Potassium Channel Contributions to Afferent Arteriolar Tone in Normal and Diabetic Rat Kidney

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ABSTRACT
We previously reported an enhanced tonic dilator impact of ATP-sensitive K⁺ channels in afferent arterioles of rats with streptozotocin (STZ)-induced diabetes. The present study explored the hypothesis that other types of K⁺ channel also contribute to afferent arteriolar dilation in STZ rats. The in vitro blood-perfused juxtamedullary nephron technique was utilized to quantify afferent arteriolar lumen diameter responses to K⁺ channel blockers: 0.1-3.0 mM 4-aminopyridine (4-AP; KV channels), 10-100 µM barium (KIR channels), 1-100 nM tertiapin-Q (TPQ; Kir1.1 & Kir3.x subfamilies of KIR channels), 100 nM apamin (SKCa channels), and 1 mM tetraethylammonium (TEA; BKCa channels). In kidneys from normal rats, 4-AP, TEA, and Ba²⁺ reduced afferent diameter by 23±3%, 8±4% and 18±2%, respectively, at the highest concentrations employed. Neither TPQ nor apamin significantly altered afferent diameter. In arterioles from STZ rats, a constrictor response to TPQ (22±4% decrease in diameter) emerged, and the response to Ba²⁺ was exaggerated (28±5% decrease in diameter). Responses to the other K⁺ channel blockers were similar to those observed in normal rats. Moreover, exposure to either TPQ or Ba²⁺ reversed the afferent arteriolar dilation characteristic of STZ rats. Acute surgical papillectomy did not alter the response to TPQ in arterioles from normal or STZ rats. We conclude that 1) KV, KIR and BKCa channels tonically influence normal afferent arteriolar tone, 2) KIR channels (including Kir1.1 and/or Kir3.x) contribute to the afferent arteriolar dilation during diabetes, and 3) the dilator impact of Kir1.1/Kir3.x channels during diabetes is independent of solute delivery to the macula densa.

Keywords: Tertiapin-Q, Barium, TEA, apamin, 4-aminopyridine
INTRODUCTION

Diabetes is the foremost cause of end stage renal disease in the western world (17). During the early stage of Type 1 diabetes (T1D), there is an increase in glomerular filtration rate (diabetic hyperfiltration) that may contribute to the eventual development of diabetic nephropathy (21). Although renal afferent arteriolar dilation is the major vascular alteration leading to diabetic hyperfiltration (22), the precise mechanism underlying the afferent arteriolar dilation during T1D has not been established.

Preglomerular microvascular resistance is tightly linked to vascular smooth muscle membrane potential and its impact on the open probability of voltage-gated Ca\(^{2+}\) channels (VGCCs). Our laboratory has previously shown a defect in the functional responsiveness of VGCCs in afferent arterioles from rats with streptozotocin (STZ)-induced T1D (7). This phenomenon is evident as reduced VGCC-dependent \([\text{Ca}^{2+}]_i\) and lumen diameter responses to depolarization, suggesting that decreased VGCC responsiveness to membrane depolarization contributes to decreased afferent arteriolar resistance during the hyperfiltration stage of T1D. An increase in the K\(^+\) conductance of the afferent arteriolar vascular smooth muscle cell membrane could exacerbate this situation, as the resulting hyperpolarization would also reduce the open probability of VGCCs, thereby decreasing \([\text{Ca}^{2+}]_i\) and promoting vasodilation. Electrophysiological and pharmacological approaches have revealed several classes of K\(^+\) channels in preglomerular microvascular smooth muscle and intact afferent arterioles — ATP-sensitive K\(^+\) channels (K\text{ATP} channels), large- and small/intermediate-conductance Ca\(^{2+}\)-activated K\(^+\) channels (BK\text{Ca} channels and SK\text{Ca} channels, respectively), inward-rectifier K\(^+\) channels (K\text{IR} channels), and voltage-gated K\(^+\) channels (K\text{V} channels, such as the delayed-rectifier) (12, 15, 16, 18, 24, 27, 40, 58). However, there is not a clear understanding as to which K\(^+\) channels contribute substantially to afferent arteriolar tone under normal conditions or during diabetes.

We previously reported that the K\text{ATP} channels contribute little to afferent arteriolar tone under normal conditions, but exert a significant tonic dilator influence on the afferent arteriole
during the hyperfiltration stage of STZ-induced T1D (24). $K_{\text{ATP}}$ channels in vascular smooth muscle are comprised of pore-forming Kir6.1 subunits (members of the $K_{\text{IR}}$ channel family) and regulatory sulfonylurea receptor (SUR2B) subunits (56). Thus, it is conceivable that additional members of $K_{\text{IR}}$ channel family may also be involved in the afferent arteriolar dilation seen in type 1 diabetes. The modest tonic dilator impact of $BK_{\text{Ca}}$ channels on afferent arteriolar tone in the normal kidney (15) might be enhanced in T1D, perhaps via an $H_2O_2$-dependent mechanism (20) during this state of oxidative stress. To our knowledge, no information is available regarding the influence of $SK_{\text{Ca}}$ or $K_V$ channels on afferent arteriolar tone in normal or disease states, although $K_V$ channels may modestly potentiate myogenic responses of this vessel (33). Thus, the goals of the present study were to survey the tonic impact of the various afferent arteriolar $K^+$ channels on lumen diameter of this vessel in normal rats, and to test the hypothesis that increased activation of one or more of these channels is involved in the afferent arteriolar dilation evident in STZ-induced diabetes.

**MATERIALS AND METHODS**

*Induction of diabetes mellitus.* All studies utilized male Sprague-Dawley rats (Harlan, barrier 218A; Prattville, AL), which were treated according to the National Institutes of Health *Guide for the Care and Use of Laboratory Animals* using procedures approved by the University of Nebraska Medical Center Institutional Animal Care and Use Committee. Rats were housed in pairs and provided *ad libitum* access to food and water. Rats were anesthetized with 50 mg/kg ip methohexital sodium to facilitate injection of streptozocin (STZ; 65 mg/kg iv). The rats were allowed to recover from anesthesia and were housed overnight. The following day, blood glucose levels were measured (Accu-Check III model 766; Boehringer Mannheim, Indianapolis, IN) and the rats were anesthetized again to allow subcutaneous insertion via a 16-gauge needle of a 2.3×2.0 mm sustained-release insulin implant (Linplant®; Linshin Canada, Scarborough, Ontario). Rats received *ad libitum* food and water for the ensuing 3-4 weeks, during which blood
glucose measurements were made twice weekly. Acute, terminal experiments were performed 22 ± 1 days after STZ injection (range 14-30 days). Approximately age-matched, normal male Sprague-Dawley rats (n = 24) served as non-diabetic controls.

The rat in vitro blood perfused juxtamedullary nephron technique. Acute experiments were performed using the rat in vitro blood-perfused juxtamedullary nephron technique (1, 3). After anesthetization with sodium pentobarbital (50 mg/kg ip), a cannula was placed in the carotid artery. A perfusion cannula introduced via the superior mesenteric artery into the right renal artery allowed renal perfusion with Tyrode's solution containing 52 g/L bovine serum albumin and a mixture of L-amino acids (43). The renal vein was incised to drain the perfusate and the animal was exsanguinated via the carotid cannula into a heparinized syringe; however, renal perfusion was maintained throughout the ensuing dissection procedure. The kidney was removed and cut longitudinally to expose the pelvic cavity, leaving the papilla intact within the perfused dorsal two-thirds of the organ. Small incisions were made in the lateral fornices, allowing the papilla to be reflected back and retained in that position by insect pins. The pelvic mucosa, adipose and connective tissues that normally cover the inside cortical surface were removed and the veins were cut open, thus exposing the tubules and microvasculature of juxtamedullary nephrons. The portions of the vasculature that give rise to arterioles associated with these juxtamedullary nephrons were isolated with tight ligatures. Blood collected from the rat was prepared for perfusion as described previously (6, 8) and processed to remove leukocytes and platelets. The reconstituted blood was filtered through a 5-µm nylon mesh, and its pH was measured (ABL5 Blood Gas Analyzer; Radiometer America Inc, Westlake, OH) and adjusted to 7.40 to 7.42 by addition of NaHCO₃, as necessary. Thereafter, the blood was stirred continuously in a closed reservoir that was maintained under pressure from a gas tank (95% O₂/5% CO₂). This arrangement provided both tissue oxygenation and the driving force for perfusion of the kidney. The Tyrode's perfusate was then replaced by the reconstituted blood perfusate. Perfusion pressure at the cannula tip in the renal artery was measured using a P23XL transducer (Gould,
Oxnard, CA) connected to a polygraph (Grass Instruments, Quincy, MA) and was maintained at 110 mmHg throughout the experiment. The tissue surface was continuously bathed with Tyrode's solution at 37°C containing 10 g/L bovine serum albumin, approximating the composition of renal interstitial fluid. Stock solutions containing vasoactive agents were stored at –20°C until the day of the experiment, at which time they were diluted with Tyrode's bath to the appropriate final concentrations.

Videometric techniques were used to measure arteriolar lumen diameter. The tissue was transilluminated on the fixed stage of a Nikon Optiphot microscope equipped with a water-immersion objective (×40, numerical aperture 0.55). Enhanced video images of the microvessels were displayed at a magnification of ×1400 on a high-resolution monitor and recorded simultaneously on a DVD recorder. During the 10-15 min equilibration period, an afferent arteriole was selected for study based on visibility and acceptable blood flow. Only arterioles with rapid flow of erythrocytes were studied, and vessels were rejected on the basis of inadequate flow if the passage of single erythrocytes could be discerned. All experimental protocols were designed to assess arteriolar diameter at a single measurement site under several experimental conditions. In kidneys from one Normal rat and two STZ rats, two vessels could be visualized within the same field of view, thus allowing images of both vessels to be recorded simultaneously and analyzed separately during playback. Afferent arteriolar lumen diameter was monitored at sites located >100 µm upstream from the glomerulus and >80 µm downstream from the interlobular artery. Diameter was measured at 12-sec intervals, with the average diameter (in µm) during the final min of each treatment period utilized for statistical analysis. Each experiment followed one of the specific protocols detailed below.

Afferent arteriolar diameter responses to barium. The effect of Ba²⁺, a broad-spectrum Kᵢᵣ channel blocker, was used to assess the tonic impact of Kᵢᵣ channels on afferent arteriolar diameter in kidneys from STZ rats (STZ kidneys) and normal rats (Normal kidneys). Lumen diameter responses to Ba²⁺ were measured during exposure of the kidney surface to the
following solutions: 1. Tyrode’s bath alone (10 min); 2. Tyrode’s bath containing 10, 30, and 100 µM BaCl₂ (5 min each); 3. Tyrode’s bath alone (10 min). After full recovery from the Ba²⁺ treatment, the tissue was exposed to 20 mM KCl to document the absence/presence of a dilator response to the classic stimulus for Kir channel activation. Published reports indicate that 20 mM K⁺ evokes Ba²⁺-sensitive arteriolar dilation that is equal to or greater than the response to 15 mM K⁺ in various vascular beds (3, 38, 48).

Afferent arteriolar diameter responses to tertiapin-Q and tetraethylammonium. Tertiapin-Q (TPQ) reversibly blocks the Kir1.1 and Kir3.x families of inward rectifier channels (30). The effect of TPQ was measured in afferent arterioles of Normal kidneys and STZ kidneys, using a protocol involving sequential exposure to the following solutions: 1. Tyrode’s bath alone (10 min); 2. Tyrode’s bath containing 1, 10, and 100 nM TPQ (5 min each); 3. Tyrode’s bath alone (10 min). In some kidneys, recovery from the initial TPQ challenge was followed by exposure to Tyrode’s solution containing 1 mM TEA (5 min; a BKCa channel blocker), with a final recovery period at the end of the experiment (Tyrode’s bath alone; 10 min). Because Kir1.1 channels are prominently expressed in the thick ascending limb, TPQ might alter solute delivery to the macula densa, thereby influencing arteriolar tone via tubuloglomerular feedback. To address this possibility, in some kidneys recovery from the initial TPQ treatment was followed by acute surgical papillectomy to physically open the tubuloglomerular feedback loop (25, 51). Ten min later, the 1-100 nM TPQ challenge was repeated, followed by a final recovery period (Tyrode’s bath alone).

Afferent arteriolar diameter responses to 4-aminopyridine and apamin. Additional experiments were conducted to test the effects of 4-aminopyridine (4-AP; a reversible Kv channel blocker (49)) and apamin (a SKCa channel blocker (1)) on afferent arteriolar tone in STZ kidneys and Normal kidneys. Afferent arteriolar lumen diameter responses to the following drug solutions were documented: 1. Tyrode’s bath alone (10 min); 2. Tyrode’s solution containing
0.1, 0.3, 1.0 and 3.0 mM 4-AP (5 min each); 3. Tyrode’s bath alone (10 min); 4. Tyrode’s solution containing 100 nM apamin (5 min); 5. Tyrode’s bath alone (10 min).

Reagents. TPQ was purchased from Tocris Bioscience (Ellisville, MO), methohexital sodium (Brevital\textsuperscript{®}) was from Eli Lilly (Indianapolis, IN) and pentobarbital sodium (Nembutal\textsuperscript{®}) was from Ovation Pharmaceuticals (Deerfield, IL). All other chemicals and reagents were purchased from Sigma Chemical (St. Louis, MO). The Tyrode’s solution was composed of (in mM) 1.8 CaCl\textsubscript{2}•2H\textsubscript{2}O, 1 MgCl\textsubscript{2}•6H\textsubscript{2}O, 2.7 KCl, 137 NaCl, 0.4 Na\textsubscript{2}HPO\textsubscript{4}, 12 NaHCO\textsubscript{3}, and either 5.5 or 20 D-glucose (for tissue from normal and STZ, rats, respectively).

Statistical Analyses. Simple between-group comparisons were made by t-test or the Mann-Whitney rank sum test. Effects of pharmacological agents on arteriolar lumen diameter in normal and STZ rats were evaluated using two-way repeated measures ANOVA and, when appropriate, the Holm-Sidak multiple comparisons test. These analyses were accomplished using SigmaStat 3.11 software (Systat, San Jose, CA). A $P$ value $<0.05$ was considered statistically significant. All data are presented as the mean ± SEM ($n$ = number of arterioles, unless otherwise stated).

RESULTS

Table 1 provides basic information characterizing Normal and STZ rats used in this study. Blood glucose levels were significantly elevated in STZ rats, compared with representative values evident in a random sampling of the Normal rats used in this study. Body weight at the time of the terminal experiment was less in STZ rats than in age-matched Normal rats. In addition, baseline afferent arteriolar lumen diameter was significantly larger in STZ kidneys compared with Normal kidneys. These observations mirror our previous reports comparing STZ rats with rats receiving sham (vehicle) treatments.

Figure 1 compares the afferent arteriolar lumen diameter responses of Normal and STZ kidneys to treatment with increasing concentrations of Ba\textsuperscript{2+}. In these experiments, baseline
arteriolar lumen diameter averaged $16.3 \pm 1.2 \, \mu m \, (n = 6)$ in Normal kidneys and $23.0 \pm 1.7 \, \mu m \, (n = 9)$ in STZ kidneys ($P < 0.05$). In Normal kidneys, afferent diameter tended to progressively decrease in response to increasing bath Ba$^{2+}$ concentration, but this effect achieved statistical significance only during exposure to 100 µM Ba$^{2+}$ ($18 \pm 5\%$ decrease in diameter). In STZ kidneys, afferent diameter was also progressively reduced in response to increasing extracellular concentrations of Ba$^{2+}$; however, the constrictor response was significant at the lowest concentration of Ba$^{2+}$ employed (10 µM; $13 \pm 5\%$ decrease in diameter) and the responses to 30 and 100 µM Ba$^{2+}$ were significantly exaggerated relative to Normal kidneys. Overall, afferent arteriolar diameter responses to Ba$^{2+}$ in STZ kidneys were about twice the magnitude of responses evident in Normal kidneys. Ultimately, the exaggerated contractile response to Ba$^{2+}$ in STZ kidneys restored arteriolar lumen diameter to values that did not differ significantly from Normal kidneys, even during 100 µM Ba$^{2+}$ treatment. The effects of Ba$^{2+}$ were reversible, with arteriolar diameters returning to $98 \pm 2$ and $95 \pm 4\%$ of baseline in Normal and STZ kidneys, respectively, during the post-Ba$^{2+}$ recovery period. Subsequent exposure to 20 mM K$^+$ provoked a $3.1 \pm 1.2 \, \mu m$ increase in afferent arteriolar diameter in Normal kidneys ($n = 6; \, P < 0.05$ vs post-Ba$^{2+}$ recovery period), but no significant change in arteriolar diameter in STZ kidneys ($\Delta = 1.8 \pm 2.7 \, \mu m; \, n = 7; \, P > 0.52$ vs post-Ba$^{2+}$ recovery period). Thus, afferent arterioles in Normal kidneys exhibited constrictor responses to Ba$^{2+}$ and dilator responses to 20 mM K$^+$, while arterioles in STZ kidneys showed exaggerated responses to Ba$^{2+}$ and no significant effect of 20 mM K$^+$.

Figure 2 shows concentration-dependent responses of afferent arterioles from Normal and STZ kidneys to TPQ (blocks Kir1.1 and Kir3.x families of Kir channels). Baseline lumen diameter was significantly greater in STZ kidneys ($22.2 \pm 0.5 \, \mu m; \, n = 11$) than in Normal kidneys ($18.0 \pm 0.7 \, \mu m; \, n = 14$). Increasing concentrations of TPQ (1-100 nM) did not alter afferent arteriolar diameter in Normal kidneys; in contrast, 100 nM TPQ evoked a $22 \pm 4\%$ decrease in afferent arteriolar diameter in STZ kidneys. It is evident from Figure 2 that 100 nM
TPQ alleviated the afferent arteriolar diameter difference between Normal and STZ kidneys. Thus, although TPQ did not alter afferent diameter in Normal kidneys, it reversed the afferent arteriolar dilation typically evident in STZ kidneys, thereby implicating TPQ-sensitive \( K_{ir} \) channels in reduced afferent arteriolar tone during diabetes.

During the post-TPQ recovery period, afferent arteriolar lumen diameter was restored to 96 ± 2 and 98 ± 3% of baseline in Normal and STZ kidneys, respectively. In about half of the experiments comprising Figure 2, the post-TPQ recovery period was followed by acute surgical papillectomy to physically open the tubuloglomerular feedback loop. Subsequently, the TPQ challenge was repeated. Papillectomy caused a modest but statistically significant increase in afferent arteriolar lumen diameter (\( \Delta = 0.7 \pm 0.3 \mu m; n = 7; P = 0.035 \)) in Normal kidneys, but did not alter afferent diameter in STZ kidneys (\( \Delta = 0.0 \pm 0.5 \mu m; n = 6 \)). After papillectomy, responses to 1-100 nM TPQ mimicked those evident in the same vessels prior to papillectomy. Figure 3 summarizes the changes in diameter evoked by 100 nM TPQ before and after papillectomy. In Normal kidneys, 100 nM TPQ did not alter arteriolar diameter before or after papillectomy, while TPQ decreased lumen diameter in STZ kidneys by 26 ± 4% with papilla intact and 21 ± 6% after papillectomy. Thus, acute surgical papillectomy did not prevent the ability of TPQ to evoke afferent arteriolar constriction in STZ kidneys, suggesting that the phenomenon arises independent of tubuloglomerular feedback.

In the remaining experiments comprising Figure 2, the post-TPQ recovery was followed by subsequent exposure to TEA (papilla intact). Figure 4 illustrates the modest diameter response to 1 mM TEA evident in Normal kidneys (9 ± 4% decrease in diameter; \( n = 6 \)) and STZ kidneys (9 ± 5% decrease in diameter; \( n = 5 \)). Constrictor responses to TEA were comparable in afferent arterioles of normal and STZ kidneys, despite the differing baseline diameters and responsiveness to TPQ treatment evident in the same arterioles, indicating that the tonic impact of BK\(_{Ca}\) channels on afferent arteriolar tone is unaltered in diabetes.
The final set of experiments explored the contribution of $K_V$ channels and $SK_{Ca}$ channels to afferent arteriole tone in Normal and STZ kidneys having baseline diameters of $19.2 \pm 1.2 \, \mu m$ ($n = 6$) and $27.3 \pm 3.1 \, \mu m$ ($n = 6; \, P < 0.05$), respectively. Exposure of the tissue to increasing concentrations of 4-AP elicited concentration-dependent decreases in afferent lumen diameter in both Normal and STZ kidneys (Figure 5). In Normal kidneys, significant decreases in diameter were apparent at 4-AP concentrations $\geq 0.3 \, \text{mM}$, while the response in STZ kidneys only achieved statistical significance during exposure to $3 \, \text{mM}$ 4-AP. In both Normal and STZ kidneys, $3 \, \text{mM}$ 4-AP decreased afferent diameter by approximately $20\%$. This response was fully reversible upon removal of 4-AP from the bath. Two-way repeated measures ANOVA revealed no significant interaction ($P = 0.33$) between treatment group (Normal vs STZ) and the diameter response to 4-AP. Thus, while these experiments revealed a significant tonic dilator impact of 4-AP-sensitive $K_V$ channels on afferent arteriolar tone, the data fail to provide evidence that diabetes alters this phenomenon. In most of these arterioles, the post-4-AP recovery period was followed by exposure to $100 \, \text{nM}$ apamin. As illustrated in Figure 6, apamin did not change afferent arteriolar lumen diameter in either Normal or STZ kidneys. Thus, apamin-sensitive $SK_{Ca}$ channels exert no significant tonic dilator impact on afferent arteriolar tone under these conditions.

**DISCUSSION**

In our hands, rats with moderately hyperglycemic STZ-induced T1D exhibit polyuria, polydipsia, polyphagia, renal hypertrophy and glomerular hyperfiltration (37, 50). The hyperfiltration results from decreased afferent arteriolar tone (44), the mechanism of which remains largely speculative. Therefore, the present study employed a pharmacological approach to determine the role of various $K^+$ channels in determining afferent arteriolar tone in normal rat kidney and in rats with STZ-induced T1D. The data reveal that $Ba^{2+}$-, TEA-, and 4-AP-sensitive mechanisms exert a tonic vasodilator influence on the normal afferent arteriole, with no
discernible impact of TPQ- and apamin-sensitive processes under these conditions. In the diabetic animals, however, Ba$^{2+}$-sensitive regulation of afferent arteriolar tone is augmented and a TPQ-sensitive tonic dilator influence emerges. Because these pharmacological agents target various K$^+$ channel families, our observations suggest that T1D alters the relative impact of specific K$^+$ channels on membrane potential in the afferent arteriole – presumably in the resident vascular smooth muscle cells.

Although millimolar concentrations of Ba$^{2+}$ can inhibit some K$_V$ channel subtypes (19), the specific blockade of K$_{IR}$ channels achieved by micromolar concentrations of Ba$^{2+}$ has been exploited in countless physiological studies. An afferent arteriolar constrictor impact of Ba$^{2+}$ has been demonstrated by Loutzenhiser and colleagues in studies utilizing the rat isolated, perfused hydrenephrotic kidney (11, 12). Results of the present study confirm that the afferent arteriole contracts in a concentration-dependent manner in response to Ba$^{2+}$, although the contractile responses to Ba$^{2+}$ in the present study were of smaller magnitude than those evident in the hydrenephrotic kidney (12). This disparity may reflect quantitative differences in responsiveness of mid-outer cortical nephrons vs juxtamedullary nephrons, or the fact that the hydrenephrotic kidney studies were performed at low perfusion pressure (80 mmHg) to maximally dilate the vessel, thereby facilitating investigation of K$_{IR}$ involvement in the myogenic response. The higher perfusion pressure (110 mmHg) utilized in the present study, together with the impact of circulating constrictor agents in the perfusate blood (5), would provoke partial depolarization of the vascular smooth muscle and greater active tone. This situation may reduce the tonic contribution of K$_{IR}$ channels on vascular tone under our experimental conditions, compared with the isolated perfused hydrenephrotic kidney. Nevertheless, it is clear that Ba$^{2+}$-sensitive mechanisms (presumably K$_{IR}$ channels) exert a tonic vasodilator influence on the afferent arteriole. Importantly, the exaggerated contractile response to Ba$^{2+}$ evident in arterioles of STZ kidneys suggests that an increased tonic influence of K$_{IR}$ channels contributes to afferent arteriolar dilation during this stage of diabetes.
A long-recognized limitation of attempts to investigate the role of KIR channels in vascular control is the lack of pharmacological tools that distinguish between the seven KIR subfamilies, each of which has different electrophysiological characteristics, tissue distributions and regulatory mechanisms (35). Ba²⁺-sensitive Kir2.1 channels are expressed in rat afferent and efferent arterioles (11), and are widely considered to be the primary KIR channel in vascular smooth muscle (29). Kir2.1 channels are necessary to evoke the vasodilator response to increases in extracellular [K⁺] (57), such as those evident in afferent arterioles of the hydronephrotic rat kidney (11, 12) and the Normal kidneys of the present study. In some vascular beds (especially skeletal muscle), elevation of extracellular [K⁺] can elicit hyperpolarization and vasodilation by stimulating the electrogenic Na⁺/K⁺-ATPase (3, 39). However, Chilton & Loutzenhiser (12) found K⁺-induced dilation of the rat renal afferent arteriole to be ouabain-insensitive but blocked by Ba²⁺, suggesting a predominant role of Kir2.1 in this response. Noting that afferent arterioles from STZ kidneys failed to exhibit significant K⁺-induced dilation, while showing exaggerated contractile responses to Ba²⁺, it is possible that the available Kir2.1 channels are tonically open in the afferent arteriole during T1D, perhaps due to a shift in inward rectification. However, it should be noted that other KIR channel subtypes with varying Ba²⁺-sensitivities also reside in the kidney and may contribute to the exaggerated afferent arteriolar contractile response to Ba²⁺ in T1D. For example, Kir2.2 and Kir2.3 are present in descending vasa recta (4), while Kir1.1 (the ROMK channel) is highly expressed in the thick ascending limb and cortical collecting duct (54). Neither these nor other KIR channel subtypes (except Kir2.1) have been reported to reside in the afferent arteriole, but we note that no studies have addressed this possibility.

As an initial attempt to identify KIR channel subtypes that contribute to reduced afferent arteriolar tone during T1D, experiments were performed using TPQ, a bee venom toxin that inhibits Kir1.1 and Kir3.x channels (9, 30). Our observation that 100 nM TPQ decreases afferent arteriolar diameter in STZ kidneys, but not in Normal kidneys, suggests a role for TPQ-sensitive KIR channels in the vasodilation seen during the hyperfiltration stage of T1D. The literature
provides few studies implicating TPQ-sensitive events in the control of vascular function under any conditions. Specifically, 1 µM TPQ attenuates urocortin-induced relaxation of coronary artery rings and 17β-estradiol-induced relaxation of mesenteric artery rings (23, 52). In addition, 10 µM TPQ attenuates relaxant responses of mesenteric resistance arteries to endothelium-derived hyperpolarizing factor and C-type natriuretic peptide (10). Our results extend these observations to suggest that TPQ-sensitive channels can influence vascular function at the microvascular level, evident as the emergence of a tonic vasodilator influence of these channels on the afferent arteriole during T1D; however, the mechanism underlying this phenomenon remains to be determined.

TPQ inhibits K+ currents with Ki values of approximately 1 nM for Kir1.1 channels and 10 nM for Kir3.x channels, with no effects of 1 µM TPQ on Kir2.1, KATP, Kv or L-type VGCCs (31, 34). Some evidence indicates that 100 nM TPQ can inhibit BKCa channels expressed in Xenopus oocytes (32); however, the irreversibility of TPQ-induced BKCa channel blockade (even 90 min after TPQ washout) is in striking contrast to the rapidly reversible effects of TPQ on Kir1.1 currents (9). Thus, the rapidly reversible afferent arteriolar contractile response to TPQ evident in the present study likely reflects the impact of this agent on Kir1.1 and/or Kir3.x channels, rather than on BKCa channels. TPQ-sensitive Kir3.x channels are expressed in neurons, atrial myocytes and neuroendocrine cells, with tetrameric assemblies of Kir3.x subunits comprising the G protein-coupled inwardly-rectifying K channels (GIRKs) that regulate neuronal excitability and heart rate (35). Kir3.1 mRNA has been detected in aortic vascular smooth muscle, where it is unaltered during T1D (47), but we are unaware of any evidence indicating protein expression or functional Kir3.x channels in the vasculature or in the kidney. On the other hand, Kir1.1 channels are expressed at the mRNA and protein levels in the renal cortical collecting duct, connecting tubule, distal convoluted tubule, macula densa and thick ascending limb (54). In the thick ascending limb, K+-recycling across the apical membrane via Kir1.1 channels is essential for maintaining function of the Na-K-2Cl cotransporter and, hence, Na+
reabsorption. One can envision a demand for increased apical K⁺ recycling in the thick ascending limb to sustain the increased Na⁺ reabsorption by this nephron segment during T1D, thereby decreasing solute concentration in early distal tubular fluid and, presumably at the macula densa (45, 53). In this manner, Kir1.1-dependent mechanisms in the thick ascending limb could contribute to tubuloglomerular feedback-mediated afferent arteriolar dilation during T1D. However, results of the present study failed to reveal any impact of acute surgical papillectomy on the vasoconstrictor response to TPQ in STZ kidneys, thus making it unlikely that TPQ-sensitive Kir1.1 channels in the thick ascending limb influence afferent arteriolar diameter during T1D by evoking a tubuloglomerular feedback response. As papillectomy would also abolish flow of tubular fluid through the connecting tubule, it is also unlikely that changes in Kir1.1 expression/function in that nephron segment underlie afferent arteriolar dilation during T1D via the connecting tubule-glomerular feedback system (46). We cannot rule out the possibility that Kir1.1 channels in macula densa or the connecting tubule tonically influence afferent arteriolar tone during T1D independent of solute delivery. Alternatively, expression of TPQ-sensitive channels in afferent arteriolar smooth muscle or endothelial cells may be induced or gain functional relevance during T1D, thereby contributing to the preglomerular vasodilation underlying diabetic hyperfiltration. Extensive further investigation is necessary to evaluate the validity of these scenarios that may underlie emergence of a tonic afferent arteriolar dilator impact of TPQ-sensitive channels in T1D.

In addition to evaluating the impact of Kir channels on afferent arteriolar tone in kidneys from Normal and STZ rats, the present study also explored the impact of other K⁺ channels known to be expressed in the vasculature. At the concentration used in the present study (1 mM), TEA is widely employed as a potent blocker of BKCa channels (2, 28, 36), although some Kv channel subclasses expressed in the vasculature may also be influenced (Kv1.1 and Kv3.x)(13, 19). To date, the only Kv channel detected by patch clamp study of renal microvascular smooth muscle is a 46 pS channel, probably a delayed rectifier, that is insensitive to TEA (58). Intrarenal
arterial administration of TEA evokes modest vasoconstriction that is not mimicked by iberiotoxin (a highly selective BK$_{Ca}$ channel blocker), suggesting that TEA has in vivo renal vascular effects that arises independent of BK$_{Ca}$ channel blockade (41). However, in studies of afferent arteriolar function using the in vitro blood-perfused juxtamedullary nephron technique, the effects of 1 mM TEA and 100 nM iberiotoxin are virtually indistinguishable (15, 26). Thus, it is likely that the effect of TEA on afferent arteriolar diameter in this experimental setting largely reflects the impact of BK$_{Ca}$ channels in maintenance of arteriolar tone. This effect was of modest magnitude in arterioles of Normal kidneys, in accord with our previous observations (15), indicating that these channels do not play a major role in the control of basal juxtamedullary afferent arteriolar tone. Moreover, quantitatively similar effects of TEA were observed in Normal and STZ kidneys, suggesting that increased BK$_{Ca}$ channel activity does not significantly contribute to the tonic afferent arteriolar dilation during T1D.

The involvement of KV channels as determinants of afferent arteriolar tone was explored through use of 4-AP, which blocks KV channels with $K_{i}$ values in the range of 0.2-1.5 mM (19). In renal microvascular smooth muscle cells, 4-AP blocks the 46 pS KV channel and transient outward current attributed to KV channels, but has no effect on BK$_{Ca}$ channels (18, 58). As previous studies have shown that 4-AP modestly potentiates the afferent arteriolar myogenic response (33), it is not surprising to find that 4-AP constricts afferent arterioles of in vitro blood perfused juxtamedullary nephrons studied at a perfusion pressure above the autoregulatory threshold; however, the contractile response to 4-AP did not differ significantly between Normal and STZ kidneys. Thus, although these data confirm that KV channels contribute significantly to afferent arteriolar tone in the normal kidney, they provide no evidence that these channels are responsible for the dilation that occurs during T1D.

Published evidence indicates that 50 nM apamin, a selective blocker of SK$_{Ca}$ channels (1), inhibits a 68 pS Ca$^{2+}$-activated K$^{+}$ channel but not the BK$_{Ca}$ channel in preglomerular microvascular smooth muscle cells (16). Although these 68 pS channels have a higher
conductance than generally attributed to SK$_{Ca}$ channels (55), 50 nM apamin also reduced macroscopic K$^+$ current recorded from voltage-clamped renal arteriolar muscle cells by 25-30% (16), suggesting that apamin-sensitive channels may play a significant role in setting membrane potential and baseline tone in the afferent arteriole. However, results of the present study failed to reveal any impact of 100 nM apamin on juxtamedullary afferent arteriolar tone in either Normal or STZ kidneys. Thus, the role of apamin-sensitive SK$_{Ca}$ channels in control of the renal microvasculature remains speculative.

In conclusion, the main findings of this study are: 1) KV, KIR and BK$_{Ca}$ channels exert tonic dilator influences on the normal afferent arteriole, while SK$_{Ca}$ channels have no discernible tonic impact; 2) tonic activation of K$_{IR}$ channels (likely Kir2.1, as well as Kir1.1 and/or Kir3.x) contributes to the afferent arteriolar dilation during T1D; and 3) the dilator impact of Kir1.1/Kir3.x channels during T1D is evident regardless of solute delivery to the macula densa. Further investigation is necessary to establish whether these phenomena involve changes in channel activity in the endothelium and/or vascular smooth muscle. In one scenario, K$^+$ efflux through endothelial K$^+$ channels might elevate K$^+$ concentration near the vascular smooth muscle, increasing the current carried by K$_{IR}$ channels in the vascular smooth muscle to elicit hyperpolarization (14). It is not clear how T1D might trigger changes in K$^+$ channel regulation of afferent arteriolar tone. These changes may reflect effects of hyperglycemia per se, renin-angiotensin system activation, oxidative stress, protein kinase C activation, or other downstream events. It is also interesting to note that K$^+$ channel regulation of vascular tone in the renal afferent arteriole contrasts with events occurring in the basilar artery & pial arterioles studied under normal conditions, as well as after a longer-duration of T1D (42), suggesting that these events are not uniform across different vascular beds. In the renal afferent arteriole, increased tonic K channel activity (K$_{ATP}$, as previously reported (24); and K$_{IR}$ channel family members, per the present study) should favor vascular smooth muscle hyperpolarization that, together with concomitant changes in Ca$^{2+}$ channel responsiveness to membrane potential (7), can be
envisioned to act synergistically to promote afferent arteriolar dilation during the hyperfiltration stage of T1D.

ACKNOWLEDGEMENTS

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FIGURE LEGENDS

Figure 1: Effects of barium on afferent arteriolar diameter in Normal kidneys (closed symbols; \(n=6\) arterioles) and STZ kidneys (open symbols; \(n=9\) arterioles). Actual lumen diameter (left) and change in lumen diameter data (right) show vasoconstrictor responses to 10, 30, and 100 \(\mu\)M \(\text{Ba}^{2+}\). Values are mean ± SEM. *\(P<0.05\) versus baseline diameter; †\(P<0.05\) vs arterioles from Normal kidneys.

Figure 2: Effects of increasing tertiapin-Q (TPQ) on afferent arteriolar diameter in Normal kidneys (closed symbols; \(n=14\) arterioles) and STZ kidneys (open symbols; \(n=11\) arterioles). Actual lumen diameter (left) and change in lumen diameter data (right) show vasoconstrictor responses to 1, 10, and 100 nM TPQ. Values are mean ± SEM. *\(P<0.05\) versus baseline diameter; †\(P<0.05\) vs arterioles from Normal kidneys.

Figure 3: Effect of acute surgical papillectomy on afferent arteriolar responses to TPQ in Normal kidneys (\(n=7\) arterioles) and STZ kidneys (\(n=6\) arterioles) before (closed bars) and after (open bars) papillectomy. These data were obtained from a subset of the vessels used to generate Figure 2. Data are shown as the change in lumen diameter in response to 100 nM TPQ. Values are mean ± SEM. *\(P<0.05\) versus baseline diameter; †\(P<0.05\) vs response in Normal kidneys. No significant effect of papillectomy was evident in either group.

Figure 4: Effects of tetraethyl-ammonium (TEA) on afferent arteriolar diameter in Normal kidneys (closed symbols; \(n=6\) arterioles) and STZ kidneys (open symbols; \(n=5\) arterioles). These data were obtained from a subset of the vessels used to generate Figure 2. Actual lumen diameter (left) and change in lumen diameter data (right) show vasoconstrictor responses to 1 mM TEA. Values are mean ± SEM. *\(P<0.05\) versus baseline diameter; †\(P<0.05\) vs arterioles from Normal kidneys.

Figure 5: Effects of increasing concentrations of 4-aminopyridine (4-AP) on afferent arteriolar diameter in Normal kidneys (closed symbols; \(n=6\) arterioles) and STZ kidneys (open
symbols; \( n = 6 \) arterioles). Data expressed as actual lumen diameter (left) and change in lumen diameter (right) show vasoconstrictor responses to 0.1, 0.3, 1, 3 mM 4-AP. Values are mean ± SEM. *\( P < 0.05 \) versus baseline diameter; †\( P < 0.05 \) vs arterioles from Normal kidneys.

Figure 6: Effects of apamin on afferent arteriolar diameter in normal rats (closed symbols; \( n = 5 \) arterioles) and STZ rats (open symbols; \( n = 6 \) arterioles). Data expressed as actual lumen diameter (left) and change in lumen diameter (right) show the lack of any response to 100 nM apamin. Values are mean ± SEM.
REFERENCES


**Table 1:** General characteristics of STZ-treated rats and Normal rats used in the present study.

<table>
<thead>
<tr>
<th></th>
<th>Normal Rats</th>
<th>STZ Rats</th>
</tr>
</thead>
<tbody>
<tr>
<td>Initial body weight, g</td>
<td>299±3 (n=26 rats)</td>
<td>331±4* (n=26 rats)</td>
</tr>
<tr>
<td>Body weight at terminal experiment, g</td>
<td>361±10 (n=5 rats)</td>
<td>369±13* (n=26 rats)</td>
</tr>
<tr>
<td>Blood glucose concentration(^1), mg/dl</td>
<td>95± 5 (n=6 rats)</td>
<td>23.5±0.9* (n=28 arterioles)</td>
</tr>
<tr>
<td>Baseline afferent arteriolar lumen diameter, µm</td>
<td>17.8±0.6 (n=25 arterioles)</td>
<td>23.5±0.9* (n=28 arterioles)</td>
</tr>
</tbody>
</table>

\(^1\)Blood glucose concentrations represent values obtained from Normal rats on the day of the terminal experiment, and the average values for STZ rats measured over the 3 wk prior to the terminal experiment. *\(P < 0.05\) vs Normal rats.
Barium Concentration (μM)

Lumen Diameter (μm)

Δ Lumen Diameter (μm)

-9 -8 -7 -6 -5 -4 -3 -2 -1 0 1

0 10 30 100

Barium Concentration (μM)

Normal

STZ

†

*
Normal STZ
Response to 100 nM TPQ
(Δ Lumen Diameter, μm)

Intact
Papillectomy

Normal
STZ

Response to 100 nM TPQ
(Δ Lumen Diameter, μm)

Intact
Papillectomy

* †
Basal Apamin Lumen Diameter (μm)

- 12
- 14
- 16
- 18
- 20
- 22
- 24
- 26
- 28
- 30
- 32
- 34

Δ Lumen Diameter (μm)

- -5
- -4
- -3
- -2
- -1
- 0
- 1
- 2
- 3
- 4
- 5

(100 nM)

Normal STZ

- Basal
- Apamin (100 nM)

- Basal
- Apamin (100 nM)