Angiotensin II Depolarizes Podocytes in the Intact Glomerulus of the Rat

Joachim Gloy,* Anna Henger,* Karl-Georg Fischer,* Roland Nitschke,‡ Peter Mundel,§ Markus Bleich,‡ Peter Schollmeyer,* Rainer Greger,† and Hermann Pavenstädt†

Department of Medicine, Division of Nephrology, and †Department of Physiology, University of Freiburg, D-79106 Freiburg, Germany; and ‡Department of Anatomy and Cell Biology, University of Heidelberg, D-69120 Heidelberg, Germany

Abstract

The aim of this study was to examine the effects of angiotensin II (Ang II) on cellular functions of rat podocytes (pod) in the intact freshly isolated glomerulus and in culture. Membrane voltage (V_m) and ion currents of pod were examined with the patch clamp technique in fast whole cell and whole cell nystatin configuration. V_m of pod was −38 ± 1 mV (n = 86). Ang II led to a concentration-dependent depolarization of pod with an ED_{50} of 10^{-8} mol/liter. In the presence of Ang II (10^{-7} mol/liter, n = 20), pod depolarized by 7 ± 1 mV. In an extracellular solution with a reduced Cl− concentration of 32 mmol/liter, the effect of Ang II on V_m was significantly increased to 14 ± 4 mV (n = 8). The depolarization induced by Ang II was neither inhibited in an extracellular Na^+-free solution nor in a solution with a reduced extracellular Ca^{2+} (down to 1 μmol/liter). Like Ang II, the calcium ionophore A23187 (10^{-5} mol/liter, n = 9) depolarized pod by 10 ± 2 mV, whereas forskolin (10^{-5} mol/liter), 8-(4-chlorophenylthio)-cAMP and N2,2'-dibutyryl-cGMP (both 5 × 10^{-4} mol/liter) did not alter V_m of pod. The angiotensin I receptor antagonist losartan (10^{-7} mol/liter) completely inhibited the Ang II-induced (10^{-7} mol/liter) depolarization (n = 5). Like pod in the glomerulus, pod in short term culture depolarized in response to Ang II (10^{-8} mol/liter, n = 5). Our results suggest that Ang II depolarizes podocytes directly by opening a Cl− conductance. The activation of this ion conductance is mediated by an AT_{1} receptor and may be regulated by the intracellular Ca^{2+} activity. (J. Clin. Invest. 1997. 99:2772–2781.) Key words: podocytes • isolated glomeruli • angiotensin II • angiotensin I receptor • membrane voltage

Introduction

Angiotensin II (Ang II) can be locally produced in the glomerulus and it is known to cause a reduction of the ultrafiltration coefficient (K_u), but the cellular basis for this glomerular effect remains unclear (1, 2). It has been suggested that Ang II reduces K_u by a contraction of mesangial cells leading to a reduction of peripheral capillary surface area, but morphometric studies have failed to support this hypothesis and it was questioned whether the contractile state of mesangial cells could affect GFR (1–3).

Besides its hemodynamic effects, Ang II is a growth hormone and contributes to the pathogenesis of glomerulosclerosis (4, 5). It is known that in contrast with other antihypertensive drugs, angiotensin-converting enzyme inhibitors ameliorate proteinuria and the progression of glomerulosclerosis in experimental and human diseases (6, 7). Concerning the influence of Ang II on proteinuria, it has been recently reported that it leads to an increase of urinary protein excretion rate and loss of glomerular size-selective functions in the rat isolated perfused kidney (8). The pathogenesis of glomerulosclerosis is not completely understood, but podocytes seem to play a major role in its initiation and progression (9). The podocyte is the most differentiated cell type in the glomerulus, which forms an important component of the glomerular filtration barrier and stabilizes the glomerular basement membrane. Its complete biological role is not understood. It has been speculated that the contractile foot of the podocyte process might not only have a static function, but it may also regulate glomerular filtration rate by changing filtration surface area and thereby K_u. It was further suggested that the contractile state of the foot processes is controlled by vasoactive hormones like Ang II (10).

On the basis of experiments with cultured glomerular epithelial cells, it has been assumed that podocytes possess receptors for various hormones such as endothelin and Ang II (11–13). However, in view of the high grade of podocyte differentiation, which does not allow cell division under physiological conditions, it is difficult to transfer the data obtained from cultured glomerular epithelial cells to the podocyte in vivo (9, 10). In addition, in the past it has been a matter of debate whether podocytes may proliferate at all and it has been difficult to determine whether glomerular epithelial cells in culture originate from the visceral or parietal epithelium (14, 15). In this regard, the morphological and immunohistochemical properties of glomerular epithelial cells in culture markedly differ from those observed in vivo (15, 16).

To come closer to the in vivo situation, we have developed an experimental approach that allows us to study podocytes in the intact glomerulus. With the help of the patch clamp technique, we have examined the effect of Ang II on membrane voltage and ion currents of podocytes in intact freshly isolated glomeruli. Our results demonstrate that it is possible to examine functional characteristics of podocytes in these intact glomeruli and suggest that Ang II alters the contractile properties of these cells.

Methods

Isolation and preparation of glomeruli. Wistar rats of either sex (80–200 g body wt) were anesthetized by injection of 100 mg/kg thiopen-
Figure 1. Preparation of a glomerulus (interference microscopy, 400×). (A) An isolated rat glomerulus is fixed at the vascular pole by a holding pipette. (B) Removal of the Bowman’s capsule by another glass pipette after incubation with 1 g/liter collagenase IV for 1 min. (C) Decapsulated glomerulus with a patch clamp pipette.
Figure 2. (A) Electron microscopy of a section of a decapsulated glomerulus showing an intact podocyte with cell body and foot processes (9,000×). (B and C) Fluorescence images of one section of a single glomerulus, obtained by laser scanning microscopy. (B) Positive immunoflu-
tal-Na (Byk-Gulden, Konstanz, Germany) i.p., and the glomeruli were obtained by the perfusion technique with the rat placed on its right side on a heated operating table. The abdomen was opened and the aorta was prepared under a lens (40×). The left renal artery was identified and ligated near the aorta. With microscissors, the renal artery was cut and cannulated by a polyethylene catheter of appropriate size. The kidney was perfused with a cold (4°C) Ringer-like solution containing 5 mmol/liter Na+ pyruvate and 5 g/liter bovine serum albumin for 1–2 min to remove blood. After clamping the vascular pole, the kidney was excised and kept at 4°C. Rat glomeruli were obtained with the sieve technique as previously described (17). Approximately one third of the glomeruli obtained in this way possessed an intact Bowman's capsule and had diameters of 70–90 μm. The isolated glomeruli were kept in a medium without serum at 37°C under a moistened atmosphere containing 95% O2/5% CO2 for up to 4 h. For the experiments, a single glomerulus with intact capsule was transferred into the bath chamber mounted on the stage of an inverted microscope. The glomerulus was kept at 37°C on the bottom of the bath and immobilized at the vascular pole by a holding glass pipette (Fig. 1 A). After incubation with 1 g/liter collagenase IV for 1–2 min, the capsule was stripped off mechanically with a small broken glass pipette (Fig. 1, B and C). After 1–2 min of incubation, the glomerulus was superfused continuously at 37°C with a Ringer-like solution to remove the enzyme.

Characterization of the podocytes. After the glomeruli were decapsulated as described above, the cells bodies of the podocytes appeared in the light microscope (400×) as round or oval structures on the surface of the glomerular capillary loops. In three experiments, the integrity of the podocytes was further examined by electron microscopy. Glomeruli were fixed in glutaraldehyde (2 g/liter) in PBS for 24 h. Embedding and preparation of sections for electron microscopy was performed as described previously (18). Electron microscopy shows that after decapsulation the capillary loops were covered by intact podocytes that were attached to the glomerular basement membrane via numerous and intact foot processes (Fig. 2 A).

Immunofluorescence staining and confocal microscopy. In three sets of experiments, glomeruli were incubated with specific antibodies against specific podocyte markers, WT1 and pp44 (19, 20), located in the nucleus and foot processes, respectively. After rinsing with a Ringer-like solution, the glomeruli were preincubated for 30 min in a PBS buffer containing 5 g/liter gelatin and 10 g/liter bovine serum albumin to block nonspecific protein binding. Incubation with the above primary antibodies was followed by a 30-min incubation with secondary antibodies: rabbit anti-rat IgG for anti-WT1, and rabbit anti-mouse IgG for anti-pp44. The secondary antibodies were conjugated with Cy 3 and FITC, respectively. After rinsing, the glomeruli were fixed on a glass cover slip in glycerol (400 g/liter) and sealed by a cover slip. Fluorescence images were obtained using a confocal laser scanning microscope (LSM 4; Carl Zeiss, Jena, Germany) equipped with an argon laser (488 nm excitation). All emitted fluorescence light above 508 nm was collected using a plan Neofluar lens (100×/1.3 oil immersion, Carl Zeiss) and an emission pinhole setting of 30 μM, corresponding to a measured optical z-resolution of 1 μm (full width half maximum). Fluorescence images of optical sections in variable z-distances through the glomerulus were recorded and analyzed together with simultaneously recorded pseudoconfocal transmitted light differential interference contrast images. Examples are shown in Fig. 2, B and C. The location of the immunofluorescence staining in the periphery of the glomerulus indicates the presence of podocytes in their original anatomical location on the surface of the capillary loops.

Cell culture of podocytes. Differentiated podocytes were cultured according to the method described by Mundel et al. (20). In brief, isolated rat glomeruli were plated on collagen I-coated dishes in RPMI 1640 medium with 100 g/liter fetal calf serum, L-glutamine (2.5 mmol/liter), Na+ pyruvate (0.1 mmol/liter), nonessential amino acids (0.2 g/liter) (Seromed, Berlin, Germany), and insulin-transferrin-selenium supplement 5 g/liter (Boehringer Mannheim, Mannheim, Germany). After 5–7 d of primary culture, outgrowing glomerular cells were trypsinized with trypsin-EDTA (0.5/0.2 g/liter) in Ca2+/Mg2+-free PBS (Seromed) and passed over a 30-μm sieve. This maneuver removed the remaining glomerular cores consisting mainly of mesangial and endothelial cells. Only cells passing through the sieve were replated on collagen I-coated glass dishes with a diameter of 1 cm. Cells were characterized with the following antibodies: rabbit anti-rat IgG for Wilm's tumor antigen WT-1 (1:100; Santa Cruz Biotechnology, Heidelberg, Germany) and rabbit anti-mouse IgG for anti-pp44 (1:1), vimentin (1:50), desmin (1:50), cytokeratin (1:20), and Factor VIII–related antigen (1:50) for 60 min (DAKOPATTS, Hamburg, Germany). The antigen-antibody complexes were visualized with fluorescein conjugated secondary antibodies (FITC or Cy3; Santa Cruz Biotechnology). Cells showed positive staining for WT-1, pp44, vimentin, and desmin, thus indicating that they possess immunological characteristics of podocytes in vivo. For the experiments, only arborized cells between days 10 and 50 after the first subcloning were examined.

Nystatin patch clamp experiments. The patch clamp methods used in these experiments have been described in detail in previous reports (21, 22). Decapsulated glomeruli were fixed with a holding pipette at the vascular pole (see above), kept at 37°C, and perfused with a solution containing (in mmol/liter): NaCl 145, K-HPO4 1.6, KH2PO4 0.4, MgCl2 1, Ca-glucmate 1.3, d-glucose 5, pH 7.4. The patch pipettes were filled with a solution containing (in mmol/liter): K-glucmate 95, KCl 30, Na2HPO4 4.8, NaH2PO4 1.2, Ca-glucmate 0.73, MgCl2 1.03, d-glucose 5, EGTA 1, ATP 1, pH 7.2. Ca2+ activity 10–7 mol/liter. The patch pipettes had input resistances of 4–8 MΩ. A flowing (10 μl/h) KCl (2.7 mol/liter) electrode was used as a reference.

After achieving a GΩ seal, the cell membrane was broken in some experiments by application of a short lasting suction via the pipette (fast whole cell configuration), and the membrane voltage (Vm) was recorded continuously by a pen recorder (Gould, Selingenstadt, Germany). In most experiments, the cell was lost during this procedure. To overcome this problem, we investigated the high concentration of nystatin (150 mg/liter) was added to the pipette solution in the majority of the experiments. Nystatin permeabilized the cell-attached membrane by inducing a nonselective cation conductance (reference 23; slow whole cell nystatin configuration). Due to the high concentration of nystatin, the membrane was usually permeabilized within a few minutes. The Vm value was not different in either series (fast whole cell configuration, slow whole cell nystatin configuration). All experiments were performed after a control period of 3–5 min. Vm and the membrane current (I) were monitored before, during, and after addition of agonists. Membrane currents were measured in the voltage clamp mode (Vc) of the amplifier. Vc was clamped to Vm = 40.
mV in steps of 10 mV with each step lasting for 100 ms, and current was measured to obtain estimates of cell conductance (Gm) after correction for access conductance. In addition, membrane capacitance was estimated by the compensation circuitry of the patch clamp amplifier. In ion replacement experiments, NaCl was replaced isosmotically by the substituting ion. To vary the free Ca²⁺ activity, EGTA was added to the solution. The Ca²⁺ activity was calculated from a standard equation and was determined with a Ca²⁺ selective electrode (Radiometer, Copenhagen, Denmark).

The following reagents were used: angiotensin II, forskolin, A23187 (Sigma Chemical Co., Deisenhofen, Germany), 8-(4-chlorophenylthio)-cAMP, and N2,2'-dibutyryl-cGMP from Boehringer (Ingelheim, Germany). Losartan was obtained from MSD (Haar, Germany).

Statistics. The data are given as mean values ± SEM; n refers to the number of experiments. Paired t test was used to compare mean values within one experimental series. P < 0.05 was accepted to indicate statistical significance (* in the figures).

Results

Membrane voltage, input conductance, and membrane capacitance of podocytes. In 86 experiments in the fast whole cell configuration or slow whole cell nystatin configuration, a stable resting Vm of −38 ± 1 mV was obtained for the control period.

After a stable Vm was reached, the podocytes had a resting membrane capacitance of 67 ± 4 pF and access conductance of 52 ± 4 nS (n = 66).

Angiotensin II depolarizes the membrane voltage of glomerular podocytes. Addition of 10⁻⁷ mol/liter Ang II to podocytes for 1–3 min led to a long lasting and significant depolarization from −36 ± 3 mV to −28 ± 3 mV (n = 20). The depolarization occurred with some delay (22 ± 4 s after the agonist was given) and the maximum depolarization was reached after 104 ± 16 s (Fig. 3 A). The depolarization was completely reversible and Vm returned to the control value 2–3 min after the removal of Ang II. The effect was concentration dependent with an EC₅₀ of ~ 1.5 · 10⁻⁹ mol/liter Ang II (Fig. 3 B). During the depolarization, the conductances for the in- and outward current increased significantly from 27 ± 3 to 36 ± 5 nS and 25 ± 4 to 31 ± 5 nS, respectively (n = 15). In contrast with Ang II, addition of arginin vasopressin (10⁻⁶ mol/liter) for 3–5 min did not induce a depolarization of podocytes (n = 5).

Influence of a reduced extracellular chloride concentration on the depolarization induced by Ang II. Under resting conditions, a reduction of the extracellular Cl⁻ concentration (from 147 to 32 mmol/liter) for 1 min did not significantly alter Vm or Gm of podocytes. In the presence of 32 mmol/liter Cl⁻, the depolarization induced by Ang II (10⁻⁷ mol/liter) was significantly augmented to −22 ± 4 mV (paired experiments, n = 8, Fig. 4). Under these experimental conditions, the conductances for the in- and outward current were not significantly al-

![Figure 3](image-url)  (A) Effect of angiotensin II (Ang II, 10⁻⁷ mol/liter) on membrane voltage and whole cell conductance of a glomerular podocyte, measured in the whole cell nystatin configuration. (B) Concentration response curve for the depolarizing effect of Ang II. Mean values ± SEM (number of observations). *Statistically significant depolarization.

![Figure 4](image-url)  (A) Original recording (A) and summary (B) of the effect of a reduction of the extracellular Cl⁻ concentration (from 147 to 32 mmol/liter) on Vm of glomerular podocytes in the absence of the agonist and under stimulation with Ang II (10⁻⁷ mol/liter). Mean values ± SEM. Note that the depolarization of Vm induced by Ang II (⁺) was significantly increased (+) in the presence of a low extracellular Cl⁻ concentration.
Control conditions (Fig. 6), suggesting that Na\(^{+}\) influx is not responsible for the depolarization under stimulation with Ang II. The \(V_m\) response to Ang II is independent of extracellular Ca\(^{2+}\) and Na\(^{+}\). To examine whether a Ca\(^{2+}\) influx may be responsible for the \(V_m\) response to Ang II, the extracellular Ca\(^{2+}\) activity was reduced from 1 mmol/liter to 1 \(\mu\)mol/liter, and the effect of Ang II was examined in the presence of high (1 mmol/liter, control) and low (1 \(\mu\)mol/liter) extracellular Ca\(^{2+}\) activity. Reduction of extracellular Ca\(^{2+}\) for at least 1 min before addition of Ang II led to a small but not significant depolarization under control conditions. In the presence of low extracellular Ca\(^{2+}\) activity (1 \(\mu\)mol/liter), the depolarization by Ang II was unaltered compared with high Ca\(^{2+}\) conditions (Fig. 5). In addition, the small but significant increase of \(G_m\) under stimulation with Ang II was still observed. An original recording and the data of six paired experiments are shown in Fig. 5.

In another series of paired experiments, the influence of the extracellular Na\(^{+}\) concentration on the \(V_m\) response to Ang II was examined. When Na\(^{+}\) was removed from the extracellular bath solution (0 vs. 145 mmol/liter Na\(^{+}\)) for 1–3 min, the cells hyperpolarized significantly from \(-25 \pm 2\) mV to \(-45 \pm 3\) mV (\(n = 15\)) and the inward conductance fell significantly from 22 \pm 4 to 16 \pm 2 \(\mathrm{nS}\). In the absence of extracellular Na\(^{+}\), Ang II (10\(^{-7}\) mol/liter) depolarized glomerular podocytes to the same value (\(-25 \pm 1\) mV vs. \(-24 \pm 2\) mV, \(n = 7\)) as under control conditions (Fig. 6), suggesting that Na\(^{+}\) influx is not responsible for the depolarization under stimulation with Ang II.

### Figure 5

The influence of the extracellular Ca\(^{2+}\) activity ([Ca\(^{2+}\)]\(_{o}\)) on the depolarization induced by Ang II (10\(^{-7}\) mol/liter). (A) Original recording. (B) Summary of six paired experiments given as mean values \pm SEM. (C) Control (10\(^{-7}\) mol/liter [Ca\(^{2+}\)]\(_{o}\), Ca\(^{2+}\)-free, and cross-hatched bar = 10\(^{-6}\) mol/liter [Ca\(^{2+}\)]\(_{o}\)). Note that Ang II depolarizes \(V_m\) of podocytes to the same extent in the presence of low extracellular Ca\(^{2+}\). *Statistically significant effects.

The calcium ionophore A23187 depolarizes \(V_m\) of glomerular podocytes by activating a Cl\(^{-}\) conductance. A23187 (10\(^{-5}\) mol/liter) depolarized glomerular podocytes significantly from \(-31 \pm 1\) to \(-21 \pm 2\) mV (\(n = 9\), Fig. 7 A). In a solution with a reduced extracellular Cl\(^{-}\) concentration (32 mmol/liter), the depolarization induced by A23187 was increased to \(-8 \pm 5\) mV (\(n = 3\)). In this concentration (10\(^{-5}\) mol/liter), the depolarization induced by A23187 was sustained and not reversible. In contrast, forskolin (10\(^{-5}\) mol/liter), 8-(4-chlorophenylthio)-cAMP, and N2,2'-o-dibutyryl-cGMP (both 5 \(\times\) 10\(^{-4}\) mol/liter, 5-min application time) did not alter \(V_m\) significantly (Fig. 7, B–D).

### Figure 6

Effect of Ang II (10\(^{-7}\) mol/liter) on \(V_m\) of podocytes in the presence and absence of extracellular Na\(^{+}\). (A) Original recording. (B) Summary of seven paired experiments, mean values \pm SEM. (C) Control (145 mmol/liter Na\(^{+}\), 0 Na\(^{+}\) and hatched bar, removal of Na\(^{+}\) and replacement by NMDG. Removal of the extracellular Na\(^{+}\) leads to a hyperpolarization of these cells. In the absence of extracellular Na\(^{+}\), Ang II depolarizes \(V_m\) of podocytes to the same value as under control conditions. *Statistically significant effects.

**Inhibition of the Ang II response by the receptor antagonist losartan.** Pretreatment of the cells with the Ang II receptor antagonist losartan (10\(^{-7}\) mol/liter) for 2–3 min completely inhibited the voltage response to Ang II (10\(^{-7}\) mol/liter, \(n = 5\)) (Fig. 8, A and B). Ang II depolarized podocytes from \(-27 \pm 2\) mV to \(-20 \pm 2\) mV, whereas in the presence of losartan \(V_m\) was not significantly altered (\(-27 \pm 1\) vs. \(-26 \pm 1\) mV). An original recording and the summary of the data are shown in Fig. 8, A and B.

**Ang II depolarizes podocytes in culture.** Podocytes in short term culture showed positive immunofluorescence staining for WT-1, pp44, vimentin, and desmin (\(n = 8–19\)). Fig. 9 shows podocytes in culture, positively stained for the podocyte-spe-
specific protein WT-1. Addition of $10^{-8}$ mol/liter Ang II to podocytes in culture for 1–3 min resulted in a depolarization of podocytes. Mean values significantly and reversibly inhibited by 80±6% ($n = 8$). Fig. 10 A shows an original recording of the effect of Ang II in the absence and presence of losartan. Fig. 10 B summarizes the data.

**Discussion**

Glomerular visceral epithelial cells (podocytes) contribute to the initiation and progression of several glomerular diseases. Especially membranous nephropathy, minimal change disease, and focal segmental sclerosis have been related to primary or secondary podocyte injuries (24). The biological function of podocytes in vivo and their contribution to glomerular injury are poorly understood.

In this study, we have developed a new method allowing examination of electrophysiological properties of podocytes within intact glomeruli. The present data suggest that Ang II directly influences the conductive properties of these cells. Glomeruli were obtained by sieving renal cortex of rat kidneys perfused in vivo. Compared with the microdissection, this technique yields a large number of encapsulated and decapsulated glomeruli. Only the visually intact and encapsulated glomeruli were selected for the present experiments. We used only glomeruli with intact capsules because we argued that the capillary loop architecture and the podocytes would be protected by the surrounding Bowman’s capsule during the sieving procedure.

The following arguments support our conclusion that podocytes were examined in this study. (a) After decapsulation of glomeruli, cell bodies of podocytes could be visually well detected with differential interference contrast microscopy, and the patch pipette was carefully placed onto the cells. (b) Methodologically, it is not possible to perforate the glomerular basement membrane without occluding the patch pipette and obtaining a tight seal (e.g., from a mesangial cell). Electron microscopy and laser scanning microscopy pictures, which had been performed after the patch clamp experiments, revealed the existence of intact glomerular basement membranes and podocytes. (c) In case of tearing off podocytes with the patch pipette from the glomerular basement membrane, only the denuded membrane but no other glomerular cells could be observed. (d) The results presented here are highly reproducible. In addition, characteristic ion currents (a Na’ permeable and an inward rectifying K’ conductance), which are not present in cultured mesangial or endothelial cells, could be detected in podocytes in the intact glomerulus and in podocytes in culture (J. Gloy, unpublished observations). (e) The studies in primary cultures of podocytes revealing qualitatively similar results support the conclusion that intact and in situ podocytes were examined (see below).

$V_m$ and $G_m$ of podocytes were examined with a modified nystatin patch clamp technique allowing for the simultaneous recording of $V_m$ and ion currents (21). The $V_m$ value of $-38±1$ mV measured with this technique is in agreement with results from cultured human glomerular epithelial cells (25). We and others (26, 27) have previously shown that mesangial cells have a resting $V_m$ that is more negative ($-49±1$ mV), whereas $V_m$ of glomerular endothelial cells is near the equilibrium po-

**Figure 7.** Summary of the effects of the calcium ionophore A23187 ($10^{-7}$ mol/liter) (A), forskolin (For, $10^{-7}$ mol/liter) (B), 8-(4-chlorophenylthio)-cAMP (cAMP, $5 \times 10^{-4}$ mol/liter) (C), and N2,2’-o-dibutyryl-cGMP (cGMP, $5 \times 10^{-4}$ mol/liter) (D) on $V_m$ of glomerular podocytes. Mean values:±SEM. C, control. Note that A23187 leads to a significant depolarization (*), whereas forskolin, 8-(4-chlorophenylthio)-cAMP and N2,2’-o-dibutyryl-cGMP did not influence $V_m$ significantly.

**Figure 8.** The Ang II receptor antagonist losartan reversibly inhibits the $V_m$ response induced by Ang II in podocytes in the intact isolated glomerulus. (A) Original experiment of the effect of Ang II ($10^{-7}$ mol/liter) on membrane voltage ($V_m$) and whole cell conductance ($G_m$) of a podocyte in the absence and presence of $10^{-7}$ mol/liter losartan. (B) Summary of these experiments. In the presence of losartan (▼), Ang II has no effect. Mean values:±SEM; *statistical significance.
Angiotensin II Depolarizes Podocytes

2779

tential of K\(^+\) (−90 mV; J. Gloy, unpublished results). The membrane capacitance of glomerular podocytes was 67±4 pF, which is substantially higher when compared with data obtained from other epithelial or mesangial cells. It probably reflects the large surface area of podocytes with their numerous interdigitating foot processes.

Ang II led to a sustained depolarization to a value that is probably close to the Nernst equilibrium of Cl\(^−\). This is supported by the present finding that the depolarization was increased when the bath Cl\(^−\) concentration was reduced to 32 mmol/liter. Furthermore, Ang II increased the inward conductance. These three findings suggest that in glomerular podocytes the depolarization induced by Ang II is at least in part due to the activation of a Cl\(^−\) conductance. To examine the influence of other ions (Na\(^+\) and Ca\(^{2+}\)), which might contribute to the depolarization, additional series were performed. We show that the voltage response to Ang II was not altered in the absence of Na\(^+\) or when Ca\(^{2+}\) in the bath was reduced to 1 μmol/liter. Nevertheless, the cells hyperpolarized in the absence of Na\(^+\) and \(G_m\) fell significantly, suggesting that these cells possess a Na\(^+\) or a nonselective cation conductance. Whether this conductance is specific for Na\(^+\) or a nonselective cation conductance remains to be clarified.

The effect of Ang II on \(V_m\) of pod was not inhibited in the presence of low extracellular Ca\(^{2+}\) (1 μmol/liter), indicating that Ca\(^{2+}\) influx is not a prerequisite for the depolarization. On the other hand, the effect of Ang II was mimicked by the Ca\(^{2+}\) ionophore A23187. Both findings can be explained by assuming that Ang II activates the Cl\(^−\) conductance by the release of Ca\(^{2+}\) from intracellular stores. The activation of other second messengers like cAMP and cGMP by Ang II does not appear likely since forskolin, 8-(4-chlorophenylthio)-cAMP, and N\(_2\),N\(_2\)′-o-dibutyryl-cGMP did not affect \(V_m\) of glomerular podocytes. This is in contrast to findings by Sharma et al. (13),

Figure 9. Rat podocytes in short term culture (320×): immunofluorescence staining for WT-1, a podocyte-specific protein located in the nuclei of podocytes.

Figure 10. (A) Original recording of the effect of angiotensin II (Ang II, 10\(^{-8}\) mol/liter) on membrane voltage (\(V_m\)) of cultured podocytes in the absence and presence of losartan (Los, 10\(^{-8}\) mol/liter). Note that Ang II leads to a sustained and reversible depolarization of a podocyte. In the presence of Los, the effect of Ang II was reversibly inhibited. (B) Summary of the data, *statistical significance.
who described an increase of cytosolic cAMP by Ang II in cultured podocytes, leading to disaggregation of actin filaments and to an alteration of the cytoskeleton, whereas atrial natriuretic peptide, via activation of cGMP, had an opposite effect. One explanation for these differences might be the fact that cAMP, if produced by Ang II in podocytes, would not alter $V_m$. Other explanations might include a different hormonal regulation of second messengers in cultured glomerular epithelial cells as compared with podocytes in the intact glomerulus. In this regard, in the latter study, cells had not been characterized by specific antibodies against podocytes, and they had a cobblestone appearance and stained positively for cytokeratin, whereas podocytes in the glomerulus possess an extremely differentiated cell shape and are cytokeratin negative (5, 14).

The effect of Ang II in several tissues is mediated by distinct angiotensin receptors that have been classified to angiotensin 1 and 2 (AT₁ and AT₂) receptors. It is known that Ang II increases the cytosolic Ca²⁺ activity in cells via an AT₁ receptor, and this was related to the vasoconstrictive properties of Ang II. Losartan was characterized as a specific blocker of the AT₁ receptor (28). In our study, losartan, in an appropriate concentration, completely inhibited the Ang II–induced depolarization of $V_m$ of podocytes, thus indicating a specific effect of Ang II on cellular functions of podocytes via an AT₁ receptor. Recently, it has been shown that a specific AT₂ receptor antagonist inhibits Ang II–mediated modulation of glomerular capillary permeability (8).

Little is known about cellular mechanisms and actions of Ang II, the transduction pathways, and even the characterization of Ang II receptors in glomerular podocytes. In vitro autoradiography using labeled Ang II revealed a high density of Ang II binding sites in glomeruli, interpreted as a high density of Ang II receptors in mesangial cells (29). However, these data have been recently reinterpreted by Kriz et al. (10). They suggest a more widespread distribution of Ang II receptors, probably including podocytes. With respect to possible Ang II effects from the urinary space, Seikaly et al. (30) have measured Ang II concentrations in the Bowman's space of up to 1,000-fold higher than those in the vascular space and argued that this Ang II is produced by the local renin-angiotensin system.

Our present data show for the first time that Ang II influences cellular functions of podocytes in the intact glomerulus. Due to our approach, we cannot completely exclude that the depolarization induced by Ang II is indirectly influenced by some messenger or autotaxoid liberated from another cell type; e.g., mesangial cells. However, arginine vasopressin, which is known to modulate mesangial cell function in a similar way as Ang II (31), did not depolarize podocytes. To further examine whether Ang II may directly influence podocyte function, we tested the effect of Ang II on the membrane voltage of cultured rat podocytes. In the past there has been a debate whether glomerular epithelial cells in culture originate from visceral or parietal epithelium (15, 32). However, it has been shown very recently that podocytes with specific characteristic morphological and immunological properties can be propagated in cell culture (33). The cultured cells used in our experiments had a characteristic arborized morphology and stained positive for pp44, WT-1, desmin, and vimentin, but did not stain for cytokeratin or Factor VIII. Thus the cells expressed the immunological properties of podocytes in vivo (17, 33).

The resting membrane voltage of podocytes in culture did not differ from the membrane voltage of podocytes in the isolated glomerulus. Like podocytes in the intact glomerulus, cells in culture depolarized in the presence of Ang II. The AT₁ antagonist losartan inhibited the response to Ang II, indicating that cultured podocytes also possess AT₁ receptors. In comparison with the effect of Ang II on podocytes in the glomerulus, Ang II–induced depolarization of the cultured cells appeared to be stronger. This might be due to an overexpression of the AT₁ receptor in the cultured cells, but subsequent studies have to clarify the exact mechanisms for this observation.

In conclusion, our results indicate that it is possible to examine the membrane voltage and conductance properties of podocytes in intact glomeruli and in cultured podocytes. They suggest that angiotensin II directly influences cellular functions of these cells. Ang II depolarizes podocytes by opening a Cl⁻ conductance via an AT₁ receptor, and this ion conductance may be mediated by the intracellular Ca²⁺ activity.

Acknowledgments
We thank Ms. Christine Hodler for her excellent technical assistance. Losartan was a gift from MSD (Haar, Germany).
This study was supported by Deutsche Forschungsgemeinschaft Pa 483/1–2 and Gr 480/11.

References


