Kidney International, Vol. 54, Suppl. 67 (1998), pp. S-168–S-170

Angiotensin II modulates cellular functions of podocytes

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Angiotensin II modulates cellular functions of podocytes. The aim of this study was to examine the effects of angiotensin II (Ang II) on membrane voltage (V_m) and cytosolic calcium activity ($[Ca^{2+}]_i$) of rat podocytes. To approach better the *in vivo* situation, we have developed an experimental approach that allows podocytes to be studied in the intact microdissected glomerulus. Ang II depolarized podocytes in the glomerulus (EC_{50} 15 nM, $N = 49$). Like podocytes in the glomerulus, podocytes in short-term culture also depolarized in response to Ang II (10 nM, $N = 5$). Ang II increased $[Ca^{2+}]_i$ in podocytes in culture (EC_{50} 3 nM, $N = 229$). In a solution with reduced extracellular $[Ca^{2+}]$ (10 μM), Ang II-mediated $[Ca^{2+}]_i$ increase was significantly reduced by 60% ± 20% ($N = 12$). Flufenamate, an inhibitor of nonselective ion channels, inhibited Ang II-mediated increase of $[Ca^{2+}]_i$ (IC_{50} 20 μM, $N = 29$). The Ang subtype 1 (AT1) receptor antagonist losartan inhibited both Ang II-mediated depolarization and $[Ca^{2+}]_i$ increase in podocytes ($N = 5$ to 35). Our results support the concept that Ang II might influence podocyte function directly via an AT1 receptor.

Previous studies indicate that angiotensin II (Ang II) plays a crucial role in the regulation of the glomerular microcirculation: it induces renal vasoconstriction and decreases the ultrafiltration coefficient K_f [1]. Ang II is also a growth hormone and plays a role in the progression of glomerular sclerosis [2]. The pathogenesis of glomerulosclerosis is not completely understood, but much evidence suggests that the initiation and the development of glomerulosclerosis is based on podocyte injury [3]. The podocyte is a highly differentiated cell that stabilizes the glomerular basement membrane and contributes to specific characteristics of the glomerular filtration barrier. The precise role of the podocyte for the maintenance of the functional properties of the filtration barrier is unclear. It has been assumed that the contractile foot processes of the podocyte might regulate glomerular filtration rate by changing filtration surface area and hence K_f [4]. However, there is no direct evidence for this, and the knowledge of biological functions of the podocyte in the glomerulus is limited because of its unique anatomical location. In addition, until recently, it was not clear whether podocytes could be propagated at all in cell culture, and morphological and immunohistochemical properties of glomerular epithelial cells in culture differ markedly from those *in vivo* [5].

Key words: cytosolic calcium activity, glomerulus, Bowman's capsule, glomerulosclerosis, pod function, angiotensin receptor.

ANGIOTENSIN II DEPOLARIZES PODOCYTES IN THE INTACT GLOMERULUS

To study the functional properties of the podocyte in the glomerulus, we have recently developed techniques permitting investigation of the properties of podocytes both in the intact glomerulus and in short-term culture [6, 7]. Isolated glomeruli with Bowman's capsule intact were transferred into a bath chamber mounted on the stage of an inverted microscope. The glomeruli were fixed by a pipette and rinsed with collagenase IV for approximately one minute. Thereafter, the capsule could be stripped off mechanically using a small pipette. A patch pipette was then placed onto the surface of a podocyte. After achieving a GΩ seal, the membrane of the podocyte was broken by suction or was permeabilized by nystatin added to the pipette solution, and membrane voltage and ion currents were examined in the fast or slow whole-cell configuration, respectively [8]. In approximately 3% of patch-clamp trials, a stable resting membrane voltage of -38 ± 1 mV could be recorded in podocytes. Addition of Ang II (0.1 to 1000 nM) induced sustained and reversible depolarization of podocytes. The effect of Ang II was concentration dependent with an EC_{50} of ~ 15 nM.

Figure 1A shows an original recording of the effect of Ang II on membrane voltage of a podocyte. The effect of Ang II was not changed in the presence of a reduced extracellular Ca^{2+} or Na^+ but was augmented significantly by lowering extracellular Cl^- from 147 to 32 mM. Like Ang II, the Ca^{2+} ionophore A 23187 (10 μM) depolarized podocytes. Activation of the cAMP pathway by forskolin had only a slight and insignificant depolarizing effect, and also the cell permeable cGMP analog N2,2′-o-dibutyryl-cGMP did not depolarize podocytes. This suggests that an increase of the cytosolic Ca^{2+} activity ($[Ca^{2+}]_i$) may be

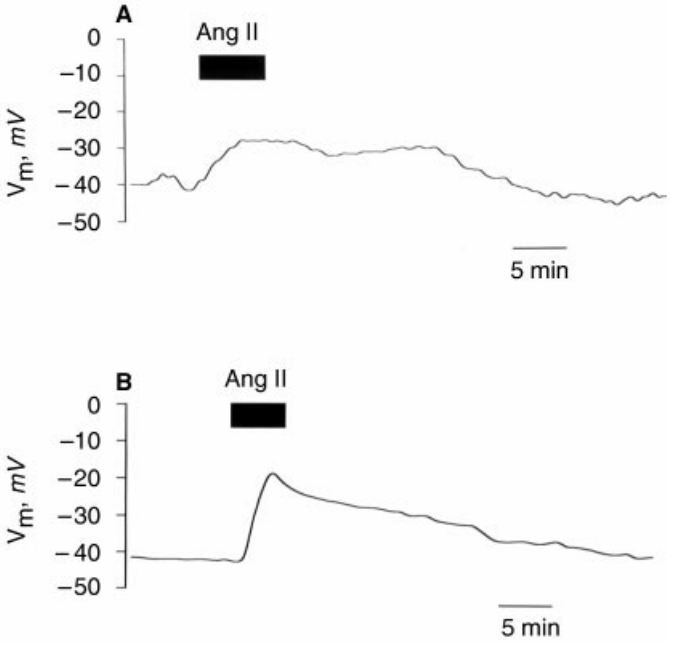

Fig. 1. Original recording of the effect of angiotensin II (Ang II, 10-7 or 10-8 M) on the membrane voltage of a podocyte in the glomerulus (*A*) or in a podocyte in cell culture (*B*).

involved in the membrane voltage response induced by Ang II. To investigate whether podocytes were morphologically intact after the patch-clamp experiments, the glomerulus was incubated with specific antibodies against the podocyte markers Wilms' tumor gene (WT1) and synaptopodin [9, 10]. Fluorescence images were obtained using confocal laser scanning microscopy and showed positive staining for WT1 and synaptopodin in the periphery of the glomerulus.

EFFECT OF ANGIOTENSIN II ON $[CA^{2+}]_I$ IN PODOCYTES IN SHORT-TERM CULTURE

It cannot be completely excluded that Ang II-induced podocyte depolarization is due to release of messengers from another glomerular cell type. We therefore investigated whether Ang II influences cellular functions of cultured podocytes. Rat podocytes with arborized morphology were propagated in short-term culture as described by Mundel et al [11]. Cells stained positively for WT1, synaptopodin, desmin, and vimentin, but not for cytokeratin or factor VIII. Only single cells with a typical arborized morphology were used in the following experiments. Like podocytes in the glomerulus, Ang II depolarized podocytes in short-term culture (passage 1; Fig. 1B). Fluorescence measurements of $[Ca^{2+}]_i$ showed a concentration-dependent increase with Ang II ($EC_{50} \sim 3$ nM) [12]. Reduction of extracellular Ca^{2+} to 1 μM reduced the $[Ca^{2+}]_i$ response to Ang II by 60%, indicating that Ang II induces both Ca^{2+} influx from the extracellular space and release from intracellular stores. Flufenamate, an inhibitor of nonselective ion channels, inhibited the $[Ca^{2+}]_i$ increase induced by Ang II, whereas the L-type Ca^{2+} channel blocker nicardipine, even at high concentrations (>1 μM), had only a small inhibitory effect.

These studies indicate that cellular functions of the podocyte can be examined in the intact glomerulus. Ang II depolarizes podocytes, most probably by activating a Ca^{2+}-dependent Cl^- conductance, via an Ang type 1 (AT1) receptor. Although Ang II reportedly binds to rat podocytes *in vivo* [13], it remains controversial whether the biological properties of podocytes are influenced by Ang II and, if so, which second messenger system is involved. In cultured rat podocytes, Ang II stimulates cAMP-dependent disaggregation of actin filaments [14]. In mouse podocytes in long-term culture, mRNA of aminopeptidase A and angiotensinogen, but not of AT1a, AT1b, and AT2 receptors, is expressed [15].

The disparate results may in part be due to the use of cells from different species and different cell culture systems. Most studies have examined properties of well-proliferating podocytes with a cobblestone appearance in long-term culture. However, the podocyte *in vivo* has a very differentiated morphology and does not proliferate [11]. Moreover, loss of hormone receptors in cells in long-term culture is not infrequent, and it is likely that certain hormone receptors are only expressed in differentiated podocytes.

The physiological relevance of Ang II-mediated changes of podocyte function for the regulation of K_f remains unclear. Ang II may alter the slit membranes by modulating the contractile apparatus of podocytes [4], but morphological studies have failed to find changes of the podocyte or filtration slit structure after infusion of Ang II [16]. We also did not observe contraction of arborized podocytes exposed to Ang II [7]. However, the results from studies on morphological changes of podocytes induced by Ang II should be interpreted cautiously. *In vivo* studies might overlook focal capillary constriction, and standard morphometric techniques might not be sufficient to detect small changes of structural changes of the podocyte [16]. In *in vitro* studies, lack of change of cell morphology is hard to interpret, as the properties of these cells are largely influenced by the cell culture conditions used [7].

ACKNOWLEDGMENTS

This article is dedicated to Prof. Dr. Dr. h.c. mult. Klaus Thurau on the occasion of his 70th birthday. R.F.G. is privileged to have been his scholar for some time and has enjoyed being a guest in his laboratory.

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