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### Title

Pharmacological gating modulation of small- and intermediate-conductance Ca<sup>2+</sup>-activated K<sup>+</sup> channels (KCa<sub>2.x</sub> and KCa<sub>3.1</sub>)

### Permalink

<https://escholarship.org/uc/item/5w73v0c9>

### Journal

Channels, 9(6)

### ISSN

1933-6950

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### Publication Date

2015-11-02

### DOI

10.1080/19336950.2015.1071748

Peer reviewed

**Pharmacological gating modulation of small- and intermediate-conductance  $\text{Ca}^{2+}$ -activated  $\text{K}^+$  channels ( $\text{K}_{\text{Ca}2.x}$  and  $\text{K}_{\text{Ca}3.1}$ )**

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*Key words:*  $\text{K}_{\text{Ca}2}$ ;  $\text{K}_{\text{Ca}3.1}$ ; SK channel; IK channel;  $\text{Ca}^{2+}$ -activated  $\text{K}^+$  channel; SKA-31, SKA-121; NS309, CyPPA; NS8593

Conflict of Interest and Financial Disclosure:

Palle Christophersen is a full time employee of Saniona A/S. Heike Wulff is named as inventor on a University of California patent claiming SKA-121 and related compounds as antihypertensives.

*Abbreviations and acronyms:*

K<sub>Ca</sub>2.x (small-conductance Ca<sup>2+</sup>-activated K<sup>+</sup> channel)

K<sub>Ca</sub>3.1 (intermediate-conductance Ca<sup>2+</sup>-activated K<sup>+</sup> channel)

ALS (amyotrophic lateral sclerosis)

Cam (calmodulin)

CamBD (calmodulin binding domain)

ICARS (International Cooperative Ataxia Rating Scale)

KCNN (potassium channel gene class N)

## *Abstract*

This short review discusses pharmacological modulation of the opening/closing properties (gating) of small- and intermediate-conductance  $\text{Ca}^{2+}$ -activated  $\text{K}^+$  channels ( $\text{K}_{\text{Ca}2}$  and  $\text{K}_{\text{Ca}3.1}$ ) with special focus on mechanisms-of-action, selectivity, binding sites, and therapeutic potentials. Despite  $\text{K}_{\text{Ca}}$  channel gating-modulation being a relatively novel field in drug discovery, efforts in this area have already revealed a surprising plethora of pharmacological sites-of-actions and channel subtype selectivity exerted by different chemical classes. The currently published positive modulators show that such molecules are potentially useful for the treatment of various neurodegenerative disorders such as ataxia, alcohol dependence, and epilepsy as well as hypertension. The negative  $\text{K}_{\text{Ca}2}$  modulators are very effective agents for atrial fibrillation. The prediction is that further unraveling of the molecular details of gating pharmacology will allow for the design of even more potent and subtype selective  $\text{K}_{\text{Ca}}$  modulators entering into drug development for these indications.

## *1. Pharmacological modulation of ion channel gating*

In the current context we define pharmacological modulation of ion channels as modulation of their activation, inactivation or deactivating mechanisms. In other words, gating modulation. Thus, we will not discuss trafficking modulation, which alters channel density on the plasma membrane or classical pore blockers, which simply obstruct the flow of ions through an open channel.<sup>1</sup> We will also not consider compounds operating at orthosteric sites (agonists), like endogenous neurotransmitters on ionotropic receptors. This article will instead focus on allosteric modulation that strengthens (positive modulator) or weakens (negative modulator) the response to the physiological stimuli for channel activation.

Pharmacological gating modulation is historically a well-established principle in drug development. The most famous example is probably the modulation of GABA<sub>A</sub> receptors by benzodiazepines: Drugs like diazepam bind to an interface pocket between the  $\alpha$  and  $\gamma$  subunits and increase the affinity for activation by GABA, an effect which strengthens inhibitory signaling in the brain and is valuable in the treatment of anxiety and seizure disorders. Importantly, the efficacy of different benzodiazepines at this binding site varies from full to partial positive modulators and comprises compounds with no efficacy, and even compounds exerting negative gating modulation.<sup>2, 3</sup> Another well-known example is the dihydropyridine binding site on L-type Ca<sup>2+</sup> channels (Ca<sub>v</sub>1.x): Nifedipine and amlodipine exert complex modulation of voltage-dependent gating parameters resulting in overall decreased Ca<sup>2+</sup>-currents at physiological membrane potentials.<sup>4</sup> These negative gating modulators are valuable drugs for the treatment of essential hypertension by their relaxing effect on vascular smooth muscles.

However, other dihydropyridines like Bay-K-8644 act as positive gating modulators via the same binding site by primarily shifting the voltage activation curve towards more negative membrane potentials.<sup>5, 6</sup>

In the following paragraphs we will review the current understanding of positive and negative gating modulation of  $\text{Ca}^{2+}$ -activated  $\text{K}^+$  channels of small- and intermediate-conductance,  $\text{K}_{\text{Ca}2.x}$  and  $\text{K}_{\text{Ca}3.1}$  (the KCNN channels), which is an emerging field in drug discovery.

## *2. Small/intermediate conductance $\text{Ca}^{2+}$ -activated $\text{K}^+$ channels*

Four KCNN genes exist in mammals.<sup>7</sup> KCNN1, KCNN2 and KCNN3 encode the small-conductance  $\text{Ca}^{2+}$ -activated  $\text{K}^+$  channels  $\text{K}_{\text{Ca}2.1}$ ,  $\text{K}_{\text{Ca}2.2}$  and  $\text{K}_{\text{Ca}2.3}$  (a.k.a. SK1, SK2 and SK3), which are prominently expressed in neurons but also found in some peripheral tissues such as cardiomyocytes and liver.<sup>8</sup> KCNN4 encodes the intermediate-conductance  $\text{K}_{\text{Ca}3.1}$  (IK, SK4) channel, which is nearly exclusively expressed on non-excitabile cells such lymphocytes, erythrocytes, fibroblasts, vascular endothelium and secretory epithelia.<sup>9</sup> However, this notion was recently challenged by a paper finding evidence for  $\text{K}_{\text{Ca}3.1}$  expression in hippocampal CA1 neurons, where the channel apparently contributes to the slow afterhyperpolarization.<sup>10</sup> All KCNN channels share a common mode-of-activation in that their gating is voltage-independent and depends exclusively on calmodulin (Cam), which is tightly bound to a Cam binding domain (CamBD) in the C-terminus of the channel (Fig. 1).<sup>11-15</sup> Upon  $\text{Ca}^{2+}$  binding to Cam the channels activate in a highly coordinated fashion following an extremely steep

Hill-equation for  $K_{Ca2}$  (and a slightly less steep curve for  $K_{Ca3.1}$ ) and  $EC_{50}$  values estimated by various groups to be in the range of 250 to 900 nM.<sup>14, 16, 17</sup> The  $Ca^{2+}$  sensitivities of KCNN channels are physiologically modulated by closely associated kinases and phosphatases: For  $K_{Ca2}$  channels casein kinase 2 (CK2) and protein phosphatase 2A (PP2A) mediate phosphorylation/dephosphorylation of Cam threonine 80, with the phosphorylated state being less  $Ca^{2+}$  sensitive (Fig. 1).<sup>8</sup> For  $K_{Ca3.1}$  the nucleoside diphosphate kinase B phosphorylates histidine 358 in the C-terminal, which is permissive for  $Ca^{2+}$  activation.<sup>18</sup>  $K_{Ca2}$  channels expressed in the soma of neurons are functionally coupled to voltage-activated  $Ca^{2+}$  channels ( $Ca_v$  channels) and give rise to  $Ca^{2+}$ -dependent afterhyperpolarizations of medium duration (mAHP) following single or trains of action potentials, which is an essential component of early spike frequency modulation in cortical and limbic neurons.<sup>19</sup> In pacemaker neurons of the basal ganglia and cerebellum, cyclic activation of  $K_{Ca2}$  channels secures highly regular firing, which is essential for muscle coordination and movement.<sup>20, 21</sup>  $K_{Ca2}$  channels expressed postsynaptically in dendritic spines are coupled to NMDA (GluN1-3) receptors and their activation hyperpolarizes the spine and accelerates voltage-dependent  $Mg^{2+}$  block of the NMDA receptor.<sup>22</sup> Thus, dendritic  $K_{Ca2}$  channels are important components of memory and learning processes at the subcellular level.<sup>23-25</sup> In contrast,  $K_{Ca3.1}$  channels in endothelial cells and cells of the immune system, such as T-lymphocytes, are activated by more global  $Ca^{2+}$  signals like  $Ca^{2+}$  released from intracellular stores or by the refilling influx through store operated  $Ca^{2+}$  channels like transient receptor potential channels (TRPs) and  $Ca^{2+}$  release activated channels (CRAC).<sup>26, 27</sup>  $K_{Ca3.1}$  is a

major component of the endothelial derived hyperpolarizing (EDH) response<sup>28, 29</sup> and important for cell proliferation and cytokine release in lymphocytes.<sup>27, 30, 31</sup>

### 3. Positive modulation of $K_{Ca2}/K_{Ca3.1}$ channels

#### 3.1 Key compounds and drugs

It can be argued that the oldest positive modulator of KCNN channels is 1-EBIO (Fig. 2), which was identified before the channels were cloned.<sup>32</sup> 1-EBIO activates all four KCNN channels. With an  $EC_{50}$  of  $\sim 30 \mu M$   $K_{Ca3.1}$  is most sensitive, while the three  $K_{Ca2}$  channels are activated at roughly 5-10 fold higher concentrations.<sup>33-35</sup> However, 1-EBIO is a tool compound for research purposes only. A pivotal finding for the field therefore was the demonstration that chlorzoxazone and zoxazolamine, centrally acting muscle relaxants used for many years to treat spasticity, also activated KCNN channels.<sup>36</sup> Another compound, riluzole, the only drug FDA approved for treatment of amyotrophic lateral sclerosis (ALS), was shown to activate  $K_{Ca3.1}/K_{Ca2.x}$  channels even more potently than 1-EBIO.<sup>37</sup> Despite having a very “rich” ion channel pharmacology, including block of voltage-dependent  $Na^+$  ( $Na_v$ ) channels (but not glutamate receptors even though its therapeutic action is often referred to as being anti-glutamatergic), the activation of KCNN channels is among riluzole’s most potent effects.<sup>38</sup> Two molecules also belonging to the benzimidazole/benzothiazole prototype  $K_{Ca}$  activator class are NS309, a very potent molecule ( $EC_{50}$  10-20 nM for  $K_{Ca3.1}$  and  $\sim 600$  nM for  $K_{Ca2}$  channels) and very useful for *in vitro* mechanistic work, and SKA-31, which was optimized from riluzole with the aim of getting better selectivity (inactive on  $Na_v$  channels) and maintaining good *in vivo* properties.<sup>38, 39</sup> The very close analogues, SKA-



111 and SKA-121, exhibits much higher  $K_{Ca3.1}/K_{Ca2}$  selectivity, and accentuate the primary role of the benzimidazole/benzothiazole series as being  $K_{Ca3.1}$  activators.<sup>40</sup>

In contrast to the above mentioned molecules, which show little selectivity between the  $K_{Ca2}$  family members, CyPPA and its more potent congener NS13001 have fundamentally different structures and also changed selectivity profiles (Fig. 2). Both compounds selectively activate  $K_{Ca2.3}$  and  $K_{Ca2.2}$  but are completely inactive on  $K_{Ca2.1}$  and  $K_{Ca3.1}$ .<sup>16, 41</sup> Still other lines of molecules are represented by GW542573X and CM-TMPF, which are selective and potent (CM-TMPF) activators of the human  $K_{Ca2.1}$  channel.<sup>42, 43</sup>

### *3.2 Mode(s) of action*

Basically, all KCNN activators conform with our definition of a positive gating modulator, since they act by shifting the  $Ca^{2+}$ -activation curve towards lower concentrations of  $Ca^{2+}$  in a concentration-dependent way (Fig. 2 bottom). This means that the determined  $EC_{50}$  values for  $Ca^{2+}$ , calculated from  $Ca^{2+}$ -concentration response curves, decreases as the concentration of modulator increases. Using fast application of different intracellular  $Ca^{2+}$  concentrations to inside-out patches, it has furthermore been shown that 1-EBIO has no effect on activation time constant, whereas it strongly reduces the deactivation constant.<sup>44</sup> Applied to a physiological situation this means that  $K_{Ca2}$  channels will not activate quicker or to a significantly higher degree during a fast neuronal action potential, but they will stay open longer after the action potential and local  $Ca^{2+}$  transient has ceased. This is exactly what is observed, since positive modulators prolong the mAHP, thereby increasing the time to the firing of the next

action potential.<sup>34, 45</sup> Given these functional data it is therefore an obvious and tempting interpretation that the mode-of-action of positive modulators simply is to decrease the  $K_d$  value for  $Ca^{2+}$  binding to Cam by decreasing the  $k_{off}$ -value for  $Ca^{2+}$ . Intriguingly, however, this is not exactly what happens! Studies of Cam mutants, which make unstable association with the CamBD, have shown that NS309 is able to stabilize the interaction, which points towards an action of NS309 in the transformation of conformational changes between the Cam and the channel.<sup>46</sup> Direct measurements of  $Ca^{2+}$  off-rates have confirmed that conclusion.

### 3.3 Site(s) of action

A chimaeric construct where the entire C-terminus (Fig. 1) was transferred from  $K_{Ca3.1}$  to  $K_{Ca2.2}$  demonstrated that the higher 1-EBIO potency on  $K_{Ca3.1}$  followed the C-terminus, suggesting that this domain (probably the CamBD) contains the binding site.<sup>44</sup> We were able to confirm and refine that conclusion for the more selective CyPPA by a mutation program with  $K_{Ca2.3}$  (active) and  $K_{Ca2.1}$  (inactive), whereas the equivalent program for the  $K_{Ca2.1}$ -selective molecules, CM-TPMF and GW542573X, gave the highly surprising result that their sites-of-action resided in transmembrane domain 5 in the pore.<sup>42, 43</sup> Recently, crystal structures for Cam/CamBD with bound 1-EBIO and NS309, respectively, have been solved.<sup>47, 48</sup> Both molecules reside in a pocket formed at the interface between Cam/CamBD (Fig. 3), but have different coordination sites on Cam and CamBD. Interestingly, NS309 interacts with an internally disorganized stretch of amino acids (called IDF, shown in red in Fig. 3), which was not visible in the 1-EBIO crystal. This interaction was speculated to explain the

disproportionately high affinity of NS309 compared to other structures in the prototype series. (In this context it is highly interesting that the regulatory membrane lipid, PIP2, which recently has been shown to be an obligatory co-agonist to  $\text{Ca}^{2+}$ , also binds with high affinity in this region, and *also* interacts with residues on both Cam and the IDF.<sup>49</sup> Detailed analyses are warranted to clarify whether NS309 possibly can substitute for PIP2 or whether it increases its affinity). Based on docking and mutations directed by the crystal structure, it was made plausible that CyPPA binds at the same or an overlapping site as NS309 and 1-EBIO. However, the crystal structure unfortunately did not give any hints as to why CyPPA is completely inactive on  $\text{K}_{\text{Ca}2.1}$ . To answer this question CyPPA, or a closely related analogue, probably needs to be co-crystallized with the  $\text{K}_{\text{Ca}2.2}$  (or  $\text{K}_{\text{Ca}2.3}$ ) CamBD and Cam.

#### *3.4 Therapeutic indications for positive modulators of $\text{K}_{\text{Ca}2}$ channels*

$\text{K}_{\text{Ca}2}$  channel expression and/or function has been reported to be down-regulated in limbic brain regions following induction of alcohol dependence and in the hippocampus following pilocarpine induced *status epilepticus*, suggesting that positive  $\text{K}_{\text{Ca}2}$  channel modulation might be particularly useful for situations where physiological  $\text{K}_{\text{Ca}2}$  channel function has been perturbed.<sup>50-53</sup> More generally, due to their ability to prolong the mAHP by increasing the apparent  $\text{Ca}^{2+}$  sensitivity of  $\text{K}_{\text{Ca}2}$  channels, positive gating modulators may also have an important role to play in the treatment of neurological diseases characterized by hyperactivity or disorganized firing. An illustrative example is episodic ataxia (EA2) caused by a partial loss-of-function of P/Q type  $\text{Ca}^{2+}$  channels ( $\text{Ca}_v2.1$ ), which make cerebellar Purkinje cell firing irregular due to

inefficient cyclic activation of  $K_{Ca2}$  channels by action potentials. Local application of 1-EBIO to the cerebellum or systemic administration of chlorzoxazone both reestablished regular pacemaking activity and partially counteracted ataxia.<sup>54, 55</sup> While this effect is straightforward to rationalize from the mode-of-action of positive modulators, it is less clear why CyPPA, NS13001, and SKA-31 exert positive effects in rodent models of spinocerebellar ataxias (SCA2 and SCA3), which are due to gain-of-function toxicity caused by glutamine prolongation of ataxins.<sup>41, 56</sup> One possibility is that diseased Purkinje cells - maybe independently of the molecular cause - go through phases where the highly regulated pacemaker firing is lost, for example through changes in climbing fiber input or denervation,<sup>57, 58</sup> leading to excessive endogenous burst firing, depolarization block, and  $Ca^{2+}$  overload, which may contribute to accelerated cell death.

Positive modulators of  $K_{Ca2}$  channels might terminate bursting by reintroducing mAHPs, reactivate depolarization blocked cells by hyperpolarization, and possibly prevent or delay Purkinje cell degeneration by relieving their metabolic burden. Recently, supportive information for a broader use in ataxia diseases has been obtained from a few smaller clinical trials. Riluzole dosed at 50 mg/kg/day b.i.d. (the treatment regimen used for ALS patients) for 8 weeks was shown to improve symptoms measured by the ICARS scale in ataxia patients of mixed etiology.<sup>59</sup> Furthermore, chlorzoxazone specifically improved downbeat nystagmus, an involuntary vertical eye movement, a devastating symptom affecting many ataxia patients.<sup>60</sup>

A new putative indication for  $K_{Ca2}$  positive modulators may be pain disorders. A recent study showed that  $K_{Ca2}$  activation with intrathecally administered NS309 can potentiate the analgesic effects of NMDA antagonists in rat pain models.<sup>61</sup> This effect

may be interpreted in the light of the tight functional coupling between NMDA receptors and  $K_{Ca2.2}$  channels (see above) in postsynaptic dendritic spines in pain pathways.

### *3.5. Indications for positive modulators of $K_{Ca3.1}$ channels*

Although  $K_{Ca2}$  and  $K_{Ca3.1}$  channels are usually not present in the same tissues, one place where  $K_{Ca2.3}$  and  $K_{Ca3.1}$  are co-expressed is the vascular endothelium, where both channels are involved in the endothelium-derived hyperpolarization (EDH) response which regulates the contractile state of the underlying smooth muscle cell layer.<sup>62, 63</sup> Mice deficient in  $K_{Ca3.1}$  and/or  $K_{Ca2.3}$  exhibit impaired EDH responses and show a ~10 mmHg increase in mean arterial blood pressure,<sup>28, 29</sup> while SKA-31 lowers blood pressure in normotensive and hypertensive mice as well as in conscious, normotensive dogs.<sup>29, 38, 64</sup> Since the blood pressure lowering effect of SKA-31 is absent in  $K_{Ca3.1}^{-/-}$  mice and higher doses of SKA-31 induce sedation and lower heart rate through central  $K_{Ca2}$  channel activation,<sup>65</sup> it seems desirable to identify  $K_{Ca3.1}$  selective positive gating modulators in order to help investigate whether such compounds could be developed into a new class of endothelial targeted antihypertensives.<sup>63</sup> This objective recently seems to have been achieved with the demonstration that the  $K_{Ca3.1}$  selective SKA-121 lowers blood pressure in normotensive and hypertensive mice without affecting heart rate.<sup>40</sup> However, SKA-121 has a short half-life (at least in rodents) and therefore does not constitute an ideal candidate compound for development.

From a therapeutic perspective an argument against positive gating modulators might be that their continued presence can lead to down-regulation of channel

expression, thereby preventing effective treatment in long term dosing regimens. This is a well-founded concern based on the known plasticity of ion channel expression in many situations. Furthermore, it has been observed that classical positive KCNN modulators like 1-EBIO, NS309, and SKA-31 can reduce the expression of  $K_{Ca}3.1$  in proliferative vascular smooth muscle cells as well as  $K_{Ca}3.1$  blockers or  $K_{Ca}3.1$  silencing.<sup>66, 67</sup> However, the phenomenon has not been reported for  $K_{Ca}2$  channels in terminally differentiated cells like neurons and cardiomyocytes, and not described for  $K_{Ca}3.1$  and  $K_{Ca}2.3$  in vascular endothelium.

#### *4. Negative modulation of $K_{Ca}2$ channels*

##### *4.1 Key compounds*

Negative gating modulation of  $K_{Ca}2$  channels is a relatively new concept, in contrast to the rich literature on peptide and small molecule blockers of both  $K_{Ca}2.x$  and  $K_{Ca}3.1$ .<sup>35</sup> (It should be noted that recent work shows that apamin, the prototypic “blocker” of  $K_{Ca}2.x$  channels, actually acts by an allosteric mechanism collapsing the outer pore.<sup>68, 69</sup> However, this is independent of gating, and therefore apamin is not counted as a negative modulator in this context). Detailed mechanistic data are mainly available for the compound that defined the concept, NS8593 (and its closest analogues), which inhibits cloned human and rat isoforms of  $K_{Ca}2$  with equal potency, while it is inactive on  $K_{Ca}3.1$ .<sup>70, 71</sup> NS8593 (Fig. 2) is a non-charged molecule with a very different structure than the small molecule blockers (“apamin mimics”).<sup>72, 73</sup> Recently, the negative modulator field has been enriched with Bu-TPMF, a  $K_{Ca}2.1$  selective

negative modulator,<sup>42</sup> and with a fluoro-trivanillic ester compound (RA-2), which inhibits both K<sub>Ca</sub>3.1 and K<sub>Ca</sub>2.3 with low nM potencies.<sup>74, 75</sup>

#### *4.2 Mode of action*

Like all negative-gating modulators NS8593 causes a concentration-dependent shift of the Ca<sup>2+</sup>-activation curve towards higher Ca<sup>2+</sup> concentrations (Fig. 2 bottom). In practical terms, if NS8593 is applied at a relatively low Ca<sup>2+</sup> concentration (low degree of channel activation) the inhibitor potency appears high, while it is essentially without effect at higher Ca<sup>2+</sup> concentrations (high degree of channel activation).<sup>70, 76</sup> The distinction between the Ca<sup>2+</sup> concentration *per se* and the degree of activation is essential here, since the effect of NS8593 also disappear at a high degree of channel activation obtained by a combination of a low Ca<sup>2+</sup>-concentration and a positive modulator like NS309 or SKA-31.<sup>45, 70, 76</sup>

#### *4.3 Site(s) of action*

In contrast to small molecule blockers, NS8593 does not displace apamin in binding studies, and the compound remains active on a channel made apamin-insensitive by specific mutations in the outer pore mouth.<sup>71</sup> Another difference is that NS8593 is able to reach its binding site both when applied from the outside and from the inside of the membrane.<sup>70</sup> Since the inhibition by NS8593 can easily be “reversed” by positive allosteric modulators and since the right shifting of the Ca<sup>2+</sup>-activation curve resembles the effect of the physiological negative gating modulation described above, we were initially convinced that the site of action would be at the Cam/CamBD region or

maybe at Cam itself. This proved not to be the case! NS8593 interacts deeply in the inner vestibule of the pore, at a site defined by just two amino acids, serine 507 and alanine 532 ( $K_{Ca2.3}$ ) positioned close to the inner side of the  $K^+$  selectivity filter (Fig. 1).<sup>76</sup> Since the physical gate of KCNN channels has - by independent measures - been located at a “deep” position in the channel,<sup>77</sup> we speculate that the mechanism behind the negative gating modulation of NS8593, may be due to its interaction with the gate itself. A crystal with bound NS8593 would be very interesting! It is noteworthy that  $K_{Ca3.1}$  inhibitors like TRAM-34 act at the equivalently positioned residues in  $K_{Ca3.1}$ , which questions whether these also act by “shutting the gate” or - as hitherto assumed - by “blocking ion flow”.<sup>78</sup> However, the inhibition by TRAM-34 shows no clear dependence on intracellular  $Ca^{2+}$  (or degree of activation) and the effect cannot be “reversed” by positive modulators.<sup>76</sup>

In contrast to NS8593, Bu-TPMF, the  $K_{Ca2.1}$  selective negative gating modulator, interacts with a site lower down in the inner pore vestibule in TM5 (Fig. 1). This site is identical to the site-of-action of the  $K_{Ca2.1}$  selective positive modulators, CM-TPMF and GW542573X (Fig. 2).<sup>42</sup> The binding site of the fluoro-di-benzoate RA-2 is currently unknown,<sup>75</sup> but based on the molecules large size and different structure, is not likely to be as high up in the inner pore as NS8593.

#### *4.4 Indications for negative gating modulators of $K_{Ca2}$ channels*

Much evidence indicates that blocking  $K_{Ca2}$  channels by apamin improves learning processes, which is easily comprehended by the close functional association between  $K_{Ca2.2}$  and NMDA receptors, as demonstrated in the hippocampus.<sup>8</sup>



Furthermore, inhibition of  $K_{Ca2.3}$  by NS8593 in dopaminergic neurons leads to increased bursting both *ex vivo* and *in vivo*, which may influence the balance between tonic and phasic dopaminergic signaling and constitute a principle for treatment of various psychiatric conditions.<sup>45, 79</sup> However, the therapeutic exploitation for CNS diseases is hitherto hampered by the low therapeutic index towards tremors, ataxia and convulsions, probably induced by  $K_{Ca2.2}$  inhibition.

The currently most promising use of negative gating modulators is as atrial selective cardiac anti-arrhythmics.<sup>80</sup> NS8593 as well as several small molecule blockers of  $K_{Ca2}$  channels have shown efficacy in protecting against and terminating atrial fibrillation both *ex vivo* and *in vivo* across a number of different species.<sup>81</sup> Intriguingly, this concept also appears to be viable after hypertension induced cardiac remodeling.<sup>82</sup>

## 5. Conclusion

Despite the strong precedence of pharmacological modulation of ion channel gating in the history of drug discovery provided by  $GABA_A$  receptor modulators, it is an often heard argument that ion channel modulation is too “messy” a principle for rational drug discovery compared to agonist/antagonists or blockers. These reservations may be founded in therapeutic considerations (such as tolerance development as discussed for positive modulators), but we suspect that the attitude is often founded on operational screening considerations, rather than an analysis of the need for specific mode of actions for treatment of various diseases. It is certainly true that the need for a well-defined partial activation of ion channels - a general prerequisite for modulator screens -

represents a challenge for quantification, but with the increasingly refined methods for medium-to-high throughput electrophysiology and spectroscopic methods these challenges can usually be handled.<sup>83-85</sup> The appearance of both positive and negative gating modulators of KCNN channels from HTS screening programs and classical medicinal chemistry efforts as described in the foregoing paragraphs shows that this is certainly possible. Furthermore, the emerging diversity in terms of mode-of-actions and subtype selectivities suggest to us that the field of KCNN gating modulators offers exciting therapeutic possibilities that currently are only beginning to be realized.

#### *Acknowledgement*

The authors wish to thank Vladimir Varov-Yarovoy in the Department of Physiology & Membrane Biology, University of California, Davis for help with Figure 3. Heike Wulff was supported by the CounterACT Program, National Institutes of Health Office of the Director (NIH OD), and the National Institute of Neurological Disorders and Stroke (NINDS), Grant Numbers U54NS079202 and R21NS072585.

## *Figure Legends*

**Figure 1.** Membrane topology of a  $K_{Ca2}$  channel and known sites of action of positive and negative gating modulators. Cam; calmodulin; CK2; casein kinase 2; PP2A; protein phosphatase 2A.

**Figure 2.** *Top*, Chemical structures of positive and negative gating modulators. *Bottom*, Schematic of effect of gating modulators on the channel  $Ca^{2+}$  concentration response curve.

**Figure 3.** Space-filled structure of NS309, calmodulin and  $K_{Ca2.2}$  C-terminus complex. The  $K_{Ca2.2}$  residues that become “ordered” following NS309 binding are shown in red.

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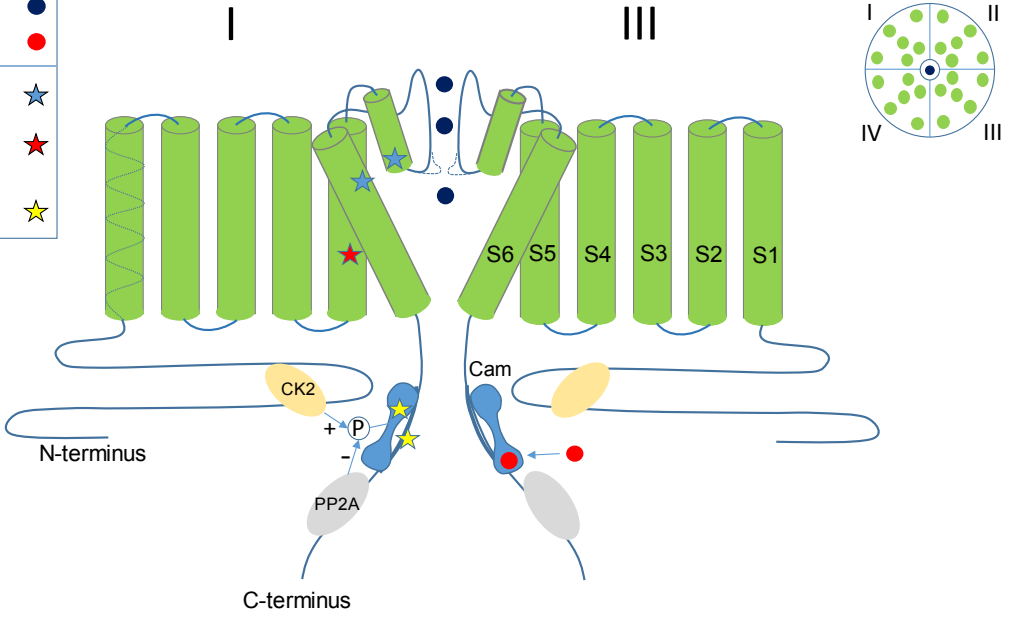


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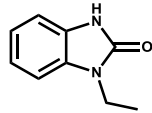
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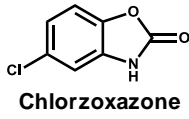
Potassium ion	●
Calcium ion	●
NS8593 and TRAM-34	★
Bu-TPMF, CM-TPMF GW542573X	★
1-EBIO, NS309, CyPPA	★



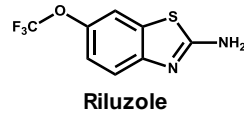
“Classical” unselective positive gating modulators



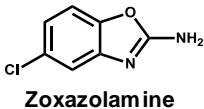
1-EBIO



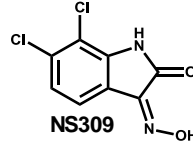
Chlorzoxazone



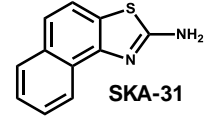
Riluzole



Zoxazolamine

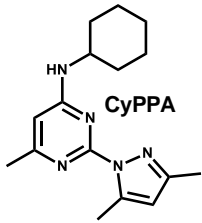


NS309



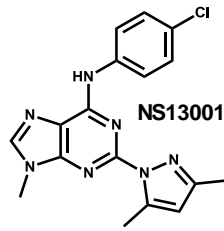
SKA-31

K<sub>Ca</sub>2 selective positive gating modulators

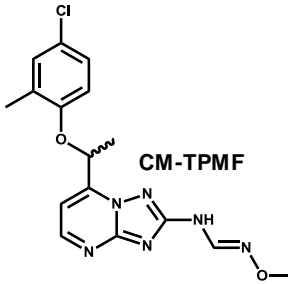


CyPPA

K<sub>Ca</sub>2.2/2.3 selective



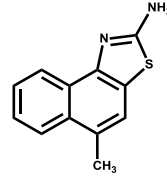
NS13001



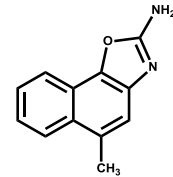
CM-TPMF

K<sub>Ca</sub>2.1 selective

K<sub>Ca</sub>3.1 selective positive gating modulators

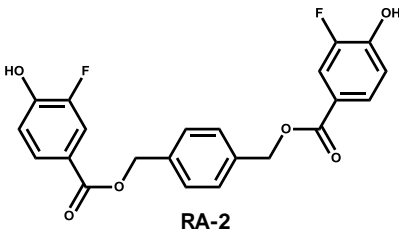


SKA-111



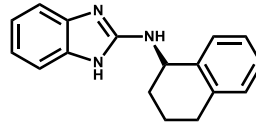
SKA-121

Unselective negative gating modulators

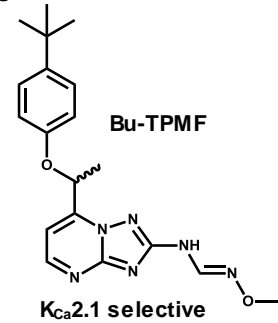


RA-2

K<sub>Ca</sub>2 selective negative gating modulators



NS8593



Bu-TPMF

K<sub>Ca</sub>2.1 selective

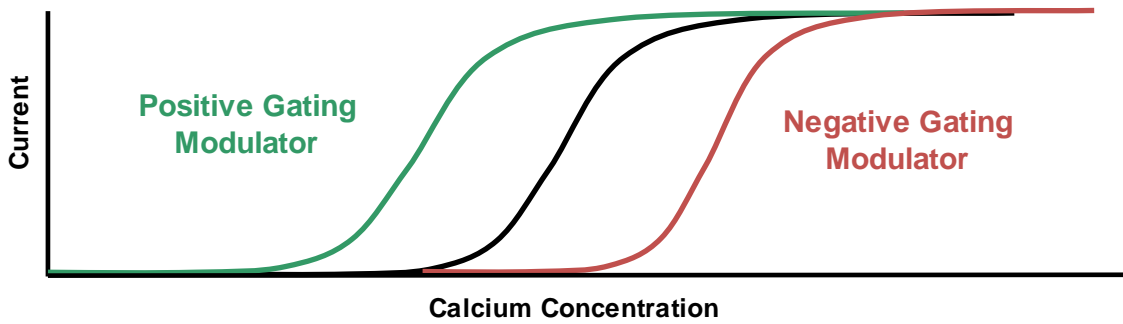


Figure 2

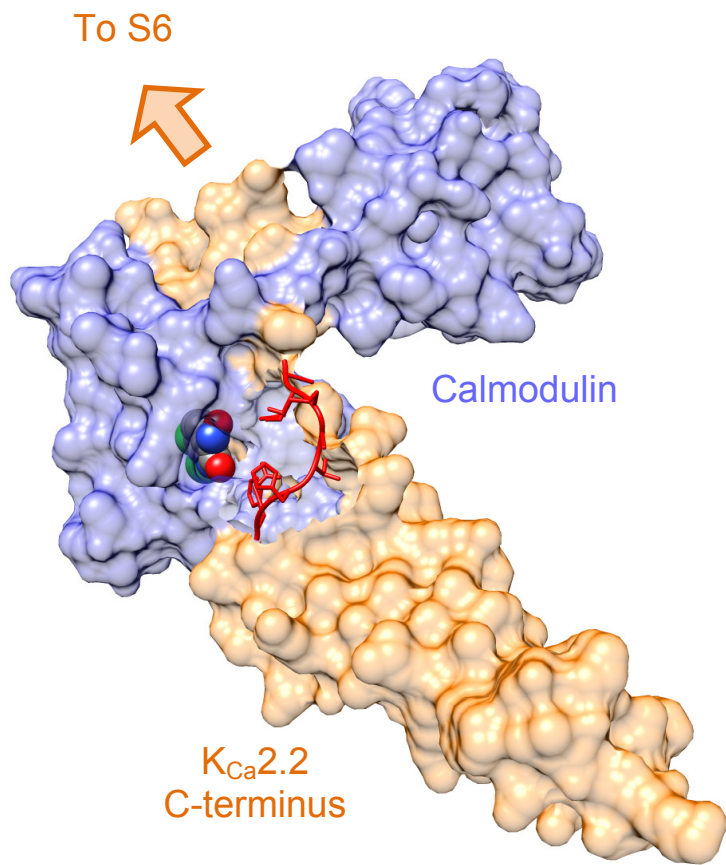


Figure 3