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Angiotensin II Type 1 receptor Regulates BK Channels Independent of G-Protein Activation in Rat Renal Arterial Smooth Muscle Cells

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In vascular smooth muscles cells (SMCs),Angiotensin II (Ang II) is known to be involved in numerous biological events, including cell contraction, proliferation, migration, and senescence, through the activation of canonical G-protein-dependent signaling pathways. We previously identified a physical interaction between expressed angiotensin II type 1 receptor (AT1R) and large-conductance calcium-activated potassium (BK) channels. In the present study, we assessed the contribution of AT1R and G-protein activation in Ang II-mediated inhibition of BK currents using losartan, an antagonist of AT1R and GDPβS, an inhibitor of G-protein activation. First, we tested the effectiveness of losartan in preventing AT1R activation in HEK293T cells by measuring ERK1/2 phosphorylation and confirmed that 10 μM losartan could fully inhibit the activation induced by 1 μM Ang II. In freshly isolated rat renal arterial smooth muscle cells (SMCs), extracellular application of 1 μM Ang II reduced whole-cell BK currents by 42.9 ± 6.5% (n=5). Supporting the role of AT1R, BK currents were unaffected after Ang II stimulation in losartan-pretreated SMCs and remained at 99.5 ± 2.8% (n=9) of their original amplitude. The full antagonistic action of losartan was also observed in Ang II pretreated HEK293T cells concurrently expressing AT1R and BK channels (n=6). On the other hand, intracellular application of 500 μM GDPβS via the pipette failed to prevent Ang II-induced inhibition of BK currents supporting a process that is independent of G-protein activation. In summary, the results demonstrate that AT1R mediates the Ang II-induced inhibition of BK currents in renal arterial SMCs, and support the hypothesis that the Ang II inhibitory effect on BK currents is mediated by an intimate interaction between AT1R and BK channels. Supported by NIH.

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Slo1 Channel Desensitization to Ethanol is Coupled to Calcium-Sensing Structures in the Channel Cytosolic Domain

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Data from native BK (*Dopico et al., 1996; Pietrzykowski et al., 2004*) and recombinant slo1 channels (*Dopico et al., 1998; Yuan et al., 2008*) consistently show that ethanol-induced increase in channel steady-state activity (NPo) disappears within several minutes of continuous alcohol exposure. Ionotropic receptor desensitization is usually a monotonic function of activating ligand (*Dopico & Lovinger, 2009*), and slo1 channels enter a low activity mode in presence of high levels of activating ligand (i.e., $Ca^{2+}{}_i$) (*Rothberg et al., 1996*). Moreover, $Ca^{2+}{}_i$ is required for ethanol to activate slo1 channels (*Liu et al., 2008*). In this work, we began to examine the $Ca^{2+}{}_i$-dependence and structural bases of slo1 channel desensitization to protracted ethanol exposure. Following BK channel-forming protein expression in membrane patches from *Xenopus laevis* oocytes, exposure to 50 mM ethanol increased NPo×1.5-2 fold within 1-2 min of drug application, a response that totally disappeared within 5 min of alcohol exposure (n=7). Experimental conditions rule out a major role of genetic adaptation, change in channel protein composition, channel internalization, cytosolic signaling and ethanol metabolism in slo1 channel desensitization to alcohol. Rather, the phenomenon must be attributed to ligand interaction(s) with the slo1 subunit and/or its immediate lipid environment. Desensitization to ethanol was observed whether the slo1 channel was studied at 0.3, 1 or 10 μM $Ca^{2+}{}_i$, indicating that slo1 channel desensitization to ethanol is not significantly modified within $Ca^{2+}{}_i$ levels that correspond to the physiological range. However, two constructs containing amino acid substitutions in the channel cytosolic domain that alter $Ca^{2+}{}_i$-sensing by slo1 (5D5N in calcium bowl; D362A/D367A in RCK1) displayed a significant delay in channel desensitization to ethanol. Thus, $Ca^{2+}{}_i$-sensing structures in the slo1 cytosolic domain are functionally coupled to ethanol-desensitization processes.

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Structural Requirements in the Steroid Lateral Chain for the Activation of β1 Subunit-Containing BK Channels by 5β-Cholanic Acid-3α-Ol Analogues

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BK channels regulate many physiological processes and thus constitute an attractive target for drug discovery. Numerous BK channel activators are available. However, these agents usually interact with the ubiquitously distributed channel-forming α subunit and cannot selectively target a particular tissue. Here, we performed a structure-activity relationship study of lithocholic acid (LCA), a cholane that was previously shown to activate BK channels via a steroid site in the accessory BK β1 subunit (*Bukiya et al., 2011*). This protein is highly abundant in smooth muscle but scarce in most other tissues (*Brenner et al., 2000*). Modifications to the LCA lateral chain length and functional group yielded a total of 14 compounds. Bath application of these LCA analogs (1-300 μM) to the cytosolic side of *Xenopus* oocyte membrane patches co-expressing BK channel-forming cbv1 and β1 subunits demonstrates that channel activation (EC_{50}~50 μM) requires the combination of a critical lateral chain length (optimal at C24), rather small volume of its functional group (65-92 $Å^3$), and net negative charge (−0.1 to −0.9 [e]). Thus, analogs having cyano or nitro groups substituting for the carboxyl at C24 in the LCA molecule constitute two novel BK channel activators that satisfy the aforementioned criteria. Data provide detailed structural information that helps us to better understand ligand recognition by the cholane steroid site present in the BK β1 protein, and will be useful to advance a pharmacophore in search of β1 subunit-selective BK channel activators. These compounds are expected to evoke smooth muscle relaxation, which would be beneficial in the pharmacotherapy of prevalent human disorders associated with increased smooth muscle contraction, such as systemic hypertension, cerebral or coronary vasospasm, bronchial asthma, bladder hyperactivity, and erectile dysfunction.

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Differential Effects of PIP2 on Slo1 BK Channels with Different β Subunits

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Phosphatidylinositol 4, 5-bisphosphate (PIP_2) regulates numerous ion channels, including large-conductance Ca^{2+}- and voltage-dependent K^+ (BK) channels (Vaithianathan et al. 2008, J Gen Physiol 132:13-28). We examined the molecular and biophysical mechanisms of the current-enhancing effect of PIP_2 on human Slo1 BK channels with different auxiliary subunits heterologously expressed in HEK cells. In the absence of auxiliary subunits, PIP_2 (10 μM) isolated from bovine brain applied to the cytoplasmic side inhibited currents through Slo1 channels recorded without Ca^{2+} by shifting GV curve by 17.8 ± 1.7 mV and decelerated the activation kinetics at positive voltages. In contrast, PIP_2 markedly increased currents through Slo1+β1 and Slo1+β4 channels by shifting the GV curves by −45.5 ± 2.2 mV and −34.0 ± 2.9 mV, respectively. PIP_2 accelerated the macroscopic activation kinetics at positive voltages and decelerated the macroscopic deactivation kinetics at negative voltages. The current-enhancing effect of PIP_2 on Slo1+β1 was observed at negative voltages and also in 100 μM Ca^{2+}. For Slo1+β2 with a deletion in the β2 N terminus to remove inactivation (Δ2-32) the GV shift by PIP_2 was significantly smaller (−28.9 ± 2.3 mV) than that observed with Slo1+β1. PIP_2 had no effect on the deactivation kinetics of Slo1+β2Δ2-32 channels. Measurements using chimeric β1-β2 subunits show that the second transmembrane domain and the C terminus of β1 are important for the large electrophysiological changes in Slo1+β1 channels caused by PIP_2. Supported in part through the NIH, DFG HE 2993/8, and Shanghai Science and Technology Commission.

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Activating Ion Determines Differential Ethanol-Sensitivity of Slo Family Channels

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Ethanol levels reached in circulation during moderate-heavy alcohol intoxication (50 mM) modify BK channel steady-state activity (NPo) eventually altering physiology and behavior. In general, ethanol decreases NPo of vascular smooth muscle BK channels while increasing NPo of neuronal BK channels. Channel subunit composition, posttranslational modification and channel lipid microenvironment all play a role in alcohol final effect (*Brodie et al., 2007*). However, ethanol action on BK NPo solely requires the channel-forming subunit (slo1) and is function of activating ion (i.e., $Ca^{2+}{}_i$) (*Liu et al., 2008; Bukiya et al., 2009*). Whether activating ion-dependence of alcohol action is unique to slo1 or, rather, extends to the other members of the slo family remains unknown. *Slo2.1* (*Slick*), *Slo2.2* (*Slack*), and *Slo3* gene products render ion channel proteins that display a phenotype common to slo1: high conductance for K^+ and dual regulation of gating by transmembrane voltage and intracellular ion-recognition, with slo2 and slo3 being activated by $Na^+{}_i$ and $OH^-{}_i$, respectively (*Salkoff et al., 2006*). Here, after expression in *Xenopus* oocytes, we probed cbv1, slick/slack and slo3 channels with 50 mM ethanol over a wide range of activating ion concentration in inside-out patches. Thus, we