

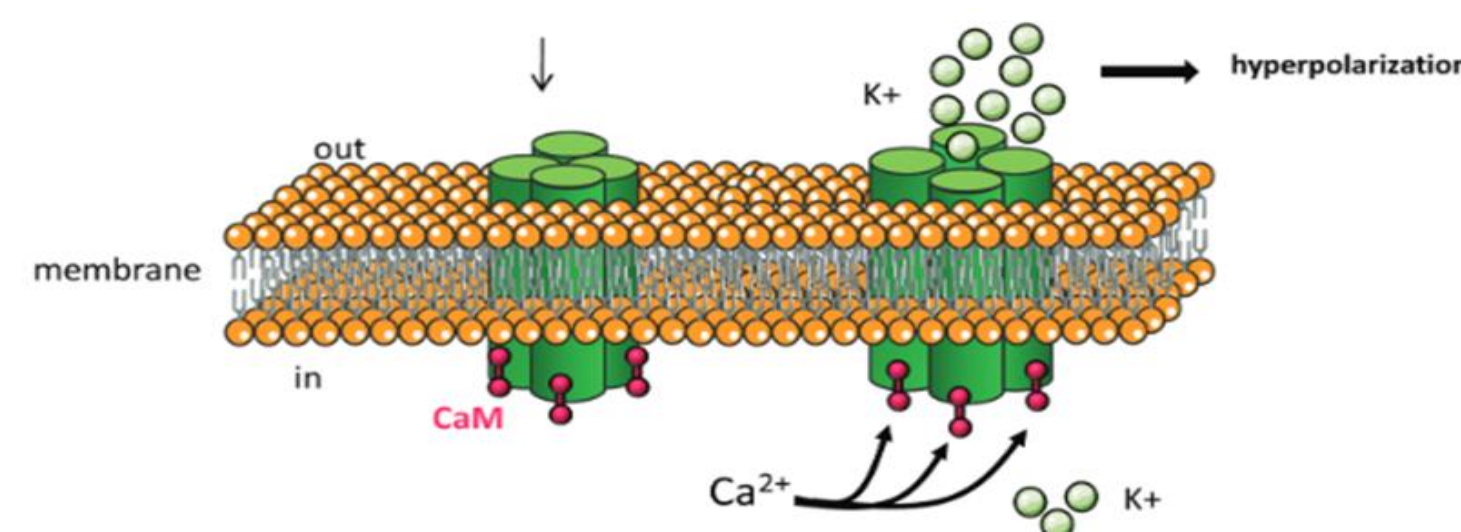
DETERMINATION OF THE AFFINITY OF MUTATED APAMINS FOR SMALL-CONDUCTANCE CALCIUM-ACTIVATED POTASSIUM CHANNELS

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Introduction

Small-conductance calcium-activated potassium (SK) channels are potassium channels that open in response to an intracellular calcium increase and are voltage-insensitive. Three subtypes are expressed in humans, namely SK1, SK2, and SK3. These channels contribute to medium-duration afterhyperpolarization of the neuronal membrane and the repolarization of the action potential in atrial cardiomyocytes¹. Blocking these channels could be beneficial in treating various pathologies, such as depression and atrial fibrillation^{2,3}. A classical blocker of these channels is apamin, a peptidic toxin from bee venom, which is known to interact in the pore region⁴ with limited selectivity for SK2 versus SK3 subtypes. Selective SK3 blockers are missing and this point remains a big challenge in medicinal chemistry.



From Girault, A. et al, *Curr Med Chem* **2012**, 19 (5), 697–713

Background

Different peptides from scorpion and bee venoms interact with SK channels in the outer pore region. The sequences of these peptides contain some similar elements (Figure A). In this figure, the presence of a short sequence called RXCQ box (in yellow) is a common element. Both arginines (RR) are the main element of the current pharmacophore^{5,6}.

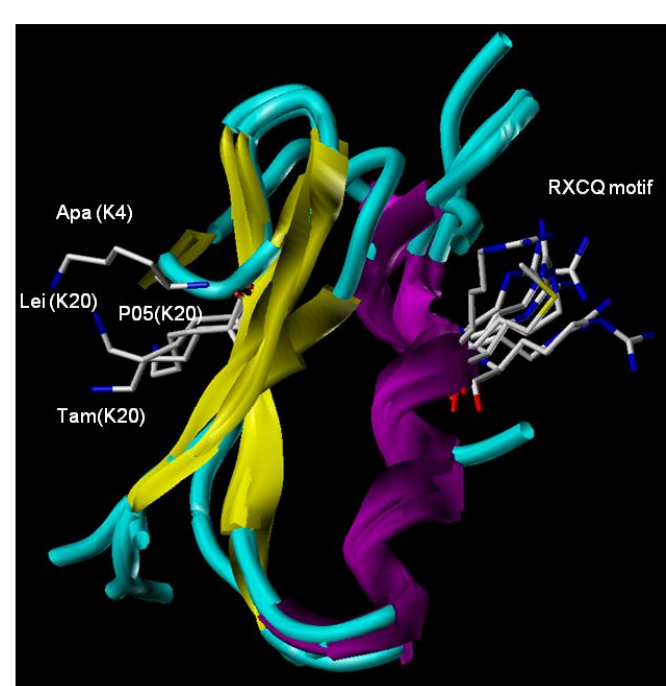
Otherwise, by superimposition of the 3D structures (Figure B), it appears that a lysine (in green) in the four peptides is in a similar spatial region.

Figure A : Peptide sequences

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Leiurotoxin 1  AFCNLRMQGLSCRSLGLLGKKGIGDKCECVKH 31
P05          TVCNLRRQGLSCRSLGLLGKKGIGVKCECVKH 31
Tamapin      AFCNLRRQGLSCRSLGLLGKKGIGEECKCVPY 31
Apamin       CNCKAPETALCARRQQH 18
  
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Figure B : Superimposition of the 3D structures of these peptides



Aim of the work

In this study, we want to determine the affinity for SK2 and SK3 channel subtypes of mutated apamins in two regions potentially important for the interaction with the channels. Regarding the lysine residue in position 4, we speculated that such residue with a protonated side chain may interact in the S3-S4 loop (see Figure C) where a triad of residues was shown to be important⁷. Five mutations were designed by introducing in replacement of the lysine an arginine, a citrulline, a diaminopropanoic acid, a diaminobutanoic acid and an aminoheptanoic acid. The corresponding side chains possess different physicochemical characters (Figure D). Regarding both arginines in 13 and 14 (RXCQ box) the residues were replaced either by two lysines or two ornithines, reducing the basicity and the length of the side chain.

Figure C: Representation of a triad of residues in the S3-S4 zone of SK channels where the Lys4 residue of apamin would potentially interact through H-bonds (full line) and π -cation bonds (dashed line).

