Herbal Liqueurs, Tactile Mouthfeel, and PIEZO2: *A Prep-HPLC based search for natural modulators of the mechanosensory ion channel PIEZO2*

Nicholas Villarino, Brandon Orzolek, Ariya Uyeno, Grace Love, Oleg Yarishkin, Eric Mulhall, Jason Lee, Jason Chen, and Ardem Patapoutian

The Scripps Research Institute, La Jolla, CA, USA

Introduction

Texture and "mouthfeel" arise from mechanical, thermal, and chemical cues acting on oral and facial tissues. Beyond taste and smell, many foods and botanicals evoke potent tactile sensations such as tingling, buzzing, or numbing, whose molecular basis is poorly understood. For chemical irritants the logic is well worked out (capsaicin → TRPV1, mustard oil → TRPA1, menthol → TRPM8), but the transducers of *tactile* chemesthesis remain largely unknown, and none have been cleanly tied to a mechanosensor *in vivo*.

Given PIEZO2's prominent role in oral and trigeminal mechanosensation, we wondered whether tactile mouthfeel might have a parallel basis: just as natural products act on TRP channels to produce chemesthetic sensations, foods and botanicals may contain chemesthetic agents that directly modulate PIEZO2. Herbal liqueurs and bitters, which are often complex mixtures of plant matter long valued for "numbing", "tingling", and other orosensory profiles, may be one untapped source of such modulators. We screened a panel of herbal liqueurs for effects on PIEZO2 and found that Chartreuse Élixir Végétal, a liqueur made from 130+ botanicals, was the top hit, providing a natural starting point to ask whether its chemesthetic compounds can directly tune PIEZO2 activity and contribute to mechano-related mouthfeel.

Methods

FM 1-43 functional screen. PIEZO2 activity was assayed by FM 1-43 dye uptake, a PIEZO2-dependent functional readout (Villarino et al., 2023), in HeLa cells stably expressing PIEZO2. Cells were loaded with FM 1-43 plus test agent or vehicle, and uptake was quantified by flow cytometry , expressed as mean fluorescence intensity.

Activity-guided preparative HPLC. Liqueurs were fractionated on a Waters prep C18 system (water/acetonitrile gradient, mass-triggered collection) into 46 timed fractions per run, which were dried, screened by FM 1-43, and prioritized by effect size. Active fractions were identified by NMR (Scripps Automated Synthesis Facility).

Electrophysiology. Mechanically activated PIEZO2 currents were recorded by automated patch clamp (SyncroPatch 384, M-Stim 4x-hole chips) in HeLa Piezo1 KO cells stably expressing human PIEZO2. Dose-response data were fit with a Hill equation (slope = −1.0); analogs were screened on the same platform, normalized to vehicle (DMSO).

Von Frey mechanical sensitivity. C57BL/6J mice received a 20 µL intraplantar injection of 500 µM Coumarin #1 or vehicle (10% DMSO, 2% PEG400, 0.5% Tween80 in saline). Paw withdrawal across von Frey forces (0.008 to 4 g) was measured at 30 and 60 min using a force-response scheme;.

Results

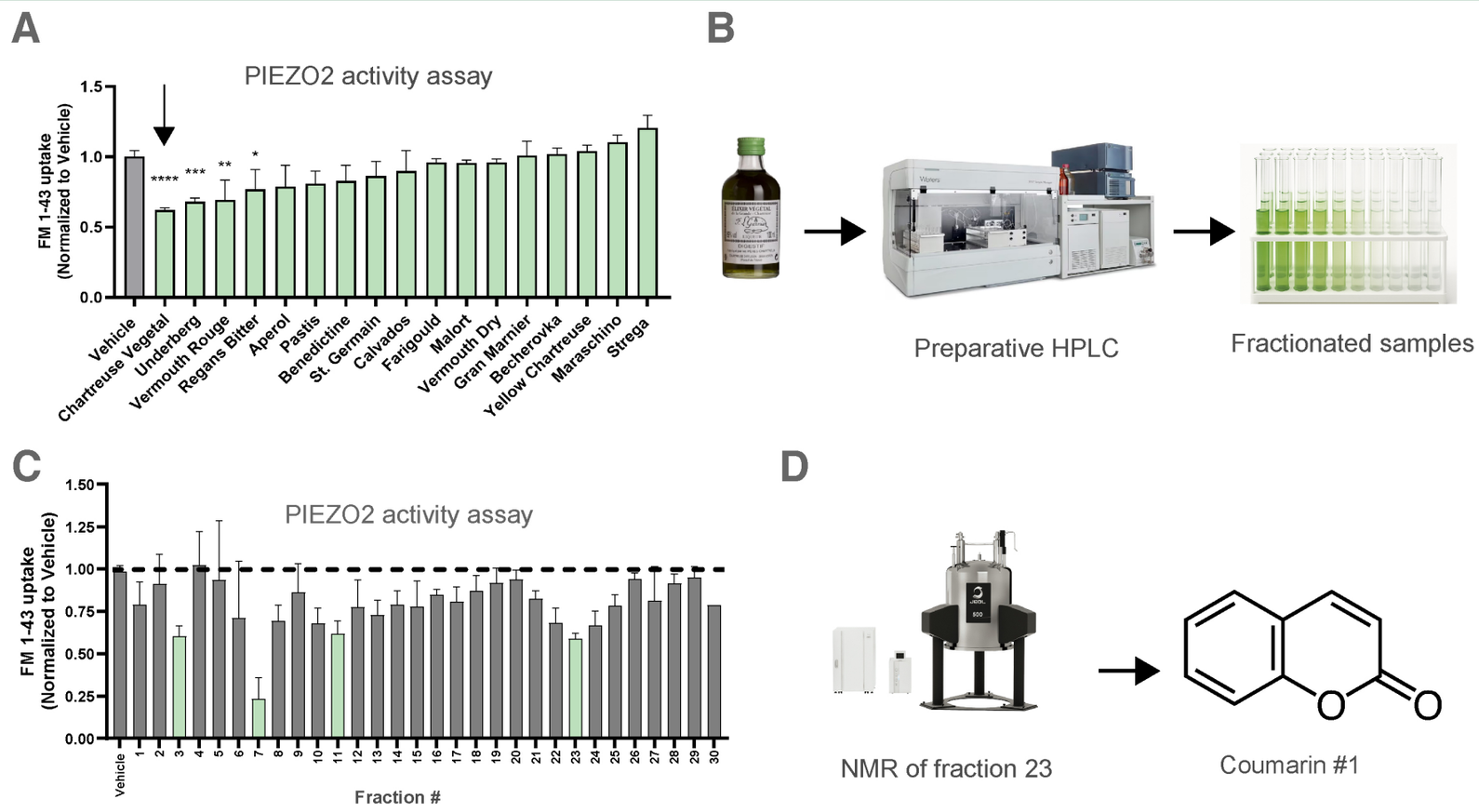

Figure 1: Identification of Piezo2 modulators in herbal liqueurs. (A) FM 1-43 based assay of PIEZO2 activity in HeLa cells stably expressing PIEZO2 (B) Schematic diagram of preparative HPLC (C18 column) for generation of fractions from Chartreuse Élixir Végétal. (C) Evaluation of bioactivity of fractions using the FM 1-43 PIEZO2 assay. (D) NMR of fraction 23 revealed Coumarin #1 as the most abundant chemical species.

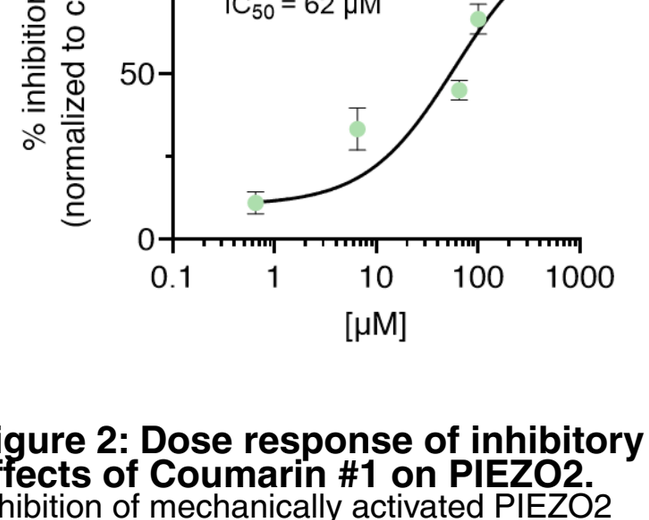

Figure 2: Dose response of inhibitory effects of Coumarin #1 on PIEZO2. Inhibition of mechanically activated PIEZO2 current (SyncroPatch 384, M-Stim; HeLa P1KO stably expressing human PIEZO2) versus Coumarin #1 concentration, fit with a Hill equation (slope = −1.0). Mean ± SD, n = 4–6 per concentration.

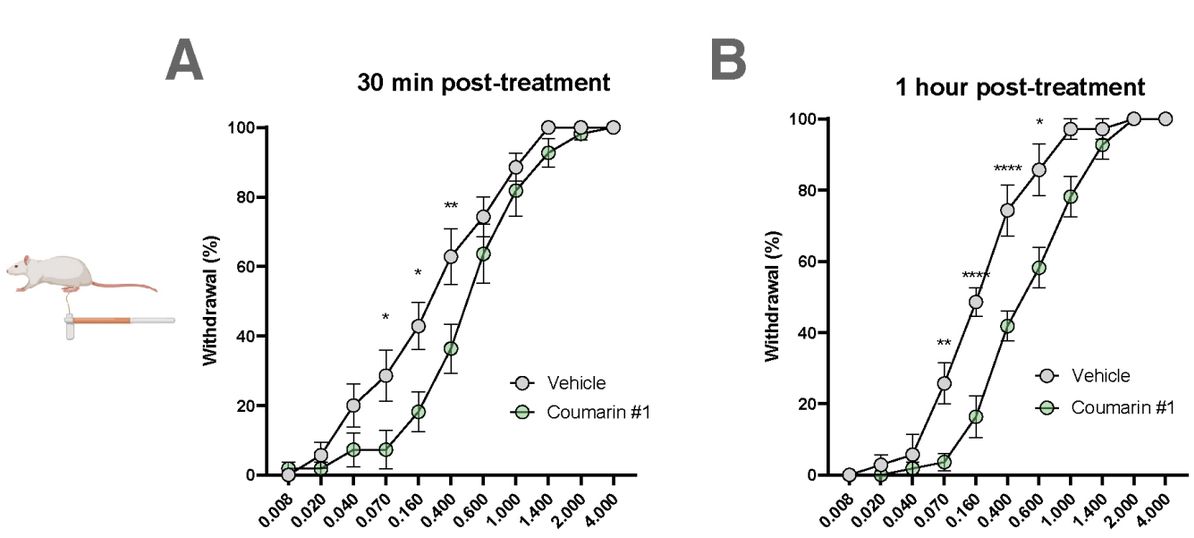

Figure 3. Coumarin #1 reduces mechanical sensitivity in vivo. Hind-paw withdrawal across von Frey forces (0.008 to 4 g) after intraplantar 500 µM Coumarin #1 or vehicle, at (A) 30 min and (B) 1 h. Withdrawal was reduced at low-to-intermediate forces, more so at 1 h. Mean ± SEM; n = 7 vehicle, 11. Coumarin #1; two-way ANOVA (* p<0.05, ** p<0.01, **** p<0.0001).

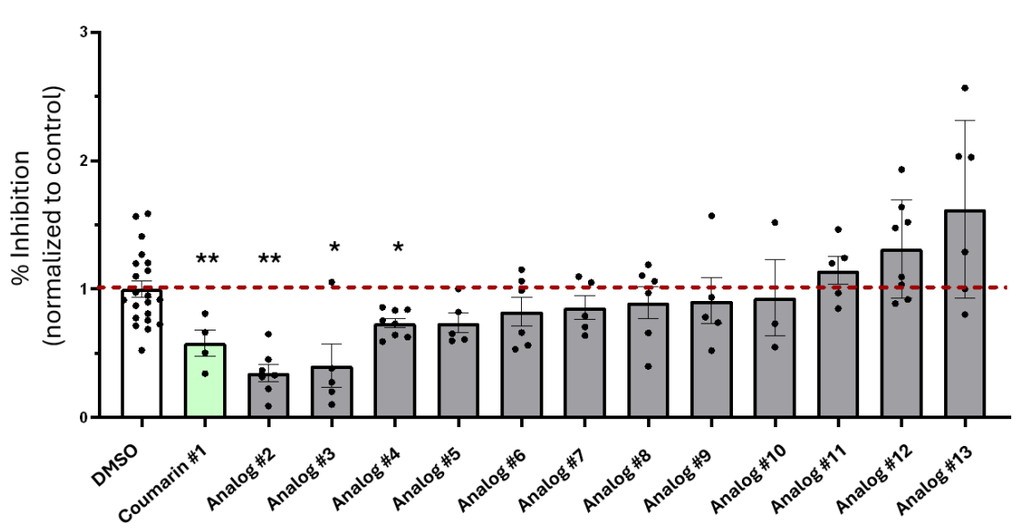

Figure 4. Structure-activity relationship across natural analogs. Normalized PIEZO2 current (SyncroPatch 384, M-Stim) for DMSO, Coumarin #1, and analogs #2–13. Inhibition was non-uniform: Bars, mean ± SEM; dots, replicates (* p<0.05, ** p<0.01 vs. DMSO).

Summary

- A functional FM 1-43 screen identified Chartreuse Élixir Végétal as a top hit for PIEZO2 inhibition
- Activity-guided preparative HPLC and NMR identified Coumarin #1 as the most abundant active in Fraction 23
- Coumarin #1 inhibits mechanically activated PIEZO2 current, IC_{50} = 62 µM
- In mice, Coumarin #1 reduced touch sensitivity
- Inhibition is shared by some, but not all, related natural analogs, pointing to specific structural determinants

Future Directions

- Human psychophysics: R-index and lingual forced-choice test (John Haye/Helene Hopfer at PSU)
- Mechanism of inhibition: Direct binding, membrane-mediated mechanism, etc.
- SAR to improve potency
- Exploratory screening: Several other active sources with bidirectional effects on PIEZO2 activity