Ex vivo human models of extracellular acidification in inflammatory pain states for enabling translational research and drug discovery

Anh-Tuan Ton, Yannick Miron, Tim Indersmitten, Phachareeya Ratchada, Katrina Sweat, Guy Page, Paul Miller and Andre Ghetti

AnaBios Corp., San Diego, CA 92109, USA

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Early Human Insights

Introduction

The discovery and development of new pain therapeutics has been complicated by the lack of reliable preclinical models. To address this challenge we have established a novel preclinical discovery strategy, which relies on the *ex vivo* interrogation of human sensory neurons isolated from organ donors. In the current study, we describe the use of this model for studying the potential of new compounds to treat different types of pain. By varying the culture conditions to model inflammatory or neuropathic states, we investigate how the physiological as well as the pharmacological properties of the cells are altered in different pain modalities.

Methods

Cell isolation & culture. Ethically consented human DRGs (hDRGs) were collected within 2 hrs. post-mortem and immediately transferred to AnaBios' cold preservation solution. Cells were enzymatically dissociated and plated on PDL-coated glass coverslips and cultured in DMEM-F12 in the presence of 10 ng/mL NGF and 10 ng/mL GDNF, at 37°C with 5% CO_2 .

Fluorescence calcium imaging. Cells were loaded with Fluo-8-AM and were excited at 480nm while emission was collected at 100 Hz at 520 nm with a pcoEDGE sCMOS camera mounted on an inverted microscope.

Electric field stimulation (EFS). A pair of carbon fiber rods was used as stimulating electrodes; trains of biphasic pulses were used (10 ms duration, at 5 Hz). Stimulus intensity was set at 7.5 V/cm to 15 V/cm.

Electrophysiology. Individual hDRG neurons are stimulated at different frequencies (0.1, 1, 3 and 10 Hz) with trains of 120 depolarization pulses (20 ms) delivered at 150% above rheobase. Inflammatory agents (500 nM Prostaglandin E_2 (PGE_2) and 50 nM bradykinin (BK)) were incubated for 72hrs.

Compounds used. PF-05089771 and A-803467 at 0.01, 0.03 and 0.1 µM. Raxatrigine at 0.3, 1 and 3 µM. Carbamazepine at

Results

EFS recordings of human DRG neurons

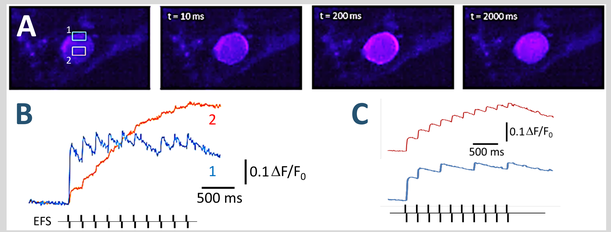

Figure 1. Intracellular calcium transients elicited by EFS in human DRG neurons. A. Time lapse images from a hDRG neuron. **B**. Ca^{2+} transients at submembrane location (ROI/Trace #1) and cytoplasmic location (ROI/Trace #2) in the cell in (A). **C.** Total whole-cell Ca^{2+} transients measured in 2 hDRG neurons. *Red trace*: neuron following the entire stimulus train. *Blue trace*: neuron partially following the stimulus train.

Inflammation alters human DRG physiology

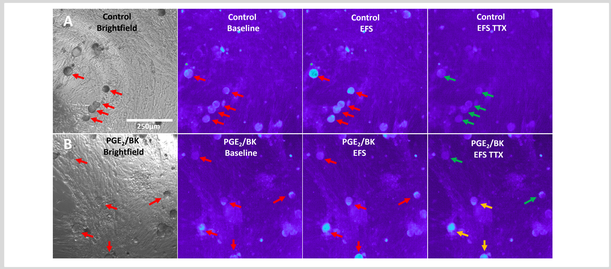

Figure 2. EFS recordings of human DRG neurons under control conditions and after treatment with PGE_2/BK.
A. Example of EFS recordings under control conditions. **B.** Example of EFS-induced responses following treatment with PGE_2/BK (PGE_2 : 1 µM and BK : 100 nM for 2 hrs.) : compared to the control, a smaller number of cells exhibit sensitivity to TTX (green arrows: TTX-S; yellow arrows : TTX-R).

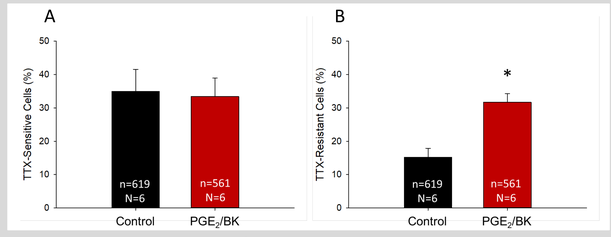

Figure 3. Effect of PGE_2/BK on TTX sensitivity of human DRG neuron on EFS-induced responses. A. Proportion of TTX-S neurons is unchanged by treatment with PGE_2/BK for 2 hrs. **B.** Treatment with PGE_2/BK increase proportion of TTX-R neurons. (n = number of cells; N = number of donors).

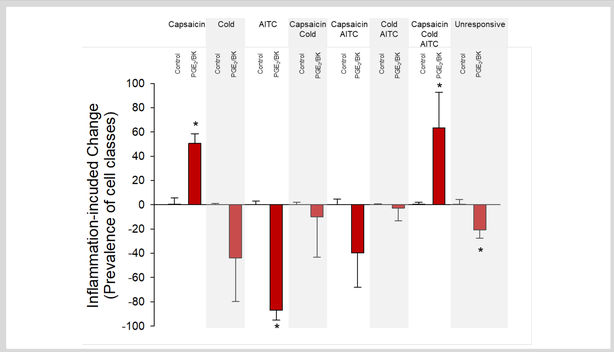

Figure 4. Effect of PGE_2/BK on human DRG responses to capsaicin, AITC and cold buffer. hDRG neurons were incubated for 2 hrs. in PGE_2 (1 µM) and BK (100 nM). Cells were challenged, sequentially, with Capsaicin (100 nM), AITC (50 mM) and ~13°C cold buffer. Change in the prevalence of cells with selective (single agonist) response or responsive to multiple agonists, is reported (Control: n = 306; Inflammation: n = 260 from 4 donors).

Current-clamp recordings of human DRG neurons

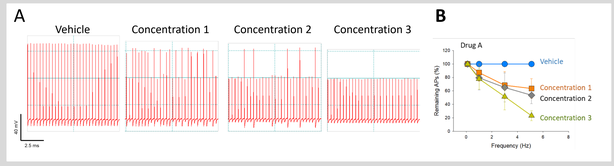

Figure 5. Dose and frequency-dependence of action potential inhibition in human DRG neurons. Individual hDRG neurons are stimulated at different frequencies in current-clamp mode with trains of 120 depolarization pulses delivered at 150% above threshold **(A)** to investigate frequency- and state-dependence of analgesics **(B)**. Rheobase is measured to determine changes in hDRG excitability (not shown).

Inflammation modulates Na_V-blocker efficacy

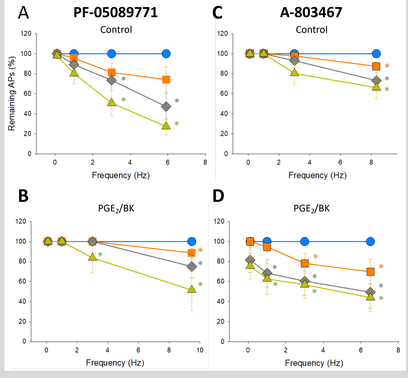

Figure 6. hDRG action potential inhibition by $Na_V1.7$-selective blocker PF-05089771 and $Na_V1.8$-selective blocker A-803467. The inhibitory activity of PF-05089771 (left) was tested in hDRG neurons cultured in control conditions (n = 12) **(A)**, or in the presence of 500 nM PGE_2 and 50 nM BK for 72 hrs. (n = 9) **(B)**. Inhibitory activity of A-803467 (right) was tested in control n = 9 **(C)**, or in inflammation, n = 10 **(D)**.

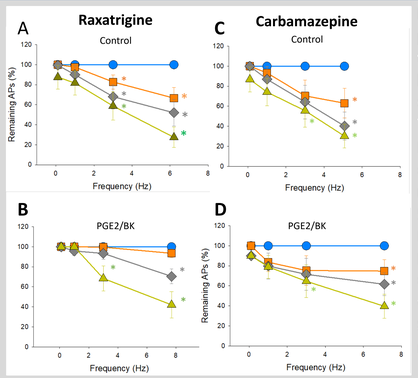

Figure 7. hDRG action potential inhibition by the non-selective Na_V-blockers Raxatrigine Carbamazepine.
The inhibitory activity of Raxatrigine (left) in control conditions (n = 9) **(A)**, or in the presence of 500 nM PGE_2 and 50 nM BK for 72 hrs. (n = 10) **(B)**. Inhibitory activity of Carbamazepine (right) was tested in control n = 8 **(C)**, or in inflammation, n = 10 **(D)**.

Inflammatory acidosis can decrease drug efficacy

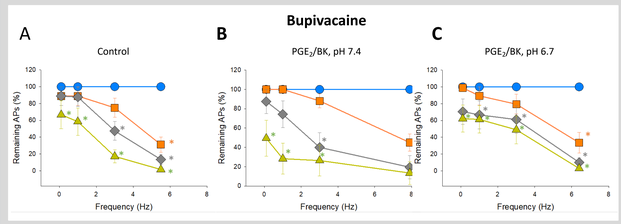

Figure 8. hDRG action potential inhibition by Bupivacaine.
Inhibitory activity of Bupivacaine in control conditions (n = 8) **(A)**, in the presence of 500 nM PGE_2 and 50 nM BK for 72 hrs. with a physiological pH of 7.4 (n = 9) **(B),** or during acidification with pH of 6.7 (n = 9) **(C)**.

Conclusion

1- EFS / fluorescent-based Ca^{2+} imaging enables the efficient profiling of the phenotype and pharmacological sensitivity of hDRG neurons.
2- *Ex vivo* treatments can model pathological states (such as chronic inflammatory-neuropathy), leading to changes in neuronal properties.
3- Pathological changes in hDRG properties result in different pharmacological sensitivity to target-specific selectivity. The two classes of cells exhibit different responses depending on pathological states.
Finally, this approach allows human-specific profiling of new drug candidates in functionally defined sub-populations of cells, improving the ability to predict the therapeutic efficacy in different clinical conditions.