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Adamantanes as building blocks for novel TRPC5 channel modulators

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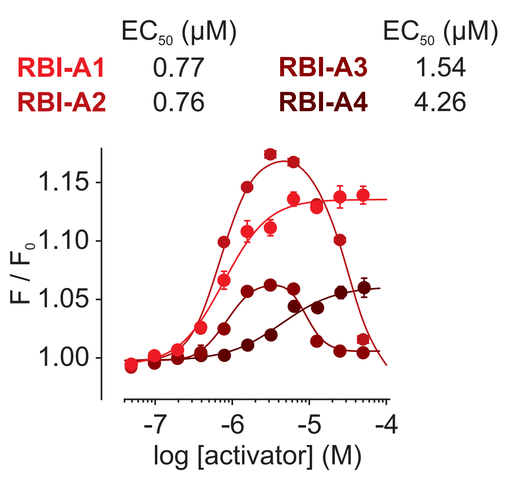
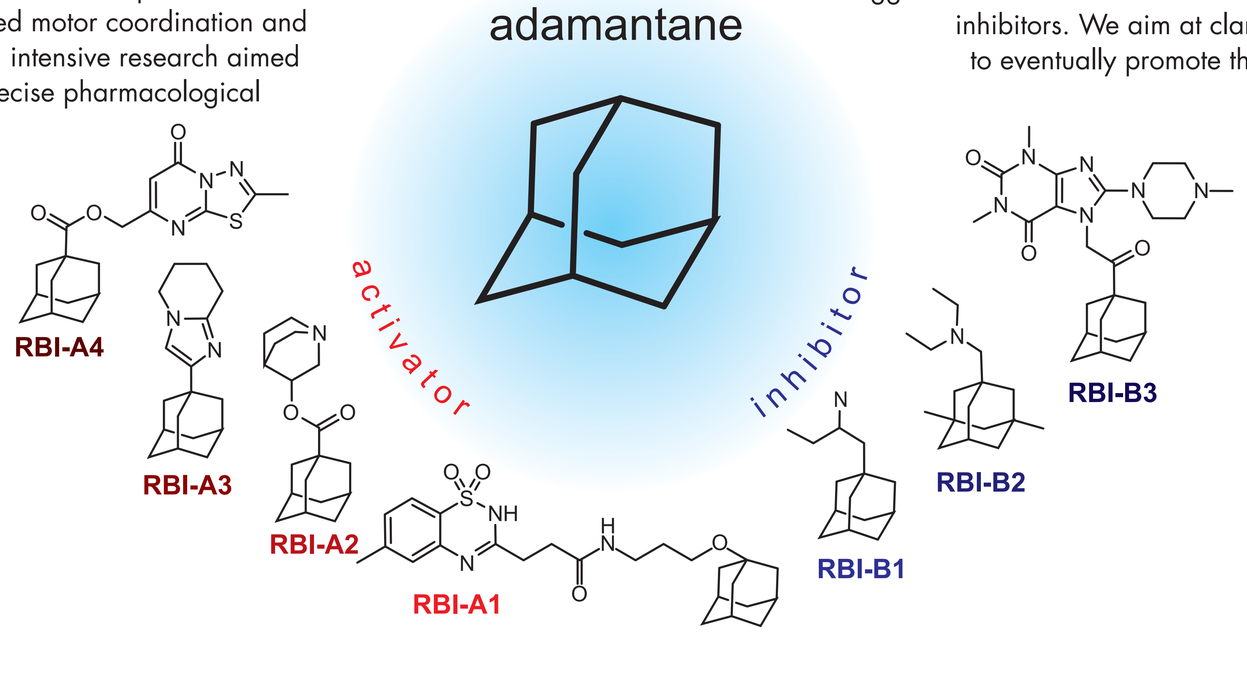
Introduction

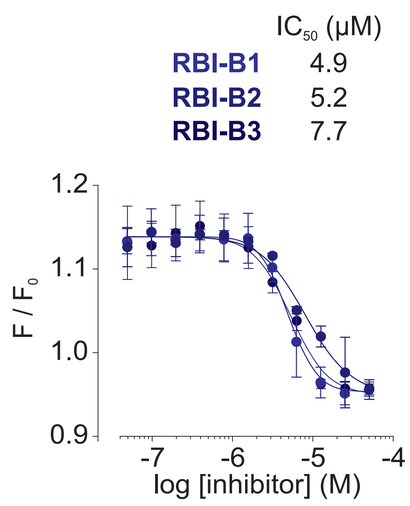
The transient receptor potential (TRP) channels are a family of non-selective cation channels, comprising 28 members in mammals. They are involved in a plethora of physiological and pathophysiological processes [1]. Among these, the canonical TRP channel 5 (TRPC5) is almost exclusively found in neuronal tissues. TRPC5 is involved in neuronal plasticity and axonal pathfinding in hippocampal neurons and has been shown to influence the formation of memory [2,3]. Furthermore, TRPC5 may interfere with the reproductive axis in rodents. TRPC5-deficient transgenic animals show impaired motor coordination and reduced fear behavior [4,5,6]. In the past years, intensive research aimed at identifying novel small molecules to enable precise pharmacological modulation of TRPC5 channels.

Key findings

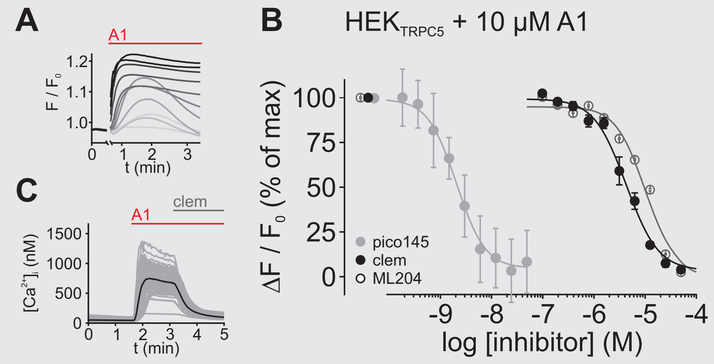
The screening of a compound library containing almost 17,000 different chemical structures revealed seven small molecules that modulate TRPC5 channel activity. These diverse chemical structures all share adamantane-substituents and acted either as channel activators or channel inhibitors. Adamantanes without prominent side chains were selective, but poorly potent inhibitors of TRPC5 and TRPC4 channels (IC_{50} of 3-10 µM), while those with bigger substitutions tend to be activators (EC_{50} of 0.7-5 µM) or mixed activators/inhibitors. We aim at clarifying adamantane effects on TRPC5 channels to eventually promote the development of ready-to-use small molecules to treat TRPC5-associated disorders.



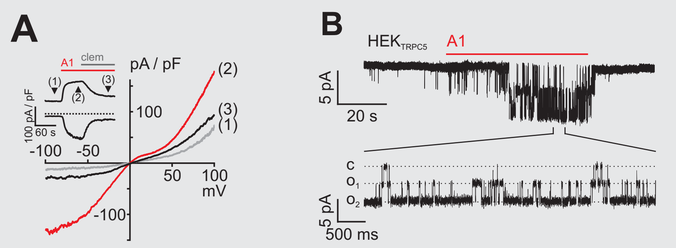




RBI-A1



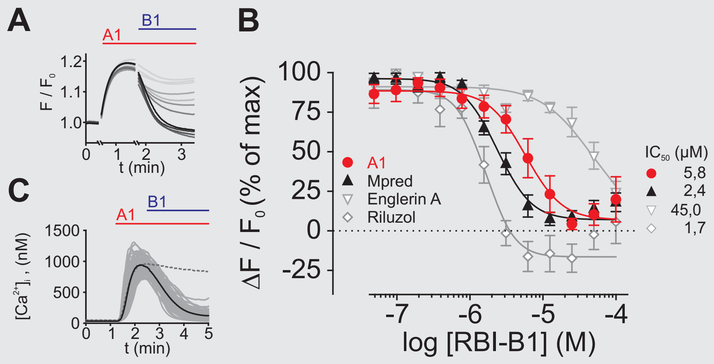
Fluorometric Ca^{2+} assay in TRPC5 overexpressing HEK293 cells (HEK_{TRPC5}). Fluo-4-loaded cells (A-B) or Fura-2-loaded cells (C) were treated with different concentrations of A1 as well as established TRPC5 inhibitors as indicated.



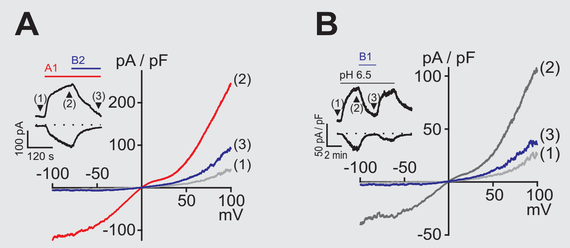
Electrophysiologic recordings of TRPC5 currents in whole cell (A) or inside-out (B) configurations. Cells or membrane patches were treated with 10 µM A1 as indicated. A1 elicits TRPC5-like currents.

Fluorometric Ca^{2+} assay with HEK293 cells that stably express various TRP channels. Cells were treated with 100 µM of A1 or a respective channel activator as indicated.

RBI-B1



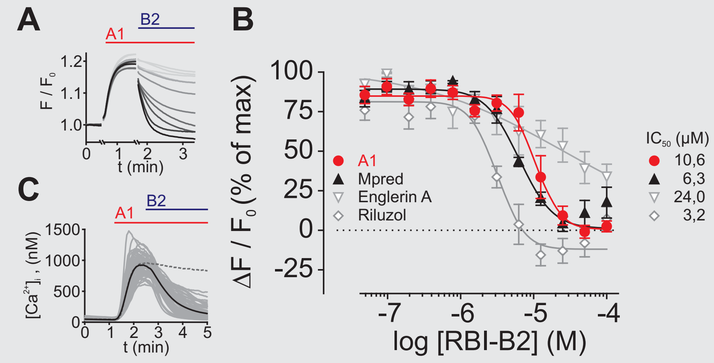
Fluorometric Ca^{2+} assay in TRPC5 overexpressing HEK293 cells (HEK_{TRPC5}). Fluo-4-loaded cells (A-B) or Fura-2-loaded cells (C) were treated with different concentrations of B1 as well as established TRPC5 activators as indicated.



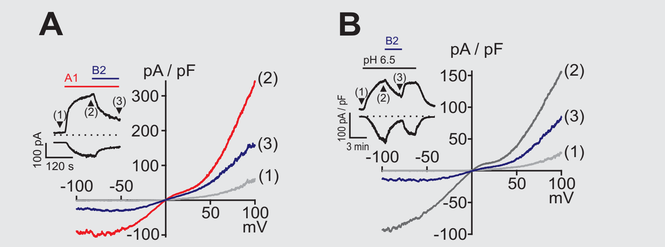
Electrophysiologic recordings of HEK_{TRPC5} cells in the whole cell configuration. Cells were activated either with 10 µM A1 or by change in pH as indicated in the inset. Inhibition was achieved by applying 10 µM B1.

Fluorometric Ca^{2+} assay with HEK293 cells that stably express various TRP channels. Cells were treated with 100 µM of B1 and respective channel activators as indicated.

RBI-B2



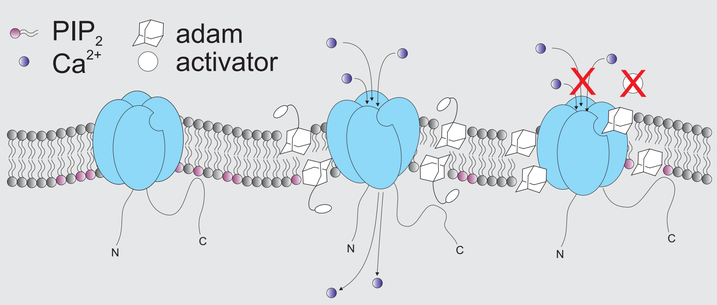
Fluorometric Ca^{2+} assay in TRPC5 overexpressing HEK293 cells (HEK_{TRPC5}). Fluo-4-loaded cells (A-B) or Fura-2-loaded cells (C) were treated with different concentrations of B2 as well as established TRPC5 activators as indicated.



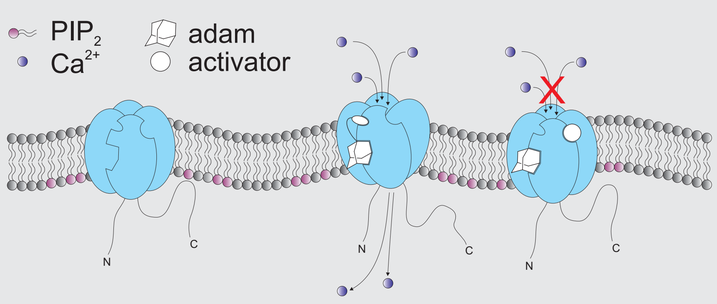
Electrophysiologic recordings of HEK_{TRPC5} cells in the whole cell configuration. Cells were activated either with 10 µM A1 or by change in pH as indicated in the inset. Inhibition was achieved by applying 20 µM B2.

Fluorometric Ca^{2+} assay with HEK293 cells that stably express various TRP channel. Cells were treated with 100 µM of B2 and respective channel activators as indicated.

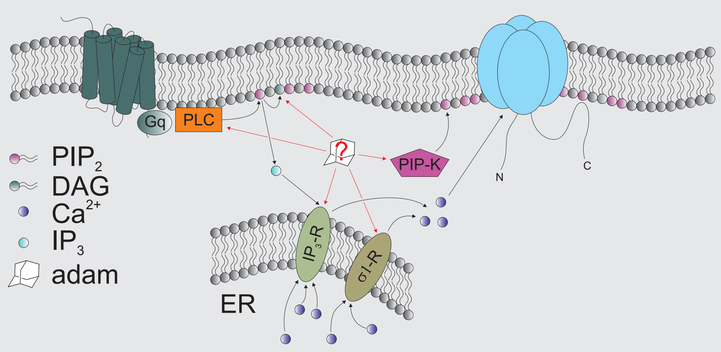
1 change in the channel surrounding



2 direct channel interaction



3 interaction with mechanisms upstream of the channel



Unless otherwise stated, standard concentrations used were: 10 µM (A1, B1) and 20 µM (B2, clem). Concentration response analyses were run in two-fold dilution steps, typically starting at 100 µM.

[1] Nilius & Owsianik (2010); Pflugers Arch. 460: 437-450
[2] Greka et al. (2003); Nature Neurosc. 6: 837-845
[3] Bröker-Lai et al. (2017); The EMBO Journal 36: 2261-2807
[4] Beck et al. (2017); Endocrinology 158: 887-902.
[5] Puram et al. (2011); Genes Dev. 25: 2659-2673.
[6] Riccio et al. (2009); Cell 137: 761-772.