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Novel Methodology to Identify TRPV1 Antagonists Independent of Capsaicin Activation

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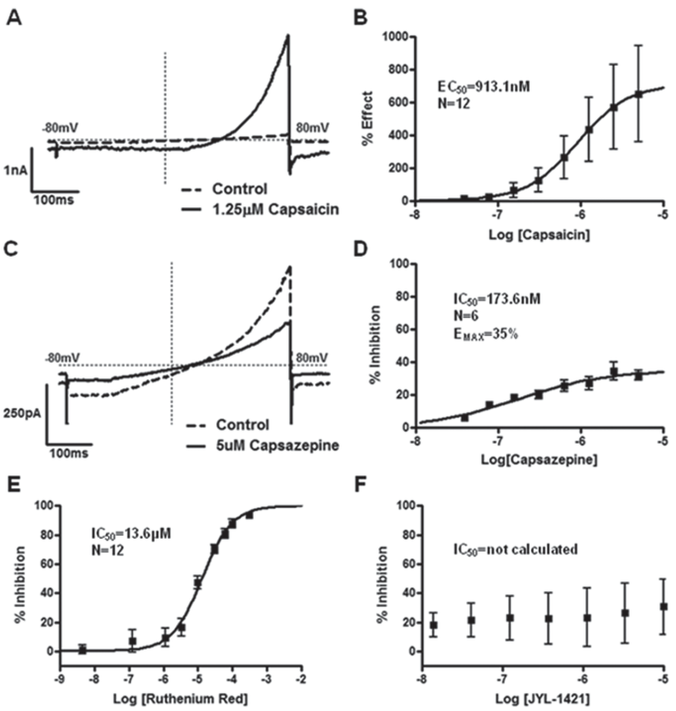
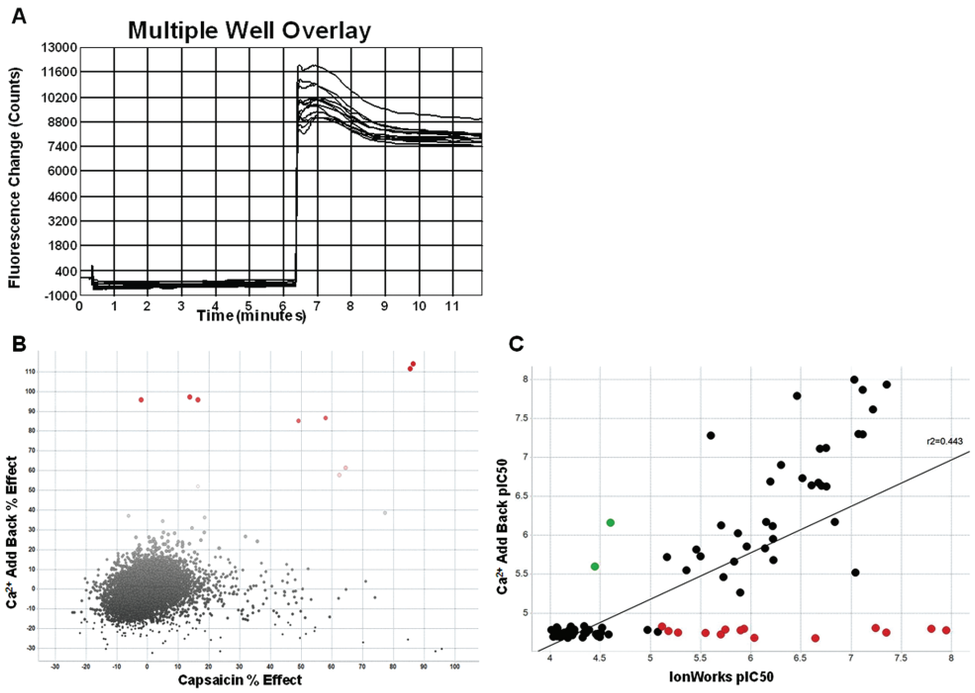
Abstract

TRPV1 was originally characterized as an integrator of various noxious stimuli such as capsaicin, heat, and protons. TRPV1- null mice exhibit a deficiency in sensing noxious heat stimuli, suggesting that TRPV1 is one of the main heat sensors on nociceptive primary afferent neurons and a candidate target for heat hypersensitivity in chronic pain. Several different potent and selective TRPV1 antagonists have been developed by more than 50 companies since the characterization of the receptor in 1997. A consequence of this competitive interest is the crowding of patentable chemical space, because very similar in vitro screening assays are used. To circumvent this issue and to expand our understanding of TRPV1 biology, we sought to take advantage of recent advancements in automated patch-clamp technology to design a novel screening cascade. This SAR-driving assay identified novel modulators that blocked the depolarization-induced activation of outwardly-rectifying TRPV1 currents independent of agonist stimulation, and we correlated the pharmacology to three other innovative assays for higher-throughput screening. Ultimately, we have identified a screening paradigm that would have good predictive value for future TRPV1 drug discovery projects and novel chemical space with a higher probability of gaining intellectual property coverage.

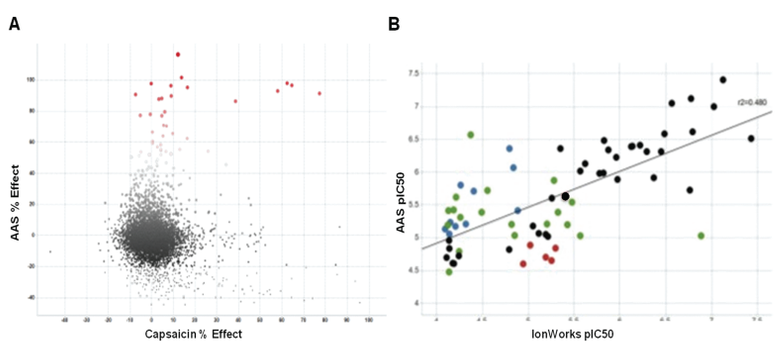
Results



(A) Current response to voltage-ramp stimulus on hTRPV1-CHO/Trex cell in the absence (dotted line) and presence of 1.25µM capsaicin (solid line). (B) Dose-response relationship of capsaicin applied to hTRPV1-expressing cells using a voltage-ramp stimulus to activate the channels. The peak current amplitude at +80 mV was used to measure capsaicin potency. (C) Basal response (dotted line) and in the presence of 5 µM capsazepine (solid line) for voltage-ramp stimulus on hTRPV1-expressing cell. (D) Dose-response relationship for capsazepine using the current amplitude at +80 mV as a measure of efficacy. Capsazepine was able to completely inhibit the voltage-evoked TRPV1 currents. Dose-response relationships for ruthenium red (E) and JYL-1421 (F) using the voltage-ramp protocol. Ruthenium red completely inhibited voltage-gated TRPV1 currents, whereas JYL-1421 had no effect.



(A) Example of responses elicited by the addition of 4.8 mM CaCl to hTRPV1-HEK/T-rex–expressing cells that were loaded with a Fluo-4 Ca2+-sensing dye in the virtual absence of Ca2+. (B) Comparison of the percentage inhibition of responses elicited when a high concentration of CaCl (4.8 mM) is applied to hTRPV1-HEK/Trex–expressing cells with the percentage inhibition on 100 nM capsaicin-elicited responses. Both are measured with a Fluo-4 Ca2+-sensing fluorophore. A single concentration of 10 µM was used for all of the compounds tested. (C) Correlation between the pIC50 values for inhibiting the calcium add-back responses and the agonistindependent voltage-gated responses measured with an IonWorks automated patch-clamp system. The compounds tested were a mix of known TRPV1 modulators and compounds identified in A with a >50% inhibition on the calcium add-back assay. Compounds highlighted in red are considered false-negatives, blue are false-positives, and green are the novel compounds never identified by a previous capsaicinbased assay.



(A) Comparison of the percentage inhibition of spontaneous rubidium influx into hTRPV1-CHO/Trex–expressing cells measured by atomic absorption spectroscopy with the percentage inhibition of 100 nM capsaicin-elicited responses measured with a Fluo-4 Ca2+-sensing fluorophore. A single concentration of 10 µM was used for all the compounds tested. (B) Correlation between the pIC50 values for inhibiting the atomic absorption spectroscopy responses and the agonist-independent voltage-gated responses measured with an IonWorks automated patch-clamp system. The compounds tested were a mix of known TRPV1 modulators and compounds identified in A with a >50% inhibition on the atomic absorption spectroscopy assay. Compounds highlighted in red are considered falsenegatives, blue are false-positives, and green are the novel compounds never identified by a previous capsaicin-based assay.

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