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Angiotensin II has acute effects on TRPC6 channels in podocytes of freshly isolated glomeruli

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A key role for podocytes in the pathogenesis of proteinuric renal diseases has been established. Angiotensin II causes depolarization and increased intracellular calcium concentration in podocytes; members of the cation TRPC channels family, particularly TRPC6, are proposed as proteins responsible for calcium flux. Angiotensin II evokes calcium transient through TRPC channels and mutations in the gene encoding the TRPC6 channel result in the development of focal segmental glomerulosclerosis. Here we examined the effects of angiotensin II on intracellular calcium ion levels and endogenous channels in intact podocytes of freshly isolated decapsulated mouse glomeruli. An ion channel with distinct TRPC6 properties was identified in wild-type, but was absent in TRPC6 knockout mice. Single-channel electrophysiological analysis found that angiotensin II acutely activated native TRPC-like channels in both podocytes of freshly isolated glomeruli and TRPC6 channels transiently overexpressed in CHO cells; the effect was mediated by changes in the channel open probability. Angiotensin II evoked intracellular calcium transients in the wild-type podocytes, which was blunted in TRPC6 knockout glomeruli. Pan-TRPC inhibitors gadolinium and SKF 96365 reduced the response in wild-type glomerular epithelial cells, whereas the transient in TRPC6 knockout animals was not affected. Thus, angiotensin II-dependent activation of TRPC6 channels in podocytes may have a significant role in the development of kidney diseases.

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Nephrotic syndrome is a group of kidney diseases characterized by heavy proteinuria, hypoalbuminemia, edema, and dyslipidemia. Urinary losses of macromolecules in nephrotic syndrome reflect a dysfunction of the highly permselective glomerular filtration barrier. In the past decade, genetic studies have led to the identification of proteins that have a crucial role in slit-diaphragm signaling and maintenance of podocyte integrity and functions.[1] Particularly, the gene encoding transient receptor potential canonical channel 6 (TRPC6) was identified as the basis for an autosomal dominant form of focal segmental glomerulosclerosis (FSGS).[2,3]

Interstitial angiotensin II (Ang II), a major bioactive product of the renin–angiotensin system, is found to be the key mediator of renal inflammation and fibrosis in progressive chronic nephropathies.[4] It was shown that the expression of Ang II and its receptor is increased in patients with progressive glomerulopathies.[5] It was also demonstrated that Ang II application increased intracellular calcium ($[Ca^{2+}]_i$) in the podocytes.[6–8] As TRPC6 channel mutations were found in patients with FSGS, members of the TRPC family emerged as prime candidates for this increase in $[Ca^{2+}]_i$.

Ang II can act through two different types of receptors, AT_1 and AT_2, which are both involved in regulation of intracellular signals in podocytes.[9] However, the majority of Ang II actions in the glomerulus are mediated by AT_1. It was shown that increased AT_1 signaling in podocytes leads to proteinuria and FSGS.[10] Studies in models of chronic hypertension and protein-induced renal damages revealed that inhibition of AT receptors is effective against proteinuria.[11] AT_1 receptor antagonist candesartan ameliorates the peak level of proteinuria by preventing a reduction in the expression of slit-diaphragm functional molecules.[12] Human trials demonstrated that the inhibition of AT_1 receptors delayed disease progression in patients with diabetic kidney disease.[13,14]

Recent studies demonstrated that Ang II enhances albuminuria by activating TRPC6 channels.[15] Furthermore, Zhang *et al.*[16] showed that alteration of TRPC6 expression and Ca^{2+} influx is involved in Ang II-induced apoptosis.

Besides, it was highlighted that the deleterious effects of Ang II on podocytes and its pathogenic role in glomerular diseases coincides with enhanced TRPC6 expression[17] and that Ang II activation of TRPC6 channels in rat podocytes requires the generation of reactive oxygen species.[18] However, the exact mechanisms of action of Ang II in intact glomeruli remain unclear. Furthermore, it is not clear whether this hormone mediates changes in the number of channels at the plasma membrane and/or channel gating.

We demonstrate here that Ang II upregulates TRPC6 activity in intact podocytes of freshly isolated glomeruli and that this channel's activation further results in extensive Ca^{2+} flux in podocytes. For these experiments, recently developed single-channel analysis of TRPC channels[19,20] and calcium measurements[21] in their native setting, freshly isolated glomeruli, were performed. Transient over-expression of TRPC6 channels together with AT_1 receptor in Chinese hamster ovary (CHO) cells was also used to test the effects of Ang II. Altogether, these techniques were used to establish the effects of Ang II on TRPC channels in the podocytes of the glomeruli, and allowed hypothesizing that TRPC6 blockade and/or inhibition of AT receptors may be of therapeutic benefit in the treatment of the nephrotic syndrome and particularly FSGS.

RESULTS

TRPC6 channel recordings in the freshly isolated mouse glomeruli

We have recently established a novel approach allowing us to perform single-channel analysis of native TRPC-like channels in the podocytes of freshly isolated glomeruli.[19,20] After the glomeruli are isolated from the kidneys of the mice, cell bodies of the podocytes appear in the light microscope as oval structures on the surface of the glomerular capillary loops. Single-channel analysis was used to assess TRPC activity in the podocytes in freshly isolated glomeruli of mice. TRPC channels typically show low levels of constitutive activity.[22] Figure 1a demonstrates the activity of a channel recorded in cell-attached configuration in symmetric chloride solutions at different potentials. The channel has distinct TRPC family properties, including reverse potential close to zero, kinetics, slight voltage dependency, and conductance of ~22 pS. The summarized current–voltage dependence for this channel is shown in Figure 1b. TRPC channel activity was also tested in the TRPC6 knockout mice; with the current solutions and conditions we were unable to record the activity of the channels similar to those recorded in the wild-type mice.

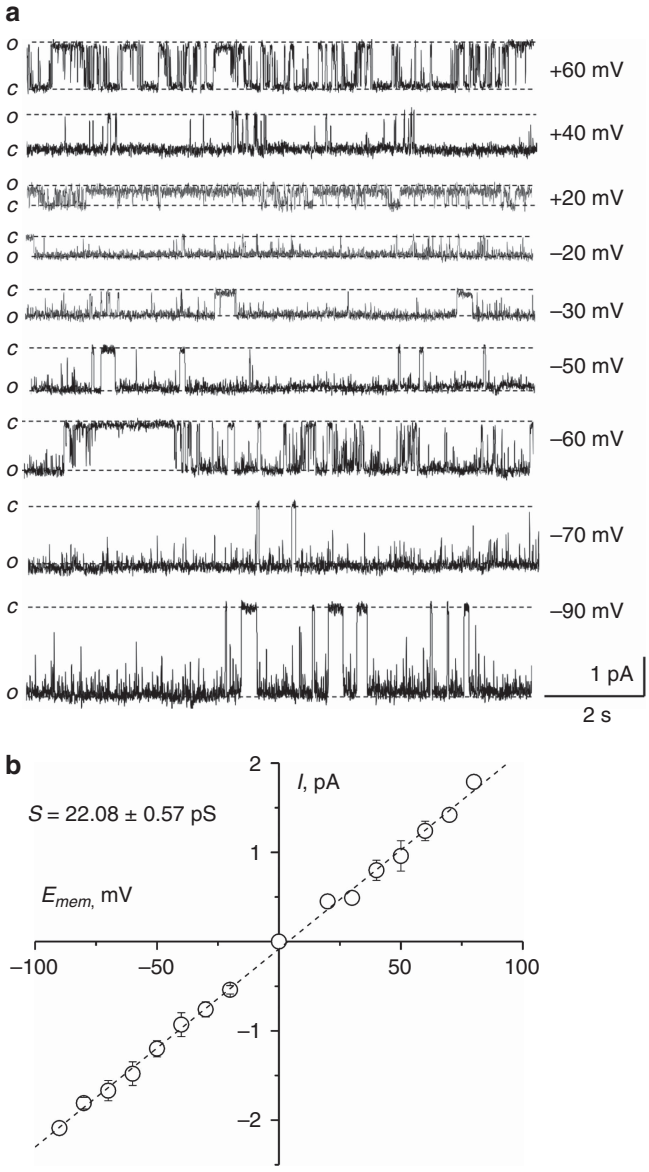

Figure 1 | Native transient receptor potential canonical channel 6 (TRPC6) channels in the freshly isolated mouse glomeruli. (**a**) Representative current traces from the podocytes of the freshly isolated wild-type mouse glomeruli. The activity of the identified TRPC6 channels is shown at different potentials. *c* and *o* denote closed and open states of the channels. (**b**) A summarized current–voltage dependency of the identified TRPC6 channel in the podocytes of the freshly isolated glomeruli. Conductance (*S*) is shown on the graph. Each point is the mean of at least six independent observations made on five animals.

Ang II activates TRPC6 channels in freshly isolated mouse glomeruli and in transfected CHO cells

Figure 2a illustrates the time course of TRPC6 channel activity in the isolated glomerulus following the addition of Ang II (1 μmol/l). As summarized in Figure 2b, application of Ang II resulted in the acute increase in the channel open probability (P_o) in this native preparation. Ilatovskaya *et al.*[20] and others[3,7,22,23] previously demonstrated that multiple members of the TRPC family are expressed in podocytes, but only TRPC6 is known as a cause of FSGS.[2,3,24] Thus, we tested an effect of Ang II specifically on the TRPC6 channel. For these experiments, we analyzed the activity of endogenous channels in response to treatment with Ang II in TRPC6 knockout mice. We did not observe any similar ion channel activity in the podocytes of the $TRPC6^{-/-}$ mice.

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