Intracellular ATP Can Regulate Afferent Arteriolar Tone via ATP-sensitive K^+ Channels in the Rabbit

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Abstract

Studies were performed to assess whether ATP-sensitive K^+ (K_{ATP}) channels on rabbit preglomerular vessels can influence afferent arteriolar (AA) tone. K^+ channels with a slope conductance of 258±13 (n = 7) pS and pronounced voltage dependence were demonstrated in excised patches from vascular smooth muscle cells of microdissected preglomerular segments. Channel activity was markedly reduced by 1 mM ATP and in a dose-dependent fashion by glibenclamide (10^{-9} M to 10^{-6} M), a specific antagonist of K_{ATP} channels. 10^{-5} M diazoxide, a K^+ channel opener, activated these channels in the presence of ATP, and this effect was also blocked by glibenclamide. To determine the role of these K_{ATP} channels in the control of vascular tone, diazoxide was tested on isolated perfused AA. After preconstriction from a control diameter of 13.1±1.1 to 3.5±2.1 μm with phenylephrine (PE), addition of 10^{-5} M diazoxide dilated vessels to 11.2±0.7 μm, which was not different from control. Further addition of 10^{-5} M glibenclamide reconstricted the vessels to 5.8±1.5 μm (n = 5; $P < 0.03$). In support of its specificity for K_{ATP} channels, glibenclamide did not reverse verapamil induced dilation in a separate series of experiments. To determine whether intracellular ATP levels can effect AA tone, studies were conducted to test the effect of the glycolytic inhibitor 2-deoxy-D-glucose. After preconstriction from 13.4±3.2 to 7.7±1.3 μm with PE, bath glucose was replaced with 6 mM 2-deoxy-D-glucose. Within 10 min, the arteriole dilated to a mean value of 11.8±1.4 μm (n = 6; NS compared to control). Subsequent addition of 10^{-5} M glibenclamide significantly reconstricted the vessels to a diameter of 8.6±0.5 μm ($P < 0.04$). These data demonstrate that K_{ATP} channels are present on the preglomerular vasculature and that changes in intracellular ATP can directly influence afferent arteriolar tone via these channels. (*J. Clin. Invest.* 1992. 90:733–740.) Key words: kidney • glibenclamide • diazoxide • 2-deoxy-D-glucose • patch clamp

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Introduction

ATP-sensitive potassium channels (K_{ATP})[1] were first described in cardiac muscle (1), and have since been reported and characterized in skeletal muscle, pancreatic β-cells, vertebrate axons, and, most recently, in vascular smooth muscle cells (2–5). Since these channels are very sensitive to intracellular concentrations of ATP, they provide an important potential link between cell metabolism and electrical excitability (2–4). Recent evidence suggests that these channels may play an important role in the pharmacologic and physiologic regulation of vascular smooth muscle tone. For example, several vasodilators known to hyperpolarize vascular smooth muscle cells, notably minoxidil, cromakalim, and diazoxide, have been found to activate ATP-sensitive K^+ channels, and the action of these drugs can be reversed by the sulfonylurea glibenclamide, which has been shown to be a selective inhibitor of K_{ATP} channels in vascular smooth muscle cells (5–7). K_{ATP} channels on vascular smooth muscle cells therefore may represent an important regulatory mechanism for the control of vascular tone.

In addition to being sensitive to the intracellular ATP concentration, K_{ATP} channel activity can also be influenced by a wide variety of other physiological variables including ADP and other nucleotide concentration, intracellular pH, divalent cation concentration, and G-protein activity (3). There is also indirect evidence that phosphorylation of certain types of ATP-sensitive K^+ channels is necessary to retain the open state (4, 8). Finally, evidence strongly suggests that certain endogenous vasodilator peptides, including vasoactive intestinal polypeptide (5), endothelium derived hyperpolarizing factor (9), and calcitonin gene related peptide (10), produce relaxation at least in part by activating these K^+ channels. In spite of this supporting evidence, there is still debate concerning the actual importance of ATP in controlling the activity of these channels. The inhibitory constant of ATP for these channels is very low, suggesting that at normal physiologic levels of intracellular ATP channel activity would be completely absent (4). Thus, in spite of the pharmacologic evidence regarding the role of these channels, there has been no direct evidence to support the concept that actual changes in intracellular ATP concentration can influence vascular smooth muscle tone via ATP-sensitive K^+ channels.

The aim of the present study was to determine whether K_{ATP} channels are present on the preglomerular microvasculature of the rabbit kidney using both the patch clamp technique and the isolated perfused afferent arteriole technique and to assess whether manipulation of cell ATP levels affect vascular tone through these channels. Single channel responses to diaz-

1. *Abbreviations used in this paper:* K_{ATP}, ATP-sensitive potassium; PE, phenylephrine.

oxide, a K^+ channel opener, and glibenclamide, a specific blocker of K_{ATP} channels, were determined on excised membrane patches obtained from preglomerular vascular smooth muscle cells of the rabbit kidney. Functional correlates to these single channel events were then assessed by measuring the effects of these agents on the diameter of isolated afferent arterioles. To determine whether intracellular ATP can influence preglomerular resistance by interacting directly with these channels, the afferent arteriolar response to intracellular ATP depletion was tested, as well as the ability of glibenclamide to modify this response. The studies establish the presence of ATP-sensitive K^+ channels on the afferent arteriole and suggest that modulation of cell ATP levels may affect renal blood flow, in part through an effect on these channels.

Methods

Patch clamp studies

Animals and tissue preparation. All experiments were performed on renal vascular smooth muscle cells obtained from New Zealand white rabbits weighing 0.8–1.5 kg and maintained on a standard rabbit chow. After euthanasia, the left kidney was removed, decapsulated, and sliced transversely through the papilla. The slices were placed in ice-cold DME containing 3% fetal calf serum, and dissection was done at 4°C. The dissection medium was bubbled with 5% CO_2 and 95% O_2, and its pH was adjusted to 7.4 immediately before use. The outer cortical portions of interlobular vascular trees were closely examined, and terminal branches of an interlobular artery, with several attached afferent arterioles, were dissected free using sharpened forceps. Glomeruli were gently removed using a razor blade. After dissection, vascular segments were incubated for 20 min at 32°C in Hanks' solution containing: (in milligrams per milliliter) collagenase (0.5; 115 U/ml); trypsin inhibitor (0.3); papain (0.4; 5.2 U/ml); dithiothreitol (0.3); BSA (7.5); pH, 7.4. Specimens were then washed and placed in normal Hanks' solution at room temperature and used within 2 h.

Patch clamp technique. Single channel currents were obtained using standard patch clamp techniques as described by Hamill et al. (11). All measurements were made at room temperature on excised patches in the inside-out configuration. Isolated vascular smooth muscle cells were visualized using an inverted microscope. Micropipettes were fabricated from capillary tubes and had a tip resistance of 5–10 Mohm. Seal resistances were in the range of 5–20 Gohm. Single channel currents were recorded through a patch clamp amplifier (8900; Dagan Corp., Minneapolis, MN). Amplifier output was filtered with an 8-pole Bessel filter set at a corner frequency of 3.4 kHz, digitized, and stored on videotape.

Bath and pipette filling solutions of the following composition were used for most observations (in millimolars): 145 KCl, 10 Hepes (*N*-[2-hydroxyethyl] piperazine-*N'*-[2-ethanesulfonic acid]), 10 glucose, and KOH to bring pH to 7.4. A calcium-buffered bath solution with the following composition was used for experiments in which calcium or ATP levels were varied (free calcium < 10 nM) (millimolar): 113 KCl, 2.02 EGTA, 20.2 K_2Pipes (Piperazine-*N*-*N'*-bis [2-ethanesulfonic acid]); pH 7.0. Calcium concentration was varied by addition of $CaCl_2$, with free calcium determined from the EGTA dissociation constant. ATP was added as the K salt (Sigma Chemical Co., St. Louis, MO), and free calcium concentration was adjusted by addition of $CaCl_2$, with correction for the ATP-dissociation constant. All drugs were added to the bath solution. Vehicles were tested independently.

Protocol. After formation of the gigaseal to establish the on-cell configuration, the pipette tip was passed briefly through the air-liquid interface to destroy the cell body and leave an inside-out patch of membrane. In this configuration the properties such as conductance, voltage, and divalent ion dependency, ATP-sensitivity, selectivity of the potassium channel present were determined. Potassium channels inactivated by ATP were characterized further by their response to bath addition of the K_{ATP} antagonist glibenclamide (Sigma Chemical Co.). in concentrations from 10^{-9} to 10^{-6} M, and/or the K_{ATP} activator, diazoxide (Quad Pharmaceuticals Inc., Indianapolis, IN) at a concentration 10^{-5} M.

Isolated perfused arteriole studies

Animals and technique. All experiments were performed on isolated afferent arterioles obtained from New Zealand white rabbits as described above. After selecting an afferent arteriole and its attached glomerulus from the outer cortical portion of an interlobular tree, tubular fragments were carefully removed using sharpened forceps. Care was taken not to stretch the vessels or to touch the vascular structures at the glomerular hilum. After dissection, the interlobular artery was cut at a site just before the branch point of the afferent arteriole, so that the final specimen consisted of a segment of interlobular artery, the afferent arteriole, the glomerulus, and a fragment of the efferent arteriole. Inspection of the specimen under high power usually revealed the presence of the macula densa cells. Specimens were not accepted if the total dissection time exceeded 60 min.

After dissection, specimens were transferred into a thermoregulated chamber placed on the stage of an inverted microscope (Olympus IMT-2) for cannulation as previously described (12). Briefly, using concentric pipettes mounted on a moveable track system (13), the proximal end of the interlobular artery was aspirated into a holding pipette. The perfusion pipette was then advanced through the lumen of the interlobular artery into the afferent arteriole. The perfusate was driven by a hydraulic pressure head of 110 cm H_2O. After establishing vessel perfusion, a small cover slip was placed over the top of the bath creating a small, closed, and stable chamber with a total volume of $\sim$ 100 μl. The bath temperature was then elevated to 38°C. Fresh bath medium was supplied by a syringe pump at a rate of 200 μl/min and removed continuously by aspirating the effluent. Initial spontaneous contractions with varying intensity were observed during the warmup period in the majority of vessels. These contractions in general subsided during an equilibration period of 60 min. Before studies were started, specimens were inspected for cellular integrity and the presence of macula densa cells using differential interference contrast optics (Olympus IMT-2-DIC) at magnifications of 600.

Protocols

Series 1: diazoxide. In five experiments, the ability of diazoxide to dilate preconstricted arterioles was tested. In all of the following experiments, a phenylephrine (PE) dose-response curve for each vessel was first determined with doses from 10^{-7} to 10^{-5} M, and a dose of PE was selected to preconstrict the vessel by 50–80%. After preconstriction with PE, diazoxide was added to the bathing medium at a concentration of 10^{-5} M. When a stable response to diazoxide had been obtained, usually after 3–4 min, 10^{-5} M glibenclamide was also added to the bathing medium to test its ability to counteract the affect of diazoxide. After 5 min, all agents were removed from the bath to determine recovery. In a separate series of control experiments, the time course of PE constriction was determined over a 10-min period, first in the presence of diazoxide vehicle and then in the presence of glibenclamide vehicle.

Series 2: verapamil. To test the specificity of the constrictor effect of glibenclamide, studies were performed to determine its ability to reverse the effect of verapamil. After preconstricting arterioles with PE, verapamil was added to the bath at a concentration of 10^{-5} M, and steady-state vasodilation was achieved in $\sim$ 3 min. Glibenclamide was then added to the bath at 10^{-5} M for a 5-min period, which was followed by a recovery period.

Series 3: 2-deoxy-D-glucose. All experiments were performed using RPMI 1640 glucose-deficient medium rather than DME as the bathing medium so that glucose could be directly varied. To determine whether decreasing intracellular levels of ATP can dilate afferent arterioles, the effect of replacing glucose with 2-deoxy-D-glucose was tested. Substitution of this analogue for glucose has been shown to decrease the intra-

cellular concentration of ATP (14). Before the beginning of this protocol, the luminal perfusate solution was exchanged for an identical solution without glucose. Afferent arterioles were first preconstricted with a half-maximal dose of PE in the presence of 100 mg% glucose. The bathing medium was then replaced with an identical solution in which the glucose had been completely replaced with 100 mg% 2-deoxy-D-glucose. When a stable response had been achieved in the presence of PE and 2-deoxy-D-glucose, usually within 10 min, 10^{-5} M glibenclamide was then added to the bath for a period of 5 min, which was followed by a final recovery period.

Solutions and reagents. Dissection and bath media were prepared from DME containing Ham's nutrient mixture F-12 with the addition of 1.2 g/liter $NaHCO_3$ (Sigma Chemical Co.). Before use this solution was aerated with 95% O_2/5% CO_2 for 45 min and its pH was adjusted to 7.4. When used as dissection medium, fetal calf serum (Gibco Laboratories, Grand Island, NY) was added to a final concentration of 3%. When used as bath medium, 1 mM $CaCl_2$ (final [Ca] = 2 mM) was added, and bovine serum albumin (Sigma Chemical Co.) was added to a concentration of 0.5%. No significant difference in bath medium pH was noted between delivered and collected samples. Vessel perfusion fluid was a modified Krebs-Ringer-HCO_3 buffer containing (millimolar): 115 NaCl, 25 $NaHCO_3$, 0.96 NaH_2PO_4, 0.24 Na_2HPO_4, 5 KCl, 1.2 $MgSO_4$, 2 $CaCl_2$, and 5.5 glucose. The buffer was gassed with 95% O_2/5% CO_2 and pH adjusted to 7.4 and bovine serum albumin was added to a final concentration of 1%. In experiments requiring glucose-free medium (series 3), RPMI 1640–deficient medium without glucose (Sigma Chemical Co.) was used instead of DME. Glucose or 2-deoxy-D-glucose was added where necessary, and the medium was otherwise treated identically to the DME.

Hanks' solution contained: (millimolar) 140 NaCl, 5.4 KCl, 0.44 KH_2PO_4, 0.42 NaH_2PO_4, 4.17 $NaHCO_3$, 0.1 $CaCl_2$, 5 Hepes, 5.55 glucose; pH adjusted to 7.4 with NaOH. PE (Sigma Chemical Co.), diazoxide (Quad Pharmaceuticals), and 2-deoxy-D-glucose (Sigma Chemical Co.), were dissolved in a stock solution of saline and diluted 100-fold in the final bath medium. Glibenclamide (Sigma) was dissolved in DMSO and diluted 1,000-fold in the final bath medium. ATP (Sigma) for patch clamp studies was dissolved in distilled water.

Measurements and statistics

Vascular diameter changes were recorded on videotape at a magnification of 600 using differential interference contrast optics. Intraluminal vascular diameters were measured on the video images using an image analysis system (Cue 2; Olympus Corp.). Since the luminal diameter of these vessels typically displayed a prominent heterogeneity along their length, measurements were made at several sites. Values reported in the text reflect the diameters measured at the most responsive segment observed for each vessel. Measurements were made on image frames taken after a steady-state diameter had been achieved for each treatment.

Statistical analysis of the data was performed on diameter measurements (expressed in micrometers) obtained directly from the image analysis system which had been previously calibrated using a slide micrometer. In all perfusion studies, statistical analysis was performed using a single-factor, within-subjects analysis of variance (ANOVA), and the Newman-Keuls multiple range test was used to supplement the analysis, where necessary, to compare individual means. Values in the text and figures represent means±SEM and differences were regarded as significant at a probability of $P < 0.05$.

Results

Patch clamp studies

Although channel activity was rarely seen in the on-cell configuration, potassium channel activity of some type appeared in ~ ⅓ of the cells examined upon conversion to the inside-out configuration. Of the excised patches exhibiting activity, ~ ⅓ contained a nonrectifying, large conductance potassium channel with a unitary conductance, measured with 145 mM $[K]_o$/145 mM $[K]_i$, averaging 258±13 pS ($n = 7$). As shown in Fig. 1, the activity of these large K^+ channels was voltage dependent between +20 and −60 mv. In previous reports in vascular smooth muscle cells somewhat lower conductance estimates and weaker voltage dependence have been reported; for example, in mesenteric arteries, K_{ATP} channels with a conductance value of 135 pS were observed with 60 mM $[K]_o$/120 mM $[K]_i$ (4). The difference in conductance probably reflects conditions of measurement, although differences in channel properties in different vascular smooth muscle cell types cannot be excluded.

As Fig. 2 *A* illustrates, channel activity in these patches ($n = 6$) was almost completely inactivated by 1 mM ATP and this effect was reversible. Mean current in the presence of 1 mM ATP was reduced to 17.8±3.2% of control ($n = 6$). The inhibition of channel activity by ATP could be partially reversed by 10 μM diazoxide as illustrated in Fig. 3 ($n = 3$). Finally, separate experiments demonstrated that the activity of these chan-

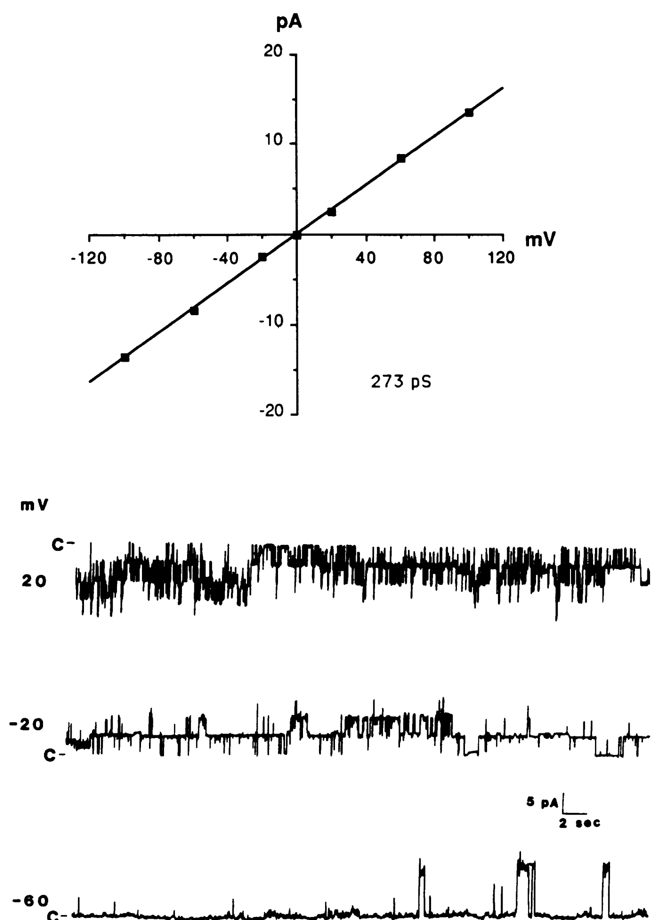

Figure 1. (*Top panel*) Current-voltage plot for a single K^+ channel. Unitary conductance for this channel was 273 pS. Excised patches obtained from enzymatically dissociated vascular smooth muscle cells from the rabbit afferent arteriole were studied in the inside-out configuration. (*Bottom panel*) Original tracings from an excised membrane patch showing voltage dependence at membrane potentials of 20, −20, and −60 mv.

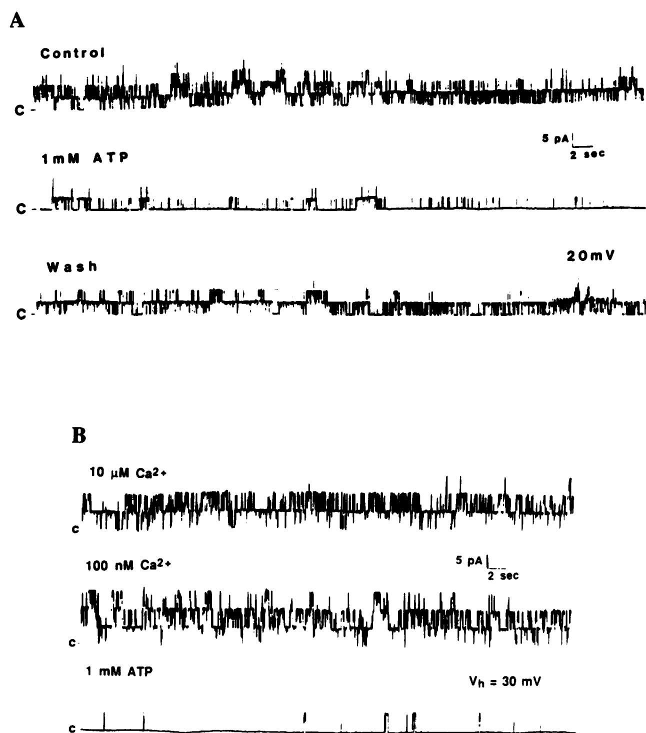

Figure 2. (*A*) Original tracings from an excised membrane patch in the inside-out configuration showing ATP sensitivity of the K^+ channel. In the absence of ATP (*top*), channel activity is high. When 1 mM ATP is added to the bath (cytosolic side), channel activity is markedly inhibited (middle tracing: recorded 1 min after addition of ATP). After washing, channel activity returns toward control levels (bottom tracing: recorded 2 min after washing). (*B*) Original tracings from an excised membrane patch in the inside-out configuration showing the lack of an effect of changing bath Ca^{++} concentration on the open probability of the ATP-sensitive K^+ channel.

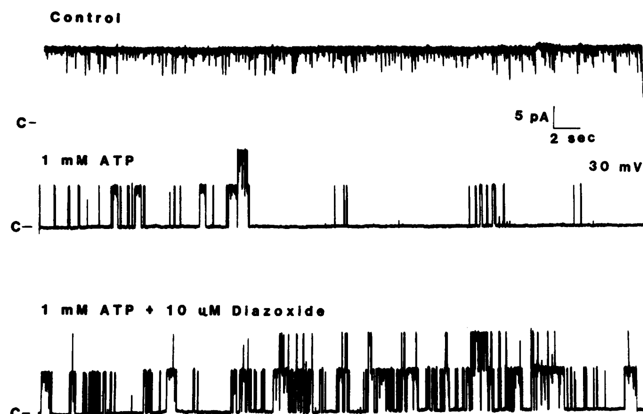

Figure 3. Original tracings from an excised membrane patch in the inside-out configuration showing that the K^+ channels are opened by diazoxide. Under control conditions, channels are almost constantly open (*top*). Addition of 1 mM ATP to the bath greatly reduces channel activity (middle tracing: recorded 1 min after addition of ATP). Further addition of 10 μM diazoxide increases K^+ channel activity (bottom tracing: recorded 1 min after addition of diazoxide).

nels could be inhibited by glibenclamide in a concentration-dependent manner such that channel activity was nearly abolished at 1 μM (Fig. 4; $n = 3$). This effect was not reversible.

Channel activity was not affected by free calcium concentration in the range from < 10 nM to 10 μM (see Fig. 2 *B*). In addition, in some excised patches from these cells, a large conductance (301±9.3 pS, $n = 3$) K channel was observed that was calcium dependent; consistent with previous reports (15), these Ca-sensitive channels were not affected by ATP or 1 μM glibenclamide.

Isolated perfused arteriole studies

Series 1. Diazoxide. The result of a single representative perfused arteriole experiment with diazoxide is shown in the upper panel of Fig. 5 (*open circles*). After constriction with 10^{-6} M phenylephrine, addition of 10^{-5} M diazoxide to the superfusing bath resulted in a vasodilation to nearly control diameter within 3 min. Subsequent application of the sulfonylurea glibenclamide at 10^{-5} M reconstricted the arteriole to a diameter of ~ 30% of control. Diameter returned to control levels after removal of all agents. The bottom panel of Fig. 5 shows the mean results for five such experiments (*solid bars*). After preconstriction of the arterioles from a control level of 13.1±1.1 μm to 3.5±2.1 μm ($P < 0.01$) with phenylephrine, addition of 10^{-5} M diazoxide dilated the vessels to a diameter of 11.2±0.7 μm ($P < 0.01$), which was not significantly different from control. Further addition of 10^{-5} M glibenclamide reconstricted vessels to 5.8±1.5 μm ($P < 0.01$ compared to control). Recovery diameter was 14.4±1.3 μm. In separate phenylephrine time control experiments (*hatched bars*, $n = 5$), maximum contraction was achieved within the first 2 min of PE application as

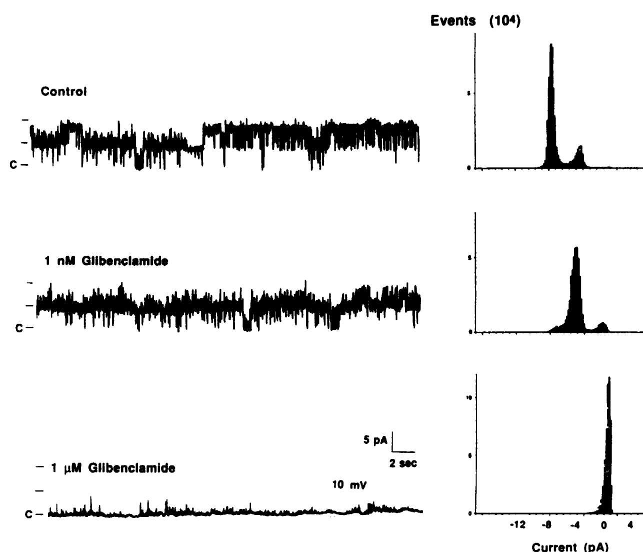

Figure 4. (*Top panel*) Original tracings from an excised membrane patch in the inside-out configuration showing dose-dependent channel sensitivity to glibenclamide. Activity is high during control conditions (*top*). In the presence of 1 nM glibenclamide, K^+ channel activity is markedly reduced (middle tracing: recorded 1 min after addition of 1 nM glibenclamide). In the presence of 1 μM glibenclamide K^+ channel activity is nearly absent (bottom tracing: recorded 1 min after addition of 1 μM glibenclamide). Adjacent to each recording is the current amplitude histograms derived from each 10-s period shown.

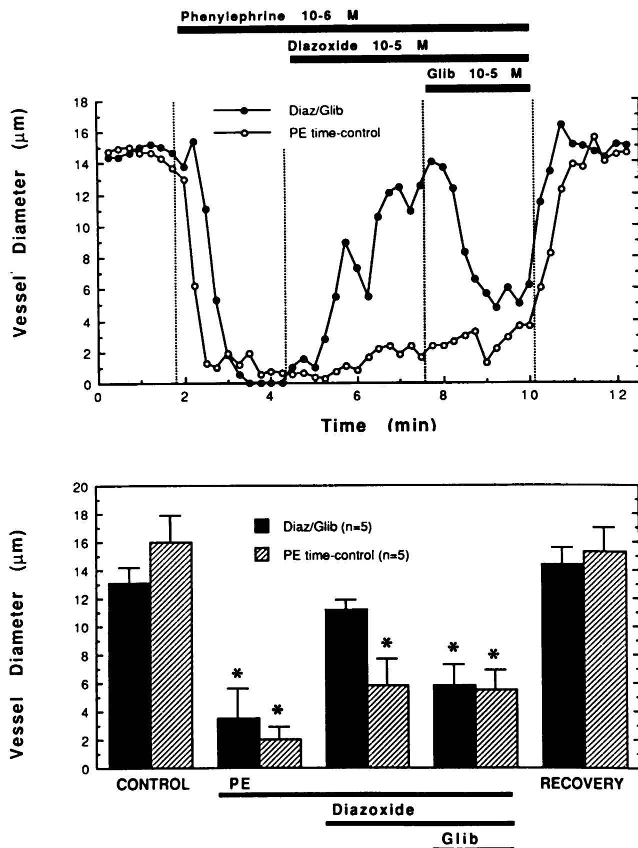

Figure 5. Effect of diazoxide and glibenclamide on afferent arterioles preconstricted with phenylephrine. (*Top*) Closed circles represent the results a single experiment in which an afferent arteriole was serially exposed to PE, diazoxide, and glibenclamide in the bath. Open circles represent a single experiment in which an afferent arteriole was exposed to PE alone for the duration of the experiment; diazoxide and glibenclamide vehicle were added at appropriate times. (*Bottom*) Mean stable diameters for five diazoxide/glibenclamide experiments (*solid bars*) and for five PE time-control experiments (*hatched bars*). $*P < 0.01$ compared to control period in the same group.

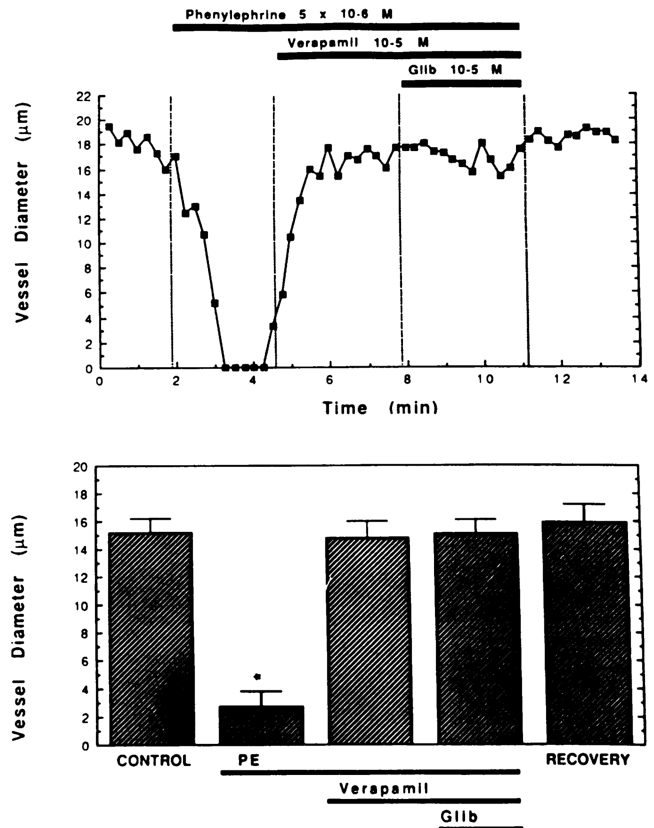

Figure 6. Effect of verapamil and glibenclamide on afferent arterioles preconstricted with phenylephrine. (*Top*) Results of a single experiment in which an afferent arteriole was serially exposed to PE, verapamil, and glibenclamide in the bath. (*Bottom*) Mean stable diameters for six verapamil/glibenclamide experiments. $*P < 0.001$ compared to control period.

diameter decreased from 16.0±1.9 μm to 2.0±1.0 μm ($P < 0.01$). During application of diazoxide vehicle, the PE contraction waned slightly, but not significantly, as diameter increased to 5.8±1.9 μm. Thereafter, the diameter remained stable at 5.5±1.4 μm during glibenclamide vehicle application, and diameter returned to 15.3±1.7 μm during recovery. It should be noted that the vessel diameter during the experimental glibenclamide period was not significantly different from that during the glibenclamide vehicle period (5.8±1.5 vs 5.5±1.4 μm). A representative experiment showing the maintenance of the phenylephrine response is shown by the open circles in the upper panel of Fig. 5.

Series 2. Verapamil. The upper panel in Fig. 6 illustrates the results from a single verapamil experiment. Following constriction with phenylephrine, addition of 10^{-5} M verapamil resulted in vasodilation to control values, and this effect was not further modified by the addition of glibenclamide. The means of six of these experiments are illustrated in the lower panel of Fig. 6. Arterioles were constricted from 15.2±1.0 μm to 2.7±1.1 μm ($P < 0.001$) with PE, and subsequent addition of 10^{-5} M verapamil dilated vessels to 14.8±1.2 μm, which is not significantly different from control. Subsequent addition of 10^{-5} M glibenclamide did not effect vessel diameter, which then averaged 15.1±1.0 μm. Recovery diameter was 15.9±1.3 μm.

Series 3. 2-deoxy-D-glucose. The upper panel of Fig. 7 shows the results from a single experiment with 2-deoxy-D-glucose. After preconstriction with phenylephrine, exposure of the vessel to 100 mg% 2-deoxy-D-glucose in the absence of glucose resulted in a gradual vasodilation over a period of 10 min. This dilation was partially reversed by the subsequent addition of 10^{-5} M glibenclamide. Photomicrographs taken from one of these experiments are shown in Fig. 7 *B,* and letters (*a–e*) on each panel correspond to the letters labeling each treatment period in the upper panel of Fig. 7 *A*. The results of six such experiments are shown in the lower panel of Fig. 7. Phenylephrine constricted vessels from a mean control diameter of 13.4±1.3 μm to 7.7±1.3 μm ($P < 0.01$). Substitution of glucose with 2-deoxy-D-glucose caused a gradual dilation which was complete within 10–15 min. After 3–5 min of exposure to 2-deoxy-D-glucose, the mean diameter had decreased only slightly to 6.7±2.0 μm (NS). After 6–9 min, diameter had increased to 9.3±2.0 μm, but this change was also not significant. After 10–15 min, the diameter had increased to 11.7±1.4 μm which was significantly greater than the PE-preconstriction

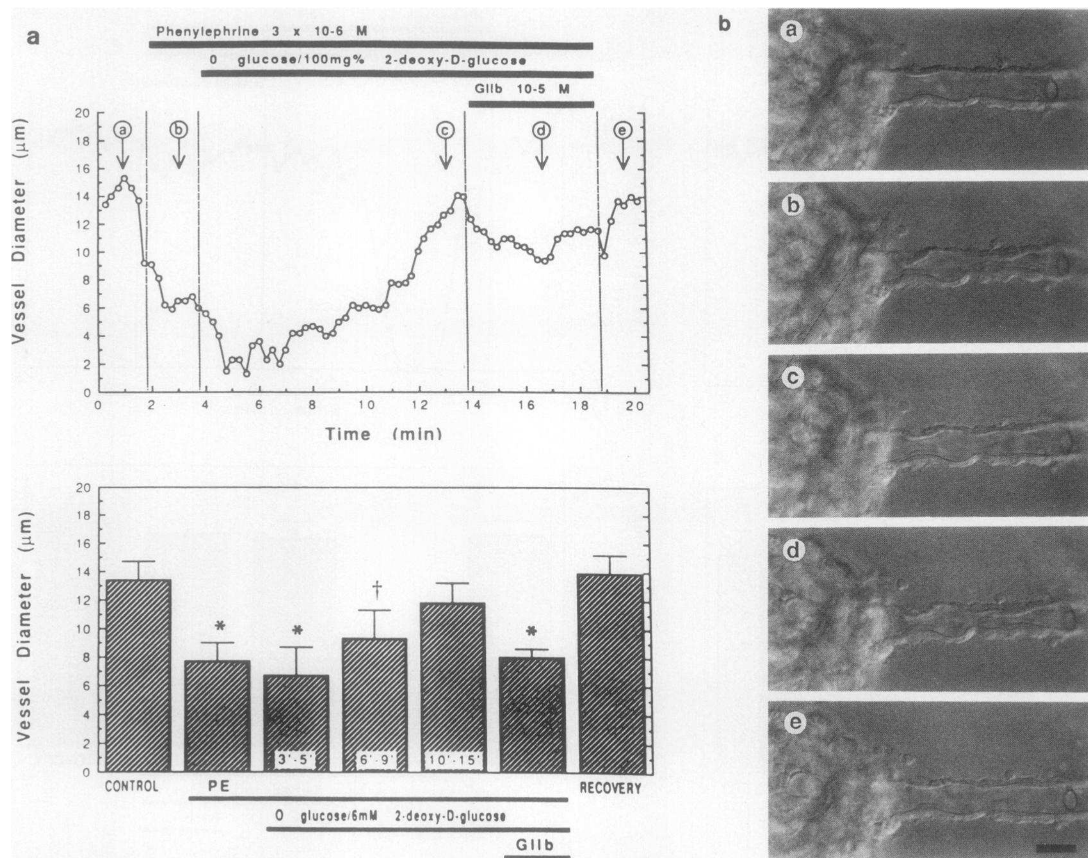

Figure 7. (*a*) Effect of glycolytic inhibition with 2-deoxy-D-glucose on preconstricted arterioles. (*Top*) Results of a single experiment in which an arteriole was serially exposed to PE, 0 glucose/6 mM 2-deoxy-D-glucose, and glibenclamide in the bath. Letters *a–e* refer to photomicrographs shown in Fig. 8. (*Bottom*) Mean diameters for six 2-deoxy-D-glucose/glibenclamide experiments. The PE + 2-deoxy-D-glucose period lasted 10–15 min and mean diameters were calculated at the ⅓ (3′–5′), ⅔ (6′–9′), and final (10′–15′) time point within that period. $^*P < 0.01$, and $^‡P < 0.05$ compared to control. (*b*) Photomicrograph showing the effects of glycolytic inhibition and of glibenclamide on preconstricted afferent arterioles. Letters *a–e* correspond to the labeled time points in the top panel of Fig. 7: *a,* control; *b,* 5×10^{-6} M PE; *c,* PE + 0 glucose and 6 mM 2-deoxy-D-glucose; *d,* PE + 0 glucose and 6 mM 2-deoxy-D-glucose + 10^{-5} M glibenclamide; *e,* recovery.

level ($P < 0.05$) and not different from control. Further addition of 10^{-5} M glibenclamide caused a vasoconstriction to a mean diameter of 8.0±0.6 μm ($P < 0.05$ compared to the previous period). Recovery diameter was 13.9±1.3 μm.

Discussion

ATP-sensitive K^+ channels, first described in cardiac myocytes (1), have now been demonstrated in a wide variety of cell types (2–4). Although the properties of ATP-sensitive K^+ channels appear to vary somewhat between cell types, all ATP-sensitive K^+ channels are highly selective for potassium and are inhibited by intracellular ATP and by sulfonylureas such as glibenclamide (3). Recent studies have established that channels in this family are also present on vascular smooth muscle cells. Using patch clamp methods on rabbit mesenteric artery smooth muscle cells, Standen and co-workers (5) documented large conductance K^+ channels (135 pS in 60 mM $[K^{++}]_o$/120 mM $[K^+]_i$) which were inactivated either by 1 mM ATP or 20 μM glibenclamide and activated by 1 μM cromakalim. ATP-sensitive K^+ channels have also been demonstrated by patch clamp studies on smooth muscle cells from rat tail arteries (16). Sulfonylureas have been shown to reverse the vasodilation produced by agents such as diazoxide, minoxidil, cromakalim, and pinacidil, supporting the conclusion that these agents act in part by opening K_{ATP} channels (4, 6, 7).

The results of the present study extend these observations to the resistance vessels of the rabbit kidney. In patch clamp studies using enzymatically dissociated cells from microdissected preglomerular vessels of the rabbit kidney, we detected the presence of a K^+ channel with a unitary conductance of ~ 260 pS under symmetrical K^+ concentrations of 150 mM. These K^+ channels were inactivated by 1 mM ATP and by glibenclamide in a dose-dependent fashion from 10^{-9} to 10^{-6} M. In addition, single channel recordings showed that, in the presence of millimolar ATP, application of 10^{-5} M diazoxide can reopen these K^+ channels, providing direct evidence for the molecular mechanism of this agent. These data, therefore, provide electrophysiological evidence for the presence of a potassium channel on the preglomerular vasculature of the rabbit with physiologic and pharmacologic properties consistent with a ATP-sensitive K^+ channel.

Functional support for a role of these channels in the control of preglomerular resistance was obtained in experiments using isolated perfused afferent arterioles preconstricted with phenylephrine. The afferent arteriole was used for study be-

cause it is a resistance vessel critical for regulation of renal blood flow and glomerular filtration rate. In the first experimental series, addition of the K^+ channel opener, diazoxide, produced vasodilatation which could be reversed by addition of glibenclamide. In the second experimental series, glucose uptake and glycolytic production of ATP were inhibited by bathing perfused vessels in glucose-free medium containing 2-deoxy-D-glucose; this produced a vasodilatation which could also be reversed by addition of glibenclamide. In separate control experiments, phenylephrine produced sustained vasoconstriction, unaffected by the application of the drug vehicles, and vasodilation produced by verapamil was not modified by glibenclamide, supporting the specificity of this agent. Taken together with the patch clamp studies, these data suggest that activation of K_{ATP} channels results in cell hyperpolarization and vasodilation, and permit the conclusion that ATP-sensitive K^+ channels can contribute significantly to the control of vascular smooth muscle tone in the rabbit afferent arteriole. Previous studies have provided evidence that K_{ATP} channels play a role in hypoxic vasodilation in coronary vessels (17). Our observations suggest that variation in glucose availability in intact vascular smooth muscle cells also influences vascular tone through an effect on ATP-sensitive K^+ channels.

Patch clamp studies have reported that the inhibitory constant for K_{ATP} channels in inside-out patches from vascular smooth muscle is between 10 and 200 μM (3), and the patch clamp results reported here are consistent with this estimate. Since cytosolic ATP concentrations are normally quite high, in the millimolar range, this low inhibitory constant would predict channel closure under resting conditions. In the present study two observations suggest that ATP-sensitive K^+ channels on the vascular smooth muscle cells were largely closed or inactive during resting conditions. First, channel activity could not be detected before cell detachment in the patch clamp experiments, and second, treatment with glibenclamide in resting afferent arterioles had no vasoactive effect (unreported observation). The acute vasodilation observed in the presence of 2-deoxy-D-glucose appears at first somewhat surprising because such manipulation is unlikely to lower average intracellular ATP concentration below the reported inhibitory constant (14, 18). There are several possible explanations for this apparent discrepancy. ATP may not be the sole factor in determining K_{ATP} channel activity. In both β-cells (19) and cardiac muscle cells (20), the inhibitory constant of ATP on these channels is significantly increased in the presence of ADP, suggesting that the ATP:ADP ratio may actually determine channel activity in the intact cell. In support of this notion, a recent study reported that, in cerebral microvessels, glycolysis is required to maintain normal values of both ATP and ATP:ADP ratio (18). Channel activity has also been reported to be sensitive to cell pH, and the levels of other nucleotides (21–23). Furthermore, as a number of investigators have observed, the high conductance of these channels and their relative abundance on many cell types has the consequence that small changes in the relative open probability may have substantial impact on cell membrane potential (4, 24, 25).

An additional factor, which may be particularly relevant for vascular smooth muscle cells, is that levels of ATP near the plasma membrane may not be the same as those in other intracellular locations. Such heterogeneity may arise as a result of the compartmentation of glucose metabolism described in the vascular smooth muscle cell (26). Vascular smooth muscle cells exhibit a distinctive set of characteristics, largely elucidated by work on muscle energetics, which make it likely that the availability of glucose has an important effect on cell function. First, intracellular glucose concentration in vascular smooth muscle is very low (27), so that glucose transport would appear to be rate limiting in intracellular glucose metabolism. Second, the energy metabolism of vascular smooth muscle is unusual in that the rate of glycolysis is substantial even under aerobic conditions. Approximately 25% of ATP synthesis is provided by the glycolytic breakdown of glucose (28). Third, ATP and glucose metabolism in vascular smooth muscle shows extensive compartmentation. The contractile function of the cell appears to be primarily fueled by oxidative metabolism, while membrane functions are dependent on glycolytic metabolism (26, 28). Thus, glycolytic inhibitors such as 2-deoxy-D-glucose, which, in the absence of glucose, lowers ATP levels in a variety of cells (29) including vascular smooth muscle cells (14, 30, 31), would be expected to have a predominant effect on membrane related functions. In support of this notion, Weiss and co-workers have reported that ATP-sensitive K^+ channels in cardiac myocytes are more closely dependent on glycolytic than on oxidative metabolism (32). It should be noted that treatment with 2-deoxy-D-glucose may lead to a number of related metabolic effects that can influence tone independent of the effect on ATP-sensitive K^+ channels. For example, low ATP levels might alter intracellular Ca^{++} through a direct effect on Ca^{++} channels (33), it might directly affect the rate of myosin light chain kinase phosphorylation (31), and it might directly effect the release of vasoactive substances from the endothelium (34). The demonstration that the vasodilation produced by 2-deoxy-D-glucose could be promptly reversed by addition of the specific ATP-sensitive K^+ channel inhibitor glibenclamide, supports the participation of K_{ATP} channels in the change in vessel tone. These observations are consistent with the hypothesis that reduction in the levels of glycolysis-derived ATP in the vascular smooth muscle cell can influence tone through a direct effect on K_{ATP} channels. Since our perfused vessel preparations include both endothelial cells and vascular smooth muscle cells, an alternate possibility is that removal of glucose and substitution of 2-deoxy-D-glucose resulted in release from the endothelial cell of a factor which activated the vascular K_{ATP} channels. There is some evidence that endothelial cells produce a hyperpolarizing factor acting through this class of channels (5, 35). In the only studies to address directly the effects of 2-deoxy-D-glucose on endothelial factors, however, this agent applied to rabbit aorta reduced release of endothelial derived relaxing factor, and produced directionally opposite changes in vascular tone from that predicted by this proposal (34).

In summary, the present experiments demonstrate the presence of ATP-sensitive potassium channels on vascular smooth muscle cells from small caliber resistance vessels of the rabbit kidney. The properties of these channels are similar to those previously reported from patch clamp studies on larger arteries (5, 10). Functional experiments using the isolated perfused rabbit afferent arteriole showed that these channels can play a role in the control of arteriolar tone, and demonstrate for the first time that manipulation of glucose availability alters vascular tone through a direct effect on ATP-sensitive K^+ channels. These data support the hypothesis that ATP-sensitive K^+ chan-

nels participate in the metabolic control of preglomerular resistance and, by inference, of glomerular filtration rate and renal blood flow.

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