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Are there superagonists for calcium-activated potassium channels?

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Similar to $GABA_A$ 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.[1] However, in contrast to $GABA_A$ 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 $GABA_A$ 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.[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 β-subunit, is constitutively associated with the calmodulin binding domain of the channels,[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,[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 (= increased open channel probability) at a given Ca^{2+} concentration.

Both benzimidazole-type activators like EBIO and NS309 and naphthothiazole/oxazole-type activators like SKA-31 and SKA-121 (Fig. 1) have been shown to bind in this interface pocket either through co-crystallization of calmodulin in complex with the calmodulin-binding domain of $K_{Ca}2.2$,[3,4] or, more recently, by our own group using a combination of electrophysiology and site-directed mutagenesis.[5] The latter study was guided by homology modeling of the $K_{Ca}2.3$ and $K_{Ca}3.1$ interface pocket and docking studies using the RosettaLigand computational modeling software. While the crystallography studies[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 $K_{Ca}3.1$ than $K_{Ca}2$ channels.[5] The presence of R362 creates an extensive "background" hydrogen-bond network in the $K_{Ca}3.1$ interface pocket that stabilizes the main contacts NH_2-substituted K_{Ca} activators make with M51 and E54 in calmodulin (Fig. 1). The three $K_{Ca}2$ 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 $K_{Ca}3.1$
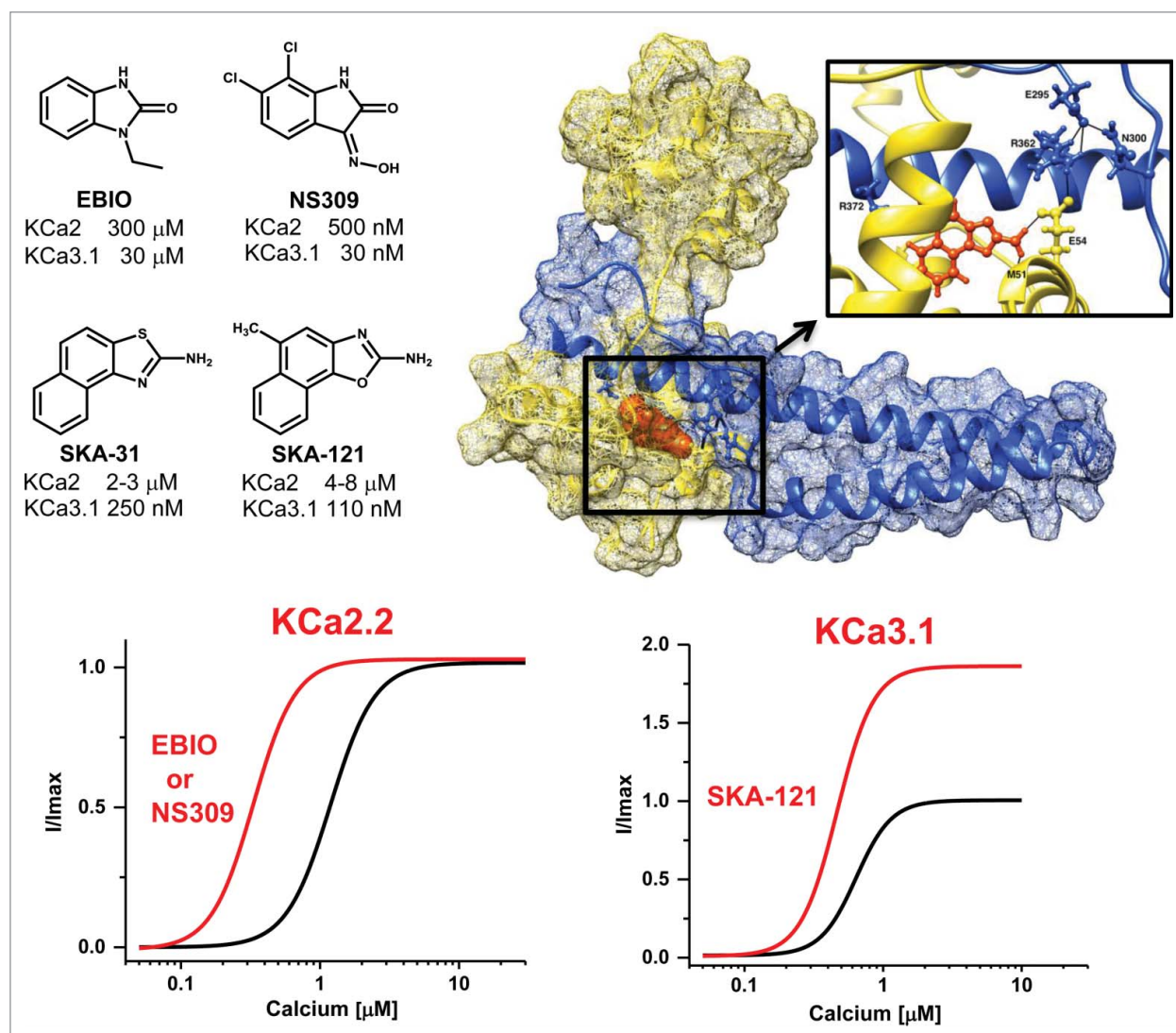
Figure 1. Top, Chemical structures of the KCa channel activators and Rosetta model of SKA-121 (orange) docked into the interface between the $K_{Ca}3.1$ calmodulin-binding domain (blue) and calmodulin (yellow). See Brown et al.[5] for details. Bottom, Cartoon of the effect of EBIO or NS309 on the calcium-response curve of $K_{Ca}2.2$ and of SKA-121 on the calcium-response curve of $K_{Ca}3.1$.

and less potent on $K_{Ca}2.3$ 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 $K_{Ca}3.1$.

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 $K_{Ca}2.2$ show a "clean" left-ward shift without any increase in maximal effect (Fig. 1). SKA-121, in contrast, doubles $K_{Ca}3.1$ currents even in the presence of 10 μM of free intracellular calcium.

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 $P_o(max)$ of $K_{Ca}3.1$. However, it becomes harder to explain for $K_{Ca}2.3$, where SKA-121 is also still able to further potentiate currents in the presence of even 30 μM free calcium[5] despite the fact that $K_{Ca}2$ 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, how does the calmodulin mediated gating of the channels actually work? The dimer-of-dimers model suggested by the C-terminal crystal structures,[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.[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. Second, 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_2) has recently been shown to regulate channel activity by binding to the $K_{Ca}2.2$ calmodulin-binding domain/calmodulin complex.[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[1] that bind in the pore domain?

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No potential conflicts of interest were disclosed.

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