Title:

Are there Superagonists for Calcium-activated Potassium Channels?

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Key words:

Gating modulator, small-conductance Ca$^{2+}$-activated K$^+$ channel, intermediate-conductance Ca$^{2+}$-activated K$^+$ channel, $K_{Ca3.1}$, $K_{Ca2.2}$, EBIO, NS309, SKA-31, SKA-121
Similar to GABA_\text{A} receptor-channels the calcium-mediated gating of the small-conductance K_{Ca}2 and the intermediate-conductance K_{Ca}3.1 channels can be positively or negatively modulated by small molecule drugs, which, in analogy to the GABA field, have been termed positive (PAM) or negative allosteric modulators. While positive gating modulators like EBIO, NS309, SKA-31 and SKA-121 shift the calcium-response curve of these voltage-independent, calmodulin-gated channels to the left and apparently increase their sensitivity to calcium, negative gating modulators decrease calcium sensitivity.\textsuperscript{1} However, in contrast to GABA_\text{A} receptors, where the binding site for the endogenous ligand GABA is located on the extracellular side and where allosteric modulation by benzodiazepines, neurosteroids and barbiturates has been studied in exquisite detail, only a small number of studies have been performed for K_{Ca} channels. One reason is of course the lower level of pharmacological interest. While GABA_\text{A} receptors are firmly established as clinically used drug targets, no K_{Ca}2 or K_{Ca}3.1 channel modulators have yet reached the clinic despite their undeniable therapeutic potential for neurological, cardiovascular and inflammatory diseases.\textsuperscript{1} Another reason is the technical challenge involved in studying K_{Ca} channel gating. The gating apparatus is located at the intracellular C-terminus, where calmodulin, which functions as a calcium-sensing \(\beta\)-subunit, is constitutively associated with the calmodulin binding domain of the channels,\textsuperscript{2} necessitating the performance of inside-out patch-clamp recordings when aiming to work at defined intracellular calcium concentrations. Nevertheless, a few studies, including some exquisite X-ray crystallography,\textsuperscript{3,4} have been performed and it is currently hypothesized that K_{Ca} channel PAMs bind at the interface between the calmodulin N-lobe and the calmodulin-binding domain of the channels and thus “facilitate” mechanical opening (= increase open channel probability) at a given Ca\textsuperscript{2+} concentration.

Both benzimidazole-type activators like EBIO and NS309 and naphthothiazole/oxazole-type activators like SKA-31 and SKA-121 (Figure 1) have been shown to bind in this interface pocket either through co-crystallization of calmodulin in complex with the calmodulin-binding domain of K_{Ca}2,\textsuperscript{3,4} or, more recently, by our own group using a combination of
electrophysiology and site-directed mutagenesis.\textsuperscript{5} The later study was guided by homology modeling of the $K_{Ca}\text{2.3}$ and $K_{Ca}\text{3.1}$ interface pocket and docking studies using the RosettaLigand computational modeling software. While the crystallography studies\textsuperscript{3,4} afforded the first insight into the atomistic mechanism of action of $K_{Ca}$ activators, our molecular modeling study provides a plausible explanation for why $K_{Ca}$ channel activators in general are 5-10-fold more potent in activating $K_{Ca}\text{3.1}$ than $K_{Ca}\text{2}$ channels.\textsuperscript{5} The presence of R362 creates an extensive “background” hydrogen-bond network in the $K_{Ca}\text{3.1}$ interface pocket that stabilizes the main contacts NH$_2$-substituted $K_{Ca}$ activators make with M51 and E54 in calmodulin (Figure 1). The three $K_{Ca}\text{2}$ channels have shorter N or S residues in the corresponding position and therefore cannot form this hydrogen-bond network. The Rosetta models further suggested an explanation for why the 5-position methyl substituted SKA-121 is more potent on $K_{Ca}\text{3.1}$ and less potent on $K_{Ca}\text{2.3}$ than its parent compound SKA-31 by identifying an increased number of hydrophobic interactions in the “back” of the interface pocket for SKA-121 in the most frequently sampled lowest energy binding poses in $K_{Ca}\text{3.1}$.

While these homology models are certainly helpful for explaining selectivity or for attempting structure based drug design, they fail to explain the experimentally observed ability of SKA-121 to further potentiate $K_{Ca}$ currents at saturating Ca$^{2+}$ concentrations. All previously published calcium-response curves for EBIO or NS309 on $K_{Ca}\text{2.2}$ show a “clean” left-ward shift without any increase in maximal effect (Figure 1). SKA-121, in contrast, doubles $K_{Ca}\text{3.1}$ currents even in the presence of 10 $\mu$M of free intracellular calcium. While this potentiation above the effect of the endogenous ligand, which is reminiscent of the superagonism observed on extrasynaptic GABA$_A$ receptors, could potentially be explained by the assumed relatively low Ca$^{2+}$-dependent $P_{o}(\text{max})$ of $K_{Ca}\text{3.1}$, it becomes harder to explain for $K_{Ca}\text{2.3}$, where SKA-121 is also still able to further potentiate currents in the presence of even 30 $\mu$M free calcium\textsuperscript{5} despite the fact that $K_{Ca}\text{2}$ channels are supposedly already fully open.
Future studies of $K_{Ca}$ channel gating and the mechanism of action of $K_{Ca}$ activators therefore will have to address several questions. First of all, how does the calmodulin mediated gating of the channels actually work? The dimer-of-dimers model suggested by the C-terminal crystal structures,\textsuperscript{3,4,6} which all show two anti-parallel $K_{Ca}.2.2$ fragments and two anti-parallel calmodulins forming a dimeric complex, has been questioned in favor of a model with four-fold rotational symmetry.\textsuperscript{7} This debate is unlikely to be resolved before a full-length structure of a $K_{Ca}.2$ or $K_{Ca}.3.1$ channel becomes available. 2) How do small molecules affect the gating and do they have the same effects on $K_{Ca}.3.1$ and $K_{Ca}.2$ channels? Up to now our laboratory is the only group that published $K_{Ca}.3.1$ calcium-response curves in the presence of a $K_{Ca}$ activator raising the question whether there are intrinsic differences between $K_{Ca}.3.1$ and $K_{Ca}.2$ channels, for which phosphatidylinositol 4,5-bisphosphate (PIP\textsubscript{2}) has recently been shown to regulate channel activity by binding to the $K_{Ca}.2.2$ calmodulin-binding domain/calmodulin complex.\textsuperscript{8} 3) Are there superagonists and partial agonists for $K_{Ca}$ channels? And lastly, how different are $K_{Ca}$ agonists that bind in the C-terminal interface pocket from $K_{Ca}$ agonists\textsuperscript{1} that bind in the pore domain?
Figure 1

**Top,** Chemical structures of the KCa channel activators and Rosetta model of SKA-121 (orange) docked into the interface between the KCa3.1 calmodulin-binding domain (blue) and calmodulin (yellow). See Brown et al.\(^5\) for details. **Bottom,** Cartoon of the effect of EBIO or NS309 on the calcium-response curve of KCa2.2 and of SKA-121 on the calcium-response curve of KCa3.1.

References

1. Christophersen P, Wulff H. Pharmacological gating modulation of small- and intermediate-conductance Ca\(^{2+}\)-activated K\(^+\) channels (KCa2.x and KCa3.1). Channels (Austin) 2015; 9:336-343. PMID: 26217968; doi: 10.1080/19336950.2015.1071748.

2. Kaczmarek LK, Aldrich RW, Chandy KG, Grissmer S, Wei AD, Wulff H. International Union of Basic and Clinical Pharmacology. C. Nomenclature and properties of calcium-activated and sodium-activated potassium channels. Pharmacol Rev 2017; 69:1-11. PMID: 28267675; doi: 10.1124/pr.116.012864.

3. Zhang M, Pascal JM, Schumann M, Armen RS, Zhang JF. Identification of the functional binding pocket for compounds targeting small-conductance Ca\(^{2+}\)-activated potassium channels. Nat Commun 2012; 3:1021. PMID: 22929778; doi: 10.1038/ncomms2017.

4. Zhang M, Pascal JM, Zhang JF. Unstructured to structured transition of an intrinsically disordered protein peptide in coupling Ca\(^{2+}\)-sensing and SK channel activation. Proc Natl Acad Sci USA 2013; 110:4828-4833. PMID: 23487779; doi: 10.1073/pnas.1220253110.

5. Brown BM, Shim H, Zhang M, Yarov-Yarovoy V, Wulff H. Structural determinants for the selectivity of the positive KCa3.1 gating modulator 5-methylnaphtho[2,1-d]oxazol-2-amine (SKA-121). Mol Pharmacol 2017; PMID: 28760780; doi: 10.1124/mol.117.109421.

6. Halling DB, Kenrick SA, Riggs AF, Aldrich RW. Calcium-dependent stoichiometries of the KCa2.2 (SK) intracellular domain/calmodulin complex in solution. J Gen Physiol 2014; 143:231-252. PMID: 24420768; doi: 10.1085/jgp.201311007.

7. Schumacher MA, Rivard AF, Bächinger HP, Adelman JP. Structure of the gating domain of a Ca\(^{2+}\)-activated K\(^+\) channel complexed with Ca\(^{2+}\)/calmodulin. Nature 2001; 410:1120-1124. PMID: 11323678; doi: 10.1038/35074145.

8. Zhang M, Meng XY, Zhang JF, Cui M, Logothetis DE. Molecular overlap in the regulation of SK channels by small molecules and phosphoinositides. Sci Adv 2015; 1:e1500008. PMID: 26366439; doi: 10.1126/sciadv.1500008.
EBIO
KCa2 300 µM
KCa3.1 30 µM

NS309
KCa2 500 nM
KCa3.1 30 nM

SKA-31
KCa2 2-3 µM
KCa3.1 250 nM

SKA-121
KCa2 4-8 µM
KCa3.1 110 nM

KCa2.2

KCa3.1

I/Imax vs Calcium [µM]

I/Imax vs Calcium [µM]

EBIO or NS309

SKA-121