Carbamazepine and CNV1427400 exhibit similar pharmacology in recombinant systems, rat and human native DRGs

S. N. TATE1, F. RUGIERO1, D. DERJEAN1, V. A. PANCHENKO2, D. OWEN1, A. GHEITI2, K. BANNISTER3, M. THAKUR3, A. H. DICKENSON3, P. MILLER2, *V. MORISSET1
1Convergence Pharmaceuticals Ltd, Cambridge, United Kingdom; 2AnaBios Corp., San Diego, CA; 3NPP, Univ. Col. London, London, United Kingdom

Introduction
Anticonvulsants decrease the hyperactivity of the pain network by targeting the ionic channels involved in the initiation and propagation of neuronal firing, such as voltage-gated sodium channels. However, current therapies are often associated with inconsistent efficacy and poor tolerability. In recent years there has been a huge effort from the pharmaceutical industry to develop new sodium channel blocking agents with improved pharmacology and safety profiles. It is therefore very important to confirm that the pharmacology and mechanism of action determined in recombinant human reagents or native rodent cells will translate to human native nerves. We have used whole cell patch-clamp electrophysiology to investigate the in vitro properties of Carbamazepine and CNV1427400, a novel sodium channel blocker, in human recombinant sodium channels, rat DRGs and human native DRGs from healthy donors.

Methods

Rat DRGs:
DRGs were obtained from neonatal rats (P7-10). Animals were euthanized in accordance with the 1986 Animals (Scientific Procedures) Act (Schedule 1). DRGs were dissected and dissociated by a prolonged collagenase treatment (0.1% in HBSS for 50 minutes at 37°C), followed by mechanical dissociation of the cells. They were then plated onto Laminin-coated coverslips (50,000 cells/ml).

Human DRGs:
All human tissues used for the study were obtained by legal consent from organ donors in the US. Donor DRGs were harvested using AnaBios proprietary surgical techniques. DRGs were then further dissected in cold neuropilic solution to remove all connective tissue and fat. The ganglia were enzymatically digested and the isolated neurons put in culture in DMEM F-12 supplemented with Glutamine 2mM, FBS 10%, HNGF (10ng/ml), hGDNF (10ng/ml) and Penicillin / Streptomycin. Cells from 3 donors were used in this study.

Results

CNV1427400 and Carbamazepine are state-dependent blockers of human recombinant Nav1.7, Nav1.2 and Nav1.6

External solution (in mM): NaCl 124, KCl 3, CaCl2 2, MgCl2 1, HEPES 10, Glucose 10 and CRE0 0.25. Internal solution (in mM): NaCl 130, CsF 30, GABA 2, CRE0 0.25. External solution (in mM): NaCl 130, CsF 30, GABA 2, CRE0 0.25. Internal solution (in mM): NaCl 130, CsF 30, GABA 2, CRE0 0.25. Recordings made at 37°C. Cells were obtained from 3 donors.

Functional consequence of state-dependent block by CNV1427400: Inhibition of firing is frequency-dependent in rat DRGs

CNV1427400 shows a similar frequency-dependent inhibition of firing in human native DRGs

External solution (in mM): NaCl 124, KCl 3, MgCl2 1, CaCl2 2, Glucose 10 and HEPES 10. External solution (in mM): NaCl 130, CsF 30, GABA 2, CRE0 0.25. Recordings made at 37°C. Cells were obtained from 3 donors.

Conclusions

- In human native and recombinant Navs, Carbamazepine and CNV1427400 show similar potency and state-dependent mechanism of block, translating into a similar frequency-dependent inhibition of action potential firing in rat and human native DRGs.
- Extending in vitro electrophysiology studies to human DRGs is a critical technological breakthrough and adds great value from a translational point of view when considering clinical dose prediction for the development of new analgesics.