Abstract. The aim of this study was to investigate the influence of adrenoceptor agonists on the intracellular calcium activity ([Ca\(^{2+}\)]\(_{i}\)) in single podocytes. Noradrenaline and the \(\alpha_1\)-adrenoceptor agonist phenylephrine induced a reversible and concentration-dependent biphasic increase of [Ca\(^{2+}\)]\(_{i}\) in podocytes (EC\(_{50}\) \(\approx 0.1\ \mu\text{M}\) for peak and plateau), whereas the \(\alpha_2\)-adrenoceptor agonist UK 14.304 did not influence [Ca\(^{2+}\)]\(_{i}\). The [Ca\(^{2+}\)]\(_{i}\) response induced by noradrenaline was completely inhibited by the \(\alpha_1\)-adrenoceptor antagonist prazosin (10 \text{nM}). In a solution with a high extracellular K\(^+\) (72.5 mM), [Ca\(^{2+}\)]\(_{i}\) was unchanged and the [Ca\(^{2+}\)]\(_{i}\) increase induced by noradrenaline was not inhibited by the L-type Ca\(^{2+}\)-channel blocker nicardipine (1 \muM). \(V_m\) and \(G_m\) were examined with the patch-clamp technique in the slow whole-cell configuration. Isoproterenol, phenylephrine, and noradrenaline depolarized podocytes and increased \(G_m\). The order of potency for the adrenoceptor agonists was isoproterenol (EC\(_{50}\) \(\approx 1\ \text{nM}\)) > noradrenaline (EC\(_{50}\) \(\approx 0.3\ \mu\text{M}\)) > phenylephrine (EC\(_{50}\) \(\approx 0.5\ \mu\text{M}\)). The \(\beta_2\)-adrenoceptor antagonist ICI 118.551 (5 to 100 nM) inhibited the effect of isoproterenol on \(V_m\). Stimulation of adenylate cyclase by forskolin mimicked the effect of isoproterenol on \(V_m\) and \(G_m\) (EC\(_{50}\) \(\approx 40\ \text{nM}\)). Isoproterenol induced a time- and concentration-dependent increase of cAMP in podocytes. The effect of isoproterenol was unchanged in the absence of Na\(^+\) or in an extracellular solution with a reduced Ca\(^{2+}\) concentration, whereas it was significantly increased in an extracellular solution with a reduced Cl\(^-\) concentration (from 145 to 32 mM). The data indicate that adrenoceptor agonists regulate podocyte function: They increase [Ca\(^{2+}\)]\(_{i}\) via an \(\alpha_1\)-adrenoceptor and induce a depolarization via a \(\beta_2\)-adrenoceptor. The depolarization is probably due to an opening of a cAMP-dependent Cl\(^-\) conductance. (J Am Soc Nephrol 9: 335–345, 1998)

Catecholamines are important regulators of many renal functions. They increase renal vascular resistance, modulate renin secretion, and stimulate Na\(^+\) and water reabsorption in the proximal tubule (1). Within the glomerulus, noradrenaline may also be involved in the regulation of glomerular ultrafiltration. It appears to increase the hydraulic pressure of glomerular capillaries and decrease the glomerular ultrafiltration coefficient \((K_f)\) in spontaneously hypertensive rats (2,3). On the cellular level, it is known that noradrenaline stimulates prostaglandin E\(_2\) synthesis and increases the uptake of Ca\(^{2+}\) in glomerular rat mesangial cells (4). Furthermore, noradrenaline contracts mesangial cells and thus might decrease \(K_f\) by a reduction of the capillary surface area (5,6). The understanding of the influence of catecholamines on cellular functions of glomerular endothelial cells or podocytes is limited. The podocyte represents the most differentiated cell type in the glomerulus, which forms the outer part of the filtration barrier (7). Recent studies indicate that podocytes play a central role in stabilizing capillary diameters (8). Furthermore, it has been speculated that the podocyte, not the mesangial cell, may regulate \(K_f\) by contraction or dilation of its foot processes (8,9). Most of our knowledge concerning the function of glomerular cells is based on studies from cultured cells. In the past, to evaluate biological functions of podocytes, researchers tried to obtain podocytes in culture. However, the cells used in these studies had a cobbled appearance, whereas the podocyte in vivo has a very complex architecture (10–12). In addition, the immunologic properties of the cultured cells did not completely fit those of podocytes in vivo (13). Very recently, it has been suggested that cultured podocytes with a cobbledstone appearance convert into arborized, differentiated podocytes (14). In a subsequent study, a conditionally immortalized arborized podocyte cell line, which expresses markers of differentiated podocytes, was established (15). This cell line might, better than previous cultures, mimic the in vivo situation of the podocyte inasmuch as it offers the advantage to study biological events in differentiated cells. The aim of this study was to investigate the influence of catecholamines on cellular functions of these cells.
Materials and Methods

**Generation and Culture of Podocyte Cell Lines**

Isolated glomeruli from 10-wk-old heterozygous H-2K\(b\)-tsAS58 transgenic mouse kidneys (16) were grown at 37°C on collagen-I-coated dishes with RPMI 1640 containing 10% fetal calf serum (Boehringer Mannheim, Mannheim, Germany), 100 U/ml penicillin, 100 \(\mu\)g/ml streptomycin (both Life Technologies, Eggenstein, Germany), and 100 U/ml recombinant mouse γ-interferon (Sigma, Munich, Germany) to induce synthesis of the immortalizing T-antigen. After 5 d of primary culture, outgrowing cells were trypsinized and passed through a 33-μm pore size sieve to remove remaining glomerular cores consisting mainly of mesangial and endothelial cells. Cells were replated and propagated at 33°C in medium of identical composition. Cells grown at 33°C exhibited an undifferentiated morphology and proliferate. From the initial pool of outgrowing glomerular cells, 10 individual cell lines were cloned by limiting dilution. Cells stained positive for WT-1 (Wilms' tumor antigen, a nuclear protein, which in the adult kidney is only found in podocytes; reference 17). Only WT-1 protein-positive clones were selected and used for additional studies. After 2 mo, the concentration of γ-interferon was gradually reduced to 10 U/ml without a significant change of cell proliferation or phenotype. Subcultivation of confluent cells was performed with trypsin at 37°C after approximately 7 d (1.5 dilution). After trypsinization, cells were thermostatted to 37°C and maintained in a medium without γ-interferon to initiate differentiation. These cells were grown in RPMI 1640 with 10% fetal calf serum and L-glutamine (2.5 mM; Seromed, Berlin, Germany). Inactivation of the T-antigen by culturing cells at 37°C leads to the differentiation of these cells, i.e., they exhibit an arborized morphology and stop proliferating after approximately 10 d. Arborized cells were immunologically characterized and stained positive for WT-1 and pp44, a characteristic marker for podocyte foot processes (18). Three different cell clones were used in the study. Cells were used between passages 6 and 30. Only cells that were grown for more than 14 d at 37°C and that exhibited an arborized morphology were examined. After each experiment, cells were replaced and new cells were examined.

**Patch-Clamp Experiments**

The patch-clamp method used in these experiments has been described in detail (19,20). In brief, podocytes were mounted in a bath chamber on the stage of an inverted microscope, kept at 37°C, and superfused with a phosphate-buffered Ringer-like solution. To vary free Ca\(^{2+}\) activity, the solutions were prepared according to established techniques with ethylene glycol-bis(\(\beta\)-aminoethyl ether)-\(\mathrm{N,N'\text{-}}\)tetra-acetic acid and nitrilotriacetic acid as Ca\(^{2+}\) buffers. The Ca\(^{2+}\) complex of 224 nmoI/L (37°C) was assumed. The given concentration of the Ca\(^{2+}\) ionophore ionomycin (5 μmol/L) and low and high Ca\(^{2+}\) buffers as described previously in detail (21). [Ca\(^{2+}\)], was calculated from the fluorescence ratio according to the equation described by Grynkiewicz et al. (22). A \(K_D\) for the Fura-2-Ca\(^{2+}\) complex of 224 mmol/L (37°C) was assumed. The given concentrations for the [Ca\(^{2+}\)], peak refer to the highest magnitude of the fluorescence ratio. The given [Ca\(^{2+}\)], plateaus refer to the fluorescence ratio 60 s after the [Ca\(^{2+}\)], peak.

**Measurement of Intracellular Calcium Activity**

Measurements of intracellular calcium activity ([Ca\(^{2+}\)]\(_i\)) with the Ca\(^{2+}\)-sensitive dye Fura-2 AM (5 μM; Sigma, Deisenhofen, Germany) were performed in single podocytes with an inverted fluorescence microscope setup recently described (21). In brief, the light from a 75-W xenon lamp was directed through a water filter, which blocked the infrared light, to avoid thermal damage of the three excitation filters mounted in a motor-driven filter wheel (10 cycles/s). The excitation filters were bandpass filters with transmission maxima at 340, 360, and 380 nm (Laser Components, Gröbenzell, Germany). A dichroic mirror (FT 425, Zeiss, Oberkochen, Germany) and a bandpass filter (500 to 530 nm; Lys und Optik Laboratories, Lyngby, Denmark) were used in the emission light pass. The fluorescence field of the phototube could be chosen by means of an adjustable rectangular diaphragm (50 to 150 × 30 μm). After amplification, the photocurrent of the phototube was digitized with a 12-bit analog-to-digital converter (MPC 64, Zeiss, Oberkochen, Germany) and recorded continuously on the hard disc of an AT computer. The fluorescence values for the three excitation wavelengths for each turn of the wheel were computed. In 20 experiments, the calibration of the Fura-2 fluorescence signal could be performed at the end of the protocol, using the Ca\(^{2+}\) ionophore ionomycin (5 μmol/L) and low and high Ca\(^{2+}\) buffers as described previously in detail (21). [Ca\(^{2+}\)], was calculated from the fluorescence ratio according to the equation described by Grynkiewicz et al. (22). A \(K_D\) for the Fura-2-Ca\(^{2+}\) complex of 224 mmol/L (37°C) was assumed. The given concentrations for the [Ca\(^{2+}\)], peak refer to the highest magnitude of the fluorescence ratio. The given [Ca\(^{2+}\)], plateaus refer to the fluorescence ratio 60 s after the [Ca\(^{2+}\)], peak.

**Measurement of Intracellular cAMP**

Podocytes were cultured in 6-well plates. They were kept at 37°C and rinsed with a physiologic Ringer-type solution. After preincubation with 0.5 M 3-isobutyl-1-methylxanthine (Research Biochemicals International, Cologne, Germany) for 5 min, cells were exposed to the added agents. To terminate the assay, the supernatants were rapidly removed, and cells were rinsed with ice-cold ethanol (70 g/L). After an ethanol extraction, cAMP concentrations were measured with an enzyme-linked immunosorbent assay (Amersham Buchler, Braunschweig, Germany).

**Chemicals**

The following agents were used: noradrenaline, phenylephrine, isoproterenol, prazosin, metoprolol, ICI 118.551, UK 14.303, forskolin, (all Research Biochemicals International), nicardipine, A 23187 (Sigma, Deisenhofen, Germany).

**Statistical Analyses**

The data are given as mean ± SEM, where \(n\) refers to the number of experiments. The average of the effect of the agonist before and after the experiment was taken as control. Paired t test was used to compare mean values within one experimental series. A \(P\) value ≤0.05 was considered statistically significant.
Results
Noradrenaline and the $\alpha_1$ Agonist Phenylephrine
Increase [Ca$^{2+}$]$_i$ in Podocytes

Addition of noradrenaline ($n = 56$) or phenylephrine ($n = 24$) to single podocytes loaded with Fura-2 resulted in a fast transient [Ca$^{2+}$]$_i$ peak, which was followed by a [Ca$^{2+}$]$_i$ plateau. Noradrenaline (1 $\mu$M, $n = 35$) and phenylephrine (1 $\mu$M, $n = 5$) increased [Ca$^{2+}$]$_i$ from $49 \pm 11$ to $262 \pm 19$ nM, and $76 \pm 25$ to $294 \pm 54$ nM, respectively. After removal of the agonists and rinsing podocytes with Ringer-like solution, [Ca$^{2+}$]$_i$ returned to baseline values. Figure 1A shows a typical fluorescence measurement obtained from a single podocyte exposed to noradrenaline (1 $\mu$M). The biphasic [Ca$^{2+}$]$_i$ response to noradrenaline and phenylephrine was concentration-dependent, with an EC$_{50}$ of approximately 0.1 $\mu$M for both agonists (Figure 1B).

The Effect of Noradrenaline on [Ca$^{2+}$]$_i$ in Podocytes Is Mediated by an $\alpha_1$-Adrenoceptor

Ten nanomolar of the $\alpha_1$-adrenoceptor antagonist prazosin inhibited completely and reversibly the noradrenaline-induced [Ca$^{2+}$]$_i$ increase ($n = 5$). Figure 2A shows an original recording of the effect of 10 nM prazosin on noradrenaline-mediated [Ca$^{2+}$]$_i$ increase. In contrast, the $\alpha_2$-adrenoceptor agonist UK 14.304 (0.1 $\mu$M, $n = 5$) and the $\beta$-adrenoceptor agonist isoproterenol (1 $\mu$M, $n = 5$) did not influence [Ca$^{2+}$]$_i$ in podocytes. Figure 2B summarizes the data.

Figure 1. Noradrenaline and phenylephrine increase the cytosolic calcium activity ([Ca$^{2+}$]$_i$) in podocytes. (A) Original recording of the effect of 1 $\mu$M noradrenaline (Nor) on [Ca$^{2+}$]$_i$. (B) Concentration–response curve of the agonists. Averaged [Ca$^{2+}$]$_i$ peak responses (mean ± SEM) to noradrenaline and phenylephrine are shown ($n =$ the number of experiments).

Figure 2. The noradrenaline-induced [Ca$^{2+}$]$_i$ increase in podocytes is mediated by an $\alpha_1$-adrenoceptor. (A) Original recording of the effect of the $\alpha_1$-adrenoceptor antagonist prazosin (Prazo; 10 nM) on the noradrenaline (Nor; 1 $\mu$M)-mediated [Ca$^{2+}$]$_i$ increase. (B) Summary of the effect of prazosin on the noradrenaline-induced [Ca$^{2+}$]$_i$ increase and the effects of the $\alpha_2$-adrenoceptor agonist UK 14.314 (0.1 $\mu$M) and the $\beta$-adrenoceptor agonist isoproterenol (Iso; 1 $\mu$M) on [Ca$^{2+}$]$_i$ in podocytes (both $n = 5$). * indicates statistical significance. In this and in subsequent experiments, the average of the effect of the agonist before and after addition of the antagonist was taken as control.
Effect of a Low Extracellular Ca\(^{2+}\) Concentration and the L-type Ca\(^{2+}\) Channel Antagonist Nicardipine on the Noradrenaline-Induced [Ca\(^{2+}\)]\(_i\) Increase

In a solution with a reduced bath Ca\(^{2+}\) (from 1 mM to 1 \(\mu\)M), the noradrenaline (1 \(\mu\)M)-mediated [Ca\(^{2+}\)]\(_i\) plateau was reduced, whereas the [Ca\(^{2+}\)]\(_i\) peak was unchanged \((n = 6)\). Figure 3A shows an original recording of the noradrenaline-induced increase of the [Ca\(^{2+}\)]\(_i\) plateau with normal and reduced bath Ca\(^{2+}\). Figure 3B summarizes six paired experiments. To examine whether the [Ca\(^{2+}\)]\(_i\) plateau was due to a Ca\(^{2+}\) influx through an L-type Ca\(^{2+}\) channel, we tested the effect of the L-type Ca\(^{2+}\) channel antagonist nicardipine (1 \(\mu\)M) on the noradrenaline (1 \(\mu\)M)-induced [Ca\(^{2+}\)]\(_i\) increase. Nicardipine did not inhibit the increase of the [Ca\(^{2+}\)]\(_i\) plateau with normal and reduced extracellular Ca\(^{2+}\) (Ca\(^{2+}\) : 1 mM) solution (Figure 3B). To further investigate whether the [Ca\(^{2+}\)]\(_i\) plateau was due to a Ca\(^{2+}\) influx through an L-type Ca\(^{2+}\) channel, we tested the effect of the L-type Ca\(^{2+}\) channel antagonist nicardipine (1 \(\mu\)M) on the noradrenaline (1 \(\mu\)M)-induced [Ca\(^{2+}\)]\(_i\) increase. Nicardipine did not inhibit the increase of the [Ca\(^{2+}\)]\(_i\) plateau induced by noradrenaline \((n = 5)\) (Figure 4). To further investigate whether podocytes in culture possess L-type Ca\(^{2+}\) channels, two additional sets of experiments were performed: \(V_m\) and [Ca\(^{2+}\)]\(_i\) of podocytes were measured separately in a solution with a reduced bath Ca\(^{2+}\) concentration of 1 mM. The effect of 10 \(\mu\)M noradrenaline on [Ca\(^{2+}\)]\(_i\) was investigated in a solution with normal and reduced bath Ca\(^{2+}\). Figure 3A shows an original recording of the noradrenaline-induced [Ca\(^{2+}\)]\(_i\) increase. The thapsigargin-induced [Ca\(^{2+}\)]\(_i\) plateau was completely inhibited in a solution with a reduced bath Ca\(^{2+}\) (1 \(\mu\)M, data not shown). Addition of 1 \(\mu\)M noradrenaline into the thapsigargin-induced [Ca\(^{2+}\)]\(_i\) plateau did not cause an additional increase, but did cause a small and significant decrease of [Ca\(^{2+}\)]\(_i\) by 18 ± 6 nmol/L \((n = 6)\). Figure 4B shows the effect of noradrenaline in the presence of thapsigargin.

Isoproterenol, Phenylephrine, and Noradrenaline Depolarize the \(V_m\) and Increase the \(G_m\) of Podocytes

The resting \(V_m\) value obtained in podocytes was -56 ± 1 mV \((n = 220)\). After a stable \(V_m\) was reached, podocytes had a resting membrane capacitance \((C_r)\) of 34 ± 2 pF and an input conductance \((G_{is})\) of 67 ± 5 nS \((n = 139)\). Isoproterenol (10 nM) depolarized podocytes significantly from -58 ± 2 mV to -41 ± 2 mV and led to a simultaneous and significant increase of the inward and outward conductance from 11 ± 2 to 15 ± 3 nS and 9 ± 1 to 13 ± 2 nS, respectively \((n = 27)\). The depolarization lasted as long as the agonists were present. Figure 5 shows an original recording of the effect of isoproterenol (10 nM) on \(V_m\) and \(G_m\). Like isoproterenol, phenylephrine and noradrenaline caused a reversible and concentration-dependent depolarization \((n = 31)\). The rank order of potency for the adrenoceptor agonists in depolarizing podocytes was isoproterenol \((EC_{50} ≈ 1 nM) >\) noradrenaline \((EC_{50} ≈ 0.3 \mu M) >\) phenylephrine \((EC_{50} ≈ 0.5 \mu M)\). Figure 6 shows the concentration-response curves for the effect of isoproterenol, noradrenaline, and phenylephrine on \(V_m\) (Panel A) and \(G_m\) (Panel B), respectively.

Isoproterenol Depolarizes Podocytes Via a \(\beta_2\)-Adrenoceptor

The specific \(\beta_2\)-adrenoceptor antagonist ICI 118.551 inhibited the isoproterenol-induced depolarization with an IC\(_{50}\) of approximately 8 nM. ICI 118.551 \((0.1 \mu M)\) completely inhibited the \(V_m\) response to 10 nM isoproterenol \((n = 8)\), whereas 1 \(\mu\)M of the \(\beta_1\)-adrenoceptor antagonist metoprolol inhibited the effect of isoproterenol on \(V_m\) only by 16 ± 3\% \((n = 7)\), data not shown). Figure 7 shows an original recording of the effect of the ICI 118.551-induced inhibition of the effect of isoproterenol on \(V_m\) (Panel A) and the concentration–response curve (Panel B).

Pretreatment of podocytes with ICI 118.551 \((0.1 \mu M)\) or prazosin \((10 nM)\) inhibited phenylephrine-mediated depolarization by 75 ± 5\% \((n = 5)\) or 36 ± 7\%. \((n = 7)\), respectively. The effect of phenylephrine was completely inhibited in the presence of both antagonists \((n = 5)\). Figure 8 summarizes the effect of ICI 118.551 and prazosin on the phenylephrine-induced depolarization. The \(\alpha_2\)-adrenoceptor agonist UK 14.304 \((0.1 \mu M)\) did not depolarize podocytes \((n = 7)\), data not shown).
Isoproterenol Activates a Cl⁻ Conductance in Podocytes

Reduction of the extracellular Cl⁻ concentration from 145 to 32 mM had no significant effect on $V_m$. In the presence of 32 mM Cl⁻, the depolarization induced by 10 nM isoproterenol was significantly augmented to $-30 \pm 6$ mV ($n = 5$). In addition, in the presence of 32 mM Cl⁻, the inward current induced by isoproterenol was reduced by $69 \pm 30\%$ ($n = 3$). In contrast, a reduction of the extracellular Ca²⁺ (from 1 mM to 1 μM, $n = 7$) or of the extracellular Na⁺ concentration (from 145 mM to 0 mM, $n = 8$) did not significantly influence the isoproterenol-induced depolarization of podocytes. These data are summarized in Figure 9.

Forskolin Mimics the Isoproterenol-Induced $V_m$ Response in Podocytes

Stimulation of cAMP production by forskolin resulted in a sustained and concentration-dependent depolarization and an increase of $G_m$ in podocytes (EC$_{50}$ ≈ 40 nM). Figure 10, A and B, depicts the concentration–response curves for the effect of forskolin on $V_m$ and $G_m$. In the presence of a low extracellular Cl⁻ concentration (32 mM), the forskolin (1 μM)-induced depolarization (from $-52 \pm 4$ mV to $-38 \pm 5$ mV) was significantly augmented (from $-52 \pm 6$ to $-31 \pm 5$ mV, $n = 5$, data not shown).

The Ca²⁺ Ionophore A 23187 Depolarizes Podocytes in Culture

To further examine whether the depolarization induced by noradrenaline and phenylephrine may be mediated at least in part by an increase of [Ca²⁺] in podocytes ($n = 5$). Mean ± SEM. Figure 4. (A) Nicardipine (Nica; 1 μM) did not inhibit the [Ca²⁺] increase induced by noradrenaline (Nor; 1 μM). Left Panel, Original experiment. Right Panel, Summary of the experiments ($n = 5$). Mean ± SEM. The last column of the right panel is a summary of the lacking effect of a high extracellular K⁺ (72.5 mM) on [Ca²⁺] in podocytes ($n = 15$). Mean ± SEM. (B) In the presence of 20 nM thapsigargin, noradrenaline induced a small decrease of [Ca²⁺] in podocytes. Left Panel, Original experiment. Right Panel, Summary of the experiments ($n = 6$). plat., plateau. Mean ± SEM. * indicates statistical significance.

Isoproterenol, Phenylephrine, and Forskolin Increase cAMP Concentrations in Podocytes

Addition of isoproterenol, phenylephrine, and forskolin to podocytes resulted in a time- and concentration-dependent increase of cAMP ($n = 6$) (Figure 11, A and B). The rank order of potency for the agents in increasing cAMP was isoproterenol (EC$_{50}$ ≈ 1 nM) > forskolin (EC$_{50}$ ≈ 0.2 μM) > phenylephrine (EC$_{50}$ ≈ 0.4 μM).

Discussion

Stimulation of renal sympathetic nerves produces a frequency-dependent fall in GFR that is due to an increase in glomer-
Figure 5. Isoproterenol (Iso) causes a sustained depolarization and an increase of the whole-cell conductance \( (G_m) \) in podocytes. To obtain \( G_m \), the voltage of the respective cell was clamped to the resting membrane voltage \( (V_m) \). Starting at this value, the whole-cell current was measured by depolarizing or hyperpolarizing \( V_m \) in steps of 10 mV to ±40 mV. Original recording of the effect of isoproterenol (10 nM) on \( V_m \) (top panel) and \( G_m \) (bottom panel).

ular arteriolar resistance, a decrease in glomerular hydraulic pressure gradient, and glomerular ultrafiltration coefficient \( (K_f) \) (24,25). Morphologic studies have shown that sympathetic nerve stimulation results in a decrease of the diameter of the glomeruli (26). Therefore, the decrease of \( K_f \) induced by sympathetic nerve stimulation may be due at least partially to a decrease of glomerular surface area (26). The podocyte contributes to the regulation of capillary wall tension, and it has been assumed that it plays an active role in the regulation of \( K_f \) (8,9).

By using new culture techniques, it has been possible to culture differentiated podocytes from a mouse carrying a transgene for a thermosensible variant of the simian virus 40-T-antigen. Podocytes from this mouse grown at 37\(^{\circ}\)C exhibit a differentiated and arborized morphology. In addition, they express specific immunologic markers of podocytes in vivo (14,15). Only cells with a characteristic arborized phenotype, resembling that of the in vivo situation, were used in this study.

The present results indicate that noradrenaline increases the \([Ca^{2+}]_i\) in podocytes. The \([Ca^{2+}]_i\) increase induced by noradrenaline was biphasic, with a fast transient \([Ca^{2+}]_i\) peak, which was followed by a \([Ca^{2+}]_i\) plateau. The plateau, but not the peak, was absent in an extracellular, nominally \(Ca^{2+}\)-free solution, indicating that noradrenaline released \(Ca^{2+}\) from intracellular stores, but also stimulated \(Ca^{2+}\) influx from the extracellular space. Similar observations have been reported in smooth muscle cells (27). In mesangial cells, a noradrenaline-induced \(Ca^{2+}\) uptake has been observed, but the precise mechanisms of the \(Ca^{2+}\) influx are not known (4). There is a great body of evidence that the \(Ca^{2+}\) influx in podocytes is probably not mediated by an \(L\)-type \(Ca^{2+}\) channel, but may be due to the opening of a receptor-operated or store-controlled \(Ca^{2+}\) or a nonselective cation channel: The \(L\)-type \(Ca^{2+}\) channel antagonist nicardipine did not inhibit the \(Ca^{2+}\) influx mediated by noradrenaline. In addition, an increase in extracellular \(K^+\) concentration, which depolarized \(V_m\) and should therefore lead to an influx of \(Ca^{2+}\) via \(L\)-type \(Ca^{2+}\) channels, did not increase \([Ca^{2+}]_i\). In the presence of thapsigargin, noradrenaline did not increase, but decreased \([Ca^{2+}]_i\) in podocytes. Similar effects of agonists on \([Ca^{2+}]_i\) in the presence of thapsigargin have been reported in other cell types, and it has been suggested that agonists not only stimulate a \([Ca^{2+}]_i\) increase, but also induce an export of \([Ca^{2+}]_i\) (28). Whether this is the case in the present cells needs to be clarified in future studies.

Catecholamines exert their influence on renal hemodynamics, \(Na^+\) and water balance, and renin secretion by activating distinct multiple subtypes of \(\alpha\)- and \(\beta\)-adrenoceptors (29). In the renal vasculature, the predominating adrenoceptors are of the \(\alpha_1\)-adrenoceptor subtype (30). The present data indicate that the effect of noradrenaline on \([Ca^{2+}]_i\) was in fact mediated by an \(\alpha_1\)-adrenoceptor. Compared with noradrenaline, the \(\alpha_1\)-adrenoceptor agonist phenylephrine elicited an equipotent increase of \([Ca^{2+}]_i\), whereas the \(\alpha_2\)-agonist UK 14.304 (31) and the \(\beta\)-agonist isoproterenol did not influence \([Ca^{2+}]_i\) in podocytes. In addition, the \([Ca^{2+}]_i\) increase induced by noradrenaline was completely inhibited by 10 nM of the \(\alpha_1\)-adrenoceptor antagonist prazosin.

The direct influence of noradrenaline on podocyte function not only may play a role under physiologic conditions, but also may participate in the pathogenesis of renal diseases such as acute renal failure. The precise pathophysiologic mechanisms of acute renal failure are not clearly understood, but the fol-
following events have been implicated: (1) renal vasoconstriction; (2) tubular obstruction; (3) back leak of filtrate; and (4) decrease in glomerular capillary permeability (32). Regarding the latter mechanism, infusion of noradrenaline into the renal artery of dogs caused acute renal failure, which was not due to vasoconstriction, tubular leakage, or tubular obstruction. Noradrenaline-induced acute renal failure causes an alteration of the filtration barrier and marked abnormalities of the podocytes. Flattening of cell body and obliteration of foot processes occur (33). Therefore, noradrenaline-induced changes of podocyte function may be the key event in this experimental model of acute renal failure. Our results support the concept that noradrenaline might directly influence podocyte function.

In many cell types (e.g., muscle cells), noradrenaline is known to increase [Ca\(^{2+}\)]\(_i\) and to depolarize \(V_m\). These cellular events, which are known to be mediated by the \(\alpha_1\)-adrenocep-
tor, lead to cell contraction (34). Phenylephrine, noradrenaline, and the \(\beta\)-agonist isoproterenol depolarized podocytes. In the presence of the \(\alpha_1\)-adrenoceptor antagonist prazosin, the Ca\(^{2+}\) transient was abolished and approximately one-third of the phenylephrine-induced depolarization was inhibited, indicating that a part of the depolarization was mediated by an \(\alpha_1\)-adrenoceptor. The Ca\(^{2+}\) ionophore depolarized podocytes by activating a Cl\(^-\) conductance. This suggests that the depolarizing effect of phenylephrine is mediated by an increase of [Ca\(^{2+}\)]\(_i\). It has been shown that [Ca\(^{2+}\)]\(_i\)-mobilizing agonists also depolarize human podocytes by activating a Cl\(^-\) conductance (35).

Two-thirds of the effect of phenylephrine was inhibited by the selective \(\beta_2\)-adrenoceptor antagonist ICI 118.551 (36), indicating that phenylephrine also acted via a \(\beta_2\)-adrenoceptor. An \(\alpha_2\)-adrenoceptor apparently is not involved in the phenylephrine response, because the \(\alpha_2\)-adrenoceptor agonist UK 14.304 did not depolarize podocytes. Compared with phenyl-
ephedrine and noradrenaline, the β-adrenoceptor agonist isoproterenol was the most potent depolarizing agent. To characterize the β-adrenoceptor involved in the isoproterenol-induced depolarization, the effect of β₁- and β₂-adrenoceptor blockers have been examined. Low concentrations of the β₂-adrenoceptor blocker ICI 118.551 inhibited the response to isoproterenol, whereas high concentrations of the β₁-adrenoceptor antagonist metoprolol only had a small effect on isoproterenol-induced depolarization. The data strongly indicate that the depolarization induced by isoproterenol was mediated by a β₂-adrenoceptor.

An isoproterenol-induced depolarization has been reported in epithelial cells such as β₁-intercalated cells (37), whereas in muscle cells isoproterenol induces a hyperpolarization (38). Stimulation of the β₂-adrenoceptor is known to activate adenylyl cyclase resulting in an increase of cAMP (39). Forskolin, a direct activator of the adenylyl cyclase, mimicked the effect of isoproterenol on $V_m$ and on ion conductances, and the concentration–response curves for cAMP production and depolarization were very similar. These data indicate that the depolarization by β₂-adrenoceptor agonists in podocytes is mediated by an increase of cAMP.

The depolarizing effect of isoproterenol was not altered in a solution with a lowered extracellular Ca²⁺ or Na⁺ concentration, whereas in the presence of a low extracellular Cl⁻, the isoproterenol-induced depolarization was significantly increased. This indicates that the depolarization was due to the activation of a Cl⁻ conductance. An isoproterenol-induced activation of a Cl⁻ conductance has also been reported in β₁-intercalated cells (37). As with isoproterenol, the effect of forskolin on $V_m$ was augmented in a solution with a lowered extracellular Cl⁻ concentration, indicating that the Cl⁻ conductance is activated by cAMP. Similar results have been observed in epithelial cells (40), whereas in mesangial cells, a cAMP-mediated hyperpolarization has been reported (41).

The functional consequences of the β₂-adrenoceptor-mediated conductance changes in podocytes are not yet clear. Direct infusion of isoproterenol into the renal artery did not change glomerular hemodynamics (42). However, these experiments have been performed in rats, and infusion of isoproterenol into the kidney activates different hormone systems, such as renin secretion, which might antagonize a direct effect of isoproterenol on podocyte function. Under pathophysiologic conditions, changes of glomerular responses to isoproterenol have been reported: Spontaneously diabetic rats show an attenuated cAMP increase in response to glomerular β-adrenergic agonists, but not to forskolin (43). Thus, the podocyte, which...
seems to be involved in the pathogenesis of diabetic nephropathy, may participate in the defective response to isoproterenol occurring in diabetes mellitus (44).

In summary, the effect of catecholamines on cellular functions of podocytes with characteristic morphologic and immunologic properties was examined in this study. Catecholamines increase \([\text{Ca}^{2+}]_i\) and cAMP in podocytes via an \(\alpha_1\)- and \(\beta_2\)-adrenoceptor, respectively. Stimulation of the receptors led to an activation of a \(\text{Cl}^-\) conductance, resulting in a depolarization of the cells.

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