Human sensory neurons: Excitability, sensitization, and multiple potassium channel conductances

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Rationale

Biological differences in sensory processing between human and model organisms may present significant obstacles to translational approaches in treating chronic pain. Such obstacles may include functional differences in target receptor pharmacology and signaling or fundamental differences in neuronal physiology.

We propose that target validation for novel analgesics can be enhanced by examining human sensory neuron physiology in vitro at the basic science stage. We believe that enhancing preclinical target validation will promote higher success rates in future clinical trials. However, little is currently known about human sensory neuron physiology.

Therefore, we recorded from >150 human DRG neurons in vitro in an attempt to characterize their electrophysiological profiles, ask whether they respond to noxious chemicals and inflammatory mediators that can induce peripheral sensitization, and explore the possibility of blocking sensitization in hDRG neurons with a pharmacological approach targeting group II metabotropic glutamate receptors. Finally, we began to explore the various conductances in voltage clamp important in maintaining and modulating the excitability of these neurons.

Methods

Human DRGs were isolated from U.S. organ donors with full legal consent for use of tissue for research. DRG from thoracic levels were dissected and enzymatically digested before dissociated cells were seeded onto glass coverslips pre-coated with poly-D-lysine.

Culture media: DMEM F-12 (Lonza) w/ 10% horse serum (Thermo Fisher Scientific), 2 mM glutamine, 25 ng/mL GDNF (Peprotech), 25 ng/mL NGF (Cell Signalling), Penicillin/Streptomycin (Thermo Fisher Scientific).

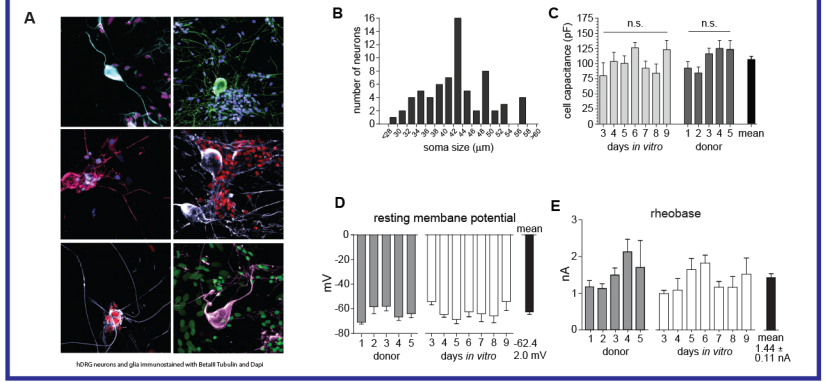
Electrophysiology: Whole-cell recording in current clamp with 2-4 MΩ pipettes. External solution (in mM): 145 NaCl, 3 KCl, 2.5 $CaCl_2$, 1.2 $MgCl_2$, 10 HEPES, 7 Glucose, pH 7.4. Internal solution (in mM): 130 K-gluconate, 5 KCl, 5 NaCl, 3 Mg-ATP, 0.3 EGTA, 10 HEPES, pH 7.3. For K+ channel isolation: External solution contained (in mM): 130 choline chloride, 10 sodium chloride, 3 potassium chloride, 1.8 calcium chloride, 1.2 magnesium chloride, 0.2 cadmium chloride, 10 HEPES, 10 glucose, adjusted to pH 7.2 with KOH and 310 mOsm with sucrose. Internal solution (in mM): 140 K-gluconate, 0.1 cadmium chloride, 1 calcium chloride, 1.8 magnesium chloride, 10 EGTA, 10 HEPES, 2 Mg-ATP, 0.4 Mg-GTP, adjusted to pH 7.4 and 325 mOsm.

Chemicals: Bradykinin (100 nM), PGE_2 (1 μM), AITC (30 μM), ATP (100 μM), histamine (100 μM), chloroquine (100 μM); 4-Aminopyridine (4mM), Tetraethylammonium (60mM); all from Sigma.

	Age	Sex	BMI	Ethnicity	Cause of Death
Donor 1	21	Male	22.9	Caucasian	Anoxia
Donor 2	13	Male	20.0	Caucasian	Head trauma
Donor 3	19	Male	26.9	Asian	Head trauma
Donor 4	19	Female	26.1	Caucasian	Stroke
Donor 5	19	Male	25.3	Hispanic	Stroke

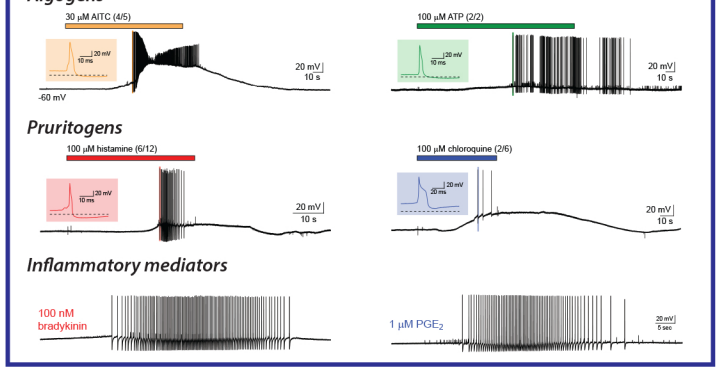
Results

1 Physical characteristics and electrical properties of human sensory neurons *in vitro*

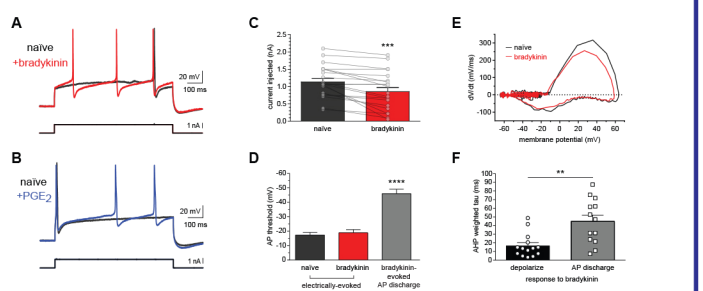


hDRG neurons and glia immunostained with Betatit Tubulin and Dapi

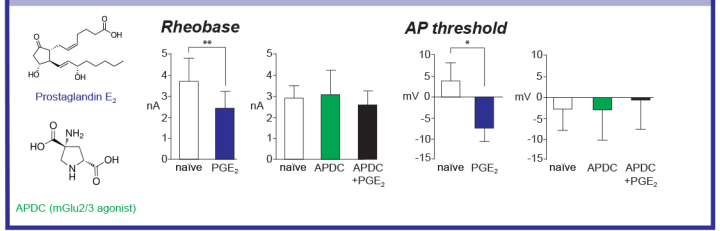
2 Responses of hDRG neurons to chemicals that induce pain, itch, and inflammation



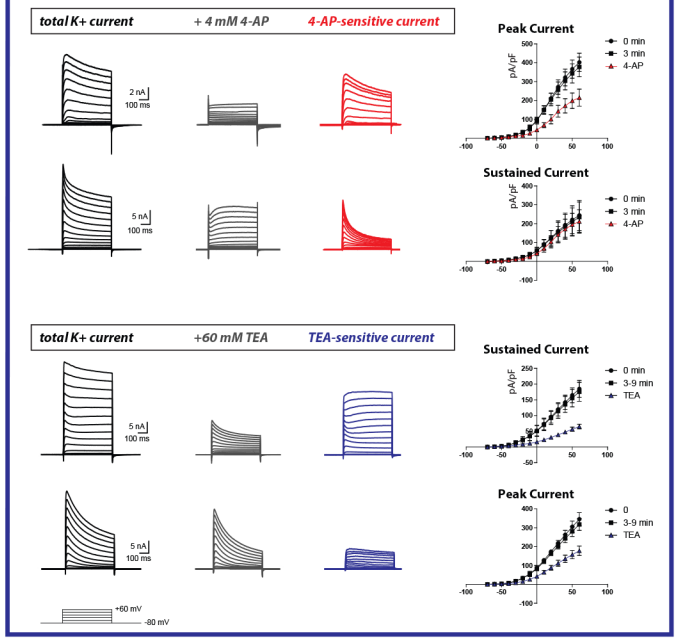
3 Senstization of hDRG neurons by bradykinin and prostaglandin E2



4 PGE_2-induced sensitization is blocked in hDRG neurons by group II mGluR activation



5 Native hDRG potassium currents possess blockable transient and sustained components



Key Points

- Target validation in human sensory neurons can enhance analgesic discovery by confirming or refuting functional similarities between animal models and human and may provide a better understanding for dosing in humans.
- Human sensory neurons can respond to AITC, ATP, histamine, and chloroquine indicating a robust chemosensitivity that may exceed that of rodents.
- Sensitization by bradykinin and prostaglandin E_2 occurs in human sensory neurons. Bradykinin reduced rheobase (but not threshold) and altered the action potential waveform. PGE_2 reduced both rheobase and threshold.
- The effects of PGE_2 on the excitability of human sensory neurons could be blocked by the group II metabotropic glutamate receptor agonist APDC. Activation of group II mGluRs in naïve hDRG neurons produced no changes to excitability.
- hDRG neurons possess transient and sustained types of outward, voltage-dependent potassium currents, blockable with 4-AP and TEA, respectively.

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