977]

8. Gordienko DV, Clausen C, Goligorsky MS. Ionic currents and endothelin signaling in smooth muscle cells from rat renal resistance arteries. *Am. J. Physiol. Renal Fluid Electrolyte Physiol* 1994;266:F325–F341.
9. Hansen PB, Jensen BL, Andreassen D, Friis UG, Skott O. Vascular smooth muscle cells express the α_{1A} subunit of a P-/Q-type voltage-dependent Ca^{2+} channel, and it is functionally important in renal afferent arterioles. *Circ. Res* 2000;87:896–902. [PubMed: 11073885]
10. Lorenz JN, Schnermann J, Brosius FC, Briggs JP, Fursan PB. Intracellular ATP can regulate afferent arteriolar tone via ATP-sensitive K^{+} channels in the rabbit. *J. Clin. Invest* 1992;90:733–40. [PubMed: 1522228]
11. Loutzenhiser RD, Parker MJ. Hypoxia inhibits myogenic reactivity of renal afferent arterioles by activating ATP-sensitive K^{+} channels. *Circ. Res* 1994;74:861–9. [PubMed: 8156633]
12. Gebremedhin D, Kaldunski M, Jacobs ER, Harder DR, Roman RJ. Coexistence of two types of Ca^{2+} -activated K^{+} channels in rat renal arterioles. *Am. J. Physiol. Renal Fluid Electrolyte Physiol* 1996;270:F69–F81.
13. Zou A-P, Fleming JT, Falck JR, Jacobs ER, Gebremedhin D, Harder DR, Roman RJ. Stereospecific effects of epoxyeicosatrienoic acids on renal vascular tone and K^{+} -channel activity. *Am. J. Physiol. Renal Physiol* 1996;270:F822–F832.
14. Zou A-P, Fleming JT, Falck JR, Jacobs ER, Gebremedhin D, Harder DR, Roman RJ. 20-HETE is an endogenous inhibitor of the large-conductance Ca^{2+} -activated K^{+} channel in renal arterioles. *Am. J. Physiol. Regulatory Integrative Comp. Physiol* 1996;270:R228–R237.
15. Fallet RW, Bast JP, Fujiwara K, Ishii N, Sansom SC, Carmines PK. Influence of Ca^{2+} -activated K^{+} channels on rat renal arteriolar responses to depolarizing agonists. *Am. J. Physiol. Renal Physiol* 2001;280:F583–F591. [PubMed: 11249849]
16. Scholz H, Kurtz A. Differential regulation of cytosolic calcium between afferent arteriolar smooth muscle cells from mouse kidney. *Pflugers Arch* 1995;431:46–51. [PubMed: 8584417]
17. Carmines PK. Segment-specific effect of chloride channel blockade on rat renal arteriolar contractile responses to angiotensin II. *Am. J. Hypertens* 1995;8:90–4. [PubMed: 7734105]
18. Takenaka T, Epstein M, Forster H, Landry DW, Iijima K, Goligorsky MS. Attenuation of endothelin effects by a chloride channel inhibitor, indanyloxyacetic acid. *Am. J. Physiol. Renal Fluid Electrolyte Physiol* 1992;262:F799–F806.
19. Jensen BL, Skott O, Temdrup C. Blockade of chloride channels by DIDS stimulates renin release and inhibits contraction of afferent arterioles. *Am. J. Physiol. Renal Fluid Electrolyte Physiol* 1996;270:F718–F727.
20. Takenaka T, Kanno Y, Kitamura Y, Hayashi K, Suzuki H, Saruta T. Role of chloride channels in afferent arteriolar constriction. *Kidney Int* 1996;50:864–72. [PubMed: 8872961]
21. Jensen BL, Ellekvist P, Skott O. Chloride is essential for contraction of afferent arterioles after agonists and potassium. *Am. J. Physiol. Renal Physiol* 1997;272:F389–F396.
22. Carmines PK, Fowler BC, Bell PD. Segmentally distinct effects of depolarization on intracellular $[\text{Ca}^{2+}]$ in renal arterioles. *Am. J. Physiol. Renal Fluid Electrolyte Physiol* 1993;265:F677–F685.
23. Conger JD, Falk SA. KCl and angiotensin responses in isolated rat renal arterioles: effects of diltiazem and low-calcium medium. *Am. J. Physiol. Renal Fluid Electrolyte Physiol* 1993;264:F134–F140.
24. Helou CM, Marchetti J. Morphological heterogeneity of renal glomerular arterioles and distinct $[\text{Ca}^{2+}]_i$ responses to ANG II. *Am. J. Physiol. Renal Physiol* 1997;273:F84–F96.
25. Carmines PK, Navar LG. Disparate effects of Ca channel blockade on afferent and efferent arteriolar responses to ANG II. *Am. J. Physiol. Renal Fluid Electrolyte Physiol* 1989;256:F1015–F1020.
26. Edwards RM, Trizna W, Ohlstein EH. Renal microvascular effects of endothelin. *Am. J. Physiol. Renal Fluid Electrolyte Physiol* 1990;259:F217–F221.
27. Loutzenhiser R, Chilton L, Trottier G. Membrane potential measurements in renal afferent and efferent arterioles: actions of angiotensin II. *Am. J. Physiol. Renal Physiol* 1997;273:F307–F314.
28. Loutzenhiser K, Loutzenhiser R. Angiotensin II-induced Ca^{2+} influx in renal afferent and efferent arterioles: Differing roles of voltage-gated and store-operated Ca^{2+} entry. *Circ. Res* 2000;87:551–7. [PubMed: 11009559]

29. Ohishi K, Okwueze MI, Vari RC, Carmines PK. Juxtamedullary microvascular dysfunction during the hyperfiltration stage of diabetes mellitus. *Am. J. Physiol. Renal Fluid Electrolyte Physiol* 1994;267:F99–F105.
30. Hayashi K, Epstein M, Loutzenhiser R, Forster H. Impaired myogenic responsiveness of the afferent arteriole in streptozotocin-induced diabetic rats: Role of eicosanoid derangements. *J. Am. Soc. Nephrol* 1992;2:1578–86. [PubMed: 1610978]
31. Vallon V, Blantz RC, Thomson S. Homeostatic efficiency of tubuloglomerular feedback is reduced in established diabetes mellitus in rats. *Am. J. Physiol. Renal Fluid Electrolyte Physiol* 1995;269:F876–F883.
32. Parving H-H, Kasstrup H, Smidt UM, Andersen AR, Feldt-Rasmussen B, Christiansen JS. Impaired autoregulation of glomerular filtration rate and renal plasma flow in short-term insulin-dependent diabetics. *Diabetologia* 1981;20:451–6. [PubMed: 7016638]
33. Jourdon P, Feuvray D. Calcium and potassium currents in ventricular myocytes isolated from diabetic rats. *J. Physiol. (Lond.)* 1993;470:411–29. [PubMed: 8308734]
34. Wang DW, Kiyosue T, Shigematsu SJ, Arita M. Abnormalities K^+ and Ca^{2+} currents in ventricular myocytes from rats with chronic diabetes. *Am. J. Physiol. Heart Circ. Physiol* 1995;269:H1288–H1296.
35. Iwashima Y, Pugh W, Depaoli AM. Expression of calcium channel mRNAs in rat pancreatic islets and downregulation after glucose infusion. *Diabetes* 1993;42:928–55.
36. Lee SL, Ostadalova I, Kolar F, Dhalla NS. Alterations in Ca^{2+} -channels during the development of diabetic cardiomyopathy. *Mol. Cell. Biochem* 1992;109:173–9. [PubMed: 1320733]
37. Ogawa T, Kashiwagi A, Kikkawa R, Shigeta Y. Increase of voltage-sensitive calcium channels and calcium accumulation in skeletal muscles of streptozotocin-induced diabetic rats. *Metabolism* 1995;44:1455–61. [PubMed: 7476334]
38. Nishio Y, Kashiwagi A, Ogawa T, Asahina T, Ikebuchi M, Kodama M, Shigeta Y. Increase in [3H] PN200-100 binding to cardiac muscle membrane in streptozotocin-induced diabetic rats. *Diabetes* 1990;39:1064–9. [PubMed: 2143487]
39. Bank N, Lahorra MA, Aynedjian HS. Acute effect of calcium and insulin on hyperfiltration of early diabetes. *Am. J. Physiol. Endocrinol. Metab* 1987;252:E13–E20.
40. Williams BW, Schrier RW. Effect of elevated extracellular glucose concentrations on transmembrane calcium ion fluxes in cultured rat VSMC. *Kidney Int* 1993;44:344–51. [PubMed: 8397316]
41. Carmines PK, Ohishi K, Ikenaga H. Functional impairment of renal afferent arteriolar voltage-gated calcium channels in rats with diabetes mellitus. *J. Clin. Invest* 1996;98:2564–71. [PubMed: 8958219]
42. Bast JP, Carmines PK. Diabetes mellitus curtails the Ca^{2+} influx response to depolarization of isolated preglomerular microvascular smooth muscle cells (Abstract). *FASEB J* 2000;14:A136.
43. Bucala R, Vlassara H, Cerami A. Advanced glycosylation endproducts: Role in diabetic and non-diabetic vascular disease. *Drug Dev. Res* 1994;32:77–89.
44. Williams B. Glucose-induced vascular smooth muscle dysfunction: The role of protein kinase C. *J. Hypertens* 1995;13:477–86. [PubMed: 7561003]
45. Williamson JR, Chang K, Frangos M, Hasan KS, Ido Y, Kawamura T, Nyengaard JR, Van den Enden M, Kilo C, Tilton RG. Hyperglycemic pseudohypoxia and diabetic complications. *Diabetes* 1993;42:801–13. [PubMed: 8495803]
46. Chiamvimonvat N, O'Rourke B, Kamp TJ, Kallen RG, Hofmann F, Flockerzi V, Marban E. Functional consequences of sulfhydryl modification in the pore-forming subunits of cardiovascular Ca^{2+} and Na^+ channels. *Circ. Res* 1995;76:325–34. [PubMed: 7859379]
47. Ikenaga H, Bast JP, Fallet RW, Carmines PK. Exaggerated impact of ATP-sensitive K^+ channels on afferent arteriolar diameter in diabetes mellitus. *J. Am. Soc. Nephrol* 2000;11:1199–207. [PubMed: 10864575]
48. Marcus RG, England R, Nguyen K, Charron MJ, Briggs JP, Brosius FC III. Altered renal expression of the insulin-responsive glucose transporter GLUT4 in experimental diabetes mellitus. *Am. J. Physiol. Renal Fluid Electrolyte Physiol* 1994;267:F816–F824.
49. Sobey CG. Potassium channel function in vascular disease. *Arterioscler. Thromb. Vasc. Biol* 2001;21:28–38. [PubMed: 11145930]

Fig. 1. Intracellular $[\text{Ca}^{2+}]$ responses to K^+ -induced depolarization of renal arterioles isolated from rabbit kidney. The disparate response patterns indicate functional expression of VGCCs in afferent arterioles, but not in efferent arterioles.

Fig. 2. Impact of streptozotocin-induced DM on juxtamedullary afferent arteriolar lumen diameter responses to activation of VGCCs. Arterioles from diabetic rats (DM) exhibited diminished responsiveness to pharmacological activation of VGCCs (BAY K 8644), as well as membrane depolarization (40 mM K⁺). * $P < 0.05$ vs Sham. Data from Carmines et al.⁴¹

Fig. 3. Suppressed intracellular $[Ca^{2+}]_i$ responses to K^+ -induced depolarization in afferent arterioles from rats with streptozotocin-induced DM. * $P < 0.05$ vs Sham. Data from Carmines et al.⁴¹

Fig. 4.

Disparate effects of pharmacological manipulation of K_{ATP} channel activity on afferent arterioles from normal (Sham) and diabetic (DM) rats. Glibenclamide (300 μ M, which yields $\sim$ 30 μ M free glibenclamide in the albumin-containing bathing solution) evoked significant constriction only in arterioles from diabetic rats. Pinacidil (300 μ M) evoked an exaggerated vasodilation in arterioles from diabetic rats. * P <0.05 vs Sham. Data from Ikenaga et al.⁴⁷

Fig. 5. Postulated impact of diabetic hyperglycemia on renal arteriolar vascular smooth muscle activation processes. Alterations in electromechanical regulation of smooth muscle tone are postulated to underlie the selective afferent arteriolar dilation that engenders diabetic hyperfiltration.